NanoPass Technologies Ltd.
Delivering Precision

MicronJet™

Scientific Publications Booklet

The **MicronJet™** device has been tested in multiple clinical studies and peer-reviewed publications, highlighting its safety and efficacy, as well as its groundbreaking MEMS (Micro-Electro-Mechanical Systems) technology.

This booklet presents some of the scientific evidence supporting the device's precise, minimally invasive intradermal delivery, demonstrating its unparalleled accuracy and reliability. It further underscores the device's suitability for diverse therapeutic, medical, and aesthetic applications.

Section 1

Vaccines

MicronJet™ is an easy-to-use, consistent, and efficient intradermal delivery device for virtually pain-free vaccination.

MicronJet™ has demonstrated improved immunogenicity and/or dose sparing compared to subcutaneous and intramuscular delivery.

MicronJet™ has demonstrated improved immunogenicity and/or potency compared to other intradermal devices.

PRODUCT REVIEW

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Intradermal vaccination using the novel microneedle device MicronJet600: Past, present, and future

Yotam Levin[1,*], Efrat Kochba[1], Ivan Hung[2], and Richard Kenney[3]

[1]NanoPass Technologies; Nes Ziona, Israel; [2]State Key Laboratory for Emerging Infectious Diseases; Carol Yu's Center for Infection and Division of Infectious Diseases; The University of Hong Kong; Queen Mary Hospital; Hong Kong Special Administrative Region; Hong Kong, China; [3]Immune Design Corp; San Francisco, CA USA

Keywords: dose-sparing, immunogenicity, influenza vaccine, intradermal, Mantoux, microneedles, vaccine delivery, vaccine device

Abbreviations: ID, intradermal; BCG, Bacillus Calmette–Guérin; PPD, Purified protein derivative; HBV, hepatitis B virus; WHO, World Health Organization; SAGE, Strategic Advisory Group of Experts; IPV, inactivated polio vaccine; SQ, subcutaneous; IM, Intramuscular; BD, Becton Dickinson; GMT, geometric mean titer; HIV, Human immunodeficiency virus; DTP, diphtheria, pertussis and tetanus; HPV, human papilloma virus; MEMS, Micro Electro Mechanical System; FDA, Food and Drug Administration; EMEA, European Medicines Agency; HA, hemagglutinin; AE, adverse event; CDC, Center of Disease Control; icddr,b, International Center for Diarrheal Disease Research, Bangladesh

Intradermal immunization has become a forefront of vaccine improvement, both scientifically and commercially. Newer technologies are being developed to address the need to reduce the dose required for vaccination and to improve the reliability and ease of injection, which have been major hurdles in expanding the number of approved vaccines using this route of administration. In this review, 7 y of clinical experience with a novel intradermal delivery device, the MicronJet600, which is a registered hollow microneedle that simplifies the delivery of liquid vaccines, are summarized. This device has demonstrated both significant dose-sparing and superior immunogenicity in various vaccine categories, as well as in diverse subject populations and age groups. These studies have shown that intradermal delivery using this device is safe, effective, and preferred by the subjects. Comparison with other intradermal devices and potential new applications for intradermal delivery that could be pursued in the future are also discussed.

A Brief History of Intradermal Vaccination

Intradermal (ID) immunization dates back to the advent of vaccines. Variolation (applying scabs or fluids from infected smallpox lesions onto healthy individuals) was practiced in many areas of the world for hundreds of years before the pioneering work of Edward Jenner, who used cowpox scarification for smallpox at the turn of the 19th century.[1-6]

Further major milestones were achieved over a century later by Calmette and Guérin,[7] who developed the BCG vaccine for tuberculosis circa 1921. Tuberculin (PPD) and the Mantoux technique[8] of intradermal injection, which typically uses a standard 25G-27G, 5/8-1.0 (16-25mm) needle for shallow (5–15 degrees) injection into the skin, were developed around the same time.

Importantly, the uptake of the standard ID Mantoux technique is still limited, some hundred years later, to a very narrow list of vaccines (**Table 1**). The Mantoux technique is neither simple nor reliable[9-11] and very often delivers the antigen too deep or it leaks out, failing on occasion to produce the typical 6–10 mm white bleb,[12] thereby limiting adoption of perhaps the most natural and physiological route of delivery of vaccines.

Benefits of ID Vaccination

ID vaccination has primarily been explored for its ability to generate equivalent antibody responses at lower doses, a phenomenon typically described as "dose-sparing".[46] The importance of dose-sparing is most evident in high-surge situations, such as in pandemic[47] and seasonal flu,[48,49] where large populations are at risk and a new set of strains can be required each year.[50] Dose sparing is also important in increasing capacity and reducing the expense of a vaccine dose, especially in cost-sensitive global-health indications where the price of the vaccine limits its use and coverage, as in the case of polio.[51,52] Exploring the intradermal approach was recommended at a recent meeting of the World Health Organization Strategic Advisory Group of Experts (SAGE),[53] as a means to reduce dose prices to make injectable polio vaccines (IPV) affordable for successful eradication of the disease in the Polio End Game[54] A limitation of many of the studies, however, lies in the fact that they have not evaluated equivalent low-dose IM or SQ vaccination groups.[55]

The most recently registered indication for intradermal vaccination is influenza, where the ID approach has actually been pursued since the 1930's.[56,57] This vaccine (Intanza®, Sanofi Pasteur), is a 5-fold concentrated form of Fluzone[58] (inactivated influenza split-virus vaccine) delivered with an intradermal prefilled syringe (BD Soluvia™ Micro Injection System, Becton Dickinson and Company) that uses a 1.5 mm needle to provide a lower (9 μg HA/strain) or a standard dose (15 μg HA/strain), depending on the population and approved indication.[35,58] Another example of an ID vaccine is rabies. Rabies is a zoonosis that occurs in over 100 countries and is invariably fatal once symptomatic. The cost of a full-dose rabies vaccine limits its widespread use in many areas. ID administration of the vaccine offers an equally safe and immunogenic alternative that requires only 20% of the dose for post-exposure prophylaxis, which could reduce the direct cost of the vaccine by 60–80%. ID regimens have been successfully introduced for post-exposure rabies prophylaxis in India, the Philippines, Sri Lanka and Thailand.[59]

Despite limited clinical data, ID vaccination also holds the promise to enhance immune responses using equivalent, rather than fractional, doses. Efforts have been made to improve influenza immunization by concentrating the formulation and delivering an equivalent dose of 15 μg HA/strain. A Phase II study administering ID with the BD 30-gauge 1.5 mm short needle[60,61] demonstrated that an equivalent dose of 15 μg in elderly patients above 60 induced GMT ratios about 1.5-1.7-fold higher, compared with the same dose IM. This study was later confirmed in a Phase III study,[62] demonstrating that equivalent dose (15 μg HA/strain) given ID can produce superior GMT's and seroprotection at 21 d post-vaccination. However, Intanza15 has not yet been shown to have superior clinical efficacy in terms of reducing mortality and morbidity, although a large retrospective study suggests a reduction in influenza related hospitalizations.[63,64]

Improving immunogenicity of various vaccines in immunocompromised hosts via the intradermal route is extremely important. Hepatitis B virus (HBV) vaccine has a 3–5% failure rate of non-seroconversion and there is a significant improvement in this after ID injection.[65] Studies have demonstrated that in patients on dialysis or in patients with HIV, the intradermal route was more immunogenic than standard intramuscular delivery with the HBV vaccine. ID vaccine recipients had significantly better seroconversion rates compared with the standard dose intramuscular group,[66] which was also demonstrated in ID HBV vaccination of dialysis patients.[67]

Table 1. Approved and pipeline vaccines delivered intradermally

Approved for ID delivery	Positive Clinical Data	Mixed Results
*BCG[13]	Hepatitis A[14,15]	HBV[16-21]
Rabies[22-26]	Pandemic influenza[27]	Measles[28-30]
Influenza[31-36]	Yellow Fever[37-39]	Inactivated Polio[40-43]
	Tick-Borne encephalitis[44]	
	Smallpox[45]	

* Intradermal delivery is the standard route for delivery for BCG.

Adverse Effects of ID Vaccination

Overall, intradermal vaccination has been demonstrated to be very safe. Studies have shown that ID vaccination may be associated with a greater incidence of local reactogenicity, including primarily mild pain, swelling, and redness, but not systemic adverse events. These events typically resolve quickly, as was noted in a meta-analysis[68] comparing the safety and immunogenicity of a large number of intradermal versus intramuscular influenza vaccines. ID vaccination was not associated with a greater incidence of any systemic adverse events examined and was associated with a lower incidence of myalgia. There was evidence of heterogeneity for most adverse events.

Devices for ID Vaccination

To address the unmet clinical and usability needs, various devices have been developed over the years. These are conceptually grouped into liquid delivery devices, including needles, mini-needles, and hollow microneedles, as well as needle adaptors and jet injectors, and solid delivery devices, such as solid microneedles, particle-injectors, and patches with coated microprojections or dissolvable needles (**Table 2**).

The most clinically advanced approach is the mini-needle technology, represented by the Intanza-Soluvia influenza vaccine combination (Sanofi Pasteur), which is commercially available. In its Intanza9 version, the 1.5 mm mini-needle demonstrated relative dose-sparing, at least non-inferior immunogenicity to standard unadjuvanted influenza vaccines, and high acceptability.[102-104] Another licensed ID vaccine delivery device that may have been used with the largest number of vaccine types is a disposable hollow microneedle (<1 mm) device known as the MicronJet600™, which is the focus of this review.

The MicronJet600 has 3 pyramid-shaped microneedles of 0.6 mm (600 μm) length (**Fig. 1**) and the device can be attached to any standard luer tip or luer-lock syringe. The needles are

Table 2. Devices for ID delivery of vaccines[69]

Type of delivery	Type of device	Vaccine fields evaluated clinically
Liquid administration	Needle and syringe (Mantoux)	Flu[46,70-74], Rabies[22-26,59], BCG[13], Polio[75,76]
	Hollow mini and microneedles	Flu[36,62], Rabies[77], Anthrax[78], Japanese encephalitis, DNA-encoding reporter genes (preclinical only)[79-83]
	Tattoo devices	HPV[84-89]
	Jet injectors	Smallpox[99], BCG[90], DTP[91], Polio[43,92], Tetanus[93], Typhoid[94-96], Rabies[97], Influenza[98], Yellow Fever[99]
Solid administration	Solid arrays	HPV[100]
	Dissolvable patches	Flu[101]

*Correspondence to: Yotam Levin; Email: yotam@nanopass.com
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fabricated as a single 3-dimensional crystal silicon chip[105] that is etched in a pattern to produce micro-pyramid-shaped microneedles, each having a very sharp tip that penetrates the epidermis followed by a conduit or through-channel for liquid delivery, and is produced using Micro Electro Mechanical System (MEMS) fabrication technology[106] in a semiconductor fabrication house. The microneedle chip is integrated with a plastic hub or female luer that attaches to any male luer tip or luer lock syringe, thereby enabling the delivery of liquid formulations from any standard (prefilled or disposable) syringe directly into the skin. The integrated device forms a direct fluid channel from the syringe or container through the microneedles, in order to deliver a vaccine where the dendritic cells are most prevalent in the superficial dermis of the skin. Injection with the MicronJet600 is characterized by an intradermal bleb or wheal, which is the hallmark of an acceptable ID injection.[107] It is registered with the US FDA (510 k), the EMEA (CE Mark), Canada, Hong Kong, and in other countries.

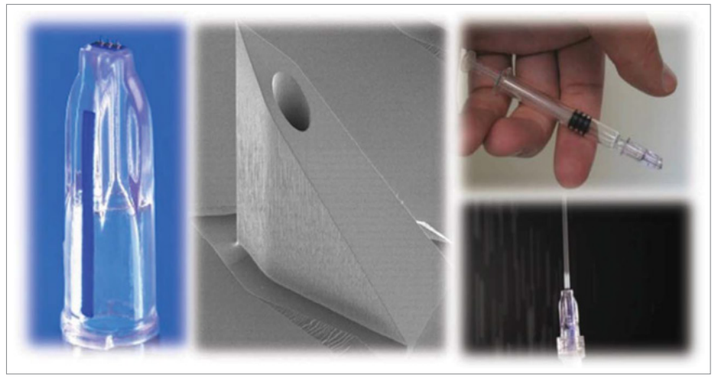

Figure 1. The MicronJet600 microneedle and attachment to a standard syringe. Left: Close-up of the MicronJet600; Middle: SEM picture (~×100) of a single microneedle prior to dicing, on wafer; Top Right: needle attached to a pre-filled syringe; Bottom Right: direction of injection flow.

Prior to the development and commercialization of the MicronJet600, an older (original) version of the MicronJet was used in the clinical trials conducted in 2007–2008. This device included 4 microneedles that were 450 μm in length, made in a very similar design. The performance characteristics of the original model were similar, but the insertion technique was less intuitive, requiring insertion at about 60 degrees and lowering the syringe while in skin to about 30 degrees. The MicronJet600 was developed to improve ease of use, requiring insertion at a more natural angle of about 45 degrees with no subsequent adjustment of position.

The Past: Clinical Results with the Original MicronJet Device

The MicronJet device was tested both in immune-competent healthy adults and in an elderly population that was considered to be relatively immunocompromised. A first-in-man study was conducted to demonstrate effective dose-sparing, safety, and user preference, using a commercially available influenza vaccine, Fluarix 2006/2007 (GSK, Belgium)[33] in healthy adults. This Phase I/II study used the original model MicronJet microneedle, described above. Groups received intradermal doses with 20% (3 μg HA/strain) or 40% (6 μg HA/strain) of the usual dose using the MicronJet device, or a 100% dose (15 μg HA/strain) given IM with a standard 26 G needle and syringe. Local reactogenicity was more frequent with ID vaccination, but was generally mild and transient. The low-dose ID groups had immune responses that were similar to those in the IM control group, demonstrating the potential for up to 5-fold dose-sparing. The regulatory criteria for re-licensure of seasonal influenza vaccines were met in full in all study groups. Recipient acceptance and discomfort was assessed using a questionnaire and demonstrated less pain and intimidation with the device compared to the IM injection (data on file).

A second study had a similar design using the A/2009/H1N1 strain and was the first intradermal vaccination study of pandemic influenza.[27] The study, which was conducted mostly in the elderly population in Hong Kong, demonstrated 5-fold dose-sparing as well, with a safety profile that was comparable to the previous study. There was a similar incidence of systemic adverse events (AEs) such as fever and arthralgia, and a higher incidence of local AEs such as erythema and edema, which is consistent with other ID influenza vaccine studies.[60,61,68]

A study in the elderly compared fractional-dose ID delivery to the full IM dose of the unadjuvanted influenza vaccine (Fluvirin™, Novartis), as well as to MF59-adjuvanted formulations with various antigen and adjuvant doses.[108] This study showed that the unadjuvanted ID approach yielded significantly higher immunogenicity at 6 μg HA/strain than unadjuvanted IM formulations at 15 μg or 30 μg HA/strain, in at least the A/H1N1 strain, with non-inferior GMTs in the other strains. One study arm (12 μg HA/strain ID) was also higher with the A/H3N2 strain compared to the unadjuvanted IM formulations. In addition, the study showed that formulations adjuvanted with MF59 yielded significantly higher GMTs than the unadjuvanted ID formulation in the A/H1N1 and B strains, but not for A/H3N2. However, the adjuvanted formulation included 15 μg HA/strain (and 30 μg HA for A/H3N2), which was 2.5-fold higher than the unadjuvanted ID groups, so a direct dose-for-dose comparison of ID (unadjuvanted) with IM (MF-59-adjuvanted) was not established.

Another seasonal influenza study evaluated various ID or IM doses of a virosomal influenza vaccine (Inflexal V™, Crucell, BV).[36] This study was unique in that it included a head to head comparison of the use of the MicronJet device with the same formulation and dose using a 25 G 16 mm (5/8 in.) length needle and syringe with the Mantoux technique (typically using a 15 degree injection angle). This study showed that ID delivery of the low dose virosomal vaccine (3 μg HA/strain) with the MicronJet achieved statistically significant higher GMT fold-increases for the H1N1 and B strains as compared with the same dose ID using Mantoux (84.2 vs. 37.8 [$P < 0.05$] and 28.5 vs. 6.9 [$P < 0.01$], respectively). Superior immunogenicity was also demonstrated for the H3N2 strain compared to IM delivery of the full dose (15 μg HA/strain) vaccine, despite using 1/5th of the dose (39.9 vs. 16.9 [$P < 0.05$]). The improved immunogenicity results observed with the MicronJet600 could potentially be due to the consistent delivery of the influenza vaccine primarily to the superficial dermis and the epidermis, where Dendritic Cells (DCs), and Langerhans cells, (LCs) are respectively abundant. Injection site for all influenza studies was the deltoid area.

The Present: Demonstrating Improved Immunogenicity with the MicronJet600

Improving the immunogenicity of vaccines is an important unmet clinical need that might even be more important than mere dose-sparing. Theoretically, using higher or equivalent doses of an antigen intradermally (instead of reducing the dose due to volume constraints) may enhance such immunogenicity, and with it, potentially, vaccine efficacy. Intradermal delivery of high doses of the antigens may require concentration, which may result in some additional manufacturing costs.

A Phase II clinical study was conducted in 2010 at Hong Kong University to evaluate the ability of ID delivery to enhance the immunogenicity of seasonal influenza vaccines with Intanza9 2009/2010 as the source of antigen.[109] The study included 2 experimental ID groups using the MicronJet device to give either 20% (3 μg HA/strain) or 60% (9 μg HA/strain) of the usual IM dose and 2 control arms dosed ID with either Intanza9 (9 μg HA/strain) or IM with Fluzone (15 μg HA/strain). The doses selected for the study were based on the available vaccines on the market. A direct comparison between 3 μg using the MicronJet to the same dose with Intanza was not done, as this dose was not tested for Intanza and the 6 μg dose did not show non-inferiority in previous studies. The study demonstrated that the typical reduction in immunogenicity of the 2009 H1N1 strain could be overcome and was significantly higher with ID vaccination when compared with the IM vaccination, with the highest seroprotection rate and GMT fold increase value generated by the lowest dose of 3 ug (20%) HA vaccine delivered by the MicronJet600. The H3N2 strain seroconversion rates were also significantly higher in the ID groups compared with the IM group. There was no significant difference in immune response between the ID groups.

Additional promising results demonstrating very significant dose-sparing, as well as improved immunogenicity, have been recently released by Merck & Co, for live attenuated herpes zoster vaccine (NCT01385566). Further detailed information is pending publication.

Table 3 outlines various published clinical studies using the MicronJet device models for the delivery of vaccines, along with a summary of results, benefits and references.

The Future of ID Delivery of Vaccines and Immunotherapeutics: Promise and Challenges

Despite many years of clinical development and the very promising early-stage trials described above, there are still significant challenges facing the ID delivery approach, for the MicronJet600 or any other device. For instance, late stage clinical trials are still required to validate superior immunogenicity and vaccine efficacy, especially in challenging populations like the elderly.[110] In addition to having a low response to vaccination at a young age (below 6 months),[111] the pediatric population also poses specific mechanical challenges, due to their thin skin, making them unsuitable for immunization with certain delivery technologies.[112] However, the MicronJet600 device was recently utilized in a large Phase III inactivated polio vaccine (IPV) study in 6–14 week-old infants sponsored by the US CDC and the International Center for Diarrheal Disease Research, Bangladesh (icddr, b) (NCT01813604). The device performed very well in this setting (publication in preparation). Additional validation of ID delivery is required in order to expand the list of applicable

Table 3. Published clinical studies using the MicronJet and MicronJet600

Field	Study ID	Phase	N	Device used	Benefit demonstrated*
Seasonal Influenza	EudraCT number 2007-001160-77	Pilot	180	MicronJet	Dose sparing[33]
Seasonal Influenza	ISRCTN 33950739	Phase II	280	MicronJet	Dose sparing and superior immunogenicity[36]
Seasonal Influenza	NCT00848848	Phase I	450	MicronJet	Superior immunogenicity[108]
Pandemic Influenza	NCT01049490	Phase I	37	MicronJet600	Dose sparing[27]
Seasonal Influenza	NCT01304563	Phase II	282	MicronJet600	Dose sparing and superior immunogenicity[109]

*Compared to a standard dose of the unadjuvanted vaccine

vaccines beyond BCG, PPD, rabies, and influenza. Another phase I of ID iPV using Microniet600 was conducted in HIV positive adults (NCT01686503).[113]

The use of ID delivery with immunotherapeutics holds future promise, coupled with unique challenges, in the settings of allergy (in Phase III clinical trials),[114] cancer immunotherapy, and Type 1 Diabetes (in preclinical studies).[115] Of most interest perhaps, is antigen-specific cancer immunotherapy, which despite past failures[116,117] is still the most vibrant vaccine field to undergo clinical evaluation of the ID approach. There are over 30 clinical programs today with ID delivery of cancer vaccines (at least one of which with the MicronJet600) and likely many more to come. The ability to enhance the skin's potent immune system with ID immunization, to directly target its Dendritic and Langerhans cells,[118,119] and to potentiate the response against cancer cells, remains one of the great challenges and promises of the 21st century.[120]

Disclosure of Potential Conflicts of Interest

Dr. Yotam Levin and Dr. Efrat Kochba are permanent employees of NanoPass Technologies, Ltd.

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Brief report

Clinical evaluation of a novel microneedle device for intradermal delivery of an influenza vaccine: Are all delivery methods the same?☆

Yotam Levin[a,*], Efrat Kochba[a], Richard Kenney[b,1]

[a] NanoPass Technologies Ltd., 3 Golda Meir Street, Nes Ziona 7403648, Israel
[b] Crucell Holland B.V., Archimedesweg 4, 2333 CN Leiden, The Netherlands

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ABSTRACT

The skin provides the largest immune barrier to infection and is a readily accessible site for vaccination, although intradermal (ID) injection can be challenging. The MicronJet™ microneedle is a novel device that consistently injects antigens very close to the skin's dendritic cells. A dose-sparing ID injection study was conducted in 280 healthy adult volunteers using trivalent virosomal adjuvanted influenza vaccine. ID injection of 3 μg using the MicronJet™ was well tolerated and showed a statistically higher geometric mean fold rise than the same dose ID using a conventional needle (Mantoux technique) for the H1N1 and B strains or a 15 μg intramuscular (IM) injection for the H3N2 strain. Thus, the immune response appears to partially depend on the delivery device and route of injection. The MicronJet™ may allow dose-sparing, yet give a superior response in influenza vaccination and warrants further clinical evaluation.

1. Introduction

Even with decades of efforts to improve influenza vaccination, influenza virus infection remains an annual cause of substantial illness and mortality, associated with pronounced clinical, logistical, and policy-making challenges to healthcare organizations globally. Immunization rates are disappointing within the public sector [1] and even for healthcare workers [2]. This continues despite major governmental efforts and the fact that vaccination remains the best global strategy for reducing influenza morbidity and mortality [3,4]. Annual strain matching remains mediocre at times and overall effectiveness rates, whenever tested, appear moderate [5]. One of the major issues for vaccine developers continues to be the timely production of the influenza vaccine in large volumes. Improving its immunogenicity, especially in low-responder populations like the elderly, the immunocompromised, and young children, remains a high developmental priority [6].

Multiple approaches are being evaluated to achieve better influenza vaccine immunogenicity and reduce the dose required for vaccination. Intradermal delivery has been proposed as a means of dose sparing in adults [7–9], children [10], and infants [11], as well as in the elderly [12]. Studies have demonstrated mixed results using the intradermal route, with some showing potentially inferior results compared to full dose IM delivery [13], whereas others show equivalence or non-inferiority [9,14] and a few show superior immunogenicity [14,15]. While there are multiple differences between the studies with respect to the populations, vaccine products, and study designs, it appears that one of the dominant factors might be the device in use. Several studies have been reported where conventional needles are used with the Mantoux technique [9,12], which is neither easy to perform nor reliable at providing consistently shallow injections [16]. More recent studies have used novel technologies that incorporate mini- (1.5 mm) and micro- (<1 mm) needle approaches. A recent Phase II clinical study comparing the minineedle technology delivering partial doses of a seasonal influenza vaccine demonstrated superior immunogenicity to the full-dose conventional IM injection for the influenza A strains [14], yet these needles tend to deliver the antigens well below the superficial dermis and epidermis. Most of the dendritic cells (DCs) that migrate from the bone marrow into the skin reside in this superficial space and perform the dual role of immune surveillance and antigen presentation [17]. Intradermal techniques

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* Corresponding author. Tel.: +972 8 9462905; fax: +972 508966787.
E-mail addresses: yotam@nanopass.com (Y. Levin), efrat@nanopass.com (E. Kochba), richard.kenney@immunedesign.com (R. Kenney).
[1] Present address: Immune Design Corp, 601 Gateway Blvd., Suite 1020, South San Francisco, CA 94080, United States.

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Table 1
Description of groups included in the clinical trial.

	N	Dose	Formulation/dose (active ingredients)	Route of administration[a]
Group A1	56	0.1	3 μg HA antigen of each strain	ID–regular needle and syringe (Mantoux)
Group A2	56	0.1	4.5 μg HA antigen of each strain	ID–regular needle and syringe (Mantoux)
Group A3	56	0.1	6 μg HA antigen of each strain	ID–regular needle and syringe (Mantoux)
Group B	56	0.5	15 μg HA antigen of each strain	IM
Group C	56	0.1	3 μg HA antigen of each strain	ID–MicronJet^{TM} microneedle device

[a] All ID administrations were made over the deltoid muscle and the IM administrations made into it.
Results for Groups A and B have been reported separately [19].

theoretically need to target these specialized epidermal cells to provide improved immunogenicity.

We report a clinical trial directly evaluating two methods of intradermal delivery: the century-old Mantoux technique using a conventional needle [18], compared to a novel microneedle device (MicronJet^{TM}, NanoPass Technologies Ltd, Israel). The MicronJet^{TM} contains highly precise silicon needles that are only 0.45 mm long and are manufactured using semiconductor technology to deliver vaccine specifically to the epidermis and shallow dermis. We report here additional results from a Phase II clinical study comparingthe safety and immunogenicity of various doses of the virosomalinfluenza vaccine and by different routes of administration [19].

2. Materials and Methods

In order to evaluate the feasibility of using this novel microneedle device we included a treatment arm of 1/5th (3 μg hemagglutinin [HA]/strain in 0.1 ml) of the standard virosomal influenza vaccine (Crucell Switzerland AG [Crucell]), delivered intradermally (ID) via the MicronJet^{TM} device (Group C). This was compared with the full dose (15 μg HA/strain in 0.5 mL) Inflexal® V (Crucell) commercial vaccine delivered intramuscularly (IM) that provided a positive control (Group B), and with reduced doses of the virosomal influenza vaccine (Crucell) delivered ID in 0.1 mL volumes using the conventional Mantoux technique with a 25 G 16 mm (5/8 in.) length needle (3 μg, 4.5 μg or 6 μg HA/strain in Groups A1, A2, and A3, respectively). The study was conducted under cGMP in a Phase I Unit in Basel, Switzerland between September and November 2007 and was sponsored by Crucell (http://www.controlled-trials.com/ISRCTN33950739). All injections were performed by a single experienced nurse and given into the deltoid muscle or the adjacent skin. Table 1 summarizes the vaccine formulations and study groups. The vaccine used was the 2007/2008-season virosomal adjuvanted influenza vaccine, containing purified viral surface antigens of A/Solomon Islands/3/2006 (H1N1)-like, A/Wisconsin/67/2005 (H3N2)-like, and B/Malaysia/2506/2004-like virus, as recommended by the WHO and EMA/CHMP.

Subjects were randomized to receive a single low dose ID vaccination (Groups A1 [*N* = 56], A2 [*N* = 56] or A3 [*N* = 56]) using the Mantoux technique or a full-dose IM vaccination (Group B [*N* = 56]) using a standard needle. A fifth group was added after randomization and given a single low dose ID vaccination with the MicronJet^{TM} device (Group C [*N* = 56]). Groups of this size provide the ability to distinguish about a 2-fold difference in comparing immunogenicity results. Antibody titers were measured using hemagglutination-inhibition (HI) assays according to standard methods at Crucell [19] at baseline and 21 days after vaccination; HI analysis was done using standard EMEA definitions [20]. Safety was assessed using a solicited adverse event checklist and a 4-day diary. Comparisons between groups were performed in the according-to-protocol (ATP) population based on *t*-test, *F*-test

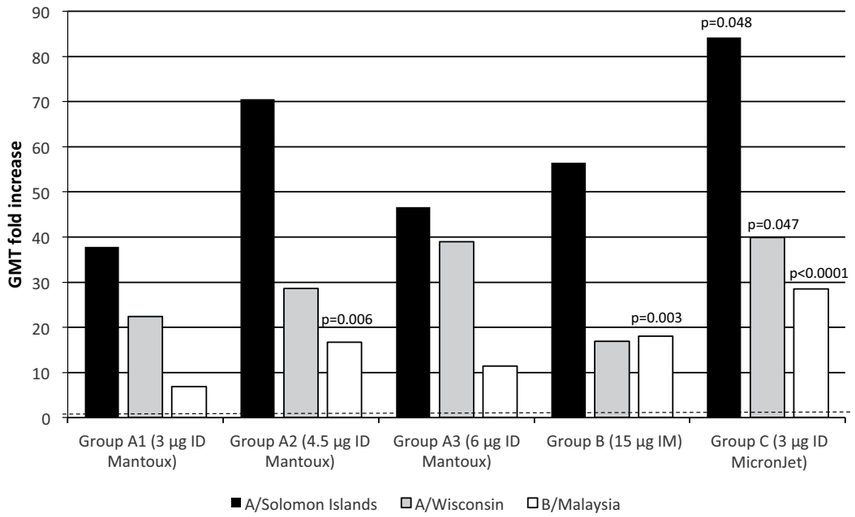

Figure 1. GMT fold increase. Significant p-values are noted compared to Group A1 (for the A/Solomon Islands [H1N1] and B/Malaysia strains) and compared to Group B (for the A/Wisconsin [H3N2] strain) (horizontal line indicates EMEA criteria threshold).

Table 2
Immunogenicity results (ATP population).

Group and Dose	**A1 (ID 3 μg) [*N* = 55]**	**A2 (ID 4.5 μg) [*N* = 53]**	**A3 (ID 6 μg) [*N* = 55]**	**B (IM 15 μg) [*N* = 54]**	**C (ID-MJ 3 μg)[a] [*N* = 54]**
Seroconversion	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)
A/Solomon Islands	47 (85.5)	44 (83.0)	46 (83.6)	47 (87.0)	49 (90.7)
A/Wisconsin	33 (60.0)	38 (71.7)	43 (78.2)	38 (70.4)	47 (87.0)**
B/Malaysia	28 (50.9)	36 (67.9)	33 (60.0)	44 (79.6)**	40 (74.1)*
Seroprotection	pre (% pre) post (% post)	pre (% pre) post (% post)	pre (% pre) post (% post)	pre (% pre) post (% post)	pre (% pre) post (% post)
A/Solomon Islands	20 (36.4) 53 (96.4)	19 (35.8) 51 (96.2)	16 (29.1) 49 (89.1)	19 (35.2) 52 (96.3)	16 (29.6) 52 (96.3)
A/Wisconsin	29 (52.7) 53 (96.4)	27 (50.9) 52 (98.1)	23 (41.8) 54 (98.2)	27 (50.0) 51 (94.4)	28 (51.9) 53 (98.1)
B/Malaysia	11 (20.0) 36 (65.5)	5 (9.4) 44 (83.0)*	5 (9.1) 40 (72.7)	7 (13.0) 46 (85.2)*	2 (3.7)* 45 (83.3)*
GMT	(pre) post fold increase	(pre) post fold increase	(pre) post fold increase	(pre) post fold increase	(pre) post fold increase
A/Solomon Islands	(27.4) 1034.5 37.8	(25.4) 1788.3$^{\cdot}$ 70.5	(16.4) 765.3 46.6	(20.9) 1180.4 56.5	(22.8) 1924.6$^{\cdot\cdot}$ 84.2$^{\cdot}$
A/Wisconsin	(52.9) 1182.8 22.4	(45.2) 1293.0$^{\&}$ 28.6	(34.0) 1324.1$^{\&}$ 39.0	(40.9) 691.8 16.9	(38.3) 1529.2$^{\&\&}$ 39.9$^{\&}$
B/Malaysia	(10.5) 72.8	(9.0) 149.9*	(8.0) 91.4	(8.5) 152.9*	(6.4*) 183.0$^{**,\cdot}$
	6.9	16.7**	11.4	18.0**	28.5$^{**,\cdot,\cdot\cdot}$

[a] ID: intradermal using the Mantoux technique, IM: intramuscular, both by conventional 25 G, 16 mm (5/8 in.) length needle, ID-MJ: intradermal using the MicronJet needle
* $p < 0.05$ compared to the response in Group A1.
** $p < 0.01$ compared to A1.
$^{\cdot}$ $p < 0.05$ compared to A3.
$^{\cdot\cdot}$ $p < 0.01$ compared to A3.
$^{\&}$ $p < 0.05$ compared to B.
$^{\&\&}$ $p < 0.01$ compared to B.

or, X^2-test, whenever applicable, and expressed as *p*-values of the investigated contrast. The analysis of HI antibodies was performed on $\log_{10}$-transformed data; adverse events (secondary endpoints) are reported descriptively.

3. Results

A total of 280 subjects were enrolled, of which 279 concluded the study. Baseline demographics between the five groups were well matched, except that Group C was slightly younger (mean of 34.1 years old) compared to the two oldest groups A1 and A3 (39.5 and 39.6 years old, respectively). No deaths or serious adverse events were reported. The local symptom of post-vaccination pain in the 4 days following vaccination was more common in the IM Group B (38.9%) than in the ID Group C using the MicronJet^{TM} device (10.9%), whereas induration was more common in Group C (50.9%) than Group B (16.7%) and erythema was more common in the ID groups (61.8%, 52.8%, 74.5%, and 89.1% in Groups A1, A2, A3, and C, respectively). Other local and systemic symptoms were reported in similar frequency in both groups.

Immunogenicity (Fig. 1 and Table 2) was higher in the 3 μg ID group using the MicronJet^{TM} needle (Group C) compared to the ID groups using the conventional Mantoux needle (Groups A1, A2, and A3) and the IM group (Group B). Group C had a higher GMT fold increase for the A/Solomon Islands (H1N1) and the B/Malaysia strains compared to Group A1 ($p = 0.048$ and $p < 0.001$, respectively), a higher A/Wisconsin (H3N2) increase compared to Group B ($p = 0.047$), and a higher B/Malaysia increase compared to Group A3 ($p = 0.004$). Groups A2 and B also had a higher increase for the B/Malaysia strain compared to Group A1 ($p = 0.006$ and 0.003, respectively). All other GMT increases appeared to be equivalent between the various groups. No dose-response curve was identified in the conventional ID delivery arms [19]. Importantly, IM delivery of the full vaccine dose did not show higher immunogenicity than the 1/5th dose ID delivery group using the MicronJet^{TM} device.

Seroconversion rates for the three strains ranged from 50.9% to 85.5% in the ID groups using the conventional needle with 3, 4.5, or 6 μg per strain (Groups A1, A2, or A3, respectively), from 70.4% to 87.0% in Group B (IM 15 μg), and from 74.1% to 90.7% in Group C (ID 3 μg using the MicronJet^{TM} device). Group C had a significantly higher seroconversion rate than Group A1 for the H3N2 and B strains ($p = 0.002$ and 0.018, respectively). After ID vaccination by conventional needle (Groups A1, A3, or A3), subjects had seroprotection rates of 65.5% to 98.2%. Seroprotection rates following intramuscular vaccination (Group B) ranged from 85.1% to 96.3%. After intradermal vaccination with the MicronJet^{TM} device (Group C), subjects had seroprotection rates of 83.3% to 98.1%. The response in Group C was somewhat greater than the response in Group A1 for the B/Malaysia strain ($p = 0.048$).

4. Discussion

This study was designed to gather information on whether a reduced ID dose of Inflexal® V could achieve comparable results to IM full dose administration in healthy adults. As the group dosed with the MicronJet^{TM} device was added at the same study site soon after randomization, the groups are comparable and in addition were dosed with the same vaccine, so comparisons with the original groups can provide useful insights. Inflexal® V administered ID at reduced doses or IM at the full dose fulfilled the individual annual relicensure parameters set by EMEA for influenza vaccines [20] in all study groups, except that seroprotection in ID Group A1, which was dosed at 3 μg using a conventional needle, was below the EMEA threshold for the B strain. Intradermal vaccination using the MicronJet^{TM} device induced significantly higher antibody

responses than a comparable dose injected with the Mantoux technique using a conventional needle for the H1N1 and B strains.

This study suggests that the immunogenicity of seasonal influenza vaccine may be dependent on the administration route (ID vs. IM) and delivery device, in addition to the effect of the vaccine dose and influenza strain in any individual year. The benefits seen with the MicronJet^{TM} device could be due to the precise delivery of the influenza vaccine primarily to the superficial dermis, where DCs are abundant, compared to deeper intradermal delivery. Injections in this study were performed by a highly experienced nurse in a professional Phase I unit; the authors hypothesize that the differences could be further pronounced in larger (Phase III) or field studies, when multiple users having varied levels of experience and expertise are performing the injections. Further data is required to evaluate the differences between delivery methods in similar studies, and particularly for low-responder populations such as the elderly and the very young.

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Conflict of interest statement: Drs. Levin and Kochba are employed by and have a financial interest in NanoPass Technologies. Dr Kenney was employed by and had a financial interest in Crucell.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2014.03.024.

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Dose sparing intradermal trivalent influenza (2010/2011) vaccination overcomes reduced immunogenicity of the 2009 H1N1 strain

Ivan F.N. Hung[a,b,*], Yotam Levin[c], Kelvin K.W. To[a], Kwok-Hung Chan[a], Anna Jinxia Zhang[a], Patrick Li[a], Clara Li[a], Ting Xu[a], Tin-Yan Wong[a], Kwok-Yung Yuen[a]

[a] *State Key Laboratory for Emerging Infectious Diseases, Carol Yu's Centre for Infection and Division of Infectious Diseases, The University of Hong Kong, Queen Mary Hospital, Hong Kong Special Administrative Region*
[b] *Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong Special Administrative Region*
[c] *NanoPass Technologies Ltd., Israel*

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ABSTRACT

Background: We hypothesized that low dose intradermal vaccination of the trivalent influenza vaccine (TIV) delivered by the MicronJet600^{TM} (NanoPass Technologies, Israel) would be non-inferior to the full dose intramuscular and mid dose Intanza® vaccination in the elderly and the chronically ill adults.
Methods: We performed a prospective randomized trial on elderly and chronically ill adults. Subjects were randomly assigned into 4 groups. Groups ID3 and ID9 received reduced dose ID TIV (3 μg and 9 μg of hemagglutinin (HA) per strain respectively) delivered by MicronJet600^{TM} (NanoPass Technologies, Israel). Group INT9 received reduced dose ID TIV (9 μg) delivered by Becton Dickinson's Soluvia^{TM} device (Intanza®9, Sanofi-Pasteur, France). Control group IM15 received a full dose IM TIV (15 μg). We measured antibody titers by hemagglutination inhibition (HAI) and microneutralization (MN) assays at baseline and day 21.
Results: Baseline characteristics for all groups were similar (group and sample sizes: ID3 = 63; ID9 = 68; INT9 = 65; and IM15 = 66). At day 21 post vaccination, the GMT ratio and the seroconversion rates difference for all three strains of the ID vaccine groups were non-inferior to the IM vaccine group. The seroconversion rate, seroprotection rate, and the GMT of the H1N1 strains by HAI and MN assays were significantly higher in the ID groups compared with the full dose IM vaccine group. The seroconversion rates of the H3N2 strain by HAI assay were also significantly higher in the ID groups when compared with the full dose IM group. Direct comparison among the three ID groups showed no significant differences. No serious adverse events related to vaccination were reported.
Conclusion: Dose-sparing ID TIV can overcome reduced immunogenicity of the H1N1 strain, and according to some measures, for the H3N2 strain. At risk subjects indicated for the TIV should be considered for intradermal immunization to compensate for reduced immunogenicity.

1. Introduction

Influenza presents a substantial public health threat and a significant burden on health authorities worldwide, even in non-pandemic years [1]. Seasonal influenza is estimated to infect between 5% and 20% of the population annually, resulting in over 200,000 hospitalizations and about 36,000 deaths in the US alone [2]. Influenza infection can cause life-threatening pneumonia and extrapulmonary complications. In addition, it can lead to substantial "non-infectious" morbidity and mortality [3]. Influenza vaccination has recently been shown [4,5] to prevent both respiratory and vascular complications in the elderly and patients with chronic illness. Furthermore, neuraminidase inhibitors, the only antiviral licensed for clinical use were not very effective in the clearance of virus in the late presenters [6]. Prevention via vaccination is considered the most important means to combat against influenza [7].

Elderly subjects present a particular challenge for immunization against seasonal and pandemic influenza due to the unfortunate combination of the reduced ability to mount protective response to vaccine due to immunosenescence [8] on one hand, and their increased vulnerability to morbidity and mortality due to influenza virus and its complications [9] on the other hand. About 86% of the all-cause mortality attributed to seasonal influenza occurs in the elderly [10]. The need to improve the immunization of the elderly is well established [11].

* Corresponding author at: Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Pokfulam Road, Pokfulam, Hong Kong Special Administrative Region. Tel.: +852 22554049; fax: +852 28186474.
E-mail address: ivanfn@gmail.com (I.F.N. Hung).

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Recent report suggested possibly reduced effectiveness of the 2009 H1N1 component of live attenuated influenza vaccine (LAIV) for the 2010–2011 influenza season [12] corresponding with an increased number of influenza cases among military recruits who received the LAIV. Low immunogenicity of the intramuscular non-adjuvanted 2009 H1N1 monovalent vaccine was also reported [13]. Dose-sparing intradermal (ID) vaccination with different delivery devices have demonstrated non-inferior immunogenicity in seasonal influenza vaccination compared with conventional intramuscular vaccination before the pandemic H1N1 2009 [14–17]. However, this strategy has not been tested for the trivalent influenza vaccine (TIV) after this pandemic. We therefore performed a prospective randomized controlled study to compare the safety and immunogenicity between conventional full dose intramuscular (IM) and reduced dose ID immunization delivered by two different devices.

The two intradermal injection devices used in this study include the BD Soluvia™ microinjection and the MicronJet600™ systems. The Intanza® (Sanofi-Pasteur) with the BD Soluvia™ microinjection system consists of a prefilled trivalent influenza vaccine, with a single 1.5 mm needle penetrating perpendicularly to the skin [14–16]. On the other hand, the MicronJet600™ system consists of an array of three microneedles each 0.6 mm in length, puncturing obliquely into the skin. The BD Soluvia™ is currently the only prefilled intradermal device licensed for influenza vaccine.

2. Materials and methods

A prospective randomized, open-label, single-center trial was conducted at Queen Mary Hospital from 25 November 2010 to 24 February 2011. We compared the safety and immunogenicity of a single low-dose (3 μg and 9 μg HA, respectively) ID TIV administration with a single full-dose (15 μg) IM administration. The vaccine used was Intanza® (Sanofi-Pasteur) for the ID groups and Fluzone® (Sanofi-Pasteur) for the IM group. The TIV used was an inactivated, non-adjuvanted vaccine formulated to contain 15 μg of HA of influenza A/California/07/2009 (H1N1)-like virus, influenza A/Perth/16/2009 (H3N2)-like virus and influenza B/Brisbane/60/2008-like virus. We recruited elderly and chronically ill adults aged ≥21 years who satisfied the WHO recommendation for annual vaccination against influenza. The study was approved by the Institutional Review Board of the Hospital Authority of Hong Kong and is registered with the ClinicalTrials.gov, number NCT01304563.

Subjects were assigned by a randomization list. Groups ID3 and ID9 received a reduced dose ID TIV (3 μg and 9 μg of HA per strain, respectively) with MicronJet600™. Group INT9 received a reduced dose ID TIV (9 μg) with BD Soluvia™ device (Intanza®9). Group IM15 received the full-dose standard IM TIV (15 μg). All patients recruited gave written informed consent. Patients with clinically significant immune-related diseases, recent co-morbidities and history of allergy to the components of the vaccine were excluded.

Safety was evaluated by asking the subjects to remain in the clinic premise for 30 min for observation post immunization. An immediate adverse event checklist was filled before discharge, covering the period for severe anaphylactic reaction. A diary was given to the subjects to document symptoms of local and systemic adverse events presented within the first 7 days post-vaccination. Systemic symptoms included fever (body temperature ≥ 37.5 °C), headache, malaise, myalgia and arthralgia, and local symptoms included redness, swelling, induration, pain and ecchymosis were documented as solicited events. The diaries were collected upon follow-up on day 21-post vaccination.

