Supplementary Materials

Supplementary Data Table 1: All related adverse events from baseline to ATI start, by grade (ordered by decreasing frequency for systemic AEs).

PT	Duration* (days)		Grade 1				Grade 2				Grade 3				Total			
	Placebo	Vaccine	Placebo		Vaccine		Placebo		Vaccine		Placebo		Vaccine		Placebo		Vaccine	
			N pat (%)	N AE	N pat (%)	N AE	N pat (%)	N AE	N pat (%)	N AE	N pat (%)	N AE	N pat (%)	N AE	N pat (%)	N AE	N pat (%)	N AE
Any AEs	**1 (1 - 29)**	**2 (1 - 93)**	**15 (100)**	**97**	**30 (100)**	**258**	**7 (46.7)**	**14**	**20 (66.7)**	**70**	**0 (0)**	**0**	**1 (3.3)**	**1**	**15 (100)**	**111**	**30 (100)**	**329**
Local AES	**1 (1 - 29)**	**2 (1 - 7)**	**6 (40)**	**12**	**29 (96.7)**	**92**	**0 (0)**	**0**	**12 (40)**	**16**	**0 (0)**	**0**	**0 (0)**	**0**	**6 (40)**	**12**	**29 (96.7)**	**108**
Injection site erythema	1 (1 - 1)	3.5 (1 - 5)	0 (0)	0	3 (10)	3	0 (0)	0	1 (3.3)	1	0 (0)	0	0 (0)	0	0 (0)	0	3 (10)	4
Injection site inflammation	1 (1 - 1)	2 (1 - 7)	0 (0)	0	3 (10)	3	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	3 (10)	3
Injection site pain	1 (1 - 29)	2 (1 - 7)	6 (40)	12	28 (93.3)	86	0 (0)	0	11 (36.7)	15	0 (0)	0	0 (0)	0	6 (40)	12	29 (96.7)	101
Systemic AES	**1 (1 - 8)**	**1 (1 - 93)**	**15 (100)**	**85**	**27 (90)**	**166**	**7 (46.7)**	**14**	**18 (60)**	**54**	**0 (0)**	**0**	**1 (3.3)**	**1**	**15 (100)**	**99**	**27 (90)**	**221**
Asthenia/Fatigue	1 (1 - 5)	1 (1 - 15)	11 (73.3)	29	21 (70)	62	4 (26.7)	4	7 (23.3)	10	0 (0)	0	1 (3.3)	1	11 (73.3)	33	22 (73.3)	73
Headache	1 (1 - 4)	2 (1 - 10)	10 (66.7)	21	9 (30)	22	4 (26.7)	6	12 (40)	19	0 (0)	0	0 (0)	0	12 (80)	27	18 (60)	41
Myalgia	1 (1 - 1)	2 (1 - 4)	3 (20)	4	18 (60)	22	0 (0)	0	8 (26.7)	13	0 (0)	0	0 (0)	0	3 (20)	4	19 (63.3)	35

Diarrhoea	1.5 (1 - 3)	2 (1 - 10)	6 (40)	9	11 (36.7)	17	1 (6.7)	1	2 (6.7)	2	0 (0)	0	0 (0)	0	6 (40)	10	13 (43.3)	19
Hyperhidrosis	2.5 (1 - 7)	1 (1 - 2)	5 (33.3)	5	10 (33.3)	10	1 (6.7)	1	2 (6.7)	3	0 (0)	0	0 (0)	0	6 (40)	6	11 (36.7)	13
Nausea	1 (1 - 4)	1 (1 - 3)	6 (40)	6	8 (26.7)	10	0 (0)	0	1 (3.3)	1	0 (0)	0	0 (0)	0	6 (40)	6	8 (26.7)	11
Decreased appetite	1.5 (1 - 3)	2 (1 - 3)	4 (26.7)	4	5 (16.7)	7	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	4 (26.7)	4	5 (16.7)	7
Pyrexia	1 (1 - 1)	1 (1 - 2)	0 (0)	0	5 (16.7)	5	0 (0)	0	1 (3.3)	1	0 (0)	0	0 (0)	0	0 (0)	0	6 (20)	6
Abdominal pain	2 (1 - 4)	2 (1 - 3)	2 (13.3)	2	3 (10)	3	1 (6.7)	1	1 (3.3)	1	0 (0)	0	0 (0)	0	3 (20)	3	3 (10)	4
Vomiting	2 (2 - 2)	1 (1 - 1)	1 (6.7)	1	2 (6.7)	2	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	1 (6.7)	1	2 (6.7)	2
Abdominal distension	1 (1 - 1)	1 (1 - 1)	0 (0)	0	1 (3.3)	1	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	1 (3.3)	1
Cough	1 (1 - 1)	2 (2 - 2)	0 (0)	0	1 (3.3)	1	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	1 (3.3)	1
Dizziness	1 (1 - 1)	1 (1 - 1)	0 (0)	0	1 (3.3)	1	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	1 (3.3)	1
Flatulence	1 (1 - 1)	3 (3 - 3)	0 (0)	0	1 (3.3)	1	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	1 (3.3)	1
Hordeolum	1 (1 - 1)	3 (3 - 3)	0 (0)	0	1 (3.3)	1	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	1 (3.3)	1
Odynophagia	5 (5 - 5)	3 (3 - 3)	0 (0)	0	1 (3.3)	1	1 (6.7)	1	0 (0)	0	0 (0)	0	0 (0)	0	1 (6.7)	1	1 (3.3)	1
Oral herpes	5 (5 - 5)	11 (11 - 11)	1 (6.7)	1	0 (0)	0	0 (0)	0	1 (3.3)	1	0 (0)	0	0 (0)	0	1 (6.7)	1	1 (3.3)	1

Pruritus	1 (1 - 1)	93 (93 - 93)	0 (0)	0	0 (0)	0	0 (0)	0	1 (3.3)	1	0 (0)	0	0 (0)	0	0 (0)	0	1 (3.3)	1
Rash	1 (1 - 1)	2 (2 - 2)	0 (0)	0	0 (0)	0	0 (0)	0	1 (3.3)	1	0 (0)	0	0 (0)	0	0 (0)	0	1 (3.3)	1
Upper respiratory tract infection	1 (1 - 1)	5 (5 - 5)	0 (0)	0	0 (0)	0	0 (0)	0	1 (3.3)	1	0 (0)	0	0 (0)	0	0 (0)	0	1 (3.3)	1
Arthralgia	1 (1 - 1)	1 (1 - 1)	1 (6.7)	1	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	1 (6.7)	1	0 (0)	0
Dermatitis acneiform	1 (1 - 1)	1 (1 - 1)	1 (6.7)	1	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	1 (6.7)	1	0 (0)	0
Hepatic enzyme increased	8 (8 - 8)	1 (1 - 1)	1 (6.7)	1	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	1 (6.7)	1	0 (0)	0

Local: local pain, inflammation and site erythema occurred in the site of injection. Systemic: rest of AE.
SOC: System Organ Class and PT: Preferred Term, according to MedDRA v20.1.
N pat (%): Absolute number of participants reporting one AE is shown, and percentages are calculated as the number of participants (N) reporting an AE of a specific severity divided by the total number of Safety Population participants.
Grading was performed according to the DAIDS grading table ('1 - Mild', '2 - Moderate', '3 - Severe', '4 - Life-threatening, '5 – Death'). In case various severities were reported from start to end date of an AE, the highest grade was registered.
An AE ended the day the patient restored his/her baseline condition. When a given AE ended and occurred the next day again, it was recorded as a new AE.
No related grade 4 was reported.
The duration of any AE is computed as: end date - start date + 1; AE that lasted <24h are recorded as 1 day of duration.

Supplementary Data Table 2: All adverse events related to the three DNA.HTI/Placebo administrations in the DDDMM/PPPPP regimen (ordered by decreasing frequency for systemic AEs in the vaccine group)

PT	Duration* (days)		Grade 1				Grade 2				Total			
	Placebo	Vaccine	Placebo		Vaccine		Placebo		Vaccine		Placebo		Vaccine	
			N pat (%)	N AE	N pat (%)	N AE	N pat (%)	N AE	N pat (%)	N AE	N pat (%)	N AE	N pat (%)	N AE
Any AEs	**1 (1 - 29)**	**1 (1 - 15)**	**15 (100)**	**56**	**23 (76.7)**	**72**	**4 (26.7)**	**6**	**6 (20)**	**6**	**15 (100)**	**62**	**24 (80)**	**78**
Local AES	**1 (1 - 29)**	**1 (1 - 7)**	**5 (33.3)**	**7**	**11 (36.7)**	**15**	**0 (0)**	**0**	**0 (0)**	**0**	**5 (33.3)**	**7**	**11 (36.7)**	**15**
Injection site pain	1 (1 - 29)	1 (1 - 7)	5 (33.3)	7	11 (36.7)	15	0 (0)	0	0 (0)	0	5 (33.3)	7	11 (36.7)	15
Systemic AES	**1 (1 - 7)**	**1 (1 - 15)**	**13 (86.7)**	**49**	**18 (60)**	**57**	**4 (26.7)**	**6**	**6 (20)**	**6**	**13 (86.7)**	**55**	**19 (63.3)**	**63**
Asthenia/Fatigue	1 (1 - 5)	1 (1 - 15)	9 (60)	17	13 (43.3)	21	2 (13.3)	2	0 (0)	0	9 (60)	19	13 (43.3)	21
Headache	1 (1 - 4)	2 (1 - 5)	7 (46.7)	11	6 (20)	11	2 (13.3)	2	2 (6.7)	2	9 (60)	13	8 (26.7)	13
Diarrhoea	1 (1 - 3)	2 (1 - 10)	5 (33.3)	5	8 (26.7)	10	0 (0)	0	2 (6.7)	2	5 (33.3)	5	10 (33.3)	12
Nausea	1 (1 - 4)	1 (1 - 3)	4 (26.7)	4	4 (13.3)	4	0 (0)	0	0 (0)	0	4 (26.7)	4	4 (13.3)	4
Myalgia	1 (1 - 1)	1 (1 - 1)	2 (13.3)	2	3 (10)	3	0 (0)	0	0 (0)	0	2 (13.3)	2	3 (10)	3
Abdominal pain	2.5 (1 - 4)	2 (1 - 3)	2 (13.3)	2	2 (6.7)	2	0 (0)	0	0 (0)	0	2 (13.3)	2	2 (6.7)	2

Abdominal distension	1 (1 - 1)	1 (1 - 1)	0 (0)	0	1 (3.3)	1	0 (0)	0	0 (0)	0	0 (0)	0	1 (3.3)	1
Decreased appetite	2.5 (2 - 3)	2 (2 - 2)	2 (13.3)	2	1 (3.3)	1	0 (0)	0	0 (0)	0	2 (13.3)	2	1 (3.3)	1
Flatulence	1 (1 - 1)	3 (3 - 3)	0 (0)	0	1 (3.3)	1	0 (0)	0	0 (0)	0	0 (0)	0	1 (3.3)	1
Hordeolum	1 (1 - 1)	3 (3 - 3)	0 (0)	0	1 (3.3)	1	0 (0)	0	0 (0)	0	0 (0)	0	1 (3.3)	1
Oral herpes	1 (1 - 1)	11 (11 - 11)	0 (0)	0	0 (0)	0	0 (0)	0	1 (3.3)	1	0 (0)	0	1 (3.3)	1
Pyrexia	1 (1 - 1)	1 (1 - 1)	0 (0)	0	1 (3.3)	1	0 (0)	0	0 (0)	0	0 (0)	0	1 (3.3)	1
Rash	1 (1 - 1)	2 (2 - 2)	0 (0)	0	0 (0)	0	0 (0)	0	1 (3.3)	1	0 (0)	0	1 (3.3)	1
Vomiting	2 (2 - 2)	1 (1 - 1)	1 (6.7)	1	1 (3.3)	1	0 (0)	0	0 (0)	0	1 (6.7)	1	1 (3.3)	1
Arthralgia	1 (1 - 1)	1 (1 - 1)	1 (6.7)	1	0 (0)	0	0 (0)	0	0 (0)	0	1 (6.7)	1	0 (0)	0
Dermatitis acneiform	1 (1 - 1)	1 (1 - 1)	1 (6.7)	1	0 (0)	0	0 (0)	0	0 (0)	0	1 (6.7)	1	0 (0)	0
Hyperhidrosis	3.5 (2 - 7)	1 (1 - 1)	3 (20)	3	0 (0)	0	1 (6.7)	1	0 (0)	0	4 (26.7)	4	0 (0)	0
Odynophagia	5 (5 - 5)	1 (1 - 1)	0 (0)	0	0 (0)	0	1 (6.7)	1	0 (0)	0	1 (6.7)	1	0 (0)	0

**Median (Min – Max).*

Local: local pain, inflammation and site erythema occurred in the site of injection. Systemic: rest of AE.

SOC: System Organ Class and PT: Preferred Term, according to MedDRA v20.1.

N pat (%): Absolute number of participants reporting one AE is shown, and percentages are calculated as the number of participants (N) reporting an AE of a specific severity divided by the total number of Safety Population participants.

Grading was performed according to the DAIDS grading table ('1 - Mild', '2 - Moderate', '3 - Severe', '4 - Life-threatening, '5 – Death'). In case various severities were reported from start to end date of an AE, the highest grade was registered.
An AE ended the day the patient restored his/her baseline condition. When a given AE ended and occurred the next day again, it was recorded as a new AE.
No related grade 3 or 4 were reported.
The duration of any AE is computed as: end date - start date + 1; AE that lasted <24h are recorded as 1 day of duration.

Supplementary Data Table 3: All adverse events related to the two MVA.HTI/Placebo administrations in the DDDMM/PPPPP regimen (ordered by decreasing frequency for systemic AEs in the vaccine group)

PT	Duration* (days)		Grade 1				Grade 2				Total			
			Placebo		Vaccine		Placebo		Vaccine		Placebo		Vaccine	
	Placebo	Vaccine	N pat (%)	N AE	N pat (%)	N AE	N pat (%)	N AE	N pat (%)	N AE	N pat (%)	N AE	N pat (%)	N AE
Any AEs	**1 (1 - 3)**	**2 (1 - 10)**	**5 (33.3)**	**10**	**28 (93.3)**	**110**	**3 (20)**	**4**	**18 (60)**	**41**	**5 (33.3)**	**14**	**28 (93.3)**	**151**
Local AES	**1 (1 - 1)**	**2 (1 - 7)**	**1 (6.7)**	**1**	**24 (80)**	**39**	**0 (0)**	**0**	**10 (33.3)**	**13**	**1 (6.7)**	**1**	**27 (90)**	**52**
Injection site erythema	1 (1 - 1)	4.5 (4 - 5)	0 (0)	0	1 (3.3)	1	0 (0)	0	1 (3.3)	1	0 (0)	0	2 (6.7)	2
Injection site inflammation	1 (1 - 1)	4.5 (2 - 7)	0 (0)	0	2 (6.7)	2	0 (0)	0	0 (0)	0	0 (0)	0	2 (6.7)	2
Injection site pain	1 (1 - 1)	2 (1 - 7)	1 (6.7)	1	23 (76.7)	36	0 (0)	0	9 (30)	12	1 (6.7)	1	27 (90)	48
Systemic AES	**1 (2 - 3)**	**1 (1 - 10)**	**4 (26.7)**	**9**	**24 (80)**	**71**	**3 (20)**	**4**	**14 (46.7)**	**28**	**4 (26.7)**	**13**	**25 (83.3)**	**99**
Asthenia/Fatigue	1 (1 - 1)	1 (1 - 10)	2 (13.3)	3	20 (66.7)	29	1 (6.7)	1	6 (20)	6	3 (20)	4	22 (73.3)	35
Myalgia	1 (1 - 1)	1.5 (1 - 3)	0 (0)	0	12 (40)	13	0 (0)	0	5 (16.7)	7	0 (0)	0	14 (46.7)	20
Headache	1 (1 - 3)	1.5 (1 - 10)	2 (13.3)	2	4 (13.3)	6	3 (20)	3	9 (30)	12	4 (26.7)	5	13 (43.3)	18

Hyperhidrosis	2 (2 - 2)	1 (1 - 2)	1 (6.7)	1	5 (16.7)	5	0 (0)	0	2 (6.7)	2	1 (6.7)	1	6 (20)	7
Diarrhoea	1.5 (1 - 2)	2 (1 - 2)	2 (13.3)	2	4 (13.3)	5	0 (0)	0	0 (0)	0	2 (13.3)	2	4 (13.3)	5
Decreased appetite	1 (1 - 1)	2 (1 - 2)	0 (0)	0	2 (6.7)	3	0 (0)	0	0 (0)	0	0 (0)	0	2 (6.7)	3
Nausea	1 (1 - 1)	1 (1 - 2)	1 (6.7)	1	3 (10)	3	0 (0)	0	0 (0)	0	1 (6.7)	1	3 (10)	3
Pyrexia	1 (1 - 1)	1 (1 - 1)	0 (0)	0	3 (10)	3	0 (0)	0	0 (0)	0	0 (0)	0	3 (10)	3
Abdominal pain	1 (1 - 1)	2 (2 - 2)	0 (0)	0	1 (3.3)	1	0 (0)	0	1 (3.3)	1	0 (0)	0	2 (6.7)	2
Cough	1 (1 - 1)	2 (2 - 2)	0 (0)	0	1 (3.3)	1	0 (0)	0	0 (0)	0	0 (0)	0	1 (3.3)	1
Odynophagia	1 (1 - 1)	3 (3 - 3)	0 (0)	0	1 (3.3)	1	0 (0)	0	0 (0)	0	0 (0)	0	1 (3.3)	1
Vomiting	1 (1 - 1)	1 (1 - 1)	0 (0)	0	1 (3.3)	1	0 (0)	0	0 (0)	0	0 (0)	0	1 (3.3)	1

**Median (Min – Max).*
Local: local pain, inflammation and site erythema occurred in the site of injection. Systemic: rest of AE.
SOC: System Organ Class and PT: Preferred Term, according to MedDRA v20.1.
N pat (%): Absolute number of participants reporting one AE is shown, and percentages are calculated as the number of participants (N) reporting an AE of a specific severity divided by the total number of Safety Population participants.
Grading was performed according to the DAIDS grading table ('1 - Mild', '2 - Moderate', '3 - Severe', '4 - Life-threatening, '5 – Death'). In case various severities were reported from start to end date of an AE, the highest grade was registered.
An AE ended the day the patient restored his/her baseline condition. When a given AE ended and occurred the next day again, it was recorded as a new AE.
No related grade 3 or 4 were reported.
The duration of any AE is computed as: end date - start date + 1; AE that lasted <24h are recorded as 1 day of duration.

