Austin Journal of Infectious Diseases

Open Access

Austin Publishing Group

Review Article

Development of Newer Smallpox Vaccines

Michael LJ*
Department of Preventive Medicine, Emory University School of Medicine, Atlanta

***Corresponding author:** Michael LJ, Department of Preventive Medicine, Emory University School of Medicine, 869 Clifton Road NE, Atlanta

Received: June 12, 2016; **Accepted:** August 04, 2016; **Published:** August 11, 2016

Abstract

The potential use of smallpox as a weapon for bioterrorism has created a need for more and better smallpox vaccines. The first generation vaccines such as Dr Yvax were produced by crude methods that would not allow licensure today. Second generation vaccines, grown in modern safe tissue cultures but employing seed virus from first generation vaccines of known effectiveness have been developed. One, ACAM2000, has been licensed and added to the US National Stockpile. These second generation vaccines can produce the same complications as first generation vaccines and myopericarditis has been well documented as associated with ACAM2000. This has created advocacy for third and fourth generation smallpox vaccines.

Third generation vaccines are those which have been attenuated either by serial passage in non-human cells or by careful deletions of genes in the laboratory. Two of these, MVA (Modified Vaccinia Ankara) and LC16m8 (a derivative of Lister Strain Vaccinia) have been tested in various human trials. These seem to be ready to apply for licensure if there proves to be a market.

Fourth generation vaccines created as subunits of full strength vaccinia or fully engineered non-replicating molecules which express various known epitopes of vaccinia and/or smallpox, have also been developed. It may be difficult or impossible to prove the efficacy of these vaccines because smallpox no longer exists and there is no animal model that accurately reflects the human disease. These fourth generation vaccines include several large stable DNA viruses into which immunogens from others agents such as HIV and malaria can be inserted, so they may have a future as vector vaccines for a variety of other agents besides smallpox.

Keywords: Smallpox; Vaccines; ACAM2000; MVA; LC16m8; Immunity; National strategic stockpile

Introduction

After 9/11/2001 and given the claims made by the former head of the Soviet Biowarfare program [1-3], public health and political observers declared a need for a safer yet fully effective improved vaccine against smallpox. Shortly after 9/11/2001 the Department of Health and Human Services created BARDA, the Biomedical Advanced Research and Development Authority. BARDA's mission is to encourage and fund the development of vaccines, antibiotics and antivirals for agents which might become weapons of biowarfare. It was also given funds to purchase such products and create a National Stockpile for them. Smallpox was high on the list of agents which occupied BARDA's early interest, in part because only a scant few million doses of (mostly outdated) Wyeth Dryvax were available for use.

The last 15 years have seen a great deal of modern virological work to this end and funds have been made available to stock newer vaccines in the US Government's National Strategic Stockpile. This stockpile now contains enough smallpox vaccine to immunize every man woman and child in the United States. This paper will briefly review this effort. Readers who wish a more complete exposition of the virological and genetic information about candidate third and fourth generation vaccines becoming available are directed to the chapter on Smallpox and Vaccinia in the 6th Edition of Plotkin, Orenstein and off its textbook *Vaccines* [4].

First Generation Smallpox Vaccines

After the Second World War first generation smallpox vaccines in the United States were largely preparations of the New York City Board of Health (NYCBOH) strain of vaccinia. These vaccines were of proven effectiveness, although there was never a placebo controlled trial of any vaccine against smallpox. They were administered by scratch or multiple pressures and after 1965 largely by using the bifurcated needle.

