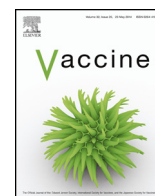




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Safety and immunogenicity of modified vaccinia Ankara as a smallpox vaccine in people with atopic dermatitis[☆]

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ABSTRACT

Background: Following vaccination with traditional smallpox vaccines or after exposure to vaccinated individuals, subjects with atopic dermatitis (AD) can develop eczema vaccinatum, a severe disease with disseminated eruption of pustular contagious lesions. Alternative smallpox vaccines with an improved safety profile would address this unmet medical need.

Methods: An open-label controlled Phase I clinical trial was conducted to investigate the safety and immunogenicity of modified vaccinia Ankara (MVA) in 15 healthy subjects compared to 45 subjects with either mild allergic rhinitis, a history of AD or presenting with mild active AD. MVA was given (Week 0 and 4) by a subcutaneous injection during a 28-week observation period.

Results: No serious adverse event was reported and vaccinations with MVA did not lead to any clinically relevant skin reactions in AD subjects. Unsolicited administration site reactions did not show any trends compared to the healthy subject group. The majority of adverse reactions were mild to moderate, and all reactions were transient and resolved without intervention. The majority of vaccinees had seroconverted by ELISA (80–93%) and PRNT (69–79%) already two weeks after the first vaccination, increasing to 100% after the second immunization, with peak GMT above 1000 and 145 for ELISA and PRNT, respectively.

Conclusions: MVA was equally well tolerated and immunogenic in all enrolled subjects with mild to moderate pain and redness at the injection site being the most frequent adverse reactions. There were no differences in the safety or immunogenicity profile of MVA in healthy subjects or those with AD or allergic rhinitis. The study has confirmed MVA as a promising smallpox vaccine candidate and demonstrated in a small study population that the vaccine has a similar safety and immunogenicity profile in healthy subjects and people with active AD.

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1. Introduction

With traditional smallpox vaccines, severe and potentially life-threatening side effects such as progressive vaccinia, generalized vaccinia and eczema vaccinatum (EV) have been observed, all associated with the replicating nature of the various vaccinia virus (VACV) strains used in the vaccine [1,2]. EV is caused by localized or systemic viral dissemination that can be lethal. Individuals with a history of or active atopic dermatitis (AD), or other eczematous skin diseases are at a higher risk of developing EV following smallpox vaccination or when coming into contact with recently

vaccinated individuals [3,4]. AD is a genetically determined pruritic chronic inflammatory skin disease thought to be caused by a panel of immune abnormalities in the skin [5,6]. Although EV is normally confined to the skin and self-limiting, it can progress to a systemic disease and historical mortality rates were as high as 6% in vaccinees and 4% in secondary contacts [7–9]. Despite attempts to avoid high risk individuals, serious adverse events associated with traditional smallpox vaccines have been recorded in military personnel and other first line responders recently vaccinated due to the fear of a deliberate spread of the variola virus [10].

Aside from identifying subjects at high risk of serious side effects caused by traditional smallpox vaccines in a pre-event vaccination scenario, another challenge is to minimize inadvertent spread from vaccinees to close contacts, especially people at higher risks such as subjects with AD [11]. There are estimates that 25–50% of the population should be excluded from smallpox vaccination, because of the potential risks associated with smallpox vaccination either to themselves, or from coming into contact with a high-risk individual [12,13].

MVA was used during the 1970s in more than 120,000 people, including children with immune deficiencies, for priming prior to administration of a traditional smallpox vaccine [14,15]. MVA was derived by more than 570 passages of the parental VACV, chorioallantoic vaccinia Ankara, in primary chicken embryo fibroblast (CEF) cells and essentially became replication restricted to avian and certain mammalian cells [16]. The attenuated properties have led to the development of MVA as a safer smallpox vaccine and studies have shown MVA to be well tolerated in healthy adults and to induce comparable immune responses as traditional smallpox vaccines [17–19,24]. These clinical data are supported by findings that MVA induces comparable [20,21], or even superior efficacy [22,23] compared to traditional smallpox vaccines in a variety of animal models.