Supplementary Data Table 4: All adverse events related to the CCM/PPP regimen, by grade (ordered by decreasing frequency for systemic AEs in the vaccine group)

	Duration* (days		Grade 1				Grade 2				Grade 3				Total			
PT	Placebo	Vaccine	Placebo		Vaccine		Placebo		Vaccine		Placebo		Vaccine		Placebo		Vaccine	
			N pat (%)	N AE	N pat (%)	N AE	N pat (%)	N AE	N pat (%)	N AE	N pat (%)	N AE	N pat (%)	N AE	N pat (%)	N AE	N pat (%)	N AE
Any AEs	**1 (1 - 8)**	**2 (1 - 93)**	**10 (66.7)**	**31**	**22 (81.5)**	**76**	**2 (13.3)**	**4**	**8 (29.6)**	**23**	**0 (0)**	**0**	**1 (3.7)**	**1**	**11 (73.3)**	**35**	**22 (81.5)**	**100**
Local AES	**1.5 (1 - 3)**	**2 (1 - 4)**	**4 (26.7)**	**4**	**20 (74.1)**	**38**	**0 (0)**	**0**	**3 (11.1)**	**3**	**0 (0)**	**0**	**0 (0)**	**0**	**4 (26.7)**	**4**	**21 (77.8)**	**41**
Injection site erythema	0 (0 - 0)	2 (1 - 3)	0 (0)	0	2 (7.4)	2	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	2 (7.4)	2
Injection site inflammation	0 (0 - 0)	1 (1 - 1)	0 (0)	0	1 (3.7)	1	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	1 (3.7)	1
Injection site pain	2 (1 - 3)	2 (1 - 4)	4 (26.7)	4	20 (74.1)	35	0 (0)	0	3 (11.1)	3	0 (0)	0	0 (0)	0	4 (26.7)	4	21 (77.8)	38
Systemic AES	**1 (1 - 8)**	**2 (1 - 93)**	**10 (66.7)**	**27**	**16 (59.3)**	**38**	**2 (13.3)**	**4**	**7 (25.9)**	**20**	**0 (0)**	**0**	**1 (3.7)**	**1**	**11 (73.3)**	**31**	**18 (66.7)**	**59**
Asthenia/Fatigue	1 (1 - 4)	2 (1 - 8)	7 (46.7)	9	8 (29.6)	12	1 (6.7)	1	4 (14.8)	4	0 (0)	0	1 (3.7)	1	7 (46.7)	10	11 (40.7)	17
Myalgia	1 (1 - 1)	2 (1 - 4)	2 (13.3)	2	5 (18.5)	6	0 (0)	0	4 (14.8)	6	0 (0)	0	0 (0)	0	2 (13.3)	2	8 (29.6)	12
Headache	1 (1 - 3)	2 (1 - 6)	6 (40)	8	5 (18.5)	5	1 (6.7)	1	4 (14.8)	5	0 (0)	0	0 (0)	0	7 (46.7)	9	9 (33.3)	10
Hyperhidrosis	1 (1 - 1)	1 (1 - 2)	1 (6.7)	1	5 (18.5)	5	0 (0)	0	1 (3.7)	1	0 (0)	0	0 (0)	0	1 (6.7)	1	6 (22.2)	6

Nausea	1 (1 - 1)	1 (1 - 3)	1 (6.7)	1	3 (11.1)	3	0 (0)	0	1 (3.7)	1	0 (0)	0	0 (0)	0	1 (6.7)	1	4 (14.8)	4
Decreased appetite	1 (1 - 1)	1 (1 - 3)	2 (13.3)	2	3 (11.1)	3	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	2 (13.3)	2	3 (11.1)	3
Diarrhoea	2 (1 - 2)	3 (1 - 4)	2 (13.3)	2	2 (7.4)	2	1 (6.7)	1	0 (0)	0	0 (0)	0	0 (0)	0	3 (20)	3	2 (7.4)	2
Pyrexia	0 (0 - 0)	2 (1 - 2)	0 (0)	0	1 (3.7)	1	0 (0)	0	1 (3.7)	1	0 (0)	0	0 (0)	0	0 (0)	0	2 (7.4)	2
Dizziness	0 (0 - 0)	1 (1 - 1)	0 (0)	0	1 (3.7)	1	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	1 (3.7)	1
Pruritus	0 (0 - 0)	93 (93 - 93)	0 (0)	0	0 (0)	0	0 (0)	0	1 (3.7)	1	0 (0)	0	0 (0)	0	0 (0)	0	1 (3.7)	1
Upper respiratory tract infection	0 (0 - 0)	5 (5 - 5)	0 (0)	0	0 (0)	0	0 (0)	0	1 (3.7)	1	0 (0)	0	0 (0)	0	0 (0)	0	1 (3.7)	1
Abdominal pain	2 (2 - 2)	1 (1 - 1)	0 (0)	0	0 (0)	0	1 (6.7)	1	0 (0)	0	0 (0)	0	0 (0)	0	1 (6.7)	1	0 (0)	0
Hepatic enzyme increased	8 (8 - 8)	1 (1 - 1)	1 (6.7)	1	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	1 (6.7)	1	0 (0)	0
Oral herpes	5 (5 - 5)	1 (1 - 1)	1 (6.7)	1	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	1 (6.7)	1	0 (0)	0

**Median (Min – Max).*
Local: local pain, inflammation and site erythema occurred in the site of injection. Systemic: rest of AE.
SOC: System Organ Class and PT: Preferred Term, according to MedDRA v20.1.
N pat (%): Absolute number of participants reporting one AE is shown, and percentages are calculated as the number of participants (N) reporting an AE of a specific severity divided by the total number of Safety Population participants.
Grading was performed according to the DAIDS grading table ('1 - Mild', '2 - Moderate', '3 - Severe', '4 - Life-threatening, '5 – Death'). In case various severities were reported from start to end date of an AE, the highest grade was registered.
An AE ended the day the patient restored his/her baseline condition. When a given AE ended and occurred the next day again, it was recorded as a new AE.
No related grade 4 was reported.

Supplementary Data Table 5: Flow Cytometry. T cell lineage, phenotype, activation and exhaustion surface markers in $CD4^+$ and $CD8^+$ T cells after completion of last series of vaccination (week 28). Results are presented by median (min-max). Wilcoxon-Mann-Whitney is used for comparison between groups of treatment. Adjustment for multiple comparisons was not considered.

Surface markers	Placebo (n=12)	Vaccine (n=20)	p-value
Memory/Naive			
CD4+TCM: CD45RA-CCR7+	20.85 (11.35 - 35.3)	19.73 (11.55 - 44.5)	0.9160
CD4+TNaive: CD45RA+CCR7+	40.5 (18.35 - 64.1)	34.53 (13.9 - 70.7)	0.6110
CD4+TEMRA: CD45RA+CCR7-	3.77 (1.42 - 10.2)	3.89 (0.675 - 7.82)	0.8631
CD4+TEM: CD45RA-CCR7-	35.58 (12.8 - 55.9)	36.28 (9.54 - 67.4)	0.7372
CD8+TCM: CD45RA-CCR7+	4.83 (2.01 - 8.58)	6.2 (1.52 - 11.7)	0.2677
CD8+TNaive: CD45RA+CCR7+	35.75 (18.7 - 74.15)	32.05 (10.75 - 79.6)	0.7156
CD8+TEMRA: CD45RA+CCR7-	22.25 (7.61 - 37.05)	18.88 (6.59 - 33.35)	0.3456
CD8+TEM: CD45RA-CCR7-	30 (13.3 - 57.35)	37.78 (8.545 - 61.15)	0.1953
TFC (T follicular helper)			
CD4+TFC:CXCR5+PD1hi	2.15 (1.2 - 4.7)	2.65 (1.6 - 5.5)	0.0591
CD8+TFC:CXCR5+PD1hi	8 (2.2 - 13.4)	7.55 (3.6 - 22)	0.6383
Activation & Exhaustion			
CD4+CD69+	7 (4.9 - 10.6)	7.75 (3.8 - 17)	0.2508
CD4+HLADR+	5.7 (3.5 - 9.6)	5.65 (1.9 - 21.5)	0.8555
CD4+PD1+	3.1 (1.2 - 4.3)	3.3 (1.5 - 8.2)	0.4022
CD4+TGIT+	14.6 (6.5 - 25)	14.6 (7.3 - 22.3)	0.9465
CD8+CD69+	4.25 (2.2 - 7.7)	4.35 (2.9 - 10.7)	0.6245
CD8+HLADR+	11.7 (5.1 - 24.4)	14.45 (2.2 - 35.2)	0.4028
CD8+PD1+	2.4 (0.9 - 3.4)	2.35 (0.8 - 6.5)	0.6107
CD8+TGIT+	33.9 (13.1 - 55.7)	36.3 (14.7 - 58.1)	0.7811

Supplementary Data Table 6: All adverse events reported during ATI phase (week 0 to week 24), by grade (ordered by decreasing frequency for systemic AEs)

PT	Grade 1				Grade 2				Total			
	Placebo		Vaccine		Placebo		Vaccine		Placebo		Vaccine	
	N pat (%)	N AE	N pat (%)	N AE	N pat (%)	N AE	N pat (%)	N AE	N pat (%)	N AE	N pat (%)	N AE
Any AEs	**8 (53.33)**	**14**	**12 (46.15)**	**25**	**7 (46.67)**	**8**	**11 (42.31)**	**17**	**11 (73.33)**	**22**	**18 (69.23)**	**42**
Special Interest	**2 (13.33)**	**2**	**2 (7.69)**	**2**	**1 (6.67)**	**1**	**3 (11.54)**	**3**	**3 (20)**	**3**	**5 (19.23)**	**5**
Retroviral rebound syndrome	1 (6.67)	1	1 (3.85)	1	0 (0)	0	1 (3.85)	1	1 (6.67)	1	2 (7.69)	2
Affective/mood disorders	1 (6.67)	1	0 (0)	0	0 (0)	0	1 (3.85)	1	1 (6.67)	1	1 (3.85)	1
Sexually transmitted infections	0 (0)	0	1 (3.85)	1	1 (6.67)	1	1 (3.85)	1	1 (6.67)	1	2 (7.69)	2
Systemic AES	**7 (46.67)**	**12**	**11 (42.31)**	**23**	**6 (40)**	**7**	**10 (38.46)**	**14**	**10 (66.67)**	**19**	**16 (61.54)**	**37**
Headache	3 (20)	3	5 (19.23)	7	0 (0)	0	3 (11.54)	3	3 (20)	3	6 (23.08)	10
Abdominal distension	0 (0)	0	1 (3.85)	1	0 (0)	0	1 (3.85)	1	0 (0)	0	1 (3.85)	2
Asthenia/Fatigue	2 (13.33)	2	2 (7.69)	2	0 (0)	0	0 (0)	0	2 (13.33)	2	2 (7.69)	2
Odynophagia	0 (0)	0	1 (3.85)	1	0 (0)	0	1 (3.85)	1	0 (0)	0	2 (7.69)	2
Rhinitis	0 (0)	0	2 (7.69)	2	1 (6.67)	1	0 (0)	0	1 (6.67)	1	2 (7.69)	2
Anal fissure	0 (0)	0	0 (0)	0	0 (0)	0	1 (3.85)	1	0 (0)	0	1 (3.85)	1
Anogenital warts	1 (6.67)	1	1 (3.85)	1	0 (0)	0	0 (0)	0	1 (6.67)	1	1 (3.85)	1
Bronchitis	0 (0)	0	0 (0)	0	0 (0)	0	1 (3.85)	1	0 (0)	0	1 (3.85)	1
Cough	0 (0)	0	0 (0)	0	1 (6.67)	1	1 (3.85)	1	1 (6.67)	1	1 (3.85)	1
COVID-19	0 (0)	0	0 (0)	0	0 (0)	0	1 (3.85)	1	0 (0)	0	1 (3.85)	1
Diarrhoea	0 (0)	0	1 (3.85)	1	0 (0)	0	0 (0)	0	0 (0)	0	1 (3.85)	1
Drug hypersensitivity	0 (0)	0	1 (3.85)	1	0 (0)	0	0 (0)	0	0 (0)	0	1 (3.85)	1
Dysuria	0 (0)	0	1 (3.85)	1	0 (0)	0	0 (0)	0	0 (0)	0	1 (3.85)	1

Gastroenteritis	0 (0)	0	0 (0)	0	1 (6.67)	1	1 (3.85)	1	1 (6.67)	1	1 (3.85)	1
Gastroenteritis shigella	0 (0)	0	0 (0)	0	0 (0)	0	1 (3.85)	1	0 (0)	0	1 (3.85)	1
Iron deficiency anaemia	0 (0)	0	0 (0)	0	0 (0)	0	1 (3.85)	1	0 (0)	0	1 (3.85)	1
Limb injury	0 (0)	0	1 (3.85)	1	0 (0)	0	0 (0)	0	0 (0)	0	1 (3.85)	1
Myalgia	0 (0)	0	1 (3.85)	1	0 (0)	0	0 (0)	0	0 (0)	0	1 (3.85)	1
Nausea	0 (0)	0	1 (3.85)	1	0 (0)	0	0 (0)	0	0 (0)	0	1 (3.85)	1
Osteitis	0 (0)	0	0 (0)	0	0 (0)	0	1 (3.85)	1	0 (0)	0	1 (3.85)	1
Papule	0 (0)	0	1 (3.85)	1	0 (0)	0	0 (0)	0	0 (0)	0	1 (3.85)	1
Pyrexia	0 (0)	0	1 (3.85)	1	0 (0)	0	0 (0)	0	0 (0)	0	1 (3.85)	1
Tooth abscess	0 (0)	0	0 (0)	0	0 (0)	0	1 (3.85)	1	0 (0)	0	1 (3.85)	1
Upper respiratory tract infection	2 (13.33)	2	1 (3.85)	1	0 (0)	0	0 (0)	0	2 (13.33)	2	1 (3.85)	1
Abdominal pain	1 (6.67)	1	0 (0)	0	0 (0)	0	0 (0)	0	1 (6.67)	1	0 (0)	0
Eczema	1 (6.67)	1	0 (0)	0	0 (0)	0	0 (0)	0	1 (6.67)	1	0 (0)	0
Nasopharyngitis	0 (0)	0	0 (0)	0	1 (6.67)	1	0 (0)	0	1 (6.67)	1	0 (0)	0
Oral herpes	0 (0)	0	0 (0)	0	1 (6.67)	1	0 (0)	0	1 (6.67)	1	0 (0)	0
Parasitic gastroenteritis	0 (0)	0	0 (0)	0	1 (6.67)	1	0 (0)	0	1 (6.67)	1	0 (0)	0
Pharyngitis	1 (6.67)	1	0 (0)	0	0 (0)	0	0 (0)	0	1 (6.67)	1	0 (0)	0
Sciatica	0 (0)	0	0 (0)	0	1 (6.67)	1	0 (0)	0	1 (6.67)	1	0 (0)	0
Tonsillitis	1 (6.67)	1	0 (0)	0	0 (0)	0	0 (0)	0	1 (6.67)	1	0 (0)	0

Special interest: acute retroviral syndrome, affective/mood disorders, and sexually transmitted infections. Systemic: rest of AE.
Affective/mood disorders corresponds to Anxiety (Vaccine, grade 2) and Anxiety (Placebo, grade 1).
Sexually transmitted infections correspond to Syphilis (Placebo, grade 2), Genital Herpes (Vaccine, grade2), and Urethritis (Vaccine, grade 1).
SOC: System Organ Class and PT: Preferred Term, according to MedDRA v20.1.
N pat (%): Absolute number of participants reporting one AE is shown, and percentages are calculated as the number of participants (N) reporting an AE of a specific severity divided by the total number of Safety Population (ATI) participants.

Grading was performed according to the DAIDS grading table ('1 - Mild', '2 - Moderate', '3 - Severe', '4 - Life-threatening, '5 – Death'). In case various severities were reported from start to end date of an AE, the highest grade was registered.
An AE ended the day the patient restored his/her baseline condition. When a given AE ended and occurred the next day again, it was recorded as a new AE.
No grade 3 or 4 were reported.

Supplementary Data Table 7. Reasons for ART resumption during the ATI

Reason (n=35[ᵀ])	Placebo	Vaccine
Completed 24 weeks of ATI	**1**	**4**
Premature ART resumption (<24 weeks of ATI)	**12**	**18**
Clinical		
ARS	0	0
Virological		
pVL of HIV-1 RNA >100,000 copies/mL	9	16[§]
pVL of HIV-1 RNA > 10,000 copies/mL for 8 weeks	1	0
Immunological		
CD4 count <350 cells/mm^3 for 2 consecutive determinations	0	1[§]
Others*	2	2

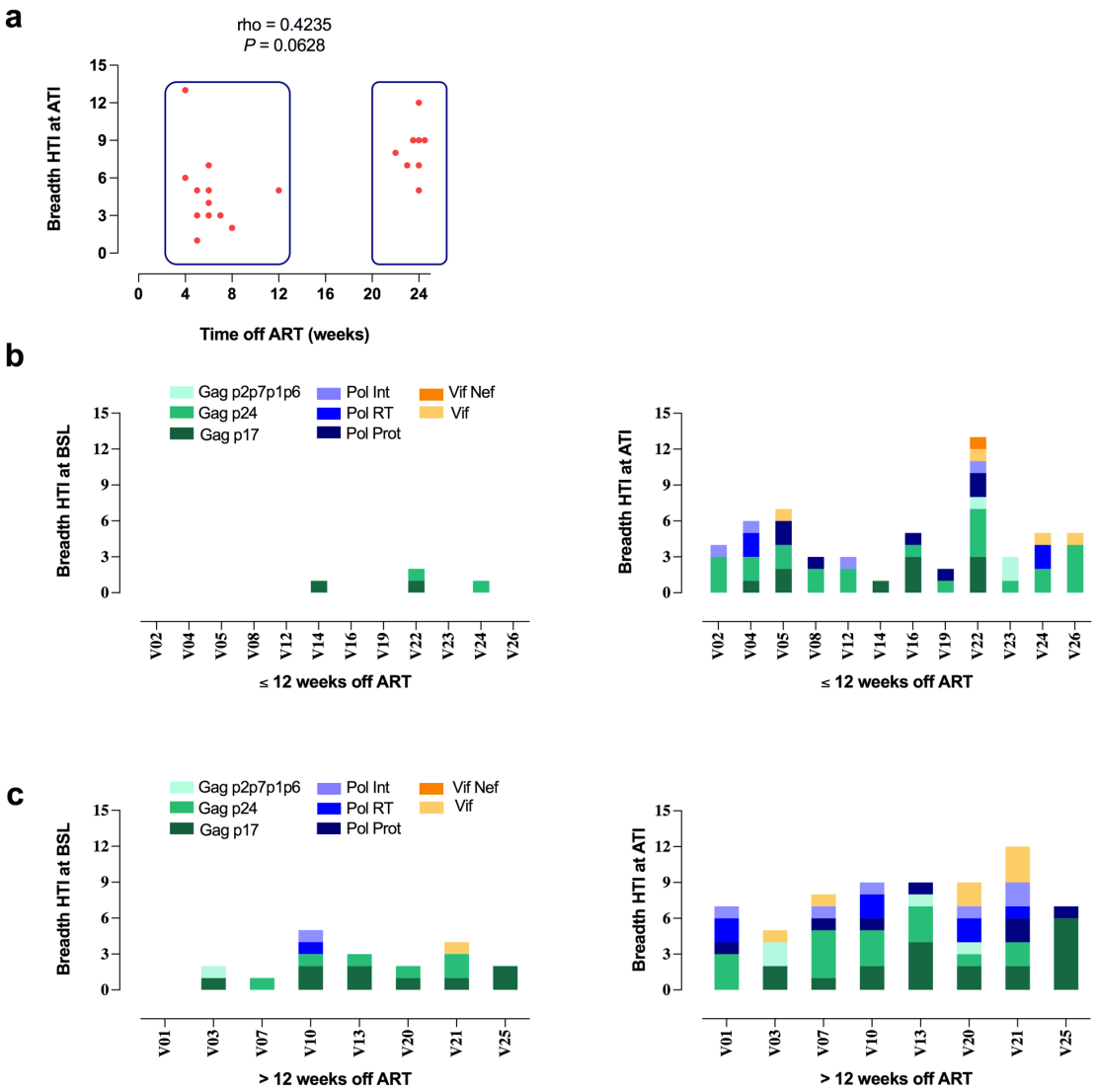
[ᵀ] N=6 did voluntarily not resume ART after completion of 24 weeks of ATI and entered into an ATI-extension phase.

[§]One participant (Vaccine) resumed ART fulfilling the virological and immunological criteria.

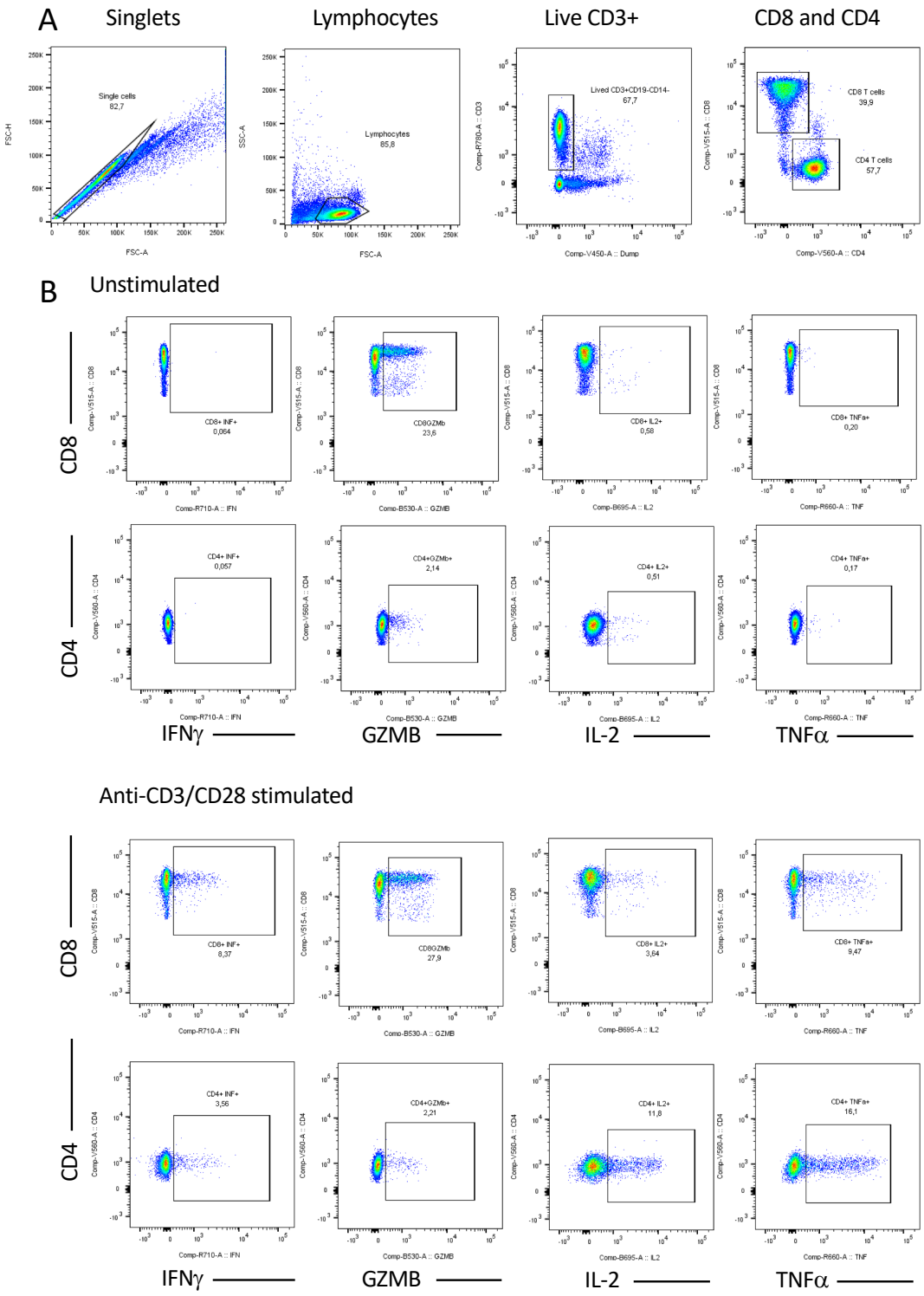
**In the context of COVID19 pandemic: 2 participants opted to resume ART (1 vaccine recipient and 1 placebo recipient at weeks 23 and 9), 1 sponsor recommendation (participant with asthma as a baseline condition and sustained pVL >10.000 for 6 weeks who refused home visits for personal reasons, Placebo, at week 12), 1 confirmed case of mild SARS-CoV-2 infection (Vaccine, week 22).*

ATI, analytical treatment interruption; ART, antiretroviral treatment; ARS, acute retroviral syndrome; pVL, plasma viral load.

