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Workshop report: Nucleic acid delivery devices for HIV vaccines: Workshop proceedings, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland, USA, May 21, 2015

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ABSTRACT

On May 21st, 2015, the U.S. National Institute of Allergy and Infectious Diseases (NIAID) convened a workshop on delivery devices for nucleic acid (NA) as vaccines in order to review the landscape of past and future technologies for administering NA (e.g., DNA, RNA, etc.) as antigen into target tissues of animal models and humans. Its focus was on current and future applications for preventing and treating human immunodeficiency virus (HIV) infection and acquired immune deficiency syndrome (AIDS) disease, among other infectious-disease priorities. Meeting participants presented the results and experience of representative clinical trials of NA vaccines using a variety of alternative delivery devices, as well as a broader group of methods studied in animal models and at bench top, to improve upon the performance and/or avoid the drawbacks of conventional needle-syringe (N–S) delivery. The subjects described and discussed included (1) delivery targeted into oral, cutaneous/intradermal, nasal, upper and lower respiratory, and intramuscular tissues; (2) devices and techniques for jet injection, solid, hollow, and dissolving microneedles, patches for topical passive diffusion or iontophoresis, electroporation, thermal microporation, nasal sprayers, aerosol upper-respiratory and pulmonary inhalation, stratum-corneum ablation by ultrasound, chemicals, and mechanical abrasion, and kinetic/ballistic delivery; (3) antigens, adjuvants, and carriers such as DNA, messenger RNA, synthesized plasmids, chemokines, wet and dry aerosols, and pollen-grain and microparticle vectors; and (4) the clinical experience and humoral, cellular, and cytokine immune responses observed for many of these target tissues, technologies, constructs, and carriers. This report summarizes the presentations and discussions from the workshop (https://web.archive.org/web/20160228112310/https://www.blsmeetings.net/NucleicAcidDeliveryDevices/), which was webcast live in its entirety and archived online (http://videocast.nih.gov/summary.asp?live=16059).

1. Opening (0:00:00)[a]

Jeffrey K. Pullen, PhD (*Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA*), workshop convener, welcomed participants and summarized workshop goals to explore current and emerging alternative vaccination-delivery technologies.

2. Overviews

2.1. Alternative vaccine delivery systems – past, present and future (0:02:30)

Bruce G. Weniger, MD, MPH, workshop chair (*Research Institute for Health Sciences, Chiang Mai University, Chiang Mai, Thailand*), reviewed briefly the pros and cons of conventional needle-syringe (N-S) devices, a key advantages of which is sure and certain

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[1] Members of the Workshop Group are listed at the end of the report (see Appendix).

[a] Starting times within the total 7-h, 27-min, 8-s length of the archived web broadcast at http://videocast.nih.gov/summary.asp?live=16059 are provided in parentheses, in the format of hours:minutes:seconds, for each speaker and discussion in order more easily to find and view their individual presentation slides, verbal presentation, and questions and answers.

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delivery (in contrast to oral doses that might be expelled, nasal sprays that are sneezed or blocked by rhinitis, patches improperly applied or prematurely removed, and jet injectors that fail to reach targeted tissue). N-S disadvantages include risky unsterile reuse, needlesticks, needle phobia, excessive cold-chain volumes, and tedious filling for campaigns. Alternative non-needle methods for vaccine delivery that target cutaneous or mucosal tissues can mitigate these problems, and may also be more immunogenic than conventional intramuscular (IM) or subcutaneous (SC) N-S injection, and thus *dose-sparing* to use less antigen to similar or better effect [1].

Five classifications for non-oral delivery methods were described:

1. **Cutaneous (skin) vaccination**, typically painless and bloodless when delivered into epidermis lacking nerves and vessels. Its nomenclature is a Tower of Babel [2]. "Cutaneous" is suggested for all methods delivering antigen into or onto the skin, reserving "classical intradermal" for liquid delivery to form a bleb, as for BCG and tuberculin testing.
   Various devices break through the skin's tough, dead barrier of *stratum corneum*. Techniques include mechanical scraping with micro-projections, injecting via hollow mini-needles ($\geq$1 mm), poking solid or dissolving microneedles (MN, <1000 μm), creating pores or paths by heat or light, peeling cellophane tape or cyanoacrylate "super glue", applying direct current to carry charged antigens, or chemicals to weaken cell-cell connections.
2. **Jet injectors** (JI) squirt pressurized liquid through a tiny orifice, like a child's water pistol. Multi-use-nozzle jet injectors (MUNJIs) capable of 600–1000 injections per hour, developed for mass vaccination in the 1950s, were removed in the 1990s for risk of blood-borne pathogen transmission. A new generation of safe disposable-syringe jet injectors (DSJIs) is in use or study for intramuscular (IM), subcutaneous (SC), and cutaneous delivery. Local reactions are often greater than for N-S, although usually tolerable.
3. **The intranasal spray** was likely the earliest technique for vaccination, for variolation in the 10th Century. Currently, BD's Accuspray™, in use since 2003, delivers FluMist® influenza vaccine.
4. **Pulmonary inhalation** is pursued for wet or dry aerosols, pioneered by Albert Sabin. Nebulizers typically require electricity and crushed ice to use off-the shelf licensed vaccine. Delivery of dried powder is promising.
5. **"Kinetic" or "ballistic" deposition** is often by supersonic gas carrying antigenic microparticles into skin, like DNA-coated gold beads or milled vaccine proteins.