Antibody titers were measured using hemagglutination-inhibition (HAI) and microneutralization (MN) assays according to standard methods as described previously, at baseline and 21 days after vaccination [18,19].

Specific study personnel who did not take part in the subsequent assessment of safety or immunogenicity performed all vaccinations. The primary outcome measure is the immunogenicity by seroconversion rate, defined as the percentage of subjects with an HAI antibody titer < 10 at baseline and a post-vaccination titer of ≥40 or a titer > 10 at baseline and at least a four-fold increase in titer post-vaccination on day 21. Secondary outcome measures included geometric mean titer (GMT) fold increases in antibody titer and adverse events of 30 min post vaccination. Seroprotection rate was also reported as defined by percentage of subjects with HAI and MN antibody titer ≥ 40 on day 21.

Based on previous study of the seroconversion rate of 82% for the intradermal seasonal influenza vaccination with a dosage of 3 μg HA per strain and 70% seroconversion rate for the regular 15 μg HA per strain intramuscular vaccination, we calculated that a total sample of 40 subjects per group would be needed to demonstrate non-inferiority [14], based on a two-sided test, Type 1 error rate of 5%, 80% power and a non-inferiority tolerance margin of 1.5. The protocol proposed recruiting 60 subjects per group, with a threshold of at least 50 to allow for 25% drop out rate. Demographic parameters and adverse reactions were compared by Fisher's exact test for categorical variables and by Kruskal–Wallis test for continuous variables. Student's *t*-test was used to compare the GMT and GMT folds increases between each of the study and control groups. Non-inferiority of each of the ID vaccine group against the intramuscular vaccine group was assessed by the day 21 post-vaccination GMT ratio and the seroconversion rates for all three strains [20,21]. Non-inferiority was defined as the upper limit of the 2-sided 95% CI of the GMT ratio (intramuscular vaccine/intradermal vaccine) not exceeding 1.5 and the upper limit of the 2-sided 95% CI for the difference in seroconversion rates (intramuscular vaccine minus intradermal vaccine) not exceeding 10% for all three strains [20,21]. Fisher's exact test and logistic regressions were conducted to compare seroconversion and seroprotection rates among the 4 groups. Correlation between post-vaccination swelling and subsequent GMT value and fold increase, seroconversion/seroprotection rate on day 21 was analyzed by Spearman rho. SPSS 18.0 for Windows (SPSS Inc., Chicago, IL) was used for statistical computation. *P* value < 0.05 was considered to represent significant difference.

3. Results

3.1. Subjects

A total of 282 subjects were enrolled of which 262 completed the study. Sixty-three subjects (ID3) received a reduced dose ID TIV (3 μg of HA per strain) with MicronJet600™, 68 subjects (ID9) received a reduced dose ID TIV (9 μg) with MicronJet600™, 65 subjects (INT9) received a reduced dose ID TIV (9 μg) with BD's Soluvia™ device (Intanza®9), and 66 subjects (IM15) received the full-dose standard IM TIV (15 μg). Twenty subjects were lost to follow-up. Dropout rates were similar among the groups ($p = 0.535$) and related to compliance, rather than specific adverse events. The four groups did not differ in terms of baseline demographics including age, gender, background diseases and vaccination history (Table 1). Majority of the patients have had hypertension only as past medical history. None of the patients enrolled were on long-term immunosuppressants. Forty-three patients (16.4%) received IM monovalent H1N1 2009 vaccine in the previous year. This vaccination history was not significantly different amongst the four groups.

Table 1
Baseline demographics.

	ID3 ($n = 63$)	ID9 ($n = 68$)	INT9 ($n = 65$)	IM15 ($n = 66$)	*P*-Value
Age					
Median (IQR)	72 (68–77)	73.5 (69.3–78)	74(68.5–78.5)	72 (66–78)	0.668
Gender					
Male	40	45	46	36	0.347
Hypertension	31	33	36	41	0.390
Diabetes mellitus	15	22	19	21	0.633
Ischemic heart disease	23	14	17	23	0.205
Cancer	10	9	6	8	0.757
Stroke	4	2	2	2	0.725
Chronic renal failure	2	1	2	5	0.272
COPD	2	4	3	1	0.571
Atrial fibrillation	2	6	10	10	0.072
Monovalent H1N1 2009 vaccine	11	9	13	10	0.744

COPD, chronic obstructive pulmonary disease.

3.2. Safety

No deaths or serious adverse events related to vaccination were reported (Tables 2 and 3). Incidence of systemic adverse events was infrequent. Although systemic symptoms (malaise, myalgia and arthralgia) occurred more frequently in ID vaccinees compared with those vaccinated IM, the difference remained insignificant (Table 2) ($p > 0.05$). Leakage events (wet injections) were more common among the ID3 and ID9 groups ($p = 0.025$). Local symptoms of post vaccination redness and swelling (grade 2 or 3) were significantly more common in the ID (ID3, ID9 and INT9) groups ($p < 0.001$) when compared with the IM15 control group. All events were only mild to moderate in intensity and invariably transient. Intradermal blebs were formed in 99.2% of the MicronJet600™ injections and in only 46% of the Soluvia™ injections ($p < 0.001$). None of the cases were taken out of the statistical analysis and all 262 subjects represent the per protocol results.

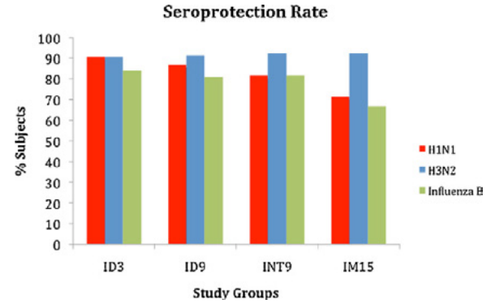

Fig. 2. Day 21 seroprotection rate of the four study groups by HAI assay.

3.3. Immunogenicity

At day 21, non-inferiority in immunogenicity of the ID groups compared with the IM group in all three strains was demonstrated (Table 4). The day 21 GMT and GMT fold increase, seroconversion and seroprotection rates of the H1N1 strains by HAI and MN (Tables 5 and 6; Figs. 1 and 2) were significantly higher in the ID groups when compared with the IM15 control group. The day 21 GMT, GMT fold increase, seroconversion and seroprotection rates by HAI were highest in the dose sparing ID3 group (Figs. 3 and 4). Seroconversion rates of the H3N2 strain by HAI were also significantly higher in all three ID groups when compared with the IM15 group. Head-to-head comparison showed no difference in immunogenicity among the three ID groups (Tables 7 and 8), other than a number of findings using the MN method, in which INT9 was inferior to ID3 (H3N2 GMT, $p = 0.023$; H3N2 seroconversion, $p = 0.02$).

There was a strong correlation between post-vaccination swelling and subsequent day 21 GMT (Spearman rho correlation 0.3; $p < 0.001$), seroconversion rate (Spearman rho correlation 0.152; $p = 0.014$), seroprotection rate (Spearman rho correlation 0.181; $p = 0.003$), and GMT fold increases (Spearman rho correlation 0.183; $p = 0.003$) for the H1N1 strain. Similar correlation was found for the day 21 GMT (Spearman rho correlation 0.126; $p = 0.04$), seroconversion rate (Spearman rho correlation 0.16; $p = 0.01$) and GMT

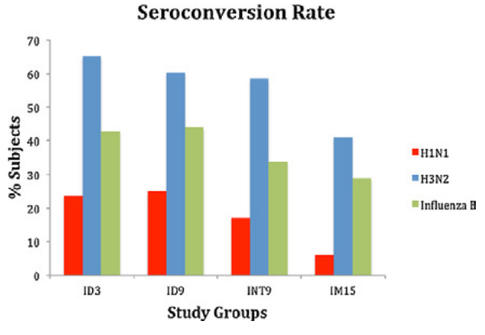

Fig. 1. Day 21 seroconversion rate of the four study groups by HAI assay.

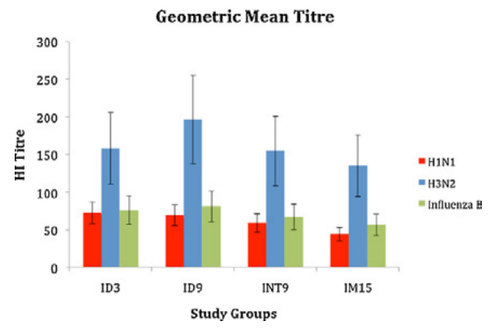

Fig. 3. Day 21 geometric mean titer by HAI assay.

Table 2
Incidence of systemic adverse events.

	ID3 ($n=63$)	ID9 ($n=68$)	INT9 ($n=65$)	IM15 ($n=66$)	*P*-Value
Fever	0	2	0	0	0.124
Headache	6	4	8	5	0.596
Malaise	13	12	13	6	0.263
Myalgia	9	11	10	4	0.279
Arthralgia	8	5	7	4	0.536
Severe adverse events	0	0	0	0	1.0

Fever: body temperature ≥ 37.5 °C.

Table 3
Incidence of local adverse events.

	ID3 ($n=63$)	ID9 ($n=68$)	INT9 ($n=65$)	IM15 ($n=66$)	*P*-Value
Redness					
Grade 1	15	22	9	1	<0.001
Grade 2 or 3	13	14	6	0	<0.001
Swelling					
Grade 1	54	62	31	1	<0.001
Grade 2 or 3	0	2	1	0	0.318
Induration					
Grade 1	0	1	0	0	0.413
Grade 2 or 3	0	0	0	0	NA
Ecchymosis					
Grade 1	0	1	2	1	0.579
Grade 2 or 3	0	0	0	0	NA
Pain					
Grade 1	2	2	4	1	0.544
Grade 2 or 3	0	0	0	0	NA

Redness, swelling, induration and ecchymosis were graded based on size: Grade 1, under 20 mm; Grade 2, 20–50 mm; and Grade 3, over 50 mm.
Pain was graded as follows: Grade 1, pain on touch, Grade 2, pain when arm is moved; and Grade 3, spontaneous pain or pain that prevents normal activity.

fold increases (Spearman rho correlation 0.167; $p=0.007$) for the H3N2 strain, and also for the day 21 GMT (Spearman rho correlation 0.125; $p=0.04$) and GMT fold increases (Spearman rho correlation 0.168; $p=0.006$) for the B strain. No correlation was found between those with history of monovalent pandemic influenza vaccination in the previous year and subsequent TIV immunogenicity ($p>0.05$).

4. Discussion

We report the results of the first ID TIV study after the pandemic of H1N1 2009, comparing the immunogenicity of TIV delivered by two different ID devices at lower doses. Immunogenicity of the H1N1 strain was significantly higher by ID vaccination delivered

Table 4
Non-inferiority analysis for day 21 geometric mean titer (GMT) ratio and seroconversion rate difference between the IM and ID devices.

Vaccine strain	GMT ratio (IM15/ID3) value	Lower 95% CI	Upper 95% CI	GMT ratio (IM15/ID9) value	Lower 95% CI	Upper 95% CI	GMT ratio (IM15/INT9) value	Lower 95% CI	Upper 95% CI
HAI									
California (H1N1)	0.67	0.72	0.63	0.69	0.68	0.68	0.89	0.87	0.86
Perth (H3N2)	0.84	0.58	0.94	0.47	0.43	0.48	0.78	1.74	0.67
Brisbane (B)	0.86	0.68	0.91	1.03	0.54	1.32	0.97	0.83	1.00
MN									
California (H1N1)	0.59	0.56	0.57	0.57	0.55	0.58	0.59	0.62	0.55
Perth (H3N2)	1.06	1.00	1.10	0.84	0.84	0.86	1.09	1.23	1.05
Brisbane (B)	1.00	0.84	1.06	0.96	0.96	0.96	1.26	1.13	1.32
Vaccine strain	SC rate diff (IM15/ID3) value	Lower 95% CI	Upper 95% CI	SC rate diff (IM15/ID9) value	Lower 95% CI	Upper 95% CI	SC rate diff (IM15/INT9) value	Lower 95% CI	Upper 95% CI
HAI									
California (H1N1)	−0.18	−0.30	−0.06	−0.19	−0.31	−0.07	−0.11	−0.22	0.01
Perth (H3N2)	−0.24	−0.41	−0.07	−0.19	−0.36	−0.03	−0.18	−0.35	−0.01
Brisbane (B)	−0.14	−0.31	0.03	−0.15	−0.32	0.01	−0.05	−0.21	0.09
MN									
California (H1N1)	−0.21	−0.37	−0.06	−0.13	−0.27	0.02	−0.16	−0.30	−0.01
Perth (H3N2)	−0.02	−0.18	0.05	0.04	−0.12	0.09	0.03	0.02	0.07
Brisbane (B)	0.04	−0.12	0.08	−0.02	−0.19	0.06	0.04	−0.02	0.08

GMT, geometric mean titer; SC rate diff, seroconversion rate difference; HAI: hemagglutination-inhibition; MN, microneutralization; and CI, confidence interval.
Non-inferiority was defined as the upper limit of the 2-sided 95% CI of the GMT ratio (IM/ID vaccines) not exceeding 1.5 and the upper limit of the 2-sided 95% CI for the difference in seroconversion rates (IM minus ID vaccines) not exceeding 10% for all three strains.

Table 5
Head-to-head comparison of the immunogenicity by HAI between the IM and ID devices.

		IM15 ($n=66$)	ID3 ($n=63$)	p^a	ID9 ($n=68$)	p^b	INT9 ($n=65$)	p^c
California (H1N1)								
GMT values (95% CI)	Day 0	32.4 (27.5–49)	38.9 (32.4–46.8)	0.182	38 (32.4–44.7)	0.248	39.8 (30.9–50.1)	0.253
	Day 21	44.2 (36.8–55)	72.4 (60.3–87.1)	**<0.001**	69.2 (57.5–85.1)	**0.001**	59.1 (47.5–73.4)	**0.029**
CPMP criteria (day 21)	Seroconversion (%)	6.1	23.8	**0.004**	25	**0.002**	16.9	**0.050**
	Seroprotection (%)	71.2	90.5	**0.005**	86.8	**0.027**	81.5	0.167
	GMT fold increase value (95% CI)	1.6 (1.3–1.9)	2.4 (1.8–3)	**0.018**	2.3 (1.9–2.8)	**0.011**	1.8 (1.5–2.2)	0.331
Perth (H3N2)								
GMT values (95% CI)	Day 0	42.7 (30.2–56.2)	34.7 (26.9–45.7)	0.282	34.7 (26.9–44.7)	0.239	40.7 (30.2–56.2)	0.737
	Day 21	135 (95.3–191.2)	158.1 (117.7–212.8)	0.324	196.3 (141.3–269.2)	0.115	154.6 (111.5–214.5)	0.571
CPMP criteria (day 21)	Seroconversion (%)	40.9	65.1	**0.006**	60.3	0.025	58.5	**0.045**
	Seroprotection (%)	92.4	90.5	0.695	91.2	0.794	92.3	0.980
	GMT fold increase value (95% CI)	7.7 (3.3–12)	9.2 (5.7–12.7)	0.580	16.3 (7.6–25)	0.081	9.9 (1.9–17.9)	0.628
Brisbane (B)								
GMT values (95% CI)	Day 0	30.6 (24.1–38.9)	29.9 (22.8–39.2)	0.895	29.6 (23.3–37.6)	0.935	32.1 (24.5–42.1)	0.789
	Day 21	56.4 (43–73.8)	76.2 (59–98.6)	0.108	81.4 (62.7–105.7)	0.053	67.2 (52.1–86.7)	0.346
CPMP criteria (day 21)	Seroconversion (%)	28.8	42.9	0.097	44.1	0.066	33.8	0.536
	Seroprotection (%)	66.7	84.1	**0.022**	80.9	0.062	81.5	0.053
	GMT fold increase value (95% CI)	3.7 (1.5–5.8)	4.3 (2.2–6.4)	0.667	3.6 (2.8–4.4)	0.940	3.8 (1.8–5.8)	0.907

GMT, geometric mean titer; CPMP, Committee for Proprietary Medicinal Products. CPMP guideline: at least one of the following criteria must be met for the viral strain in the vaccine: GMT fold increase > 2.5, seroconversion rate > 40% and seroprotection rate > 70%; HAI, hemagglutination-inhibition [significant *p* values in bold].
[a] For the IM15 group versus the ID3 group.
[b] For the IM15 group versus the ID9 group.
[c] For the IM15 group versus the INT9 group.

Table 6
Head-to-head comparison of the immunogenicity by MN between the IM and ID devices.

		IM15 ($n=66$)	ID3 ($n=63$)	p^a	ID9 ($n=68$)	p^b	INT9 ($n=65$)	p^c
California (H1N1)								
GMT values (95% CI)	Day 0	12 (10.7–13.6)	15.5 (13.1–18.3)	**0.048**	11.3 (9.9–12.8)	0.445	13.1 (11.1–15.6)	0.413
	Day 21	23.1 (20.4–26.2)	45.4 (36.1–57.2)	**<0.001**	37.4 (31.5–44.5)	0.694	34.7 (27.9–43.1)	0.126
CPMP criteria (day 21)	Seroconversion (%)	15.2	52.4	**<0.001**	54.4	**<0.001**	47.7	**<0.001**
	Seroprotection (%)	34.8	76.2	**<0.001**	76.4	**<0.001**	64.6	**0.001**
	GMT fold increase value (95% CI)	2.4 (1.8–2.9)	4.1 (3.2–5.1)	**0.007**	4.2 (3.3–5)	0.274	4.1 (2.9–5.3)	0.084
Perth (H3N2)								
GMT values (95% CI)	Day 0	10.9 (9.1–13.1)	12.9 (10.6–15.8)	0.450	11.3 (9.3–13.8)	0.981	12.4 (9.9–15.6)	0.302
	Day 21	27.9 (21.4–36.4)	33.8 (27–42.2)	0.926	32.5 (25.8–40.9)	0.945	26 (19.9–34)	0.981
CPMP criteria (day 21)	Seroconversion (%)	33.3	41.3	0.851	33.8	0.628	32.3	0.943
	Seroprotection (%)	45.4	47.6	0.909	45.6	0.877	43.1	0.632
	GMT fold increase value (95% CI)	3.6 (2.7–4.6)	3.4 (2.7–4.2)	0.204	4.3 (3.2–5.4)	0.471	3.3 (2.2–4.4)	0.112
Brisbane (B)								
GMT values (95% CI)	Day 0	28.7 (21–39.3)	30.3 (22–41.5)	0.677	37.9 (27.4–52.4)	0.164	37.8 (26.9–53.1)	0.176
	Day 21	74.9 (52.6–106.6)	84.4 (59–120.9)	0.789	97.9 (69.1–138.5)	0.388	80.7 (56.2–115.8)	0.897
CPMP criteria (day 21)	Seroconversion (%)	37.9	39.7	0.511	47.1	0.854	38.5	0.421
	Seroprotection (%)	72.7	74.6	0.725	79.4	0.766	75.4	0.801
	GMT fold increase value (95% CI)	4.8 (2.6–7)	4.8 (3.1–6.6)	0.358	5 (2.7–7.3)	0.435	3.8 (2.3–5.3)	0.123

GMT, geometric mean titer; CPMP, Committee for Proprietary Medicinal Products. CPMP guideline: at least one of the following criteria must be met for the viral strain in the vaccine: GMT fold increase > 2.5, seroconversion rate > 40% and seroprotection rate > 70%; MN, microneutralization [significant *p* values in bold].
[a] For the IM15 group versus the ID3 group.
[b] For the IM15 group versus the ID9 group.
[c] For the IM15 group versus the INT9 group.

Table 7
Head-to-head comparison of the immunogenicity by HAI between among the ID devices.

		INT9 (n = 65)	ID3 (n = 63)	p^a	ID9 (n = 68)	p^b
California (H1N1)						
GMT values (95% CI)	Day 0	39.8 (30.9–50.1)	38.9 (32.4–46.8)	0.992	38 (32.4–44.7)	0.836
	Day 21	59.1 (47.5–73.4)	72.4 (60.3–87.1)	0.212	69.2 (57.5–85.1)	0.327
CPMP criteria (day 21)	Seroconversion (%)	16.9	23.8	0.337	25	0.257
	Seroprotection (%)	81.5	90.5	0.148	86.8	0.413
	GMT fold increase value (95% CI)	1.8 (1.5–2.2)	2.4 (1.8–3)	0.089	2.3 (1.9–2.8)	0.080
Perth (H3N2)						
GMT values (95% CI)	Day 0	40.7 (30.2–56.2)	34.7 (26.9–45.7)	0.497	34.7 (26.9–44.7)	0.445
	Day 21	154.6 (111.5–214.5)	158.1 (117.7–212.8)	0.685	196.3 (141.3–269.2)	0.299
CPMP criteria (day 21)	Seroconversion (%)	58.5	65.1	0.445	60.3	0.831
	Seroprotection (%)	92.3	90.5	0.714	91.2	0.815
	GMT fold increase value (95% CI)	9.9 (1.9–17.9)	9.2 (5.7–12.7)	0.885	16.3 (7.6–25)	0.280
Brisbane (B)						
GMT values (95% CI)	Day 0	32.1 (24.5–42.1)	29.9 (22.8–39.2)	0.706	29.6 (23.3–37.6)	0.729
	Day 21	67.2 (52.1–86.7)	76.2 (59–98.6)	0.487	81.4 (62.7–105.7)	0.297
CPMP criteria (day 21)	Seroconversion (%)	33.8	42.9	0.298	44.1	0.228
	Seroprotection (%)	81.5	84.1	0.701	80.9	0.924
	GMT fold increase value (95% CI)	3.8 (1.8–5.8)	4.3 (2.2–6.4)	0.744	3.6 (2.8–4.4)	0.809

GMT, geometric mean titer; CPMP, Committee for Proprietary Medicinal Products. CPMP guideline: at least one of the following criteria must be met for the viral strain in the vaccine: GMT fold increase > 2.5, seroconversion rate > 40% and seroprotection rate > 70%; HAI, hemagglutination-inhibition [significant p values in bold].
[a] For the INT9 group versus the ID3 group.
[b] For the INT9 group versus the ID9 group.

Table 8
Head-to-head comparison of the immunogenicity by MN among the ID devices.

		INT9 (n = 65)	ID3 (n = 63)	p^a	ID9 (n = 68)	p^b
California (H1N1)						
GMT values (95% CI)	Day 0	13.1 (11.1–15.6)	15.5 (13.1–18.3)	0.176	11.3 (9.9–12.8)	0.150
	Day 21	34.7 (27.9–43.1)	45.4 (36.1–57.2)	0.067	37.4 (31.5–44.5)	0.296
CPMP criteria (day 21)	Seroconversion (%)	47.7	52.4	0.497	54.4	0.720
	Seroprotection (%)	64.6	76.2	0.108	76.4	0.205
	GMT fold increase value (95% CI)	4.1 (2.9–5.3)	4.1 (3.2–5.1)	0.801	4.2 (3.3–5)	0.307
Perth (H3N2)						
GMT values (95% CI)	Day 0	12.4 (9.9–15.6)	12.9 (10.6–15.8)	0.721	11.3 (9.3–13.8)	0.300
	Day 21	26 (19.9–34)	33.8 (27–42.2)	0.023	32.5 (25.8–40.9)	0.033
CPMP criteria (day 21)	Seroconversion (%)	32.3	41.3	0.020	33.8	0.090
	Seroprotection (%)	43.1	47.6	0.112	45.6	0.114
	GMT fold increase value (95% CI)	3.3 (2.2–4.4)	3.4 (2.7–4.2)	0.315	4.3 (3.2–5.4)	0.026
Brisbane (B)						
GMT values (95% CI)	Day 0	37.8 (26.9–53.1)	30.3 (22–41.5)	0.342	37.9 (27.4–52.4)	0.991
	Day 21	80.7 (56.2–115.8)	84.4 (59–120.9)	0.891	97.9 (69.1–138.5)	0.467
CPMP criteria (day 21)	Seroconversion (%)	38.5	39.7	0.889	47.1	0.320
	Seroprotection (%)	75.4	74.6	0.919	79.4	0.582
	GMT fold increase value (95% CI)	3.8 (2.3–5.3)	4.8 (3.1–6.6)	0.404	5 (2.7–7.3)	0.411

GMT, geometric mean titer; CPMP, Committee for Proprietary Medicinal Products. CPMP guideline: at least one of the following criteria must be met for the viral strain in the vaccine: GMT fold increase > 2.5, seroconversion rate > 40% and seroprotection rate > 70%; MN, microneutralization [significant p values in bold].
[a] For the INT9 group versus the ID3 group.
[b] For the INT9 group versus the ID9 group.

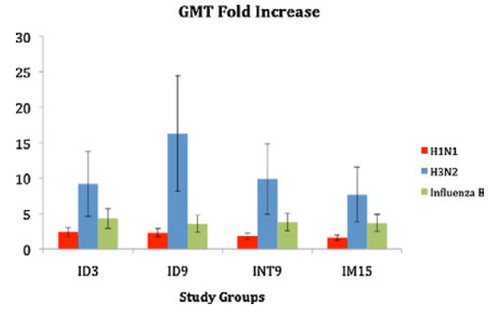

Fig. 4. Day 21 geometric mean titer fold increase by HAI assay. GMT, geometric mean titer.

by both devices when compared with the IM vaccination, with the highest seroprotection rate and GMT fold increase value generated by the lowest dose of 3 μg (20%) HA vaccine delivered by the MicronJet600™. Seroconversion rates of the H3N2 strain by HAI were also significantly higher with the ID groups. Non-inferiority in immunogenicity of the ID vaccines was demonstrated for the B strain as illustrated in Section 3 and Table 4. Post vaccination redness and swelling were significantly more prevalent among the ID groups when compared with the IM15 group, consistent with many previous reports [13–15]. Presence of swelling correlated well with the subsequent immunogenicity but with no long-term sequelae, and should be explored in the future as a marker for effective ID vaccination, especially in the elderly and the hard to immunize populations. No serious adverse events related to vaccination were found in any of the groups.

Suboptimal response to the monovalent 2009 H1N1 vaccine via intradermal or intramuscular routes has been reported [13].

Recent report also suggested possible reduced effectiveness of the 2009 H1N1 component of the LAIV in the young immunocompetent hosts for the 2010–2011 influenza season, corresponding with an increased number of influenza cases among military recruits who received the LAIV [12]. This study confirmed the reduced immunogenicity of the H1N1 2009 component of the TIV in older adults when compared to the H3N2 and B strains. Some studies have shown that prior administration or co-administration of TIV is associated with a reduced response to the monovalent pandemic influenza vaccine [22,23]. One possible explanation is the phenomenon of "original antigenic sin" in which stimulation of the immune system by previous vaccines reduces the immune response to the current vaccine. Nevertheless, result from our study showed no correlation between receipt of monovalent pandemic influenza vaccine in the previous years and subsequent TIV immunogenicity. Intradermal vaccination however, could overcome this reduced immunogenicity of the H1N1 2009 strain, and save doses. Furthermore, two recent surveys performed in Australia and Europe have demonstrated well acceptance of the ID vaccination for seasonal influenza by the Intanza®9, both by adult vaccinees and prescribers [24,25]. Small needle size and high immunogenicity were the two attractive factors. Importantly, the ability to reduce the dose by a five-fold (as demonstrated in the ID3 group), which enables five-times dose sparing, is also important to consider in high demand situations such as pandemics. All of the features above have important implications to national vaccination policy.

Poor immunogenicity of influenza vaccination by conventional intramuscular route of delivery among the elderly and immunocompromised is well known. The poor immune response might be related to low serum albumin level secondary to poor nutritional status and concomitant diseases [26]. Immunosenescence of the innate immune system is associated with decreased number of Langerhans cells, decreased capacity of dendritic cells to present antigen, defective or reduced expression of Toll-like receptors and MHC class I and II molecules. Moreover immunosenescence of the adaptive immune system is associated with decreased production of mature naive T cells by the thymus. As expected, low pre-vaccination HAI titer and advanced age were associated with earlier decline of HAI titers [27]. Emergence and dominance of the novel H1N1 2009 strain also contributed to the low pre-vaccination titer against the H1N1 strain. Prevalence of seroprotection against the pandemic H1N1 virus after the 2009 pandemic was particularly low among people aged 50–79 years [28]. Their poor response may be overcome by ID vaccination due to enhanced Langerhans cells response [29] and more rapid recall immune responses against the influenza virus. Previous studies have demonstrated non-inferiority in immunogenicity of reduced dose ID influenza vaccination among healthy adults and children when compared with full-dose IM vaccination [14,30,31]. The superior immunogenicity by the ID systems as demonstrated in this study could be explained by the relative poor immunogenicity of the H1N1 2009 strain. This is different from previous studies conducted where the A/New Caledonia/20/99 IVR-116 (H1N1) component of the trivalent seasonal influenza vaccine showed no immunogenicity difference when compared to the H3N2 or the B components [30]. More recent study has further suggested trends for higher immunogenicity of ID influenza vaccination in renal transplant patients who were non-responders to conventional influenza vaccination [32].

Vaccination by both intradermal devices resulted in superior immunogenicity when compared to intramuscular vaccination for the H1N1 strain. Comparison of the two intradermal devices (Tables 7 and 8) showed no differences statistically, other than in MN H3N2 GMT and seroconversion, which were higher in the ID3 group despite using 1/3 of the Intanza® dosage. The day 21 immunogenicity of the H1N1 strain with the dose-sparing ID3 was highest among the three ID groups. This could be explained by the difference of the two microneedles delivery systems. Vaccination of the INT9 group was delivered by the BD Soluvia™ microinjection system with a single 1.5 mm needle penetrating perpendicularly to the skin [15,17]. This resulted in a substantial proportion of the vaccination injected into the subcutaneous space, as evidenced by 53.8% of INT9 cases in which the typical intradermal bleb was not formed as compared with only 0.8% for the ID3/ID9 groups (data not shown). On the other hand, vaccination of the ID3 and ID9 groups were delivered by the MicronJet600™ system [14], with an array of three microneedles each 0.6 mm in length, puncturing obliquely into the skin. This provides the shallowest injection method available. To the best of our knowledge shallow delivery correlates with effective immunization as it reaches the appropriate immune cells in skin [14,15,17,32], thus allowing the dose-sparing effect and higher immunogenicity. Nevertheless, the BD Soluvia microinjection system was associated with significantly fewer leakage events and less frequent post vaccination redness or major swelling when compared with the MicronJet600™ system, which may be an indication of effective immunogenicity as well. The non-superior immunogenicity of ID9 when compared to ID3 despite a higher dosage could be related to more frequent leakage and also antigen saturation of the intradermal Langerhan cells as a limiting factor. In terms of their ease of use, the Intanza® with the BD Soluvia™ microinjection system has the advantage of being a prefilled vaccine and the injection given at a perpendicular angle, whereas for the MicronJet600™ system the injection has to be given obliquely into the skin.

The limitation of this study is that the majority of the recruited patients were elderly subjects. Long-term immunogenicity beyond 6 months was also not available for comparison. Nevertheless we would expect an even higher and more sustained immunogenicity if young healthy adults receive ID TIV vaccination. In conclusion, there is reduced immunogenicity of the H1N1 strain of the TIV. Immunogenicity of the dose-sparing ID TIV was significantly better than the IM vaccination of the H1N1 and to certain extent of the H3N2 strains. All at risk subjects indicated for the TIV may receive intradermal immunization to improve immunogenicity or to compensate for occasional reduced immunogenicity of influenza vaccines.

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The second author Yotam Levin would like to declare that he is currently an Executive with NanoPass Technologies Ltd.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2012.08.014. These data include Google maps of the most important areas described in this article.

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MAJOR ARTICLE

Comparison of the Immunogenicity of Various Booster Doses of Inactivated Polio Vaccine Delivered Intradermally Versus Intramuscularly to HIV-Infected Adults

Stephanie B. Troy,[1] Diana Kouiavskaia,[3] Julia Siik,[1] Efrat Kochba,[5] Hind Beydoun,[2] Olga Mirochnitchenko,[3] Yotam Levin,[5] Nancy Khardori,[1] Konstantin Chumakov,[3] and Yvonne Maldonado[4]

[1]Department of Internal Medicine and [2]Graduate Program in Public Health, Eastern Virginia Medical School, Norfolk; [3]Center for Biologics Evaluation and Research, Food and Drug Administration, Silver Spring, Maryland; [4]Department of Pediatrics, Stanford University School of Medicine, California; and [5]NanoPass Technologies, Nes Ziona, Israel

Background. Inactivated polio vaccine (IPV) is necessary for global polio eradication because oral polio vaccine can rarely cause poliomyelitis as it mutates and may fail to provide adequate immunity in immunocompromised populations. However, IPV is unaffordable for many developing countries. Intradermal IPV shows promise as a means to decrease the effective dose and cost of IPV, but prior studies, all using 20% of the standard dose used in intramuscular IPV, resulted in inferior antibody titers.

Methods. We randomly assigned 231 adults with well-controlled human immunodeficiency virus infection at a ratio of 2:2:2:1 to receive 40% of the standard dose of IPV intradermally, 20% of the standard dose intradermally, the full standard dose intramuscularly, or 40% of the standard dose intramuscularly. Intradermal vaccination was done using the NanoPass MicronJet600 microneedle device.

Results. Baseline immunity was 87%, 90%, and 66% against poliovirus serotypes 1, 2, and 3, respectively. After vaccination, antibody titers increased a median of 64-fold. Vaccine response to 40% of the standard dose administered intradermally was comparable to that of the standard dose of IPV administered intramuscularly and resulted in higher (although not significantly) antibody titers. Intradermal administration had higher a incidence of local side effects (redness and itching) but a similar incidence of systemic side effects and was preferred by study participants over intramuscular administration.

Conclusions. A 60% reduction in the standard IPV dose without reduction in antibody titers is possible through intradermal administration.

Keywords. intradermal; fractional dose; inactivated polio vaccine; HIV; polio; vaccine.

Globally, paralytic poliomyelitis rates from wild poliovirus have dropped by >99% since 1988, with 406 cases reported in 2013, and only 3 countries remain with uninterrupted endemic transmission [1]. Much of this success is due to oral polio vaccine (OPV), which is used for polio vaccination in most of the developing world. However, as a live virus, OPV can mutate into forms capable of causing paralytic poliomyelitis, such as vaccine-derived poliovirus (VDPV), which caused 6 outbreaks of paralytic poliomyelitis in 2013 alone [2]. Because of these risks from OPV, the recent Polio Eradication and Endgame Strategic Plan 2013–2018 proposed by the World Health Organization (WHO) includes initiating at least 1 dose of inactivated polio vaccine (IPV) for children in all countries and subsequently phasing out OPV [3]. In addition, 2 recent studies have demonstrated that a booster dose of IPV results in significantly higher humoral and mucosal polio

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Correspondence: Stephanie B. Troy, MD, Eastern Virginia Medical School, 825 Fairfax Ave, Ste 572, Norfolk, VA (amadapaah@gmail.com).

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immunity than a booster dose of OPV in children who received OPV as their primary regimen [4, 5]. IPV has also been shown to significantly increase seroconversion rates in children who did not respond to OPV [6]. Consequently, IPV may also have a role as a booster dose in supplementary immunization campaigns to control outbreaks of wild poliovirus or VDPV infection.

One difficulty with these plans is that IPV is currently too expensive for many developing countries, costing approximately 20-fold more than OPV per dose [7]. A potential method to make IPV more affordable is to decrease the dose by using intradermal instead of intramuscular administration. The skin has a particularly high concentration of dendritic cells, and it has been possible to reduce the dose of other vaccines to 20%–60% of the standard intramuscular dose without decreasing immunogenicity through intradermal administration [8]. For influenza vaccines, some studies have shown superior immunogenicity despite using fractional intradermal doses [9, 10]. However, past clinical studies of fractional-dose intradermal IPV have only used 20% of the standard dose and have all resulted in significantly lower antibody titers, compared with full-dose intramuscular IPV [7, 11–15].

To determine whether a booster of intradermal IPV using a fractional dose >20% of the standard dose can be equally effective as the full standard dose of intramuscular IPV, we conducted a randomized, controlled clinical trial comparing booster doses of 40% fractional-dose intradermal IPV, 20% fractional-dose intradermal IPV, full-dose intramuscular IPV, and 40% fractional-dose intramuscular IPV. Because this was a proof-of-concept study and the first time 40% fractional-dose intradermal IPV had been tested in humans, we elected to enroll adult volunteers at our home institution. Because all past studies of intradermal IPV were in healthy children and adults, we chose to limit enrollment to subjects infected with human immunodeficiency virus (HIV).

HIV-infected adults have a reduced immunologic response to most vaccines. Although some of the decreased immunologic response seen in these individuals can be explained by the low number of $CD4^+$ T cells associated with advanced HIV infection, the decreased immunologic response to vaccines persists even in HIV-infected individuals who are receiving antiretroviral therapy and have $CD4^+$ T-cell counts in the normal range [16, 17]. This has been shown for multiple vaccines, including the pneumococcal vaccines [17], hepatitis B vaccines [18, 19], and influenza vaccines [16]. We have previously shown that HIV infection significantly reduces the immunologic response to OPV in Zimbabwean infants [20]. Prior studies evaluating the effect of HIV infection on the immunologic response to IPV were all conducted prior to the development of combined antiretroviral therapy, and all used full IPV doses administered intramuscularly [21–26]. In general, these studies show that advanced HIV infection decreased the response to intramuscular IPV but that HIV-infected subjects with higher $CD4^+$ T-cell counts had similar seroprotection rates, although lower antibody titers, after intramuscular IPV vaccination, compared with uninfected controls. Since the countries with the highest rates of HIV infection primarily use OPV and will need to transition to IPV with the new WHO polio eradication plan, it is important to know whether fractional-dose intradermal IPV would be effective even in immunocompromised populations.

MATERIALS AND METHODS

Study Design and Population

This study was conducted at the Eastern Virginia Medical School (EVMS) HIV clinic in Norfolk, Virginia, and followed the principles of the Declaration of Helsinki and good clinical practice guidelines. The study was approved by the EVMS Institutional Review Board and was registered with ClinicalTrials.gov (NCT01686503). Of note, in the United States, IPV (the older formulation) was licensed and its widespread use began in both infants and older children in 1955 [27]. OPV was licensed in 1961, rapidly replacing IPV, and then enhanced IPV replaced OPV in 2000. Wild poliovirus was prevalent in the United States prior to the introduction of IPV in 1955, but annual cases of paralytic poliomyelitis had dropped to <150 by the early 1960s, and the last case of naturally occurring paralytic poliomyelitis due to wild poliovirus in the United States was in 1979.

We enrolled 231 subjects between 7 September 2012 and 8 July 2013. Inclusion criteria included documented HIV infection, age >18 years, and an HIV load of <400 copies/mL at the most recent measurement. Exclusion criteria included current acute illness, pregnancy, or history of allergic reaction to any component of IPV. Written informed consent was obtained from all subjects.

At the enrollment visit, a blood specimen was collected, and subjects were randomly assigned to one of 4 study groups in a ratio of 2:2:2:1, based on a computer-generated randomization scheme done in 3 blocks of 77 to ensure even distribution over the enrollment period. Group 1 received 40% (0.2 mL) of the standard dose of IPV intradermally (66 subjects), group 2 received 20% (0.1 mL) of the standard dose intradermally (66 subjects), group 3 (the control group) received the full dose (0.5 mL) intramuscularly (66 subjects), and group 4 received 40% (0.2 mL) of the standard dose intramuscularly (33 subjects). The IPV used was IPOL (Sanofi Pasteur), containing 40 D antigen units of serotype 1, 8 D antigen units of serotype 2, and 32 D antigen units of serotype 3 poliovirus per 0.5 mL. Intramuscular injections were done into the deltoid muscle, and intradermal injections were done into the skin over the deltoid muscle. Intradermal injections were done using the NanoPass MicronJet600 device, a Food and Drug Administration (FDA)–approved microneedle-based device for intradermal injection. Occurrences of major leakage (defined as a visible drop >2 mm in diameter on the skin) and bleb formation were recorded after intradermal administration. Subjects also completed a questionnaire, and information, including laboratory data, medications, and comorbidities, was extracted from the medical records.

Subjects were given a diary to record adverse events during the first week and were called by the study coordinator within a week of enrollment, who asked about adverse reactions. The second study visit occurred 4–6 weeks after the enrollment visit and included collection of a second blood specimen and a follow-up questionnaire.

Sample Analysis

On the day of collection, blood samples were centrifuged, and the serum was stored at −80°C. After the study visits had been completed, aliquots of frozen serum samples were shipped on dry ice to Dr Konstantin Chumakov's laboratory at the FDA, where poliovirus neutralizing antibody assays were done in a blinded fashion according to the World Health Organization method [28]. The antibody titer was defined as the reciprocal of the highest dilution of serum that neutralized the virus, and all serum samples were diluted until the highest dilution was determined.

Immunity, or seroprotection, was defined as an antibody titer of ≥8. Vaccine response was defined as seroconversion in subjects not immune at baseline or as a ≥4-fold rise in titer in subjects immune at baseline.

Sample Size Calculations

Sample sizes were calculated to show equivalency in vaccine response between groups 1 and 3 (56 subjects were required in each group), a 15% increase in vaccine response in group 1 versus group 2 (42 subjects were required in each group), and a

Table 1. Demographic Characteristics of Study Subjects

Characteristic	Group 1, 40% ID (n = 66)	Group 2, 20% ID (n = 66)	Group 3, 100% IM (n = 66)	Group 4, 40% IM (n = 33)	*P* Value
Age, y					
Mean ± SD	45 ± 10	45 ± 11	46 ± 11	46 ± 11	.96
Range	24–61	23–70	21–63	21–63	
Female sex	36 (24)	36 (24)	32 (21)	21 (7)	.42
Race					.68
White	29 (19)	26 (17)	29 (19)	33 (11)	
Black	71 (47)	73 (48)	71 (47)	64 (21)	
Other	0 (0)	2 (1)	0 (0)	3 (1)	
Born in the US	98 (65)	95 (63)	91 (60)	94 (31)	.38
Lived or traveled internationally	27 (18)	29 (19)	44 (29)	36 (12)	.16
Received all childhood vaccines	97 (64)	97 (64)	91 (60)	94 (31)	.43
Year of HIV infection diagnosis, mean[a]	2001	2002	2001	2001	.90
Currently receiving ART	97 (64)	97 (64)	100 (66)	100 (33)	.90
$CD4^+$ T-cell count in cells/mm^3, mean ± SD					
Most recent	669 ± 361	630 ± 331	676 ± 354	569 ± 260	.44
Lowest in the medical record	328 ± 269	287 ± 221	294 ± 294	322 ± 260	.8
Most recent HIV load (copies/mL), mean ± SD	111 ± 95	82 ± 86	85 ± 78	99 ± 69	.67
Diagnosis of AIDS in the record	58 (38)	50 (33)	59 (39)	52 (17)	.69
Ever homeless	39 (26)	33 (22)	21 (14)	21 (7)	.08
Current smoker	58 (38)	47 (31)	35 (23)	39 (13)	.06
Coinfected with hepatitis C virus	18 (12)	15 (10)	9 (6)	15 (5)	.48
Coinfected with hepatitis B virus	11 (7)	6 (4)	3 (2)	0 (0)	.29
History of hypertension	42 (28)	38 (25)	42 (28)	45 (15)	.89
History of depression or bipolar disorder	33 (22)	33 (22)	29 (19)	27 (9)	.87
IPV lot no. received					.92
H1452–1	9 (6)	9 (6)	14 (9)	12 (4)	
H1604–1	29 (19)	26 (17)	21 (14)	21 (7)	
H1605–2	62 (41)	65 (43)	65 (43)	67 (22)	

Data are % (no.) of subjects, unless otherwise indicated.

Abbreviations: ART, antiretroviral therapy; HIV, human immunodeficiency virus; ID, intradermal; IM, intramuscular; IPV, inactivated polio vaccine; SD, standard deviation.

[a] The SD for groups 1–3 was 8 years, and the SD for group 4 was 9 years.