Vaccination technique

Most first generation vaccines used in the United States after 1965 were lyophilized for better shelf life, which enhanced their effectiveness when taken into the field in tropical areas. A bifurcated needle is dipped into liquid vaccine (lyophilized vaccine after diluent has been added). Capillary action draws a droplet of vaccine into the crotch of the needle. The vaccinator then uses 15 firm but gentle downward strokes onto the skin of the arm around the insertion of the deltoid muscle. The first stroke dislodges the droplet of vaccine and the subsequent strokes through this droplet abrade the skin and allow entry of the vaccinia virus into the Malpighi and layer of the skin. A tiny droplet of blood is often visible at the site, but frank bleeding indicates technique that is too vigorous. The site may be covered by a loose dressing, which helps avoid transfer of the virus to other sites [5,6].Good technique with fully potent vaccines caused a major skin

Citation: Michael LJ. Development of Newer Smallpox Vaccines. Austin J Infect Dis. 2016; 3(2): 1025.

reaction to develop by about 7 days, with a central lesion surrounded by visible inflammation, the so-called "take". This is a viral infection and frequently there is some mild fever and discomfort around 6 to 8 days after inoculation [5,6].

Immunity

Immunity following vaccination has components of humoral antibodies and cellular immunity. Vaccinia and variola are the largest viruses that infect humans. Their structure and functions is complex. The relationship between the many circulating antibodies against vaccinia virus antigens and the several cell-mediated immune responses evoked, are complex and controversial. This is an area of active research, with many animal species utilized and hundreds of HLA class I and II epitopes identified [4,7]. Unfortunately no studies have been accomplished that definitively show what level of antibodies, or what form of cellular immunity is fully protective. We thus also don't know precisely how long immunity lasts. Currently the most accepted measure of neutralizing antibodies is PRNT [3,4,6-8]. These antibodies become detectable by about the 6th day after vaccination and seem to last for several decades. Cellular immunity has been measured in several ways and different researchers employ somewhat different tests for quantifying it. These are also serological evidence that such immunity lasts for several decades [4,6].

Epidemiological evidence also suggests that there is some residual immunity for decades after a single primary vaccination. While patients several years away from vaccination sometime acquire smallpox, the disease is mild and death rates are low until about 5 decades post vaccination [7,9,10].

Complications and contraindications for smallpox vaccination

Anyone who has had a documented face-to-face contact with a smallpox patient should be vaccinated. Smallpox is always worse than vaccinia, so there are no contraindications to vaccinating such patients. Vaccinia is a viral infection and there are several complications that can arise, so that patients without direct contact with smallpox cases must be screened for contraindications [11-13].

In common with many viral infections, vaccinia can rarely be followed by post-vaccinial encephalitis. This has an incidence of 1 to 2 cases per million primary vaccines and is more common in infants than in older patients. Infants should therefore not be vaccinated.

Patients with atopic dermatitis (eczema) or a history of atopic dermatitis are at risk of developing eczema vaccinatum. This can be fatal, particularly in infants, who have a greater body surface area in relation to their size than adults and in whom eczema vaccinatum can act like a serious burn with loss of protein and electrolytes. Patients with atopic dermatitis should not be vaccinated nor should family members who have close contact with atopic individuals.

Patients with diseases or conditions which jeopardize the immune systems or who are taking immunosuppressive medications such as steroids are at risk for developing progressive vaccinia. This condition is often fatal with vaccinia virus growing out of control and often spreading throughout the body.

While vaccinia does not seem to increase fetal wastage or cause prematurity, since it is a viral infection pregnant women should not be vaccinated unless they have had direct contact with smallpox patients.

The vaccination process leaves live virus on the skin surface and the developing Jennerian vesicle shed copious amount of virus. Infants often scratch the lesions and transfer vaccinia to unintended areas such as the eye. Covering the area with a loose dressing helps reduced such spread.

Photographs of patients with serious complications of smallpox vaccination can be found in Fenner [6] or at the smallpox section of the CDC website [14].

With no cases of smallpox occurring in the US after 1949 and given the frequency of serious complications to the vaccinia virus [11-13] vaccination in the United States was largely abandoned after 1971-1972 [15].