Supplementary Fig. 1. (**a**) Correlation between cumulative breadth of HTI-specific responses after CCM and time off ART in vaccine recipients without beneficial HLA (n=20). *Spearman's correlation is used.* Distribution within different HIV-1 subproteins included in the HTI immunogen of the HTI responses vaccine recipients without beneficial HLA that remained off ART ≤12 (**b**) or >12 weeks (**c**) at AELIX-002 entry (left) and after CCM (right). Different HIV-1 subproteins included in the HTI immunogen are color coded. *OLP:* overlapping peptides, *C:* ChAdOx1.HTI, *M:* MVA.HTI, *ART:* antiretroviral treatment.



Supplementary Fig. 2. Gating strategy followed to determine T-cell subpopulations, activation, exhaustion and cytokine production. (**a**) A forward scatter height (FSC-H) vs forward scatter area (FSC-A) plot was used to exclude doublets. Then an FSC-A vs side scatter area (SSC-A) plot was used to select PBMCs. Live CD3+ cells were gated with the dump channel, which allowed exclusion of dead cells, monocytes (CD14), and B cells (CD19). Within the live CD3+ cell population, expression of CD4 and CD8 was then determined. The evaluation of cytokine production (INF-γ, GzmB, IL-2, and TNFα) (**b**) in $CD4^+$ and $CD8^+$ T cell populations from unstimulated and anti-CD3/CD28 stimulated conditions in one representative participant is shown.


A
Singlets
Lymphocytes
Live CD3+
CD8 and CD4
Single cells
82,7
FSC-H
FSC-A
SSC-A
Lymphocytes
85,8
Lived CD3+CD19-CD14-
67,7
Comp-R780-A :: CD3
Comp-V450-A :: Dump
CD8 T cells
39,9
CD4 T cells
57,7
Comp-V515-A :: CD8
Comp-V560-A :: CD4
B
Unstimulated
CD8
CD4
CD8+ INF+
0,064
CD8GZMb
23,6
CD8+ IL2+
0,58
CD8+ TNFa+
0,20
Comp-R710-A :: IFN
Comp-B530-A :: GZMB
Comp-B695-A :: IL2
Comp-R660-A :: TNF
CD4+ INF+
0,057
CD4+GZMb+
2,14
CD4+ IL2+
0,51
CD4+ TNFa+
0,17
IFNγ
GZMB
IL-2
TNFα
Anti-CD3/CD28 stimulated
CD8+ INF+
8,37
CD8GZMb
27,9
CD8+ IL2+
3,64
CD8+ TNFa+
9,47
CD4+ INF+
3,56
CD4+GZMb+
2,21
CD4+ IL2+
11,8
CD4+ TNFa+
16,1

Supplementary Data S1. Study Protocol

A Phase I, Randomized, Double-Blind, Placebo-Controlled Safety, Tolerability and Immunogenicity Study of Candidate HIV-1 Vaccines DNA.HTI, MVA.HTI and ChAdOx1.HTI in Early Treated HIV-1 Positive Individuals

Code: AELIX-002

Version 7.0, 3rd February 2020

EudraCT: 2017-000532-34

Sponsor:

AELIX Therapeutics
Parc Científic de Barcelona
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Coordinating Investigator: Beatriz Mothe Pujadas, MD, PhD

The information contained in this document is confidential and must not be revealed to third persons without authorization as contemplated by Law.

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Dr. Ian McGowan, AELIX Therapeutics, will be invited as an ad-hoc Non-Voting Consultant, as required.

STUDY MEDICATION PRODUCTION

DNA.HTI: NHSBT Clinical Biotechnology Centre, Bristol, UK

MVA.HTI: IDT Biologika GmbH, Germany.

ChAdOx1.HTI: Advent, Italy

Placebo: Pharmacy, Hospital Universitari Germans Trias i Pujol, Badalona, Spain

QP RELEASE TO TRIAL

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Clinical Development Director aleselbaum@aelixtherapeutics.com Margarida Garcia Clinical Study Manager (blinded) mgarcia@aelixtherapeutics.com	

SIGNATURES

The coordinating investigator and the sponsor of the study:

A Phase I, Randomized, Double-Blind, Placebo-Controlled Safety, Tolerability and Immunogenicity Study of Candidate HIV-1 Vaccines DNA.HTI, MVA.HTI and ChAdOx1.HTI in Early Treated HIV-1 Positive Individuals

Declare that this study will be conducted in compliance with the protocol, Good Clinical Practices (GCP) and the applicable regulatory requirements.

Modifications to this protocol must be submitted prior agreement of the coordinator investigator and sponsor.

Coordinating Investigator: Beatriz Mothe Pujadas, MD, PhD

Signature and Date: 05/02/2020

Sponsor: Anne Leselbaum, MD

Signature and Date: 5 Feb 2020

1 GENERAL INFORMATION

1.1 FULL TITLE

A Phase I, Randomized, Double-Blind, Placebo-Controlled Safety, Tolerability and Immunogenicity Study of Candidate HIV-1 Vaccines DNA.HTI, MVA.HTI, and ChAdOx1.HTI in Early Treated HIV-1 positive Individuals

1.2 SHORT TITLE & CODE

Safety and immunogenicity study of DNA.HTI, MVA.HTI and ChAdOx1.HTI in HIV-1-positive participants (AELIX-002)

1.3 LAY TITLE

Study of the safety and immune response to a combination of DNA, MVA and ChAdOx1 HIV vaccines in HIV-1- positive individuals.

1.4 PROTOCOL VERSION AND DATE

Version 7.0, 3rd February 2020

Any modification of the protocol must also bear the amendment number and date.

1.5 SPONSOR

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Person authorized by the sponsor to sign the protocol and amendments:
Anne Leselbaum, MD

1.6 MONITOR

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1.8 SITES

Single-site study. The trial will be performed at Unitat Polivalent d'Investigació Clínica (UPIC), Hospital Universitari Germans Trias i Pujol, Badalona.

1.9 TECHNICAL SERVICES INVOLVED

Core Laboratory: Biochemistry, hematology, serologies, quantitative HIV-1 RNA levels, HLA typing, and CD4 counts will be performed at the biochemistry, hematology, microbiology and immunology departments of Hospital Universitari Germans Trias i Pujol.

All other procedures including immunomonitoring and sample storage will be performed in the IrsiCaixa AIDS Research Institute (responsible of sample distribution Dr. Beatriz Mothe, bmothe@irsicaixa.es, T. +34 93 465 63 74).

IMP administrations and clinical visits from all participants will be performed at Unitat Polivalent d'Investigació Clínica (UPIC), Phase I unit located at Hospital Universitari Germans Trias i Pujol. Contact person: Dr. Ana María Barriocanal, abarrio.germanstrias@gencat.cat, T. +34 93 497 84 88.

Study product reception, storage, randomization and dispensing will be performed at the Pharmacy Service of Hospital Universitari Germans Trias i Pujol. Contact person: Adrià Siles, asiles.germanstrias@gencat.cat, T: +34 934 978 874. Study product preparation and blinding in Phase B and C will be allowed to be performed at the UPIC of Hospital Universitari Germans Trias i Pujol by Fundació Lluita contra la Sida personnel.

During the Analytical Treatment Interruption (ATI) period, participants will be offered follow-up visits at BCN-Checkpoint community center (contact details Dr. Pep Coll and Michael Meulbroek, pcoll@irsicaixa.es, T. +34 934 656 374 and mmeulbroek@hispanosida.com, T. +34 933 182 056).
Analyses for potential viral shedding in body secretions will be performed at Covance CRS Ltd, in UK. Contact person: Dr Andreas Claas, Principal Scientist, CMC Genomics, Andreas.Claas@covance.com, T. +44 (0)1480 893016

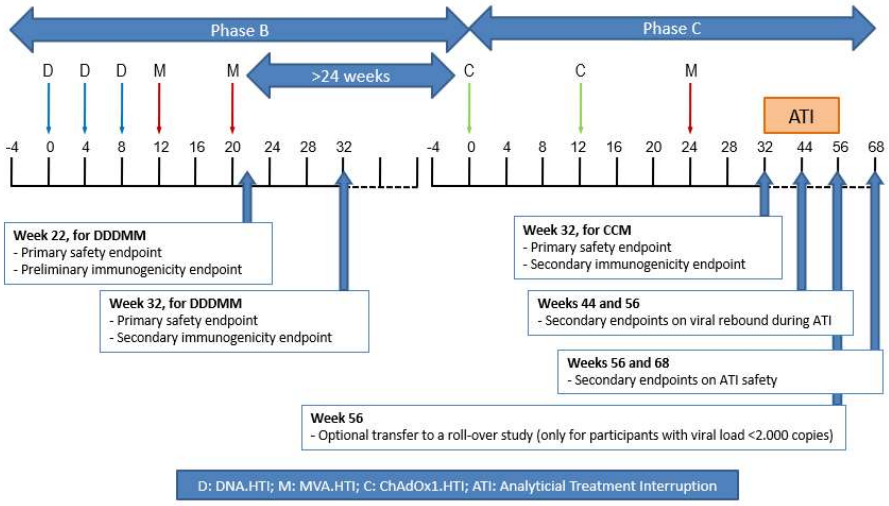
Participants will be offered a free shuttle service through the company Bonotaxi (contact person: María Sánchez, maria.sanchez@bonotaxi.com, T. +34 630 036 654).

Electronic Capture Data Form (eCRF) and data management will be performed by Dynamic Solutions, (contact persons: Alex Miñarro (IT Director) and Nuria Pajuelo (Statistics and Data management Director), T. +34 933 51 16 15.

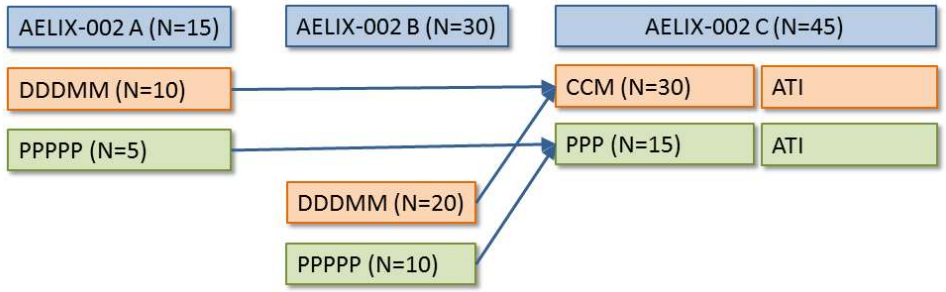
CONTENTS

2 SYNOPSIS

Title	A Phase I, Randomized, Double-Blind, Placebo-Controlled Safety, Tolerability and Immunogenicity Study of Candidate HIV-1 Vaccines DNA.HTI, MVA.HTI and ChAdOx1.HTI in Early Treated HIV-1 Positive Individuals.
Clinical Phase	Phase I
Trial Design	Randomised, double-blinded, placebo-controlled study, recruited sequentially in 2 phases: AELIX-002 Phase A (15 participants) and AELIX-002 Phase B (30 participants) to evaluate safety, immunogenicity and efficacy of three novel HIV-1 vaccines administered in a heterologous prime-boost regimen DDDMM and CCM, followed by an ATI period (Phase C).
Study Population	Early treated HIV-1 positive males and females, 18-60 years of age.
Sample size	Phase A: 15 Phase B: 30 Phase C: 15 + 30 = 45
Trial period	Phase A: Q1 2017 – Q4 2017 Phase B: Q1 2018 – Q1 2019 Phase C: Q1 2019 – Q2 2021
Primary Objective	To evaluate the safety of DNA.HTI administered alone and as part of an heterologous prime-boost regimen with MVA.HTI and ChAdOx1.HTI in early treated HIV-1 positive individuals (DDDMM and CCM).
Primary Endpoints	Proportion of participants that develop Grade 3 or 4 local reactions. Proportion of participants that develop Grade 3 or 4 systemic reactions. A descriptive summary of any local and systemic events, including laboratory abnormalities, including severity, durability and relationship to study product in vaccine and placebo recipients. For DDDMM prime-boost regimen, the primary endpoint will be assessed from first DNA.HTI/placebo administration up to visits Phase A/B week 22 and Phase A/B week 32 For CCM booster regimen, the primary endpoint will be assessed from first ChAdOx1.HTI/placebo administration up to visit Phase C week 32.

	
Secondary Objectives	To evaluate the immunogenicity of DNA.HTI, MVA.HTI and ChAdOx1.HTI vaccines as part of heterologous prime-boost regimens (DDDMM and CCM) in early treated HIV-1 positive individuals. To evaluate whether the heterologous prime-boost vaccination of DNA.HTI, MVA.HTI and ChAdOx1.HTI vaccines is able to prevent or delay viral rebound, induce post-rebound viral control, and/or prevent or delay the need for resumption of antiretroviral therapy during an analytical treatment interruption (ATI) of antiretroviral therapy in early treated HIV-1 positive individuals. To assess the safety of an ATI period after heterologous prime-boost vaccination (DDDMM and CCM) in early treated HIV-1 positive individuals.
Secondary Endpoint	*T-cell Immunogenicity:* - Proportion of participants that develop de-novo T cell responses to HTI-encoded regions as determined by IFNγ ELISPOT assay in vaccine and placebo recipients. - Breadth and magnitude of total vaccine induced HIV-specific responses measured by IFNγ ELISPOT in vaccine and placebo recipients. *Viral rebound during an ATI period (from Phase C week 32 to Phase C week 56)* - Percentage of participants with viral remission, defined as plasma viral load (pVL) <50 copies/mL at 12 and 24 weeks after ATI (visits Phase C week 44 and week 56). - Percentage of participants with viral control, defined as a pVL <2,000 copies/mL at 12 and 24 weeks after ATI (visit Phase C week 44 and week 56). - Time to viral detection, defined as the time from ATI start (visit Phase C week 32) to first occurrence of detectable pVL (≥50 copies/mL). - Time to viral rebound, defined as the time from ATI start (visit Phase C week 32) to first occurrence of pVL ≥ 10,000 copies/mL. - Percentage of participants who remain off cART at 12 and 24 weeks after ATI (visits Phase C week 44 and week 56). - Time off cART, defined as time to cART resumption since ATI start (visit Phase C week 32).

	Safety of an ATI Intervention (from Phase C week 32 to Phase C week 56): - Proportion of participants who develop symptoms compatible with acute retroviral syndrome (ARS). - Proportion of participants who develop new mutations not present in the pre-cART genotype conferring clinically-significant resistance to antiretroviral drugs (out of the individuals not reaching viral re-suppression 12 weeks after cART resumption). During the post cART resumption safety follow-up period of 12 weeks (from Phase C week 56 to Phase C week 68): - Proportion of participants who suppress pVL to <50 copies/mL 12 weeks after cART resumption. In those participants not reaching viral re-suppression 12 weeks after cART resumption an ART genotype will be analysed from the ATI sample to address if new drug-resistance mutations have emerged.
Exploratory Objectives	To evaluate the effect on the HIV-1 reservoir (proviral HIV-1 DNA in CD4+ T cells) of DNA.HTI, MVA.HTI and ChAdOx1.HTI vaccines as part of heterologous prime-boost regimen (DDDMM and CCM) in virologically-supressed early-treated HIV-1 positive individuals. To further characterise immunological, viral and microbiological responses to the DNA.HTI, MVA.HTI and ChAdOx1.HTI vaccines. To further evaluate safety and vaccine-immunogenicity of DNA.HTI and MVA.HTI at the long-term (Roll-Over Phase before entering Phase C).
Exploratory Endpoints	*Viral reservoir*: - Change in total proviral HIV-1 DNA per 10^6 CD4+ T cells from baseline (visit Phase A/B week 0) to ATI start (visit Phase C week 32). PBMC, plasma and faeces will be stored for other exploratory assays to further characterise vaccine-expanded T cell populations such as in-vitro viral suppressive capacity, polyfunctionality, functional avidity, induction of anti-vector antibodies, innate immune markers, as well as to address potential changes in the viral reservoir with alternative assays to proviral DNA, cell-associated RNA, epigenetic and microbiome studies. Long-term safety and vaccine-immunogenicity of DNA.HTI and MVA.HTI will be further evaluated during the Roll-over Phase of the study before entering Phase C.
IMPs	DNA.HTI plasmid DNA, attenuated poxvirus MVA.HTI, chimpanzee adenovirus ChAdOx1.HTI or placebo (saline solution).
Dose	DNA.HTI: 4mg (D) MVA.HTI: $2x10^8$pfu (M) ChAdOx1.HTI: $5x10^{10}$ Vp (C)
Route	Intramuscular (IM) needle injection

Study Design	 *D - DNA.HTI IM 4mg; M - MVA.HTI IM 2x10^{8} pfu; C - ChAdOx1.HTI IM 5x10^{10} Vp;* *P – placebo IM; ATI: Analytical treatment interruption.*
Follow-up Duration	In Phase A/B: A period of 8 weeks will be allowed from the screening to the baseline visit (first DNA.HTI/placebo administration). Follow up will be of 32 weeks after the first IMP administration. Roll-over Phase: After visit Phase A/B week 32, there will be an extension period, with regular visits every three months up to start of Phase C. Phase C: all participants in Phase A/B will be offered enrolment in to Phase C, at least 24 weeks after the second MVA.HTI/placebo dose in Phase A/B. Participation in Phase C will be offered sequentially: first to Sentinel participants in Phase A (Group 1), secondly, to Non-sentinel participants in Phase A (Group 2), and, lastly, to participants in Phase B (Group 3). Participants will be followed up for a maximum of 68 weeks after the first Phase C IMP administration.

3 ABBREVIATIONS

AE	Adverse event
AEMPS	Agencia Española de Medicamentos y Productos Sanitarios
AIDS	Acquired immune deficiency syndrome
ALT	Alanine aminotransferase
AR	Adverse reaction
ATI	Analytical treatment interruption
BL1	Biological Level 1
BL2	Biological Level 2
BP	Blood pressure
CSTD	Closed system transfer device
CAC	Community Advisory Committee
cART	Combined Antiretroviral Therapy
CBC	Complete blood count
ChAdOx1	Chimpanzee adenovirus Oxford 1
CMO	Chief Medical Officer
CRF	Case Report Form
CRO	Clinical Research Organisation
CTA	Clinical Trial Agreement
CTL	Cytotoxic T Lymphocyte
DAIDS	Division of acquired immune deficiency syndrome
DNA	Deoxyribonucleic Acid
DSUR	Development update safety report
DLT	Dose limiting toxicity
EC	Ethics Committee
ECG	Electrocardiogram
eCRF	Electronic CRF
ELISPOT	Enzyme-linked immunospot
ELISA	Enzyme-linked immunosorbent assay
FLS	Fundació Lluita contra la SIDA
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
GMO	Genetically modified organism
HBsAg	Hepatitis B virus surface antigen
HBV	Hepatitis B virus

HCV	Hepatitis C virus
HDPE	High density poly-ethylene
HGTIP	Germans Trias i Pujol Hospital
HIV	Human immunodeficiency virus
HLA	Human Leukocyte Antigen
HTI	HIVACAT T-cell Immunogen
HTS	Heterosexual
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference of Harmonisation
IFN-γ	Interferon gamma
IM	Intramuscular
IMP	Investigational medicinal product
INR	International normalized ratio (coagulation parameter)
IUD	Intra-uterine device
LOCF	Last Observation Carried forward
LOPD	Ley Orgánica de protección de datos
MVA	Modified virus Ankara
NGO	Non-governmental organizations
OLP	Overlapping peptides
PBMC	Peripheral Blood Mononuclear Cell
PT	Prothrombin time (coagulation parameter)
PIS	Patient information sheet
SMC	Safety Monitoring Committee
SmPC	Summary of product characteristics
SAE	Serious adverse event
SUSAR	Serious unexpected adverse reaction
SOP	Standard Operating Procedure
TCR	T cell Receptor
TPE	Thermoplastic elastomer
UPIC	Unitat Polivalent d'Investigació Clínica
ULN	Upper limit of normal
UNAIDS	United Nations Program on HIV/AIDS

4 BACKGROUND INFORMATION

4.1 INTRODUCTION

Increased access to highly active combination antiretroviral therapy (cART) has resulted in a dramatic decrease in morbidity and mortality associated with infection by the human immunodeficiency virus (HIV). However, despite having new classes of antiretroviral drugs, currently available cART regimens are not able to eradicate HIV from the body. Consequently, cART cessation in participants maintaining undetectable viral load is followed by a fast rebound in viremia[1,2]. This reflects the inability of the standard cART in eliminating a viral reservoir formed by latently infected cells in which the integrated provirus remains quiescent and very stable since very early stages of infection, and the inability of the immune response to effectively contain viral rebound after treatment interruption.