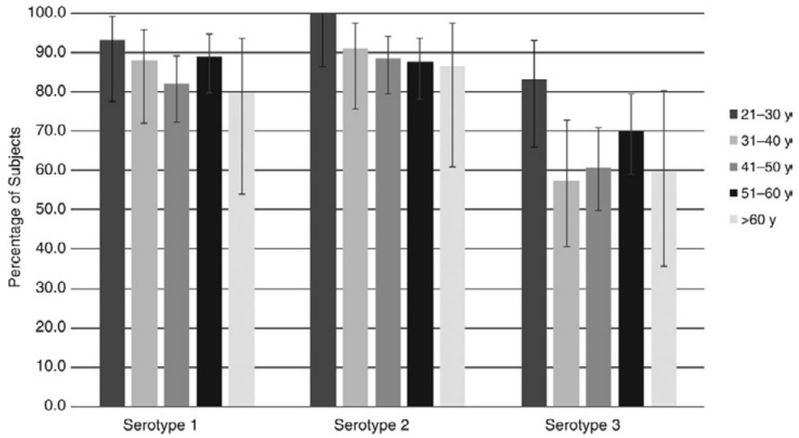

Figure 1. Baseline polio immunity, by age group in years. There were 30, 33, 79, 74, and 15 subjects aged 21–30, 31–40, 41–50, 51–60, and >60 years, respectively. The 95% confidence intervals of each proportion were calculated using the modified Wald method. A 2-tailed Fisher exact test revealed that the only significant differences in baseline immunity between age groups were for serotype 3 between the group aged 21–30 years and the groups aged 31–40 and 41–50 years ($P = .03$ and .04, respectively). Of note, the first 3 age groups (21–50 years) likely received oral polio vaccine as children and likely were not exposed to wild poliovirus, and the last 2 age groups (>51 years) likely received inactivated polio vaccine as children and may have been exposed to wild poliovirus.

25% increase in vaccine response in group 1 versus group 4 (21 subjects were required in each group), with an α level of 0.05, a β level of 0.20, and a 2-sided test for the equivalency study. Predicted vaccine response was based on the assumption that HIV infection would lead to a decrease of ≥5% in the vaccine response of approximately 85% reported in other studies (published by 2011, when our study was planned) investigating an IPV booster in HIV-uninfected subjects who received an OPV primary regimen [29, 30]. To compensate for an estimated 15% drop-out rate, enrollment in each group exceeded the required level by at least 18%.

Statistical Analysis

Descriptive and univariate analyses were performed using SAS, version 9.3 (SAS Institute, Cary, North Carolina). Baseline demographic characteristics were compared using *t* tests for continuous and χ^2 tests for categorical variables. One-way analysis of variance tests with Bonferroni-corrected pairwise comparisons were used to assess associations between study groups and continuous outcomes (fold-rise in titer and geometric mean titers at baseline and after receipt of booster), and χ^2 tests for categorical outcomes (immunity, vaccine response, and presence of side effects). Two-sided statistical tests were conducted at an α level of 0.05.

Post hoc, noninferiority for postbooster antibody titers was concluded if the lower limit of the 95% confidence interval of the difference between the $\log_2$ postbooster geometric mean titer (GMT) of the experimental group (minuend) and the control group (full-dose intramuscular IPV) did not exceed −1 for all 3 serotypes [11].

RESULTS

A total of 240 subjects consented to the study, of whom 9 did not meet screening criteria because of lack of laboratory data in the past 6 months or an HIV load of >400 copies/mL. We enrolled 231 subjects, of whom 97% completed both study visits (65, 63, 64, and 32 in groups 1, 2, 3, and 4, respectively). Demographic variables were not significantly different among the 4 groups (Table 1).

Although 95% of subjects reported having received all their childhood vaccinations, most could not remember which specific polio vaccines they had received. However, 95% of subjects were born in the United States, and their vaccination history and wild poliovirus exposure can be estimated by their age (see "Materials and Methods" section for remarks on the history of polio vaccination in the United States). The 142 subjects (61%) who were 21–50 years old at enrollment were likely vaccinated with OPV and were likely not exposed to wild poliovirus. The 89 subjects (39%) who were 51–70 years old were likely vaccinated with IPV and may have been exposed to wild poliovirus (indeed, a 61-year-old subject reported history of paralytic poliomyelitis as a child). Of note, no subjects reported receiving polio vaccine as an adult for international travel.

Table 2. Polio Immunity and Vaccine Response 1 Month After Inactivated Polio Vaccine (IPV) Booster Receipt by Study Group

		Postbooster Immunity, by Serotype			Vaccine Response, by Serotype		
Group, IPV Formulation	Subjects, No.	1	2	3	1	2	3
Group 1, 40% ID	65	98 (64)	98 (64)	97 (63)	91 (59)	92 (60)	91 (59)
Group 2, 20% ID	63	100 (63)	100 (63)	98 (62)	84 (53)	84 (53)	87 (55)[a]
Group 3, 100% IM	64	100 (64)	100 (64)	100 (64)	92 (59)	94 (60)	98 (63)
Group 4, 40% IM	32	100 (32)	100 (32)	100 (32)	94 (30)	94 (30)	97 (31)

Data are % (no.) of subjects in each group for each serotype who were immune (defined as a polio neutralizing antibody titer of ≥8) after booster vaccination or who responded to the vaccine (defined as seroconversion in subjects who were not immune at baseline or at least a 4-fold rise in titer in subjects who were immune at baseline). Differences in postbooster immunity and vaccine response were not statistically significant between study groups, unless otherwise indicated.

Abbreviations: ID, intradermal; IM, intramuscular.

[a] $P = .01$, compared with group 3.

There were no significant differences in baseline polio immunity between study groups: 87%, 90%, and 66% of all subjects were immune to serotypes 1, 2, and 3, respectively. Baseline immunity rates against serotypes 1 and 2 were both significantly higher than that against serotype 3 ($P < .0001$ for both comparisons). Baseline immunity, stratified by age group, is shown in Figure 1.

There were no significant differences in rates of polio immunity 1 month after receipt of the IPV booster between study groups (Table 2). With the exception of a 61-year-old outlier in group 1 who had no measurable immunity to any serotype either before or after vaccination, every subject was immune to serotypes 1 and 2 after the IPV booster. All but 3 subjects were immune to serotype 3 after the IPV booster. Vaccine response for serotype 3 was significantly lower for group 2 (20% intradermal dose) versus group 3 (full intramuscular dose; $P = .01$; Table 2). Other differences were not significant.

The baseline geometric mean titers (GMTs) and 1 month postbooster GMTs are shown in Table 3. There were no significant differences for baseline GMTs for any serotype. The postbooster GMTs were highest in group 1 (40% intradermal dose), but not significantly so. The fold-rises in titer were robust, with overall median fold-rises of 32, 42, and 161 for serotypes 1, 2, and 3, respectively. The fold-rise in titer for serotype 2 was significantly higher for group 1 (40% intradermal dose) versus group 2 (20% intradermal dose), but no other differences were significant.

All experimental groups (groups 1, 2, and 4) were noninferior to the control group (group 3), based on postbooster antibody titers. For serotypes 1, 2, and 3, the 95% confidence intervals for the $\log_2$ postbooster GMTs of the experimental group, minus the control group, were −.24 to 1.16, −.18 to 1.29, and −.57 to 1.39, respectively, for group 1; −.96 to .25, −.79 to .69, and −.97 to .81, respectively, for group 2; and −.72 to .90, −.47 to 1.23, and −.86 to 1.28, respectively, for group 4.

Intradermal administration was preferred by most subjects who received IPV intradermally (54% preferred intradermal administration, 3% preferred intramuscular administration, and 42% did not care). Major leakage occurred with 12 of the 132 intradermal injections (9%), but all but one of these injections still had good bleb formation. Major leakage was not associated with lower antibody titers or the date of study enrollment. There were no significant differences between systemic side effects in the intradermal versus intramuscular groups, but the intradermal groups had higher rates of transient local effects, such as redness or itching at the injection site (Table 4). The only serious adverse event, which occurred 1 month after enrollment in a subject from group 1 and was considered unlikely to be related

Table 3. Poliovirus Neutralizing Antibody Geometric Mean Titers (GMTs) at Baseline and After Receipt of Inactivated Polio Vaccine (IPV) Booster

	Serotype 1, GMT (95% CI)		Serotype 2, GMT (95% CI)		Serotype 3, GMT (95% CI)	
Group, IPV Formulation	Baseline	After Booster	Baseline	After Booster	Baseline	After Booster
Group 1, 40% ID	44 (31–64)	1715 (1174–2504)	33 (24–44)	2188 (1507–3178)	14 (10–20)	2375 (1423–3963)
Group 2, 20% ID	42 (29–59)	976 (730–1304)	53 (37–76)	1438 (984–2101)	20 (14–28)	1698 (1114–2588)
Group 3, 100% IM	42 (30–58)	1249 (916–1705)	36 (26–51)	1489 (1041–2128)	16 (11–21)	1792 (1133–2835)
Group 4, 40% IM	34 (20–56)	1328 (795–2219)	44 (29–66)	1938 (1232–3047)	11 (7–16)	2075 (1225–3514)

There were data on baseline GMT for 66, 66, 66, and 33 subjects and on postbooster GMT for 65, 63, 64, and 32 subjects for groups 1, 2, 3, and 4, respectively. There were no significant differences between study groups for either baseline or postbooster GMTs.

Abbreviations: CI, confidence interval; ID, intradermal; IM, intramuscular.

Table 4. Frequency of Adverse Events in the Week Following Vaccination, by Study Group

Adverse Event	Group 1, 40% ID (n = 65)	Group 2, 20% ID (n = 63)	Group 3, 100% IM (n = 64)	Group 4, 40% IM (n = 32)
Any[a]	51 (33)	46 (29)	28 (18)	31 (10)
Fever	0 (0)	3 (2)	0 (0)	3 (1)
Rash	2 (1)	2 (1)	6 (4)	6 (2)
Redness at injection site[b]	29 (19)	35 (22)	6 (4)	9 (3)
Swelling at injection site	8 (5)	11 (7)	5 (3)	6 (2)
Tenderness at injection site	15 (10)	13 (8)	17 (11)	16 (5)
Itching at injection site[c]	11 (7)	6 (4)	0 (0)	0 (0)

Data are (%) (no.) of subjects. Differences were not statistically significant, unless otherwise indicated.

Abbreviations: ID, intradermal; IM, intramuscular.

[a] $P = .008$ for group 1 vs group 3, and $P = .04$ for group 2 vs group 3.

[b] $P = .0007$ for group 1 vs group 3, $P \leq .0001$ for group 2 vs group 3, $P = .03$ for group 1 vs group 4, and $P = .008$ for group 2 vs group 4.

[c] $P = .007$ for group 1 vs group 3, and $P = .04$ for group 2 vs group 3.

to the study, was hospitalization for chest pain and electrolyte abnormalities that were attributed to alcohol withdrawal.

DISCUSSION

We report the results from the first human trial using a fractional intradermal dose of IPV that was >20% of the standard dose and the first trial of intradermal IPV in HIV-infected subjects. The 40%-dose intradermal IPV group achieved the highest postbooster antibody titers, compared with the other 3 groups (20%-dose intradermal IPV, full-dose intramuscular IPV, and 40%-dose intramuscular IPV), but the difference did not reach significance. Baseline and postbooster immunity were similar between the 4 study groups and for serotypes 1 and 2 for vaccine response, but the 20%-dose intradermal group had a significantly lower vaccine response to serotype 3, compared with the full-dose intramuscular group. Surprisingly, we found that adults with well-controlled HIV infection maintain high levels of polio immunity decades after polio vaccination and also have a robust memory response to booster IPV vaccination administered either intradermally or intramuscularly. Intradermal administration was well tolerated and preferred by a majority of subjects.

Among prior published randomized, controlled trials comparing seroconversion rates after 20%-dose intradermal IPV with those after full-dose intramuscular IPV in children, 3 showed significantly inferior seroconversion rates in the intradermal group [12–14], and 2 showed similar seroconversion rates [7, 15]. However, all prior trials showed significantly lower antibody titers after 20%-dose intradermal IPV, compared with full-dose intramuscular IPV [7, 11–15]. Our results are consistent with these studies but are the first to demonstrate that a booster of 40%-dose intradermal IPV results in antibody titers that are not only noninferior to full-dose intramuscular IPV but are actually higher, although not significantly so. The clinical significance of lower antibody titers that are above the threshold for seroprotection remains unclear. However, studies have suggested that high antibody titers (≥128) after IPV immunization are needed for reduction of fecal transmission [31], and 2 recent clinical trials showed that lower antibody titers at the time of an OPV challenge are associated with significantly higher OPV shedding [4, 5]. Because IPV as a primary regimen is known to induce less intestinal immunity than OPV [32], and because reducing fecal transmission is essential to stopping community circulation of poliovirus, choosing an IPV vaccination strategy that results in high antibody titers would be beneficial.

The high levels of baseline polio immunity in our HIV-infected subjects, even up to 5 decades after they should have last received a polio vaccination, is encouraging for the global polio eradication effort. We have previously shown that HIV-infected infants have a significantly lower immunologic response to OPV than uninfected infants [20]. However, the results from this current study suggest that, for the 33 million HIV-infected adults globally [33], most of whom were infected with HIV years after polio vaccination, such as the subjects in this study, polio immunity levels may remain high. Of note, these high levels of baseline polio immunity were evident even though most of our study subjects had a history of AIDS and even though OPV has not been used in the United States since 2000, so there would have been no recent boosting in our subjects due to community spread of OPV. Although the polio eradication effort has primarily focused on polio immunity in children, the large 2010 outbreak of wild poliovirus infection in the Republic of the Congo primarily affected adults (74% of cases) [34]. In this outbreak, older age was associated with a 7-fold higher risk of death [35]. A study using mathematical modeling suggested that the contribution of older children and adults to the spread of wild poliovirus in this outbreak was also significant [36]. Consequently, it is reassuring that polio immunity can remain high for decades despite HIV infection as an adult. Our data are consistent with 4 smaller studies from the 1990s that evaluated whether HIV-infected adults maintain polio seroprotection [24–26, 37]. These studies found seroprotection rates of 73%–80%, 73%–95%, and 54%–87% against poliovirus serotypes 1, 2, and 3, respectively. Lower immunity to serotype 3, compared with serotypes 1 and 2, following polio vaccination has been well documented and is a reason behind the OPV formulation change in the early 1990s [27] and the IPV formulation change in 1987 [38].

Surprisingly, the 40%-dose intramuscular IPV group had a similar vaccine response and antibody titers to both the full-dose intramuscular IPV group and the 40%-dose intradermal IPV group. Our preclinical study in rats showed that dose response was more consistent with intradermal IPV, compared with intramuscular IPV, in that antibody titers increased with increasing intradermal doses up to the maximum intradermal dose tested (40%), whereas antibody titers with increasing intramuscular doses were erratic [39]. It is possible that, given the high levels of preexisting immunity in our cohort, the maximal intramuscular response plateaued at a lower booster dose. However, our results suggest that in populations with high baseline levels of immunity, a full booster dose of IPV may not be needed even with intramuscular administration.

Intradermal administration of fractional IPV doses of up to 40% seems to be safe and well tolerated. Although the overall rate of adverse events was significantly higher in the intradermal groups, this was due to transient local adverse events such as redness and itching at the injection site. Rates of systemic adverse events such as fever and rash were low overall and did not differ significantly between groups. The majority of subjects who received intradermal vaccination said that they preferred intradermal to intramuscular administration. This is consistent with past intradermal IPV studies in infants that used needle-free intradermal delivery devices [7, 12]. In these studies, transient local adverse events were also significantly higher in the intradermal group, but the parents strongly preferred intradermal over intramuscular administration because it was less likely to make their infants cry.

We conducted this study in HIV-infected adults because they have suboptimal responses to many vaccines even with well-controlled HIV infection [16, 17, 19], a finding considered to be related to chronic immune activation [40]. Consequently, we felt that this population could function as a surrogate for populations in the developing world with suboptimal vaccine responses. However, the fold-rise in titers and postbooster GMTs in our study population were quite high and were actually comparable to those from a Dutch study investigating booster intramuscular and intradermal IPV doses in healthy adults who received IPV as children [11]. This similar booster response may have been because most of our subjects likely received OPV, not IPV, as children. However, it still suggests that while well-controlled HIV infection may impair the primary response to a vaccine, it might not impair the boosting response to a vaccine first received as a child prior to HIV infection.

Our study has limitations. The booster responses were much higher than anticipated, so our predetermined sample sizes may have been too low to detect differences. The polio vaccination history was not known for individual subjects but could only be assumed on the basis of US vaccination policies when the subjects were children. Finally, HIV-infected adults in the United States are only a surrogate for the groups in whom intradermal IPV may be most relevant.

Despite these limitations, we demonstrate that a 40% fractional dose of IPV administered intradermally results in at least noninferior antibody titers, compared with the full dose administered intramuscularly, and that it results in higher antibody titers than a 20% intradermal dose. Intradermal IPV administration at a fractional dose of >20% should be considered as a means to make IPV more affordable for developing countries, balancing sufficient immunity with cost reduction.

Notes

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S. B. T. had full access to all of the data and is responsible for the content of the article.

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Early priming with inactivated poliovirus vaccine (IPV) and intradermal fractional dose IPV administered by a microneedle device: A randomized controlled trial

Abhijeet Anand[a,*], K. Zaman[b], Concepción F. Estíviariz[a], Mohammad Yunus[b], Howard E. Gary[a], William C. Weldon[a], Tajul I. Bari[c], M. Steven Oberste[a], Steven G. Wassilak[a], Stephen P. Luby[d], James D. Heffelfinger[a,b], Mark A. Pallansch[a]

[a] *Centers for Disease Control and Prevention, 1600 Clifton Road, Atlanta, GA 30033, United States*
[b] *International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), 68 Shahid Tajuddin Sarani, Dhaka 1212, Bangladesh*
[c] *Expanded Program on Immunization and Surveillance, Mohakhali, Dhaka 1212, Bangladesh*
[d] *Stanford University, Stanford, CA 94305, United States*

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ABSTRACT

Introduction: Inactivated poliovirus vaccine (IPV) introduction and phased oral poliovirus vaccine (OPV) cessation are essential for eradication of polio.
Methods: Healthy 6-week old infants in Bangladesh were randomized to one of five study arms: receipt of trivalent OPV (tOPV) or bivalent OPV (bOPV) at ages 6, 10 and 14 weeks, intramuscular IPV or intradermal one-fifth fractional dose IPV (f-IPV) at ages 6 and 14 weeks, or f-IPV at ages 6 and 14 weeks with bOPV at age 10 weeks (f-IPV/bOPV). All participants received tOPV at age 18 weeks.
Results: Of 975 infants randomized, 95% (922) completed follow-up. Type 1 seroconversion after 3 doses at 6, 10 and 14 weeks was higher with bOPV compared with tOPV (99% vs 94%, $p = 0.019$). Seroconversions to types 1 and 3 after 2 IPV doses at ages 6 and 14 weeks were no different than after 3 doses of tOPV or bOPV at ages 6, 10 and 14 weeks. A priming response, seroconversion 1 week after IPV at 14 weeks among those who did not seroconvert after IPV at 6 weeks, was observed against poliovirus types 1, 2 and 3 in 91%, 84% and 97%, respectively. Compared with IPV, f-IPV failed non-inferiority tests for seroconversion with 1 or 2 doses and priming after 1 dose.
Discussion: The findings demonstrate considerable priming with IPV at age 6 weeks, comparable immunogenicity of tOPV and bOPV, and inferior immunogenicity of one-fifth f-IPV compared with IPV. If IPV induced priming at age 6 weeks is similar to that at age 14 weeks, IPV could be administered at a younger age and possibly with a higher coverage.

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1. Introduction

Oral poliovirus vaccines (OPV) consist of live attenuated poliovirus strains that can revert and cause paralysis, that is indistinguishable from paralysis caused by wild polioviruses (WPV), either due to vaccine-associated paralytic polio (VAPP) or circulating vaccine-derived polioviruses (cVDPV), in which the reverted vaccine virus also acquires the ability to circulate [1]. Since the last type 2 WPV (WPV2) was reported in 1999 in India [2] and about 87% of VDPVs during 2000–2013 were type 2 [3], the strategic advisory group of experts on immunization (SAGE) has recommended a phased cessation of OPV starting with type 2 OPV [4]. In countries using trivalent OPV (tOPV), a mixture of types 1, 2 and 3 OPV, in routine immunization (RI), SAGE has recommended a switch to bivalent OPV (bOPV), a mixture of OPV types 1 and 3 following introduction of 1 dose of inactivated poliovirus vaccine (IPV) generally at age ≥14 weeks [5]. It is expected that delaying IPV administration to age ≥14 weeks is likely to maximize IPV immunogenicity [5]; however, compared with vaccinating at age 6 weeks, vaccination at age ≥14 weeks is likely to be associated with lower vaccination coverage in some high-risk countries [6].

The principal objective of introducing IPV with bOPV is to mitigate the risk associated with increased susceptibility to WPV2 or cVDPV2. For IPV, priming is defined as a seroconversion response

* Corresponding author at: Global Immunization Division, Centers for Disease Control and Prevention, MS A04 Atlanta, GA 30333, United States. Tel.: +1 404 639 1970.
E-mail address: aanand@cdc.gov (A. Anand).

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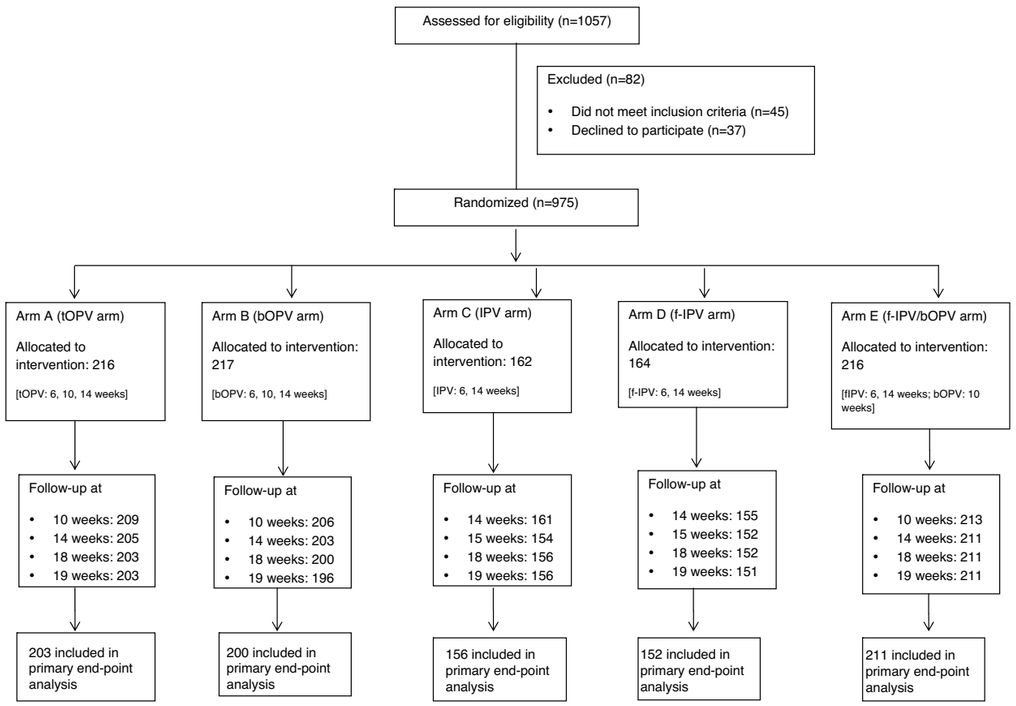

Fig. 1. Trial profile with number of subjects followed by study time-point.

1 week after a second dose of IPV among those who did not seroconvert after the first IPV dose. One clinical trial in Cuba reported considerable immunogenicity (seroconversion [63%] and priming [35%]) with 1 dose of IPV at age 4 months [7]. The absence of immunogenicity data by age, including priming response after IPV, is the chief limitation in assessing the optimal age for IPV administration in RI. In 2012, SAGE also recommended collecting additional immunogenicity data on intradermal (ID) one-fifth dose of IPV (0.1 ml fractional IPV [f-IPV]) as a potential substitute for intramuscular IPV (0.5 ml) [8].

2. Methods

2.1. Randomization and masking

We conducted an open-label 5-arm randomized controlled trial from 27 November 2012 to 30 November 2013 in Mirpur, an urban neighborhood in Dhaka, Bangladesh. The trial enrolled participants from 5 different sections of Mirpur. During the duration of the trial no polio vaccination campaigns were conducted in or around the study site. Infants were assigned randomly to one of five arms using a block randomization scheme of 65 blocks with a block size of 18 and an allocation ratio of 4:4:3:3:4 (Fig. 1). The tOPV arm received tOPV at ages 6, 10 and 14 weeks; the bOPV arm received bOPV at ages 6, 10 and 14 weeks; the IPV arm received IPV at ages 6 and 14 weeks; the f-IPV arm received f-IPV at ages 6 and 14 weeks; and the f-IPV/bOPV arm received f-IPV at ages 6 and 14 weeks with bOPV at age 10 weeks. All participants received tOPV at age 18 weeks (Table 1 in Supplementary Appendix).

2.2. Study objectives

The study's three primary objectives were to compare immunogenicity of (1) f-IPV and bOPV with bOPV alone; (2) 3 doses of tOPV with 3 doses of bOPV; and (3) 2 doses of intramuscular IPV with 2 doses of f-IPV.

2.3. Study design and procedures

Infants were recruited at age 6–7 weeks (42–51 days), if the parents were willing to participate, comply with study procedures, and provide written informed consent. Exclusion criteria included (1) receipt of any polio vaccine before enrollment; (2) diagnosis or suspicion of immunodeficiency or a bleeding disorder; (3) known allergy to polio vaccines or constituents; (4) any acute illness such as vomiting, diarrhea or infection immediately before enrollment; and (5) an infant who was part of a multiple birth. Enrolled participants were withdrawn from the study if requested by their parents or if they received polio vaccine outside of the study.

Study physicians administered all study vaccines and routine non-polio vaccines for infants as recommended by the Bangladesh Ministry of Health and Family Welfare. Intramuscular IPV (0.5 ml) was administered using a standard needle and syringe. Intradermal f-IPV (0.1 ml) was administered using NanoPass MicronJet 600

(MJ600), a microneedle device with three microneedles (0.6 mm in length) that attaches to an intradermal syringe. Multiple clinical trials have been conducted using MJ600 [9–12]. IPV and f-IPV were administered in the anterolateral thigh, opposite the side used for routine immunization of injectable vaccines.

Blood samples (1 ml) were obtained by venipuncture at ages 6, 14, and 18 weeks from all participants and at age 15 weeks from participants assigned to IPV or f-IPV arms before administering any scheduled study vaccine. Sera were stored at −20 °C and tested for antibodies to poliovirus types 1, 2 and 3 at the Centers for Disease Control and Prevention (CDC), Atlanta, USA using microneutralization assay. Titers below a dilution of 1:8 were considered negative for presence of poliovirus antibodies and the highest measurable titer was 1:1448. Parents were asked to collect a stool specimen (8 g) from participants 1 week after tOPV administration at age 18 weeks. Stool specimens were stored at −20 °C and tested at CDC for presence of poliovirus by type [13].

2.4. Analysis

No published studies were found to have administered f-IPV with bOPV, or 3 doses of bOPV. Therefore, for sample size calculations based on limited evidence, we assumed seroconversions of 85% for types 1 and 3 with f-IPV and bOPV and 95% with 3 doses of bOPV [14,15]. For tOPV, we assumed sero-conversions of 75% for type 1 and 65% for type 3 [16]. Therefore, a sample size of 207 per arm would be sufficient to obtain a power of 90% with two-sided α of 0.05 to detect a difference in seroconversion of at least 10% when comparing 3 doses of bOPV with 3 doses of tOPV, and 2 doses of f-IPV and 1 dose of bOPV with 3 doses of bOPV. No published studies were found to have reported immunogenicity of IPV or f-IPV with two doses 8 weeks apart at ages 6 and 14 weeks. For a non-inferiority comparison, we assumed a sero-conversion of 90% with both IPV and f-IPV with a non-inferiority margin of 10% [14,17]. For this comparison a sample size of 155 per arm is required for a power of 90% with one-sided α of 0.05. Hence, the effective sample size for the trial was 931, with an enrollment target of 1170 assuming 20% attrition (Table 1 in Supplementary Appendix).

Seroconversion was defined as either conversion from seronegative to seropositive or a four-fold increase in antibody titers between two specimens after adjusting for decay of maternal antibodies. The half-life of maternal antibodies was assumed to be 28 days [14,18]. The primary analytical approach was intent-to-treat for participants with serological results. The primary end-point was seroconversion at age 18 weeks. To compare immunogenicity across study arms, the proportion of participants who seroconverted were compared using Fisher's exact test (two-tailed). Priming was defined as a seroconversion response at age 15 weeks after receipt of the second IPV/f-IPV dose among those who did not seroconvert by age 14 weeks after one IPV/f-IPV dose at age 6 weeks. Reverse cumulative distribution curves, which are constructed by representing on the vertical axis the percent of subjects with antibody titers equal to or greater than that marked in x-axis, were used to compare distribution of antibody titers by study arms [19].

2.5. Study oversight

The study protocol was reviewed by icddr,b's Institutional Review Board (IRB). The study was conducted in compliance with good clinical practice guidelines. UNICEF assisted in the procurement of vaccines used in this study. OPV was manufactured by Sanofi Pasteur and IPV was manufactured by the Netherlands Vaccine Institute (NVI). NanoPass Technologies Ltd. donated the supplies of MJ600. UNICEF, Sanofi Pasteur, NanoPass, and NVI had no role in the study design, implementation, data analysis, or interpretation of study results. The study was registered with Clinicaltrials.gov (NCT01813604). Adverse events data were reviewed by the Data Safety Monitoring Board (DSMB) of icddr,b.

2.6. Role of funding source

The study was funded by the Global Immunization Division of the Centers for Disease Control and Prevention. CDC staff participated in the study design, sample testing, data analysis and decision to submit for publication.

3. Results

3.1. Baseline characteristics

The study enrolled and randomized 975 participants and of these, 922 (95%) with blood specimens available at ages 6 and 18 weeks were included in the primary end-point analysis (Fig. 1). Enrollment was stopped after enrolling 975 participants as the study had achieved its effective sample size due to lower than anticipated study attrition. No statistically significant differences were observed at baseline among participants who completed the study compared with those who did not (data not shown) except that median type 2 antibody titers at baseline were lower for those who completed the study (1:28 vs 1:41, Kruskal–Wallis = 0.036). No other significant differences in baseline characteristics, including seroprevalence to polioviruses, were observed among study arms (Table 1).

3.2. Humoral immunogenicity

The median bleb diameter after intradermal injection with MJ600 was 10 mm and 99% of the participants had no residual liquid present on the skin following the injection.

Seroconversion to poliovirus type 1 (PV1) after 2 and 3 doses was higher in the bOPV arm compared with the tOPV arm (2 doses: 93% vs 87%, $p = 0.047$; 3 doses: 99% vs 94%, $p = 0.019$; Table 2). PV1 seroconversion with 2 doses of IPV (95%) was statistically no different from that observed with 3 doses of tOPV or bOPV. PV1 seroconversion with 2 doses of f-IPV and 1 dose of bOPV was higher than that observed with 2 doses of f-IPV alone ($p = 0.005$) and no different from that with 3 doses of tOPV or bOPV.

Seroconversion at 18 weeks to PV2 was higher with 3 doses of tOPV compared with 2 doses of IPV $p = 0.002$) or f-IPV in either f-IPV arms ($p < 0.001$). Seroconversion to PV3 was statistically no different with 3 doses of tOPV (95%) compared with 3 doses of bOPV (94%), 2 doses of IPV (97%) or f-IPV (89%), or 2 doses of f-IPV with 1 dose of bOPV (94%).

Compared with IPV, f-IPV failed the non-inferiority test for all serotypes for seroconversion observed with 1 or 2 doses (Fig. 2). Additionally, compared with IPV, f-IPV failed the non-inferiority test for all serotypes for priming response observed at 15 weeks.

Reverse cumulative distribution curves for antibody titers by study arm at age 18 weeks show that the highest titers were reached for PV1 in the bOPV arm, PV2 in the tOPV arm and PV3 in the IPV arm (Fig. 3). f-IPV was associated with the lowest titers for all three poliovirus types among those receiving type specific vaccines. One dose of IPV or f-IPV was not associated with a substantial change in distribution of antibody titers, despite the high degree of priming with 1 dose; however, within a week of the second dose of IPV or f-IPV, a rapid rise in antibody titers was observed (Fig. 1 in Supplementary Appendix).

3.3. Intestinal mucosal immunity

One week after receiving tOPV at age 18 weeks, 15%, 6%, and 8% of participants in the tOPV arm were excreting PV 1, 2, and

Table 1
Baseline characteristics among those who completed the study by study arms.

Baseline characteristics	A tOPV (n = 203)		B bOPV (n = 200)		C IPV (n = 156)		D f-IPV (n = 152)		E f-IPV/bOPV (n = 211)		p-value[a]
Median age in days (range)	44 (41, 53)		44 (41, 53)		44 (42, 53)		44 (41, 52)		44 (41, 53)		0.662
Male n (%)	95	46.8%	99	49.5%	79	52.1%	79	52.0%	110	52.1%	0.828
Mother's education n (%)											
No formal school	36	17.7%	27	13.5%	29	19.0%	24	15.8%	40	19.0%	0.869
Primary school	88	43.4%	92	46.0%	68	41.2%	70	46.1%	87	41.2%	
Middle school	36	17.7%	42	21.0%	30	23.2%	25	16.5%	49	23.2%	
High school	33	16.3%	34	17.0%	27	15.2%	28	18.4%	32	15.2%	
Graduate	10	4.9%	5	2.5%	2	1.4%	5	3.3%	3	1.4%	
Type 1 seroprevalence n (%)	99	48.8%	97	48.5%	78	47.4%	77	50.7%	100	47.4%	0.976
Median (range)[b]	28 (9, 1448)		18 (9, 1448)		23 (9, 1448)		23 (6, 1448)		23 (9, 724)		0.947
Type 2 seroprevalence n (%)	118	58.1%	118	59.0%	95	58.8%	82	54.0%	124	58.8%	0.796
Median (range)[b]	28 (9, 1448)		36 (9, 1448)		28 (9, 1448)		28 (9, 1448)		23 (9, 1448)		0.884
Type 3 seroprevalence n (%)	51	25.1%	56	28.0%	43	30.8%	38	25.0%	65	30.8%	0.698
Median (range)[b]	23 (9, 1448)		23 (9, 1024)		23 (9, 1448)		18 (9, 362)		18 (9, 1448)		0.643
Wasting present n (%)	31	15.3%	38	19.0%	28	16.1%	33	21.7%	34	16.1%	0.545
Stunting present n (%)	40	19.7%	37	18.5%	28	23.7%	31	20.4%	50	23.7%	0.661
Exclusive breastfeeding n (%)	180	88.7%	170	85.0%	134	82.9%	130	85.5%	175	82.9%	0.465

tOPV, trivalent oral poliovirus vaccine (OPV); bOPV, bivalent OPV; IPV, inactivated poliovirus vaccine; f-IPV, fractional IPV.
[a] Fisher's exact test. Kruskal–Wallis test used for mother's education and rank test for medians.
[b] Among those with titers ≥8.

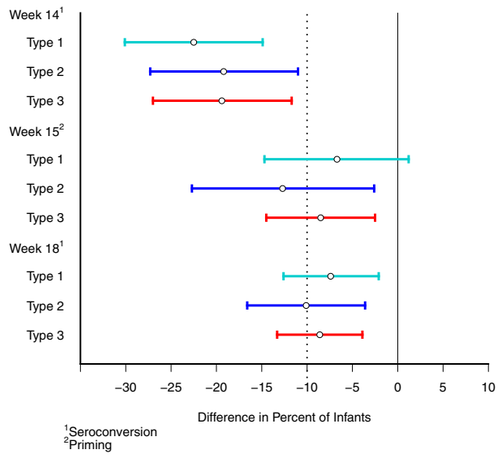

Fig. 2. Differences in seroconversion and priming between fractional intradermal inactivated poliovirus vaccine (f-IPV) arm and intramuscular IPV arm by poliovirus type. f-IPV fails to pass the test of non-inferiority if the lower limit of the 90% confidence interval crosses −10%.

3, respectively (Table 2). Among participants in the bOPV arm, 61% were excreting PV2 1 week after receiving tOPV. The percent of participants excreting type 1 poliovirus was statistically lower in the bOPV arm compared with the f-IPV/bOPV arm (4% vs 13%, Fischer's exact = 0.001). The percent excreting PV3 was statistically lower in the bOPV arm compared with f-IPV/bOPV arm (6% vs 14%, Fischer's exact = 0.013). No statistically significant differences in percent excreting polioviruses by type were observed between IPV and f-IPV arms.

3.4. Adverse events

No adverse events (AE) were reported among participants 30 min after receiving the study vaccine. During follow-up (age 6–19 weeks), 68 AE were reported among participants; 11 were considered serious AE (SAE), including hospitalization or death (Table 2 in Supplementary Appendix). Three infants died during follow-up: two in the sequential f-IPV/bOPV arm and one in the f-IPV arm. No AE/SAE were attributed to trial vaccines or MJ600 by the DSMB.

4. Discussion

The study demonstrated that considerable priming can be achieved with 1 dose of IPV at age 6 weeks. Cumulatively, 90% of children had either seroconverted or were primed against type 2 poliovirus with 1 dose of IPV at age 6 weeks. These results are particularly relevant for current policy considerations regarding global polio eradication. In November 2013, SAGE recommended introduction of at least 1 dose of IPV at age ≥14 weeks in RI in countries where IPV has not been introduced, in advance of a global implementation of the switch from tOPV to bOPV. With removal of type 2 OPV, the objective of IPV introduction is to maximize type 2 population immunity, which is a product of IPV immunogenicity and coverage. If the considerable priming noted in this study at age 6 weeks is similar to the priming noted at age 14 weeks, IPV vaccination at age 6 weeks will likely lead to higher population immunity compared with vaccination at age 14 weeks

Table 2
Humoral and intestinal immunogenicity by study arm.

	A tOPV		B bOPV		C IPV*		D f-IPV*		E f-IPV/bOPV		Fisher's exact test (a priori)	Fisher's exact test (post hoc)**
Type 1												
Seroconversion by 14 weeks: n (%)	178/205	86.8%[b,e]	189/203	93.1%[a,b,f]	57/161	35.4%[e,f]	20/155	12.9%[c,d,f]	173/211	82.0%[a,d]	[a] p = 0.001 B vs E; [b] p = 0.047 A vs B; [c] p < 0.001 A vs D; [d] p < 0.001 D vs E; NS: A vs E	[e] p < 0.001 A vs C; [f] p < 0.001 B vs C
Priming response by 15 weeks: n (%)†	–	–	–	–	78/86	90.7%	91/109	83.5%	–	–		
Cumulative effect of one dose (seroconversion and priming): n (%)†	–	–	–	–	124/132	93.9%	110/128	85.9%	–	–		
Seroconversion by 18 weeks: n (%)	190/203	93.6%[a]	197/200	98.5%[a]	148/156	94.9%	133/152	87.5%[b]	202/211	95.7%[b]	[a] p = 0.019 A vs B; [b] p = 0.005 D vs E; NS: B vs E; A vs E; A vs D	NS: A vs C; B vs C
Poliovirus shedding at 19 weeks: n (%)	31/203	15.3%	7/196	3.6%[a]	77/156	49.4%	73/151	48.3%	28/211	13.3%[a]	[a] p = 0.001 B vs E; NS: C vs D	
Type 2												
Seroconversion by 14 weeks: n (%)	190/205	92.7%[b,c,d,e]	14/203	6.9%[a,b,f]	62/161	38.5%[e,f]	30/155	19.4%[d]	53/211	25.1%[a,c]	[a] p < 0.001 B vs E; [b] p < 0.001 A vs B; [c] p < 0.001 A vs E; [d] p < 0.001 A vs D; NS: D vs E	[e] p < 0.001 A vs C; [f] p < 0.001 B vs C
Priming response by 15 weeks: n (%)†	–	–	–	–	66/79	83.5%	73/101	72.3%	–	–		
Cumulative effect of one dose (seroconversion and priming): n (%)†	–	–	–	–	119/132	90.2%	100/128	78.1%	–	–		
Seroconversion by 18 weeks: n (%)	200/203	98.5%[b,c,d,e]	28/200	14%[a,b,f]	142/156	91%[e,f]	123/152	80.9%[d]	172/211	81.5%[a,c]	[a] p < 0.001 B vs E; [b] p < 0.001 A vs B; [c] p < 0.001 A vs E; [d] p < 0.001 A vs D; NS: D vs E	[e] p = 0.002 A vs C; [f] p < 0.001 B vs C
Poliovirus shedding at 19 weeks: n (%)	12/203	5.9%	119/196	60.7%	89/156	57.1%	99/151	65.6%	122/211	57.8%	NS: C vs D	
Type 3												
Seroconversion by 14 weeks: n (%)	174/205	84.9%[b,c,e]	181/203	89.2%[a,f]	54/161	33.5%[e,f]	22/155	14.2%[c,d]	153/211	72.5%[a,b,d]	[a] p < 0.001 B vs E; [b] p = 0.003 A vs E; [c] p < 0.001 A vs D; [d] p < 0.001 D vs E; NS: A vs B	[e] p < 0.001 A vs C; [f] p < 0.001 B vs C
Priming response by 15 weeks: n (%)†	–	–	–	–	84/87	96.6%	94/107	87.9%	–	–		
Cumulative effect of one dose (seroconversion and priming): n (%)†	–	–	–	–	129/132	97.7%	115/128	89.8%	–	–		
Seroconversion by 18 weeks: n (%)	192/203	94.6%	188/200	94.0%	152/156	97.4%	135/152	88.8%	198/211	93.8%	NS: B vs E; A vs B; A vs E; A vs D; D vs E	NS: A vs C; B vs C
Poliovirus shedding at 19 weeks: n (%)	16/203	7.9%	12/196	6.1%[a]	50/156	32.1%	64/151	42.4%	29/211	13.7%[a]	[a] p = 0.013 B vs E; NS: C vs D	

NS, not significant; tOPV, trivalent oral poliovirus vaccine (OPV); bOPV, bivalent OPV; IPV, inactivated poliovirus vaccine; f-IPV, fractional IPV.
* Test comparison of IPV (Arm C) and f-IPV (Arm D) presented in Fig. 2.
** Bonferroni correction. Significance at p < 0.0125.
† Analysis restricted to those with serological results at 6, 14, 15 and 18 weeks.

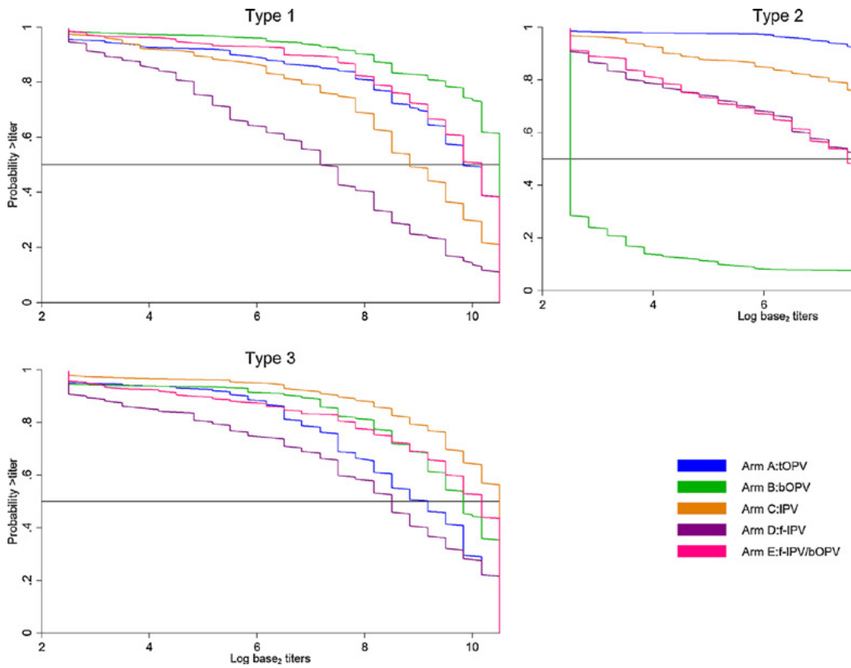

Fig. 3. Reverse cumulative antibody titers at 18 weeks of age by study arm.

as vaccination coverage in many high-risk countries is higher at age 6 weeks compared with age 14 weeks [6].

The study confirms that bOPV is more immunogenic than tOPV for poliovirus types 1 and 3 [15]; however, after 3 doses, the differences in seroconversion are small and high titers of antibodies were observed after administration of both vaccines. Prior field assessments of tOPV have reported substantially lower effectiveness though those estimates have been based on parental report of the number of vaccine doses received [20,21]. This study demonstrates a high immunogenicity of tOPV in a developing country with a tropical climate [22–24].