Based on these favorable biological features of MVA we performed a Phase I open-label clinical trial to investigate the safety and immunogenicity of MVA in 18–40 year old vaccinia-naïve (previously not vaccinated against smallpox) subjects with AD.

2. Materials and methods

2.1. Vaccine

MVA was produced by IDT Biologika GmbH (Dessau-Roßlau, Germany) according to Good Manufacturing Practice and was provided by Bavarian Nordic A/S (Kvistgaard, Denmark) as a refrigerated freeze-dried product. The vaccine was reconstituted in 0.5 ml sterile water to deliver at least 1×10^8 TCID₅₀/ml (i.e. at least 5×10^7 TCID₅₀/dose).

2.2. Participants and vaccination schedule

The trial was approved by the relevant Independent Ethics Committees and all study related procedures followed the Declaration of Helsinki principles, Good Clinical Practice and the requirements of the German Drug Law. Male or female subjects aged 18–40 years with an acceptable medical history were considered eligible for participation. After participants provided written Informed Consent, screening and dermatological examinations were initiated at the Department of Dermatology and Allergy, Biederstein, Technische Universität München, Munich, Germany. The assessment included the medical history, review of the eligibility criteria, physical examination with special attention to the skin, and baseline safety lab. Women were neither pregnant nor breast-feeding and used effective birth control during the study. Since cardiac events (cases of myo-/pericarditis) have been reported after vaccinations with

traditional smallpox vaccines [26], an Electrocardiogram (ECG) was conducted and cardiac risk factors evaluated. Subjects from all groups with pre-existing immunity against VACV identified at baseline by ELISA were excluded. The assignment of 15 subjects into each of the four parallel study groups was planned as follows:

Group 1: Healthy subjects without any history of, or current signs and symptoms of atopic disease.

Group 2: Subjects having documentation of at least one allergic rhinitis event during the previous year.

Group 3: Subjects having documentation of a history of AD.

Group 4: Subjects presenting active AD and having an individual Scoring AD (SCORAD) value between 1 and 15 [27]. The SCORAD, used to grade the severity of AD and calculated based on extent and intensity, ranges from 0 to 83 using objective items, with mean scores <15, 15–40 and >40 indicating mild, moderate and severe AD, respectively.

Subcutaneous MVA vaccinations (0.5 ml) were administered at the Department of Infectious Diseases and Tropical Medicine, Ludwig-Maximilians-Universität, Munich, Germany, in the non-dominant upper arm on Visit 1 (day 0) and Visit 3 (Week 4). After enrollment and first vaccination, study subjects returned to the study center for five assessment visits (Weeks 2, 4, 6, 8 and 28) to conduct safety evaluations including dermatological evaluations and blood collection for safety and immunogenicity analysis. The study was conducted between June 2004 and December 2005 (including follow-up).

2.3. Safety assessments

Safety and tolerability of MVA was determined by the incidence and grade of local and systemic reactions according to standardized scales and by routine clinical and laboratory examinations. Occurrence, relationship and intensity of any unsolicited adverse events (AE) at any time were recorded and documented at each visit until four weeks after the last vaccination. Serious adverse events (SAE) were recorded until the end of the 28-week follow-up period. All subjects completed diaries for documentation of solicited local injection site symptoms (erythema, swelling, induration and pain), body temperature and solicited general symptoms (headache, myalgia, chills, nausea, fatigue) during the 8-day period post-vaccination including the day of vaccination. ECGs were performed two weeks after each vaccination and at the last visit of the main study phase (Week 8). An Independent Safety Monitor heading a Safety Monitoring Committee surveyed the safety of the trial.

2.4. Immunogenicity

Poxvirus specific antibody responses were measured at all study visits using a validated enzyme-linked immunosorbent assay (ELISA) using MVA as antigen [19] and Plaque reduction neutralization test (PRNT) using VACV as the antigen as previously described [34]. Total and neutralizing titers below the assay cut-off value (50 and 10, respectively) were assigned an arbitrary value of 1 for the purpose of calculations, while an ELISA value ≥ 50 and a PRNT value ≥ 10 were considered seropositive. Geometric Mean Titers (GMT) were calculated using the antilogarithm of the mean of the log₁₀ titer transformation.