Production of first generation vaccines

These first generation vaccines were produced by methods that would not be permitted today [6]. The shaved skin of animals (usually cows or sheep) was widely inoculated with seed virus. The resulting inflammatory exudate was then scraped off about 7 days after inoculation. This produced a vaccine that contained animal proteins, bacteria from the animal skin and possibly an unknown animal virus. Vaccines produced in this manner would not now be licensed by the FDA. Thus in addition to wanting a safer vaccine, there is a need for vaccines produced by methods that could pass modern standards of good practice.

An ideal new vaccine

An ideal new smallpox vaccine would be a live virus vaccine with a long shelf life, lyophilized and administered with a bifurcated needle. It would produce a visible skin lesion so that a successful major reaction ("take") could be documented without laboratory work. It should be produced in readily available cell cultures. Such candidate vaccines must have reasonable data available showing fewer and less serious complications than the first generation NYCBOH vaccines.

Unfortunately the efficacy of new smallpox vaccines is difficult to prove. There is no simple serological marker for full effectiveness. A normal primary vaccination in a non-immune individual produces an array of circulating antibodies and a complex group of markers of cellular immunity [4,6,7]. Given the eradication of smallpox the absence of any human cases makes field trials of efficacy impossible. The FDA has therefore developed the "Two Animal Rule" [16]. This requires that new vaccine candidates for licensure must show efficacy in two animals in which infection with an orthopox closely related to smallpox is a reasonable mimic of human's infection with Variola major. (An ideal animal model would be an infection with live Variola virus that produces a disease quite similar to human smallpox). While several orthopox viruses are virologically similar to Variola (monkeypox, ectromelia, buffalopox, vaccinia) they do not produce the pathophysiology in mammals such as non-human primates that is similar to Variola in humans [17-20]. Even Jahrling's work using large intravenous innocula of live Variola virus in monkeys, while producing lesions on the skin similar to smallpox does not closely mimic the widespread replication of the virus in reticuloendothelial tissues [18]. Animal studies to fulfill the two animal rules are costly and higher primates are particularly expensive and difficult to work

with requiring special lab facilities. Animal studies employing live Variola virus can only be performed in the high security lab at CDC in Atlanta and require permission from the World Health Organization.

Second Generation Smallpox Vaccines

Second generation vaccines are vaccinia strains that are clones of first generation strains of vaccinia of known effectiveness, but are grown on tissue culture and are thus free of bacteria and animal proteins. There are several such vaccines but only one, ACAM2000, has been subject to non-inferiority trials comparing it directly with the first generation vaccine Wyeth's Dryvax (a New York City Board of Health vaccinia vaccine that was used extensively in Africa and Asia during the Smallpox Eradication Program) [7]. Dryvax is now known to be a soup of closely related vaccinia strains, a single one of which was picked to yield ACAM2000.

Straight forward non-inferiority trials allowed licensure. Non-inferiority of ACAM2000 to DRYVAX was shown by measurement of neutralizing antibodies, rates of major reactions ("takes") and measures of cellular immunity [7,21]. Such head to head comparison trials employing Dryvax or other first generation vaccines are no longer possible because of the documentation of myopericarditis following vaccination with both first generation vaccines and ACAM2000 [22-25]. ACAM2000 is now licensed in the United States and BARDA has added several hundred million doses to the United States Government's National Strategic Stockpile [26,27].

There continue to be safety concerns about the use of first and second generation smallpox vaccines. There has been an increase in the prevalence of eczema since the studies of complications of vaccination performed in the 1960's [28]. There has been a dramatic increase in the prevalence of immunocompromised patients, given HIV, oncological treatments, organ transplants and other conditions which jeopardize the immune system [28]. Patients with severe immunological defects are at risk for developing progressive vaccinia in which vaccinia virus continues to grow unchecked, usually resulting in death. These concerns have led to vigorous efforts to develop third generation vaccines.