IPV demonstrated a higher immunogenicity compared with f-IPV for priming with one dose and seroconversion with one or two doses. These results address a prior identified information need by SAGE to collect more evidence on the comparative immunogenicity of f-IPV and IPV [8]. Also these results are consistent with other studies that have reported lower immunogenicity of a one-fifth IPV dose compared with IPV [7,14,25]. The findings of this study confirm the safety of NanoPass MJ-600 in intradermal f-IPV administration, a device that had not been previously used for f-IPV administration.

The stool excretion results demonstrate a minimal reduction in type 2 excretion with IPV and f-IPV recipients compared with bOPV recipients, who did not receive any type 2 vaccine. Also a vaccination schedule of f-IPV/bOPV reduced the percent of participants who excreted type 1 or 3 polioviruses 1 week after receiving tOPV compared to the use of IPV or f-IPV alone. Although the percent excreting poliovirus in the f-IPV/bOPV arm was significantly higher than those in the bOPV arm, the absolute difference was not large. A prior study with tOPV demonstrated the substantial reduction in excretion of polioviruses with 1–2 doses of tOPV with minimal reduction with additional doses [26]. These findings taken together with noteworthy priming associated with IPV at age 6 weeks support evaluating polio vaccination schedules with IPV only as the first poliovirus vaccine followed by OPV.

This study has notable limitations. First, transmission of OPV received by other children in the community was observed. However, the effect of community transmission was low with only 14% type 2 seroconversion over 12 weeks in the bOPV arm [23,27]. Second, in the assessment of priming, the primary as well as secondary (challenge at 14 weeks) vaccines had different routes of administration and dosage between IPV and f-IPV arms, which limits comparison. Lastly, assessment of MJ600 performance was limited to safety and injection quality associated with the device and we could not compare immunogenicity of IPV administered by MJ600 with standard needle and syringe for intradermal administration.

Overall, findings from this study address several previously identified information gaps with regard to primary routine polio vaccine performance and could help simplify and expand polio vaccination policy options. The study supports the safety and comparable immunogenicity of tOPV and bOPV for types 1 and 3 poliovirus and demonstrates the lack of non-inferiority of one-fifth f-IPV to IPV. Most importantly, the study shows the promising degree of priming with an early (6 week) dose of IPV. A useful next step would be to compare priming at age 6 weeks to that with the SAGE-recommended IPV schedule at age ≥14 weeks.

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Contributors

AA prepared the first draft of the manuscript and all authors reviewed and approved the manuscript. AA, CFE, HG, MAP, SW, MSO and WW contributed to the design of the study. The design team jointly developed the trial implementation strategy with KZ, SPL, JDH, MY and TBI.

WW and MSO contributed to laboratory testing. AA and HG contributed to data analysis. All authors contributed to interpretation of study results.

Conflict of interest

All authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2015.09.039.

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Immune response and reactogenicity of intradermal administration versus subcutaneous administration of varicella-zoster virus vaccine: an exploratory, randomised, partly blinded trial

Chan R Beals, Radha A Railkar, Andrea K Schaeffer, Yotam Levin, Efrat Kochba, Brian K Meyer, Robert K Evans, Eric A Sheldon, Kenneth Lasseter, Nancy Lang, Adriana Weinberg, Jennifer Canniff, Myron J Levin

Summary

Background The licensed live, attenuated varicella-zoster virus vaccine prevents herpes zoster in adults older than 50 years. We aimed to determine whether intradermal administration of zoster vaccine could enhance vaccine immunogenicity compared with conventional needle subcutaneous administration.

Methods In this randomised, dose-ranging study, adults aged 50 years or older who had a history of varicella or who had resided in a country with endemic varicella-zoster virus infection for 30 years or more were eligible. Participants received the approved full or a 1/3 dose of zoster vaccine given subcutaneously or one of four intradermal doses (full, 1/3, 1/10, or 1/27 dose) using the MicronJet600 device. The two subcutaneous doses and the four intradermal doses were randomised (1·5:1:1:1:1:1) by computer generated sequence with randomisation stratified by age (50–59 years or 60 years or older). The primary immunogenicity endpoint was the change from baseline in IgG antibody to varicella-zoster virus-specific glycoproteins (gpELISA) measured at 6 weeks. All patients were included in the primary and safety analyses. This study is registered with ClinicalTrials.gov, number NCT01385566.

Findings Between Sept 2, 2011, and Jan 13, 2012, 224 participants were enrolled from three clinics in the USA and 223 were randomly assigned: 52 to receive the full dose subcutaneous zoster vaccine, 34 to receive the 1/3 dose subcutaneous zoster vaccine, 34 to receive the full dose intradermal zoster vaccine, 35 to receive the 1/3 dose intradermal zoster vaccine, 34 to receive the 1/10 dose intradermal zoster vaccine, and 34 to receive the 1/27 dose intradermal zoster vaccine. Full dose zoster vaccine given subcutaneously resulted in a gpELISA geometric mean fold-rise (GMFR) of 1·74 (90% CI 1·48–2·04) at 6 weeks post-vaccination compared with intradermal administration which resulted in a significantly higher gpELISA GMFR of 3·25 (2·68–3·94; p<0·0001), which also remained high at 18 months. An apparent dose–response relation was observed with intradermal administration (1/3 dose subcutaneous GMFR 1·64 [90% CI 1·36–1·99], 1/3 dose intradermal 2·58 (2·13–3·13), 1/10 dose intradermal 2·22 [1·83–2·69], and 1/27 dose intradermal 1·64 [1·35–2·00]). Each partial dose of zoster vaccine given intradermaly had a gpELISA GMFR comparable to that of full dose zoster vaccine given subcutaneously. Transient erythema and induration were more common after intradermal administration (31% erythema for full subcutaneous dose and 77% for intradermal dose).

Interpretation Intradermal zoster vaccine showed a greater increase in varicella-zoster virus gpELISA antibody compared with subcutaneous zoster vaccine at comparable doses. Larger and longer studies of intradermal administration of live, attenuated zoster vaccine are needed to provide convincing evidence of improved cell mediated immunity.

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Merck & Co, Kenilworth, NJ, USA (C R Beals MD, R A Railkar PhD, A K Schaeffer MSc, B K Meyer PhD, R K Evans PhD); **NanoPass Technologies Ltd, Nes Ziona, Israel** (Y Levin MD, E Kochba MD); **Miami Research Associates, Miami, FL, USA** (E A Sheldon MD); **Clinical Pharmacology of Miami, Miami, FL, USA** (K Lasseter MD); **and Pediatric Infectious Diseases, University of Colorado, Denver Anschutz Medical Campus, Aurora, CO, USA** (N Lang BSN, Prof A Weinberg MD, J Canniff BS, Prof M J Levin MD)

Correspondence to:
Dr Chan Beals, Merck & Co, PO Box 1000, RY34-A500, Kenilworth, NJ 07033, USA
chanbeals@gmail.com

Introduction

Herpes zoster is an unilateral, usually painful, vesicular cutaneous eruption in a dermatomal distribution, which results from the reactivation of latent varicella-zoster virus that resides in sensory ganglia following varicella (chickenpox), and can result in persistent pain (post-herpetic neuralgia).[1,2] The incidence of herpes zoster and post-herpetic neuralgia increases significantly after 50 years of age, and more so as people become older, reflecting the age-related decline in varicella-zoster virus-specific cell-mediated immunity that is essential to prevent or limit reactivation of latent varicella-zoster virus.[1,3,4]

The Shingles Prevention Study,[5] which resulted in the licensure of the herpes zoster vaccine, showed that vaccination reduced the incidence of herpes zoster by 51% (95% CI 44–58) in immune competent individuals aged 60 years or older. In adults aged 50–59 years, zoster vaccine reduced the incidence of herpes zoster by 69·8% (95% CI 54·1–80·6).[6] Further reduction in the incidence and severity of herpes zoster by vaccination, particularly in the advanced elderly population, is desirable.

Research in context

Evidence before this study
In October, 2015, we searched PubMed with the search terms "intradermal", "herpes zoster", "vaccine", and "adult". There were no completed studies written in English that were pertinent to our investigation. The efficacy and safety of the licensed live, attenuated herpes zoster vaccine has been studied with subcutaneous administration. Vaccination reduced the incidence of herpes zoster by 51% (95% CI 44–58) in individuals aged 60 years or older. Further protection from herpes zoster is desirable, particularly in older individuals.

Added value of this study
This is the first randomised study to compare subcutaneous and intradermal zoster vaccine immunisation across a dose range. We noted higher antibody fold-rise and titres to varicella zoster virus glycoproteins with the full dose of zoster vaccine delivered intradermally compared with subcutaneous administration. Equivalent antibody responses were observed with partial intradermal doses. Transient erythema and induration were more common after intradermal administration, as expected.

Implications of all the available evidence
The higher fold-rise in varicella-specific antibodies has previously been shown to be a correlate of protection of this vaccine. This study lays the groundwork for additional studies to show that improved immunogenicity from intradermal vaccination translates to additional protection from herpes zoster.

Studies with a variety of vaccines have shown superior immunogenicity after intradermal administration compared with subcutaneous or intramuscular administration, and equivalent responses were achieved with reduced antigen dose (dose sparing).[7–9] Enhanced immunogenicity could be a consequence of efficient vaccine delivery to potent antigen-presenting dendritic cells, which are highly abundant in the dermis and support efficient antigen presentation and sculpting of the adaptive immune response.[10–12] This exploratory study aimed to assess the immunogenicity and safety of intradermal zoster vaccination compared with the conventional subcutaneous route.

Methods

Study design and participants

In this exploratory, randomised, partly blinded, parallel group study, participants were recruited at three clinics (Aurora, Colorado, and two in Miami, Florida) in the USA. Individuals who were aged 50 years or older, had a history of varicella or who had resided in a country with endemic varicella-zoster virus infection for 30 years or more, had a temperature less than 38°C on day of vaccination, and were in good health were enrolled. Women of reproductive potential had a negative pregnancy test just before vaccination and agreed to use two acceptable methods of birth control for 3 months post-vaccination. Participants were excluded if they had either: a previous history of herpes zoster, received varicella vaccine, recent exposure to systemic immune suppressants, immune dysfunction, recent live virus vaccinations, antiviral drugs active against varicella-zoster virus, or immune suppressed household members. Additional exclusion criteria included history of hypersensitivity reactions to any vaccine component, household exposure to pregnant women who had not had chickenpox and had not been vaccinated against varicella, household or workplace exposure to children 18 months and younger who have not been vaccinated against varicella, received immune globulin or blood products from 5 months before vaccination, receipt of inactivated vaccine from 7 days before study vaccine to 7 days postvaccination, except for inactivated influenza vaccine, not ambulatory, pregnant or breastfeeding, and active untreated tuberculosis.

The protocol was approved by the institutional review board of each study centre. Participants provided written informed consent.

Randomisation and masking

Participants received the approved full or a 1/3 dose of zoster vaccine given subcutaneously or one of four intradermal doses: full, 1/3, 1/10, or 1/27 dose. The two subcutaneous doses and the four intradermal doses were randomised (1·5:1:1:1:1:1) by a computer generated sequence (table 1). Randomisation was stratified by age (1:1; 50–59 years and ≥60 years). To assess the local tolerability of the intradermal vaccination, 39 participants across all treatment groups were randomly selected to receive roughtly 0·1 mL intradermal saline, whereas recipients of the full dose intradermal vaccine received two roughly 0·15 mL intradermal injections of saline. The study staff did not inform the participants of the dose of zoster vaccine or whether zoster vaccine or saline was injected into a given arm, but the method of the delivery was not concealed.

Procedures

Zoster vaccine is a lyophilised preparation (ZOSTAVAX, Merck & Co Inc, Kenilworth, NJ, USA) of live, attenuated varicella-zoster virus (Oka/Merck) stored frozen before reconstitution. Subcutaneous doses were given in either 0·65 mL (full dose) or approximate 0·22 mL (1/3 dose) with a needle and syringe. Intradermal injection used the NanoPass MicronJet600 device (NanoPass, Nes Ziona, Israel), which is equipped with three silicon microneedles, each 0·60 mm in length.[13] Intradermal doses were reconstituted in the diluent used for subcutaneous administration except for the 1/27 dose, which was reconstituted

	Full subcutaneous	1/3 subcutaneous	Full intradermal	1/3 intradermal	1/10 intradermal	1/27 intradermal
Study population						
Vaccine recipients	52	34	34	35	34	34
Concomitant placebo	9*	6*	6†	6†	6†	6†
Vaccine volume (mL)	0·65	0·22	0·15 × 2‡	0·1	0·1	0·1
Sex						
Women	31 (60%)	20 (59%)	17 (50%)	17 (49%)	16 (47%)	24 (71%)
Men	21 (40%)	14 (42%)	17 (50%)	18 (51%)	18 (53%)	10 (29%)
Age (years)						
50–59	26 (50%)	16 (47%)	17 (50%)	16 (46%)	16 (47%)	16 (47%)
≥60	26 (50%)	18 (53%)	17 (50%)	19 (54%)	18 (53%)	18 (53%)
Mean (SD)	60 (8)	61 (8)	62 (9)	61 (9)	62 (8)	60 (7)
Median	59·5	60·0	59·5	60·0	60·0	60·0
Range	50–83	50–83	51–86	50–81	50–76	50–74
Race						
Asian/African-American	3 (6%)	0	2 (6%)	3 (9%)	3 (9%)	2 (6%)
European	49 (94%)	34 (100%)	32 (94%)	32 (91%)	31 (91%)	32 (94%)
Ethnicity						
Hispanic or Latino	39 (75%)	25 (74%)	27 (79%)	24 (69%)	21 (62%)	25 (74%)
Not Hispanic or Latino	13 (25%)	9 (26%)	7 (21%)	11 (31%)	13 (38%)	9 (26%)

39 participants across all groups received concomitant saline placebo in the shoulder. *0·1 mL intradermal injection of placebo (saline). †Two intradermal injections of saline placebo spaced roughly 5 cm apart. ‡Two 0·15 mL intradermal injections of vaccine spaced roughly 5 cm apart.

Table 1: **Participant characteristics and dosing**

with the sterile normal saline, because reconstituting in diluent would cause the dose to be too hypotonic. The intradermal full dose was given with two injections of roughly 0·15 mL spaced approximately 5 cm apart. The 1/3, 1/10, and 1/27 intradermal doses were given in roughly 0·1 mL. In vitro tests showed that the live attenuated varicella-zoster virus retained viability using these reconstitution conditions. All doses were given over the deltoid muscle of the non-dominant arm. In 39 participants, saline was given in the dominant arm with the MicronJet600 device to provide a measure of the safety of the device.

A blood sample was obtained before vaccination and 6 weeks later. After 42 days, participants returned a completed vaccine report card, which records injection site reactions and systemic safety. Participants who had not received full dose subcutaneous zoster vaccine were then eligible to receive a full subcutaneous dose. After 18 months, participants who were randomly assigned to the full subcutaneous dose, and participants who were randomly assigned to other treatments and refused optional immunisation with full subcutaneous dose zoster vaccine at day 42, were invited to provide blood for antibody testing.

Flow cytometry of thawed peripheral blood mononuclear cells (PBMCs) with viability 70% or greater was done. Peripheral blood mononuclear cells were cultured in the presence of infectious varicella-zoster virus at a concentration of 8×10^4 plaque forming units/mL, or medium control, for 3 days. Brefeldin A (Sigma-Aldrich, St Louis, MO, USA) was added for the last 16 h. Viable cells were stained with fluorochrome conjugated antibodies to cell surface markers or intracellular proteins. $1{\cdot}5–2{\cdot}0\times10^4$ viable lymphocytes were analysed with a Gallios instrument (Beckman Coulter, Indianapolis, IN, USA) and Kaluza software (Beckman Coulter). Varicella-zoster virus-specific memory and effector T cells were expressed as percentages of the parent CD4+ or CD8+ lymphocyte populations. The gating strategy is shown in the appendix (pp 1–3).

The percentage of participants who reported redness, swelling, and pain or tenderness are given as descriptive statistics. Additional adverse experiences, serious adverse experiences, and non-injection site varicella-like rash are presented as descriptive statistics.

Outcomes

The primary outcome measure was change from baseline in IgG antibody to varicella-zoster virus-specific glycoproteins (gpELISA) measured at 6 weeks, determined by methods previously optimised and validated.[14] Varicella-zoster virus-specific interferon γ-producing peripheral blood mononuclear cells were enumerated and cryopreserved with an ELISPOT method previously optimised and validated.[15]

Secondary objectives were to compare the subcutaneous and intradermal injection experience by questionnaire, and to examine leakage from the injection site.

Vaccine report cards recorded local reactions from each injection site for 5 days and systemic reactions for 42 days. Intradermal vaccinees completed a questionnaire on the intradermal device experience.

See Online for appendix

Statistical analysis

The primary immunogenicity endpoint was the geometric mean fold rise (GMFR) of gpELISA at 6 weeks over baseline. The full subcutaneous dose group was expected to show a GMFR of 2, a natural log scale SD of 1, and a correlation of 0·7 between baseline and week 6 gpELISA based on previous studies.[16] To have 80% power to detect a GMFR greater than 1·4 with a one-sided α=0·05 test, 51 evaluable participants were required in the full subcutaneous dose group. The hypothesis that GMFR increased in the full subcutaneous dose group was tested. With 34 participants in one of the intradermal dose groups, there is 80% power to detect a difference of 1·5 fold in the GMFR from the full subcutaneous dose group, with a one-sided α=0.05 test, deemed a signal of potential interest. Comparisons between vaccine groups were not subject to multiplicity adjustments.

All participants who received the study vaccine and had valid serology results were included in the immunogenicity analysis. The CIs for the means (mean differences) were constructed on the natural log scale and referenced the t-distribution. Exponentiating the least-squares means (mean differences) and lower and upper limits of these CIs yielded estimates for the population geometric-means (geometric mean ratios) and corresponding CIs (prespecified at 90%) on the original scale.

To compare the GMFR of gpELISA between the different vaccination groups, a constrained longitudinal data analysis method described by Liang and Zeger[17] was used. This model assumed a common mean across treatment groups at baseline and a different mean for each treatment at each of the post-vaccination timepoints. The response vector consisted of the baseline and week 6 natural log gpELISA antibody titres. No restriction was imposed on the trajectory of the means over time. An unstructured covariance matrix was used to model the correlation among repeated measurements. Normality of the log titres is also assumed by the model, which was nearly the case. A similar analysis was done to compare the GMFR of ELISPOT at week 6 between the different vaccination groups.

The gpELISA geometric mean titre (GMT) and the ELISPOT geometric mean counts (GMC) at baseline and week 6 post-vaccination for each vaccination group were also computed. 90% CIs were prespecified for this exploratory study. A post-hoc ANCOVA was done to explore whether, in addition to treatment group, factors such as age, sex, and baseline gpELISA titer affected the gpELISA titre at day 42 post-vaccination. The post-hoc analysis of flow cytometry data is explained in the appendix (p 4).

Data were analysed with SAS version 9 (Cary, NC, USA).

This study is registered with ClinicalTrials.gov, number NCT01385566.

Role of the funding source

The study was funded by Merck and Co Inc. The study design, data collection, data interpretation, and writing the report was a collective effort of all the authors, some of whom are employees of Merck (CRB, RAR, AKS, BKM, RKE). The corresponding author had full access to all the data in the study and had the final responsibility for the decision to submit for publication.

Results

Between Sept 2, 2011, and Jan 13, 2012, 224 participants were screened, of whom 223 were randomly assigned; 52 to receive the full dose subcutaneous zoster vaccine, 34 to receive the 1/3 dose subcutaneous zoster vaccine, 34 to receive the full dose intradermal zoster vaccine, 35 to receive the 1/3 dose intradermal

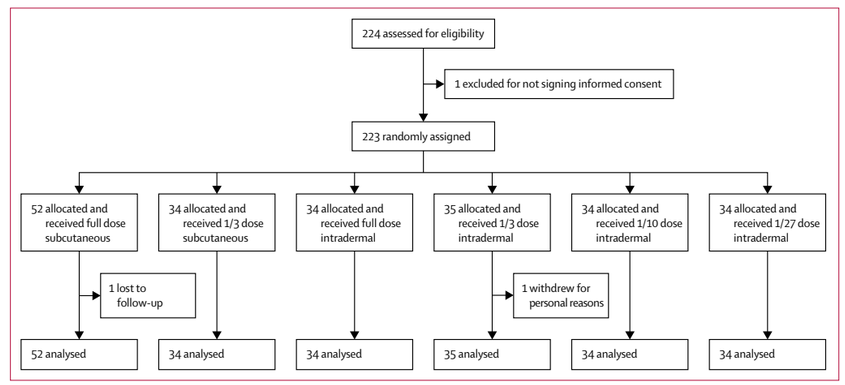

Figure: **Trial profile**

zoster vaccine, 34 to receive the 1/10 dose intradermal zoster vaccine, and 34 to receive the 1/27 dose intradermal zoster vaccine (figure). One participant was excluded because they declined to sign the informed consent. All randomised participants except two completed the 6 week study. One participant randomised to full subcutaneous dose was lost to follow-up. One participant randomised to 1/3 intradermal zoster vaccine withdrew for personal reasons. Two participants contributed baseline samples but not 6 week samples, but the baseline values for these participants were included in the model from which the GMFRs were calculated.

The age distribution (50–86 years) was balanced across dose groups (table 1). An imbalance in the sex distribution was noted, with 24 (71%) women in the 1/27 dose intradermal group compared with 16–31 (47–60%) women in other dose groups. 94% of participants identified their race as European and 72% identified their ethnicity as Hispanic or Latino.

The varicella-zoster virus antibody responses measured by gpELISA at 6 weeks after zoster vaccine and the fold-rise from baseline responses for each dose and route of administration are shown in table 2. Participants who received full dose intradermal vaccination had significantly higher GMT (p<0·0001) and GMFR (p<0·0001) in varicella-zoster virus antibody titres post-vaccination than did those who received full subcutaneous dose. Participants who received 1/3 intradermal dose had a GMT comparable to that of participants receiving full subcutaneous dose and had a significantly higher GMFR (p=0·007). The lower intradermal zoster vaccine doses induced GMTs and GMFRs comparable to that of full dose zoster vaccine given subcutaneously. An apparent dose-response relation in GMT and GMFR after intradermal zoster vaccine administration was observed. In a post hoc analysis, an ANCOVA model indicated that dose (p<0·0001), route of administration (p=0·0005), and baseline gpELISA titre (p<0·0001) were determinants of gpELISA titre at 6 weeks post-vaccination, but age and sex were not (data not shown).

To assess the durability of gpELISA responses a convenience sample of vaccinees who were randomised to receive full dose subcutaneous zoster vaccine and of vaccinees who declined the offer of supplemental subcutaneous zoster vaccine at 6 weeks, had additional blood drawn at about 18 months. The sample size for each dose group was roughly a third that initially enrolled (table 3). As expected, a 37–55% decline in gpELISA titre was noted in all dose groups (compare 6 weeks in table 2 and 18 months in table 3), but the size of the decline was generally comparable across the dose groups. Thus, the gpELISA GMFRs in the full intradermal dose and 1/3 intradermal dose groups remained significantly higher than those in the full subcutaneous dose group at 18 months (p=0·002 and p=0·005, respectively).

Administration of zoster vaccine by either route significantly increased the varicella-zoster virus-specific interferon-γ ELISPOT responses at full dose or 1/3 dose (table 4). The GMCs of the intradermal doses were less than for comparable subcutaneous doses, but the GMFRs were similar at these two doses by either route of administration. Interpretation is complicated by the small sample sizes and the differences in baseline GMC in the treatment groups. No correlation between gpELISA

	n	gpELISA (90% CI) GMT		GMFR (90% CI)
		Baseline	Week 6	
Full dose subcutaneous	52	181 (146–224)	327 (275–389)	1·74 (1·48–2·04)
1/3 dose subcutaneous	34	183 (137–246)	310 (246–391)	1·64 (1·36–1·99)
Full dose intradermal	34	260 (183–369)	737 (574–945)*	3·25 (2·68–3·94)†
1/3 dose intradermal	35	143 (100–206)	441 (318–610)	2·58 (2·13–3·13)‡
1/10 dose intradermal	34	241 (189–308)	483 (389–601)	2·22 (1·83–2·69)
1/27 dose intradermal	34	194 (152–247)	319 (257–396)	1·64 (1·35–2·00)

One participant in the full dose subcutaneous group did not have 6 week sample submitted and one participants in the 1/3 intradermal group withdrew consent, but the baseline values for these participants were included in the model from which the GMFRs were calculated. GMT=geometric mean titre. GMFR=geometric-mean fold-rise. *p<0·0001 for the comparison of GMT at week 6 between full intradermal versus full subcutaneous. †p<0·0001 for the comparison of GMFR at week 6 between full intradermal versus full subcutaneous. ‡p=0·007 for the comparison of GMFR at week 6 between 1/3 intradermal versus full subcutaneous.

Table 2: **gpELISA varicella-zoster vaccine antibody titre and fold-rise in titre from baseline at week 6 post vaccination by treatment group**

	n	GMT		GMFR (90%CI)
		Baseline	Month 18	
Full dose subcutaneous	22	166 (119–232)	157 (112–220)	0·93 (0·77–1·12)
1/3 dose subcutaneous	16	207 (140–307)	225 (152–334)	1·13 (0·91–1·41)
Full dose intradermal	10	116 (71–191)	224 (136–367)	1·72 (1·31–2·26)*
1/3 dose intradermal	13	95 (62–147)	174 (112–268)	1·56 (1·22–1·98)†
1/10 dose intradermal	15	334 (223–501)	341 (228–512)	1·21 (0·96–1·51)
1/27 dose intradermal	14	194 (128–296)	235 (155–358)	1·24 (0·98–1·56)

GMT=geometric mean titre. GMFR=geometric mean fold-rise. *p=0·002 for full intradermal versus full subcutaneous. †p=0·005 for 1/3 intradermal versus full subcutaneous.

Table 3: **gpELISA varicella-zoster vaccine antibody titre and fold-rise in titre from baseline at month 18 post vaccination by treatment group**

	n	Interferon γ ELISPOT (90% CI) GMC		GMFR (90% CI)	One-sided p value
		Baseline	Week 6		
Full dose subcutaneous	52	73·9 (56·5–96·9)	98·9 (74·7–130·9)	1·51 (1·22–1·88)	0·001
1/3 dose subcutaneous	34	49·3 (30·5–79·6)	79·9 (57·0–112·0)	1·62 (1·25–2·11)	0·001
Full dose intradermal	34	33·9 (23·1–49·8)	75·2 (54·3–104·1)	1·97 (1·51–2·55)	<0·001
1/3 dose intradermal	35	37·1 (24·0–57·3)	64·4 (42·3–98·2)	1·58 (1·22–2·06)	0·002
1/10 dose intradermal	34	45·2 (29·9–68·2)	67·0 (43·9–102·2)	1·44 (1·11–1·87)	0·011
1/27 dose intradermal	34	56·7 (35·3–91·0)	60·8 (37·6–98·4)	1·14 (0·88–1·49)	0·205

Estimates are least squares means based on a constrained longitudinal data analysis model with unstructured covariance. Four (2%) of 223 enrolled participants had non-evaluable ELISPOT values either at day 1 (baseline) or day 42. GMC=geometric mean counts. GMFR=geometric mean fold-rise.

Table 4: **Varicella-zoster virus interferon γ ELISPOT GMC and fold-rise in GMC at week 6 post vaccination by treatment group**

	Full dose subcutaneous (n=52)	1/3 dose subcutaneous (n=34)	Full dose intradermal (n=34)	1/3 dose intradermal (n=35)	1/10 dose intradermal (n=34)	1/27 dose intradermal (n=34)	Placebo (n=39)
≥1 injection site adverse events	27 (52%)	7 (21%)	27 (79%)	22 (63%)	19 (56%)	19 (56%)	5 (13%)
Erythema	16 (31%)	5 (15%)	26 (77%)	21 (60%)	16 (47%)	18 (53%)	4 (10%)
Pain	15 (29%)	4 (12%)	8 (24%)	9 (26%)	5 (15%)	6 (18%)	0
Swelling	13 (25%)	4 (12%)	13 (38%)	8 (23%)	6 (18%)	7 (21%)	2 (5%)
Induration	5 (10%)	2 (6%)	12 (35%)	12 (34%)	11 (32%)	10 (30%)	1 (3%)
Pruritus	1 (2%)	2 (6%)	4 (12%)	4 (12%)	1 (3%)	1 (3%)	0
Haematoma, anesthesia, rash, scab*	3 (6%)	0	2 (6%)	1 (3%)	0	0	0

Data are n (%). Any vaccinee with one or more adverse event is counted once for each category of adverse event. *Two haematoma with full dose subcutaneous; one anaesthesia with 1/3 dose intradermal; one rash with full dose subcutaneous; and one rash with full dose intradermal; one scab with full dose intradermal.

Table 5: **Participants with injection site adverse events**

measurements at 6 weeks post-vaccination and interferon-γ ELISPOT measurements were noted.

At the Denver clinical site (because of technical expertise), the effect of route of administration on varicella-zoster virus-specific PBMC was examined by flow cytometry. Because the number of vaccinees was small, all doses of zoster vaccine were combined for the subcutaneous or intradermal route for comparison. 6 weeks after vaccination, intradermal recipients had significantly higher proportions of CD4+ central memory cells (CD4+ CD69+ CD27+ CD28+ CD45RO+) among varicella-zoster virus-specific circulating CD4+ cells than subcutaneous recipients as identified by the expression of CD69 after in vitro varicella-zoster virus stimulation (p=0·0449; appendix p 4). The mean change in the proportion of CD4+ central memory cells among total varicella-zoster virus-specific CD4+CD69+ cells in PBMC was –8·17% for the subcutaneous route and 0·16% for the intradermal route (p=0·045). The difference in the proportion of varicella-zoster virus-specific CD4+ effector memory cells (CD4+CD27+CD28-CD45RO+) 6 weeks after intradermal immunisation compared with subcutaneous immunisation was not statistically significant (p=0·0954). The mean change in the proportion of varicella-zoster virus-specific CD4+ effector memory cells was –0·87% for the subcutaneous route and 1·06% for the intradermal route (p=0·095). Of note, the numbers represent cell proportions in varicella-zoster virus-stimulated conditions after subtraction of the proportions in medium-stimulated conditions, which explains why some of the numbers are negative. Other varicella-zoster virus-specific CD4+ and CD8+ subsets did not differ by route of administration. These included CD4+ differentiated effectors, all CD8+ memory and effector subsets, and both CD4+ and CD8+ cell populations that produced interleukin 21, interleukin 2, or perforin upon varicella-zoster virus stimulation (appendix p 4).

A larger percentage of participants who received intradermal vaccine reported injection site erythema and induration or swelling than did those who received subcutaneous vaccine (table 5), but injection site pain was comparable between the routes of administration. Intradermal saline given with the MicronJet600 device caused very few injection site adverse events. A lesion sample from the only participant reporting a varicella-zoster virus-like rash had no detectable varicella-zoster virus DNA.[18] No serious adverse events were reported, and there were no temperatures greater than 38°C through to day 42.

Leakage from the vaccine site occurred in three (6%) of 52 participants in full dose subcutaneous group, none of 34 in 1/3 dose subcutaneous group, 15 (44%) of 34 in full dose intradermal group (which had two injections), six (17%) of 35 in 1/3 dose intradermal group, ten (29%) of 34 in 1/10 dose intradermal group, and eight (25%) of 34 in 1/27 intradermal group.

121 (81%) of 149 questionnaire respondents would prefer the MicronJet600 device for future immunisations for themselves over hollow steel needles and 114 (77%) of 149 would prefer it for their children's vaccinations. 114 (77%) of 149 respondents rated the experience as painless, whereas six (4%) of 149 suggested it was more painful than the standard needle (appendix p 5).

Discussion

Intradermal zoster vaccine either at the full dose recommended for subcutaneous administration or 1/3 of that dose induced a significantly greater boost in varicella-zoster virus-specific antibody than did a full dose given subcutaneously, as measured by GMFR (primary endpoint) or GMT. A dose-response relation was apparent with intradermal administration. Moreover, the relative difference in GMFR responses after the full and 1/3 intradermal doses compared with the full subcutaneous dose persisted for 18 months after immunisation. Improved immunogenicity could imply better clinical efficacy via intradermal vaccination. Similar varicella-zoster virus-specific antibody boosts from lower intradermal doses than from the full subcutaneous dose indicate that intradermal administration might be dose

sparing, which could be potentially useful to expand vaccine supply or reduce cost.

The more than three-fold GMFR in gpELISA from intradermal delivery is promising compared with previous results with subcutaneous vaccination. GMFR at 6 weeks was 2·31 (95% CI 2·20–2·43) in 50–59 year olds[19] and 1·7 fold (1·6–1·8) in participants older than 60 years.[16] Over time, gpELISA titres diminish, remaining 10–20% above placebo-corrected levels at 1, 2, and 3 years of follow-up.[19] Despite these small persistent changes, protection from herpes zoster was shown across the median 3·2 years of the pivotal study.[5]

Varicella-zoster virus-specific antibodies measured by gpELISA are unlikely to be mechanistically deterministic in preventing varicella-zoster virus reactivation because their levels remain steady with age, while the risk of herpes zoster increases.[1] These antibodies are a non-mechanistic correlate of protection, because the magnitude of GMFR was shown to correlate with the occurrence of herpes zoster in 50–59-year-old zoster vaccine-recipients at 6 weeks post-vaccination compared with participants who developed herpes zoster.[19] An exploratory analysis of these data concluded that GMFR is a correlate of protection for zoster virus.[20] The superior GMFR in gpELISA imply our results might translate to improved clinical efficacy.

The varicella-zoster virus cell mediated immunity responses measured by ELISPOT GMFR did not differ between the intradermal and the subcutaneous route. The absence of improved ELISPOT responses could be due to small sample sizes, because this assay has greater variability than the gpELISA antibody assay. Inequality of baseline varicella-zoster virus cell mediated immunity levels in the treatment groups might also contribute to these results. However, flow cytometry in a subsample of vaccinees suggests that intradermal administration of zoster vaccine expands varicella-zoster virus-specific CD4+ memory cells, which typically contribute to long-term protection conferred by T cells.

Intradermal administration of zoster vaccine more commonly produced erythema and induration or swelling than did subcutaneous administration, but these reactions were considered mild and transient. Moreover, most intradermal recipients indicated that the pain of injection was less than the standard subcutaneous needle injection and indicated that they would prefer this route of administration for themselves and for their children for future injections. The higher incidence of local adverse events (but not systemic adverse events like fever) are consistent with many other intradermal vaccination studies.[8,13,21]

We hypothesised the zoster vaccine might show improved immunogenicity if it was efficiently delivered to potent antigen-presenting dendritic cells, because the quality of the interaction of vaccine antigens and dendritic cells are important determinants of the size and profile of the response to vaccination.[8,9,11] Other existing vaccines have shown improved immunogenicity with intradermal vaccination.[8,9,13] The MicronJet600 device has been used in several studies showing improved immunogenicity or reduced dose of intradermal vaccination.[13]

Intradermal vaccination could overcome immune senescence, which is a well characterised limitation of influenza, pneumococcal, and zoster vaccines.[5,22,23] Intradermal vaccination places antigen in proximity to the dense, extended network of antigen-presenting dendritic cells in the epidermis and dermis. Varicella-zoster virus infects monocytes and dendritic cells, and activates NOD and Toll-like receptor 2, yielding pro-inflammatory cytokines.[24,25] The mild, prevalent local skin inflammation following intradermal vaccination might contribute to the efficiency of dendritic cell priming of the adaptive immune response. Novel vaccines could be another approach to overcome the risk of zoster in the elderly population.[26]

This study has limitations. First, the 18 month comparative result is based on a convenience sample of small sample size. Second, the differential advantage of intradermal administration was readily shown for the antibody endpoint, but not for the cell-mediated immunity endpoint. Finally, the sample studied, and the number of participants of advanced age, were limited. We have shown that a full dose of intradermal live, attenuated herpes zoster vaccine results in higher fold-rise in varicella-zoster virus-specic antibody than the recommended subcutaneous injection. These antibodies are a correlate of protection from herpes zoster. Larger and longer studies of intradermal administration of zoster vaccine will need to provide convincing evidence of improved cell mediated immunity and ultimately, protection from herpes zoster.

Contributors

EAS, KL, NL, AW, JC, and MJL enrolled the participants, collected and interpreted the data, and prepared the report. CRB, RAR, AKS, YL, BKM, RKE, EAS, KL, and MJL conceived and designed the study, analysed and interpreted the data, and prepared the report. EK analysed and interpreted the data and prepared the report.

Declaration of interests

This study was funded by Merck & Co Inc. Although the sponsor formally reviewed a penultimate draft, the opinions expressed are those of the authorship and may not necessarily reflect those of the sponsor. All co-authors approved the final version of the report. CRB, RAR, AKS, BKM, and RKE are employees of Merck & Co Inc; employees may hold stock and/or stock options in the company. KL, EAS, and MJL are investigators for the sponsor. MJL is a consultant to the sponsor and shares intellectual property rights on Zostavax. YL and EK are employees of NanoPass Technologies Ltd, the provider of the MicronJet600 device

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Short communication

Ameliorated immunity elicited by intradermal inoculation in individuals vaccinated with inactivated SARS-CoV-2 vaccine

Shengtao Fan, Dandan Li, Heng Zhao, Li Yu, Pingfang Cui, Lichun Wang, Ying Zhang, Yun Liao, Xingli Xu, Guorun Jiang, Qihan Li *

Institute of Medical Biology, Chinese Academy of Medical Sciences & Peking Union Medical College, Yunnan Key Laboratory of Vaccine Research and Development on Severe Infectious Diseases, Kunming 650118, China

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ABSTRACT

In clinical trials, antibodies against SARS-CoV-2 were almost eliminated in participants six months after immunization with an inactivated SARS-CoV-2 vaccine. The short duration of antibody persistence is an urgent problem. In this study, the problem was solved by intradermal inoculation with trace antigen. Within 72 h after intradermal inoculation, slight inflammatory reactions, such as redness and swelling, were observed at the inoculation site of the participants. On the 7th, 60th and 180th days after inoculation, the antibodies of the participants were detected, and it was found that the neutralizing antibody and ELISA (IgGs) anti-S antibody levels rapidly increased and were maintained for 6 months. These results indicate that there was a SARS-CoV-2-specific immune response in the participants immunized with an inactivated SARS-CoV-2 vaccine, which could be quickly and massively activated by intradermal trace antigen inoculation to produce an effective clinically protective effect.

1. Introduction

The number of individuals with coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection has exceeded 221 million worldwide (as of September 08, 2021, COVID-19.who.int). The world's economic and social development is facing unprecedented challenges. To date, coronavirus disease is currently developing, and the disease prevention and control situation is still urgent [1]. Vaccines are the most effective way to prevent acute infectious diseases [2]. Since the COVID-19 outbreak, many countries have made great efforts to develop an effective vaccine against SARS-CoV-2 [3]. To date, there have been four types of SARS-CoV-2 vaccines: inactivated vaccines [4], adenovirus vector vaccines [5], recombinant protein vaccines [6] and mRNA vaccines [7], which cause different immune responses and antibody persistence effects [8]. There are no methods to evaluate SARS-CoV-2 vaccine effectiveness. Based on previous research, it is generally believed that neutralizing antibodies is still the gold standard to evaluate vaccines, but for SARS-CoV-2 in particular, ELISA (IgGs) anti-S/N antibodies are also important in vaccine evaluation [9]. As SARS-CoV-2 is novel, little is known about the induced immune response. Compared with the production induced by conventional vaccines [10,11], the production of SARS-CoV-2 antibodies induced by intramuscular inoculation decreases too fast [9]. In this study, we will provide insight into how to elicit antibodies from humans immunized with an inactivated SARS-CoV-2 vaccine.

2. Methods

The study protocol was approved by the Ethics Committee of the West China Second University Hospital, Sichuan University (approval number: Y2020008). Fifty participants were randomly selected and immunized with inactivated SARS-CoV-2 vaccine via the intramuscular route. The SARS-CoV-2 inactivated vaccine was developed by the Institute of Medical Biology (IMB), Chinese Academy of Medical Sciences (CAMS). Briefly, the KMS-1 strain (MT226610.1) was inoculated into Vero cells. Dual inactivation was performed with formaldehyde (1:4000) to partially disrupt the viral membrane, followed by beta-propiolactone (1:2000) to disrupt the structure of the viral genome. The viral antigen content was measured via enzyme-linked immunosorbent assay [12]. In all, 150 U of SARS-CoV-2 antigens and 0.125 mg of aluminium adjuvant were contained in a 0.5 ml/dose. A booster immunization was performed 14 days after the first dose. On the 14th, 28th and 180th days after immunization, serum samples were collected to detect neutralizing antibodies and ELISA (IgGs) anti-S/N antibodies. In brief, inactivated serum was serially diluted 2-fold and incubated with the KMS-1 strain (100 lgCCID$_{50}$/well) for 2 h at 37 °C, followed by inoculation into Vero cells for cytopathic effect (CPE) observation. The neutralizing antibody titers of the serum were defined by CPE assay. ELISAs were conducted with antibodies against the S protein and the N protein that were developed by this institute. S and N proteins were used to coat 96-well ELISA plates at a concentration of 5 μg/well and then incubated with serum samples. The OD values were measured using an ELISA plate reader [12]. On the 186th day after the two intramuscular injections, 20 participants were randomly assigned to a group inoculated with 10 U of SARS-CoV-2 antigen (0.1 ml/dose) via the intradermal route with a MicronJet 600 Microneedle (NanoPass Technologies, Ltd.) according to the manufacturer's instructions. The occurrence time and diameter of redness were recorded. On the 7th, 60th and 180th days after intradermal immunization, serum samples were collected to detect neutralizing antibodies (NAb) and ELISA (IgGs) anti-S/N antibodies (Fig. 1).

3. Results

In the phase I clinical trial of an inactivated vaccine, the antibody level of healthy adults aged 18–59 years reached a relatively high level on days 14 and 28 after immunization according to the 0- and 14-day two-dose intramuscular immunization procedure, including that of neutralizing (34.1 and 29.3) (Fig. 2A), ELISA (IgGs) anti-S (2700 and 2314) (Fig. 2B), and ELISA (IgGs) anti-N (457 and 400) (Fig. 2C) antibodies, but there was no significant difference between the two time points. In a follow-up investigation, we found that the antibody level was not durable and was in a state of rapid decline. On the 180th day after immunization, both neutralizing and ELISA (IgGs) anti-S antibody levels decreased to a relatively low level, especially those of neutralizing antibodies, and ELISA (IgGs) anti-S antibody levels were significantly different from those on the 14th and 28th day after immunization (Fig. 2).

To find an effective way to stimulate the immune response, 20 randomly selected participants were intradermally immunized. Twenty-four hours after inoculation, the inoculation site of all participants turned red (diameter: 0.93 ± 0.03 cm). Over time, the redness area gradually increased. At 36 h (diameter: 1.38 ± 0.13 cm), 48 h (diameter: 1.83 ± 0.08 cm) and 72 h (diameter: 0.83 ± 0.03 cm) (Fig. 3), the temperature was normal for all the participants, the inoculation site did not hurt or itch, and there was no adverse reaction.

On the 7th, 60th and 180th days after intradermal immunization, the neutralizing antibody and ELISA (IgGs) anti-S antibody levels of all participants were determined again. On the 7th day after intradermal inoculation, the neutralizing antibody level was 12.5, which was 8 times that before inoculation ($P < 0.01$); on the 60th day after antigen stimulation, the neutralizing antibody level reached 53.3, which was 33 times that before antigen stimulation ($P < 0.0001$). Furthermore, on the 180th day after antigen stimulation, the neutralizing antibody level reached 13.3, which was 8.5 times that before antigen stimulation ($P < 0.01$) (Fig. 4A). On the 7th day after antigen stimulation, the ELISA (IgGs) anti-S antibody level was 2200, 10 times higher than that before antigen stimulation ($P < 0.05$); on the 60th day after antigen stimulation, the ELISA (IgGs) anti-S antibody level was 21333, 97 times higher than that before antigen stimulation ($P < 0.0001$). On the 180th day after antigen stimulation, the ELISA (IgGs) anti-S antibody level was 2667, 12 times higher than that before antigen stimulation ($P < 0.05$) (Fig. 4B). On the 7th day after antigen stimulation, the ELISA (IgGs) anti-N antibody level was 2400, 8 times higher than that before antigen stimulation ($P < 0.05$); on the 60th day after antigen stimulation, the ELISA (IgGs) anti-S antibody level was 17600, 59 times higher than that before antigen stimulation ($P < 0.0001$). On the 180th day after antigen stimulation, the ELISA (IgGs) anti-N antibody level was 2667, 9 times higher than that before antigen stimulation ($P < 0.05$) (Fig. 4C). These results indicate that the memory immune response was activated rapidly and maintained for 6 months after a single intradermal injection.