T cell immunogenicity elicited by MVA was measured at all study visits by Intracellular Cytokine Staining (ICS) for interferon-gamma (IFN- γ) producing T cells using cryopreserved peripheral blood mononuclear cells (PBMC) as described previously [19].

2.5. Statistical methods

Data were analyzed descriptively in an explorative way and all statistical analyses were performed using SAS software. The safety analysis was based on the full analysis set (FAS) consisting of all subjects who had received at least one dose of study vaccine. The immunogenicity analysis was performed on the per-protocol analysis set (PPS) comprising of all subjects without major protocol violations. Antibody seroconversion was defined as the appearance of a positive titer in subjects with no titer prior to vaccination. Seroconversion rates between groups were compared at each visit using Fisher's exact test. Confidence intervals were calculated for the GMT under the assumption that the $\log_{10}$ titers have a normal distribution. Differences between groups were tested for using a one-way ANOVA on the $\log_{10}$ titers at each visit. Differences between CD8 measurements were tested using a non-parametric Kruskal–Wallis test at each visit.

3. Results

3.1. Participants and demographic data

A total of 31 male (51.7%) and 29 female (48.3%) vaccinia-naïve subjects between 19 and 34 years were enrolled into four study groups (Fig. 1). The majority were of Caucasian origin (95%), with one Black (1.7%) and two Oriental/Asian (3.3%) individuals. Demographic characteristics did not show significant differences between the four groups. Active eczema was detected in 14 of 15 subjects (93.3%) in Group 4 (active AD) on the day of screening and all subjects in this group had a baseline SCORAD value of 3 or higher, with a mean value of 9.3 ($SD \pm 4.15$). There were no significant changes in AD status during the study. All subjects in Group 2 had at least one documented allergic rhinitis episode during the previous year. All subjects that received at least one dose of MVA were included in the FAS population ($N = 60$) for safety analysis. All