Multiple strategies have been evaluated to try to achieve an optimal control of HIV infection in the absence of cART. These have included early treatment initiation within the first 6 months after HIV acquisition, cART intensification, immunotherapies including interleukin administrations (IL-2, IL-7, IL-10, IL-12, and IL15), treatment with cyclosporine, mycophenolate, hydroxyurea, thalidomide, passive administration of antibodies, etc. and a wide range of therapeutic vaccines designed to expand the response mediated by cytotoxic T lymphocytes[3–6]. Minimal clinical effect has been observed after a vaccination strategy with an autologous dendritic-cell vaccination approach, which was able to demonstrate transient 1 log reduction in the viral setpoint of vaccinated compared to unvaccinated patients after discontinuation of treatment[7]. In addition, recent data from a pilot study suggests that re-education of T cells towards conserved regions of HIV by therapeutic vaccines in early treated patients (<6 months of HIV acquisition) may contribute to durable HIV control in a considerable proportion of participants after treatment cessation (Mothe B *et al*, CROI 2017, 119LB). Both sets of results, although suboptimal, set the stage for improved therapeutic vaccine concepts.

To date, the most relevant cause of therapeutic vaccines' failure probably lies in the composition of the antigen insert (immunogen) expressed. In particular, the inclusion of whole HIV proteins as antigens limits the immunogenic effect of the vaccine towards a nonspecific CTL expansion; a CTL response pattern which, in natural HIV infection, has been proved ineffective in controlling viral replication in most individuals[8,9]. In this regard, there is a growing consensus in the need to improve the immunogen design by selecting viral sequences able to induce T cell responses which are more beneficial to the host[10–12].

AELIX Therapeutics in collaboration with IrsiCaixa has developed a novel immunogen, termed HTI which was designed as an immunogen for T cells. HTI is a chemically synthesized chimeric protein consisting of 16 continuous segments of HIV-1 each between 11 and 78 amino acids in length and encoding critical targets of viral proteins Gag (45%), Pol (44%), Vif (8%) and Nef (3%). The function of the immunogen HTI is the induction of an immune response by HIV-1-specific T cells directed against the regions included in the insert HTI, which can control HIV-1 effectively[12]. The HTI immunogen is administered through an heterologous prime-boost vaccination that includes three components, a DNA vaccine prime (DNA.HTI) and a viral vaccine boost (MVA.HTI and ChAdOx1.HTI). The aim of the sequential administration of the therapeutic vaccines is to achieve a so-called "functional cure", in which HIV-infected participants could control viral replication in the absence of anti-retroviral treatment.

AELIX-002 is a double-blind, randomised first-in-human study in 45 participants with HIV infection to assess safety, tolerability, and immunogenicity of the vaccine components.

4.2 T CELL RESPONSES IN HIV CONTROL

HIV-1 infection induces strong and broadly directed HLA class I and class II restricted T-cell responses, for which some specific epitopes and restricting HLA alleles have been associated with relative in vivo virus control or lack thereof [13–15]. Among these, CD8+ cytotoxic T lymphocytes (CTL) responses to HIV-1 Gag have most consistently been associated with reduced viral loads in both HIV-1 clade B- and C-infected cohorts [14,16]. CD4+ T-cell responses to Gag have also been associated with relative HIV-1 control [17,18]. In addition, the elevated level of conservation of Gag across viral isolates [19] and the severe fitness reductions caused by CTL escape variants [20–24] may provide Gag-specific T-cell responses with a particular advantage. At the same time, it is also clear that not all Gag-specific responses exert the same antiviral activity, suggesting that a rational selection of Gag components could help focus vaccine induced responses onto the most protective targets. The same likely applies for all other viral proteins as well, as they may contain some regions that are of particular value for inclusion in a vaccine while other regions or proteins may induce less useful T cell responses. As such, effective vaccine design should probably aim to induce broad and evenly distributed responses to conserved and vulnerable sites of the virus while avoiding the induction of responses to regions that can be highly immunogenic but that may act as potential "decoy" targets and divert responses away from more relevant targets [11,25–29].

The failure of various T-cell vaccine candidates expressing entire HIV-1 proteins in large human clinical trials and data from post-trial analyses suggesting a sieve effect on the infecting viral strains, indicate the urgent need to improve vaccine immunogen design [30–33]. HTI represents a novel approach to design an HIV-1 T cell immunogen aimed to contribute to an effective HIV-1 control.

4.3 HTI DESIGN. RATIONALE

HTI is a chemically synthesized chimeric protein consisting of 16 continuous segments of HIV-1 each between 11 and 78 amino acids in length and encoding critical targets of viral proteins Gag (45%), Pol (44%), Vif (8%) and Nef (3%) The function of the immunogen HTI is the induction of an immune response by HIV-1-specific T cells directed against the regions included in the insert HTI, which aim to control HIV-1 effectively.

A comprehensive screening of large cohorts of clade B and C HIV-1-infected participants identified viral targets associated with relative HIV-1 control [8,34]. These earlier analyses in aggregate identified 26 regions in HIV-1 Gag, Pol, Vif and Nef proteins that (i) were preferentially targeted by participants with low viral loads and largely independent on beneficial HLA class I genotypes, (ii) turned out to be more conserved than the rest of the proteome, and (iii) elicited responses of higher functional avidity and broader variant cross-reactivity than responses to other regions.

The 26 beneficial OLP were aligned and if located in close proximity (<4 amino acid residues between one end and the start of an adjacent beneficial OLP) fused using the naturally occurring clade B consensus sequence residues. This resulted in 16 continuous segments with the precise start and end positions defined after considering additional residues up- and down-stream of the identified 26 OLP for inclusion. The final sequence comprised 16 segments ranging from 11–78 amino acids in length (total length of 529 aa). Linkers between segments consisted of either single, dual or triple alanine residues, and were included with the aim of inducing preferential proteolytic cleavage between segments and to avoid premature epitope digestion[35,36].

The final linear HTI sequence includes a high density of both $CD8^+$ and $CD4^+$ T-cell epitopes across all protein subunits restricted by at least 42 different HLA alleles (n = 55 well-characterized optimal defined CTL epitopes[37] and 6 most frequently targeted $CD4^+$ T helper epitopes in Gag[38]. Of note, 14 Gag $CD4^+$ T cell 'promiscuous' epitopes (recognized in the context of two or more DRB alleles)[17] are covered as well. Importantly, there is no overrepresentation of HLA-B27 or HLA-B57 supertype-restricted epitopes, which is in line with the unbiased general population screening that provided the data for the identification of beneficial OLP signal.

In conclusion, the HTI polypeptide sequence in a novel immunogen designed to a) contain epitope-rich regions in the context of a broad HLA class I and class II allele coverage, b) induce responses to subdominant epitopes associated with viral control and c) focus the vaccine-induced response onto the most vulnerable targets in the viral proteome. The rational design of this sequence also differs conceptually from other approaches that have either been based on full protein sequences [30–32], on very short, conserved segments of the virus [39,40], select conserved CD8 T cell epitopes[41] or other approaches that attempt to cover viral diversity [42–45] and which did not incorporate human immune reactivity data as the base of their designs.

4.4 VACCINE VECTORS

A plasmid DNA vaccine prime (DNA.HTI) and two viral vaccines boost based on modified vaccinia virus Ankara (MVA.HTI) and chimpanzee adenovirus (ChAdOx1.HTI) are used in AELIX-002 as vaccine vectors to express the HTI immunogen in an heterologous prime-boost vaccine regimen.

4.5 DNA.HTI VACCINE

DNA.HTI is a circular and double stranded deoxyribonucleic acid (DNA) plasmid vector of 5,676 base pairs derived from the pCMVkan expression vector backbone that contains the DNA encoding the 529 amino acid (aa) sequence for HTI. The HTI plasmid DNA contains the expression-optimised HTI open reading frame inserted into a pCMVkan vector comprising a plasmid backbone optimized for growth in bacteria, the human cytomegalovirus (CMV) promoter without any introns, the HTI gene, the bovine growth hormone (BGH) polyadenylation site, and the kanamycin resistance gene. The HTI protein is composed of 16 individual segments arranged linearly and linked via 1–3 alanine amino acid linkers and is preceded by the human GM-CSF signal peptide for better secretion.

The DNA.HTI DS is manufactured, quality-control tested and released in accordance with the requirements of good manufacturing practice (cGMP) by the Clinical Biotechnology Centre (CBC), Bristol Institute for Transfusion Sciences, University of Bristol, UK.

4.6 MVA.HTI VACCINE

MVA.HTI (Modified Vaccinia Virus Ankara) is a live, attenuated recombinant vaccinia (pox) virus attenuated by serial passages in cultured chicken embryo fibroblasts (CEF) that contains six large deletions from the parental virus genome. A transgene coding for the insert HTI has been inserted within the MVA in order to induce an HIV-1 specific T cell immune response. The size of MVA.HTI after the insertion is estimated to be approximately 7,290 kbp.

It is intended to be administered as a solution for vaccine boost by two i.m. injections eight weeks apart and starting 4 weeks after the last DNA.HTI administration. The production of the recombinant virus MVA.HTI is carried out by the German company IDT Biologika and is based on a system of 'seed virus', in which a 'master seed virus' (MSV) and a working virus (WSV) is prepared. All the preparation, verification of the genetic stability and MSV and WSV storage is done at IDT under cGMP conditions and according to EU regulations.

4.7 CHADOX1.HTI VACCINE

ChAdOx1.HTI is a replication-defective recombinant chimpanzee adenovirus (ChAd) vector based on a chimpanzee adenoviral isolate Y25[46] that encodes the HTI sequence. ChAdOx1.HTI was derived by sub-cloning the HTI antigen sequence into the generic ChAdOx1 BAC in order to induce HIV-1 specific T-cell immune response. The plasmid resulting from this sub-cloning (pC255; 40,483 kbp) was linearized and transfected into commercial HEX293A T-REx® cells to produce the vectored vaccine ChAdOx1.HTI.

ChAdOx1.HTI is formulated as a suspension for i.m. injection, and it is intended to be administered as a boosting vaccine in two sequential doses 12 weeks apart, starting at least 24 weeks after the second dose of the MVA.HTI dose of a vaccination regimen with DNA.HTI and MVA.HTI vaccines in the DDDMM regimen. HTI sub-cloning and generation of first ChAdOx1.HTI batch for non-clinical use have been performed at the University of Oxford (UK), whereas large scale amplification and purification of ChAdOx1.HTI have been performed at ReiThera/Advent (Italy) according to cGMP.

4.8 SUMMARY OF PRE-CLINICAL DATA WITH DNA.HTI, MVA.HTI AND ChAdOx1.HTI.

There is no relevant species for modelling HIV infection or for adequately assessing the development of the immune response. C57BL/6 mice and Indian Rhesus macaques have been chosen for the *in vivo* pharmacology assessment since mice are known to respond to the immune modulator effect of the vaccines, and Rhesus macaques of Indian origin are the best characterized and most utilized nonhuman primate model for the acquired immune deficiency syndrome (AIDS). The main route of administration in the nonclinical studies is the intended clinical route of administration, i.e. the intramuscular route.

In vivo non-clinical studies have been carried out in order to investigate the performance of the final HTI sequence, in some cases compared to other vaccines expressing full-length proteins, and to determine the effective dose and the regime of administration. Studies were carried out either with each vaccine alone, or with sequential administrations of DNA.HTI, MVA.HTI and/or ChAdOx1.HTI.

Pharmacodynamic (PD) studies were conducted in mouse (C57BL/6 mice: Study ID 174, Study C1022-1023, Study C1054-1058, and Study C1336-1339, and Study C1447) and in non-human primate models (Rhesus macaque: Study R679-681). Briefly[12], HTI immunogen was able to induce robust and broadly distributed T-cell responses in DNA.HTI vaccinated mice, comparable in their magnitude to a mixture of plasmids expressing full-length proteins. In addition, HTI was able to prevent the strong Gag dominance that was induced by the full-length immunogen approach. A subsequent MVA.HTI boost after two or three DNA prime vaccinations led to higher responses with significantly higher magnitudes (median total magnitude of 777 vs 2,865 IFN-γ SFC/million mice splenocytes after DDD and DDDM, respectively, p = 0.0087). ChAdOx1.HTI, administered in doses ranging from 107 to 1010 viral particles (Vp), induced a broad and strong immune response to HTI with all subunits of HTI targeted by T-cell responses.

In macaque studies, three (R678, R679, and R680) out of the four vaccinated animals induced HTI-specific responses mediated primarily by CD8+ effector memory (EM) T cells (EM; CD28− CD95+ cells) with significant contributions by the CD4+ and CD8+ central memory (CM) T cell subsets (CM; CD28+CD95+ cells). Induced responses persisted over several months and were still in the range as measured after the DDD vaccination (range 0.1-0.6% IFN-γ T cells), supporting the longevity of the DNA-vaccine induced immunity using an IM/Electroporation approach[47,48]. At four months after DDD, first MVA.HTI boost vaccination led to a 3- to 20-fold increase in the response, reaching 0.4-3.2% IFN-γ T cells. In two of animals (R678, R680) the levels were higher than the peak response after three DNA immunizations. The responses persisted over the 3 months of follow-up and were successfully expanded in all 4 animals by a 2nd MVA.HTI administration. Importantly, the majority of induced HTI-specific IFN-γ+ CD8+ T cells were double positive for CD107a+ and GzmB+, both after DNA prime and MVA boost vaccinations. Similar to the memory subsets, these cytotoxic phenotypes did not significantly change over time.

The safety and toxicity profile of the proposed heterologous vaccine was also characterized in non-clinical studies with a repeat dose study in mice conducted at higher dose and a higher frequency and number of administrations of each product than will be used in clinical studies. The toxicity profile of ChAdOx1.HTI has been assessed in the GLP PD study CP-2018013 and in the GLP toxicity study TX05CW. Both studies, performed at doses of 10^8 and 10^{10} Vp, respectively, have finished the experimental stage, although no data have been published yet

According to the *WHO guidelines on nonclinical evaluation of vaccines* (World Health Organization (WHO) No. 927 - Annex 1, 2005), the schedule of administration should mimic the intended clinical regimen. The LS18WJ study was conducted at Envigo (Alconbury Huntingdon Cambridgeshire, United Kingdom) with GLP pilot batches of both DNA.HTI and MVA.HTI products. This was a 15-week study, where the heterologous prime-boost vaccine was administered intramuscularly to C57BL/6 mice. The study included a 4-week recovery period. In the subsequent GLP study CP-2018027, the three products of the heterologous boost vaccine were assessed: DNA.HTI, MVA.HTI, and ChAdOx1.HTI. In addition to a control group treated with vehicle, both studies included groups treated with DNA.HTI, MVA.HTI and the corresponding heterologous vaccination. In the case of study CP-2018027, in addition to the heterologous vaccination with DNA.HTI and MVA.HTI, three groups were treated with the heterologous vaccination including DNA.HTI, MVA.HTI, and ChAdOx1.HTI; one of them mirrored the complete vaccination sequential administration established in the current version of the study protocol (i.e., DDDMMCCM, i.m.).

Regarding toxicological doses, heterologous vaccinations of study LS18WJ were performed at 0.390 mg of DNA.HTI and 8.4 x 10^6 pfu of MVA.HTI, which correspond to approximately 25 and 11 times the proposed human dose based on body surface. In the case of ChAdOx1.HTI, the administered dose was 1 x 10^8 Vp in the heterologous regimen and 1x10^{10} Vp in the repeated dose regimen, the last corresponding to 52 times the proposed human dose. Consequently, the toxicological doses and dose regimen of this repeat dose study are far above the starting doses and regimen of what is planned in clinical trials. For all groups, there were no clinical signs that were attributable to treatment, no reaction to treatment at the dose site and there were no unscheduled deaths during the study.

4.9 SUMMARY OF CLINICAL EXPERIENCE WITH DNA, MVA AND ChAdOx1-VECTORED VACCINES

Vectored vaccines DNA.HTI and MVA.HTI were first administered in humans in the setting AELIX-002 (Phase A and B) clinical trial. An interim report including the 15 first participants in the study showed that DNA.HTI and MVA.HTI administrations were safe when administered as a regimen of 3 DNA.HTI prime vaccinations followed by 2 MVA.HTI booster vaccinations. No treatment-related AEs of grade 3, grade 4 or SAE were reported. IMP-related adverse events were reported in 12 (80.0%) participants. Most frequent related AEs were flu-like symptoms such as headache (n=12, 80.0%), asthenia/fatigue (n=10, 66.7%), myalgia (n=6, 40.0%) and diarrhoea (n=4, 26.7%), all resolved spontaneously (internal data). There is significant experience with ChAd-derived vaccines in the setting of vaccine research, while ChAdOx1.HTI has not been administrated in humans before.

Numerous Phase I/IIa and II trials have been conducted with a range of plasmids and antigens for different conditions, including cancer. All DNA vaccines used so far were well tolerated with no local or systemic serious adverse effects[49]. DNA.HTI was generated using a pCMVkan vector from Vical pVR1012/pVR1332. A high similar vector backbone which was also derived from Vical pVR1012/pVR1332 has been used by the Vaccine Research Center VRC/NIAID for HIV clinical trials with DNA (i.e., the HIV-1 gag plasmid VRC4401; GenBank CS070894). VR4401 has been administered in clinical trials as a part of development of the HIV-1 DNA vaccine (HVTN204). This vaccine has been assessed in 10 different clinical trials in HIV infected and uninfected individuals.

Regarding MVA.HTI, several large clinical trials have demonstrated its safety and tolerability profile. Phase I/IIa clinical trials using highly similar candidates, such as MVA.HIVconsv or MVA.HIVA, have been completed (NCT01712425, NCT02099994, NCT01151319, NCT00982579 and NCT01371175) in combination with other candidates such as ChAdV63.HIVconsv, and DNA plasmids such as pSG2.HIVconsv and pTHr.HIVA.

MVA was originally developed in the 1970s as a vaccine against smallpox. MVA smallpox vaccination was administered in more than 120,000 participants in Germany including high-risk participants such as participants with nervous system disorder, allergy or skin disease, chronic disease, infants and children. Contrary to what had been observed with other vaccinia strains, no serious adverse events

were reported during the vaccination campaign with MVA. Only mild or moderate side effects were associated with the use of this vaccine, such as local reaction (redness), fever (in ~ 2% of vaccinees), "flulike" symptoms (in ~4% of vaccinees)[50]. MVA has since then been evaluated in extensive preclinical studies in animal models and in human studies (under normal or immune-suppression conditions) and was found to be safe and immunogenic without developing clinical disease[51–54].

The use of MVA as a recombinant HIV vaccine expressing HIV-1 antigens is also extensive. MVA-B, expressing a clade B HIV-1 immunogen (Bx08 gp120 and IIIB gag/pol/nef) has been tested in more than 300 volunteers in several Phase I/IIa studies without special safety concerns[55,56]. Similarly, MVA.HIVconsv, an MVA-vectored vaccine expressing an immunogen based on the 14 most conserved subprotein domains has been tested in HIV- population in the HIV-CORE 002 trial (NCT01151319) in Oxford[57] and also in Africa in the HIV-CORE 004 (NCT02099994)[58]. In HIV+ population MVA.HIVconsv vaccines have been tested in the recently finalized BCN01 and BCN02 trials (Early treated HIV+,NCT01712425[59] & NCT02616874) in Barcelona and in the HIV-CORE 001 trial in Oxford (Chronic HIV+, NCT01024842[60]) without safety concerns.

In the last few years, vectors derived from Chimpanzee adenoviruses (termed ChAd) have emerged as promising candidates for vectored vaccines[61]. Vectored vaccines based on simian adenoviruses have the advantage of lacking pre-existing anti-vector immunity that can limit vaccine performance, resulting in high and persistent immune responses. Owing to these advantages, ChAd viral vaccines against malaria, Ebola, HIV, influenza, respiratory syncytial virus, HCV, tuberculosis, and prostate cancer have all progressed to Phase I clinical trials, showing a good safety and immunological profile[61].

The ChAdOx1 variant, modified from *Pan troglodytes* Y25[46], has been tested as delivery vector in four completed trials investigating therapeutic vaccines against tuberculosis (NCT01829490), malaria (NCT03203421), and Influenza (NCT01818362 and NCT01623518), all of them as part of a prime/boost vaccination regimen with the corresponding MVA-derived vaccines. To date, only data on the vaccine ChAdOx1 NP+M1 expressing conserved Influenza A antigens have been reported. The safety record of this study, from 15 participants, showed no serious adverse reactions following administration with the ChAdOx1-derived vaccine at any of the investigated doses[62]. Local adverse reactions included pain, redness, selling, itching, and warmth, all of them mild. The most common systemic adverse reactions were fatigue (67% of volunteers), malaise (58%), and headache (58%). Seventy-one percent of systemic adverse reactions resolved within 48 hours.

In the area of HIV vaccination, two ChAd-derived vaccines have been investigated. On one hand, a set of tHIVconsvX, vectored by ChAdOx1, have shown high immunogenic capacity in two strains of mice. On the other hand, the vaccine ChAdV63.HIVconsv has progressed to a Phase I trial investigating safety and immunogenicity of a prime/booster vaccination regimen of ChAdV63.HIVcons and MVA.HIVcons vaccines in HIV – individuals in the HIV-CORE 02 trial in Oxford NCT01151319[57], and in early treated HIV+ in the BCN01 trial (NCT01712425). Both trials were successfully completed without safety concerns.