Third Generation Smallpox Vaccines

Several third generation vaccine candidates have been developed [4]. During the 1960's the Germans produced a vaccine called Modified Vaccinia Ankara (MVA). This was developed by 570+ serial passages of a first generation vaccinia strain in chick embryo fibroblasts. While MVA is a live virus, it does not replicate in human tissues and therefore functions somewhat like a killed virus vaccine. It does not produce a visible skin lesion. MVA has been shown by modern genetic methods to have lost several genes from the parent vaccinia strain [29]. The MVA strain developed by Bavarian Nordic, IMVAMUNE, has gone through several trials in humans to demonstrate safety. The vaccine has a potency of 10^8 TCID after reconstitution. Optimal immunity requires two doses of 0.5 ml reconstituted vaccine delivered subcutaneously. While the many trials have included patients with HIV, patients with a history of eczema and have employed several dosing schedules, none have included young children [30-38].

MVA is not a good option for control of smallpox outbreaks. Optimal immunity requires two doses of MVA administered subcutaneously roughly 4 weeks apart. IMVAMUNE is supplied in individual vials, one for each dose with 0.5 ml of vaccine containing 10^8 TCID per dose. It requires refrigeration up to the time of use. It must be injected with a needle and syringe and therefore should be administered by a doctor or nurse. It does not produce a visible skin lesion and thus meticulous records must be kept because health workers cannot tell at a glance whether an individual has been vaccinated. Since optimal levels of immunity require two doses, contacts of cases may not be protected after initial processing and their first inoculation. IMVAMUNE may be the best vaccine to use in situations where there is no smallpox, but people with contraindications to vaccination with second generation vaccines require vaccination. These would include laboratory workers exposed to orthopox viruses and medical workers who might form teams of caregivers in the event of an actual smallpox outbreak.

The Japanese have for several years worked with a third generation vaccine developed from first generation Lister strain vaccinia. This third generation vaccine is named LC16m8. While there are fewer published trials than with MVA, the Japanese have used LC16m8 extensively and apparently have experienced few serious complications [39-42]. This vaccine would be good for outbreak control. It is lyophilized and thus can be taken into the field without refrigeration. It is administered with a bifurcated needle. It produces a visible major skin reaction at the site of vaccination, so that a successful vaccination can be documented at a glance.

Fourth Generation Smallpox Vaccines

Many new potential vaccinia-derived strains have been developed by genetic engineering techniques. Several candidate third and fourth generation vaccines have been created by careful deletion of genes from vaccinia or from creating preparations that express epitopes common to variola or vaccinia [4,43,44]. These are under development in the laboratory, with a few that have progressed to animal experiments, generally using animal models employing small mammals and viruses such as vaccinia or ectromelia.

Barriers to Developing Newer Smallpox Vaccines

While it would be good to have a safe and effective new vaccine to supplement or replace ACAM2000, development of such a vaccine is doubtful despite excellent viral generic and immunological work. In the absence of actual smallpox or credible threats of bioterrorist attacks, there probably is no market for such a vaccine and indeed funding for research in this area is limited. Large scale production facilities capable of producing large lots do not exist and would be costly to create and operate. Since such vaccines might be added to the National Strategic Stockpile, they should maintain their potency for a very long shelf life. Given the need for vaccines against Ebola, SARS, Zika and other viruses with serious potential as public health problems, it is difficult to justify diverting the funds and expertise to create and actually produce an improved smallpox vaccine.

While first generation Dryvax cost less than a penny a dose during the Smallpox Eradication Program, new vaccines might be much more costly. The newer second and third generation vaccines that have been purchased by BARDA for the National Strategic Stockpile

would be free to the public and only used after a documented need for vaccination. ACAM2000 and IMVAMUNE prices are not available, but from the amounts bought by BARDA for the National Strategic Stockpile, we can estimate that their cost is between $4 and $17 per dose.

Work on development of third and fourth generation vaccines will probably progress. Vaccinia and its many artificial variants such as MVA and NYVAC are large stable DNA viruses, relatively safe and easy to work with [4,41,42]. Given their safety in humans, they may be excellent vectors for other vaccine antigens. In animal models, MVA vaccines are immunogenic and protective against various infectious agents including HIV, simian immunodeficiency virus, influenza, parainfluenza, measles, malaria, tuberculosis and several cancers. An NYVAC based vaccine against HIV shows promise [45-47].