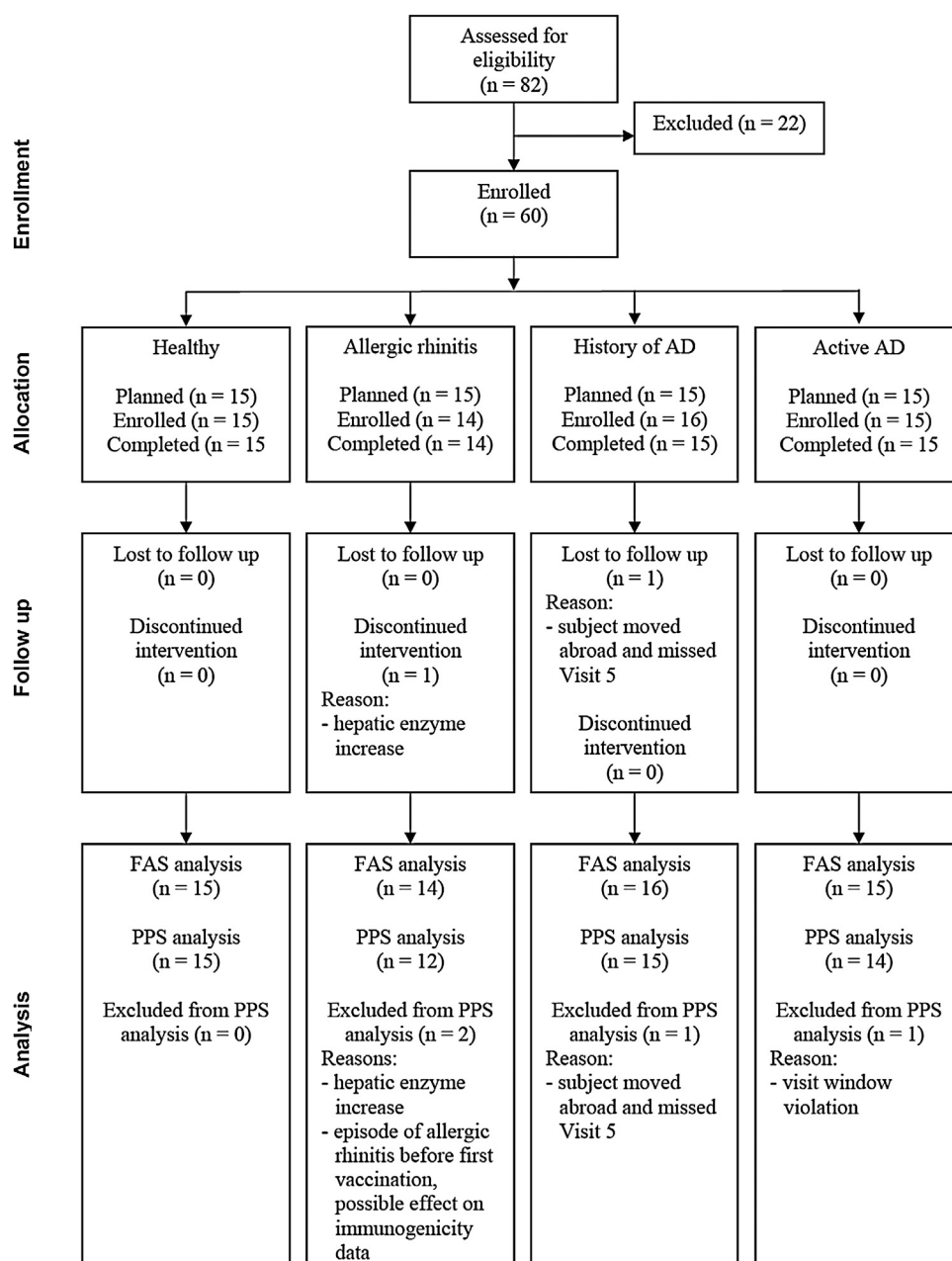


Fig. 1. Disposition of subjects and data sets analyzed.

subjects, with the exception of one from the group with a history of AD, completed the study, including the 28 weeks follow-up visit.

Four subjects did not fulfill the criteria for inclusion in the PPS ($N=56$), due to the following four major protocol violations: AE before the first vaccination, withdrawal of consent due to moving abroad, blood draw out of visit window, withdrawal from second vaccination due to an AE (Fig. 1).

3.2. Safety and reactogenicity

No SAE and no case of EV were reported. No ECG changes or other signs indicating myo-/pericarditis were observed.

Regarding solicited AE, nearly all subjects reported mild to moderate pain and redness at the administration site. However, local as well as general solicited AE with an intensity grade ≥ 3 were uncommon (Table 2).

Unsolicited administration site reactions did not show any trends between the groups (Table 1). Single cases of unsolicited AE were injection site burning, injection site nodule, flushing, urticaria, dysaesthesia, arthralgia, and vertigo. One subject in the allergic rhinitis group experienced an AE classified as possibly related (“hepatic enzyme increase”) preventing the subject from receiving the second vaccination, but had returned to normal at the next visit 14 days later. Except for one other subject with an elevated liver function test (AD history group), no clinically significant changes in hematology or biochemistry values were observed.

All adverse reactions were transient and no medical intervention was necessary. Dermatological examinations did not reveal clinically relevant unfavorable reactions to MVA. In two subjects with active AD, a slight worsening of AD status reflected by a SCORAD increase from 13 to 18 and from 14 to 21 respectively was

observed at the visit performed two weeks after the second vaccination (Table 1). Due to the temporal relationship, the investigator assessed both exacerbations to be possibly related to the vaccination. These resolved two weeks later when the investigator assessed the SCORAD values of both subjects to be below their baseline value.

