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Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Schneider M, Narciso-Abraham M, Hadl S, et al. Safety and immunogenicity of a single-shot live-attenuated chikungunya vaccine: a double-blind, multicentre, randomised, placebo-controlled, phase 3 trial. *Lancet* 2023; published online June 12. https://doi.org/10.1016/S0140-6736(23)00641-4.

Appendix

Supplementary Table 1: Inclusion and Exclusion Criteria for Participants

Inclusion Criteria

Participants who meet all of the following criteria are eligible for this study:

1. Participant is 18 years of age or above[1] on the day of screening (Visit 0);
2. Participant has an understanding of the study and its procedures, agrees to its provisions, and voluntarily gives written informed consent prior to any study-related procedures;
3. Participant is **generally healthy**[2] as determined by the Investigator's clinical judgement based on medical history, physical examination and screening laboratory tests;
4. If participant is of childbearing potential:
    a) Participant has practiced an adequate method of contraception (see below) during the 30 days before screening (Visit 0);
    b) Participant has a negative serum or urine pregnancy test at screening (Visit 0) or Visit 1, respectively;
    c) Participant agrees to employ adequate birth control measures for the first three months post-vaccination (i.e. until day 85, Visit 4). This includes one of the following measures:
        - Hormonal contraceptives (e.g. implants, birth control pills, patches);
        - Intrauterine hormone-release systems and intrauterine device;
        - Barrier type of birth control measure (e.g. diaphragms, cervical caps);
        - Vasectomy in the male sex partner ≥ 3 months prior to first vaccination;
        - Sexual abstinence;
        - Same sex relationships.

Exclusion Criteria

Participants who meet any of the following criteria are not eligible for this study:

1. Participant has had a CHIKV infection in the past, including suspected CHIKV infection; is taking medication or other treatment for unresolved symptoms attributed to a previous CHIKV infection; or has participated in a clinical study involving an investigational CHIKV vaccine;
2. Participant has an acute or recent infection (and who is not symptom-free in the week prior to the Screening Visit (Visit 0);
3. Participant tests positive for human immunodeficiency virus (HIV), hepatitis B surface antigen (HBsAg) or hepatitis C virus (HCV);
4. Participant has received another live virus vaccine within 28 days or inactivated vaccine within 14 days prior to vaccination in this study or plans to receive a vaccine within 28 days or 14 days after vaccination, respectively;
5. Participant has abnormal findings in any required study investigations (including medical history, physical examination, and clinical laboratory) considered clinically relevant by the Investigator which pose a risk for participation in the study based on his/her judgement;
6. Participant has a medical history of or currently has acute or progressive, unstable or uncontrolled clinical conditions (e.g. cardiovascular, respiratory, neurologic, psychiatric, or rheumatologic conditions) that poses a risk for participation in the study, based on Investigators clinical judgement. Examples include individuals with poorly controlled or unstable disease, ongoing suspected or active