4.10 DOSING SCHEDULE JUSTIFICATION

The proposed clinical trial AELIX-002 is a double-blind, randomised first-in-human study in 45 HIV-1 positive participants to assess safety and tolerability of the vaccine components. Immunogenicity measures will also be assessed for facilitating the design of the subsequent clinical development program. Participants will receive a fixed dose of DNA.HTI (4 mg) with fixed doses of MVA.HTI ($2x10^8$pfu) and ChAdOx1.HTI (5×10^{10} Vp) administered as booster regimen. No dose escalation is envisioned in this protocol.

The rational for the doses and regimen proposed are based on the doses and schedule supported by toxicity studies LS18WJ, C1447, and CP-2018027 (please refer to IMPD Nonclinical section) and given that these doses are equivalent to effective doses in animal studies, therefore are expected to have some benefit. The toxicological doses of repeat dose study were in excess of the starting dose to be

administered in the Phase I clinical trial, since it is more than 12-fold higher than the calculated human equivalent dose for DNA.HTI, MVA.HTI, and ChAdOx1.HTI. The vaccinations envisioned in the clinical trial are also administered at a reduced frequency (i.e., once every 4 weeks and the last dose at 8 weeks instead of every two weeks for DNA.HTI and MVA.HTI, and 12 weeks apart for ChAdOx1.HTI) as used in the repeat dose study.

In addition, and according to literature data, the safety and tolerability of sequential administration of plasmid DNA and MVA vaccines in about 400 healthy HIV-uninfected volunteers and in HIV-1 infected participants has been demonstrated in several clinical trials[63,64]. Please also see the original Table 3 in the IMPD clinical sections for an overview of clinical doses with other products.

The safety and tolerability of DNA plasmid has been demonstrated for doses of 0.5 up to 8 mg (intramuscularly) as a prime of the booster with MVA in healthy HIV-uninfected volunteers and in HIV-1 infected volunteers in several clinical trial[63–70]. Moreover, several clinical trials have assessed the safety and tolerability of DNA plasmids at 8 mg dose for the treatment of HIV (NCT01922284, NCT00069030[66], NCT02431767, the latest is currently recruiting participants) among other indications (NCT01138410, NCT00513968, NCT00072605, NCT00711997).

According to literature data, the safety and tolerability of MVA vectors has been evaluated from 5 x 10^7 pfu to 2.5 x 10^8 pfu given mainly by the intradermal route as a booster of DNA vaccination or alone in several clinical trials[71]. Moreover, the safety and tolerability of MVA subtype C vaccine at 5 x 10^7 pfu given intramuscularly has also been demonstrated in HIV-1-uninfected individuals[64]. MVA has also been injected at 1 x 10^8 pfu/dose in 1 mL for three separate doses in another trial of a related HIV vaccine[72]. MVA.HIVconsv administered as well at 2 x 10^8 pfu intramuscularly.

The standard dose for ChAd vectors across all serotypes is generally 5 x 1010. As described in the previous section, clinical experience with ChAdOx1 vectors is limited. However, i.m. doses tested in completed Phase I/II trials with ChAdOx1-vectored vaccines ranged from 1.0 x 109 to 1.0 x 1010 Vp (NCT01829490, NCT03203421, NCT01818362, and NCT01623518), with acceptable safety results. Finally, the Phase I trials NCT01712425 and NCT02336074 assessing safety and immunogenicity of HIVconsv-based vaccines in early-treated HIV individuals, used 5 x 1010 Vp of ChAdV63.HIVconsv as part of an heterologous regimen with MVA.HIVconsv. No IMP-related serious adverse event was observed in these studies.

Overall, similar doses of DNA, MVA and ChAd vectors used in previous Phase I/II studies investigating vectored vaccines for HIV and other viremias have shown to be safe and immunogenic. This design will allow us to generate preliminary immunogenicity data on DNA, MVA, and ChAdOx1 vaccines, and determine whether further doses or combination with other products need to be explored as part of the development pathway in larger proof-of-concept studies.

4.11 ANALYTICAL TREATMENT INTERRUPTION

Antiretroviral treatment interruptions were first used in the setting of routine clinical practice with the aim of reducing the exposure of HIV+ participants to antiretroviral drugs[73]. However, the Strategies for Management of Antiretroviral Therapy (SMART) trial showed that such therapeutic approach not only failed to reduce the risk of adverse reactions but increased the risk of opportunistic diseases or death from any cause[74].

Although cART interruptions are no longer recommended in clinical practice, this strategy has remained in the research setting of HIV. With the advent of immunogenic therapies to achieve cART-free remission of HIV infection, analytical treatment interruptions (ATI) have become a cornerstone in the efficacy assessment of curative strategies for HIV[75]. The most common design of first ATI studies were based on a pre-established duration of cART interruption up to 24 weeks, with the viral load set point, CD4+ cell count or cART resumption as the primary efficacy outcomes[7,76,77]. Despite the overall safety outcomes reported in most of these studies, ATI based on a fixed time period raised concerns regarding

the replenishment of HIV reservoirs, immune damage, and clinical risks[75]. Consequently, new approaches for performing ATI in the context of clinical trials in which cART is resumed based on periodical assessments of plasma viral load (pVL) and CD4+ count have been proposed[56,78–80].

The risk of treatment interruptions were first described in the SMART study[74]. However, short-term ATI conducted under close virologic monitoring has been considered to be clinically safe[81]. Potential risks associated with ATI include the expansion of the HIV reservoir, persistent immune activation/damage, development of an acute retroviral syndrome, HIV rebound in the central nervous system, emerging resistance to antiretroviral drugs, mostly in participants taking nonnucleoside reverse- transcriptase inhibitors (NNRTI), and eventual HIV transmission to serodiscordant partners[82].

Aside shortening the time the participant is exposed to high-level viremia, preservation of CD4+ counts has been identified as an important measure for minimizing some of the risks associated with ATI. In fact, a secondary analysis of SMART which included participants with preserved CD4+ counts showed that cART interruption up to 16 weeks was not associated with significant increased risk of clinical events[83]. This finding was supported by subsequent eradication studies in HIV using an ATI approach[7,76,77]. Although there is no consensus regarding the optimal pVL and CD4+ thresholds for cART resumption[82], previous studies consistently considered CD4+ < 350 cells/mm[356,78–80,84]. Regarding the pVL threshold, criteria are heterogeneous across studies, ranging from 10,000 to 300,000 copies/mL. None of these studies reported significant adverse events associated with ATI or the presence of symptoms consistent with acute retroviral syndrome. Moreover, short-term ATI does not necessarily lead to expansion of the persistent HIV reservoir nor irreparable damages to the immune system in the peripheral blood, and the size of the HIV reservoirs as well as immune parameters, including markers of exhaustion and activation, return to pre-ATI levels 6–12 months after cART resumption[85].

Concerning the risk of HIV transmission during the ATI period, traditional prophylactic measures (e.g. the use of condoms and avoiding sharing injection devices) become of high relevance in these studies. Additionally, the use of pre-exposure prophylaxis (PrEP) may actively contribute to preventing new HIV infections. PrEP consists of a combination of tenofovir disoproxil fumarate plus emtricitabine (TDF/FTC), and it has been approved by the European Medicines Agency to reduce the risk of sexually acquired HIV-1 infection in adults and adolescents at high risk[86], although its routine use has not been implemented by Spanish authorities yet.

In addition to the aforementioned safety concerns, a debate exists regarding the optimal design and endpoints of ATI for assessing the efficacy of potentially curative strategies. Studies investigating the dynamics of viral rebound after cART interruption have shown that most patients experience a viral rebound soon after cART cessation, with median times to rebound ranging from 4 to 8 weeks[85,87,88] . However, some individuals who started cART soon after HIV infection have shown other response profiles: a) the absence of post-ATI rebound [89–92], b) a delayed viral rebound [93] , and c) a post-ATI viral control, defined as a sustained suppression of viremia after an initial rebound [79,94]. According to this heterogeneity, premature cART resumption during ATI might hamper the assessment of viral outcomes in some participants. Considering the time course of HIV–specific T-cell responses developed during acute infection to decrease viremia before a viral set point is established, 24 weeks of cART interruption should be enough to capture both a delay in viral rebound and a post-peak control of viremia.

4.12 SAMPLE SIZE JUSTIFICATION

AELIX-002 is a first-in-human exploratory Phase I study to evaluate safety of three novel vaccine candidates for HIV-1. A total of 45 participants will be included sequentially, in two recruitment periods: the first 15 participants will be included in Phase A, and 30 additional participants will be included in Phase B (only after all participants in Phase A have reached week 22 follow-up visit and a favourable safety report from the SMC has been released). After all participants in Phase B have reached week 21

follow-up visit and a favourable safety report from the SMC has been released, all Phase A and Phase B participants will be offered to participate in Phase C.

The cohort size of Phase A is proposed to be the minimum required to the study objectives as stated on Guideline on Requirements for First-in-human clinical trials for potential high-risk medicinal products (EMEA/CHMP/SWP/28367/2007).

An interim safety report is envisioned after the last participant in Phase A reaches the w22 visit of the trial (see section 10). Transition to Phase B and recruitment of 30 additional participants will occur only after a favourable SMC report. Immune data available at the interim report will not be taken into consideration to allow for the transition from Phase A to Phase B.

As a means to characterize the statistical properties of this study for the safety primary endpoint, the table below presents the probability of observing zero, at least one, and two or more adverse events among the 30 participants in the vaccine arm for various "true" event rates (table 1):

Table 1. Analysis of safety event frequency

Event rate	P (0 events | n=30)	P (≥1 events | n=30)	P (≥2 events | n=30)
1%	80%	26%	3.6%
5%	21.5%	78.5%	44.6%
10%	4.2%	95.8%	81.6%
15%	0.8%	99.2%	95.2%
25%	0%	100%	99.8%

P= probability

In terms of the chances of observing an AE, 30 participants in the active group will provide a high probability (78.5%) that this study will observe at least 1 event if the event occurs in the population with a true rate of 5%.

This sample size is similar to other clinical trials evaluating the safety of comparable HIV vaccines [70] .

There is no power calculation for this descriptive study. The sample size is proposed to provide preliminary safety information on the vaccine regimen (primary objective); and to explore (ii) the immunogenicity response (secondary objective); and (iii) whether the combination of DNA.HTI, MVA.HTI and ChAdOx1.HTI vaccines are able to prevent/delay viral rebound or induce a sustained viral control during following an analytical treatment interruption (ATI) (secondary objective). The inclusion of placebo arm will ensure that any potential for bias in the analysis of immune responses is minimised and will give greater confidence to assignations of the causality of any adverse reactions observed in this study and will help to maintain blinding of the participants.

4.13 CLINICAL TRIAL DESIGN

The AELIX-002 Phase I study will evaluate the safety, immunogenicity and efficacy of DNA.HTI, MVA.HTI and ChAdOx1.HTI vaccines in HIV-1 positive participants on suppressive antiretroviral treatment who started cART within first 6 months of confirmed HIV-1 acquisition.

Participants will be included in two recruitment periods: the first 15 will be included in Phase A, and 30 additional participants will be included in Phase B.

During Phase A/B, participants will be randomized to receive active vaccine (DDDMM) or placebo (PPPPP) in a double blinded fashion. There will be a Sentinel group of three participants of Phase A (Group 1); two of them will receive active vaccine and one will receive placebo (0.9% normal saline). Only one Sentinel participant will be enrolled per day. Two weeks later and in the absence of any related SAE or ≥ Grade 3 adverse event lasting >72h after IMP administration in any of the 3 Sentinel individuals, six of the remaining cohort will be enrolled (in blocks of 3 participants per day) and the final six participants one week later, also in blocks of 3 participants per day (Group 2). On each IMP administration day, 2 will receive active IMP and 1 will receive placebo (2:1).

An interim report is envisioned after the last included participant reaches the week 22 study visit (see section 10). After a favourable SMC report, transition to Phase B will be allowed, and 30 additional participants will be recruited sequentially and randomized 2:1 (Group 3) based on randomization list pre-established (see section 6.3.1).

At visit Phase A/B week 32, all participants will be invited to participate in a Roll-over Phase where clinical and laboratory follow-up visits will be performed every 12 weeks to explore long-term safety and immunogenicity of DNA.HTI and MVA administrations until start of Phase C.). No interventions are envisioned during this extension Roll-over Phase.

Treatment allocation will remain blind for all participants until the blind is open after the End-of-ATI analysis is completed.

An interim report is envisioned after the last included participant in Phase B reaches the visit Phase B week 21 (see section 10). After a favourable SMC report, transition to Phase C will be allowed. During Roll-over Phase participants in Phase A/B will be offered to participate in Phase C. Participants who received active treatment (DDDMM) in Phase A/B will continue to receive active treatment (CCM) in Phase C, while participants who received placebo in Phase A/B will continue to receive placebo (PPP). Treatment allocation will remain blind. Eight weeks after the third MVA.HTI/placebo administration, all participants will undergo an ATI of up to 24 weeks of duration and will be followed for safety 4 and 12 weeks (Phase C week 68, End-of-study visit) after cART resumption.
Participants who maintain HIV-1 pVL <2,000 copies/ml after 24 weeks on ATI (visit Phase C week 56) will be offered to end the study and stay off cART within a roll-over investigator initiated study out of the scope of the present protocol.

4.14 TARGET POPULATION

According to the "*Note for guidance on the clinical evaluation of vaccines*" (CHMP/VWP/164653/2005), although pilot studies sometimes have to be performed in healthy adults, data should be obtained as early as possible in the clinical development programme in the target population.

Heterologous vaccination with DNA.HTI and MVA.HTI vaccines have been designed as 'therapeutic vaccines' for chronically HIV-1 positive participants on stable combination antiretroviral therapy (cART) who are virologically suppressed who can be immunized to modulate/increase their cytotoxic T lymphocyte (CTL) response to HIV that will enable them to control/prevent the virus to rebound when antiretroviral treatment is stopped.

The AELIX-002 study will be conducted in HIV-1 positive participants as this is the population for whom the products being evaluated will be ultimately used. The key focus of the study is to assess the safety, immunogenicity and efficacy of the DNA.HTI, MVA.HTI and ChAdOx1.HTI vaccines. DNA and MVA vectors have previously been evaluated and found to be safe in HIV-1-infected participants (NCT01378156). Regarding ChAdOx1, various studies have evaluated its immunogenic effect in the setting of other infections - including viral infections - (NCT01829490, NCT03203421, NCT01818362, and NCT01623518) without remarkable safety concerns, but only pre-clinical data exist on HIV. The HTI

immunogen (expressed in an mRNA vector) has also been evaluated and shown to be safe in HIV-1-infected participants under cART in the iHIVARNA Phase I trial (NCT02413645). Based on available data it seems reasonable to conduct the first evaluation of the heterologous prime-boost vaccination regimen of DNA.HTI, MVA.HTI and ChAdOx1.HTI in HIV-1-infected participants. In addition, immunogenicity in HIV-1-uninfected participants may not correlate with similar immunogenicity in HIV-infected participants; HIV-1- positive participants will have pre-existing T cell immune responses to HIV. The evaluation of vaccine induced T cell responses will be critical in determining whether the DNA and MVA vaccines have the potential to refocus T cell responses towards more beneficial epitopes that might expect to correlate with the capacity of the regimen to maintain viral suppression following discontinuation of antiretroviral therapy.

Over the last few years, new data have been published regarding the benefits of early cART in acute/recent HIV-1 infection not only improving immune recovery[95,96], but also reducing the incidence of AIDS and non-AIDS related diseases[97], limiting immune escape[98] and latent reservoir size[99,100] and preserving T cell functionality (Rosas, CROI 2017 # 271). Recent data, , also shows that vaccine-induced T cell levels in early treated population are closer to those observed in HIV-1-negative individuals and considerably stronger than responses reported in past therapeutic vaccine trials in chronically suppressed participants[4,56]. These data suggest that early cART initiation contribute critically to a preserved T cell functionality that might allow for an effective refocus and boost of T cell responses to relevant T cell viral targets after therapeutic vaccination. We therefore aim to perform the first study assessing DNA.HTI, MVA.HTI and ChAdOx1.HTI vaccines in a population of recently HIV-1 infected participants who have received early antiretroviral treatment within the first 6 months after confirmed HIV-1 acquisition.

4.15 HYPOTHESES

1. Administration of 3 x DNA.HTI (4 mg) with 2 x MVA.HTI ($2x10^8$pfu) intramuscularly in HIV-1 positive participants will be safe and well tolerated.
2. Administration of 2 x ChAdOx1.HTI ($5x10^{10}$ Vp) with 1 x MVA.HTI ($2x10^8$pfu) intramuscularly in HIV-1 positive participants who previously received 3 x DNA.HTI (4 mg) with 2 x MVA.HTI ($2x10^8$pfu) will be safe and well tolerated.
3. An heterologous prime-boost vaccination with 3 x DNA.HTI (4 mg) plus 2 x MVA.HTI ($2x10^8$pfu) will induce HTI-encoded specific T cell responses detectable by an ex-vivo IFNɣ ELISPOT assay.
4. A booster vaccination with 2 x ChAdOx1.HTI ($5x10^{10}$ Vp) with 1 x MVA.HTI ($2x10^8$pfu) after an heterologous prime-boost vaccination with 3 x DNA.HTI (4 mg) plus 2 x MVA.HTI ($2x10^8$pfu) will induce HTI-encoded specific T cell responses detectable by an ex-vivo IFNɣ ELISPOT assay.
5. An heterologous prime-boost vaccination with 3 x DNA.HTI (4 mg) plus 2 x MVA.HTI ($2x10^8$pfu) followed by a booster vaccination with 2 x ChAdOx1.HTI ($5x10^{10}$ Vp) with 1 x MVA.HTI ($2x10^8$pfu) will prevent/delay viral rebound and induce viral control HIV-1 positive participants during a short interruption of cART (ATI)
6. An ATI after heterologous prime-boost regimens (DDDMM and CCM) in virologically-supressed early-treated HIV-1 positive individuals will be clinically safe.
7. An heterologous prime-boost vaccination with 3 x DNA.HTI (4 mg) plus 2 x MVA.HTI ($2x10^8$pfu) followed by a booster vaccination with 2 x ChAdOx1.HTI ($5x10^{10}$ Vp) with 1 x MVA.HTI ($2x10^8$pfu) will change the HIV-1 reservoir (proviral HIV-1 DNA in CD4+ T cells) in virologically-supressed early-treated HIV-1 positive individuals.

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5 TRIAL OBJECTIVE AND ENDPOINTS

5.1 PRIMARY OBJECTIVE AND ENDPOINTS

Primary Objective:

To evaluate the safety and tolerability of candidate HIV-1 vaccine DNA.HTI administered IM alone and as part of a heterologous prime-boost regimen with MVA.HTI and ChAdOx1.HTI in early treated HIV-1 positive individuals (DDDMM and CCM).

Primary Endpoints:

Safety of vaccines will be determined by analysis of local and systemic reactogenicity and biochemical and hematological data. The data will be expressed as:

1. Proportion of participants who develop a Grade 3 or 4 local reaction.
2. Proportion of participants who develop a Grade 3 or 4 systemic reaction.
3. A descriptive summary of any local and systemic events, including laboratory abnormalities, including severity, durability and relationship to study product in vaccine and placebo recipients.

For DDDMM prime-boost regimen, the primary endpoint will be assessed from first DNA.HTI/placebo administration up to visits Phase A/B week 22 and Phase A/B week 32.

For CCM booster regimen, the primary endpoint will be assessed from first ChAdOx1.HTI/placebo administration up to visit Phase C week 32.

The grading system is based on the HIV Vaccine Trials Network Table for Grading Severity of Adverse Experiences or DAIDS scale where grade 4 is life-threatening and grade 5 is death (Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Paediatric Adverse Events (Version 2.1, March 2017)a. This is a standard and accepted system for assessing vaccine AEs and safety in an HIV-infected population.

5.2 SECONDARY OBJECTIVES AND ENDPOINTS

Secondary Objectives:

To evaluate the immunogenicity of DNA.HTI, MVA.HTI and ChAdOx1.HTI vaccines as part of heterologous prime-boost regimens (DDDMM and CCM) in early treated HIV-1 positive individuals.

To evaluate whether the heterologous prime-boost vaccination of DNA.HTI, MVA.HTI and ChAdOx1.HTI vaccines is able to prevent or delay viral rebound, induce post-rebound viral control, and/or prevent or delay the need for resumption of antiretroviral therapy during an analytical treatment interruption (ATI) of antiretroviral therapy in early treated HIV-1 positive individuals.

To assess the safety of an ATI period after heterologous prime-boost vaccination (DDDMM and CCM) in early treated HIV-1 positive individuals.

Secondary Endpoints on immunogenicity:

Vaccine immunogenicity will be expressed as:

1. Proportion of participants that develop de-novo T cell responses to HTI-encoded regions as determined by IFNγ ELISPOT assay in vaccine and placebo recipients.

[a] U.S. Department of Health and Human Services, National Institutes of Health, National Institute of Allergy and Infectious Diseases D of A. Table for Grading the Severity of Adult and Pediatric Adverse Events. Division of AIDS (DAIDS). 2017. p. Version 2.1

2. Breadth and magnitude of total vaccine induced HIV-specific responses measured by IFNγ ELISPOT in vaccine and placebo recipients.

Secondary endpoints on immunogenicity will be assessed from first DNA.HTI/placebo administration visit to visit Phase A/B week 32 for DDDMM regimen, and from first ChAdOx1.HTI/placebo administration to visit Phase C week 32 for CCM regimen.