3.3. Antibody responses

As illustrated in Fig. 2, the kinetics of the total and neutralizing antibody responses induced following vaccinations with MVA were highly comparable between all groups, regardless of the subjects AD status. No statistical differences in either GMT or seroconversion rates were observed between groups at any visit by ELISA. Already two weeks after the first MVA vaccination the majority (80–93%) of the previously vaccinia-naïve subjects had seroconverted by ELISA (Fig. 2A) with GMT ranging from 63 to 118 (Fig. 2B). These high total antibody responses progressed to 100% seroconversion rates in all study groups four weeks after the first immunization. At this time the GMT after a single vaccination with MVA were in the same range for all study groups with a peak of 251 reached in subjects with a history of AD. Two weeks following the second vaccination with MVA, an additional 5- to 7-fold rise in the GMT were observed with values above 1000 in all four study groups. With the exception of one subject in the active AD group, all remaining subjects (54 of 55 analyzed subjects, 98.2%) tested seropositive 26 weeks after the last vaccination, with GMT ranging from 95 to 174 in all 4 groups (Fig. 2B).

Two weeks after the first vaccination, 69–79% of all study subjects appeared seropositive by PRNT with neutralizing GMT ranging from 11 to 36, with the lowest value in the active AD group and the highest GMT in subjects with allergic rhinitis (Fig. 2C and D). Two