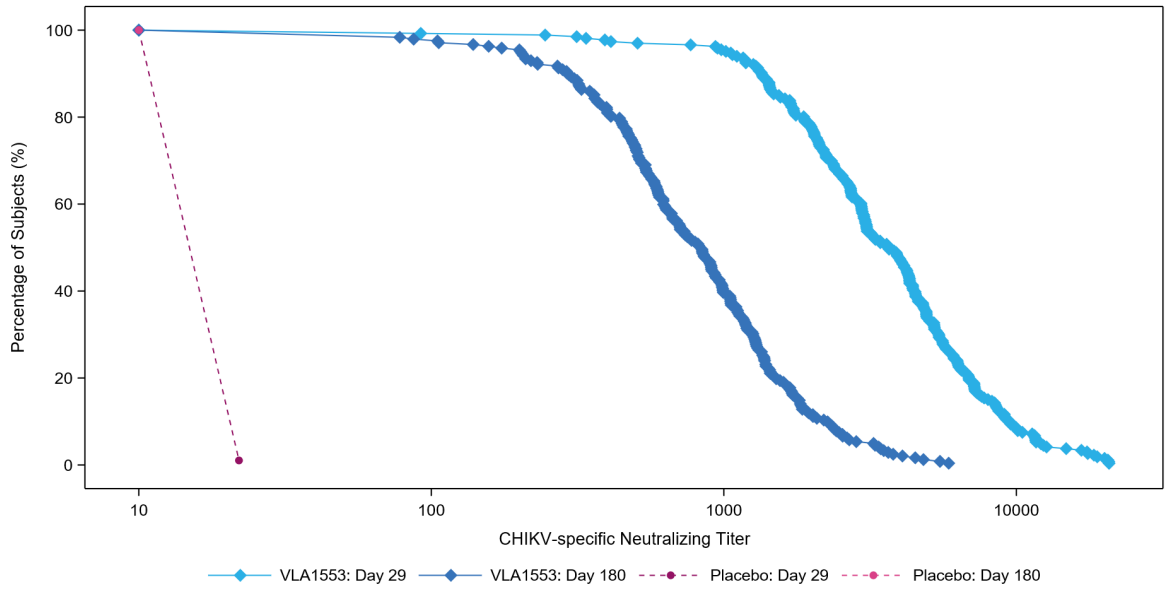
[1] From the 18th birthday or above.

[2] Participants are considered **generally healthy** if (1) any chronic illness/condition, e.g. hypertension, type 2 diabetes mellitus, or hyperlipidemia is stable and well-controlled on therapy for the past 6 months, and (2) they do not have a disease that is identified as an exclusion criterion.

inflammation, or poor compliance with pharmacologic treatment, or presence of high risk comorbidities (e.g. significant cardiopulmonary disease);

7. Participant has a history of immune-mediated or clinically relevant arthritis/arthralgia;
8. Participant has a history of malignancy in the past 5 years other than squamous cell or basal cell skin cancer. If there has been surgical excision or treatment more than 5 years ago that is considered to have achieved a cure, the participant may be enrolled. A history of hematologic malignancy is a permanent exclusion. Participants with a history of skin cancer must not be vaccinated at the previous tumor site;
9. Participant has a known or suspected defect of the immune system, such as participants with congenital or acquired immunodeficiency, including infection with HIV, status post organ transplantation or immuno-suppressive therapy within 4 weeks prior to Visit 1. Immuno-suppressive therapy is defined as administration of chronic (longer than 14 days) prednisone or equivalent ≥ 0.05 mg/kg/day within 4 weeks prior to study entry, radiation therapy or immunosuppressive cytotoxic drugs/ monoclonal antibodies in the previous 3 years; topical and inhaled steroids are allowed.
10. Participant has a history of any vaccine related contraindicating event (e.g., anaphylaxis, allergy to components of the candidate vaccine, other known contraindications);
11. Participant presents with clinical conditions representing a contraindication to intramuscular vaccination and blood draws;
12. Participant is pregnant (positive serum or urine pregnancy test at screening or Visit 1, respectively), has plans to become pregnant during the first three months post-vaccination or lactating at the time of enrollment;
13. Participant has donated blood, blood fractions or plasma within 30 days or received blood-derived products (e.g. plasma) within 90 days prior to vaccination in this study or plans to donate blood or use blood products until day 180 of the study;
14. Participant has a rash, dermatological condition or tattoos that would, in the opinion of the Investigator, interfere with injection site reaction rating;
15. Participant has a known or suspected problem with alcohol or drug abuse as determined by the Investigator;
16. Participant has any condition that, in the opinion of the Investigator, may compromise the participants well-being, might interfere with evaluation of study endpoints, or would limit the participant's ability to complete the study;
17. Participant is committed to an institution (by virtue of an order issued either by the judicial or the administrative authorities);
18. Participant has participated in another clinical study involving an investigational medicinal product (IMP) or device within 30 days prior to study enrollment or is scheduled to participate in another clinical study involving an IMP, or device during the course of this study;
19. Participant is a member of the team conducting the study or in a dependent relationship with one of the study team members. Dependent relationships include close relatives (i.e., children, partner/spouse, siblings, parents) as well as employees of the Investigator or site personnel conducting the study.