Secondary Endpoints on viral rebound during an ATI period (from Phase C week 32 to Phase C week 56):

- Percentage of participants with viral remission, defined as plasma viral load (pVL) <50 copies/mL 12 and 24 weeks after start of ATI (visits Phase C week 44 and week 56).
- Percentage of participants with viral control, defined as a pVL <2,000 copies/mL at 12 and 24 weeks after ATI (visit Phase C week 44 and week 56).
- Time to viral detection, defined as the time from ATI start (visit Phase C week 32) to first occurrence of detectable pVL (≥50 copies/mL).
- Time to viral rebound, defined as the time from ATI start (visit Phase C week 32) to first occurrence of pVL ≥ 10,000 copies/mL.
- Percentage of participants who remain off cART at 12 and 24 weeks after ATI (visits Phase C week 44 and week 56).
- Time off cART, defined as time to cART resumption since ATI start (visit Phase C week 32).

Secondary endpoint on safety of an ATI Intervention (from Phase C week 32 to Phase C week 56):

- Proportion of participants who develop symptoms compatible with acute retroviral syndrome (ARS).
- Proportion of participants who develop new mutations not present in the pre-cART genotype conferring clinically-significant resistance to antiretroviral drugs (out of the individuals not reaching viral re-suppression 12 weeks after cART resumption).

During the post cART ressumtion safety follow-up period of 12 weeks (from Phase C week 56 to Phase C week 68):

- Proportion of participants who suppress pVL to <50 copies/mL 12 weeks after cART resumption. In those participants not reaching viral re-suppression 12 weeks after cART resumption an ART genotype will be analysed from the ATI sample to address if new drug-resistance mutations have emerged.

5.3 EXPLORATORY OBJECTIVES AND ENDPOINTS

Exploratory Objectives

To evaluate the effect on the HIV-1 reservoir (proviral HIV-1 DNA in CD4+ T cells) of DNA.HTI, MVA.HTI and ChAdOx1.HTI vaccines as part of heterologous prime-boost regimens (DDDMM and CCM) in virologically-supressed early-treated HIV-1 positive individuals.

To further characterise immunological, viral and microbiological responses to the DNA.HTI, MVA.HTI and ChAdOx1.HTI vaccines. To further evaluate safety and vaccine-immungenicity of DNA.HTI and MVA.HTI at the long-term (Roll-Over Phase up to week 80 before entering Phase C).

Exploratory Endpoints on viral reservoir:

- Change in total proviral HIV-1 DNA per 10^6 CD4+ T cells from baseline (visit Phase A/B week 0) to ATI start (visit Phase C week 32).

PBMC, plasma and faeces will be stored for other exploratory assays to further characterise vaccine-expanded T cell populations such as in-vitro viral suppressive capacity, polyfunctionality, functional avidity, induction of anti-vector antibodies, innate immune markers, as well as to address potential changes in the viral reservoir with alternative assays to proviral DNA, cell-associated RNA, epigenetic and microbiome studies. Long-term safety and vaccine-immunogenicity of DNA.HTI and MVA.HTI will be further evaluated during the Roll-over Phase of the study, before entering to Phase C.

As this is a first in human study for DNA.HTI, MVA.HTI and ChAdOx1.HTI vaccines, primary endpoint is safety and tolerability. This trial has been designed as a pilot study the sample size has been chosen that will only allow detection of large response differences. Thus, it is expected to yield data which are primarily descriptive. The inclusion of placebo arm will ensure that any potential for bias in the analysis of immune responses is minimised and will give greater confidence to assignations of the causality of any adverse reactions observed in this study and will help to maintain blinding of the participants.

6 TRIAL DESIGN

At the time of release of version 7.0 of the study protocol, Phases A/B have been performed and Phase C is still ongoing but this version of protocol has been worded as planned for all phases of the trial.

6.1 TYPE OF TRIAL

AELIX-002 is a Phase I/proof of concept, first in human randomized, double-blinded, placebo-controlled study, to evaluate safety, immunogenicity and efficacy of three novel HIV-1 vaccines administered in a heterologous prime-boost regimen DDDMM and CCM, followed by an ATI period. The inclusion will be performed in two recruitment periods: 15 participants in Phase A and 30 participants in Phase B, after all participants in Phase A have reached week 22 follow-up visit and a favourable safety report from the SMC has been released. After all participants in Phase B have reached week 21 follow-up visit and a favourable safety report from the SMC has been released, all Phase A and Phase B participants will be offered to participate in Phase C.

6.2 DESCRIPTION OF THE DESIGN

Table 2. Study Design

Study phase	Group	N	Phase A/B					Phase C					Safety follow-up
			W0	W4	W8	W12	W20	W0*	W12	W24	W32	W56** EOA visit	W68 EOS visit
			D1	D2	D3	M1	M2	C1	C2	M3	-	-	-
Phase A	Group 1 (Sentinel)	2	D	D	D	M	M	C	C	M	ATI	Resume cART – ATI Last visit	-Last visit
		1	P	P	P	P	P	P	P	P	ATI	Resume cART- ATI Last visit	- Last visit
	Group 2 (Non-sentinel)	8	D	D	D	M	M	C	C	M	ATI	Resume cART- ATI Last visit	-Last visit
		4	P	P	P	P	P	P	P	P	ATI	Resume cART- ATI Last visit	-Last visit
Phase B	Group 3 (Non-sentinel)	20	D	D	D	M	M	C	C	M	ATI	Resume cART- ATI Last visit	-Last visit
		10	P	P	P	P	P	P	P	P	ATI	Resume cART- ATI Last visit	-Last visit

C – ChAdOx1.HTI IM 5 x 10^{10} Vp; D - DNA.HTI IM 4mg; M - MVA.HTI IM 2x10^{8} pfu; P - placebo IM; EOA – End of ATI; EOS – End of Study
Time is shown in weeks after first vaccine administration within each phase.

**Participants will be enrolled in Phase C after at least 24 weeks since second MVA.HTI/placebo administration in Phase A/B.*
***cART will be resumed according to criteria pre-specified in the study protocol. EOS visit for participants with pVL<2.000 copies/ml and willing to participate in a roll-over study.*

The primary objective of this study is to assess the safety and tolerability of the vaccine components. A double-blind, randomized, placebo-controlled study design has been chosen to minimize bias in the reporting of safety and immunogenicity. According to *ICH E10 Choice of Control Group and Related Issues in Clinical Trials (ICH E10),* the use of placebo is to enable the most optimal comparison of the safety and immune response.

Participants will be included in two recruitment periods: the first 15 will be included in Phase A, and 30 additional participants will be included in Phase B.

During Phase A/B, participants will be randomized to receive active vaccine (DDDMM) or placebo (PPPPP) in a double blinded fashion. There will be a Sentinel group of three participants of Phase A (Group 1); two of them will receive active vaccine and one will receive placebo (0.9% normal saline). Only one Sentinel participant will be enrolled per day. Two weeks later and in the absence of any related SAE or ≥ Grade 3 adverse event lasting >72h after IMP administration in any of the 3 Sentinel individuals, six of the remaining cohort will be enrolled (in blocks of 3 participants per day) and the final six participants one week later, also in blocks of 3 participants per day (Group 2). On each IMP administration day, 2 will receive active IMP and 1 will receive placebo (2:1).

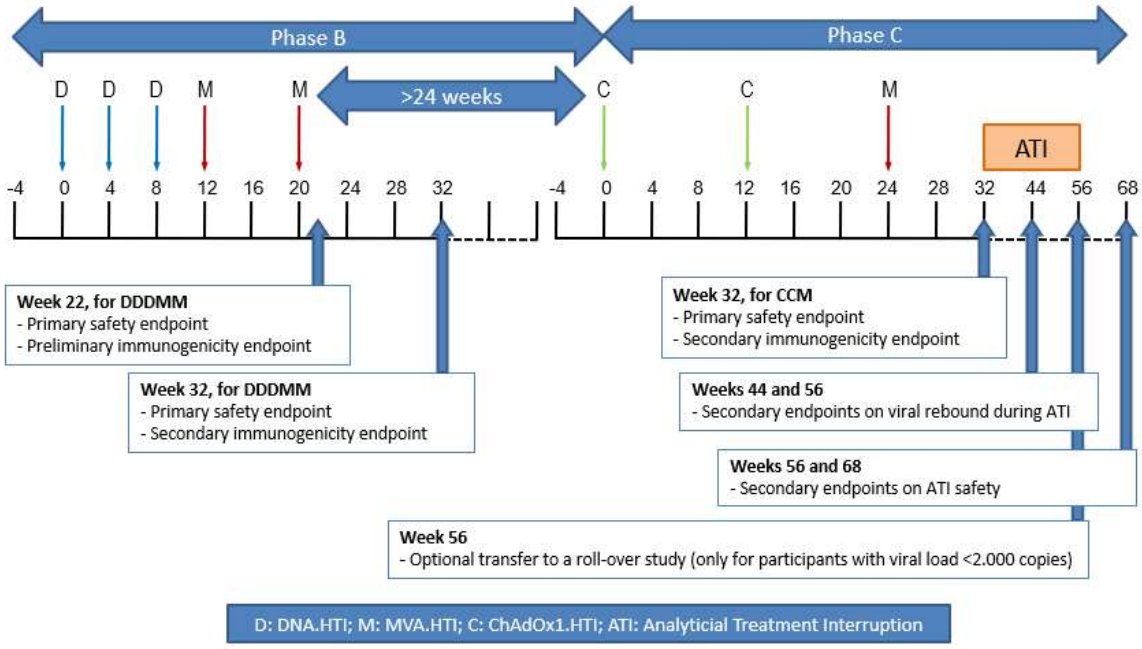
An interim report is envisioned after the last included participant reaches the week 22 study visit (see section 10). After a favourable SMC report, transition to Phase B will be allowed, and 30 additional participants will be recruited sequentially and randomized 2:1 (Group 3) without following blocks of pre-defined number of vaccinees and placebos per immunization day.

At visit Phase A/B week 32, all participants will be invited to participate in a Roll-over Phase where clinical and laboratory follow-up visits will be performed every 12 weeks to explore long-term safety and immunogenicity of DNA.HTI and MVA administrations until start of Phase C. No interventions are envisioned during this extension Roll-over Phase.

Treatment allocation during the extension Roll-over Phase will remain blind for all participants until the blind is open after the End-of-ATI analysis is completed.

An interim report is envisioned after the last included participant in Phase B reaches the visit Phase B week 21 (see section 10). After a favourable SMC report, transition to Phase C will be allowed. During Roll-over Phase participants in Phase A/B will be offered to participate in Phase C. Participants who received active treatment (DDDMM) in Phase A/B will continue to receive active treatment (CCM) in Phase C, while participants who received placebo in Phase A/B will continue to receive placebo (PPP). Treatment allocation will remain blind. Eight weeks after the third MVA.HTI/placebo administration, all participants will undergo an ATI of up to 24 weeks of duration and will be followed for safety 4 and 12 weeks (Phase C week 68, End-of-study visit) after cART resumption.
Participants who maintain HIV-1 pVL <2,000 copies/ml after 24 weeks on ATI (visit Phase C week 56) will be offered to end the study and stay off cART within a roll-over investigator initiated study out of the scope of the present protocol.

Figure 1. IMP administration schedule



6.2.1 Safety and tolerability assessment: Sentinel and Non-sentinel groups.

The Sentinel group (Group 1) will consist of 3 participants included in Phase A of the study: 2 administered with vaccine and 1 with placebo (2:1 ratio) in a random, double-blind allocation. The rest of participants will be part of the Non-sentinel groups: Group 2 (n=12) also included in Phase A and Group 3 (n=30) included in Phase B.

<u>During Phase A, for Sentinel participants in Group 1 safety and tolerability will be evaluated after each IMP administration at:</u>

a) Day +1 (24h post IMP administration): In the absence of any related SAE or ≥ Grade 4 adverse event, the next Sentinel participant will receive his/her IMP administration. In cases where related Grade 3 adverse event are reported and confirmed by the site investigator, IMP administrations will not be paused and close monitoring (either in-person or by phone contact) will be performed by the study team every 24h to address evolution of the adverse event.

b) Day +7: for clinical exam, adverse events monitoring and safety laboratory test.

Two weeks after each IMP administration (Day 14) and in the absence of any related SAE or ≥ Grade 3 adverse event lasting >72h after first IMP administration confirmed by the site investigator in any of the 3 Sentinel participants, six of the remaining cohort (Group 2) will be enrolled (in blocks of 3 participants per day) and the final six participants one week later, also in blocks of 3 participants per day. Each IMP administration block will consist of 3 participants: 2 administered with vaccine and 1 placebo (2:1 ratio) in a random, double-blind allocation.

Vitals and the injection sites will be monitored during 2h after each IMP administration.

<u>During Phase A, for Non-sentinel participants in Group 2 safety and tolerability will be evaluated after each IMP administration at:</u>

a) Day +7: for clinical exam, adverse events monitoring and safety laboratory test.

Vitals and the injection sites will be monitored during 2h after each IMP administration.

After all participants in Group 1 and Group 2 have reached week 22 visit and a favourable SMC report has been released, the 30 additional participants from Phase B will be enrolled (Group 3). Participants will be recruited sequentially and without following blocks of pre-defined number of vaccinees and placebos per immunization day. A randomization list with 20 vaccines and 10 placebos random allocations will be provided to the pharmacy team before Phase B commences.

During Phase B, participants in Group 3 safety and tolerability will be evaluated at:

a) Day +7 after 1st and 2nd DNA.HTI/placebo administration: remotely (by phone contact) for adverse events monitoring, with especial assessment of any related SAE or ≥ Grade 3.

   In cases where ≥ Grade 3 adverse event are reported, close monitoring (either in-person or by phone contact) will be performed by the study team to address evolution of the adverse event.

b) Day +7 after 3rd DNA.HTI/placebo and 1st and 2nd MVA.HTI/placebo administrations: in-person for clinical exam, adverse events monitoring and safety laboratory test.

Vitals and the injection sites will be monitored during 30 minutes after each IMP administration.

At visit Phase A/B week 32, all participants will be invited to participate in an extension sub-study (Roll-over Phase).

During Roll-over Phase, long-term safety, tolerability and immunogenicity will be evaluated at:

a) Every 12 weeks: for clinical exam, adverse events monitoring and safety laboratory test. At weeks 44 and 68, a full clinical routine laboratory panel including a complete lipid profile, and STD screening (HCV and syphilis serology and urinary multiplex PCR) will be included.

After all participants in Phase B (Group 3) have reached week 21 visit and a favourable SMC report has been released, transition to Phase C will be performed. Participation in Phase C will be offered at least 24 weeks after the 2nd MVA.HTI/placebo administration.

During Phase C, participation will be offered sequentially: first to Sentinel participants in Phase A (Group 1), secondly, to Non-sentinel participants in Phase A (Group 2), and, lastly, to participants in Phase B (Group 3).

Safety and tolerability in Phase C will be evaluated following the same procedures as those described in Phases A and B for each Group of participants (if no related SAE or ≥ Grade 3 AE after IMP administration are present in any of the 15 participants in Groups 1 and 2 vitals and the injection sites in Group 3 will be monitored during 30 minutes; otherwise, Group 3 will follow same procedures as in Groups 1 and 2 during Phase A, and as a consequence vitals and the injection sites in Group 3 will be monitored 10 minutes, 30 minutes and 2 hours post-administration).

Pharmacovigilance procedures, expedited reporting and Safety Monitoring Committee (SMC) evaluations are described in Section 9.

6.2.2 Safety assessment: Viral shedding

Body secretions samples will be collected to determine ChAdOx1.HTI vector shedding. To minimize any risks associated with vector shedding, participants will be educated to follow precautionary measures

to reduce the risk of exposure to partners or household members or unnecessary transmission to the environment.

Participants will be educated and required the use of the following **precautionary measures** during the study:

- Not to share towels with others;
- To practice proper toilet hygiene and hand washing following urination and defecation;
- To cover the mouth and nose upon sneezing;
- To cover each injection site with a light bandage for 3 days starting on the day the IMP was administered;
- To use barrier method contraception during intercourse (this is already a requirement of the study);
- To seek medical attention (preference with study physicians) for all open skin wounds to ensure adequate cleaning, disinfecting and wound covering;

Additional precautionary measures:

- During sneezing or coughing episodes, or during an episode of allergic rhinitis or of an upper/lower respiratory tract infection, participants should stay home from work, should avoid public places and should avoid intimate contact with others. Use of a disposable protective face mask (for example a surgical mask) to cover the nose and mouth during episodes of coughing or sneezing is recommended. Frequent handwashing is recommended.

The following samples will be collected for **ChAdOx1.HTI viral shedding assessment**:

- Skin swab at each injection site
- Saliva sample
- Nasal swab in each nostril
- Urine sample (1st morning void urine sample)
- Stool sample (when possible)

At the following timepoints:

- Before each ChAdOx1.HTI/placebo administration (on the same day of the IMP administration)
- 1 day after each ChAdOx1.HTI/placebo administration
- 7 days after each ChAdOx1.HTI/placebo administration

6.2.3 Safety assessments and cART resumption criteria during ATI period

Safety will be evaluated during Phase C ATI for all participants:

a) Weekly: clinical exam adverse events monitoring (including symptoms suggestive of acute retroviral syndrome), CD4 counts and pVL test.
b) Monthly: in addition to weekly assessment, safety laboratory test, plasma/PBMCs storage.

Participants will resume cART if they meet any of the following criteria:

- pVL of HIV-1 RNA is ≥ 10,000 copies/mL on 2 tests (at least 1 week apart) and does not decrease to <10,000 by 8 weeks after the first test.
- pVL of HIV-1 RNA >100,000 copies/mL
- CD4 count is confirmed to be <350 cells/mm3 (confirmed per repeat test 1 week after initial result)

A participant's ART also may be restarted at the discretion of the investigator based on other clinical criteria.

cART regimen will be at the discretion of the study physician, according to clinical criteria (no changes on participant's previous cART regimen are envisioned as no emergence of drug resistances is expected).
To address that viral re-suppression is achieved, all participants will be assessed on a safety follow-up at weeks 4 and 12 after cART resumption (i.e., end-of-study visit)

Participants who maintain HIV-1 pVL <2,000 copies/ml 24 weeks after ATI start (visit Phase C week 56) will be offered to end the study and stay off cART within a roll-over investigator initiated study out of the scope of the present protocol.

6.3 MEASURES TO AVOID BIAS

6.3.1 Randomization

Once eligibility has been confirmed at baseline (week 0, 1st DNA.HTI/placebo administration) the participants will be assigned vaccine or placebo through a randomisation schedule based on the randomisation plan using dedicated computer software. Participants will be randomized into 2 arms in a 2:1 (vaccine:placebo) ratio.
Allocation to active treatment or placebo will be maintained throughout the study (Phase A/B/C).

6.3.2 Stratification

Given the small size of this Phase I study no stratification will be undertaken.

6.3.3 Blinding

Laboratory personnel, clinical investigators, study nurses administering the IMP, data entry personnel, and participants will be blinded with respect to the allocation of vaccine or placebo. Only pharmacy staff and independent personnel preparing the IMPs will not be blinded.

Blinding of the investigators will be ensured by shielding vaccine vials (*ALX002-SOP04-IMP Masking*). Briefly, appropriate syringes will be labelled for administration using validated labels that comply with applicable regulatory requirement(s) and that protect the blinding by covering the entire syringe. Sterile saline injections will be prepared and labelled using the same protocol.

The statistician carrying out the randomisation will have no direct contact with participants nor blinded site/CRO personnel and sponsor blinded team members.

There will be designated clinical research associates (CRAs) in charge of monitoring unblinded procedures (randomization, dispensing, IMP preparation and masking). Mechanisms to guarantee the blinding within the CRA team are detailed in the Monitoring Manual (*ALX002- Manual03-Monitoring*).

6.3.4 Unblinding Procedures

Unblinding of an individual participant is indicated in the event of a medical emergency where the clinical management of the participant would be altered by knowledge of the IMP assignment. The decision to unblind will be immediately communicated to the Sponsor and SMC. Procedures and contact details for unblinding are defined in *ABX002-SOP02-Unblinding*. The site personnel will ensure

that the reasons for unblinding are documented in the electronic health record and in the unblinding register (*ALX002-Form06-Unblinding*). Participants will also be given contact details of trial staff to be contacted in an emergency (Appendix 8: *ALX002.ID Card*).

Note to mention that, in order to ensure study blindness and to avoid notifying sensitive details, the study team has a list of sensitive variables (*ALX002-MO2-sensitive variables*) and a contingency action plan to facilitate a quick resolution of IMP-related incidences that may occur as well as a detailed notification flow-chart to all blinded study and Sponsor members (*ALX002-M03-Contingency actions list for IMP-related incidences*).

Treatment allocation will remain blind for all participants along the entire study (Phase A/B/C) and until End-of-ATI analysis is completed.