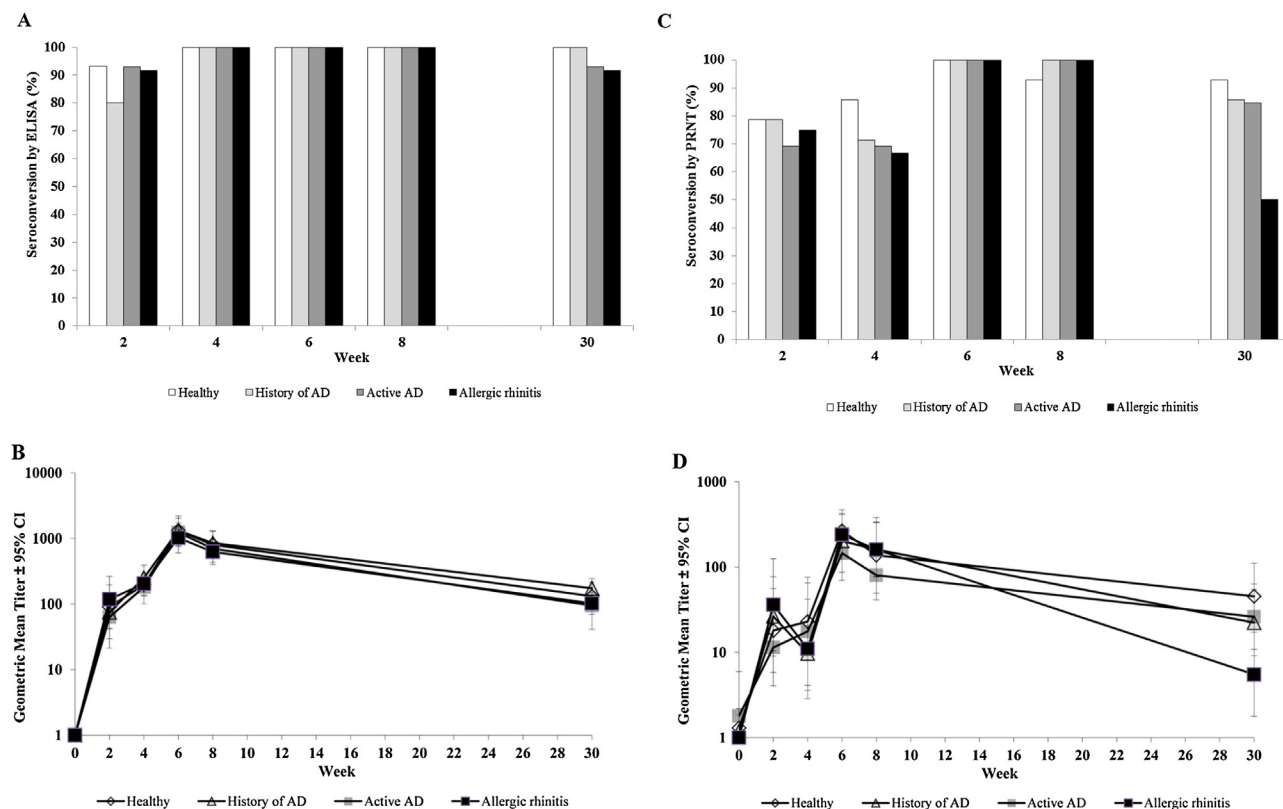


Fig. 2. Kinetics of humoral immune responses following vaccination with MVA. Total and neutralizing antibody responses were analyzed by an ELISA (panels A and B) and PRNT (panels C and D) following vaccination with MVA on Week 0 and 4. The graphs illustrate the seroconversion rates (A: ELISA, C: PRNT) and the geometric mean titers (GMT), calculated by the antilogarithm of the mean $\log_{10}$ titer, together with the 95% CI (B: ELISA, D: PRNT) from the PPS ($N=56$). Seroconversion was defined as post-vaccination titers $\geq$ the assay cut-off (50 in ELISA, 10 in PRNT).

Table 1

Unsolicited adverse reactions during the 28-day follow-up period after both vaccinations with an occurrence a preferred term higher than one single case in the study population (Full Analysis Set, N = 60).

System organ class Preferred term	Healthy (N = 15)			Allergic rhinitis (N = 14)			History of atopic dermatitis (N = 16)			Active atopic dermatitis (N = 15)		
	n	(%)	n*	n	(%)	n*	n	(%)	n*	n	(%)	n*
<i>Administration site conditions</i>												
Inj. site pruritus	3	(20.0)	5	1	(7.1)	1	4	(25.0)	5	5	(33.3)	7
Inj. site discoloration	2	(13.3)	3	1	(7.1)	1	0	(0.0)	0	1	(6.7)	1
Inj. site lymphadenopathy ^A	0	(0.0)	0	0	(0.0)	0	1	(6.3)	1	0	(0.0)	0
Inj. site warmth	0	(0.0)	0	0	(0.0)	0	2	(12.5)	2	1	(6.7)	1
<i>Skin and subcutaneous tissue disorders</i>												
Pruritus (generalized)	0	(0.0)	0	0	(0.0)	0	0	(0.0)	0	2	(13.3)	3
Dermatitis atopic	0	(0.0)	0	0	(0.0)	0	0	(0.0)	0	2	(13.3)	2
<i>Investigations</i>												
Hepatic enzyme increased	0	(0.0)	0	1	(7.1)	2	1	(6.3)	1	0	(0.0)	0
<i>Blood and lymphatic system disorders</i>												
Lymphadenopathy ^A	0	(0.0)	0	1	(7.1)	1	0	(0.0)	0	0	(0.0)	0
Eye disorders												
Conjunctivitis	0	(0.0)	0	1	(7.1)	1	0	(0.0)	0	1	(6.7)	1

N = number of subjects; n (%) = number (percentage) of subjects with at least one report of the specific AE; n* = number of events; ^A“lymphadenopathy” was documented only once each in two different system organ classes (“administration site conditions” and “blood and lymphatic system disorders”). Both events were rated as similar events and therefore displayed in the table.

weeks after the second vaccination, 100% of the subjects in all study groups had seroconverted by PRNT and a 7- to 14-fold rise in GMT was observed. Peak GMT values ranged between 266 in the healthy subject group and 145 in the active AD group. PRNT seroconversion rates measured at the half-year follow-up visit had dropped to values between 93.3% and 54.5% in the four groups. With the exception of a significantly lower GMT at Visit 6 for the allergic rhinitis group ($p = 0.0160$), there were no other significant differences in GMT or seroconversion rates between all groups by PRNT.

The overall results obtained for neutralizing antibody responses were comparable to those determined by ELISA and a correlation

analysis revealed a statistically significant correlation between the two assays ($p < 0.0001$ for the results of study visits at 2, 4 and 6 weeks and $p < 0.05$ for study visits at 8 and 28 weeks).