4. Discussion

To effectively solve the problem that antibody levels cannot be maintained after vaccination with a SARS-CoV-2 vaccine, inspired by the classical tuberculin test [13], we adopted the skin test method to detect the cellular immune response to the SARS-CoV-2-specific antigen. If the skin test results are positive, there are immune cells specific to the tested antigen, such as sensitized Th1 cells [14] and antigen-specific B cells [15]. In our experiment, all the participants had mild inflammatory reactions, such as local redness at the inoculation site. According to the classical immunology theory [16–18], the specific immune reaction in all the participants was activated, and there were SARS-CoV-2-specific antibodies in their bodies. In particular, neutralizing antibodies and ELISA (IgGs) anti-S antibodies are produced in large quantities in a short period of time and can be maintained for a long time, which may be due to the rapid activation of antigen-specific B cells [19]. These results further verify our conjecture. Of course, this method of inducing rapid activation of the immune response has been tested for only inactivated vaccines, and whether it functions similarly with other types of vaccines still needs further verification.

In this work, we conducted a preliminary study on how to induce or strengthen the immune response of the body and provided an idea and solution to the problem that antibody levels cannot be maintained after vaccination with an inactivated SARS-CoV-2 vaccine, which has important guiding significance for the establishment and maintenance of herd immunity after mass vaccination with SARS-CoV-2 vaccines in the future.

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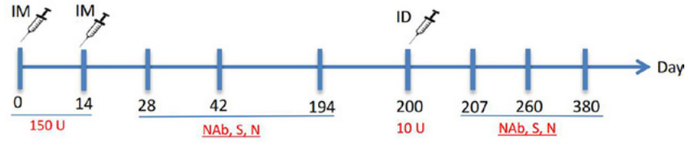

Fig. 1. Schematic depicting the immunization schedule.

* Corresponding author.
E-mail address: liqihan@imbcams.com.cn (Q. Li).

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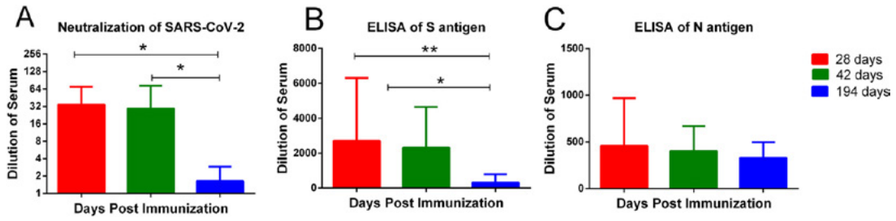

Fig. 2. Immune response induced by intramuscular immunization with an inactivated SARS-CoV-2 vaccine in adults. Neutralizing antibodies (A), ELISA (IgGs) anti-S antibodies (B) and ELISA (IgGs) anti-N antibodies (C) whose production was induced by an inactivated vaccine in a clinical trial in participants assigned to the 0- and 14-day schedule at 28, 42 and 192 days after intramuscular immunization. Statistical significance was assessed by unpaired t tests (*p < 0.05, **p < 0.01).

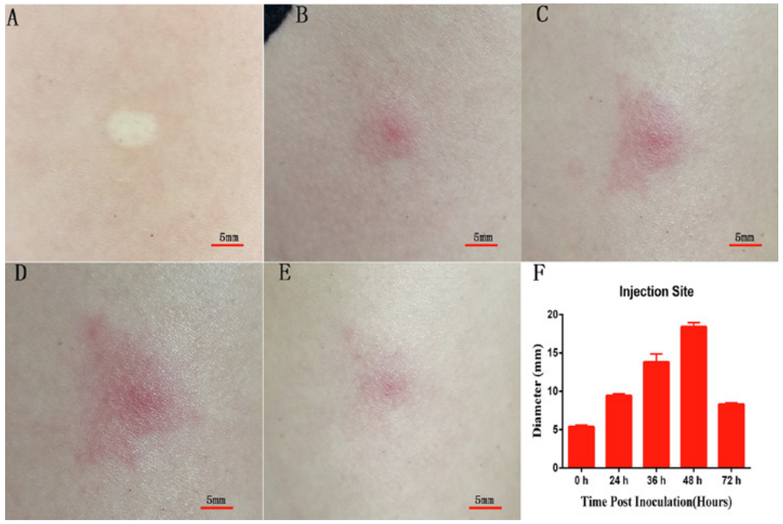

Fig. 3. Clinical observations at the inoculation site in participants Clinical observation of redness in the appearance of the skin at 0 h (A), 24 h (B), 36 h (C), 48 h (D) and 72 h (E) post inoculation. The red scale is 0.5 cm. Statistical analysis of skin redness in participants at 0 h, 24 h, 36 h, 48 h and 72 h post inoculation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

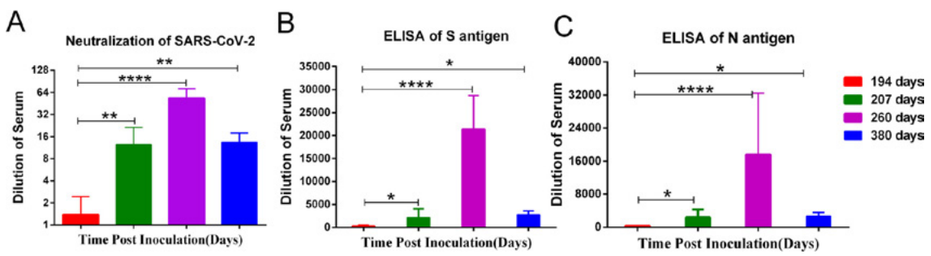

Fig. 4. Immune response induced by trace antigen in individuals vaccinated with an inactivated SARS-CoV-2 vaccine Neutralizing antibodies (A), ELISA (IgGs) anti-S antibodies (B) and ELISA (IgGs) anti-N antibodies (C) whose production was induced by an inactivated vaccine in individuals 7, 60 and 180 days after intradermal injection. Statistical significance was assessed by unpaired t tests (*p < 0.05, **p < 0.01, ****p < 0.0001).

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Section 2

Skin Test

MicronJet™ provides an easy-to use, reliable, and virtually pain-free intradermal delivery device for allergy skin testing (e.g., tuberculin skin test, allergy testing).

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Safety and efficacy of tuberculin skin testing with microneedle MicronJet600™ in healthy adults

H-J. Lee,*† H-J. Choi,‡ D-R. Kim,§ H. Lee,† J-E. Jin,† Y-R. Kim,‡ M-S. Lee,‡ S-N. Cho,† Y. A. Kang¶

*Clinical Vaccine Research Section, International Tuberculosis Research Center, Changwon, †Brain Korea 21 Plus Project for the Medical Sciences, Department of Microbiology and Institute for Immunology and Immunological Diseases, Yonsei University College of Medicine, Seoul, ‡Clinical Research Section, International Tuberculosis Research Center, Changwon, §Development and Delivery Unit, International Vaccine Institute, Seoul, ¶Division of Pulmonology, Department of Internal Medicine, Severance Hospital, Institute of Chest Diseases, Yonsei University College of Medicine, Seoul, Republic of Korea

SUMMARY

SETTING: Intradermal injection using a syringe and needle is generally accepted as the most accurate method for the tuberculin skin test (TST). However, the Mantoux technique using a conventional needle is often difficult to perform reliably, affecting testing results and safety.

OBJECTIVE: We evaluated the efficacy and safety of a novel intradermal injection device, the MicronJet600™ microneedle, compared with conventional injection in terms of skin reactivity to the TST.

DESIGN: A prospective, open-label clinical study was conducted. The TST was administered by both methods in the same subject. For pain assessment, participants filled in a visual analogue scale (VAS) after each TST. Any side effects due to TST or injections were observed.

RESULTS: TST reaction rates (cut-off ⩾5 mm) from microneedles and needles were respectively 44.0% and 47.2%, with no significant difference between the two. Furthermore, agreement of positivity between the two methods was excellent with both 5 mm and 10 mm cut-off values. However, the level of pain experienced when microneedles were used for TST was significantly lower than with conventional needles. No adverse effects were attributed to the MicronJet device.

CONCLUSION: The novel microneedle device used for TST in this study was effective, safe and less painful in healthy adult volunteers.

KEY WORDS: microneedle device; TST; Mantoux; intradermal

TUBERCULOSIS (TB) REMAINS a major public health problem in the world. It is known that one third of the world's population has latent tuberculous infection (LTBI); these individuals have been infected with *Mycobacterium tuberculosis*, but have not yet clinically developed the disease.[1] Accurate diagnosis of LTBI, followed by proper chemoprophylaxis, might be an effective way to control TB and prevent it from spreading within a high-risk population.

The tuberculin skin test (TST) is widely used for the diagnosis of LTBI. Intradermal injection using a syringe and needle, called the Mantoux technique, is generally accepted as the most appropriate method for the TST, as the delivered purified protein derivative (PPD) dose (0.1 ml) can be precisely measured and controlled, resulting in more consistent mycobacteria-specific immunity.[2–5] However, it requires a well-trained nurse who is skilful with the technique in the field to form a wheal with an acceptable size of >6 mm in diameter, indicating proper intradermal injection of PPD into the epidermal layers of the skin.[6] The percutaneous method, using a multipuncture device, has also been introduced to overcome issues such as mass, and to facilitate the rapid and less skillful administration of the TST.[7]

A novel microneedle device for intradermal injection has recently been introduced to complement an unmet need in the intradermal delivery of vaccines and other biologics.[8] The MicronJet600™ (NanoPass Technologies Ltd, Nes Ziona, Israel) used in this study is composed of three microneedles, 0.6 mm in length, enabling controlled delivery depths with minimal pain and lowered risk associated with handling needles during injection. The device is designed to be mounted on any standard syringe and used as a substitute for a conventional needle in intradermal injection.[9] Previous studies have shown that various types of vaccines, such as the seasonal and pandemic influenza vaccines, can be delivered via the intradermal route with favourable efficacy and safety, compared with intramuscular injection.[8,10–14] In addition, MicronJet can be used for other drugs or vaccines currently delivered by intradermal injection, such as insulin, rabies, influenza and anthrax, to control injection depth, reduce injection pain and ease the need for skilled users.[15]

We conducted a study to evaluate the performance of a novel microneedle device in the intradermal injection of PPD in healthy volunteers. The aim of the study was to compare the results of TST administered using a conventional syringe and needle method and the MicronJet method, in terms of efficacy and safety.

Correspondence to: Sang-Nae Cho, Department of Microbiology, Yonsei University College of Medicine, 50-1 Yonsei-ro, Seodaemun-gu, Seoul, 120-752, Republic of Korea. Fax: (+82) 2 392 9310. e-mail: raycho@yuhs.ac

Young Ae Kang, Division of Pulmonology, Department of Internal Medicine, Severance Hospital, Institute of Chest Diseases, Yonsei University College of Medicine, 50-1 Yonsei-ro, Seodaemun-gu, Seoul, 120-752, Republic of Korea. Fax: (+82) 2 393 6884. e-mail: mdkang@yuhs.ac

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STUDY POPULATION AND METHODS

Study design and participants

This was a randomised, open-label study to evaluate the efficacy and safety of the novel MicronJet microneedle device for applying the TST in healthy adults. Healthy volunteers aged 20–60 years were recruited at a tertiary hospital, the Severance Hospital, Seoul, Republic of Korea, from November 2014 to March 2015. All participants were screened using chest X-ray (CXR), and clinical information, including history of BCG vaccination, TB, TST and other comorbidities, was collected onto clinical research forms on interview. Individuals with an abnormal CXR or any chronic illness with immune suppression, such as uncontrolled diabetic condition, chronic liver disease, taking immunosuppressive agents, or history of TB or TST, were excluded. After enrolment, a trained nurse administered the TST twice for each subject on both the left and the right arms, starting with either the conventional needle or the microneedle. The site of PPD injection using the microneedle device was assigned by block randomisation.

Approval for the clinical study using an investigational medical device was provided by the Korean Ministry of Food and Drug Safety, Seoul (Study No. 644). Ethical approval was provided by the Institutional Research Board of Severance Hospital, Seoul, Republic of Korea (IRB #1-2014-0026). All volunteers provided written informed consent to participate in the study.

Tuberculin skin test with a microneedle device and a conventional needle

For each TST, 0.1 ml of 2 tuberculin units of tuberculin PPD RT23 (Statens Serum Institut, Copenhagen, Denmark) was administered on one arm with a microneedle device and the other arm with a conventional needle in the same subject by a trained nurse. The MicronJet devices were donated by NanoPass Technologies Ltd, and were used according to the manufacturer's instructions. To assess the proper intradermal injection with 0.1 ml of PPD, the size of the white vesicle (wheal) of each injection site was measured in mm. For pain assessment, a Visual Analogue Scale (VAS) pain score graded 0 from 10 was recorded after each PPD injection. After 48–72 h, the induration diameter transverse to the long axis of the arm was measured by two trained nurses. Any side effects due to TST or injections were observed before the skin reactions were read.

Determination of the number of participants

The sample size of the study was determined by the following factors: the previously reported rate of TST reaction (⩾5 mm) in Korean adults,[16,17] significance level and power, and equivalence margin difference in rates of TST reaction between the two methods (conventional needle and microneedle). Using an equivalence test for two correlated rates and assuming a value of 40% for the TST reaction (⩾5 mm) with a significance level of 0.05, a power of 0.80 and an equivalence difference of 10%, the minimum sample size was estimated to be 152.[15]

Statistical analysis

Data analyses were performed using SAS 9.3 software (Statistical Analysis System, Cary, NC, USA). To compare the TST indurations, VAS scores and wheal sizes from the two methods of PPD administration in each study subject, the paired *t*-test was used. Comparison of the rates of TST reaction between the two methods was performed using the McNemar test. Relationships between the two methods were analysed using κ statistics and Pearson correlation coefficient. In the κ statistics, $\kappa > 0.75$ represented excellent agreement beyond chance, while κ 0.4–0.75 represented fair to good agreement beyond chance. Comparison of the primary outcome, i.e., rates of TST reaction, between the two PPD administration methods were evaluated with the lower boundary and upper boundary of one-sided 95% confidence intervals (CIs) using an SAS macro suggested by Tango based on the score method.[18,19] $P < 0.05$ was seen as significant.

RESULTS

Characteristics of participants

A total of 159 participants were enrolled in the study. There were no losses to follow-up or dropouts during the study. Of 159 participants, 63 (39.6%) were male and 96 (60.4%) female; the mean age was 34.5 years (range 20–59). From the BCG scar inspection at the site, 145 (84.3%) participants still had BCG scars on the left upper arm. The mean body mass index (BMI) was 23.3 ± standard deviation (SD) 3.2 kg/m^2; 8

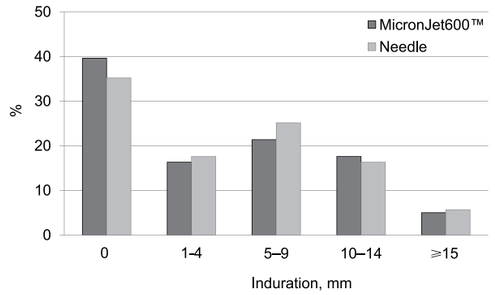

Figure Distribution of TST indurations with MicronJet600™ and the conventional injection needle ($n = 159$). TST = tuberculin skin test.

(5.0%) participants said that they had had contact with TB patients in the past.

Efficacy

In terms of skin reactivity, we evaluated the efficacy of TST using MicronJet devices compared with conventional needles in 159 participants (Figure). TST reaction positivity (cut-off ⩾5 mm) from the two methods of administration was 44.0% with MicronJet and 47.2% with the needle. Similarly, the TST positivity rates (cut-off ⩾10 mm) were 22.6% with MicronJet and 22.0% with the needle. The TST reaction with MicronJet was equivalent to that seen with the conventional needle, as the lower and upper boundary of differences measured were within the pre-defined equivalence margin of 10% (Table 1).

The mean induration sizes of the TST reaction, after excluding non-reactors (induration 0 mm in both groups), were 7.2 ± 5.4 mm with MicronJet and 7.5 ± 4.9 mm with the needle; there was no significant difference between the two methods ($n = 110$, $P = 0.062$). However, the difference in mean indurations of MicronJet and the needle approached significance, mainly affected by two outliers with respectively 0–5 mm and 0–8 mm in paired induration sizes of the MicronJet and the needle method.

When plotting the results of the TST with MicronJet and the needle, paired TST indurations performed by two methods of administration in the same subject were well correlated (correlation coefficient = 0.970, $P = 0.0001$). Agreement of TST positivity between the MicronJet and the needle methods was excellent at

Table 1 Effect of TST method on positive reaction rate

TST positivity cut-off	MicronJet600™ %	Needle %	Difference % (95%CI)*
5 mm	44.0	47.2	−3.2 (−6.6 to −0.5)
10 mm	22.6	22.0	0.6 (−2.1 to 3.5)

* One-tailed.
TST = tuberculin skin test; CI = confidence interval.

Table 2 Agreement of TST-positive reaction rates between MicronJet and the needle methods

MicronJet600™	Needle *n*			Agreement κ
Cut-off	<5 mm	⩾5 mm	Total	
<5 mm	83	6	89	0.911
⩾5 mm	1	69	70	
Total	84	75	159	
Cut-off	<10 mm	⩾10 mm	Total	
<10 mm	121	2	123	0.909
⩾10 mm	3	33	36	
Total	124	35	159	

TST = tuberculin skin test.

both 5 mm and 10 mm cut-offs (respectively κ = 0.911 and κ = 0.909) (Table 2). Using a 10 mm cut-off, two participants showed 13 and 12 mm indurations with the needle but 9.5 and 7.5 mm with MicronJet, while three participants showed 10, 10 and 12 mm with MicronJet, but 9, 8.5 and 9.5 mm with the needle, respectively.

Safety

During TST administration in 159 healthy volunteers, one mild adverse skin reaction with redness, itchiness and a blister that burst at the injection site was observed due to TST reactivity itself. However, no adverse events or safety concerns were attributed to the microneedle device or conventional needle. No breakage of microneedles was reported during the study. There were no reports of any other mechanical failures.

Usability

We evaluated the utility of the novel microneedle for PPD injection for both participants and study nurses. For study participants, we measured the relative degree of pain following injections with both microneedles and conventional needles. Among the 159 participants, the mean VAS pain score was 3.4 ± 1.7 with MicronJet and 4.9 ± 1.9 with the needle, showing that pain scores with MicronJet were significantly lower than with the other method ($P < 0.001$) (Table 3).

In addition, we measured the size of the wheals formed after each injection of 0.1 ml PPD; the wheals should be >6 mm in diameter when successful. In this study, all injections achieved the proper wheal size (100%) according to current national guidelines. However, microneedles yielded larger wheals than did conventional needles ($P < 0.0001$; mean 8.52 vs. 7.67 mm).

DISCUSSION

In this study, we found that there were no significant differences in TST reaction rates at 5 mm and 10 mm cut-off between microneedles and conventional needles. The difference in induration associated with the use of the two methods was not statistically significant, and correlations between the TST indurations performed by the two methods in the same subject were excellent. In addition, no adverse events or safety concerns were attributed to microneedle devices, with low pain response in participants and acceptable usability in study nurses.

The TST and the interferon-gamma release assay (IGRA) are currently used to diagnose LTBI worldwide. Improvement of PPD delivery by the Mantoux technique is important to TB control programmes for LTBI screening in contact investigations, among hospital employees and in national surveys. Where nurses do not have training in TST application, leakage of PPD solution at the injection site might occur, necessitating repeated skin tests.[20] Based on our results, the 0.6 mm microneedle device allows intradermal administration with minimal expertise for the nurses who apply the TST injections and minimal pain for the recipients, and shows valuable advantages over conventional needles, including reduced need for training and less needle fear, stress and discomfort associated with intradermal injections. In addition, the use of a microneedle device helps reduce any risks or injuries associated with the handling of used needles at the field site, particularly in a school setting.

To compare TST results between the two methods in our study, two trained nurses independently measured the indurations of the skin reaction, and were blinded to the injection method on each arm. Although there were two or three notable differences in readings, most were well correlated between two nurses (Spearman rank correlation = 0.96 with MicronJet, 0.95 with the needle); we thus averaged readings from the two nurses for each of the methods. One of the key factors for the interpretation of TST reading variability can be intra- and inter-observer consistency between different readers, which should be controlled and minimised by training.[21–23] With respect to the unavoidable reading variations in the TST, the small differences in TST results between the MicronJet and the needle methods in our study could be acceptable in the field.

Regarding pain assessment in this study, participants marked lower pain scores for TST using microneedles compared to conventional needles, but the difference in score (–1.4) was not as large as expected, although it was significant. This might be because our study participants were adults aged 20–60 years (mean age 34.5); this age group tends to have less needle fear than children or young adults.[24] The difference in pain scores might have been greater if we had performed the TST in children or young adults. Taking into consideration the greater needle fear in children and young adults, we expect that the microneedle device, MicronJet may be feasible for contact investigation that occurs mainly in schools.

By introducing microneedle-based delivery of PPD, specific hurdles of the TST related to injection skills, safety, and pain due to conventional needles may be overcome. However, the limitations of the TST itself, due to possible errors made by examiners, previous BCG vaccination or infection with non-tuberculous mycobacteria, remain unresolved. Although the agreement of TST reactivity between microneedles and needles was excellent in our study, we noted several participants with different sizes of induration near the 10-mm cut-off, which might affect the determination of LTBI diagnosis in the field. This suggests that without improved skills for the measurement of induration ('by definition') and the interpretation of tests in different populations, despite administration using microneedles, the TST still has limitations in the diagnosis of LTBI.

The usefulness of this microneedle-based device is not restricted to the TST. It may be extended to BCG vaccination (or novel TB vaccination with intradermal delivery) and any drug deliveries requiring intradermal injection, as shown in recent studies.[10–14] Unlike percutaneous administration by other multipuncture devices for the BCG vaccination, if validated by a clinical study, MicronJet may deliver the BCG vaccine (or novel TB vaccines) by intradermal administration, by which the delivered dose can be precisely measured and administration can be controlled, resulting in improved mycobacteria-specific immunity.[2,25,26]

CONCLUSION

In this study, we found that the use of microneedle devices did not negatively affect the result of the TST, and that an acceptable amount of PPD was administered to the Mantoux recipient with less pain compared with that of conventional needles. In addition, no adverse events or safety concerns were

Table 3 Comparison of VAS pain scores

VAS pain	MicronJet600™	Needle	Difference*	*t*-statistics	*P* value*
Mean ± SD	3.4 ± 1.7	4.9 ± 1.9	−1.4 ± 1.8	−9.86	<0.001
Median (min-max)	3 (0–9)	5 (1–10)	—	—	—

* The difference in scores was calculated by subtracting the VAS pain score for the MicronJet method from that for the needle method for each subject.
VAS = Visual Analogue Scale; SD = standard deviation.

Section 3

Systemic Delivery

MicronJet™ improves pharmacokinetics.

MicronJet™ offers potential for better safety and efficacy of systemic delivery of drugs.

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DTT

ORIGINAL ARTICLE

Improved Insulin Pharmacokinetics Using a Novel Microneedle Device for Intradermal Delivery in Patients with Type 2 Diabetes

Efrat Kochba, MD,[1] Yotam Levin, MD,[1] Itamar Raz, MD,[2] and Avivit Cahn, MD[2,3]

Abstract

Background: Currently available short-acting insulin analogs have slower absorption compared with endogenous insulin occasionally resulting in immediate postprandial hyperglycemia. Intradermal (ID) injection facilitates faster drug absorption and may result in improved insulin pharmacokinetics.
Methods: Seventeen patients with type 2 diabetes were included in this single-center, pilot, open-label crossover study. Patients received 0.2 U/kg Insulin aspart ID injections using a MicronJet (MJ) needle and subcutaneous (SC) injections, using a conventional needle in a crossover design. Thirteen patients were studied under fasting conditions and four before a standard meal test. The pharmacokinetic/pharmacodynamic (PK/PD) profile, as well as the safety and tolerability of injections, was compared.
Results: Fourteen patients completed the study per-protocol. ID versus SC injection demonstrated significantly shorter Tmax (median 35 vs. 87.5 min [$P < 0.001$]), while the Cmax did not significantly differ (median 80 vs. 55 μU/mL [$P = 0.085$]). Median insulin area under the curve (AUC; 360 min) did not differ between the groups (9914 vs. 10,936 μU/mL/min [$p = 0.077$]), yet 0–60 min insulin AUC was higher with ID versus SC injection (mean ± SD 3821 ± 1429 vs. 2534 ± 737 μU/mL/min [$p = 0.01$]) and 4–6 h AUC was lower with ID versus SC injection (mean ± SD 2054 ± 858 vs. 2929 ± 1412 μU/mL/min [$p = 0.02$]). The relative bioavailability of the ID versus the SC insulin (AUC_{ID}/AUC_{SC}) was similar (median 0.91 [95% confidence interval 0.73–1.27]).
Conclusions: ID insulin injection delivered through an MJ needle demonstrated superior PK profile compared with conventional SC administration, including shorter Tmax and higher early and lower late exposure in patients with type 2 diabetes. This may help achieve better insulin coverage of meals and lower postprandial glucose excursions.

Introduction

Insulin remains the most effective blood glucose-lowering agent.[1] Short-acting insulin analogs exhibit rather slow absorption kinetics with a Tmax of about 45–70 min, which is much longer than that of endogenic insulin in a healthy individual.[2] Matching peak insulin levels to peak postprandial glucose excursions requires delivery of the insulin injection before the meal and assessment of portion sizes before eating. This poses some limitation on the spontaneity of eating and caution is needed to prevent hypoglycemia induced by too early insulin injection or ingestion of a smaller meal size than anticipated. Moreover, the relatively delayed insulin absorption may lead to high glucose levels in the first 90–120 min after a meal, resulting in inadequate glycemic control.

To address the challenge of expediting insulin absorption and shortening the Tmax, some investigational approaches are being developed.[3–5] These include reformulation of the substance injected, that is, by the addition of EDTA, hyaluronidase, or nicotinamide and arginine, or by employment of physical methods to enhance insulin absorption such as local heating of the injection site and intradermal (ID) or inhaled delivery of insulin.[4]

ID delivery is commercially used for several indications, including vaccines (BCG, influenza), local anesthesia, and aesthetics, as well as allergy and TB testing.[6,7] The dermis is highly vascularized (Fig. 1), thereby facilitating faster drug absorption. ID delivery of vaccines is currently done using a standard metal needle (technique known as Mantoux), which requires significant expertise. The challenge of using a standard needle to directly target the dermis without injecting too deep into the subcutaneous (SC) space or leaking externally, both frequently occurring, has limited the widespread use of ID injection.[8,9]

Microneedles have been developed to facilitate reliable ID administration routes, which due to their minute size enable targeting the formulation injected into the dermis with maximal accuracy. MicronJet (MJ; NanoPass Technologies Ltd) is a microneedle device comprising four microneedles, each 0.45 mm in length, mounted on a standard syringe instead of a conventional needle (Fig. 2). Unlike the regular needle and syringe used for ID injection (Mantoux technique), the MJ device requires minimal expertise for successful ID injection, causes minimal pain during insertion, and potentially reduces the chances of trauma associated with needle handling. The safety and efficacy of ID delivery using the device were demonstrated in multiple clinical trials.[10–14] Local adverse reactions, including local edema and erythema of the skin at the injection site, have been frequently observed and are typical of ID delivery of vaccines; these injection site reactions are usually mild and transient. Device-related serious adverse events (SAEs) have not been observed, either with the MJ needle or with its successor model, the MJ 600 needle, which has 3 microneedles of 0.6 mm length.[14]

This study was designed to assess the pharmacokinetic and safety profile of ID insulin delivered through an MJ needle compared with SC delivery of insulin in patients with type 2 diabetes. We report the ID delivery of Insulin aspart (Novorapid; Novo Nordisk) with the use of the novel MJ needle versus SC delivery while evaluating the relative safety and PK/PD profile of the two insulin delivery methods in patients with type 2 diabetes.

[1]NanoPass Technologies Ltd., Nes Ziona, Israel.
[2]Diabetes Unit, Division of Internal Medicine, Hadassah Medical Organization, Jerusalem, Israel.
[3]Endocrinology and Metabolism Service, Division of Internal Medicine, Hadassah Medical Organization, Jerusalem, Israel.

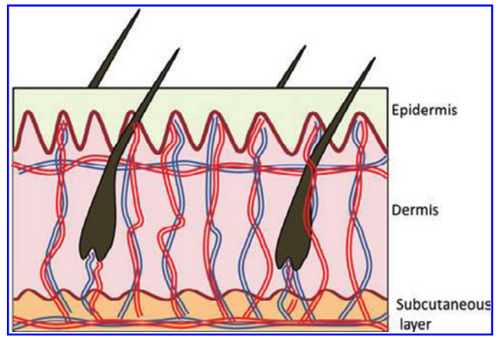

FIG. 1. An illustration of the extensive vascular network and arterial-venous shunts in the dermis.

FIG. 2. The experimental MicronJet needle device.

Research Design and Methods

Study oversight

This was an open-label, single-center, pilot crossover study designed to evaluate the pharmacokinetic/pharmacodynamic (PK/PD) profile, safety, and tolerability of ID injection of aspart using MJ needle versus SC injection using NovoPen with a conventional needle in patients with type 2 diabetes. The study was performed in the Diabetes Unit, Division of Internal Medicine; Hadassah Medical Organization (Jerusalem, Israel). The study was approved by the Institutional Ethics Board. All subjects who participated in the study provided a signed informed consent form (NCT00602914).

Study population

Eligible patients had type 2 diabetes and were aged 30–70 years, with a body–mass index (BMI) <35, HbA1c of 6.5%–10%, and were treatment naïve or treated with metformin alone. Females of childbearing potential were not included. Exclusion criteria included hypersensitivity to any drug, any disease or condition known to interfere with the absorption, distribution, metabolism, or excretion of drugs, clinically significant medical disorders (heart, lung, liver, or kidney), history of recent alcohol or other substance abuse, or positive hepatitis B, hepatitis C, or HIV serologies.

Study conduct

The study included three groups of patients with type 2 diabetes, originally planned to be of equal size with six participants in each group (Table 1). Group 1 had subjects receiving two single injections of aspart (Novo Nordisk) 0.2 U/kg, one ID using the MJ needle and one SC with NovoPen and a conventional needle. Injections were delivered in a randomized order to each individual before a standard meal. Group 2 had subjects receiving same regimen under fasting conditions. Group 3 had subjects receiving four single injections of aspart 0.2 U/kg, two ID and two SC in a randomized order under fasting conditions. Injections were conducted 4–14 days apart. Subjects taking metformin regularly did not take it on study day.

The ID injections were done using MJ, a microneedle device comprising four microneedles, each 0.45 mm in length, with width comparable with a ~30G needle, and mounted on a standard syringe. The NovoPen® 25G/1″ conventional steel needle mounted on Novopen served as the reference device. All injections were conducted in the right lower abdomen.

Blood samples for insulin and glucose were collected at the following times: 5 min before dose administration, at baseline, at 10-min intervals between 0 and 2 h, and at 30-min intervals between 2 and 6 h postdose administration. Insulin

Table 1. Study Groups

Group	ITT population	PP population	Testing condition	Treatment
1	4	3	Standard meal	Two single insulin injections: one with MJ and one with NovoPen
2	7	6	Fasting	Two single insulin injections: one with MJ and one with NovoPen
3	6	5	Fasting	Four single insulin injections: two with MJ and two with NovoPen

The trial included 17 patients in the ITT group, with 14 patients completing the study PP.
ITT, intention to treat; MJ, MicronJet; PP, per protocol.

levels were measured using the ADVIA Centaur XP Immunoassay System by Siemens.[15]

Local site reactions as well as any adverse events (AEs) or SAEs occurring on the day of study or on the subsequent visits were recorded, as well as their possible association with the intervention.

Tolerability endpoints were pain per visual analog scale (VAS) and a survey of subjects' preference parameters.

Statistical analysis

The study was designed as a pilot study and sample size determination was not planned to meet any specific significance and power requirements.

Descriptive statistics were calculated for all data. No imputation for missing values was applied. PK parameters (Tmax, Cmax, and area under the curve [AUC]) were derived from the individual insulin concentration data. The data were analyzed using SPSS software (version 20.01; SPSS, Inc.). PK analyses were carried out using PK Solutions 2.0™. PK evaluations were carried out for each individual and the average results per group are displayed. Patients included in group 3 had two measurements for each injection type and the average of their two measurements was considered when calculating the group's average. PD data were derived from the blood glucose levels measured during the study. Comparison between groups was assessed using the Mann–Whitney test. Relative bioavailability (f) was calculated by comparing insulin kinetics between the investigational MJ device and standard SC Novopen. Within-subject comparison of tolerability of ID versus SC injections was carried out using the Wilcoxon paired test.

Post hoc analyses included assessment of the time to 50% insulin Cmax in each of the groups as well as measurement of partial AUC of insulin and glucose in the early (60 and 90 min) and the late (4–6 h) postinjection times.

Table 2. Patient Demographics

	ITT population	PP population
N	17	14
Gender (male), *n* (%)	16 (94.1)	13 (92.9)
Age, years	54.4 ± 10.0	55.7 ± 9.1
Weight, kg	85.2 ± 7.3	85.6 ± 7.6
BMI, kg/m^2	28.4 ± 3.6	28.4 ± 3.3
Diabetes duration, years	6.8 ± 4.6	7.21 ± 4.5
Concomitant metformin, *n* (%)	8 (47.1)	6 (42.9)

Data are mean ± SD.
BMI, body–mass index.

Results

Patient characteristics

Seventeen subjects were enrolled and 14 completed the study per-protocol and are included in all further analyses. One patient was excluded from the trial due to protocol violation—HbA1c of 5.84% and BMI >35 kg/m^2, one dropped out by personal choice, and one was excluded as per investigator's decision following a hypoglycemic AE.

Table 2 lists demographics of all patients recruited to the trial and of those completing the trial per-protocol.

Pharmacokinetics and pharmacodynamics

ID insulin injection with the MJ needle resulted in a shorter Tmax than the SC injection (Table 3). Interpatient variability in Tmax was lower with ID versus SC injections in the overall population (inter quartile range/median 28.6% vs. 62.9%). Higher insulin Cmax values were observed with ID versus SC injection, yet this did not reach statistical significance (Table 3). Post hoc analysis of time to 50% Cmax was significantly shorter with the ID versus the SC injections (median 14.0 vs. 26.0 min, $p = 0.008$). The PK profile of insulin injections, ID versus SC, is presented in Figure 3.

Post hoc analysis revealed that the insulin AUC 60 and 90 min after insulin administration were significantly higher in ID versus SC injection; 4–6 h after insulin injection, the AUC in the ID group was significantly lower than SC insulin injection (Table 4).

The relative bioavailability of the ID versus the SC insulin (AUC_{ID}/AUC_{SC}) was similar (median 0.91 [95% confidence interval 0.73–1.27]).

Pharmacodynamic data of the glucose levels measured under fasting conditions are presented in Figure 4. The glucose AUC during 4–6 h postinjection was lower in the ID injection versus the SC injection (Table 4).

AEs and tolerability

All 17 patients recruited to the trial were included in the safety and tolerability analysis. No local AEs (injection site reactions) were reported. A total of 3 of 17 subjects experienced 5 AEs. Three subjects experienced mild hypoglycemia, which resolved with oral glucose consumption. One of them had glucose levels of 40 mg/dL at 80 min following his first SC injection, which was followed by a slight increase to 58 mg/dL at 120 min. The subject was withdrawn from the study by the investigator's decision. One additional patient noted acute gastroenteritis one day following the ID injection, with subsequent anxiety, and elected to withdraw from the study.

Table 3. Insulin Kinetics by Treatment Group

	Fasting (n = *11*)			*Standard meal test* (n = *3*)		*All patients* (n = *14*)		
Data	*ID*	*SC*	P	*ID*	*SC*	*ID*	*SC*	P
Tmax, Minutes								
Median	35	90	**<0.001**	50	70	35	87.5	**<0.001**
IQR	30–40	60–115		30–60	50–90	30–40	60–110	
Cmax, μU/mL								
Median	79	54.5	0.125	95	68	80	55	0.085
IQR	47–104	46–58		78–98	64–111	51–98	47–68	
AUC, μU/mL/min								
Median	9672.5	10,407.5	0.215	12690	13960	9913.75	10936.25	0.077
IQR	5578–12125	9280–12196		10060–17655	12640–23050	5800–13685	9305–13690	

Fourteen patients with diabetes received injections of aspart (Novorapid), both ID using a MicronJet needle and SC injections, in a crossover design. Eleven were in the fasting state and 3 received the injection before a standard meal test. Insulin pharmacokinetics is shown. *P* value was not calculated in the subjects receiving insulin poststandard meal test due to the small number of subjects.
ID, intradermal; SC, subcutaneous.
P values in boldface are statistically significant.

Pain evaluation by VAS on a range of 1–100 was done for both insertion and injection pain. There was no statistical significant difference in insertion pain between the ID and the SC injections (mean ± SD: 8.97 ± 9.97 and 6.84 ± 4.76, respectively, $p = 0.975$). Greater injection pain was noted with the ID injection versus the SC injection (mean ± SD: 15.78 ± 15.05 and 4.14 ± 4.77, respectively, $p = 0.023$). Patients in group 3 who received two injections of each type noted reduced injection pain in the second ID injection compared with the first (mean ± SD: 12.2 ± 10.6 vs. 4.5 ± 3.8, $p = 0.043$). Overall, use of the MJ device was associated with minimal discomfort, with the highest VAS score recorded lower than the 50% threshold. In a subject preference survey, answered by the 14 patients who completed the study PP, 7 patients said the MJ technology enabled painless injection, 4 were neutral, and 3 disagreed. Five patients said they would prefer the ID injection in the future, eight were neutral, and one preferred the SC injection.

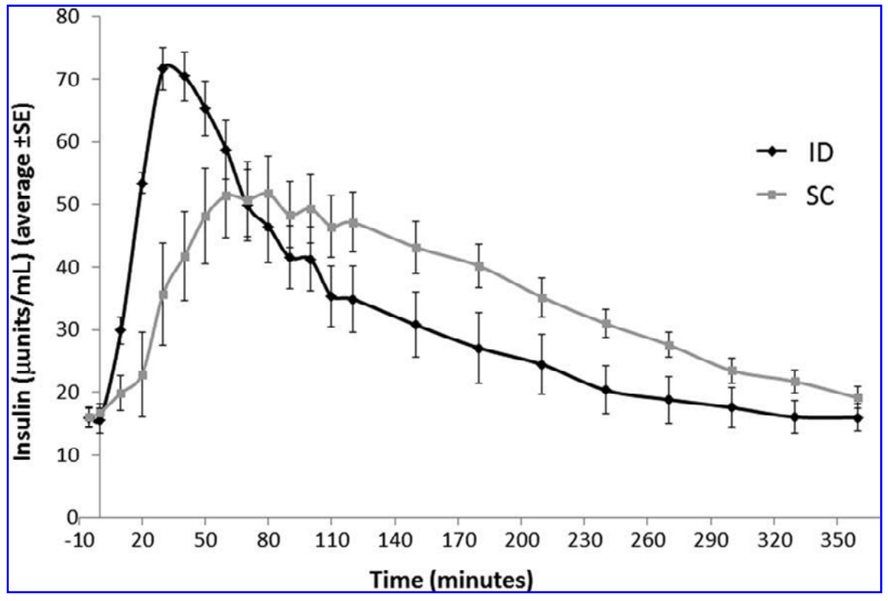

FIG. 3. PK profile of insulin: Fourteen patients with T2DM were injected 0.2U/kg aspart using an intradermal (ID) route through a MicronJet (MJ) needle or subcutaneous (SC) route using a standard needle. Three patients were injected before a standard meal test and five patients received two injections of each type. All injections were delivered in a random order, with 4–14 days between injections. The plasma insulin levels during the 6 h following the injections are displayed. Each curve represents the average of 14 patients.

Table 4. Partial Insulin and Glucose Area Under The Curve Analysis

	Intradermal (MicronJet)	*Subcutaneous*	P
Overall population ($n=14$)			
Insulin AUC 0–1	3820.9 ± 1428.6	2534.1 ± 737.1	**0.01**
Insulin AUC 0–1.5	5156.3 ± 1988.7	4035.4 ± 1255.9	**0.03**
Insulin AUC 4–6	2054.4 ± 857.7	2929.0 ± 1412.1	**0.002**
Fasting population ($n=11$)			
Insulin AUC 0–1	3695.2 ± 1593.3	2346.1 ± 609.7	**0.029**
Insulin AUC 0–1.5	4912.0 ± 2154.6	3647.7 ± 904.0	**0.033**
Insulin AUC 4–6	2027.3 ± 946.5	2903.3 ± 1571.9	**0.009**
Fasting population ($n=11$)			
Glucose AUC 0–1	9295.8 ± 2772.6	9713.0 ± 2169.3	0.110
Glucose AUC 0–1.5	12,352.4 ± 3943.6	12,814.8 ± 2664.8	0.328
Glucose AUC 4–6	12,125.2 ± 2405.2	9908.9 ± 1555.2	**0.009**

Fourteen patients with diabetes received injections of aspart (Novorapid), both ID using a MicronJet needle and SC injections, in a crossover design. Eleven were in the fasting state and 3 received the injection before a standard meal test. Partial AUC data are shown for insulin (μU/mL/min) and glucose (mg%/min) in the early (first 1 and 1.5 h postinjection) and late (4–6 h postinjection) phases. Data are mean ± SD.

AUC, area under the curve.

P values in boldface are statistically significant.

Discussion

This study demonstrates the superior pharmacological profile of ID injection of aspart with the MJ needle versus SC injection of the same insulin utilizing a conventional needle. The ID delivery of insulin demonstrated a shorter Tmax, higher early exposure, and reduced interpatient variability in Tmax. Additionally, time to 50% Cmax was significantly shorter with ID versus SC injection. Finally, late AUC glucose levels were higher with ID injection, potentially limiting late hypoglycemic events.

The ID injection of insulin by the MJ device yielded a good safety profile, with no significant additional risk compared with conventional SC administration.

ID delivery of insulin to accelerate its absorption into the systemic circulation has been evaluated in several clinical studies

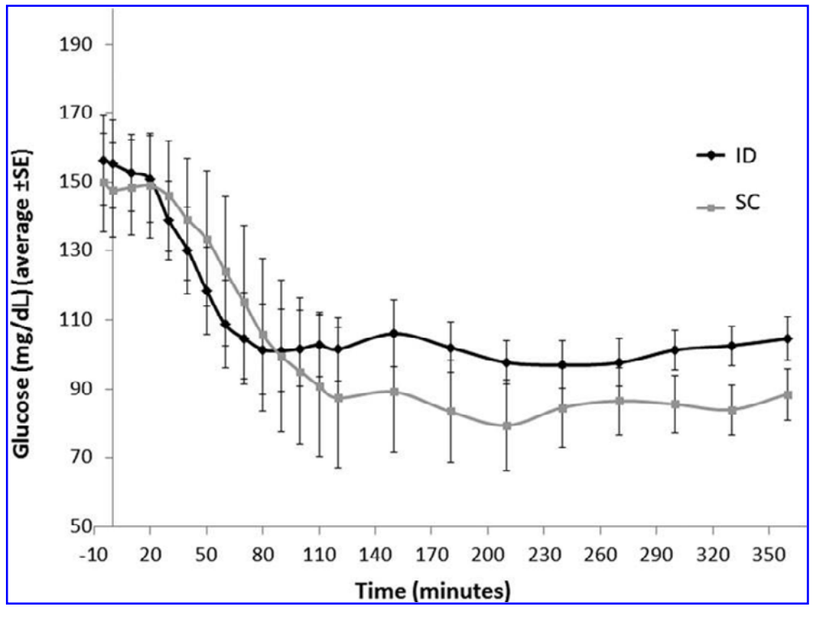

FIG. 4. PD profile of glucose in fasting patients: Legend: Eleven patients with T2DM were injected 0.2U/kg aspart using the ID route through a MicronJet (MJ) needle or SC route using a standard needle and remained fasting for 6 h following the injection. Five patients received two injections of each type. All injections were delivered in a random order, with 4–14 days between injections. The plasma glucose levels during the 6 h following the injections are displayed. Each curve represents the average of 11 patients.

of healthy volunteers or patients with type 1 diabetes.[16–23] Gupta et al. demonstrated that ID insulin administration through microneedle reached peak insulin concentrations in approximately half of the time than the catheters, resulting in a better reduction of plasma glucose levels.[16–17] ID administration of Insulin Lispro or regular human insulin by microneedles showed significantly faster uptake and time to maximum concentration, higher maximum concentration, and shorter systemic circulating duration versus SC application, both in healthy male volunteers or type 1 diabetes mellitus (T1DM) patients.[18–19] In children and adolescents with T1DM, it was found that insulin onset and offset time (defined as time to 50% Cmax during insulin onset [T 50% max rising] and offset [T 50% max falling]) was faster after microneedle delivery compared with SC delivery, and the pain was significantly lower.[20] ID delivery of insulin demonstrated safety and efficacy in continuous infusion through a microneedle-based continuous insulin infusion pump.[21]

The Tmax of the SC injection in our trial was longer compared with previously reported studies. Tmax of the SC insulin in our study was 87.5 min compared with 51.6 min measured by McVey et al.[22] and 57 min measured by Gupta et al.[17] The Tmax of the ID injection in the three trials was 35, 36, and 27 min, respectively. Slower absorption of subcutaneous insulin in patients with type 2 diabetes versus type 1 diabetes has been reported,[24] and further studies in patients with type 2 diabetes will be needed to further support this observation.