6.4 FORESEEN CALENDAR

- CTA submission: March 2017

Phase A:
- Screening period: 4 weeks
- Inclusion period: 4 weeks
- Follow-up period: 32 weeks (12 weeks after last IMP administration)
- Interim safety report: 4 weeks after last participant at week 22 visit

- Amendment SA03 submission: December 2017

Phase B:
- Pre & Screening period: 12 weeks
- Inclusion period: 4 weeks
- Follow-up period: 32 weeks (12 weeks after last IMP administration)
- Interim safety report: 4 weeks after last participant at w21

- Amendment SA04 submission: Q4 2018

Phase C:
- Inclusion period: 20 weeks
- Follow-up period: maximum 68 weeks

- Amendment SA05 submission: July 2019

- Amendment SA06 submission: February 2020

Two reports summarizing findings from Phase C will be elaborated:

- Final Report, which will include safety/immunogenicity and efficacy data until c-ART resumption (i.e., end-of-ATI visit); and

- A Follow-up safety report, which will include safety data up to 12 weeks after c-ART resumption (i.e., end-of-study visit) plus exploratory reservoir endpoints.

6.5 END OF TRIAL

The study will be paused in the event that a product related SAE or ≥ Grade 4 local or systemic adverse event, or any related Grade 3 event lasting >72h within first 7 days after IMP administration occurs. In these cases, a review by the SMC will be requested (see section 9.7.). Following this review, the SMC will make a recommendation to the Principal Investigator and Sponsor regarding continuation of the study.

In addition, if more than 50% of participants withdraw from the study, the SMC will discuss a premature stop of the trial.

Otherwise, the date of the end of the trial will be the last Phase C visit of the last participant, expected to be 12 weeks after cART resumption (visit Phase C week 68).

6.5.1 Early study suspension

If the study must be interrupted prematurely, all non-used materials should be returned to the Sponsor.

6.6 SOURCE DATA

Source documents are the participants' electronic health records including laboratory results obtained from blood tests. Also, specific forms such as DNA.HTI/placebo, MVA.HTI/placebo and ChAdOx1.HTI/placebo administration forms (Appendix 1: *ALX002-Form01-Product administration*), will be considered as source documents. The original copy of all specific forms and a copy of source documents such as laboratory results will be included in the investigator site file.

Information regarding safety and tolerability of first 7 days following DNA.HTI/placebo, MVA.HTI/placebo and ChAdOx1.HTI/placebo administrations will be collected using a participant diary card (Appendix 2: *ALX002.Diary Card*) which will be reviewed by the attending physician for grading and assessment of causality/imputability, either 1 or 4 weeks after each administration (see Appendix 5: *ALX002.Schedule of procedures and blood volumes*). Additional safety information during the trial will be gathered through scheduled medical visits and safety laboratory tests.

Study data will be collected through an electronic Case Report Form (eCRF).

All investigational results from immunogenicity analysis performed will be recorded in electronic databases, as defined in the Data Management Plan (*ALX002-Manual04-Data Management*).

6.7 EXPERIMENTAL TREATMENT

Three investigational medicinal products (IMP)s will be tested in this trial:

- Vaccine DNA.HTI 4mg, an investigational immunological product (vaccine) from a biological / biotechnological origin (plasmid DNA)

- Vaccine MVA.HTI $2x10^8$ pfu, an investigational immunological product (vaccine) from a biological / biotechnological origin that contain genetically modified organisms (modified vaccinia virus Ankara)

- Vaccine ChAdOx1.HTI $5x10^{10}$ Vp, an investigational immunological product (vaccine) from a biological / biotechnological origin that contain genetically modified organisms (chimpanzee adenovirus)

This is a placebo-controlled study. The placebo will be a commercially sterile saline solution (sodium chloride (NaCl) 0.9%)

6.8 SUPPLY, PACKAGING, LABELING AND STORAGE

All IMPs are manufactured under GMP conditions and in compliance with European Union GMP Guidelines, Annex 13 by the contract cGMP manufacturer companies (CMO) NHSBT Clinical Biotechnology Centre, IDT Biologika GmbH and Advent. The vaccines will be open-labelled and labelled under GMP and in compliance with regulatory requirements.

The container closure system for DNA.HTI consists of a 2 mL sterile cyclo-olefin copolymer vial, a thermoplastic elastomer (TPE) stopper and a cap made of high density poly-ethylene (HDPE). MVA.HTI suspension is supplied in Type 1 clear borosilicate glass injection vials of 2 mL, sealed with 13 mm grey rubber injection stoppers and 13 mm flip off seals. ChAdOx1.HTI is supplied 3 mL/16 mm sterile borosilicate glass vials of type 1 quality, sealed with a Chlorobutyl/Fluorotec rubber stopper and an aluminium cap.

Investigational products will be supplied by AELIX Therapeutics.

Release of the IMP will be in compliance with procedures required by ICH-GCP. Authorisation to ship the IMP to the site will be given in writing by the Sponsor upon confirmation that all critical documents required for shipment authorisation are completed. The IMP will be shipped to the trial site on dry ice with temperature logging. IMPs will be stored at the Pharmacy Service of Hospital Universitari Germans Trias i Pujol under validated temperature-monitored systems, where reception, units, batch number, and expiration date will be confirmed. No conditioning nor labelling will be required in the Pharmacy Service, except in case expiry date were to be extended. The placebo will be stored at room temperature.

6.8.1 DNA.HTI

The DNA.HTI is a non-marketed vaccine, and will be supplied by AELIX Therapeutics. DNA.HTI has been manufactured by NHSBT Clinical Biotechnology Centre, Bristol, UK. Storage is to be performed in a temperature-monitored freezer at -65ºC.

6.8.2 MVA.HTI

The MVA.HTI is a non-marketed vaccine, and will be supplied by AELIX Therapeutics. MVA.HTI has been manufactured by IDT Biologika GmbH in Germany. Storage is to be performed in a temperature-monitored freezer at ≤-65ºC.

6.8.3 ChAdOx1.HTI

The ChAdOx1.HTI is a non-marketed vaccine, and will be supplied by AELIX Therapeutics. ChAdOx1.HTI has been manufactured by Advent in Italy. Storage is to be performed in a temperature-monitored freezer at <-60ºC.

6.8.4 Placebo

The placebo for all vaccines will be commercially normal saline solution (0.9% NaCl) for IM injection bought by the Hospital Pharmacy on behalf of AELIX Therapeutics. Saline is stored at room temperature.

Table 3. Description of IMPs and Placebos

Vaccine/Placebo	Dosage	Formulation	Volume Injected (approximate)
DNA.HTI	4 mg	PBS pH 7.4	1ml (500 µl x 2 injections)
MVA.HTI	$2x10^8$ pfu	Tris pH 7.7	500 µl (250 µl x 2 injections)
ChAdOx1.HTI	$5x10^{10}$ Vp	L- Histidine: 10 mM NaCl: 35 mM Sucrose: 7.5 % (w/v) MgCl2: 1 mM; EDTA disodium: 0.1 mM Tween 80 (Polysorbate-80): 0.1 % (w/v) Ethanol 0.5 %: (v/v) HCl: Adjusted to pH 6.6	500 µl (250 µl x 2 injections)
Normal saline placebo	0.9% NaCl	-	matched to IMPs

6.9 DOSE, INTERVAL, ROUTE AND METHOD OF ADMINISTRATION

The IMPs will be dispensed according to a study-specific SOPs (*ALX002-SOP05-IMP Dispensing*) by the Clinical Trial Pharmacist. Administration of DNA.HTI, MVA.HTI and ChAdOx1.HTI will be performed in the Unitat Polivalent d'Investigació Clínica (UPIC) from Hospital Universitari Germans Trias i Pujol by the designated study nurse. IMPs will be prepared, masked and administered in appropriate shielded syringes from view of participants and study team to ensure blinding.

6.9.1 DNA.HTI

Dose: 4mg
Interval: at Phase A/B weeks 0, 4, and 8 (not administered in Phase C).

Method of administration:

At the time of release of version 7.0 of the study protocol, all DNA.HTI administrations have been performed. DNA.HTI is an investigational product (vaccine) and was used with appropriate handling procedures. Study staff followed specific Standard Operating Procedure for the handling, dispensing, preparation, masking and administration of HIV vaccines for the AELIX-002 trial.

Briefly, during Phase A of the study, DNA.HTI vials were thawed until completely liquid in sterile conditions in a biological level 2 (BL2) safety hood located at the hospital pharmacy. The vial was not shaken and the volume was drawn and split into two 1ml syringes using a Closed system transfer device (CSTD) (500µl in each syringe) following instructions described in *ALX002-SOP03-IMP Preparation v2.0*. Syringes were masked following instructions described in *ALX002-SOP04-IMP Masking v1.0* and dispensed to the study nurse, following *ALX002-SOP05-IMP Dispensing v1.0* for immediate administration, following *ALX002-SOP06-IMP administration v1.0*.

After completion of Phase A of the trial and considering a) the gained expertise using the CSTD system, b) the nature of DNA.HTI not fulfilling any National Institute for Occupational Safety and Health (NIOSH)

criteria for hazardous drugs[b,c,d] and c) that were not any leakage or IMP-preparation accidents, during Phase B of the study IMP were allowed to be prepared as follows:

Briefly, DNA.HTI vaccines were dispensed by the pharmacist to designated unblinded study personnel in a closed container (following *ALX002-SOP05-IMP Dispensing v2.0)*. The IMPs were transferred and prepared in a designated BL1 room for drug preparation at the UPIC. There, product was thawed and transferred to two syringes using a CSTD system in aseptic conditions (following *ALX002-SOP03-IMP Preparation v3.0*). Then, syringes were masked accordingly to guarantee study team blindness (following *ALX002-SOP04-IMP Masking v2.0)*. After the preparation, shielded syringes were transferred in a closed container from the preparation room to the administration room for immediate administration by the blinded study nurse (following *ALX002-SOP06-IMP administration v2.0*). Used DNA.HTI vials were returned to the pharmacy for drug accountability (see Section 6.10) in a closed container.

DNA.HTI was administered by IM injection into the deltoid regions of both arms. Vitals and the injection sites were observed for 2h after IMP administration during Phase A and for 30 minutes after IMP administration during Phase B. Data was logged in a specific form (Appendix 1: *ALX002-Form01-Product administration Phase A* and *ALX002-Form01-Product administration Phase B*).

During Phase C, DNA.HTI will not be administered.

6.9.2 MVA.HTI

Dose: $2x10^8$ pfu
Interval: at Phase A/B weeks 12 and 20 and at Phase C week 24.

Method of administration:

At the time of release of version 7.0 of the study protocol, all Phase A/B MVA.HTI administrations have been performed, Phase C MVA.HTI administrations are still ongoing. MVA.HTI is an investigational product (vaccine) and will be used with appropriate handling procedures. Study staff will follow specific Standard Operating Procedure for the handling, dispensing, preparation, masking and administration of HIV vaccines for the AELIX-002 trial.

Briefly, during Phase A of the study, MVA.HTI vials were thawed until completely liquid in a biological level 2 (BL2) safety hood located at the hospital pharmacy. The vial was not be shaken and the required volume was drawn and split into two 1 mL syringes using a CSTD (250 µl in each syringe), following instructions described in *ALX002-SOP03-IMP Preparation v2.0*. Syringes were masked following instructions described in *ALX002-SOP04-IMP Masking v1.0* and dispensed to the study nurse, following *ALX002-SOP05-IMP Dispensing v1.0* for immediate administration, following *ALX002-SOP06-IMP administration v1.0*.

After completion of Phase A of the trial and considering a) the gained expertise using the CSTD system, b) the nature of MVA.HTI not fulfilling any National Institute for Occupational Safety and Health (NIOSH)

[b] Connor TH, Burroughs GE, McDiarmid MA, Mead KR, Power LA, Reed LD. NIOSH Alert: preventing occupational exposures to antineoplastic and other hazardous drugs in health care settings. Atlanta DHHS Publ. 2004;1–50.
[c] Connor TH, MacKenzie BA, DeBord DG, O'Callaghan JP, Trout DB. NIOSH list of antineoplastic and other hazardous drugs in healthcare settings 2014. Dep Heal Hum Serv Centers Dis Control Prev Natl Inst Occup Saf Heal DHHS. 2016.
[d] Page MR. Independent Tests Show Key Differences in Protective Efficacy of CSTDs, with Important Implications for Pharmacists [Internet]. Pharmacy Times. 2016 [cited 2018 Jul 8]. Available from: https://www.pharmacytimes.com/publications/health-system-edition/2016/september2016/independent-tests-show-key-differences-in-protective-efficacy-of-cstds-with-important-implications-for-pharmacists

criteria for hazardous drugs[e,f,g,h,i] and c) that were not any leakage or IMP-preparation accidents, during Phase B and C of the study IMP was allowed to be prepared as follows:

Briefly, MVA.HTI vaccines will be dispensed by the pharmacist to designated unblinded study personnel in a closed container (following *ALX002-SOP05-IMP Dispensing v2.0)*. The IMPs will be transferred and prepared in a designated BL1 room for drug preparation at the UPIC. There, product was thawed and transferred to two syringes using a CSTD system in aseptic conditions (following *ALX002-SOP03-IMP Preparation v3.0*). Then, syringes will be masked accordingly to guarantee study team blindness (following *ALX002-SOP04-IMP Masking v2.0)*. After the preparation, shielded syringes will be transferred in a closed container from the preparation room to the administration room for immediate administration by the blinded study nurse (following *ALX002-SOP06-IMP administration v2.0*). Used MVA.HTI vials will be returned to the pharmacy for drug accountability (see Section 6.10) in a closed container.

MVA.HTI in Phase A/B will be administered by IM injection into the deltoid regions of both arms. Vitals and the injection sites will be observed for 2h after IMP administration during Phase A and for 30 minutes after IMP administration during Phase B. Data will be logged in a specific form (Appendix 1: *ALX002-Form01-Product administration Phase A, ALX002-Form01-Product administration Phase B).*

Data will be logged in a specific form (Appendix 1: *ALX002-Form01-Product administration Phase C*).

6.9.3 ChAdOx1.HTI

Dose: $5x10^{10}$ Vp
Interval: At Phase C weeks 0 and 12.

Method of administration:

At the time of release of version 7.0 of the study protocol, all Phase C ChAdOx1.HTI administrations have been performed. ChAdOx1.HTI is an investigational product (vaccine) and was used with appropriate handling procedures. Study staff followed specific Standard Operating Procedure for the handling, dispensing, preparation, masking and administration of HIV vaccines for the AELIX-002 trial.

After completion of Phases A and B of the trial and considering a) the gained expertise using the CSTD system, b) the nature of ChAdOx1.HTI not fulfilling any National Institute for Occupational Safety and Health (NIOSH) criteria for hazardous drugs[j,k,l,m] and c) previous test of specific-CSTD for ChAdOx1.HTI resulting in no leakage or preparation accidents, during Phase C of the study IMP was allowed to be prepared as follows:

[e] von Wyl V, Gianella S, Fischer M, Niederoest B, Kuster H, Battegay M, et al. Early Antiretroviral Therapy During Primary HIV-1 Infection Results in a Transient Reduction of the Viral Setpoint upon Treatment Interruption. Weaver EA, editor. PLoS One. 2011 Nov 15;6(11):e27463.
[f] Zuñiga R, Lucchetti A, Galvan P, Sanchez S, Sanchez C, Hernandez A, et al. Relative dominance of Gag p24-specific cytotoxic T lymphocytes is associated with human immunodeficiency virus control. J Virol. 2006 Mar;80(6):3122–5.
[g] Zhang SC, Martin E, Shimada M, Godfrey SB, Fricke J, Locastro S, et al. Aminopeptidase substrate preference affects HIV epitope presentation and predicts immune escape patterns in HIV-infected individuals. J Immunol. 2012 Jun;188(12):5924–34.
[h] Verheust C, Goossens M, Pauwels K, Breyer D. Biosafety aspects of modified vaccinia virus Ankara (MVA)-based vectors used for gene therapy or vaccination. Vaccine. 2012 Mar;30(16):2623–32.
[i] Page MR. Independent Tests Show Key Differences in Protective Efficacy of CSTDs, with Important Implications for Pharmacists [Internet]. Pharmacy Times. 2016 [cited 2018 Jul 8]. Available from: https://www.pharmacytimes.com/publications/health-system-edition/2016/september2016/independent-tests-show-key-differences-in-protective-efficacy-of-cstds-with-important-implications-for-pharmacists
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Briefly, ChAdOx.HTI vaccines were dispensed by the pharmacist to designated unblinded study personnel in a closed container (following *ALX002-SOP05-IMP Dispensing v3.0)*. The IMPs were transferred and prepared in a designated BL1 room for drug preparation at the UPIC. There, product were thawed and transferred to two syringes using a CSTD system in aseptic conditions (following *ALX002-SOP03-IMP Preparation v4.0*). Alternatively, preparations could be performed in a biological level 2 (BL2) safety hood located at the IrsiCaixa AIDS Research Institute and using an intramuscular needle instead of the vial adaptor of the CSTD to transfer the product to two syringes in aseptic conditions.

Then, syringes were masked accordingly to guarantee study team blindness (following *ALX002-SOP04-IMP Masking v3.0)*. After the preparation, shielded syringes were transferred in a closed container from the preparation room to the administration room for immediate administration by the blinded study nurse (following *ALX002-SOP06-IMP administration v3.0*). Used ChAdOx1.HTI vials were returned to the pharmacy for drug accountability (see Section 6.10) in a closed container.

ChAdOx1.HTI is administered by IM injection into the deltoid regions of both arms.

Data was logged in a specific form (Appendix 1: *ALX002-Form01-Product administration Phase C*).

6.9.4 Placebo

Dose: 0.9% sterile normal saline solution
Interval: at Phase A/B weeks 0, 4, 8, 12, 20 and at Phase C weeks 0, 12 and 24.

Method of administration:

At the time of release of version 7.0 of the study protocol, all Phase A/B placebo administrations have been performed, Phase C placebo administrations are still ongoing. Study staff will follow specific Standard Operating Procedure for the handling, dispensing, preparation, masking and administration of HIV vaccines for the AELIX-002 trial.

Briefly, sterile saline injections will be dispensed and prepared using the same CSTD system similarly (two syringes with matching volumes of saline to vaccines for which the placebo are controlling). Then, syringes will be masked accordingly to guarantee study team blindness (following *ALX002-SOP04-IMP Masking v3.0)*. After the preparation, shielded syringes will be transferred in a closed container from the preparation room to the administration room for immediate administration by the blinded study nurse (following *ALX002-SOP06-IMP administration v3.0*). Used placebo vials will be returned to the pharmacy for destruction. No accountability is done.

Data will be logged in a specific form (Appendix 1: *ALX002-Form01-Product administration Phase A, ALX002-Form01-Product administration Phase B* and *ALX002-Form01-Product administration Phase C*).

The vaccine placebo will be administered by IM injection into the deltoid regions of both arms. Vitals and the injection sites will be monitored for the same time as the matched vaccine.

6.10 EXPERIMENTAL DRUG ACCOUNTABILITY

During the study, an accountability log, dispensing log and log of used vials will be kept for all IMPs at the Pharmacy Service. These logs will be monitored according to Good Clinical Practice (GCP) guidelines.

6.11 ARM DESCRIPTION

Phase A/B:

- Vaccine: DNA.HTI at weeks 0, 4, and 8 and MVA.HTI at weeks 12 and 20.
- Placebo: normal saline solution at weeks 0, 4, 8, 12, and 20.

Phase C:
- Vaccine: ChAdOx1.HTI at weeks 0 and 12 and MVA.HTI at week 24.
- Placebo: normal saline solution at weeks 0, 12 and 24.

Allocation to active treatment or placebo in Phase C will be maintained from Phase A/B.

6.12 CONCOMITANT TREATMENTS

All treatments, including antiretrovirals, administered during the study period will be considered concomitant treatments and should be documented in the eCRF.

All participants will be discouraged to initiate any concomitant treatment, including over-the-counter medications and alternative treatments, without the knowledge and permission of the investigator from 4 weeks before first DNA.HTI/placebo administration until the end of the study.

Indications for allowed medications and doses to treat local and mild vaccine reactogenicity will be described in the participant diary card (Appendix 2: *ALX002.Diary Card*)

Participants will be excluded if taking any interferon or systemic corticosteroids or other immunosuppressive agents (only use of inhaled steroids for asthma or topical steroids for localized skin conditions are permitted).

All participants will be discouraged from receiving any vaccination (including Hepatitis B, Pneumococcal, or Flu vaccinations and other licensed vaccinations) within 2 weeks preceding any IMP administration and during the ATI period.

6.13 COMPLIANCE

DNA.HTI, MVA.HTI, ChAd.Ox1.HTI and placebo compliance is guaranteed because study medication is to be administered by designated study nurse at the clinical site.

7 SELECTION AND WITHDRAWAL OF PARTICIPANTS

Participants will be adult men and women aged 18-60 who fully comprehend the purpose and details of this study as provided in the Patient Information Sheet and are able to provide informed consent. Eligibility will depend on the results of laboratory tests, review of medical history, and physical exam results.