3.4. Cellular immune responses

When measuring the cytotoxic IFN- γ producing CD8⁺ T cell responses, 31–71% of subjects in the four study groups presented as responders two weeks after the first vaccination with a mean value of $\geq 0.20\%$ vaccinia-specific IFN- γ producing CD8⁺ T cells (Fig. 3). The magnitude of the CD8⁺ T cell response further increased after

Table 2

Overall incidence of solicited local and general adverse events (AE) respectively showing Grade ≥ 3 AE during the 8-day follow-up period after both vaccinations (Full Analysis Set, N = 60).

		Healthy (N = 15)		Allergic rhinitis (N = 14)		History of atopic dermatitis (N = 16)		Active atopic dermatitis (N = 15)	
Symptom		n	(%)	n	(%)	n	(%)	n	(%)
Local symptoms at vaccination site									
Pain	Total	14	(93.3)	14	(100.0)	14	(87.5)	15	(100.0)
	Grade ≥3 ^A	0	(0.0)	2	(14.3)	1	(6.3)	1	(6.7)
Erythema	Total	12	(80.0)	14	(100.0)	15	(93.8)	15	(100.0)
	Grade ≥3	0	(0.0)	0	(0.0)	1	(6.3)	2	(13.3)
Swelling	Total	12	(80.0)	11	(78.6)	13	(81.3)	11	(73.3)
	Grade ≥3	0	(0.0)	1	(7.1)	1	(6.3)	0	(0.0)
Induration	Total	13	(86.7)	12	(85.7)	11	(68.8)	15	(100.0)
	Grade ≥3	0	(0.0)	0	(0.0)	1	(6.3)	1	(6.7)
General symptoms									
Fatigue	Total	5	(33.3)	9	(64.3)	10	(62.5)	7	(46.7)
	Grade ≥3	0	(0.0)	1	(7.1)	0	(0.0)	0	(0.0)
Headache	Total	6	(40.0)	6	(42.9)	7	(43.8)	5	(33.3)
	Grade ≥3	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
Myalgia	Total	0	(0.0)	4	(28.6)	7	(43.8)	2	(13.3)
	Grade ≥3	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
Pyrexia	Total	1	(6.7)	0	(0.0)	2	(12.5)	3	(20.0)
	Grade ≥3	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
Nausea	Total	1	(6.7)	2	(14.3)	1	(6.3)	2	(13.3)
	Grade ≥3	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
Chills	Total	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
	Grade ≥3	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)

N = number of subjects; n (%) = number (percentage) of subjects with at least one report of the specific AE. ^AThe maximum intensity of local injection site (Grade ≥ 3) reactions was scored using the following grades: Redness, swelling, induration ≥ 50 mm; Pain: spontaneously painful, prevents normal activity; Pyrexia: $\geq 40.0^\circ\text{C}$ < 24 h; Headache and Myalgia: severe pain or disabling; Chills: not responsive to narcotic medication; Nausea: no significant intake, requiring i.v. fluids; Fatigue: severe up to bedridden/disabling.

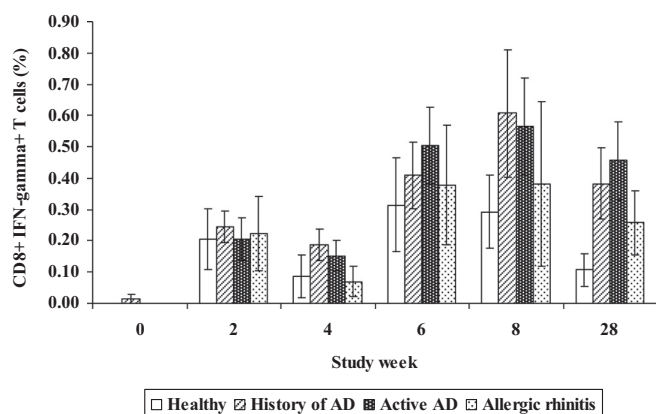


Fig. 3. CD8⁺ IFN- γ positive T cells measured by intracellular cytokine staining following vaccination with MVA.

the second vaccination and reached maximum levels at either study Week 6 (healthy and allergic rhinitis groups) or study Week 8 (active AD and history of AD groups) recording a group mean ranging from 0.31% to 0.61% vaccinia-specific IFN- γ producing CD8⁺ T cells. The response in all four study groups slowly decreased over time to lower responses in 31–57% of study subjects by Week 30. In contrast to the CD8⁺ T cell responses, the majority of the subjects in all treatment recorded no vaccinia-specific IFN- γ producing CD4⁺ T cells at any time point post vaccination (data not shown). No statistical differences in T cell responses were observed between groups at any visit.