Supplementary Figure 1: Reverse Cumulative Distribution Curve on Day 29 and Day 180 (Per-Protocol Population)



Neutralizing antibodies to the vaccine were evaluated from clinical specimen (human serum) using a microneutralization assay (µPRNT). A $\mu PRNT_{50}$ titre was defined as the serum dilution inducing a 50% plaque reduction in a micro plaque reduction neutralization test. Reverse cumulative distribution curve of CHKV-specific neutralizing antibodies on day 29 and day 180 (PP Population).

Supplementary Table 2: Demographics of the Immunogenicity Subset

Characteristic	VLA1553 (N=375)	Placebo (N=126)	Total (N=501)
Sex [n (%)]			
Female	214 (57·1)	78 (61·9)	292 (58·3)
Male	161 (42·9)	48 (38·1)	209 (41·7)
Race [n (%)]			
American Indian or Alaska Native	5 (1·3)	0	5 (1·0)
Asian	5 (1·3)	3 (2·4)	8 (1·6)
Black or African American	54 (14·4)	13 (10·3)	67 (13·4)
Native Hawaiian or other Pacific Islander	3 (0·8)	1 (0·8)	4 (0·8)
White	296 (78·9)	104 (82·5)	400 (79·8)
Other	12 (3·2)	5 (4·0)	17 (3·4)
Ethnicity [n (%)]			
Hispanic or Latino	40 (10·7)	17 (13·5)	57 (11·4)
Not Hispanic or Latino	329 (87·7)	108 (85·7)	437 (87·2)
Not Reported	5 (1·3)	1 (0·8)	6 (1·2)
Unknown	1 (0·3)	0	1 (0·2)
Age (years)			
n	375	126	501
Median	49.0	51·0	50·0
Q1, Q3	36.0, 63.0	34·0, 63·0	36·0, 63·0
Min, Max	18, 82	18, 76	18, 82
Age Group [n (%)]			
≥18 years – 64 years (stratum A)	293 (78·1)	98 (77·8)	391 (78·0)
≥65 years (stratum B)	82 (21·9)	28 (22·2)	110 (22·0)
Weight (kg)			
n	369	126	495
Median	86·50	85·95	86·30
Q1, Q3	73·08, 99·60	70·30, 98·90	72·60, 99·60
Min, Max	44·9, 179·6	50·2, 142·4	44·9, 179·6
Height (cm)			
n	370	126	496
Mean (std)	169·15 (10·09)	169·15 (9·66)	169·15 (9·97)
Min, Max	128·8, 199·4	144·7, 198·1	128·8, 199·4
BMI (kg/m2)			
n	369	126	495
Median	29·70	30·09	29·84
Q1, Q3	25·74, 35·04	25·76, 34·48	25·74, 34·92
Min, Max	16·7, 102·3	17·1, 53·7	16·7, 102·3

std=standard deviation, BMI=Body Mass Index

Supplementary Table 3: Seroconversion Rate for CHIKV-Specific Neutralizing Antibodies by Visit (Per-Protocol Population)

	18 to 64 Years		≥ 65 Years		Total	
Time Point[a] [n] Statistic	**VLA1553 (N=207)**	**Placebo (N=73)**	**VLA1553 (N=59)**	**Placebo (N=23)**	**VLA1553 (N=266)**	**Placebo (N=96)**
day 29	207	73	59	23	266	96
Participants with seroconversion [n (%)]	205 (99·0)	1 (1·4)	59 (100·0)	0	264 (99·2)	1 (1·0)
95% CI for seroconversion rate	96·6, 99·9	0·0, 7·4	93·9, 100·0	0·0, 14·8	97·3, 99·9	0·0, 5·7
p-value to placebo[b]	***<0·0001***		***<0·0001***		***<0·0001***	
day 180	184	68	58	23	242	91
Participants with seroconversion [n (%)]	181 (98·4)	0	57 (98·3)	0	238 (98·3)	0
95% CI for seroconversion rate	95·3, 99·7	0·0, 5·3	90.8, 100·0	0·0, 14·8	95·8, 99·5	0·0, 4·0
p-value to placebo[b]	***<0·0001***		***<0·0001***		***<0·0001***	