MVA and other engineered fourth generation viruses such as NYVACprobably have more of a future as engineered vectors than as smallpox vaccines.

Summary and Conclusion

Given the problems of serious side effects and outmoded production methods, the first generation of smallpox vaccines, despite their proven effectiveness, are not now acceptable. Second generation vaccines whose effectiveness can be assumed because they are made with the same vaccinia strains as first generation vaccines have been created. One, ACAM2000, has shown non-inferiority to first generation vaccine and has been added to the National Strategic Stockpile.

Third generation vaccines, which are derived from first generation vaccinia strains by serial passage in non-human tissues or by genetic modification of such strains in modern viral genetic laboratories show promise as practical vaccines. MVA (Modified Vaccinia Ankara) has undergone several trails for safety in humans, including those who are HIV positive or atopic. It may be a good vaccine for use in persons who have contraindication to vaccination with first or second generation vaccines, but who require vaccination. MVA has been added to the National Strategic Stockpile. The Japanese vaccine LC16m8 seems good for outbreak control because it can be lyophilized, administered with a bifurcated needle and produces a visible major reaction on the skin that proves its "take". LC16m8 has not yet been submitted for licensure in the United States.

There are many fourth generation candidates, produced by modern immunologic and virologic techniques. These are subunits of vaccinia with several genes removed or vaccines created de novo by adding various epitopes or other immunogens from vaccinia to artificially created molecules. The cost and difficulty in proving that such vaccines are effective against smallpox may inhibit their full development as smallpox vaccines, but they may prove very good as "vector vaccines" for other infectious agents because immunogenic parts of such agents can be added to their genetic structure.