The importance of mitigating postprandial glucose excursion and minimizing glycemic variability has been extensively discussed in patients with type 1 and type 2 diabetes.[25] Multiple approaches to expedite insulin delivery in patients with type 2 diabetes to minimize postprandial glucose excursions are being explored.[5] Our study is the first to demonstrate the superior PK and PD profile of ID insulin in patients with type 2 diabetes, creating a safe and well-tolerated insulin delivery mode for this population, which carries potentially better postprandial glucose control and lower risk of hypoglycemia.

Our study carries several limitations. The dose of insulin administered was small, and patients with type 2 diabetes may often require higher doses of insulin to attain glycemic control. Further study with higher insulin doses and a dose–response study of ID absorption of insulin at higher doses are warranted. Additionally, intrasubject variability was not assessed due to the small number of patients receiving more than one injection of each type and only two injections. Furthermore, the insulin assay used detected to a similar extent human insulin and aspart. Finally, the trial included only a small number of subjects tested postprandially, which did not allow for full statistical analysis comparing the postprandial glucose PD between the ID and SC groups.

In conclusion, the PK profile of ID insulin delivery by MJ in patients with type 2 diabetes is improved—reaching earlier systemic insulin levels, lower late insulin levels, and higher late glucose levels, potentially reducing the risk for postprandial hypoglycemia. The lack of significant safety, convenience, or tolerability issues supports the use of the MJ needle in patients with diabetes.

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Disclosure Statement

I.R. reports the following: Advisory Board: AstraZeneca/Bristol-Meyers Squibb, Eli Lilly and Company, Medscape LLC, Merck Sharp & Dohme Limited, Novo Nordisk, Inc., Sanofi, Orgenesis, SmartZyme Innovation Ltd, and Labstyle Innovations Ltd; Consultant: AstraZeneca/Bristol-Myers Squibb, Insuline Medical, Gili Medical, Kamada Ltd., and FuturRx Ltd.; Speaker's Bureau: AstraZeneca/Bristol-Myers Squibb, Eli Lilly and Company, Johnson & Johnson, Merck Sharp & Dohme Limited, Novartis Pharma AG, Novo Nordisk, Inc., Sanofi, and Teva; Stock/Shareholder: Insuline Medical, Labstyle Innovations SmartZyme Innovation Ltd., Orgenesis, and Glucome Ltd.

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Address correspondence to:
Efrat Kochba, MD
NanoPass Technologies Ltd.
3 Golda Meir Street
Nes Ziona 7403648
Israel

E-mail: efrat@nanopass.com

Section 4

Immunotherapy

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Safety and feasibility of intradermal injection with tolerogenic dendritic cells pulsed with proinsulin peptide—for type 1 diabetes

Induction or restoration of immune tolerance is the holy grail in type 1 diabetes.[1] However, non-specific immunotherapies to control the T-cell dependent autoimmune response in type 1 diabetes show substantial side-effects and only temporarily modulate the course of the disease.[2] Preferably, therapy should be effective long term to selectively target and regulate β-cell autoimmunity. Tolerogenic dendritic cells (tolDCs) are considered as an attractive approach to modulate autoimmune diseases in an antigen-specific manner and to intervene in the pathogenesis of type 1 diabetes.[3] Naturally derived proinsulin peptide C19-A3 has been shown to be safe[4] and to elicit immune responses in patients with type 1 diabetes; and tolDCs presenting this peptide can induce proinsulin-specific regulatory T cells.[5,6] Therefore, we aimed to assess the clinical applicability of proinsulin peptide loaded tolDCs in a safety and feasibility trial in patients with type 1 diabetes (D-Sense trial).

Here, we present the safety and feasibility data of a first-in-man prospective, open label, placebo-controlled, dose escalation, phase 1 trial in nine patients with long-standing type 1 diabetes. TolDCs pulsed with proinsulin peptide were administered by two intradermal vaccination series (ie, 5, 10, or 20 intradermal injections depending on the dose cohort), according to the prime-boost protocol, 1 month apart (appendix p 2). Feasibility and safety (appendix pp 5, 8–9) were assessed for doses of 5×10^6, 10×10^6, and 20×10^6 tolDCs per injection series. After screening and selection (appendix p 1, 7), study participants underwent leukapheresis (duration varying between 173–376 min), to collect a sufficient number of leukocytes for $CD14^+$ monocyte selection and generation and cryopreservation of immature tolDCs (appendix p 8). Immature tolDCs were thawed 2 days before intradermal administration, and subsequently matured and loaded with proinsulin peptide C19-A3 to yield tolDC products, fulfilling all required and validated release criteria (appendix p 8). The projected doses of 5, 10, or 20 million tolDCs per injection were successfully administered in eight patients; for one patient in the highest dose group only 19 million tolDCs (instead of the 20 million) could be obtained per injection.

Patients were extensively monitored after leukapheresis and for 6 months after the tolDC injection. Besides typical and reversible leukapheresis-related discomforts, administration of tolDCs caused a mild stinging and local non-itchy redness of the skin (erythema) at the injection site. In patients receiving both vehicle and tolDC injections these symptoms were slightly more pronounced with tolDCs compared with injections containing only saline (figure 1A). The redness largely reduced in the first hour after injection and disappeared within 24 h, leaving a small bulgy blister-like injection scar of 1–3 mm in diameter. Skin reactions (erythema) were variable among patients, and more evident in patients that reported being familiar with dermatographia (skin writing). However, skin reactions did not differ depending on the tolDC dose and disappeared completely within 1–2 weeks, never requiring medical intervention. In total 13 adverse events were recorded in 7 patients: three grade 2 events and ten grade 1 events (appendix p 9). Grade 2 adverse events were allergic rhinitis, cold, and toothache which occurred 2 to 3 months after injection but were not considered related to the tolDC injection. Grade 1 adverse events such as fatigue were noted in two patients receiving the lowest tolDC dose in the time period between leukapheresis and tolDC injection. Two patients showed decreased leucocytes and mild eosinophilia in the intermediate dose group, and dry skin and arthralgia were noted in one patient in the highest dose group (appendix p 9). Registered events had resolved in the monitoring period without requiring additional intervention. Finally, minor deviations from the reference range in blood chemistry were registered (appendix p 9) at the last monitoring appointment and appeared to be reversible, and not part of any morbidity as shown outside of the study follow-up.

β-cell function and overall diabetic control remained stable during the 6 months of extensive monitoring. All patients maintained tight glycaemic control after tolDC treatment with stable HbA_{1c} values, unchanged insulin requirements, and a similar number of weakly hypoglycaemic events as before the trial, until the last follow-up visit (figure 1B). This finding was irrespective of the administered tolDC dose. Residual β-cell function was assessed by a mixed-meal tolerance test before and after the tolDC injection. Of the nine patients included in the study, three had detectable stimulated C-peptide that did not change after tolDC treatment. This low rate of residual β-cell function was expected given our safety-driven strategy of choosing only patients with long standing type 1 diabetes (on average more than 12 years with the disease) for this first-in-man trial.

Prime-boost intradermal vaccination containing up to 20 million proinsulin-epitope loaded tolDCs per injection coincided with low grade, acceptable toxicity which was not likely related to the therapy. Most importantly, there were no signs of systemic immune suppression, no induction of allergy to insulin, no interference with insulin therapy, and no accelerated loss in β-cell function in patients with the remaining C-peptide. In conclusion, generation and intradermal administration of autologous tolDCs pulsed with proinsulin peptide appears feasible and safe. Our results warrant subsequent clinical testing in patients with a shorter diagnosis of type 1 diabetes and with preserved C-peptide production, to assess whether this novel immune intervention strategy is able to delay or halt progressive loss of β-cell function. Further testing would tell whether antigen-specific immunomodulation using tolDCs protects β cells from autoimmune destruction and can act as curative therapy for type 1 diabetes.

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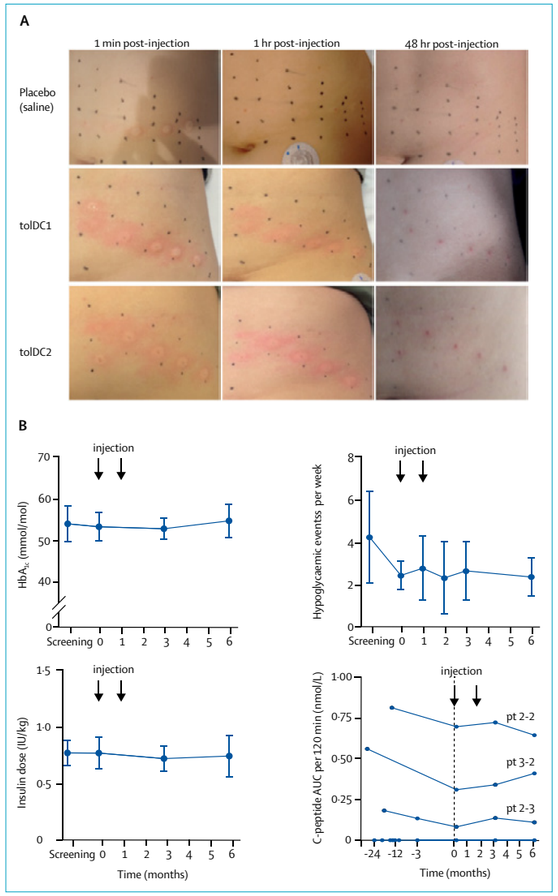

Figure: **Skin changes and diabetes parameters after intradermal injection with tolDCs pulsed with proinsulin peptide**
(A) Representative photographs of the skin from the first patient given the 5 million tolDC dose, shown at various timepoints after the placebo (saline only) injection (top row), first tolDC injection (middle row—tolDC1), and second tolDC injection (bottom row—tolDC2). Black dots delineate the area within which injections took place. Each injection dot received 200 µl of injection fluid (placebo or tolDCs), which raised the epidermis creating a bleb and some redness. This redness disappeared after 1–2h post-injection and was completely resolved within the next 48 h post-injection. Similar reactions were noted in all patients; however, the intensity did not relate to the given dose. (B) Monitoring diabetes parameters after tolDC injection. HbA_{1c}, glycemic events, and insulin dose were measured at the time of screening and during the trial. Bars represent the mean ±SD. Arrows indicate injection timepoints. None of the measured parameters changed after tolDC administration. The bottom right graph depicts stimulated C-peptide release (in a mixed-meal tolerance test) before injection, 3 months, and 6 months after tolDC administration. AUC=area under the curve. pt=patient. tolDCs=tolerogenic dendritic cells.

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*Tatjana Nikolic†, Jaap J Zwaginga†, Bas S Uitbeijerse, Nicky J Wittiez, Eelco J de Koning, Henk-Jan Aanstoot, Bart O Roep
j.j.zwaginga@lumc.nl

†Contributed equally

Immunomodulation and Regenerative Cell Therapy (TN, JJZ, NJW, BOR) and Department of Endocrinology (BSU, EJdK), Leiden University Medical Center, 2333 ZA Leiden, Netherlands; Diabeter, Center for Pediatric and Adolescent Diabetes Care and Research, Rotterdam, Netherlands (H-JA); and Department of Diabetes Immunology, Diabetes and Metabolism Research Institute, Beckman Research Institute at City of Hope, Duarte, CA, USA (BOR)

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ORIGINAL RESEARCH

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A Phase 1b Study Evaluating the Safety, Tolerability, and Immunogenicity of CMB305, a Lentiviral-Based Prime-Boost Vaccine Regimen, in Patients with Locally Advanced, Relapsed, or Metastatic Cancer Expressing NY-ESO-1

Neeta Somaiah[a], Sant P. Chawla[b], Matthew S. Block[c], John C. Morris[d], Khanh Do[e], Joseph W. Kim[f], Mihaela Druta[g], Kamalesh K. Sankhala[h], Patrick Hwu[i], Robin L. Jones[j,k,l], Sacha Gnjatic[m], Seunghee Kim-Schulze[m], Kevin Tuballes[m], Mahlet Yishak[m], Hailing Lu[n,o], Adam Yakovich[n,p], Jan Ter Meulen[n], Michael Chen[n,q], Richard T. Kenney[n,r], Chet Bohac[n,s], and Seth M. Pollack[t]

[a]Department of Sarcoma Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX, United States; [b]Sarcoma Oncology Center, Santa Monica, CA, United States; [c]Department of Medical Oncology, Mayo Clinic, Rochester, MN, United States; [d]Department of Medicine, University of Cincinnati College of Medicine, Cincinnati, OH, United States; [e]Medical Oncology, Dana-Farber Cancer Institute, Boston, MA, United States; [f]Department of Medical Oncology, Yale School of Medicine, New Haven, CT, United States; [g]Medical Oncology, Moffitt Cancer Center, Tampa, FL, United States; [h]Hematology/Oncology, Cedars-Sinai Medical Center, Beverly Hills, CA, United States; [i]Division of Cancer Medicine, The University of Texas MD Anderson Cancer Center, Houston, TX, United States; [j]Seattle Cancer Care Alliance, Seattle, WA; [k]Sarcoma Unit, Royal Marsden Hospital, London, UK; [l]Sarcoma Clinical Trials, Institute of Cancer Research, London, UK; [m]Human Immune Monitoring Center, Icahn School of Medicine at Mount Sinai, New York, NY, United States; [n]Immune Design Corp., South San Francisco, CA, United States; [o]Biomarkers and Diagnostics, Seattle Genetics, Inc, Bothell, WA, United States; [p]Medical Affairs, Replimune Group, Inc, Woburn, MA, United States; [q]*Sangamo Therapeutics, Inc., Brisbane, CA, United States; [r]Clin Reg Biologics, LLC, Potomac, MD, United States; [s]Macrogenics, Inc, Rockville, MD, United States; [t]Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA, United States

ABSTRACT

Preclinical data suggest that a "prime-boost" vaccine regimen using a target-expressing lentiviral vector for priming, followed by a recombinant protein boost, may be effective against cancer; however, this strategy has not been evaluated in a clinical setting. CMB305 is a prime-boost vaccine designed to induce a broad anti-NY-ESO-1 immune response. It is composed of LV305, which is an NY-ESO-1 expressing lentiviral vector, and G305, a recombinant adjuvanted NY-ESO-1 protein. This multicenter phase 1b, first-in-human trial evaluated CMB305 in patients with NY-ESO-1 expressing solid tumors. Safety was examined in a 3 + 3 dose-escalation design, followed by an expansion with CMB305 alone or in a combination with either oral metronomic cyclophosphamide or intratumoral injections of a toll-like receptor agonist (glucopyranosyl lipid A). Of the 79 patients who enrolled, 81.0% had sarcomas, 86.1% had metastatic disease, and 57.0% had progressive disease at study entry. The most common adverse events were fatigue (34.2%), nausea (26.6%), and injection-site pain (24.1%). In patients with soft tissue sarcomas, a disease control rate of 61.9% and an overall survival of 26.2 months (95% CI, 22.1–NA) were observed. CMB305 induced anti-NY-ESO-1 antibody and T-cell responses in 62.9% and 47.4% of patients, respectively. This is the first trial to test a prime-boost vaccine regimen in patients with advanced cancer. This approach is feasible, can be delivered safely, and with evidence of immune response as well as suggestion of clinical benefit.

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Introduction

Based on preclinical studies, therapeutic cancer vaccines designed to induce an immune response against tumor cells are a promising treatment option for cancer.[1–3] However, clinical cancer vaccine studies have resulted in only marginal efficacy to date, particularly in the advanced and metastatic settings, and identifying the optimal vaccine platform, patient (sub)population, and tumor antigen target(s) remains a challenge.[4–6] New York esophageal squamous cell carcinoma-1 (NY-ESO-1) is a cancer-testis antigen expressed only in the spermatogonia of the testis, the placenta, and in certain malignancies, and serves as an immunotherapeutic target for a wide variety of solid tumors, including melanoma, lung, and ovarian cancers.[7–10] Multiple trials targeting NY-ESO-1 in these cancers and others using both vaccine and adoptive T-cell therapy approaches have demonstrated clear clinical benefit.[11–13] In this regard, two soft tissue sarcoma (STS) subtypes, synovial sarcoma (SS) and myxoid/round cell liposarcoma (MRCL), have been of particular interest because of the very high consistency and homogeneity of their NY-ESO-1 expression.[8,14]

CMB305 was developed as a clinical prime-boost vaccine regimen Figure 1. Heterologous prime-boost regimens that use two different vaccines to first prime the immune system and then boost its response have been shown to improve the efficacy of cancer vaccines in numerous preclinical animal models,

CONTACT Seth M. Pollack spollack@fredhutch.org Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA, 98109, United States.
*Current affiliation
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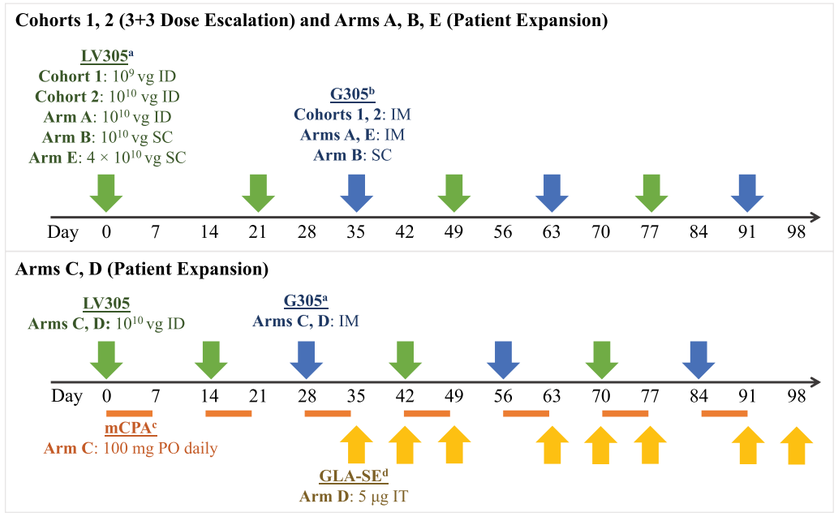

Figure 1. Dose, route, and timing of treatment administration by study arm. [a] LV305 is a NY-ESO-1 expressing, dendritic-cell tropic lentiviral vector. [b] G305 is recombinant NY-ESO-1 protein formulated in an oil-in-water stable emulsion with the synthetic TLR4 GLA. G305 dose for all study arms consisted of 250 µg NY-ESO-1 protein mixed with 5-µg GLA-SE. Patients were also given a boosting dose of G305 at each follow-up visit during the first year. [c] mCPA was only administered to patients in Arm C. It was dosed at 100 mg PO once daily for 7 days, then was not given for the next 7 days, in cycles that repeated until day 97. Patients were given a 1-week supply at each visit.[d] IT GLA-SE (5 µg/dose) was only administered to patients in Arm D and could have been injected into accessible primary tumors or distant metastases. If no accessible tumor was present at weeks 10, 11, 13, or 14, GLA-SE was not administered. Abbreviations: GLA-SE = glucopyranosyl lipid A-stable emulsion; ID = intradermal; IM = intramuscular; mCPA = metronomic cyclophosphamide; PO = oral; SC = subcutaneous; IT = intratumoral; µg = microgram; vg = viral genomes.

and lentiviral vectors as the priming component have emerged as a promising new vaccine modality.[15–19]

The priming component of CMB305 is LV305, which is a replication-incompetent, integration-deficient, improved third-generation lentiviral vector that contains RNA encoding for the full-length NY-ESO-1 protein.[20] Further, LV305 is based on the ZVex® platform, which has been shown to transduce dendritic cells through pseudotyping with an engineered Sindbis virus glycoprotein called SINVar1 that binds the C-type lectin receptor DC-SIGN (dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin) expressed on immature dendritic cells.[21,22] As a result, the vector induces direct major histocompatibility complex class I presentation of cluster of differentiation 8 (CD8) epitopes and robust CD8 T-cell immune responses. A phase I clinical trial demonstrated that LV305 is safe with evidence of inducing an anti-NY-ESO-1 CD4 and CD8 T-cell immune response, but no anti-NY-ESO-1 antibodies.[23] Dosing of LV305 led to a partial remission in one SS patient refractory to multiple lines of prior therapy.[24]

The boost component of CMB305 is G305, which is composed of full-length recombinant *E. coli*-produced NY-ESO-1 protein co-formulated with glucopyranosyl lipid A (GLA), a potent toll-like receptor 4 (TLR4) agonist as an adjuvant, in a stable squalene oil-in-water emulsion (SE). G305 can induce anti-NY-ESO-1 specific CD4 T-cell and antibody responses as a single agent and has been shown to be safe at doses ranging from 2 to 10 µg.[25] The rationale of combining LV305 and with G305 was to induce stronger T-cell responses and integrated immune responses (CD4 and CD8 T-cells, and antibodies), which preclinically resulted in improved tumor control.[15]

This phase 1b, first-in-human study of CMB305 evaluated the safety, efficacy, and immunogenicity LV305 and G305 administered in a prime-boost vaccine regimen in patients with advanced solid tumors. The CMB305 regimen was also tested in a cohort receiving metronomic cyclophosphamide (mCPA) in order to eliminate regulatory T-cell populations.[26,27] In addition, CMB305 was tested in an intratumoral "prime-pull" strategy that was designed to first stimulate (prime) the systemic innate immune response and then recruit (pull) NY-ESO-1-specific CD8 T-cells to the tumor by adding GLA dosed locally. This approach was shown in preclinical models to increase the T-cell inflammation of tumors and greatly enhance clinical efficacy.[28]

Materials and methods

Patient population

Patients aged 18 years or older with Eastern Cooperative Oncology Group (ECOG) Performance Status score of 0 or 1 who had locally advanced, relapsed, and/or metastatic solid tumors positive for NY-ESO-1 expression by immunohistochemistry staining were eligible to participate. Table 1 displays the tumor types eligible for each study arm. Key exclusion criteria were the receipt of cancer therapies ≤3 weeks prior to CMB305 dosing; prior administration of LV305, G305, or NY-ESO-1 targeting immunotherapy; and concurrent or recent immunosuppression from systemic corticosteroids or other immunosuppressive medications (the use of physiologic doses of corticosteroids may have been approved after consultation with the Sponsor).

Table 1. Eligible tumor types and rationale for each study arm.

Arm	Eligible Tumor Types	Rationale
Part 1[a]		
Cohort 1	NSCLC, ovarian, melanoma, sarcoma (any subtype)	Dose finding
Cohort 2	NSCLC, ovarian, melanoma, sarcoma (any subtype)	Dose finding
Part 2[b]		
Arm A	NSCLC, ovarian, SS, MRCL	Monotherapy of ID route
Arm B	SS, MRCL	Monotherapy of SC route
Arm C	SS, MRCL	Evaluate mCPA effect
Arm D	SS, MRCL	Evaluate IT GLA-SE effect
Arm E	Any soft tissue sarcoma	Dose finding of increased dose via SC route

Abbreviations: GLA-SE = glucopyranosyl lipid A-stable emulsion; ID = intradermal; IT = intratumoral; mCPA = metronomic cyclophosphamide; MRCL = myxoid/round cell liposarcoma; NSCLC = non-small cell lung carcinoma; SC = subcutaneous; SS = synovial sarcoma
[a]3 + 3 design.
[b]Patients in Arms C, D, and E must have had tumors accessible for biopsy and must have provided consent for biopsies.

Study design

This phase 1b, multi-center, open-label study conducted in the United States occurred from January 29, 2015 to August 3, 2019. The study (ClinicalTrials.gov identifier NCT02387125) was conducted according to the principles outlined in the Declaration of Helsinki and Good Clinical Practice guidelines. Patients were not involved in the design of the study. Informed consent was obtained from all patients prior to participation, and the Institutional Review Boards and Institutional Biosafety Committees at the participating study sites approved the study protocol and the use of the lentiviral vector LV305 (biosafety level 2).

In Part 1, dose escalation, a standard 3 + 3 design was used to study the safety of intradermal (ID) administration of 2 dose levels (10^9 and 10^{10} viral genomes [vg]) of the LV305 component of CMB305. A fixed dose of G305 (250 µg NY-ESO-1 recombinant protein mixed with 5 µg GLA-SE) was used in Part 1 and all arms in Part 2. Dosing was to be suspended at any dose level if dose-limiting toxicity (DLT) was observed in 2 or more patients. In Part 2, there were 5 separate study arms: A, B, C, D, and E. Study treatment doses, routes, and schedules for each arm are presented in Figure 1. The CMB305 vaccine regimen was administered over 91 days for the dose-escalation cohorts and all arms except Arms C and D, for which administration occurred over 84 days. Arm A included a 10^{10} vg ID dose of LV305 and intramuscular (IM) administration of G305. Arm B examined subcutaneous (SC) administration of both 10^{10} vg of LV305 and G305. Arms C and D were added in August 2016; patients in these arms received 100 mg of oral mCPA or intratumoral injections of 5 µg GLA-SE, respectively, in addition to ID administration of 10^{10} vg of LV305 and IM G305. Finally, Arm E was added in October 2017 and used a 3 + 3 design to evaluate the safety of a higher 4×10^{10} vg SC dose of LV305 with the standard dose of IM G305; dosing was to be suspended if DLTs were observed in one-third or more of subjects. The sample sizes in Part 2 were designed to provide adequate preliminary data to inform subsequent trials and to reject an indication should no clinical benefit have occurred.

The primary objective was to evaluate the safety and tolerability of CMB305 in Cohorts 1 and 2 and in Arms A, B, and E, and then CMB305 in combination with oral mCPA or intratumoral GLA in Arms C and D, respectively. Adverse events (AEs) and serious adverse events (SAEs) were reported up to 30 days after the last dose. The potential for DLTs was assessed for 42 days, based on AE severity using the National Cancer Institute Common Terminology Criteria for Adverse Events v4.03.[29] An LV305 persistence assay to evaluate for replication competent lentivirus was run using peripheral blood mononuclear cell (PBMC) pellets collected at different time points post-treatment (Day 168, Month 12, Month 24, and beyond) using a polymerase chain reaction-based assay (Molecular MD, Cambridge, MA).

The secondary objectives included evaluation of clinical responses, overall survival (OS), and progression-free survival (PFS). Tumor imaging was performed at baseline and every 8 weeks (12 weeks in Arms C and D) until confirmed disease progression per Response Evaluation Criteria in Solid Tumors (RECIST) v1.1 modified to use the immune-related response criteria (irRC).[30,31] Survival visits were completed every 3 months until the end of the study. Additional secondary objectives included evaluation of time to next treatment, time to progression, cellular and humoral immune responses to NY-ESO-1, and evaluation of pre- and post-regimen blood samples for potential biomarkers of immunogenicity and clinical tumor response. Tumor biopsies were obtained from all patients at baseline to evaluate NY-ESO-1 expression, which was done by immunohistochemistry staining at Mosaic laboratory (Lake Forest, CA).

Systemic NY-ESO-1 immune response assessment was performed on all patients with biomarker samples using methods that have been published previously.[24] Pre- and post PBMC and plasma collection occurred at baseline and pre-specified timepoints throughout the study. Assays for antibody response to NY-ESO-1 tumor antigen were evaluated by enzyme-linked immunosorbent assay using recombinant NY-ESO-1 protein and peptide pools. The induction of antibodies was defined as ≥4-fold increase in antibody titer as compared to baseline or seroconversion from negative (titer <100) to positive (titer ≥100). Cellular (T-cell) immune response to NY-ESO-1 was

evaluated by interferon gamma (IFNγ) enzyme-linked immune absorbent spot (ELISpot). After bead-guided selection, CD4 and CD8 T-cells were independently cultured with peptide pulsed, irradiated T-cell depleted PBMC (serving as antigen-presenting cells) in RPMI + 10% serum type AB (to avoid potential reactivity) supplemented with interleukin-2 (10 U/mL) and interleukin-7 (20 ng/mL) twice a week. Cells were assessed for specificity at days 10 and 20 of culture, respectively for CD8 and CD4, using autologous antigen-presenting cells pulsed with NY-ESO-1 peptides or controls (influenza nucleoprotein peptide pool or dimethyl sulfoxide). A pool of overlapping 20-mer peptides covering the entire sequence of NY-ESO-1 was used as antigen, which ensured that any naturally processed Class I and Class II-restricted epitopes were detected rather than requiring up-front selection of minimal peptides. The assay was repeated for confirmation at day 14 and day 25 in most patients. The induction of CD4 or CD8 T-cells was defined as ≥2-fold increase as compared to baseline in spots per well in ELISpot.

Statistical analysis

Safety and efficacy analyses were performed with the safety population, which included all patients who received at least one injection/dose of study drug. All statistical tests were exploratory, two-sided and tested at alpha = 0.05. The nominal *P* values were presented without multiplicity adjustment. All statistical analyses were performed using SAS® version 9.4. Throughout the study, key safety analyses were performed quarterly for the purposes of safety monitoring.

Overall survival and PFS were analyzed using the Kaplan-Meier methodology. Stepwise Cox regression analysis was used to investigate prognostic baseline factors associated with OS and PFS. Tumor response was assessed by RECIST v1.1 criteria modified to use the unidimensional measurements approach of the irRC.[30] At each tumor assessment, the response in index and new measurable lesions was defined based on the change in the sum of the longest diameters. Best overall response was defined as the best overall tumor response assessment assigned to a patient at any time-point during the study. Overall response rate was defined as percent of patients with immune-related complete response (irCR) or partial response (irPR) and the confidence interval (CI) was estimated using Clopper-Pearson exact method. Disease control rate was defined as the number of patients whose best overall response was irCR, irPR, or immune-related stable disease (irSD) divided by the number of evaluable patients. The minimum amount of time to establish irSD was 42 days (6 weeks). Median duration of response (DOR), time to next treatment, and time to progression with the corresponding 95% CIs were estimated using the Kaplan-Meier method in each treatment arm and disease type.

Results

Patient characteristics

A total of 90 patients were screened and 79 patients were enrolled at 8 sites (Appendix Figure 1). The median age of patients was 50 years (range: 20–80), and 40 (50.6%) patients were female Table 2. At study entry, 64 (81.0%) patients had sarcomas, 68 (86.1%) had metastatic disease, and 45 (57.0%) had progressive disease (PD). Twenty-eight (35.4%) patients had received ≥3 prior therapies. The highest level of NY-ESO-1 expression (>75% of tumor cells positive) was observed in 46 (58.2%) patients, while 9 (11.4%) patients had moderate (>-25–75% of cells positive) and 24 (30.4%) patients had low (≤25% of cells positive) NY-ESO-1 expression levels, respectively (Appendix Figure 2). The majority of patients with non-small cell lung carcinoma (NSCLC) and ovarian cancer had ≤25% NY-ESO-1 expression (75.0% and 72.7%, respectively), whereas most patients with STS (69.8%) had >75% expression of NY-ESO-1 (Appendix Table 1). Clinical development of CMB305 ended in early 2019 and patients participating in this trial were taken off study drug treatment and completed end of study visits regardless of their status in the protocol visit schedule.

Safety

In total, 72 (91.1%) patients who received CMB305 experienced at least 1 AE. The frequency of AEs was similar across study arms, with 3 (100%) patients experiencing AEs in Cohort 1, 2 (66.7%) in Cohort 2, and 32 (91.4%), 9 (100%), 10 (100%), 9 (90.0%), and 7 (77.8%) in Arms A, B, C, D, and E, respectively Figure 2. The most common AEs overall were fatigue (27; 34.2%), nausea (21; 26.6%), injection-site pain (19; 24.1%), decreased appetite (17; 21.5%), and dyspnea (13; 16.5%) (Appendix Table 2).

Fifty-four (68.4%) patients experienced AEs considered related to study treatment; among these, the most common AEs were fatigue (19; 24.1%), injection-site pain (18; 22.8%), influenza-like illness (11; 13.9%), myalgia (10; 12.7%), and injection-site reaction (9; 11.4%). Among patients who received CMB305 monotherapy (Cohorts 1 and 2 and Arms A, B, and E), AEs considered related to treatment occurred in 66.7%, 0%, 82.9%, 66.7%, and 55.6% of patients, respectively. In Arm C (CMB305 plus mCPA), 5 (50.0%) patients experienced AEs related to CMB305 and 5 (50.0%) related to mCPA. In Arm D (CMB305 plus GLA-SE), 7 (70.0%) patients experienced AEs related to CMB305 and 4 (40.0%) related to GLA-SE.

The majority of patients had AEs of maximum severity grade 1 (22; 27.8%) or grade 2 (27; 34.2%). Grade 3 AEs occurred in 21 (26.6%) patients; of these, 3 (3.8%) were considered related to treatment. One patient experienced two grade 4 AEs (sepsis and platelet count decreased) and one patient experienced a grade 5 AE of acute respiratory failure that resulted in death, but these events were considered not related to CMB305 treatment. There were no clinically relevant changes in laboratory parameters related to CMB305.

A total of 18 (22.8%) patients experienced SAEs. Of the SAEs reported, 2 (2.5%) were grade 3 events that were considered related to treatment: prostatic pain in a patient with metastatic SS, and pneumonitis in a patient with NSCLC who had a previous history of pneumonitis.

Adverse events that led to study treatment discontinuation occurred in 7 (8.9%) patients; 1 (1.3%; pneumonitis) was considered possibly related to treatment. Protocol-defined DLTs

Table 2. Patient demographics and baseline characteristics.

	Number (%) of Patients						
	Part 1: 3 + 3 Dose Escalation		Part 2: Patient Expansion				
	Cohort 1 10^9 vg dose of LV305 (N = 3)	Cohort 2 10^{10} vg dose of LV305 (N = 3)	Arm A CMB305 expansion (N = 35)	Arm B SC admin of CMB305 (N = 9)	Arm C CMB305 + mCPA (N = 10)	Arm D CMB305 + GLA-SE (N = 10)	Arm E CMB305 escalation (N = 9)	Total (N = 79)
Age (years), mean (SD)	62.3 (19.5)	34.0 (7.0)	53.8 (15.3)	41.8 (13.7)	48.0 (14.2)	51.6 (14.9)	48.4 (14.8)	50.4 (15.3)
Female	2 (66.7)	1 (33.3)	18 (51.4)	5 (55.6)	4 (40.0)	6 (60.0)	4 (44.4)	40 (50.6)
Race								
White	3 (100)	1 (33.3)	30 (85.7)	7 (77.8)	6 (60.0)	9 (90.0)	6 (66.7)	62 (78.5)
Asian	0	1 (33.3)	1 (2.9)	0	1 (10.0)	0	2 (22.2)	5 (6.3)
Black or African American	0	1 (33.3)	2 (5.7)	0	1 (10.0)	1 (10.0)	0	5 (6.3)
Not Reported/Other	0	0	2 (5.7)	2 (22.2)	2 (20.0)	0	1 (11.1)	7 (8.9)
Ethnicity								
Not Hispanic or Latino	2 (66.7)	3 (100)	32 (91.4)	6 (66.7)	7 (70.0)	9 (90.0)	6 (66.7)	65 (82.3)
Hispanic or Latino	1 (33.3)	0	2 (5.7)	1 (11.1)	2 (20.0)	1 (10.0)	2 (22.2)	9 (11.4)
Not reported	0	0	1 (2.9)	2 (22.2)	1 (10.0)	0	1 (11.1)	5 (6.3)
ECOG Performance Status								
0	1 (33.3)	0	20 (57.1)	3 (33.3)	0	7 (70.0)	5 (55.6)	36 (45.6)
1	2 (66.7)	3 (100)	15 (42.9)	6 (66.7)	10 (100)	3 (30.0)	4 (44.4)	43 (54.4)
Disease type[a]								
NSCLC	0	0	4 (11.4)	0	0	0	0	4 (5.1)
Ovarian	0	0	11 (31.4)	0	0	0	0	11 (13.9)
Sarcoma	3 (100)	3 (100)	20 (57.1)	9 (100)	10 (100)	10 (100)	9 (100)	64 (81.0)
MRCL	0	0	9 (25.7)	2 (22.2)	4 (40.0)	8 (80.0)	4 (44.4)	27 (34.2)
SS	2 (66.7)	1 (33.3)	11 (31.4)	7 (77.8)	6 (60.0)	2 (20.0)	4 (44.4)	33 (41.8)
Other sarcoma	1 (33.3)	2 (66.7)	0	0	0	0	1 (11.1)	4 (5.1)
Disease status			27 (77.1)	9 (100)	10 (100)	9 (90.0)	8 (88.9)	68 (86.1)
Metastatic	2 (66.7)	3 (100)	8 (22.9)	0	0	1 (10.0)	1 (11.1)	11 (13.9)
Locally advanced	1 (33.3)	0						
Progression status based on physician assessment								
Stable disease	0	0	14 (40.0)	2 (22.2)	4 (40.0)	2 (20.0)	5 (55.6)	27 (34.2)
Any tumor growth/PD	1 (33.3)	3 (100)	17 (48.6)	6 (66.7)	6 (60.0)	8 (80.0)	4 (44.4)	45 (57.0)
No evidence of disease	2 (66.7)	0	4 (11.4)	1 (11.1)	0	0	0	7 (8.9)
Current TNM stage[b]								
Stage IIIA	1 (33.3)	0	0	0	0	0	1 (11.1)	2 (2.5)
Stage IIIC	0	0	6 (17.1)	0	0	0	0	6 (7.6)
Stage IV	2 (66.7)	3 (100)	23 (65.7)	9 (100)	10 (100)	9 (90.0)	8 (88.9)	64 (81.0)
Not staged/Missing	0	0	6 (17.1)	0	0	1 (10.0)	0	7 (8.9)
NY-ESO-1 expression (% tumor cells positive)								
1–25%	1 (33.3)	0	15 (42.9)	1 (11.1)	2 (20.0)	2 (20.0)	3 (33.3)	24 (30.4)
>25–50%	0	0	3 (8.6)	0	0	0	1 (11.1)	4 (5.1)
>50–75%	0	2 (66.7)	2 (5.7)	1 (11.1)	0	0	0	5 (6.3)
>75–100%	2 (66.7)	1 (33.3)	15 (42.9)	7 (77.8)	8 (80.0)	8 (80.0)	5 (55.6)	46 (58.2)
Number of lines of prior therapy (any type)								
1	1 (33.3)	1 (33.3)	11 (31.4)	6 (66.7)	4 (40.0)	2 (20.0)	0	25 (31.6)
2	0	0	8 (22.9)	2 (22.2)	3 (30.0)	5 (50.0)	4 (44.4)	22 (27.8)
≥3	2 (66.7)	2 (66.7)	14 (40.0)	1 (11.1)	3 (30.0)	2 (20.0)	4 (44.4)	28 (35.4)
Missing	0	0	2 (5.7)	0	0	1 (10.0)	1 (11.1)	4 (5.1)
Type of prior therapy								

(*Continued*)

Table 2. (Continued).

	Number (%) of Patients						
	Part 1: 3 + 3 Dose Escalation			Part 2: Patient Expansion			
	Cohort 1 10^9 vg dose of LV305 (N = 3)	Cohort 2 10^{10} vg dose of LV305 (N = 3)	Arm A CMB305 expansion (N = 35)	Arm B SC admin of CMB305 (N = 9)	Arm C CMB305 + mCPA (N = 10)	Arm D CMB305 + GLA-SE (N = 10)	Arm E CMB305 escalation (N = 9)	Total (N = 79)
Radiotherapy	3 (100)	2 (66.7)	19 (54.3)	4 (44.4)	8 (80.0)	9 (90.0)	8 (88.9)	53 (67.1)
Immunotherapy	1 (33.3)	0	5 (14.3)	0	1 (10.0)	0	2 (22.2)	9 (11.4)
Chemotherapy	3 (100)	3 (100)	33 (94.3)	9 (100)	10 (100)	9 (90.0)	8 (88.9)	75 (94.9)
Other therapy	1 (33.3)	1 (33.3)	4 (11.4)	1 (11.1)	1 (10.0)	1 (10.0)	2 (22.2)	11 (13.9)

Abbreviations: ECOG = Eastern Cooperative Oncology Group; GLA-SE = glucopyranosyl lipid A-stable emulsion; mCPA = metronomic cyclophosphamide; MRCL = myxoid/round cell liposarcoma; NSCLC = non-small cell lung carcinoma; PD = progressive disease; SC = subcutaneous; SD = standard deviation; SS = synovial sarcoma; TNM = tumor node metastasis
[a]Patients with melanoma were eligible to participate, but no patients with melanoma enrolled in the study.
[b]No patients had a current TNM stage of 0, I, II, IIB, or IIIB.

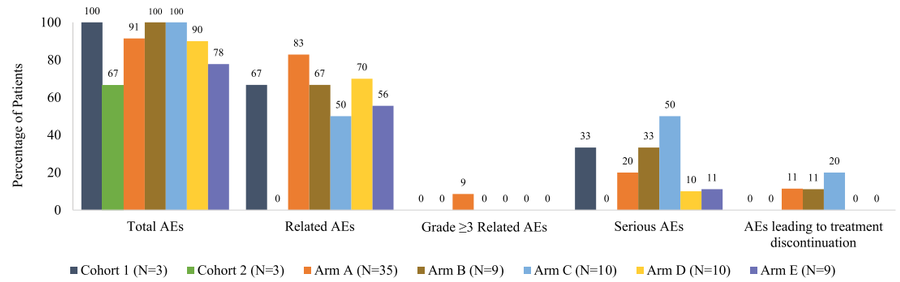

Figure 2. Summary of adverse events by study arm. Three patients experienced dose-limiting toxicities, but there were no AEs or safety concerns reported with these dose-limiting toxicities. Two patients experienced treatment-related serious AEs in Arm A (prostatic pain in a patient with metastatic synovial sarcoma, and pneumonitis in a patient with non-small cell lung carcinoma who had a previous history of pneumonitis); no other patients experienced serious AEs considered related to treatment. One patient in Arm A experienced an AE of acute respiratory failure not considered related to treatment that led to death. Abbreviation: AE = adverse event.

were reported for 3 patients (1 in Arm A and 2 in Arm B), but none prevented a patient from receiving further injections and there were no associated AEs or safety concerns reported with these DLTs. Four patients had medical events of interest: 1 patient had grade 3 vomiting considered unrelated to the study drug and 3 patients (2 in Arm A and 1 in Arm D) had non-serious events of overdose of study drug stemming from dispensing errors that did not result in any sequelae or change to dosing.

Depending on the availability of PBMC, LV305 persistence assay was performed in 51 (64.6%) patients, who all tested negative at 1 (25.3%), 2 (21.5%), or more (17.7%) timepoints tested. Twenty-eight (35.4%) patients had no LV305 persistence test performed due to death, withdrawal of consent, study termination, or unknown reasons.