CHIKV=chikungunya virus; CI=confidence interval; µPRNT$_{50}$ titre= serum dilution incuding a 50% plaque reduction in a micro plaque reduction neutralization test; PP=per protocol.

a Number of participants with non-missing titers at the specified time point.

b P-value from Fisher's Exact test.

Percentages are based on the number of participants with non-missing titres at the visit. Seroconversion was defined as µPRNT$_{50}$ titre ≥20 for µPRNT baseline negative participants (<20) or >4-fold titre increase over baseline for µPRNT baseline positive participant (≥20). Two-sided 95% exact (Clopper-Pearson) CIs presented.

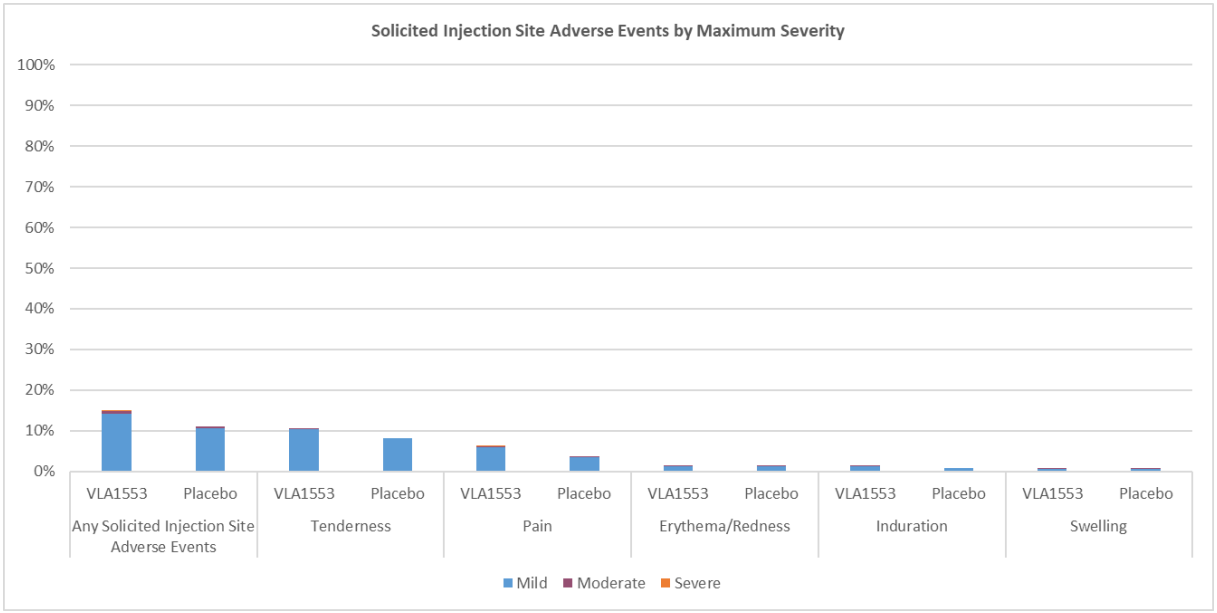
Supplementary Table 4: Summary of Fold Increase of CHIKV-Specific Neutralizing Antibody Titres by Visit (Per-Protocol Population)