References

1. Alibek K, Handelman S. Biohazard. Random House New York NY. 1999.
2. Henderson DA, Inglesby TV, Bartlett JG, Ascher MS, Eitzen E, Jahrling PB, et al. Smallpox as a biological weapon: medical and public health management. Working Group on Civilian Biodefense. JAMA. 1999; 281: 2127-2137.
3. Lane JM, Summer L. Smallpox as a Weapon for Bioterrorism. A New Dilemma for the 21st Century. Springer. New York NY. 2005; 147-167.
4. Kennedy RB, Lane JM, Henderson DA, Poland GA. Smallpox and Vaccinia. Elsevier. 2013; 718-745.
5. Fulginiti VA, Papier A, Lane JM, Neff JM, Henderson DA. Smallpox Vaccination: A Review, Part I. Background, Vaccination Technique, Normal Vaccination and Revaccination and Expected Normal Reactions. Clin Infect Dis. 2003; 37: 240-250.
6. Fenner F, Henderson DA, Arita I, Jezek Z, Ladnyi ID. Smallpox and its eradication. World Health Organization. Geneva. 1988.
7. Frey SE, Newman FK, Kennedy JS, Ennis F, Abate G, Hoft DT, et al. Comparison of the safety and immunogenicity of ACAM1000, ACAM2000 and Dryvax in healthy vaccinia-naive adults. Vaccine. 2009; 27: 1637-1644.
8. Hughes CM, Newman FK, Davidson WB, Olson VA, Smith SK, Holman RC, et al. Analysis of Variola and Vaccinia Virus Neutralization Assays for Smallpox Vaccines. Clin Vaccine Immunol. 2012; 19: 1116-1118.
9. Mack TM. Smallpox in Europe, 1950-1971. J Infect Dis. 1972; 125: 161-169.
10. Dixon CW. Smallpox. Little Brown and Company, Boston.1962.
11. Lane JM, Ruben FL, Neff JM, Millar JD. Complications of smallpox vaccination, 1968. New Eng J Med. 1969; 281: 1201-1208.
12. Lane JM, Ruben FL, Neff JM, Millar JD. Complications of smallpox vaccination, 1968: results in ten statewide surveys. J Infect Dis. 1970; 122: 303-309.
13. Fulginiti VA, Papier A, Lane JM, Neff JM, Henderson DA. Smallpox vaccination: a review, part II. Adverse events. Clin Infect Dis. 2003; 37: 251-271.
14. Centers for disease control and prevention.
15. Lane JM, Millar JD. Routine childhood vaccination against smallpox reconsidered. New Eng J Med. 1969; 281: 1220-1224.
16. Product Development under the Animal Rule Guidance for Industry. FDA. 2015.
17. Chapman JL, Nichols DK, Martinez MJ, Raymond JW. Animal Models of Orthopoxvirus Infection. Vet Pathol. 2010; 47: 852-870.
18. Jahrling PB, Hensley LE, Martinez MJ, LeDuc JW, Rubins KH, Relman DA, et al. Exploring the potential of variola virus infection of cynomolgus macaques as a model for human smallpox. Proc National Acad Sci. 2004; 101: 15196-15200.
19. Schriewer J, Buller RM, Owens G. Mouse Models for Exploring Orthopoxvirus Respiratory Infections. Methods in Molecular Biol. 2004; 264: 289-308.
20. Essbauer S, Pfeffer M, Meyer H. Zoonotic poxviruses. Vet Microbiol. 2010; 140: 229-236.
21. Greenberg RN, Kennedy JS. ACAM2000: a newly licensed cell-culture based live vaccinia smallpox vaccine. Expert Opin Investig Drugs. 2008; 17: 555-564.
22. Morgan J, Roper MH, Sperling L, Scheiber RA, Heffelfinger JD, Casey CG, et al. Myopericarditis, Pericarditis and Dilated Cardiomyopathy after Smallpox Vaccination among Civilians in the United States. Clin Infect Dis. 2008; 46: 242-250.
23. Chen RT, Lane JM. Myocarditis: the unexpected return of smallpox vaccine adverse events. Lancet. 2003; 362: 1345-1346.
24. Arness MK, Eckart RE, Love SS, Atwood JE, Wells TS, Engler RJ, et al. Myopericarditis following smallpox vaccination. Am J Epidemiol. 2004; 160: 642-651.
25. Cassimatis DC, Atwood JE, Engler RM, Linz PE, Grabenstein JD, Vernalis MN. Smallpox vaccination and myopericarditis: a clinical review. J Am Coll Cardiol. 2004; 43: 1503-1510.
26. Center for Disease Control and Prevention. Smallpox Response Plan and Guidelines. 2003.

27. Nalca A, Zumbrun EE. ACAM2000: The new smallpox vaccine for United States Strategic National Stockpile. Drug Des Devel Ther. 2010; 4: 71-79.

28. Lane JM, Goldstein J. Evaluation of 21st century risks of smallpox vaccination and policy options. Ann Int Med. 2003; 138: 488-493.

29. Meyer H, Sutter G, Mayr A. Mapping of deletions in the genome of highly attenuated vaccinia virus MVA and their influence on virulence. J Gen Virol. 1991; 72: 1031-1038.

30. Vollmar J, Arndtz N, Eckl KM, Thomsen T, Petzold B, Mateo L, et al. Safety and immunogenicity of IMVAMUNE, a promising candidate as a third generation smallpox vaccine. Vaccine. 2006; 24: 2065-2070.

31. Von Krempelhuber A, Vollmar J, Pokorny R, Rapp P, Wulff N, Petzold B, et al. A randomized, double-blind dose-finding phase II study to evaluate the immunogenicity and safety of the third-generation smallpox vaccine candidate IMVAMUNE. Vaccine. 2010; 28: 1209-1216.

32. Frey SE, Newman FK, Kennedy JS, Sobek V, Ennis FA, Hill H, et al. Clinical and immunologic responses to multiple doses of IMVAMUNE (Modified Vaccinia Ankara) followed by Dryvax challenge Vaccine. 2007; 25: 8562-8573.