2.2. RNActive® technology for potent prophylactic vaccines (0:24:30)

Mariola Fotin-Mleczek, PhD (*CureVac AG, Tübingen, Germany*) described RNActive® ribose nucleic acid (RNA) vaccines for HIV, and cancers of prostate and lung. Trials demonstrated improved survival in cancer patients. RNA is administrable without direct dendritic-cell loading, transfection reagents, nor adjuvants.

Half the RNActive® RNA is complexed with protamine, providing adjuvantation and RNAase protection. Skin showed up-regulation up to 14 h of TNF, IL-6, CXCL-10, and CXCL-9, with uptake and migration by various cells. Other findings were correct presentation as protein antigen, inducible innate and memory cells, balanced Th1/Th2 responses, and immunogenicity in pigs and non-human primates (NHP) comparable to licensed vaccines.

Discussion (0:46:35):

Q1: Which technology was used for skin delivery?
A1: Both N-S and PharmaJet jet injection.
Q2: How long is expression?
A2: Several days to one week at vaccination site.
Q3: What is ID dose volume?
A3: 50–100 μl in animals, 200 μl in humans.

2.3. Clinical landscape of nucleic acid vaccines and delivery methods (0:48:10)

Michael C. Keefer, MD (*University of Rochester Medical Center, School of Medicine and Dentistry, University of Rochester, Rochester, New York, USA*) reported cross-protocol analyses of DNA delivery methods, N-S, JI [Biojector® 2000], and electroporation [EP, CELLECTRA®]) in HIV Vaccine Trials Network studies HVTN 70, 80, 90, and soon-to-be-initiated 98.

Overall, HIV-specific CD4+/CD4+ responses were more frequent than CD8+, with more CD4+ in females than males (p = .002), without CD8+ gender difference. Better CD4+ correlated with body mass index (BMI) <25, compared to >30, independent of gender. Trials found HIV-specific CD4+ in 30–40%, others 60–70%, while CD8+ were typically <25%. Binding and/or neutralizing antibody induction was rare. JI by IM bettered N-S.

Local reactogenicity was greater by EP in HVTN 80, with or without IL-12, than by N-S in HVTN 70. Compared to EP alone, EP plus IL-12 improved CD4+/CD8+ responses, as well as CD4+ to *gag/pol* DNA. Little response was seen for *env* DNA.

HVTN 49 of DNA prime followed by envelope-protein boost found priming response was Th_1, while boosting induced high neutralization against homologous virus with DNA prime, but not by protein alone.

Discussion (1:08:45):

Q1: Why would DNA priming help protein boost?
A1: Unclear are cellular-level events that induce better antibody after protein boost. Perhaps a favorable innate response, as DNA is agonistic for toll-like-receptor-9 (TLR-9).
Q2: Was BMI analyzed further? E.g., lengthening needle to pass fat and increase IM deposition?
A2: Needle size was not changed, but lengthening might assure IM. Skin-fold thickness may better measure body composition affecting immunogenicity.
Q3: Have you looked for serum protein? How much does transfection produce? Is protein secreted by cells?
A3: We have not looked in serum for protein. Protein from IM DNA delivery is a local phenomenon. Protein by transfection has not been detected in preclinical studies.

3. Electroporation (1:13:05)

Session Chair: **Merlin Robb, MD** (Department of Vaccine Development, U.S. Military HIV Research Program, The Henry M. Jackson Foundation, Bethesda, Maryland, USA).

3.1. Clinical development of enhanced DNA vaccine and electroporation technology (1:13:30)

Amir S. Khan, PhD (*Inovio Pharmaceuticals Inc., Plymouth Meeting, Pennsylvania, USA*) explained EP involves multiple electrodes inserted around a hollow injecting needle to generate an electric field that enhances cell transfection. DNA-encoded protein produced intracellularly is taken up by APCs for migration to nodes for presentation to immune cells. In rabbits, EP enhanced naked-DNA protein expression 100–1000 times, compared to IM without EP, with 10-to-100-fold immune enhancement [3].