	18 to 64 Years		≥ 65 Years		Total	
Time Point Statistic	**VLA1553 (N=207)**	**Placebo (N=73)**	**VLA1553 (N=59)**	**Placebo (N=23)**	**VLA1553 (N=266)**	**Placebo (N=96)**
day 8						
n	198	70	53	23	251	93
Mean fold increase (std)	4·38 (30·28)	1·03 (0·18)	2·06 (3·31)	1·00 (0·00)	3·89 (26·94)	1·02 (0·15)
Median	1·00	1·00	1·00	1·00	1·00	1·00
Q1, Q3	1·00, 1·00	1·00, 1·00	1·00, 1·00	1·00, 1·00	1·00, 1·00	1·00, 1·00
day 29						
n	207	73	59	23	266	96
Mean fold increase (std)	460·27 (384·05)	1·02 (0·14)	507·70 (412·47)	1·00 (0·00)	470·79 (390·23)	1·01 (0·12)
Median	342·20	1·00	422·40	1·00	364·05	1·00
Q1, Q3	202·40, 597·90	1·00, 1·00	223·90, 672·70	1·00, 1·00	207·60, 608·40	1·00, 1·00
day 180						
n	184	68	58	23	242	91
Mean fold increase (std)	108·81 (97·24)	1·00 (0·00)	102·09 (79·43)	1·00 (0·00)	107·20 (93·17)	1·00 (0·00)
Median	80·70	1·00	92·95	1·00	82·10	1·00
Q1, Q3	48·20, 135·15	1·00, 1·00	47·30, 137·10	1·00, 1·00	48·00, 135·80	1·00, 1·00

CHIKV=chikungunya virus; std=standard deviation; µPRNT=micro plaque reduction neutralization test; NT=neutralizing antibody titer; PP=per protocol.
The fold increase from baseline in the CHIKV-specific NT was defined as [µPRNT result at nominal time point/ µPRNT result at baseline].

Supplementary Figure 2: Bar Chart of Maximum Severity of Solicited Adverse Events by Symptom (Safety Population)

A



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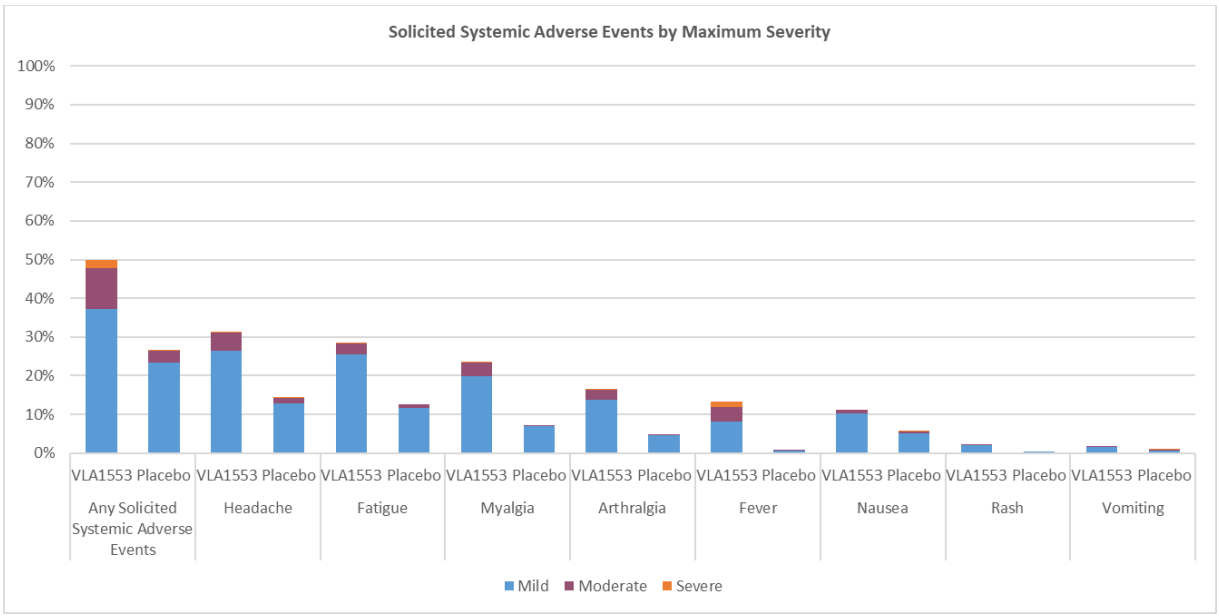

Safety and tolerability of the vaccine were assessed in the safety population. Participant eDiaries were used for the collection of daily oral body temperature, solicited injection site and systemic adverse events up to 10 days post-vaccination. Participants with solicited AEs after vaccination with VLA1553, and placebo by maximum severity.