33. Kennedy JS, Greenberg RN. IMVAMUNE modified vaccinia ankara strain as an attenuated smallpox vaccine. Expert Rev Vaccines. 2009; 8: 13-24.

34. Frey SE, Winokur PL, Salata RA, El-Kamary SS, Turley CB, Walker EB, et al. Safety and immunogenicity of IMVAMUNE smallpox vaccine using different strategies for a post event scenario. Vaccine. 2013; 31: 3025-3033.

35. Mayer H, Sutter G, Mayr A. Mapping of deletions in the genome of highly attenuated vaccinia virus MVA and their influence on virulence. J Gen Virol. 1991; 72: 1031-1038.

36. Greenberg RN, Overton ET, Haas DW, Frank I, Goldman M, von Krempelhuber A, et al. Safety, immunogenicity and surrogate markers of clinical efficacy for modified vaccinia ankara as a smallpox vaccine for HIV-infected subjects. J Infect Dis. 2013; 207: 749-758.

37. Walsh SR, Wilck MS, Dominguez DJ, Zablowsky E, Bajimaya S, Gagne LS, et al. Safety and immunogenicity of modified vaccinia ankara in hematopoic stem cell transplant recipient: a randomized, controlled trial. J Infect Dis. 2013; 207: 1988-1897.

38. Garza NL, Hatkins JM, Livingston V, Nichols DK, Chaplin PJ, Volkmann A, et al. Evaluation of the efficacy of Modified Vaccinia Ankara (MVA)/ IMMVAMUNE against aerosolized rabbitpox virus in a rabbit model. Vaccine. 2009; 27: 5496-5504.

39. Saito M, Fujii T, Kanatani Y, Saijo M, Shigera M, Yokote H, et al. Clinical and Immunological Response To Attenuated Tissue-Cultured Smallpox Vaccine LC16m8. JAMA. 2009; 301: 1025-1033.

40. Hashizume S, Yoshizawa H, Morita M, Suzuki K. Properties of Attenuated Mutant of Vaccinia Virus LC16m8, derived from Lister Strain in Quinnan CG ed Vaccinia Viruses as vectors for Vaccine Antigens: Proceeding of a Workshop held Nov 13-14, Chevy Chase Maryland. Elsevier New York NY. 1985.

41. Kidokoro M, Tashiro M, Shida H. Genetically stable and fully effective smallpox vaccine strain constructed from highly attenuated vaccine LC16m8. Proc Natl Acad Sci USA. 2005; 102: 4152-4157.

42. Morikawa S, Sakiyama T, Hasegawa H, Saijo M, Maeda A, Kurane I, et al. An attenuated LC16m8 smallpox vaccine: analysis of full-genome sequence and induction of immune protection. J Virol. 2005; 79: 11873-11891.

43. Meseda CA, Weir JP. Third generation smallpox vaccines: challenges in the absence of clinical smallpox. Future Microbiol. 2010; 5: 1367-1382.

44. Kennedy RB, Poland GA. The identification of HLA class II-restrictive T cell epitopes to vaccinia virus membrane proteins. Virology. 2010; 408: 232-240.

45. Tartaglia J, Perkins ME, Taylor J, Norton EK, Audomet JC, Cox WI, et al. NYVAC: a highly attenuated strain of vaccinia virus. Virology. 1992; 188: 217-232.

46. Cottingham MG, Carroll MW. Recombinant MVA vaccines: dispelling the myths. Vaccine. 2013; 31: 4247-4251.

47. Perreau MI, Welles HC, Harari A, Hall O, Martin R, Maillard M, et al. DNA/ NYVAC vaccine regimen induces HIV-specific CD4 and CD8 T-cell responses in intestinal mucosa. J Virol. 2011; 85: 9854-9862.

Austin J Infect Dis - Volume 3 Issue 2 - 2016
Submit your Manuscript | www.austinpublishinggroup.com

Citation: Michael LJ. Development of Newer Smallpox Vaccines. Austin J Infect Dis. 2016; 3(2): 1025.