Efficacy

In Part 1 of the study, the median OS was 19.2 (95% CI, 7.1–not available [NA]) and 23.7 (95% CI, 7.5–NA) months in Cohorts 1 and 2, respectively. In Part 2, the median OS was 28.9 months (95% CI, 13.5–33.8) for the 35 patients in Arm A and 18.4 months (95% CI, 6.9–NA) for the 9 patients in Arm B (Figure 3; Appendix Table 3). The median OS for Arms C, D, and E was not reached, with a 30-month OS rate of 50.0%, 100%, and 88.9% and a median duration of observation of 11.99, 20.42, and 9.23 months, respectively. Among patients with SS, MRCL, ovarian cancer, and NSCLC, the median OS was 26.2 (95% CI, 13.0–NA), 29.5 (95% CI, 22.1–NA), 30.3 (95% CI, 8.4–33.8), and 7.7 (95% CI, 1.2–13.5) months, respectively (Appendix Table 4; Appendix Figure 3). The median PFS in Part 1 was 14.0 months in Cohort 1 and 3.1 months in Cohort 2, and ranged from 2.0 (Arm C) to 3.7 months (Arm A) in Part 2 (Figure 3; Appendix Table 3). Patients with SS, MRCL, ovarian cancer, and NSCLC had a median PFS of 2.4 (95% CI, 2.1–5.6), 5.1 (95% CI, 2.6–7.2), 3.3 (95% CI, 1.8–3.9), and 2.3 (95% CI, 1.2–2.5), respectively. Among patients with STS, 6 patients with SS (2 in Cohort 1, 1 in Arm A, 1 in Arm B, and 2 in Arm C) remained progression-free for 12.0 to 30.4 months, and 2 patients with MRCL in Arm A remained progression-free for 23.0 and 35.1 months, respectively. In a subgroup analysis, patients with STS who had PD at screening but achieved stable disease during the study had a median PFS of 6.0 months (95% CI, 3.1–9.2).

In Part 2, disease control rates were 68.6% in Arm A, 33.3% in Arm B, 40.0% in Arm C, 90.0% in Arm D, and 66.7% in Arm E, with a total of 50 (63.3%) of patients on the study achieving irSD based on irRC. The disease control rate for patients with STS was 61.9% with a median DOR of 4.6 months (95% CI, 2.0–7.1), and 81.8% of patients with ovarian cancer and 50.0% of patients with NSCLC had irSD, with a median DOR of 1.4 (95% CI, 0.5–3.7) and 0.4 (95% CI, NA–NA) months, respectively (Appendix Table 4). No objective responses were observed. Time to next treatment and time to progression results are available in Appendix Tables 5 and 6.

Immune response

At baseline, evidence of preexisting NY-ESO-1 specific antibodies (sarcomas 28.3%, ovarian 45.5%, and NSCLC 33.3%) and T-cells (sarcomas 38.0%, ovarian 37.5%, and NSCLC 33.3%) were comparable across disease types (Appendix Figure 4). There was a weak positive correlation between NY-ESO-1 expression level (0–100%) and preexisting T-cells ($r = 0.3107$; $p = .0148$), but not preexisting NY-ESO-1 antibodies ($r = 0.1000$; $p = .3965$) (Appendix Figure 5). CMB305 induced antibody responses to NY-ESO-1 in 62.9% of patients and T-cell responses in 47.4%; a total of 22.8% of patients had both Figure 4. Appendix Figure 6 displays the time course of CD4 and CD8 T-cell and NY-ESO-1 antibody responses in a patient with an induced integrated response. No difference was observed by changing administration routes (ID LV305 and IM G305 in Arm A vs SC for both LV305 and G305 in Arm B), addition of oral mCPA (in Arm C), or addition of intratumoral GLA-SE injection (in Arm D).

This preliminary study was not powered to evaluate correlations between efficacy and immune outcomes. However, a signal indicating a potentially higher 1-year OS rate was observed in patients with SS treated with CMB305 alone when they also had preexisting NY-ESO-1 antibody (100% vs

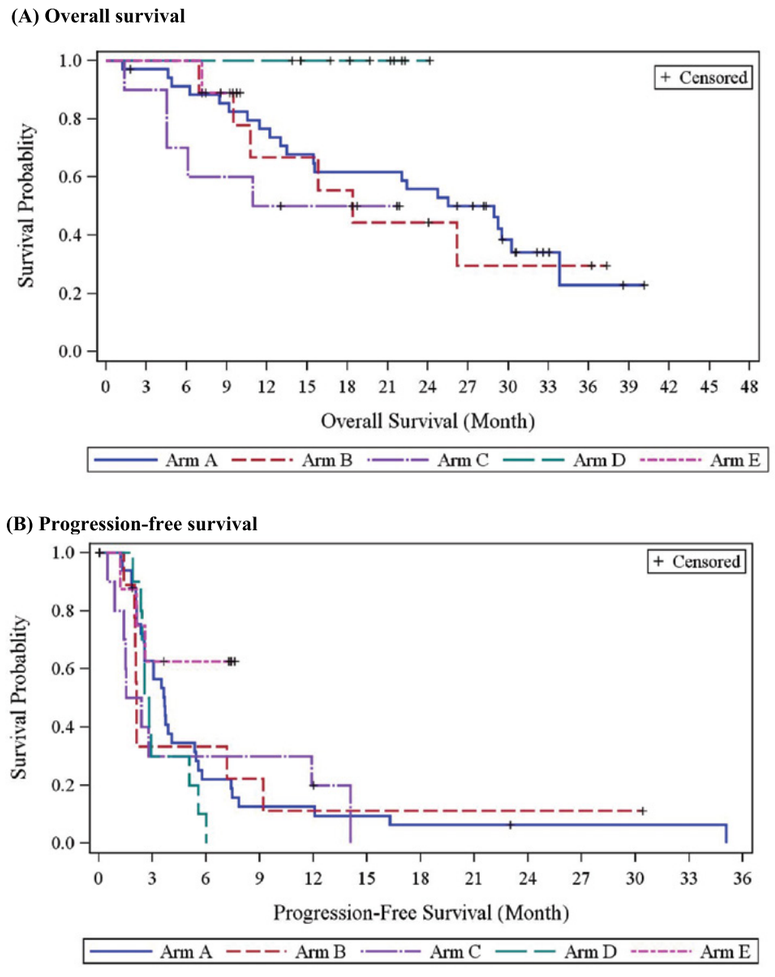

Figure 3. (a) Overall survival and (b) Progression-free survival by study arm.

69.2%, with a difference of 30.8%; 95% CI, 5.7–55.9; $p = .0162$), as well as those for whom T-cells were induced on ≥2 time points (100% vs 75.0%, with a difference of 25.0%; 95% CI, 0.5–49.5; $p = .0455$) or had an integrated response post-study treatment (100% vs 76.9%, with a difference of 23.1%; 95% CI, 0.2–46.0; $p = .0483$) (Appendix Figure 7).

Discussion

While prime-boost vaccines built around a lentiviral vector as the priming component have been evaluated in the context of infectious diseases such as HIV (human immunodeficiency virus),[32,33] to our knowledge, this phase 1b trial is the first report of a clinical study using this vaccination strategy in cancer. CMB305 treatment either alone or in combination with oral mCPA or intratumoral GLA-SE was well-tolerated in the dose-escalation phase and across tumor types in this trial, with the most common AEs being fatigue, nausea, injection-site pain, decreased appetite, and dyspnea. While 54 (68.4%) patients had AEs considered related to study treatment, most of these patients (51; 94.4%) had AEs that were only grade 1 or 2 in severity and transient. Three patients experienced protocol-defined DLTs, but they were not associated with other AEs or safety concerns and did not prevent resumption of study treatment. Overall, the safety profile of

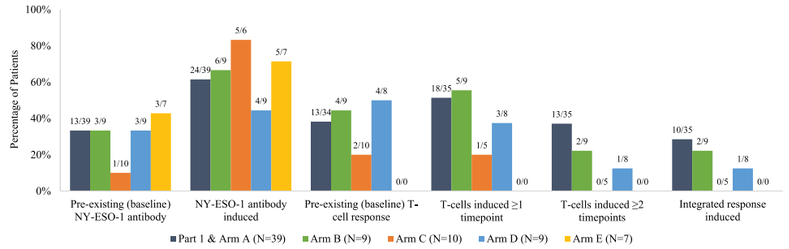

Figure 4. Immune response frequencies by study arm. Complete biomarker data were not available for all patients. The Ns for each study arm denote the total number of patients with biomarker data in that arm. Numerators and denominators are shown above each bar. Integrated response was defined as positive if both NY-ESO-1 antibody and T-cells (CD4 and CD8) were positive. T-cell analysis was not performed for patients in Arm E due to early study termination.

CMB305 appeared to be similar across treatment arms, with most patients in each study arm experiencing at least one mild to moderate adverse event. The CMB305 vaccine regimen was generally well tolerated in each arm, with expected toxicity profiles observed.

CMB305 demonstrated an ability to induce anti-NY-ESO-1 antibody and T-cell responses across treatment arms and disease types. Eighteen percent of patients experienced the induction of an integrated immune response, which has previously been linked to enhanced tumor control in melanoma patients treated with ipilimumab.[34] A signal indicating a potentially higher 1-year survival rate was observed in patients with SS treated with CMB305 alone who had preexisting NY-ESO-1 antibodies, T-cells induced at ≥2 time points, or an integrated response post-study treatment. Overall, there was no significant difference in OS between patients with and without induced NY-ESO-1 antibodies, T-cells, or an integrated immune response. In approximately half of the patients who had an induced T-cell response at the first evaluated timepoint, a response was not present at the second evaluated timepoint. These results may indicate that the induction of an immune response is not sufficient to produce durable tumor control in this population with advanced oncologic disease. The ability to interpret the efficacy data is limited by the small sample size and heterogeneity within the treatment arms and the lack of a controlled comparator group.

Previous cancer vaccines studies have had inconsistent outcomes regarding immune responses and have not led to tumor regressions, but prolonged survival has been noted.[35–39] In this study, the median OS of 26.2 and 29.5 months in patients with SS and MRCL, respectively, compares favorably with published data (OS of 11.7 to 13.5 months) for patients with advanced or metastatic STS in second-line and beyond.[40–43] In addition, a total of 51.5% of patients with SS and 74.1% of patients with MRCL experienced irSD on the study. The observed median PFS ranged from 2.0 to 3.7 months in Part 2, which is consistent with other published trials in this patient population (PFS of 1.5 to 4.6 months).[41–43] It is important to consider that evaluation of PFS in this study included clinical progression/symptomatic deterioration, which leads to shorter median PFS compared to later phase studies that include only radiological PD. Patients receiving the higher dose of LV305 in Arm E (4×10^{10} vg SC) had an OS rate of 88.9% and a PFS rate of 62.5% at the time the study was terminated, with the median OS and PFS not yet reached. The study termination and small number of patients prevent interpretation about the long-term benefit of the higher SC LV305 dose.

Several confounding factors must be considered when interpreting the clinical outcomes in this study. Patients had a relatively high level of disease burden overall, with 86.1% having metastatic disease at the start of the study. However, there was considerable heterogeneity both across and between treatment arms, with each arm having multiple tumor types, NY-ESO-1 expression levels, and types and lines of prior therapy. Combined with the small sample size and lack of a control arm, these factors limit the interpretation and generalizability of the clinical outcome findings for any specific disease type.

To enhance the clinical activity of vaccine-based approaches, strategies that combine the vaccine with checkpoint inhibitors or other immunomodulatory therapies to alleviate immunosuppression in the tumor microenvironment have been discussed.[36,39,44–46] In this study, administering the CMB305 vaccine in combination with intratumoral GLA-SE, a synthetic TLR4 agonist (Arm D), resulted in positive activity in patients with SS or MRCL. With a median follow-up of 20.4 months, 100% of the patients in Arm D were still alive at the time of study termination. Additionally, patients in Arm D achieved a disease control rate of 90.0% (95% CI, 56%–100%), even though 9 (90.0%) patients in Arm D patients had metastatic disease, 8 (80.0%) had PD at study entry, and 9 (90.0%) had prior chemotherapy, including 7 (70.0%) with ≥2 prior lines of chemotherapy. Further research is necessary to evaluate the clinical benefit of a "prime-pull" strategy combining treatments such as CMB305 with intratumoral GLA-SE in patients with advanced or metastatic SS or MRCL. While CMB305 administration with mCPA resulted in a less robust clinical response (disease control rate of 40.0%), the patients in this arm also had the lowest percentage of preexisting NY-ESO -1 antibodies and T-cells. Given that patients with preexisting NY-ESO-1 antibodies exhibited better 1-year survival rates, this may explain the reduced clinical activity demonstrated with the CMB305 and mCPA combination.

With limited treatment options and continued poor outcomes for patients with SS and MRCL, there has been growing interest in vaccine strategies to induce an immune response directed against these cancers.[36,45,47] Given that effective

therapies for patients with SS or MRCL remain inadequate despite ongoing research,[48–50] this study argues that novel vaccination strategies could potentially benefit patients with SS and MRCL and that further exploration is warranted.

Conclusions

In summary, administering a lentiviral vector as the priming component in a prime-boost vaccine regimen was feasible, safe, and well-tolerated in this Phase 1b trial of 79 patients with locally advanced, relapsed, or metastatic cancer expressing NY-ESO-1. The prime-boost regimen exhibited both clinical and immunogenic activity across study arms and disease types. This study will serve as a benchmark for future studies of vaccine trials using prime-boost regimens, as well as those using dendritic cell-targeted lentiviral agents.

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Disclosure of interest

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Section 5

Local Anesthesia

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RESEARCH ARTICLE

Intradermal injection of lidocaine with a microneedle device to provide rapid local anaesthesia for peripheral intravenous cannulation: A randomised open-label placebo-controlled clinical trial

Alexey Rzhevskiy[1]*, Andrei Popov[1], Chavdar Pavlov[2], Yuri Anissimov[3], Andrei Zvyagin[1], Yotam Levin[4], Efrat Kochba[4]

1 Center of Biomedical Engineering, Sechenov First Moscow State Medical University, Moscow, Russia, **2** Clinic of Internal Diseases Propedeutics, Sechenov First Moscow State Medical University, Moscow, Russian Federation, **3** School of Natural Sciences, Griffith University, Gold Coast, Queensland, Australia, **4** NanoPass Technologies, Nes Ziona, Israel

* rzhevskiy01@gmail.com

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Competing interests: The authors have read the journal's policy and have the following competing interests: EK and YL are paid employees of NanoPass Technologies Ltd. the developer of the MicronJet600 device. This does not alter our adherence to PLOS ONE policies on sharing data and materials. There are no patents, products in development or marketed products associated with this research to declare.

Abstract

Background

Peripheral venous cannulation is one of the most common procedures in medicine. It is associated with noticeable pain and apprehension, although in most cases it is performed without any anesthesia due to lack of a painless, cost-effective option, which would provide rapid local anesthesia with subsequent significant reduction in the experienced pain. We conducted an open-label placebo-controlled clinical trial to evaluate the efficacy and safety of a 2% lidocaine injection using the commercially available microneedle device Minron-Jet600 (NanoPass Technologies Ltd, Israel) to achieve rapid local anesthesia prior to peripheral venous cannulation.

Methods

One hundred and two subjects were randomly allocated into two groups. In the first group, 100μL of lidocaine hydrochloride (2%) was injected intradermally to subjects using the MicronJet600 device in the left arm (MJ-Lido) and 100μL of saline was injected intradermally using the device in the right arm (MJ-Saline). In the second group, 100μL of lidocaine hydrochloride (2%) was injected using the MicronJet600 device into the left arm (MJ-Lido), with no injection into the right arm of subjects (No pretreatment). In both groups the intradermal injection was performed at the cannulation site prior to insertion of a 18G cannula into a median cubital vein in both arms. As a primary variable, a score of cannulation-induced pain was indicated by subjects using a 100-point visual analog scale immediately after cannulation. As a secondary variable, subjects in Group 2 also indicated their preference to receive the anaesthetic injection with MicronJet600 in the future by using the 5-point Likert scale. Also, as a secondary variable, the duration of skin numbness after lidocaine injection was indicated by performing a superficial pin-prick with a 27G needle at 15, 30 and 45 minutes, at distances of 1, 2 and 3 centimeters from the injection site.

Results

A significant pain reduction (11.0-fold) was achieved due to the lidocaine injection compared to the cannulation without any pretreatment ($p < 0.005$). After the lidocaine injection the anesthesia was effective up to 2 centimeters from the injection site and remained for up to 30 minutes. Eighty percent of subjects from the second group preferred cannulation after the lidocaine injection over cannulation without any pretreatment. No significant side effects were identified.

Conclusion

Intradermal injection of anaesthetic with Micronjet600 was found to be a safe and effective option for providing rapid local anesthesia for peripheral intravenous cannulation.

Trial regiatration

The clinical trial was registered, before the patient enrollment began, in the Research Registry publicly accessible database (registration identifier: researchregistry4662). Also, the trial was registered in ClinicalTrials.gov (registration identifier: NCT05108714) after its completion.

Introduction

Intravenous cannulation is a common painful procedure which is, however, usually performed without local anaesthesia [1]. The simplest approach involving injection of a local anaesthetic into the skin using a regular needle is, in itself, painful therefore several techniques were previously tested for reducing pain in intravenous cannulation, with each having specific limitations which reduce convenience [2–7]. Intravenous cannulation requires local anaesthesia, which simultaneously provides an immediate effect, cost-effectiveness and simplicity, with a minimum of discomfort to the patient [8].

The use of hollow microneedles is currently one of the most promising techniques for providing local anaesthesia in superficial interventions involving skin and subcutaneous adipose tissue, in particular for peripheral venous cannulation [9]. To date, several commercially available, microneedle-based devices can be found on the market. Among them is the hollow microneedles based device, MicronJet600 (MJ600) by NanoPass Technologies Ltd, Israel, which was approved by regulatory authorities in many territories, including the United States and the European Union. MicronJet600 was primarily investigated as a device for nearly-painless [10] intradermal injection of vaccines [10–18]. The device is also considered promising for use in other intradermal applications [19], including intradermal injection of anaesthetics.

To test the efficacy of MicronJet600 to provide rapid local anaesthesia for peripheral intravenous cannulation via intradermal injection of micro-amounts of anaesthetic, with a subsequent decrease of the intervention-related pain score as a primary variable, an open-label placebo-controlled clinical trial was conducted. To assess safety of the intervention, potential side effects were estimated. Further, preference of cannulation, preceded by the intradermal

injection of anaesthetic, over the cannulation without any pretreatment, duration and area of skin numbness after the lidocaine injection, were assessed as the secondary variables.

Materials and methods

The trial was registered prior to patient enrollment in the Research Registry publicly accessible database (registration identifier: researchregistry4662, principal investigator: Chavdar Pavlov, date of registration: 29 January 2019, URL: https://www.researchregistry.com/browse-the-registry#home/registrationdetails/5c4d811ac413740862094f0f/). Also, the trail was registered in ClinicalTrials.gov (registration identifier: NCT05108714) after its completion. The authors confirm that all ongoing and related trials for this drug/intervention are registered. The study received ethical approval from the Local Ethics Committee of First Moscow State Medical University (Extract from Minutes No. 07–17 of the Local Ethics Committee meeting of 13.09.2017) and written informed consent was obtained from all subjects participating in the trial. The study was conducted in accordance with the Declaration of Helsinki (2013) protocol and CONSORT (Consolidated Standards of Reporting Trials) (Fig 1). Study subjects, healthy volunteers and patients at University's Clinical Hospital 2 (Moscow, Russia) were enrolled between January 29th, 2019 and March 15th, 2019. The recruitment ended after the number of enrolled participants exceeded the designated sample size for each group.

Study design

A single center, open-label placebo-controlled clinical trial to evaluate the efficacy and safety of 2% lidocaine injection, using the commercially available microneedle device MinronJet600 (NanoPass Technologies Ltd, Israel), to achieve rapid local anesthesia prior to peripheral venous cannulation.

Study objectives

The primary objective was to evaluate, in terms of VAS score, the efficacy of intradermal administration of low doses of lidocaine 2% solution using MicronJet600, to reduce the pain associated with peripheral venous catheter insertion. Secondary objectives included identification of potential side effects from intradermal administration of lidocaine with the

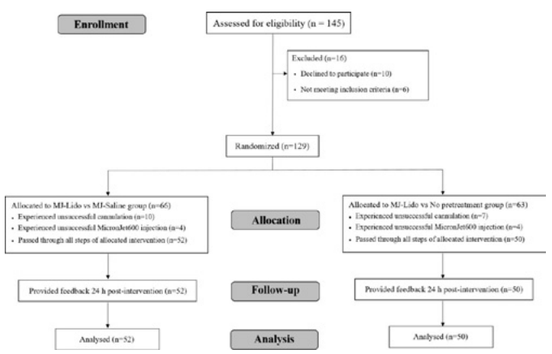

Fig 1. Consolidated standards for reporting of trials diagram.

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MicronJet600 and assessment of the area and duration of skin numbness by performing a gentle superficial pinprick with 27 G hypodermic disposable needle, at various distances from the injection site, at various time points. Subjects' preference for local anaesthetic injection with MicronJet600 prior to future cannulations was also assessed.

Participants

One hundred and two healthy volunteers were pre-screened for eligibility. Inclusion criteria included any gender, age between 18–65 years, and absence of all exclusion criteria. The main exclusion criteria included pregnancy or breast feeding, evidence of allergy to lidocaine, presence of pain of any localization and character not associated with the study, or treatment with any analgesics, any local tissue damage at the site of intervention, and serious systemic diseases. After being considered eligible for the study and signing the informed consent form, the subjects were randomly allocated into two groups by the first observer AR (Observer1). Simple randomization was performed to allocate subjects into two groups using the Microsoft Excel random number generator. The subjects who were allocated random even numbers were assigned to the first study group (MJ-Lido vs MJ-Saline) while the subjects who were allocated random odd numbers were assigned to the second study group (MJ-Lido vs No pretreatment).

Intervention

Prior to the intervention, each subject had his or her median cubital vein identified by palpitation of the cubital fossa area by a nurse, to determine the site for intravenous cannulation. Further, the cannulation site was wiped with ethanol swabs. Each subject from the MJ-Lido vs MJ-Saline group received an injection of 100 μL of 2% lidocaine hydrochloride injectable solution (Biokhimik, Russia) into the left arm at the cannulation site and an injection of 100 μL of saline solution (Biokhimik, Russia) placebo into the right arm at the corresponding site. Each injection was immediately (t = 0) followed by cannulation with an 18 G peripheral venous catheter. In the second group MJ-Lido vs No pretreatment, each subject received the injection of 100 μL of 2% lidocaine into the left arm at the cannulation site which was followed by the cannulation with an 18 G catheter, while the right arm of each subject was cannulated with an 18 G catheter without any pretreatment. Thus, each subject was his or her own control. This trial design was chosen to identify the presence or absence of a placebo-related effect.

The injections of both lidocaine and placebo were performed with MicronJet600 (Fig 2(A)) placed on a 1 mL syringe (Fig 2(B)), prefilled with a 27G needle. The injection procedure lasted approximately 4 seconds with the a flow rate of approximately 25 μl/sec. The intradermal

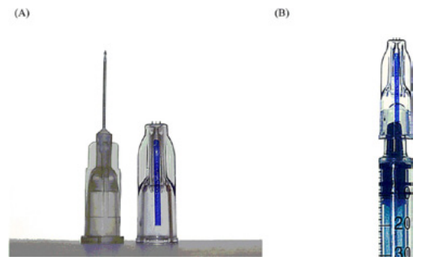

Fig 2. MicronJet600 compared to the 27 G hypodermic needle (A), and MicronJet600 placed on 1 mL syringe (B).

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injection with MicronJet600 was considered successful if a bleb, of approximately 10–15 mm in width and 3–6 mm in height, was formed at the site of injection. A cannulation was considered successful when a small amount of blood was present in the cannula's hub following the cannula insertion. Each cannulation was performed by moving the cannula only forward when inserting into the vein. In case of unsuccessful insertion, any attempt at reinsertion was prohibited. Immediately after the insertion, the cannula was removed and the site of cannula insertion was then wiped with ethanol swabs and covered with an adhesive bandage. All injections and cannulations were performed by the same highly-qualified staff nurse of the University's Clinical Hospital 2, who had previously undergone training on the proper use of MicronJet600 based on the training materials provided by NanoPass Technologies Ltd, Israel.

After each cannulation, as a primary endpoint variable, the subjects scored the pain experienced using a 100-point visual analog scale (VAS), ranging from no pain (0) to unbearable pain (100) [20], presented by the second observer AP (Observer2), and the scores were recorded. The pain experienced by subjects due to cannulation in each of the cases (following lidocaine injection, placebo injection, or without pretreatment) was also evaluated in terms of the VAS-score. Thus, in the context of the current study, VAS-score = 0 was considered as a lack of pain, VAS-score$\leq$10 as a mild pain score, VAS-score$\leq$20 as an acceptable pain score, VAS-score$>$20 as an unacceptable pain score. s a secondary endpoint variable, the duration of skin numbness due to lidocaine injection was assessed by performing a gentle superficial pinprick with a 27 G hypodermic disposable needle, perpendicularly to the arm at the distance of 1, 2 and 3 cm from the injection site in the distal direction at 15 (t = 15), 30 (t = 30) and 45 (t = 45) minutes after the injection. The pinpricks were performed by the Observer1. For each subject, a single 27 G hypodermic needle was used at each time point, and the needle disposed of after the procedure. The pain experienced due to the pinpricks was also assessed, by the subjects, in accordance with the provided 100-point VAS scale and recorded by Observer2. After the cannulations were performed in both arms of the subjects in the MJ-Lido vs No pretreatment group, the subjects were asked whether they would prefer to receive an anaesthetic injection with MicronJet600 prior to cannulations in future. After the cannulations were performed in both arms of the Group 1 subjects (MJ-Lido vs MJ-Saline), the subjects were asked whether they would prefer to receive anaesthetic injection with MicronJet600 prior to the cannulations in the future. The preference assessment was performed with the 5-point Likert scale where 1 was defined as strong disagreement, 2 as disagreement, 3 as lack of any preference, 4 as agreement and 5 as strong agreement. To assess possible side effects of the intervention, the cannulation site was examined for evidence of swelling, edema, hematoma, or hemorrhage at 60 minutes after the procedure. Further, the subjects were contacted by phone, 24 hours after the injection, and asked about any evidence of study-related adverse events. A general study scheme is presented in Fig 3.

Statistical analysis

Regression modeling and results visualization were performed using R (version 3.6.3) environment for statistical computing (R Foundation for Statistical Computing, Vienna, Austria) and third-party packages lme 4 1.1–21, clubSandwich 0.4.1 and emmeans 1.4.8 available on the CRAN repository. Linear mixed effects models (implemented in the lme4 1.1–21 package) were used to model VAS scores after interventions: assuming random intercepts, random slope for repeated measurements (corresponding to coefficients for 30 min and 45 min) for each study participant, and measurement time (15, 30 and 45 min)–distance (1, 2 and 3 cm) interaction. For all models Sandwich cluster-robust variance-covariance matrix estimators (implemented in the clubSandwich 0.4.1 package) were used to address heteroskedasticity, the

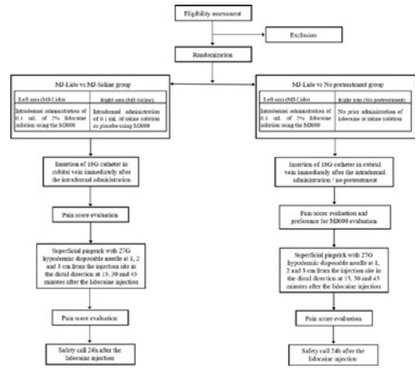

Fig 3. General study scheme.

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Satterthwaite method was used to approximate degrees of freedom and the Tukey method was used to adjust p-values obtained from pairwise comparisons. Cohen's d was used as a standardized effect size estimate.

Sample size

A sample size of 40 subjects per group was calculated to detect an effect size of (expected difference on the VAS score between two time points at a specific distance) 1 with standard deviation in the effect of 2.2, using a paired t-test with 80% power and 5% type I error rate assuming a two-sided significance testing procedure. At the same time, an additional 22 subjects (102 subjects in total) were enrolled in order to compensate for dropouts.

Results

One hundred and twenty-nine subjects gave informed consent and were enrolled in the study; 66 subjects were allocated into Group 1 (MJ-Lido vs MJ Saline) and 63 into Group 2 (MJ-Lido vs No pretreatment). Ten subjects from Group 1 and 7 subjects from Group 2 had at least one unsuccessful cannulation; these subjects were excluded from the study. Four subjects from the Group 1 and 6 subjects from Group 2 were also excluded from the study due to unsuccessful injections with MicronJet600 at the first attempt. In these cases, owing to deviations in the technique for the injection, insufficient penetration of the microneedles into the skin led to a major leakage of the injected solution onto the skin (10 out of 186 injections, 5.4%, resulted in major leakage). Thus, data from 52 subjects from MJ-Lido vs MJ Saline (Group 1) and 50 subjects from MJ-Lido vs No pretreatment (Group 2) were analyzed (Table 1).

The results from the linear mixed effects model of VAS score after the cannulation are presented in S1 Table. According to the results (Fig 4), the mean pain score of the cannulation was 3.6 (95% CI from 2.6 to 4.6) for the MJ-Lido, 41.5 (95% CI from 38.2 to 44.8) for the MJ-Saline and 39.7 (95% CI from 35.7 to 43.7) in the absence of pretreatment. The pain reduction effect caused by intradermal administration of 100 μL of 2% lidocaine compared with both saline injection and no pretreatment was statistically significant ($p < 0.0001$) with corresponding Cohen's d estimates -4.5 (95% CI from -4.9 to -4.2) and -4.3 (95% CI from -4.8 to

Table 1. Baseline demographic characteristics.

Characteristics	Treatment group	
	MJ-Lido vs MJ-Saline (N = 52)	MJ-Lido vs No pretreatment (N = 50)
Sex—no (%)		
Male	35 (67%)	29 (58%)
Female	17 (33%)	21 (42%)
Age–years (±)		
Min	18	18
Max	59	63
Mean	28.6 (±11.3)	30.2 (±13.6)
Median	24.5	28.4
Body Mass Index (BMI)		
Mean (SD)	24.8 (±3.6)	25.3 (±3.1)
Range	18.8–34.3	17.4–31.7

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-3.9). Also, no placebo-related effect was determined (Cohen's d = 0.2, 95% CI from -0.4 to 0.8, p = 0.8).

The distribution of cases between four VAS-score groups (VAS-score = 0, VAS-score≤10, VAS-score≤20 and VAS-score>20) was estimated in percentage for the scenarios with the lidocaine (n = 102) or placebo injection (n = 52) prior to cannulation, or without any pretreatment (n = 50). Thus, the distribution of cases in the scenario when lidocaine injection preceded the cannulation was 54.9%, 95.1%, 100% and 0% for VAS-score = 0, VAS-score≤10,

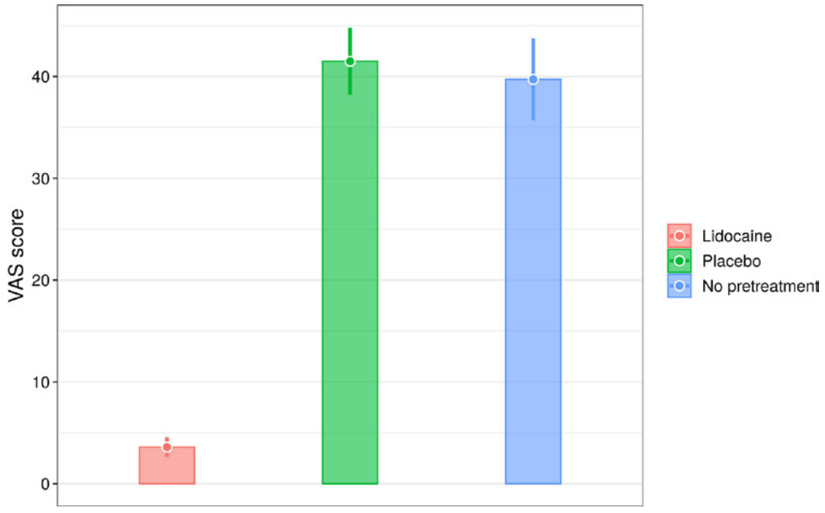

Fig 4. Mean estimates of VAS scores with corresponding 95% confidence intervals for pain experienced by the subjects after cannulations preceded by the lidocaine injection (MJ-Lido vs MJ-Saline and MJ-Lido vs No pretreatment groups (red bar), saline injection (MJ-Lido vs MJ-Saline group, green bar), or performed without any pretreatment (MJ-Lido vs No pretreatment group, blue bar).

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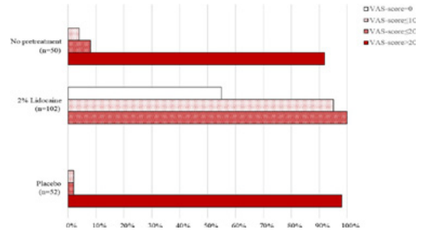

Fig 5. The bar chart shows the distribution of subjects between the groups ranked by VAS-score for the cases of the injection of 100 μL of 2% lidocaine or placebo prior to cannulation, or cannulation without any pretreatment. Thus, the non-shaded bar, slightly shaded bar, moderately shaded bar and entirely shaded red bar represents the percentage of subjects related to the groups: VAS-score = 0, VAS-score≤10, VAS-score≤20, VAS-score>20, respectively.

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VAS-score≤20 and VAS-score>20, respectively; the distribution of cases in the scenario when placebo injection preceded the cannulation was 0%, 1.9%, 0% and 98.1% for VAS-score = 0, VAS-score≤10, VAS-score≤20 and VAS-score>20, respectively; the distribution of cases in the scenario when cannulation was performed without any pretreatment was 0%, 4%, 8% and 92% for VAS-score = 0, VAS-score≤10, VAS-score≤20 and VAS-score>20, respectively (Fig 5).

The results from the linear mixed effects model of VAS score after the cannulation are presented in S2 Table. The dependence of skin numbness after the intradermal lidocaine injection was determined to be statistically significant for both predictors: distance of the pinprick from the injection site with 27G needle, time after the injection and their interaction ($p<0.0001$). As expected, skin numbness was significantly higher at t = 15 and the distance of 1 cm with the mean VAS of 4.5 (95% CI from 3.5 to 5.5) as compared to other time points and distances: 9.0 (95% CI from 8.1 to 10.0), 10.9 (95% CI from 9.7 to 12.0), 11.0 (95% CI from 9.8 to 12.2), 12.4 (95% CI from 11.0 to 13.8), 12.2 (95% CI from 10.9 to 13.6), 11.7 (95% CI from 10.6 to 12.7), 12.3 (95% CI from11.0 to 13.5), 12.1 (95% CI from 11.1 to 13.2) for t = 15 and 2cm, t = 15 and 3cm, t = 30 and 1 cm, t = 30 and 2cm, t = 30 and 3cm, t = 45 and 1cm, t = 45 and 2cm, t = 45 and 3cm, respectively (Table 2). Fig 6 depicts the alteration of the average pain scores at three time points in relation to the distance from the injection site. At the end of the study (t = 60), no subjects indicated a feeling of skin numbness at the injection site.

Adverse events of lidocaine injection with MicronJet600 were visually assessed right after the injection (t = 0) and at the end of the study (t = 60min); Thus, at t = 0, there were no adverse events indicated. A bleb (wheal) of 10–15 mm in length and 3–6 mm in height was formed in all subjects immediately after the injection, which is considered a sign of successful intradermal injection. At t = 60 min, a slight erythema of the injection site was noticeable in

Table 2. Mean estimates with corresponding 95% confidence intervals for VAS pain score due to pin-pricks with a 27G needle at three time points at 15, 30 and 45 minutes after the lidocaine injection with MicronJet600, and distances at 1, 2 and 3 centimeters from the injection site.

Time (min)	Distance (cm)		
	1	2	3
15	4.5 (95% CI: 3.5–5.5)	9.0 (95% CI: 8.1–10.0)	10.9 (95% CI: 9.7–12.0)
30	11.0 (95% CI: 9.8–12.2)	12.4 (95% CI: 11.0–13.8)	12.2 (95% CI: 10.9–13.6)
45	11.7 (95% CI: 10.6–12.7)	12.3 (95% CI: 11.0–13.5)	12.1 (95% CI: 11.1–13.2)

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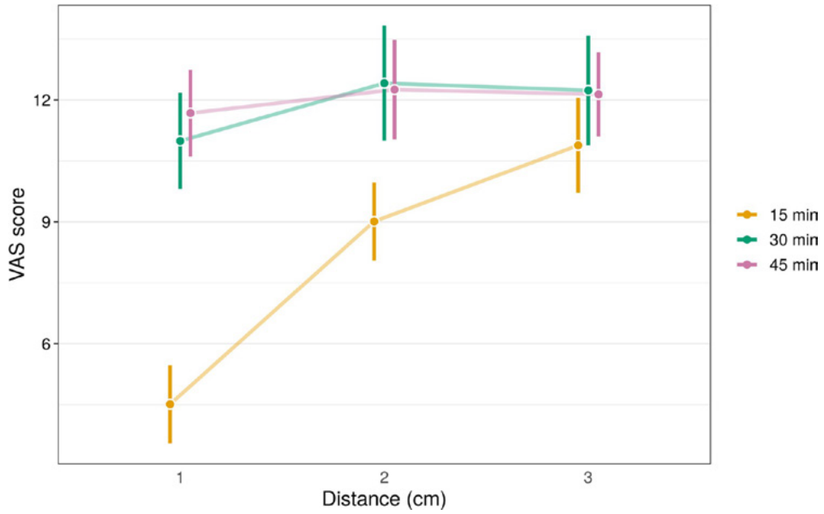

Fig 6. The multi-line chart demonstrates mean estimates of VAS scores with 95% confidence intervals for pain experienced by subjects due to the superficial pin-pricks with 27 G needle for three different time points at 15, 30 and 45 minutes after the lidocaine injection with MicronJet600, and at 1, 2 and 3 centimeters from the injection site.

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subjects with pale skin. Further, 24 hours after the study, there were no reports of erythema or swelling at the site of lidocaine injection and cannulation. At the same time, 9 subjects (8.8% in total from Group1 and Group2), 5 subjects (9.6%) and 4 subjects (8%) complained of local hematoma around the cannulation site for the scenarios when cannulation was performed after the lidocaine injection, after placebo injection, or without any pretreatment, respectively. However, local hematoma is considered a common adverse event of the cannulation itself and there was no evidence for correlation between the injections with MicronJet600 prior to cannulation and an increased prevalence of hematomas.

Discussion

Currently, there is an unmet need in clinical practice in which common painful procedures, including intravenous cannulation, are performed without proper or any anaesthesia. This can cause pain, anxiety and discomfort to patients. In this study, the efficacy and safety of intradermal administration of anaesthetic with MicronJet600 to provide local anaesthesia for peripheral intravenous cannulation was tested. According to the results of this open-label placebo controlled clinical trial, the intradermal injection of 100 μL of 2% lidocaine with MicronJet600 significantly decreased the pain score experienced by subjects due to insertion of 18 G cannula into a median cubital vein. The difference between pain scores experienced due to intravenous cannulation with and without local anaesthesia provided by the lidocaine injection substantially exceeded the average clinically significant difference of 9–13 on the 100-point VAS [21, 22]. Further, there were no statistically significant differences between the average VAS-scores due to cannulations after placebo injection and without any pretreatment, which demonstrates the absence of a placebo-related effect. Moreover, intradermal injection of 2% lidocaine with

MicronJet600 provided local anaesthesia for 15–30 minutes, and therefore can be effectively used in diverse cases of mid-term surgical intervention involving skin and subcutaneous fat. No significant adverse events from the intervention were identified.

At the same time, this study has several limitations such as the number of subjects per group; time points for pain score measurement after the intravenous cannulation; volume and concentration of the anaesthetic and type of anaesthetic. Also blinding could has been performed more rigorously, although it is difficult to blind (disguise) the use of the MicronJet600 device; the intraindividual comparisons are confounded with the application side, which has an unclear effect on the study result; since there is no evidence regarding the difference of pain sensitivity between arms in population, arms were not randomised. Further, it is worth mentioning that the comparison within Group2 is confounded with the MicronJet600 use. In addition, the study was not powered for adverse events. Finally, the study did not involve a control group whereby subjects would receive regular intradermal injection of lidocaine with a hypodermic needle.

Although no direct comparison was made between local anaesthesia with the MicronJet600 and its most competitive alternative, Jet injectors, it is anticipated that the use of MicronJet600 is more effective. In a clinical trial by Lysakowski, Dumont, Tramer, Tassoniy [23] the effectiveness of local anaesthesia with intradermal jet injection of lidocaine with J-Tip (National Medical Products Inc, CA, USA) was investigated; the average pain scores, experienced by subjects following a 18-G cannula insertion into a vein on the dorsal part of the arm and measured with 10-point Numerical Verbal Scale (NVS), were: 3.9, 4.2 and 1.7 for the scenarios of cannulation without pretreatment, cannulation after the injection of 500 μL of saline and cannulation after the injection of 500 μL of 2% lidocaine, respectively. Consequently, the average pain scores for cannulation with no pretreatment, and cannulation with the preliminary intradermal injection of the placebo, were comparable between the current study and the study by Lysakowski, Dumont, Tramer, Tassoniy [23]. Thus, the average 100-point VAS pain scores versus 10-point NVS were: 39.7 vs 3.9 for cannulation without any pretreatment, and 41.5 vs 4.2 for cannulation after the placebo injection. The reduction in the average pain score of the cannulation by intradermal administration of 2% lidocaine, however, was substantially higher in case of the MicronJet600 intradermal administration. It resulted in an 11.0-fold reduction (from 39.7 to 3.6) in VAS pain score, compared to the jet injection intradermal administration which resulted in only 2.3-fold reduction from 3.9 to 1.7 in NVS pain score. Moreover, in the current study, a significantly lower amount (100 μL) of 2% lidocaine was administered in comparison with the study by Lysakowski, Dumont, Tramer, Tassonyi (500 μL) [23] which illustrates further the greater effectiveness of MicronJet600 as a tool for providing intradermal administration of anaesthetics to achieve rapid local anaesthesia over the jet injection method.

The adverse events of cannula insertion after the lidocaine injection with MicronJet600 were insignificant. The only obvious sign of the injection was the formation of a bleb, which is considered a sign for successful intradermal injection. Additionally, as the intradermal injection of only a small amount (100 μL) of 2% lidocaine with MicronJet600 was sufficient to achieve the substantial reduction of pain, the technique is considered safe in terms of prevention of serious complications if the injection was accidentally performed in a subject with lidocaine hypersensitivity.

Conclusions

Overall, intradermal administration of low doses of lidocaine 2% solution with MicronJet600 is effective in reducing the pain associated with a peripheral venous catheter insertion procedure, providing a sufficient rate of local anaesthesia immediately post-injection. No significant

adverse events were associated with the intervention, which signifies its high safety. Further, 80% of subjects from the MJ-Lido vs No pretreatment group preferred cannulation after the lidocaine injection over the cannulation without any pretreatment.

Supporting information

S1 Checklist.
(DOC)

S1 Table. The results of the linear mixed effects model of VAS score after the cannulation.
(DOCX)

S2 Table. The results from the linear mixed effects model of skin numbness after the lidocaine injection.
(DOCX)

S1 File.
(PDF)

S2 File.
(PDF)

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Author Contributions

Conceptualization: Alexey Rzhevskiy, Yotam Levin, Efrat Kochba.

Investigation: Alexey Rzhevskiy, Chavdar Pavlov, Yuri Anissimov.

Methodology: Alexey Rzhevskiy, Efrat Kochba.

Project administration: Alexey Rzhevskiy, Chavdar Pavlov.

Resources: Chavdar Pavlov, Yotam Levin.

Software: Alexey Rzhevskiy, Yuri Anissimov.

Supervision: Yotam Levin, Efrat Kochba.

Validation: Efrat Kochba.

Visualization: Efrat Kochba.

Writing – original draft: Alexey Rzhevskiy, Andrei Popov, Chavdar Pavlov, Yuri Anissimov, Yotam Levin, Efrat Kochba.

Writing – review & editing: Alexey Rzhevskiy, Andrei Zvyagin, Yotam Levin, Efrat Kochba.

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Section 6

Aesthetic Applications

MicronJet™ demonstrated superior performance in reducing skin wrinkles, improving elasticity, and increasing hydration compared to classic needles.

MicronJet™ enables accurate intradermal delivery, ensuring the product remains in the dermis without unnecessary diffusion, leading to better efficacy.

MicronJet™ improved patient comfort and Safety, showed significantly less pain and fewer adverse effects, such as erythema and bruising, making it a safer and more comfortable option.

Patients showed quicker improvements in skin radiance, hydration, and wrinkle reduction, with noticeable effects as early as three weeks post-treatment.

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LETTERS TO THE EDITOR

Facial rejuvenation using a microneedle-based device with a revitalizing solution and free hyaluronic acid

To the Editor,

Treating delicate areas for facial rejuvenation is always a challenge as the procedure must be effective but not too aggressive because of side effects as pain. Here, we illustrated the preliminary results on the performance and tolerability of a protocol that combines an innovative device with a commercially available revitalizing solution in a real-life setting. This was a multicenter study, carried out by a panel of eight independent clinicians with different specialties in esthetic medicine.