Supplementary Table 5: Solicited Injection Site Adverse Events (Safety Population)

Category [n (%) m]	VLA1553 (N=3,082)	Placebo (N=1,033)	Total (N=4,115)
Any Solicited Injection Site Adverse Events	463 (15·0) 630	115 (11·1) 153	578 (14·0) 783
95% CI	13·8, 16·3	9·3, 13·2	13·0, 15·1
Tenderness	328 (10·6)	84 (8·1)	412 (10·0)
95% CI	9·5, 11·7	6·5, 10·0	9·1, 11·0
Pain	191 (6·2)	38 (3·7)	229 (5·6)
95% CI	5·4, 7·1	2·6, 5·0	4·9, 6·3
Erythema/Redness	46 (1·5)	15 (1·5)	61 (1·5)
95% CI	1·1, 2·0	0·8, 2·4	1·1, 1·9
Induration	44 (1·4)	8 (0·8)	52 (1·3)
95% CI	1·0, 1·9	0·3, 1·5	0·9, 1·7
Swelling	21 (0·7)	8 (0·8)	29 (0·7)
95% CI	0·4, 1·0	0·3, 1·5	0·5, 1·0

CI=confidence interval; n=Number of participants; m=Number of events; PT=preferred term; SOC=system organ class
Note: Adverse events were coded using MedDRA version 24.1. For each SOC and PT, participants were included only once, even if they experienced multiple events in that SOC or PT.

Supplementary Table 6: Solicited Systemic Adverse Events (Safety Population)

Category [n (%) m]	VLA1553 (N=3,082)	Placebo (N=1,033)	Total (N=4,115)
Any Solicited Systemic Adverse Events	1,547 (50·2) 3,990	278 (26·9) 488	1,825 (44·3) 4,478
95% CI	48·4, 52·0	24·2, 29·7	42·8, 45·9
Headache	969 (31·4)	151 (14·6)	1,120 (27·2)
95% CI	29·8, 33·1	12·5, 16·9	25·9, 28·6
Fatigue	879 (28·5)	130 (12·6)	1,009 (24·5)
95% CI	26·9, 30·2	10·6, 14·8	23·2, 25·9
Myalgia	735 (23·8)	76 (7·4)	811 (19·7)
95% CI	22·4, 25·4	5·8, 9·1	18·5, 21·0
Arthralgia	520 (16·9)	50 (4·8)	570 (13·9)
95% CI	15·6, 18·2	3·6, 6·3	12·8, 14·9
Fever	414 (13·4)	8 (0·8)	422 (10·3)
95% CI	12·2, 14·7	0·3, 1·5	9·3, 11·2
Nausea	345 (11·2)	58 (5·6)	403 (9·8)
95% CI	10·1, 12·4	4·3, 7·2	8·9, 10·7
Rash	70 (2·3)	5 (0·5)	75 (1·8)
95% CI	1·8, 2·9	0·2, 1·1	1·4, 2·3
Vomiting	58 (1·9)	10 (1·0)	68 (1·7)
95% CI	1·4, 2·4	0·5, 1·8	1·3, 2·1

CI=confidence interval; n=Number of participants; m=Number of events; PT=preferred term; SOC=system organ class
Note: Adverse events were coded using MedDRA version 24.1. For each SOC and PT, participants were included only once, even if they experienced multiple events in that SOC or PT.