4. Discussion

To minimize the risks for study participants in this first MVA trial in individuals diagnosed with AD, a limited number of 14–16 subjects per group and only subjects with a mild stage of active AD (defined as a SCORAD of ≤ 15) were enrolled. This study has provided encouraging results demonstrating that MVA appeared equally well tolerated and immunogenic in healthy subjects, as in individuals with active or a history of AD, or subjects with allergic rhinitis.

Neither any serious adverse reactions, nor cases of EV or myo/pericarditis were reported during the study. The local adverse reactions after vaccinations such as pain, redness and swelling were assessed as mild to moderate resolving within 3–4 days after the vaccination. They have been reported following MVA in previous trials in healthy subjects [18,19,29,34] and are typical following a subcutaneously administered vaccine [30].

Two subjects experienced a slight worsening of their active AD, including pruritus after the second vaccination with MVA. Although this was assessed as possibly related to the study vaccination due to the temporal relationship, flare-ups spontaneously occur in a population with active AD and a firm association with MVA can only be evaluated in larger clinical studies. Apart from these isolated events, subjects with AD did not experience any significant cutaneous reactions following vaccinations with MVA.

While AD is associated with a panel of innate and adaptive immune deficiencies in the skin, such as the reduced expression of antimicrobial peptides (e.g. cathelicidin) and an impaired T_H1 local response [5,6], there is also evidence of a systemic allergic response associated with elevated serum IgE, eosinophilia and an increased systemic T_H2 response [6]. Such a predisposition of subjects with AD to an allergic type immune response may reduce the effectiveness of a live smallpox vaccine that is associated with the induction of a predominately T_H1 immune response [18]. However, the kinetics and magnitude of the immune responses induced by MVA in this

study mirrored those previously reported in earlier clinical studies evaluating MVA in healthy subjects [31]. Indeed, there were no differences in the immunogenicity recorded in healthy subjects or any of the subjects with either a history or active AD, allowing the conclusion that MVA was equally immunogenic in all subjects enrolled into this study.

An impaired local T_H1 response and low cathelicidin levels are assumed to predispose inflamed skin for virus dissemination with an increased risk of developing EV [5,6]. Therefore, the subcutaneous route of MVA probably allows the immune abnormalities in the skin of AD subjects to be avoided, resulting in the induction of the same immune response observed in healthy subjects, which has previously been shown to be comparable to that of traditional smallpox vaccines [18,24]. This is also supported by animal data using a model mimicking AD that has demonstrated the administration of a traditional smallpox vaccine to sensitized skin resulted in satellite lesions and an impaired T_H1 response, while the administration to distant skin sites or via a parental route induced a T_H1 biased response observed in normal mice [9].

As previously reported for healthy subjects, vaccination with MVA was associated with a strong CD8⁺ T cells response and the peak 40–70% responder rates recorded in all subjects, regardless of their AD status in the present study, has previously been reported using ICS following vaccination with traditional smallpox vaccines [32]. However, while lower CD4⁺ T cells have been recorded following vaccination with MVA [19] and traditional smallpox vaccines [32], the inability to demonstrate a CD4⁺ response in this study in any of the subjects likely reflects that the quality and functionality of the PBMC may have been affected during the processing of blood [33].

The small number of study subjects enrolled in this Phase I study, as well as their mild AD condition (suggesting limited impairment of their immunologic function) requires a cautious interpretation of the results. However, the promising results of the present trial justify larger clinical trials with individuals suffering from more severe AD, which are expected to shed more light on the safety and immunogenicity of MVA in this population.

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