Skin aging can largely be attributed to dermal fibroblast dysfunction and decrease in their biosynthetic activity. Injection treatments are the most performed procedures in the cosmetic dermatology practice.[1] Accurate injection is fundamental for optimum results, and therefore, standardized procedures would be beneficial.[2] The innovative technology (Fillmed Nanosoft^{tm}) is designed to inject the product with standardized intradermal delivery allowing reliability and accuracy of injections, reduced pain and minimal bruising, mainly in delicate areas. It is characterized by 3 Silicone Pyramid-shapes micro-needles (0.6 mm) and can be adapted to all syringes. A blue line determines the correct device orientation, which should be placed at 45° angle with respect to the skin (Figure S1). In our protocol, the device is combined with a poly-revitalizing solution (NCTF® 135HA) of 59 ingredients and free non-cross-linked hyaluronic acid with a well-known efficacy and safety profile.[2,3] After the injection, the development of papules, that last no longer than 24 h, is an indication of the correct positioning of the product into the dermis (Figures S2 and S3).

Clinicians retrospectively collected data on their patients, aged between 35 and 50 years, who were treated for the first time with the solution using the Nanosoft^{tm} device. Previous injections of the product with a standard needle were allowed. The treatment must envisage fullface including upper/lower eyelids. Each patient was handled with the same needle and one vial of product for the entire treatment and underwent three sessions every 30 days and a final evaluation after 90 days. We analyzed patient's profile at baseline, post-treatment changes, and side effects. Methods were detailed in the Appendix S1.

Overall, 33 records were collected and summarized in Table S1.

Figure S4 illustrates the magnitude of the post-treatment changes. Approximately two-thirds of subjects showed a remarkable/truly remarkable improvement in wrinkles and degree of elastosis (S4-A). The effect of the intervention on lower (S4-B) and upper (S4-C) eyelids was comparable (p = 0.177) and clearly perceivable in the short term (Figures 1 and 2). Patients and clinicians substantially agreed on the effect of the protocol on the overall skin quality: Almost 70% of them simultaneously declared to observe a remarkable/truly remarkable (64%) or moderate (6%) improvement. When in disagreement, the patient's perception of the intervention (S4-D) was significantly better compared to the clinician's judgment (S4-E) (p = 0.037).

The complications included moderate edema (n = 3) and/or erythema (n = 11); pain experience was observed in only 3 patients and was correlated to the product but not to the injection device. Almost all the side effects were transient and resolved within few hours (1 erythema and 2 pain experience), a day (2 edema, 10 erythema, and 1 pain experience), or 2 days (1 edema).

All patients declared being satisfied, and almost all of them (97% = 32/33) were willing to continue the treatment.

In conclusion, our preliminary results are encouraging in supporting the use of microneedle-based devices as patient-centered technology in the everyday clinical practice. The combined protocol is effective and safe for treating facial wrinkles and delicate areas like eyelids, and its performance seems to be independent from the skin patient's profile. Larger and controlled studies are necessary to provide the best evidence.

KEYWORDS

esthetic medicine, microneedles, nanosoft

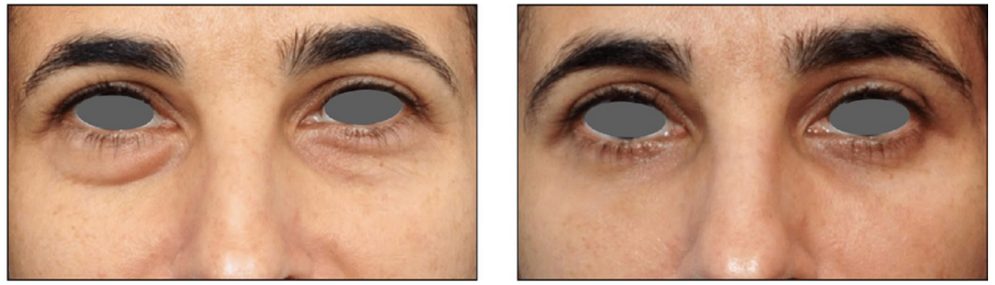

FIGURE 1 Before and after photographs at baseline (left) and at 90 days (right) showing eyelids wrinkle severity in a representative subject who underwent three monthly intradermal injections using an innovative microneedles medical device

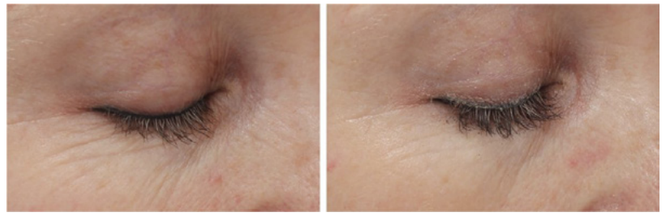

FIGURE 2 Before and after photographs at baseline (left) and at 90 days (right) showing eyelids wrinkle severity in a representative subject who underwent three monthly intradermal injections using an innovative microneedles medical device

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All listed authors have provided a significant contribution in the study by participating in design and conduct, data entering, data analysis, patient enrollment and assessment, and manuscript preparation.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

R.D. designed the research study, analyzed the data, and wrote the paper. R.D., A.G., E.V., M.B., F.M., L.L, A.R., and M.V. performed the research. All authors have read and approved the final manuscript.

ETHICAL APPROVAL

It's a multicentric study from different cities and doctors in Italy. The retrospective data analysis requires no ethical commitment.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

Roberto Dell'Avanzato MD[1]
Alessandro Gualdi MD[2]
Elena Vescovi MD[3]
Matteo Basso MD[4]
Francesco Marchetti MD[5]
Luciano Lanfranchi MD[6,7]
Alessio Redaelli MD[8]
Massimo Vitale MD[9]

[1]*Director of Aesthetic & Regerative Medicine Department - La Madonnina Clinic, Milan, Italy*
[2]*Università Vita-Salute San Raffaele, Milan, Italy*
[3]*Private Practice, Vasto, Chieti, Italy*
[4]*Private Practice, Rome, Italy*
[5]*Private Practice, Rome and Milan, Italy*
[6]*Palestro Medical Center, Milan, Italy*
[7]*Department of Plastic and Reconstructive Surgery, Centro Diagnostico Italiano, Milan, Italy*
[8]*Private practice at Visconti di Modrone Medical Centre, Milan, Italy*
[9]*Private Practice, Bologna, Italy*

Correspondence
Roberto Dell'Avanzato, Aesthetic Medicine Department at L'Albereta Relais & Chateaux – Erbusco, Brescia, Italy.
Email: info@dellavanzatoroberto.it

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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ORIGINAL ARTICLE OPEN ACCESS

Evaluation of the Performance and Safety of a New Micro-Needle Technology in Comparison With the Classic Needle on the Antiaging Effects of a Biorevitalizing Solution: A Randomized Split Face/Neck Study

Andreea Boca[1] | Ferial Fanian[2] | Riekie Smit[3] | Alessio Redaelli[4] | Ranesha Goorochurn[5] | Hanane Issa[2] | Natalia Sukmanskaya[6] | Valérie Philippon[7] | Roberto Dell' Avanzato[8]

[1]Private Clinic, Cluj, Romania | [2]Scientific Department, FILLMED Laboratories, Paris, France | [3]Private Clinic, Pertoria, South Africa | [4]Genova University, Milan, Italy | [5]Dermo Dev Partners, Paris, France | [6]FILLMED Laboratories, Paris, France | [7]Private Clinic, Paris, France | [8]Private Clinic, Milan, Italy

Correspondence: Ferial Fanian (fanian@gmail.com)

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Keywords: HA | micro-needle | multiple intradermal injection | new technology | skin aging | skin biorevitalization

ABSTRACT

Background: Skin biorevitalization involves multiple intradermal injections to enhance skin quality, but precise dermal targeting can be challenging due to variations in skin thickness smaller, less painful needles with fewer skin reactions are attractive options.

Aims: This study evaluates a new Micro-Needle device's performance and safety in comparison with the classic needle used in skin biorevitalization.

Patients/Methods: Subjects with facial and neck skin aging were enrolled. Safety outcomes, including immediate and local tolerability, were assessed. Performance outcomes measured skin radiance, wrinkles and photoaging grade, hydration, subepidermal low echogenic band, dermis thickness, and skin elasticity. Both subjects and investigators recorded Global Aesthetic Improvement Scale scores.

Results: Micro-Needle injections demonstrated superior performance compared to the classic needle, influenced by the specific skin zones and thickness. Micro-Needle was superior for skin wrinkles at D49 for periorbital zone and nasolabial folds by −14.5% ($p = 0.01$) and −15% ($p = 0.004$), respectively, and for neck by 9.6% ($p = 0.0008$). The Nanosoft device showed a faster improvement for skin hydration at D42 for the cheek zone ($p = 0.04$) and at D75 for the neck area ($p = 0.01$); and for skin radiance at D75 ($p = 0.03$) and at D120 ($p = 0.0098$). Ex vivo studies confirmed the Micro-Needle's accuracy in product placement in the dermis. Adverse events were milder with Micro-Needle and no serious adverse events occurred.

Conclusions: Both needles significantly improved skin quality, but Micro-Needle enhanced the outcomes of skin biorevitalization procedures, particularly in terms of skin wrinkle reduction, elasticity, and overall skin hydration.

1 | Introduction

The aging perception by patients is the most frequent reason for a medical consultation. Both intrinsic and extrinsic factors, participate in the aging process of the skin. In addition, photo-exposed areas, such as the face or hands, are subject to the cumulative effect of both chronological aging and environmental factors [1, 2]. Face aging of the superficial plane (skin) and that of the support structures (fat, muscles, and bones) are distinguished. At the level of the skin a reduction in cell renewal, dehydration, loss of radiance, elasticity, firmness, and the appearance of fine lines and wrinkles are observed. Structural aging (deep planes) causes atrophy of the bone structures and melting of the fats associated with a displacement of the latter toward the bottom of the face (gravity). This results in sagging skin in the middle and lower third of the face (cheeks, oval), accompanied by wrinkles and furrows increasingly marked.

Nonsurgical cosmetic solutions have enabled to offer simple and efficient treatments to patients wishing to slow down facial aging. However, as the barrier properties of skin limit the transport of molecules, various chemical and physical permeation enhancement techniques have been deployed for the delivery of active molecules through skin [3–6]. Among the most recent administration techniques and attractive treatment option, the application of microneedle-based devices is a favorable drug administration approach for skin rejuvenation [7, 8].

In this clinical trial, we use NCTF 135HA (FILLMED Laboratories, Paris, France) as a skin rejuvenation strategy thanks to its effects previously demonstrated on tissue filling of fine lines with hyaluronic acid and restructuring of the extracellular matrix by maintaining the hydration of the skin and its biochemical and biological architecture [9, 10]. NCTF 135HA is an antiaging biorevitalization solution containing 59 nutritive ingredients and 5 mg/mL of non–cross-linked hyaluronic acid.

The administration of NCTF 135HA product, mesotherapy product, with the indication of biorevitalization is defined as a minimally invasive cosmetic medical treatment, which involves the intradermal injection directly in the zone to treat of active substances, to achieve the best efficiency. In order to penetrate the epidermis and upper dermal layer of the skin, Micro-Needle devices can be presented either in a single needle—with length range of 4–6 mm for Mesoneedle and 1–2 mm for Mesogun—or an array of micron-sized needles—with length range of 0.25–2 for Needle Pen and 0.5–1.5 mm for DermaRoller.

However, the longer needles are the more adverse effects can be expected such as erythema, edema, hyperpigmentation, and scarring on the skin [8]. The literature showed that more studies are required to assess the safety profile of biorevitalization to manage and minimize the risk of potential adverse reactions, mostly for isolated cases [11, 12].

Therefore, in this study, our purpose was to determine the performance and safety of a new Micro-Needle device based on vaccine delivery clinically approved system (Nanopass Technologies) [3, 13] in the aim to better manage the risk of adverse effects linked to intradermal injections.

2 | Materials and Methods

2.1 | Subject Selection

The inclusion criteria were the male or female subjects older than 19 years old with a Fitzpatrick phototype of I to IV and a photoaging grade of 2 or 3 on Glogau scale; a Lemperle wrinkle score of 2–4 for periorbital lines and a Bazin neck wrinkle score of 2–4. Female subject accepted to do a pregnancy test. The noninclusion criteria included the participants with any allergy to the study product, history of dermal fillers during last 1 year, history of keloid scars, facial herpes, autoimmune disease, coagulation disorders, any acute inflammation/infection or any other medication, condition or disease which may interfere the results by investigator decision.

2.2 | Objectives

The main objective was to demonstrate objectively the difference between the efficacy and safety of hyaluronic acid-based solution injected bilaterally and randomly on the face and neck treated with Micro-Needle technology (Nanosoft, Micro-Needle, Nanopass Technologies Ltd., Israel) versus the other side treated with classic needle from D0 to D75 (30 days after the third and last treatment).

2.3 | Study Design

The study adapted with the declaration the Helsinki with the authorization of the local ethical committee of Medical University of "Iuliu Haţieganu" under registration number 2/1101.2019. All participants signed a written consent form, accepting not modifying their lifestyle and avoiding the sun exposure during the whole study. This study is a randomized, comparative, prospective monocenter study for 120 days.

The protocol consisted of three injection sessions, 3 weeks apart at Day 0, D21, D42 and three follow-up sessions at D49 (7 days after the last injection session), D75 and D120.

The physician disinfected the treated area with chlorhexidine and injected the biorevitalizing solution (NCTF 135HA) which was prepared in a 3 mL sterile syringe through a 32G × 4 mm classic needle on one side or through a Micro-Needle (Nanosoft) for the other side in a randomized way. The injection volume for each zone was as follows: 2 vials of 3 mL for whole face (3 mL per side) and 1 vial of 3 mL for the neck (1.5 mL per side). The treatment based on the multiple intradermal injections on whole face and neck spaced every 1–1.5 cm with a quantity of 0.05 mL on each point to produce a visible papula.

2.4 | Investigated Products

2.4.1 | Micro-Needle Nanosoft

A latex-free CE marked Micro-Needle technology with three silicon-based needles of 0.6 mm (Figure 1). The Micro-Needle enables to control intradermal delivery in any procedure which requires administration of substances to the dermal compartment. This device was used previously for vaccination and recently is introduced for the first time in aesthetic indications by FILLMED Laboratories.

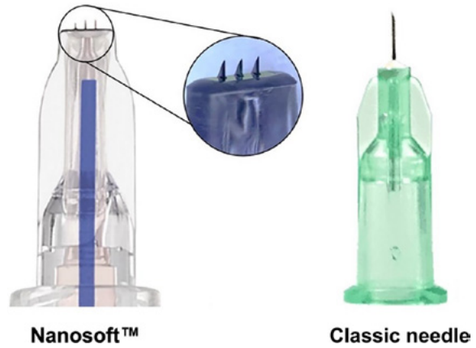

FIGURE 1 | The injector devices, Nanosoft with three silicon needles of 0.6 mm and classic needle, 32 gage with 4 mm length.

2.4.2 | Classic Needle

We used a 32-gage 4-mm (32G × 3/16″) needle, TSK Laboratory, Japan, EMERGO EUROPE (Figure 1).

2.4.3 | Biorevitalizing Solution

NCTF 135HA (FILLMED Laboratories, France) is a 3-mL vial containing 5 mg/mL of non–cross-linked sodium hyaluronate and a polyrevitalizing solution (described in Table 1).

2.5 | Pre-Clinical Evaluation of Nanosoft Versus Classic Needle

In an ex vivo study performed internally with a colored NCTF 135HA, both Nanosoft and 32G × 4 mm classic needle were evaluated for their capability to produce a papula in the dermis and the duration of its persistence. After the injection the explant was cut at 2, 6, 8, and 24 h (Figure 2). The results show that regardless of the injection method, the papules became flattened till 24 h. However, a visible diffusion of the colored injected product in the hypodermis is observed for the classic needle

TABLE 1 | Complete ingredients of NCTF135HA.

Compound class	Components
Vitamins total: 12	Ascorbic acid (vit. C), biotin (vit. B8), pantothenic acid (vit. B5), folic acid (vit. B9), inositol (vit. I), nicotinamide (vit. B3), pyridoxine (vit. B6), riboflavin (vit. B2), thiamine (vit. B1), tocopherol (vit. E), retinol (vit. A), vit. B12
Minerals total: 6	Calcium chloride, potassium chloride, magnesium sulfate, sodium acetate, sodium chloride, sodium dihydrogenophosphate
Nucleosides total: 5	Deoxyadenosine, deoxycytidine, deoxyguanosine, deoxythymidine, 5-methyl-2′-deoxycytidine
Amino acids total: 24	α-Aminobutyric acid, alanine, arginine, asparagine, aspartic acid, cystine, glutamine, glutamic acid, glycine, histidine, hydroxyproline, isoleucine, leucine, lysine, methionine, ornithine, phenylalanine, proline, serine, taurine, threonine, tryptophane, tyrosine, valine
Coenzymes total: 6	TPP (Cocarboxylase), CoA (coenzyme A), FAD (flavine adenine dinucleotide), NAD (nicotinamide adenine dinucleotide), NADP (nicotinamide adenine dinucleotide phosphate), UTP (uridine triphosphate)
Other compounds total: 6	Glutathione, polysorbate 80, glucuronic acid, glucuronic acid lactone, glucosamine, dextrose anhydrous

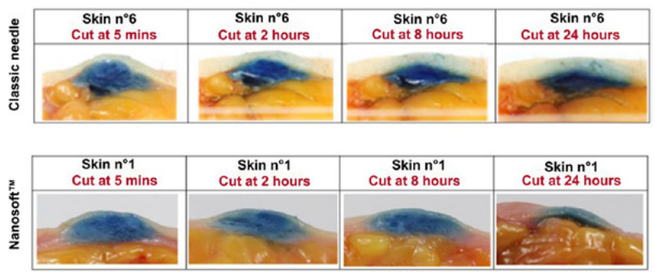

FIGURE 2 | Monitoring over time of papules formed by injection of colored NCTF 135 HA, by Nanosoft and by a 32-gage 4 mm classic needle. The skin was cut immediately, 2, 8, and 24 h after the injection.

TABLE 2 | Summary of evaluation methods.

Parameters	Scoring system	Zone(s)	Scales
Skin radiance	Skin radiance clinical scoring	Face	0: Very dull skin 1: Dull skin, lacking radiance 2: Slightly radiant skin 3: Radiant skin 4: Very radiant skin
Skin wrinkles	Lemperle score [14]	Nasolabial periorbital cheek	0: No wrinkle 1: Very shallow, still visible wrinkle 2: Shallow wrinkles 3: Moderately deep wrinkles 4: Deep wrinkles, well defined edges 5: Very deep wrinkles, redundant folds
	Bazin photographic visual score [15]	Neck	0: No wrinkle 1: Very shallow, still visible wrinkle 2: Shallow wrinkles 3: Slight wrinkles 4: Mild wrinkles 5: Deep wrinkles 6: Very deep wrinkles
Global photoaging	Glogau scale [16]	Face	I: Mild—no wrinkles, early photoaging II: Moderate—wrinkles in motion, early to moderate photoaging III: Advanced—wrinkles at rest, advanced photoaging IV: Severe—only wrinkles, severe photoaging

Parameters	Technique/device	Assessed area	Values
Skin hydration	Moisturemeter EpidD, Delfin Technology	–Face (cheeks) –Neck	Hydration level of epidermis (percentage)
Subepidermal Low Echogenic Band (SLEB) [17]	High Frequency Ultrasound imaging (DermaLab, Cortex Technology)	–Face (cheeks) –Neck	SLEB index in μm
Dermis thickness	High Frequency Ultrasound imaging (DermaLab, Cortex Technology)	–Face (periorbital zone and cheeks) –Neck	Thickness in μm
Skin Elasticity	DermaLab, Cortex Technology	–Face (periorbital zone and cheeks) –Neck	Overall elasticity (VE index) in MPa/mms

while the product remained intact in the dermis with no diffusion in the hypodermis for the skin injected by the Nanosoft (Figure 2). This diffusion could waste the product form its main target which is the superficial and deep dermis.

2.6 | Evaluation Methods

2.6.1 | Antiaging Performance Measures

The clinical assessment was performed by a visual scoring system regarding the skin radiance, skin wrinkles in different zones (face and neck), and the photoaging Glogau Scale (Table 2).

The instrumental assessment was carried out using various techniques detailed in Table 2.

The satisfaction rate was assessed by seven grades Global Aesthetic Improvement Scale (GAIS) from −3 to +3 evaluated by the investigators and also the subjects with following description (very much improved [+3], much improved [+2], improved [+1], no change [0], worse [−1], much worse [−2], and very much worse [−3]).

2.6.2 | Safety

Safety analysis includes all subjects who received at least one injection session with one of the devices under study. Immediate tolerance was assessed by measuring the pain based on an analog 10 grades visual scale from 0 for no pain to 10 for very intense pain. All local adverse events associated with the injection were recorded as well. They were scored by the investigator at each visit based on a 0–3 scale from absent to very severe from the first injection until the end of the study. These expected local adverse events are including erythema, ecchymosis, hematoma, edema, dyschromia, nodule/papule, and pruritus. In parallel, the patients recorded any local or systemic reactions or disorders on a daily log which was evaluated in each time point by investigator and recorded them in the CRF.

2.7 | Statistical Methodology

The main criterion for this study is based on 5-point scale clinical scoring (either Lemperle clinical scoring for face or Bazin clinical scoring for neck). For these scales, a decrease of at least one point demonstrates an apparent aesthetic evolution which indicated the success rate. Assuming a standard deviation of the after-before differences equal to 2, then 35 patients per group are required to have a 90% chance of detecting a difference between means of 1.0 with a significance level (alpha) of 5% (calculated with the sample size for paired *t*-test [one-tailed]). As this study is a split face/neck study, the minimum number is 35 subjects. Considering the drop off rate of patients, a total 40 subjects were included. Statistical analysis performed with Statistica Version 12, Graphpad Instat and Excel 2016. Descriptive statistics are provided for each parameter (i.e., number of observations, mean, standard-deviation [SD], minimum, maximum, median, 95% confidence interval).

The repartition of the sample size is provided for the evolution of the clinical scores in the different classes of scores under the form of n/percentage. Analyses is performed per area and per treatment.

The statistical significance of the evolution of the score between D0 and other time points was checked by a paired-series Student's *t*-test or its nonparametric equivalent and the superiority of the treatment with Micro-Needle was tested by comparing evolution observed with both treatments (one-sided Student's *t*-test or Wilcoxon test on the deltas *D0-Dx*).

3 | Results

3.1 | Study Population

Forty healthy subjects between 32 and 69 years old (mean age: 46.9 years) were enrolled in the study in Cluj, Romania including 5 male and 35 female volunteers. Twenty-one patients were included in March 2019 and 20 patients were included in August 2020; one subject voluntarily stopped the study just after one injection. Analyses were thus performed on population per protocol (PP): $N = 40$ subjects. Among them, only six had previously received aesthetic treatments (hyaluronic acid/fillers, botulinum toxin or biorevitalization) with an acceptable delay according to the noninclusion criteria.

3.2 | Skin Radiance

A significant improvement of skin radiance score was observed as early as 3 weeks after only one injection (D21). This result remains significant for all time points versus baseline till D120 for both face sides and devices ($p < 0.0001$ for all time points) (Table 3 and Figure 3). The difference between two devices is statistically significant at D75 ($p = 0.03$) and at D120 ($p = 0.0098$) in favor of Nanosoft.

3.3 | Photoaging Assessments

The data revealed a significant improvement of Glogau photoaging score after 4 months of treatment (D120) compared to the baseline (D0) for both treated sides ($p < 0.0001$ for all) (Table 3). This positive evolution allows a global photoaging improvement no matter the treatment options with no significant difference between both modes of injection. This observation is due to the direct effect of the biorevitalizing solution (NCTF 135HA) and not due to the injector.

3.4 | Skin Wrinkles

A significant improvement of skin wrinkles score was obtained for face on cheeks, periorbital area and nasolabial fold as well as on neck for all time points: at D49 (7 days after 3 injections), at D75 (1 month after 3 injections) and at D120 (2.5 months after 3 injections) versus baseline, for both face sides ($p < 0.0001$ for all) (Table 3). A statistical difference in favor of Micro-Needle was reported for every time point and for all examined sites, except at D49 for the cheek area which still show a tendency difference in favor of Micro-Needle ($p = 0.054$). The peak decrease of skin wrinkles score was obtained on the cheek at D49 compared to baseline, by 53.8% for classic needle versus 64.3% for microneedle side. The percentage of the evolution for other zones at the same time point (D49) was reported as: 52% versus 37.5% for periorbital zone ($p = 0.02$), 50% versus 35% for nasolabial fold ($p = 0.01$) and 42.9% versus 33.3% for neck wrinkles ($p = 0.0002$) (Table 3). Furthermore, the difference between the percentage of skin wrinkles with Micro-Needle and classic needle showed that the highest diminution score difference was in favor of Micro-Needle technique, for the periorbital by −14.5% ($p = 0.01$) (Figure 4), for nasolabial folds by −15% ($p = 0.004$) (Figure 5) and for neck by 9.6% ($p = 0.0008$) (Figure 6 and Table 3).

3.5 | Skin Quality

The assessment of high-frequency ultrasound and skin elasticity by DermaLab (Cortex Technology, Denmark) shows a significant improvement of dermis thickness and the overall elasticity versus baseline for both treated sides.

3.5.1 | Cheeks

The cheek area also revealed a raise of the dermis thickness at D49 with Micro-Needle technology ($p < 0.02$). Regarding skin elasticity, the side treated with Micro-Needle device showed an improvement of the overall elasticity (mean VE index) for this zone with an increase of 34% at D120 compared to the baseline ($p = 0.006$) while the evolution on classic needle was nonsignificant (Figure 7 and Table S1).

TABLE 3 | Clinical scoring of different time points for Micro-Needle or classic needle.

	Classic needle			Micro-Needle			
***n*=40**	**Mean**	**SD**	***p***	**Mean**	**SD**	***p***	**Δ*p***
Skin radiance grade							
D0	1.6	0.6	—	1.6	0.6	—	—
D21	3.0	0.6	<0.0001	3.0	0.6	<0.0001	ns
D42	3.4	0.5	<0.0001	3.5	0.5	<0.0001	ns
D49	3.5	0.6	<0.0001	3.5	0.6	<0.0001	ns
D75	3.3	0.6	<0.0001	3.4	0.6	<0.0001	0.0363
D120	3.0	0.3	<0.0001	3.1	0.5	<0.0001	0.0098
Glogau score							
D0	2.60	0.46	—	2.60	0.50	—	—
D120	2.20	0.60	<0.0001	2.10	0.50	<0.0001	ns
Skin wrinkles score							
Cheek zone							
D0	1.3	0.6	—	1.4	0.6	—	—
D49	0.6	0.5	<0.0001	0.5	0.5	<0.0001	ns
D75	0.7	0.6	<0.0001	0.6	0.5	<0.0001	0.0205
D120	0.8	0.6	<0.0001	0.7	0.5	<0.0001	0.0205
Periorbital zone							
D0	2.4	0.7	—	2.5	0.8	—	—
D49	1.5	1.2	<0.0001	1.2	1.1	<0.0001	0.0181
D75	1.7	1.0	<0.0001	1.5	1.1	<0.0001	0.0212
D120	1.8	1.0	<0.0001	1.5	1.0	<0.0001	0.0004
Nasolabial folds							
D0	2.0	0.9	—	2.0	1.0	—	—
D49	1.3	0.9	<0.0001	1.0	0.9	<0.0001	0.0041
D75	1.4	1.0	<0.0001	1.2	0.9	<0.0001	0.0178
D120	1.6	0.9	<0.0001	1.4	0.9	<0.0001	0.0286
Neck zone							
D0	2.7	0.7	—	2.8	0.9	—	—
D49	1.8	0.9	<0.0001	1.6	0.9	<0.0001	0.0008
D75	2.0	0.8	<0.0001	1.8	1.0	<0.0001	0.0002
D120	2.0	0.8	<0.0001	1.9	0.8	<0.0001	0.0017

***n*=40**	**Classic needle**			**Micro-Needle**		
	Δmean (%)			**Δmean (%)**		
	D49	**D75**	**D120**	**D49**	**D75**	**D120**
Cheek	−53.8	−46.2	−38.5	−64.3	−57.1	−50.0
Periorbital	−37.5	−30.0	−40.0	−52.0	−40.0	−40.0
Nasolabial	−35.0	−30.0	−20.0	−50.0	−40.0	−30.0
Neck	−33.3	−25.9	−25.9	−42.9	−35.7	−32.1

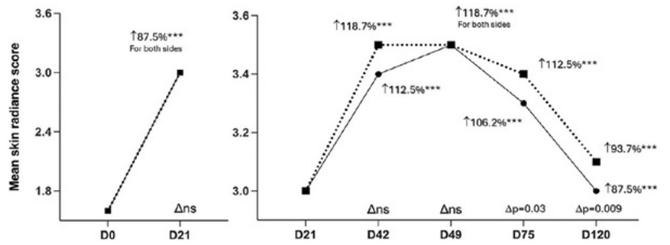

FIGURE 3 | Mean skin radiance score from very dull skin (Grade 0) to very radiant skin (Grade 4). Significance value indicate ***$p<0.001$.

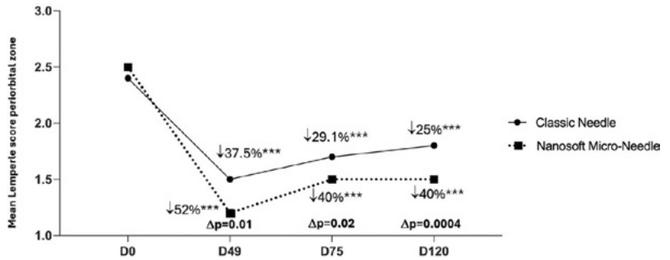

FIGURE 4 | Mean Lemperle score on periorbital zone on different time points. Significance value indicate ***$p<0.001$.

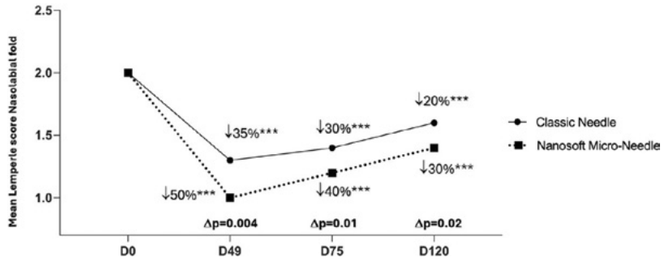

FIGURE 5 | Mean Lemperle score on nasolabial fold on different time points. Significance value indicate ***$p<0.001$.

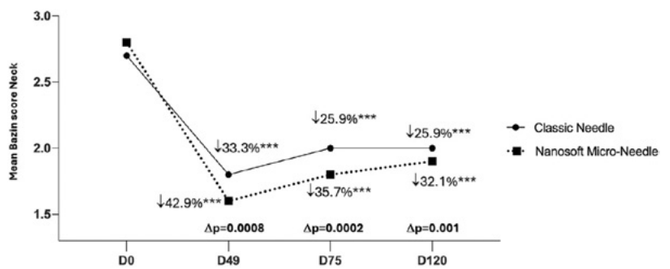

FIGURE 6 | Mean Bazin score on neck on different time points. Significance value indicate ***$p<0.001$.

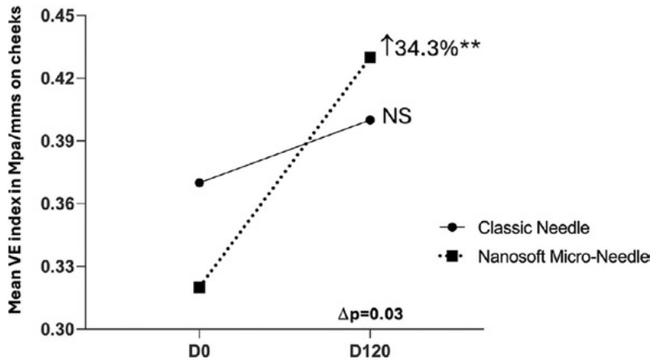

FIGURE 7 | Mean overall elasticity by VE index (in MPa/mms) assessed on the cheek. Significance value indicate $^{**}p<0.01$.

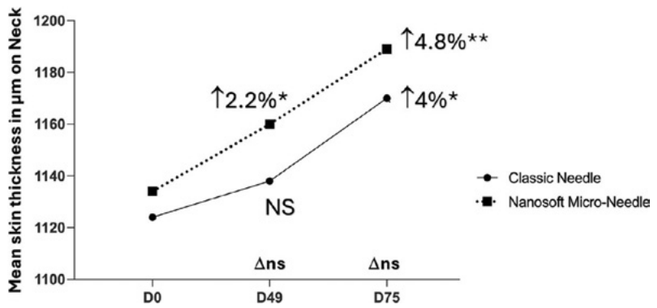

FIGURE 8 | Mean dermis thickness in µm assessed on the neck zone on different time points. Significance value indicates $^{*}p<0.05$, $^{**}p<0.01$.

3.5.2 | Periorbital Area

The data obtained for the overall elasticity indicated a significant difference of evolution between treatments in favor to Micro-Needle technology for periorbital zone after 4 months of treatment at D120 ($p<0.05$) (Table S1).

3.5.3 | Neck

The neck zone presented a significant increase of the dermis thickness only for the side treated with the Micro-Needle, 7 days and 30 days after the third and last treatment ($p<0.05$ and $p<0.002$, respectively); whereas the side treated by classic needle showed a slight increase of the dermis thickness only at 30 days after the third and last treatment ($p<0.05$) (Figure 8 and Table S1).

3.6 | Skin Hydration

The face skin hydration level measured by MoistureMeter EpiD (Delfin technology, Finland) on cheeks was significantly improved on the Nanosoft side for all time points from D21 (3 weeks after only one injection session) until D75 (1 month after 3 injections) (D21 $p=0.02$, D42 $p=0.01$, D49 $p<0.0001$, D75 $p=0.008$) while the results are significant only 7 days after 3 injections (D49) for classic needle ($p=0.0002$) (Table 4). Regarding the neck area, the side treated with Nanosoft showed a significant improvement of hydration compared to baseline at D21 (56.0 ± 4.1 vs. 53.5 ± 5.1 at D0; $p=0.01$) and D49 (57.6 ± 6.4 vs. 53.5 ± 5.1 at D0; $p<0.006$). However, the neck side treated with a classic needle only showed a better epidermal hydration level at D49 (55.4 ± 5.8 vs. 52.4 ± 4.6 at D0; $p=0.03$) (Table 4). The difference between two devices is statistically significant for Nanosoft at D42 for the cheek zone ($p=0.04$) (Figure 9) and at D75 for the neck area ($p=0.01$).

3.7 | High-Frequency Ultrasound Imaging

Moreover, at baseline (prior to injections), ultrasound showed the presence of SLEB (Subepidermal Low Echogenic Band) in all subjects, which is a reliable marker for skin photoaging grade (Figure 10).

3.7.1 | Regarding the Face Skin

The thickness measurements of SLEB highlighted few significant changes compared to baseline for both treated sides.

TABLE 4 | Biometrological parameters measured at all time points for Micro-Needle or classic needle.

	Classic needle			Micro-Needle			
***n*=19**	**Mean**	**SD**	***p***	**Mean**	**SD**	***p***	**Δ*p***
Deep hydration level of epidermis index							
Cheek zone							
D0	44.7	7.9	—	45.0	8.1	—	—
D21	48.0	9.0	ns	50.7	9.7	0.0218	ns
D42	45.9	8.0	ns	50.6	5.8	0.0135	0.0453
D49	53.5	8.1	0.0002	54.9	11.5	<0.0001	ns
D75	48.7	9.5	ns	51.7	11.8	0.0080	ns
D120	47.7	8.0	ns	47.9	9.1	ns	ns
Neck zone							
D0	52.4	4.6	—	53.5	5.1	—	—
D21	54.1	4.3	ns	56.0	4.1	0.0128	ns
D42	51.1	7.8	ns	52.3	6.6	ns	ns
D49	55.4	5.8	0.0329	57.6	6.4	0.0058	ns
D75	51.6	7.7	ns	55.3	6.5	ns	0.0166
D120	51.6	6.9	ns	52.2	6.8	ns	ns
SLEB index							
Cheek zone							
D0	46.0	89.0	—	56.0	104.0	—	—
D49	53.0	101.0	ns	49.0	94.0	ns	ns
D75	51.0	109.0	ns	60.0	119.0	ns	ns
D120	45.0	88.0	ns	47.0	107.0	ns	ns
Periorbital zone							
D0	151.6	127.5	—	130.7	118.8	—	—
D49	108.0	117.4	ns	110.5	134.6	ns	ns
D75	108.7	123.5	0.0427	111.2	113.2	ns	ns
D120	103.4	131	0.0499	116.6	120.3	ns	ns
Neck zone							
D0	70.7	86.2	—	81.3	93.7	—	—
D49	70.2	92.7	ns	53.0	73.2	0.0356	0.0191
D75	84.3	107.1	ns	63.3	89.1	ns	ns
D120	62.6	84.0	ns	69.2	84.7	ns	ns

Pain during injection score

	Classic needle			Micro-Needle		
***n*=40**	**Mean**	**SD**	**Median**	**Mean**	**SD**	**Median**
Face zone						
D0	5.3	2.1	5.5	3.2	1.7	2.8
D21	5.1	2.2	5.1	3.3	2.3	2.4

(Continues)

TABLE 4 | (Continued)

Pain during injection score						
	Classic needle			Micro-Needle		
***n*=40**	**Mean**	**SD**	**Median**	**Mean**	**SD**	**Median**
D42	4.5	2.5	4.8	3.0	1.9	2.5
Neck zone						
D0	4.4	2.3	4.2	2.7	2.1	2.4
D21	4.8	2.4	4.9	3.0	2.3	2.3
D42	4.0	2.3	3.5	2.6	2.0	2.4

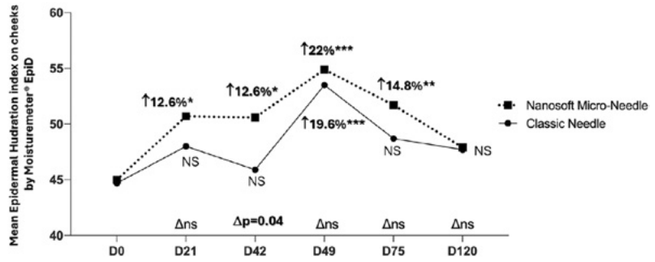

FIGURE 9 | Mean epidermal hydration level (in percentage water content PWC %) measured by MoistureMeter EpiD assessed on the cheek. Significance value indicates *$p<0.05$, **$p<0.01$, ***$p<0.001$.

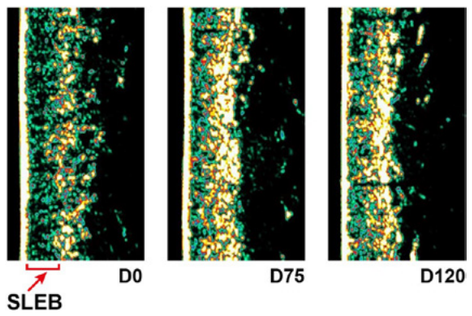

FIGURE 10 | Visible decrease on SLEB from baseline to D75 and D120.

3.7.2 | Regarding the Neck Skin

A significant diminution of SLEB thickness was obtained for Micro-Needle treatment at D49 ($p<0.04$) (Table 4). In addition, there is a significant difference of evolution between treatments in favor to Micro-Needle technology ($p<0.02$).

3.8 | Global Aesthetic Improvement Assessment

The satisfaction rate was evaluated by investigator GAIS (IGAIS) and also by subject GAIS (SGAIS) on two skin zones: neck and face (Figure S1.1 and S1.2).

3.8.1 | IGAIS

The investigator reported an excellent satisfaction rate for the face zone with 100% of improvement on both sides of the face at D42, D75, and D120. For the neck area, the same improvement rate on both sides were obtained at D42, D49, and D75.

3.8.2 | SGAIS

The patients observed an excellent improvement rate with a slight difference according to the mode of injections: 98% for the face zone from D49 to D120 with both classic needle and Micro-needle, while with Micro-Needle, the same percentage of improvement was achieved also at D42 (only after two injection sessions), which show that the results started sooner.

4 | Safety and Tolerance

No serious adverse events have been reported during the study. Eighty-one adverse events were reported by only 17% of the total population. Most of them were relating to expected adverse events that occurred frequently after dermal injections: erythema ($N=17/21\%$), burning sensations ($N=13/16\%$), irregularities on palpation ($N=10/13\%$), ecchymosis ($N=8/10\%$), pruritus ($N=2/3\%$) and pain ($N=1/1\%$). They did not last more than 11 days (mean duration of 3.1 ± 2.8 days with Classic needle and 2.4 ± 2.1 days with Micro-needle). Pain reported during injection were lower with the Micro-Needle than with the classic needle (about −2 on both mean and median values) for the face and the neck zone (Figure 11 and Table 4). Only one AE was not related to injection (COVID suspicion for one subject who stopped the study after the injection at D0).

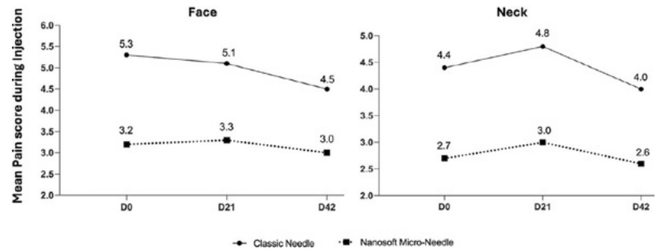

FIGURE 11 | Pain during injection assessed on the face and the neck assessed after each injection session.

5 | Discussion

In this study, various measures were performed to re-validate the performance of NCTF 135 HA and to evaluate any difference between the two injection modes: Classic needle versus Micro-Needle. Antiaging biorevitalization can be indicated for tired or lack of radiance skin with intense dehydration [18], and the study data showed a significant improvement in radiance and wrinkles as early as 30 days after the last injection on both injected sides.

As discussed in the literature, biorevitalization is a mildly invasive procedure that involves subcutaneous drug injections to stimulate fibroblasts, increase collagen and elastin production, and improve skin properties [9, 10, 19]. Our previous study has shown that the use of intradermal microinjections of NCTF 135 HA in combination with biorevitalization have significant improvements in crow's-feet wrinkles, pore size, dermatological scores, and skin tone [10].

Interestingly, Micro-Needle injections appeared to be more efficient and rapid than classic needle injections for enhancing skin radiance. The same positive results were observed for skin wrinkles, with superior effects observed on the face for periorbital wrinkles, nasolabial fold and also for neck wrinkles. These findings were further supported by instrumental measures such as skin hydration level. High-frequency ultrasound imaging provided deeper insights, revealing positive evolutions in the favor of the side treated by Micro-Needle.

Considering that the skin thickness varies across different facial zones, and our results indicated measurable improvements primarily on cheeks and the neck. As the evaluation was conducted over a period of 4 months, it is possible that the periorbital area may require more time to fully demonstrate its restorative effects.

In terms of safety, our study found that the most adverse events encountered with both injection modes were quite similar. Notably, the pain reported during injection was significantly lower with the Micro-Needle technology.

5.1 | Conclusion

Overall, these findings highlight that NCTF 135 HA, particularly when administrated using Micro-Needle injections, offers a compelling solution for antiaging biorevitalization. Micro-Needle technology seems to be a more rapid, efficient, and safe device for biorevitalizing solutions. It is a suitable device to treat the most delicate zones such as periorbital area and neck. These results could be explained by the ex vivo studies which showed the remaining of the product on the right level until 24 h [9].

Acknowledgments

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Ethics Statement

The study adapted with the declaration the Helsinki with the authorization of the local ethical committee of Medical University of 'Iuliu Haţieganu' under registration number 2/1101.2019.

Conflicts of Interest

F. F., H. I., N. S., and V. P. are the employee of FILLMED Laboratories in Paris. The other authors declare that they have no conflicts of interest in this work.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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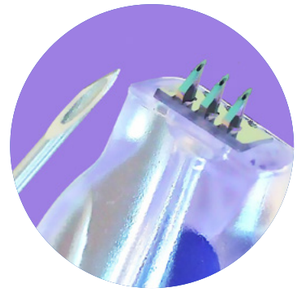
Supporting Information

Additional supporting information can be found online in the Supporting Information section.



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