Supplementary Table 7: Unsolicited and Related Unsolicited AEs by System Organ Class and Preferred Term with a frequency ≥1% in VLA1553 Study Arm (Safety Population)

System Organ Class Preferred Term [n (%)]	VLA1553 (N=3,082)	Placebo (N=1,033)	Total (N=4,115)
Any Unsolicited AE up to Day 29	687 (22·3) 1108 20·8, 23·8	138 (13·4) 219 11·3, 15·6	825 (20·0) 1327 18·8, 21·3
Any Unsolicited AE up to Day 180	933 (30·3) 1795 28·7, 31·9	248 (24·0) 430 21·4, 26·7	1181 (28·7) 2225 27·3, 30·1
Any Related Unsolicited AE	303 (9·8) 449 8·8, 10·9	48 (4·6) 68 3·4, 6·1	351 (8·5) 517 7·7, 9·4
General disorders and administration site conditions	81 (2·6) 86 2·1, 3·3	12 (1·2) 13 0·6, 2·0	93 (2·3) 99 1·8, 2·8
Chills	51 (1·7) 51 1·2, 2·2	2 (0·2) 2 0·0, 0·7	53 (1·3) 53 1·0, 1·7
Blood and lymphatic system disorders	67 (2·2) 83 1·7, 2·8	5 (0·5) 5 0·2, 1·1	72 (1·7) 88 1·4, 2·2
Neutropenia[a]	31 (1·0) 31 0·7, 1·4	1 (0·1) 1 0·0, 0·5	32 (0·8) 32 0·5, 1·1

[a] Safety Laboratory Sample only taken from Immunogenicity subset.

Supplementary Table 8: Rate of Subjects Reaching Pre-Specified Fold Increase of CHIKV-Specific Neutralizing Antibody Titers by Visit (Per-Protocol Population)

	18 to 64 Years		≥ 65 Years		Total	
Time Point Statistic	**VLA1553 (N=207)**	**Placebo (N=73)**	**VLA1553 (N=59)**	**Placebo (N=23)**	**VLA1553 (N=266)**	**Placebo (N=96)**
Day 29						
n	207	73	59	23	266	96
Subjects reaching 4-fold increase [n (%)]	205 (99·0)	0	59 (100)	0	264 (99·2)	0
95% CI[a]	96·6, 99·9	0·0, 4·9	93·9, 100·0	0·0, 14·8	97·3, 99·9	0·0, 3·8
Subjects reaching 8-fold increase [n (%)]	205 (99·0)	0	59 (100)	0	264 (99·2)	0
95% CI[a]	96.6, 99.9	0·0, 4·9	93·9, 100·0	0·0, 14·8	97·3, 99·9	0·0, 3·8
Subjects reaching 16-fold increase [n (%)]	204 (98·6)	0	59 (100)	0	263 (98·9)	0
95% CI[a]	95.8, 99.7	0·0, 4·9	93·9, 100·0	0·0, 14·8	96.7, 99.8	0·0, 3·8
Subjects reaching 64-fold increase [n (%)]	200 (96·6)	0	57 (96·6)	0	257 (96·6)	0
95% CI[a]	93·2, 98·6	0·0, 4·9	88·3, 99·6	0·0, 14·8	93·7, 98·4	0·0, 3·8
Day 180						
n	184	68	58	23	242	91
Subjects reaching 4-fold increase [n (%)]	181 (98·4)	0	57 (98·3)	0	238 (98·3)	0
95% CI[a]	95·3, 99·7	0·0, 5·3	90·8, 100·0	0·0, 14·8	95·8, 99·5	0·0, 4·0
Subjects reaching 8-fold increase [n (%)]	181 (98·4)	0	56 (96·6)	0	237 (97·9)	0
95% CI[a]	95·3, 99·7	0·0, 5·3	88·1, 99·6	0·0, 14·8	95·2, 99·3	0·0, 4·0
Subjects reaching 16-fold increase [n (%)]	177 (96·2)	0	55 (94·8)	0	232 (95·9)	0
95% CI[a]	92·3, 98·5	0·0, 5·3	85·6, 98·9	0·0, 14·8	92·5, 98·0	0·0, 4·0
Subjects reaching 64-fold increase [n (%)]	106 (57·6)	0	35 (60·3)	0	141 (58·3)	0
95% CI[a]	50·1, 64·8	0·0, 5·3	46·6, 73·0	0·0, 14·8	51·8, 64·5	0·0, 4·0

CHIKV=chikungunya virus; µPRNT=micro plaque reduction neutralization test; NT=neutralizing antibody titer; PP=per protocol.
Percentages are based on the number of participants with non-missing titers at the visit.
The fold increase from baseline in the CHIKV-specific NT is defined as [µPRNT result at nominal time point / µPRNT result at baseline].
a. Two-sided 95% exact (Clopper-Pearson) CI.

Supplementary Table 9: GMTs for CHIKV-Specific Neutralizing Antibodies by Visit (Per-Protocol Population)

	18 to 64 Years		≥ 65 Years		Total	
Time Point [n] Statistic	**VLA1553 (N=207)**	**Placebo (N=73)**	**VLA1553 (N=59)**	**Placebo (N=23)**	**VLA1553 (N=266)**	**Placebo (N=96)**
day 29 (n)	207	73	59	23	266	96
Geometric Mean	3273·7	10·1	3688·8	10·0	3361·6	10·1
95% CI for GM	2860·93, 3746·04	9·89, 10·33	2938·94, 4630·10	10·00, 10·00	2993·83, 3774·45	9·92, 10·25
n[a]	207	73	59	23	266	96
LS Mean (SE)[b]	3273·70 (1·06)	10·11 (1·10)	3688·85 (1·10)	10·00 (1·17)	3440·54 (1·06)	10·30 (1·09)
95% Confidence Interval[b]	2915·10, 3676·43	8·31, 12·29	3043·26, 4471·38	7·35, 13·61	3068·42, 3857·78	8·66, 12·26
Difference in GMT[b]						
Difference in LS Mean (SE)[c]	323·85 (1·12)		368·88 (1·20)		333·90 (1·10)	
95% Confidence Interval[c]	258·03, 406·47		256·53, 530·46		275·25, 405·05	
p-value[c]	***<0·0001***		***<0·0001***		***<0·0001***	
day 180 (n)	184	68	58	23	242	91
Geometric Mean	755·1	10·0	742·8	10·0	752·1	10·0
95% CI for GM	656·01, 869·16	10·00, 10·00	578·36, 954·00	10·00, 10·00	665·91, 849.52	10·00, 10·00
n[a]	184	68	58	23	242	91
LS Mean (SE)[b]	755·10 (1·06)	10·00 (1·11)	742·80 (1·11)	10·00 (1·18)	749·83 (1·06)	9·97 (1·09)
95% Confidence Interval[b]	669·62, 851·50	8·21, 12·19	601·33, 917·56	7·15, 13·99	667·04, 842·88	8·35, 11·90
Difference in GMT[b]						
Difference in LS Mean (SE)[c]	75·51 (1·12)		74·28 (1·22)		75·20 (1·11)	
95% Confidence Interval[c]	59·92, 95·16		49·97, 110·43		61·64, 91·74	
p-value[c]	***<0·0001***		***<0·0001***		***<0·0001***	

GM = geometric mean; std = standard deviation; LS = least squares; SE = standard error; GMT = geometric mean titer.
n = number of subjects with available result.
a. n is the number of subjects that contribute data at least once in the primary analysis model.
b. LS means, standard errors, confidence intervals, and p-values are from an analysis of covariance (ANCOVA) model with fixed factors for study arm and age stratum.
c. p-values, LS mean differences and associated confidence intervals are presented for the VLA1553 arm minus Placebo treatment arm.
Note: The ANCOVA model is applied to the log-transformed titers, and back-transformed results are displayed for the LS mean and difference. The difference in GMT is a ratio of the LS means.