7.1 INCLUSION CRITERIA PHASE A/B

1. Confirmed HIV-1 infection
2. On combined antiretroviral treatment (defined as ≥ 3 antiretroviral drugs) initiated within 6 months of estimated time of HIV-1 acquisition.
3. Willing and able to be adherent to their cART regimen for the duration of the study.
4. Optimal virological suppression for at least 1 year defined as maintained pVL below the limit of detection (based on current available assays, 20, 40 or 50 copies/ml) allowing for isolated blips (<200 cop/ml, non-consecutive, representing <10% total determinations).
5. Being on the same cART regimen for at least 4 weeks at screening visit.
6. Nadir CD4 count ≥ 200 cells per mm^3. Isolated lower counts at the moment of acute HIV-1 infection will be allowed only if appropriate immune recovery was followed after cART initiation (as is criteria 7)
7. Stable CD4 counts ≥ 500 cells per mm^3 (Phase A) or ≥ 400 cells per mm^3 (Phase B) for the last 6 months at screening visit.
8. Availability of stored biological sample (including PBMC and plasma) before any cART initiation.
9. Aged at least 18 years on the day of screening and no greater than 50 years (Phase A) or 60 years (Phase B) on the day of the first IMP administration.
10. Willing to comply with the requirements of the protocol and available for follow-up for the planned duration of the study.
11. In the opinion of the principal investigator or designee, the participant has understood the information provided and capable of giving written informed consent.
12. If heterosexually active female; using an effective method of contraception (hormonal contraception, intra-uterine device (IUD), or anatomical sterility in self or partner[1]) from 14 days prior to the first IMP administration until at least 12 weeks after the last IMP administration; all female volunteers must be willing to undergo urine pregnancy tests at time points specified in the Schedule of Procedures (Appendix 5: *ALX002.Schedule of procedures and blood volumes*).
13. If heterosexually active male; willing to use an effective method of contraception (anatomical sterility in self) or agree on the use of an effective method of contraception by his partner (hormonal contraception, intra-uterine device (IUD), or anatomical sterility[1]) from the day of the first IMP administration until 12 weeks after the last IMP administration.
14. Willing to accept blood draws and collect stool at time points specified in the Schedule of Procedures.
15. Willing to forgo donating blood during the study.

[1] *Condom use nor diaphragm are considered as an additional method of contraception only and cannot be the only method of contraception used as not been considered an effective method by the Clinical Trial Facilitation Group (CTFG) guidelines.*

7.2 EXCLUSION CRITERIA PHASE A/B

1. Pregnancy or lactating.
2. When available, pre-cART genotypic data that demonstrates the presence of clinically significant drug resistance mutations that would prevent the construction of a viable cART

regimen post-treatment interruption

3. Reported periods of suboptimal adherence to cART
4. History of past antiretroviral treatment interruptions longer than 2 weeks.
5. Participation in another clinical trial within 12 weeks of study entry (at screening visit).
6. Any AIDS-defining disease or progression of HIV-related disease.
7. History of autoimmune disease.
8. History or clinical manifestations of any physical or psychiatric disorder which could impair the subject's ability to complete the study.
9. Receipt of approved vaccines within 2 weeks of study entry and along the duration of the trial[1]
10. History of anaphylaxis or severe adverse reaction to vaccines.
11. Previous immunisation with any experimental immunogens.
12. Receipt of blood products within 6 months of study entry.
13. Treatment for cancer or lymphoproliferative disease within 1 year of study entry.
14. Any other current or prior therapy which, in the opinion of the investigators, would make the individual unsuitable for the study or influence the results of the study.
15. Current or recent use (within last 3 months) of interferon or systemic corticosteroids or other immunosuppressive agents (use on inhaled steroids for asthma or topic steroids for localized skin conditions are permitted).
16. Any laboratory abnormalities including:

    Haematology
    - Haemoglobin < 10.0 g/dl
    - Absolute Neutrophil Count (ANC) ≤ 1,000 /mm^3 (≤ 1 x 10^9 /l)
    - Absolute Lymphocyte Count (ALC) ≤ 600 /mm^3 (≤ 0.6 x 10^9 /l)
    - Platelets ≤100,000 /mm^3, ≥ 550,000 /mm^3 (≤ 100 /l, ≥ 550 /l)

    Biochemistry
    - Creatinine > 1.3 x ULN
    - Aspartate aminotransferase (AST) > 2.5 x ULN
    - Alanine aminotransferase (ALT) > 2.5 x ULN

    Microbiology
    - Positive for hepatitis B surface antigen,
    - Positive for hepatitis C antibody, unless confirmed clearance of HCV infection (spontaneous or following treatment)
    - Positive serology indicating active syphilis requiring treatment[2]

17. (Phase A participants only) Small-pox vaccination.
    - Clinical evidence of vaccinia scarification or self-reported history of vaccinia vaccination.

18. (Phase B participants only) Refusal to an eventual cART interruption within the scope of a future study objective[3].

[1]*Efforts will be made to ensure all participants included are updated on their Hepatitis A, Hepatitis B and Pneumococcal vaccinations before enrolment. Participants willing to undergo seasonal Flu vaccinations or other licensed vaccinations will be excluded if vaccination would be expected to occur throughout the duration of the trial. Administration of approved vaccines will be allowed during the Roll-over Phase.*
[2] *Cases in which positive RPR titres are detected but syphilis has been confirmed to have been properly treated will be allowed if treatment has been given >2 months prior to study entry.*

[3]A questionnaire related to participation in vaccine/cure trials and cART interruption is performed during the screening visit to be able to address all participant's expectations and worries in a timely manner to reduce the risk of participants lost to follow-up during the trial.

7.3 INCLUSION CRITERIA PHASE C

1. Participants having participated on Phases A or B.
2. Participants having received second MVA.HTI administration on Phase A/B at least 24 weeks prior to entry.
3. Willing and able to be adherent to their cART regimen for the duration of Phase C.
4. Willing to comply with the requirements of the protocol and available for follow-up for the planned duration of the study Phase C.
5. In the opinion of the investigator, the participant has understood the information provided and capable of giving Phase C written informed consent.
6. If heterosexually active female; using an effective method of contraception (hormonal contraception, intra-uterine device (IUD), or anatomical sterility in self or partner[1]) from 14 days prior to the first IMP administration until at least 12 weeks after the last IMP administration and during ATI; all female volunteers must be willing to undergo urine pregnancy tests at time points specified in the Schedule of Procedures (Appendix 5: *ALX002.Schedule of procedures and blood volumes*).
7. If heterosexually active male; willing to use an effective method of contraception (anatomical sterility in self) or agree on the use of an effective method of contraception by his partner (hormonal contraception, intra-uterine device (IUD), or anatomical sterility[1]) from the day of the first IMP administration until 12 weeks after the last IMP administration and during ATI.
8. Willing to accept blood draws and collect stool at time points specified in the Schedule of Procedures.
9. Not willing to donate blood during the study.

[1] *Condom use nor diaphragm are considered as an additional method of contraception only and cannot be the only method of contraception used as not been considered an effective method by the Clinical Trial Facilitation Group (CTFG) guidelines.*

7.4 EXCLUSION CRITERIA PHASE C

1. Pregnancy or lactating.
2. Virological failure during Phase A/B, defined as 2 consecutive determinations of pVL > 200 cop/ml.
3. Reported periods of suboptimal adherence to cART during Phase A/B.
4. History of antiretroviral treatment interruptions longer than 2 weeks during Phase A/B.
5. Any AIDS-defining disease or progression of HIV-related disease.
6. History of autoimmune disease.
7. History or clinical manifestations of any physical or psychiatric disorder which could impair the subject's ability to complete the study.
8. Receipt of approved vaccines within 2 weeks before first IMP administration in Phase C visit[1]
9. History of anaphylaxis or severe adverse reaction to vaccines.
10. Previous immunisation with any experimental immunogens other than DNA.HTI and MVA.HTI.
11. Treatment for cancer or lymphoproliferative disease within 1 year of Phase C screening visit.
12. Any other current or prior therapy which, in the opinion of the investigators, would make the individual unsuitable for the study or influence the results of the study.
13. Current or recent use (within 3 months prior to first IMP administration of Phase C) of interferon or systemic corticosteroids or other immunosuppressive agents (use on inhaled steroids for asthma or topic steroids for localized skin conditions are permitted).
14. Any laboratory abnormalities including:

Haematology

- Haemoglobin < 10.0 g/dl
- Absolute Neutrophil Count (ANC) ≤ 1,000 /mm^3 (≤ 1 x 10^9 /l)
- Absolute Lymphocyte Count (ALC) ≤ 600 /mm^3 (≤ 0.6 x 10^9 /l)
- Platelets ≤100,000 /mm^3, ≥ 550,000 /mm3 (≤ 100 /l, ≥ 550 /l)

Biochemistry

- Creatinine > 1.3 x ULN
- Aspartate aminotransferase (AST) > 2.5 x ULN
- Alanine aminotransferase (ALT) > 2.5 x ULN

Microbiology

- Positive for hepatitis B surface antigen,
- Positive for hepatitis C antibody, unless confirmed clearance of HCV infection (spontaneous or following treatment)
- Positive serology indicating active syphilis requiring treatment[2]

15. Complete refusal to cART interruption[3].

[1]*Efforts will be made to ensure all participants included are updated on their Hepatitis A, Hepatitis B and Pneumococcal vaccinations before enrolment. All participants will be discouraged from receiving any vaccination (including Hepatitis B, Pneumococcal, or Flu vaccinations and other licensed vaccinations) within 2 weeks preceding any IMP administration and during the ATI period.*
[2] *Cases in which positive RPR titres are detected but syphilis has been confirmed to be properly treated will be allowed if treatment has been given >2 months prior to study entry.*
[3]*A questionnaire related to participation in vaccine/cure trials and cART interruption was performed during the End of Phase A/B visits to be able to address all participant's expectations and worries in a timely manner to reduce the risk of participants lost to follow-up during the trial.*

7.5 SUBJECT WITHDRAWAL CRITERIA

7.5.1 Related to DNA.HTI, MVA.HTI and ChAdOx1.HTI or placebo administration

Any individual for who is being considered for discontinuation or postponement of IMP administrations will be discussed with the trial team and Sponsor in the regular trial management meetings.

Participants might be discontinued or postponed for any of the following reasons:

- Ineligibility (either arising during the study or retrospective having been overlooked at screening).

- A disease, condition or an adverse event (including clinically significant abnormal laboratory values; see below) that develops, regardless of relationship to the study products, if, in the opinion of the investigator, further vaccinations would jeopardize the safety of the participant

- Haematology

  - Haemoglobin < 10.0 g/dl
  - Absolute Neutrophil Count (ANC) ≤ 1,000 /mm^3 (≤ 1 x 10^9 /l)
  - Absolute Lymphocyte Count (ALC) ≤ 600 /mm^3 (≤ 0.6 x 10^9 /l)

- Platelets ≤100,000 /mm^3, ≥ 550,000 /mm^3 (≤ 100 /l, ≥ 550 /l)

- Biochemistry
  - Creatinine > 1.3 x ULN
  - Aspartate aminotransferase (AST) > 2.5 x ULN
  - Alanine aminotransferase (ALT) > 2.5 x ULN

- Pregnancy.
- Significant non-compliance with study requirements.
- A related serious adverse event to IMP administration.
- An adverse event which requires discontinuation of the study product or results in inability to continue to comply with study procedures.
- Loss to follow up.
- The safety of the participant would be jeopardized in the opinion of the investigator or sponsor.
- Investigator discretion.
- Significant protocol deviation.
- Consent withdrawn.

7.5.2 Early subject withdrawal

Participants will complete the clinical study before the stipulated time in the following circumstances:

- Concurrent process or illness which in the opinion of the investigator requires the withdrawal of the participant.
- Protocol deviation which in the opinion of the sponsor requires the withdrawal of the participant.
- The participant does not wish to continue in the study.

7.5.3 Medical approach to withdrawal

In all cases, 'end of study form' is to be filled. Detailed information will be given about the date and reasons of the discontinuation to the sponsor. The investigator will facilitate the necessary medical support to the participant.

7.5.4 Follow-up after early withdrawal

That is, as a general rule, all participants who discontinue the protocol prematurely during Phase A/B will undergo a clinical examination and all tests specified in Phase A/B week 32 visit. The Roll-over Phase will not be offered to these participants.

All participants who discontinue the protocol prematurely during Roll-over Phase will undergo a clinical examination and all tests specified in the Roll-over Phase week 80 visit.

All participants who discontinue the protocol prematurely during Phase C will undergo a clinical examination and all tests specified in the Phase C week 68 visit (i.e., end-of-study visit).

In case early withdrawal happens after at least one IMP administration, a follow-up of either in-person or remote visit 28 days after the last IMP dose will be performed for AEs collection.

7.5.5 Replacement of participants

Replacement of participants will be allowed only in Phase A/B in case of early withdrawal happening before participants have received first IMP. No replacement of participants will be allowed during Phase C.

In case that a withdrawal of a Sentinel participant (Group 1) happens after the first IMP administration, one Non-sentinel participant from Group 2 will be asked to be re-allocated as Sentinel in order to keep sentinel surveillance in at least 3 participants. Selection of participants to be re-allocated will be done by the pharmacist and the unblinded CRA to ensure same number of vaccine (n=2) and placebo (n=1) recipients are kept in the Sentinel group.

SMC will be called if more than 50% of participants withdraw to consider a premature stop of the trial.

7.6 PRE-RANDOMIZATION / PRE-BASELINE LOSSES

Data from participants that do not meet the selection criteria after completing the screening visit will not be considered for the analysis of the study, but the reason for not meeting selection criteria will be recorded in the screening log form in an anonymised manner, and data will also be collected in the eCRF.

8 CLINICAL PROCEDURES

8.1 RECRUITMENT AND PRE-SCREENING VISIT

Participants will be recruited at the HIV Unit from FLS/HUGTIP.

For Phases A and B, potential candidates will be contacted and offered a pre-screening visit to have an informal discussion with the Principal Investigator or collaborators, when they will be provided with information on the vaccines and the AELIX-002 study protocol and will be given a copy of the Patient Information Sheet (Appendix 3: *ALX002.PIS and IC Phase A*, *ALX002.PIS and CI Phase B*) and the Summary information sheet (Appendix 4: *ALX002.Summary PIS Phase A*, *ALX002. Summary PIS Phase B*). Candidates will have the opportunity to ask questions and to arrange an appointment for a full screening visit if they wish. In the scheduling of screening visits, it will be ensured that candidates have adequate time to decide whether to participate.

A registry will be maintained with candidates having a pre-screening visit in order to know how many candidates were contacted for potential study participation. Only the date of the pre-screening visit and whether the candidate wanted to participate in the study will be recorded. In case the candidate wants to participate, the screening visit date, reasons for exclusion, if applicable, and the participant identification number (once candidate is included) will be recorded. A screening log form will be used.

All participants of Phase A and B will be offered participation in Phase C, during a Roll-over study visit, at least 24 weeks after 2nd MVA.HTI/placebo administration. Participants will be provided with information on Phase C and will be given a copy of the Patient Information Sheet (Appendix 3: *ALX002.PIS and IC Phase C*). Participants will have the opportunity to ask questions and it will be ensured that participants have adequate time to decide whether to participate. The Roll-over visit will be accepted as screening visit for Phase C, if performed within timelines defined in the schedule of procedures (Appendix 5: *ALX002.Schedule of procedures and blood volumes*).

8.2 PATIENT INFORMATION AND INFORMED CONSENT FORM

Informed consent is the process of ensuring that participants fully understand what will and may happen to them while participating in a research study. The patient information and informed consent form documents that a participant (i) has been informed about the potential risks, benefits, and alternatives to study participation, and (ii) is willing to participate in the study. Patient information and informed consent encompasses all written or verbal study information that staff provide to the participant, before and during the trial.

The informed consent process continues throughout the study. Key study concepts should be reviewed periodically with the participants, such as the procedures and requirements for that visit and for the remaining visits. Additionally, if any new information is learned that might affect the participants' decisions to stay in the trial, this information will be shared with participants. If necessary, participants will be asked to sign revised informed consent forms.

All participants must sign a protocol-specific consent before any procedures are performed to determine eligibility.

There will be specific informed consent forms for participants to be included in Phase A, Phase B, for the Roll-over Phase and Phase C. Also, there will be a specific informed consent for pregnancies and suspected pregnancies notifications, and for PrEP use (a co-signed information sheet by the participant and his/her HIV seronegative partner).

8.3 VISIT SCHEDULE

A detailed Schedule of Procedures associated with every visit can be found in Appendix 5: *ALX002.Schedule of procedures and blood volumes*.

Study visits will take place in the clinical research unit (Unitat Polivalent d'Investigació Clínica (UPIC) at the Hospital Universitari German Trias i Pujol (HUGTIP) in Badalona, Spain. During the Phase C- ATI follow up visits at BCN-Checkpoint community centre will be allowed.

8.3.1 Phase A/B screening visit

The following procedures will be performed:

- Site personnel will obtain informed consent;
- Inclusion and exclusion criteria will be reviewed;
- Data collection: demographics, history of past and foreseen vaccinations, history of HIV infection and other medical conditions, prior and current antiretroviral and concomitant medications;
- Use of antibiotics for the last 6 months will be recorded (including antibiotic name, dose, and approximate start-end dates);
- Comprehensive clinical examination including vital signs (temperature, pulse, and blood pressure);
- ECG;
- Serology (hepatitis B, hepatitis C, syphilis);
- HIV viral load*;
- Safety bloods (sodium, potassium, urea, creatinine, estimated glomerular filtration rate (CKD-EPI), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-GT (GGT), alkaline phosphatase (AP), total bilirubin, total protein, albumin)*;
- Complete blood count (CBC): Hb, HCT, platelets, WBC, lymphocytes, & neutrophils*
- Immunology (CD4/CD8 T-cell counts)*;
- INR and PT*;
- Blood draw for HLA typing;
- Urine pregnancy test (female participants)*;
- Plasma and PBMC storage following scheme detailed in Appendix 5: *ALX002.Schedule of procedures and blood volumes**;
- Provide a calendar with all the planned visits (Appendix 6: *ALX002-Form11-Participant Calendar*);
- Pregnancy prevention assessment (female and male participants in HTS relationships);
- Fill expectations and worries questionnaire (Appendix 13: *ALX002-Form26- Questionnaire of expectations and worries screening*). Address questions with the participant;
- Schedule next visit

* All laboratory data must be obtained within a period of 8 weeks prior to enrolment. Otherwise, these lab tests must be repeated.

8.3.2 Phase A/B enrolment visit (week 0) and first IMP administration

Enrolment is simultaneous with the first administration of the study product. At the enrolment visit the following procedures are performed before IMP administration:

- Review results of laboratory tests with participant if required;
- Review inclusion and exclusion criteria;
- Urine pregnancy test (female participants);

- Assessment of any new or unresolved AEs/intercurrent illnesses;
- Assessment of concomitant medications;

Once the eligibility of the participant is confirmed, the following procedures will be performed:

- Randomisation;
- Abbreviated clinical examination, including weight, vital signs, and a symptom-directed evaluation by history and/or appropriate physical exam based on participant self-reported symptoms or complaints;
- Plasma and PBMC storage following scheme detailed in Appendix 5: *ALX002.Schedule of procedures and blood volumes*;
- Stool sample collection and storage following scheme detailed in Appendix 5: *ALX002.Schedule of procedures and blood volumes*;
- Fill dietary questionnaire (Appendix 7: *ALX002-Form12-Dietary questionnaire*);
- Intramuscular administration of study product;
- Fill in the product administration form (Appendix 1: *ALX002-Form01 Product administration*);
- Provide the participant with the diary card (Appendix 2: *ALX002.Diary Card*) and the Participant Identification card (Appendix 8: *ALX002.ID Card*);
- Give instructions to fill diary card;
- Provide a thermometer and a measuring tape;
- Schedule next visit

8.3.3 Phase A/B subsequent IMP administration visits (w4, w8, w12 and w20)

At all IMP administration visits, the following procedures are performed before IMP administration:

- Review results of laboratory tests with participant if required and provide post-test counselling as appropriate;
- Abbreviated clinical examination, including weight, vital signs, and a symptom-directed evaluation by history and/or appropriate physical exam based on participant self-reported symptoms or complaints;
- Assessment of concomitant medications;
- Assessment of any new or unresolved AEs/intercurrent illnesses;
- For Phase B participants, in visits W4 and W8, assessment of participant diary card (Appendix 2: *ALX002.Diary Card*);
- Urine pregnancy test (female participants);
- Blood draw for HIV viral load following scheme detailed in Appendix 5: *ALX002.Schedule of procedures and blood volumes;*
- Plasma and PBMC storage following scheme detailed in Appendix 5: *ALX002.Schedule of procedures and blood volumes;*
- Only at w20 blood draw for complete blood count (CBC): Hb, HCT, platelets, WBC, lymphocytes, & neutrophils and immunology (CD4/CD8 T-cell counts) will be done.

Following completion of all procedures in the preceding list, IMP administration may proceed. Clinical team confirms IMP administration to the trial pharmacist using the IMP administration prescription, IMP are prepared (*ALX002-SOP03-IMP Preparation*, *ALX002-SOP04-IMP Masking*) and transferred to study nurse for immediate administration (*ALX002-SOP06-IMP administration*). The following procedures will be performed:

- Intramuscular administration of study product;
- Fill in the product administration form (Appendix 1: *ALX002-Form01 Product administration*).

Immediately following IMP administration, the participant remains in the clinic for observation. Vital signs are collected at 10, 30 minutes and 2 hours post administration for Phase A participants, and 10 and 30 minutes post administration for Phase B participants. An initial reactogenicity assessment is made at a target of 30 minutes after injection, with an acceptable range of 25-60 minutes and at the end of the visit. Before leaving the clinic, the participant is given the post-administration symptom diary card and is instructed on how to complete it. A list of allowed medications to treat reactogenicity symptoms is provided in the participant diary card (Appendix 2: *ALX002.Diary Card*).

The following procedures will be performed at all Phase A/B IMP administration visits. These procedures may be performed prior to or following IMP administration:

- Pregnancy prevention assessment (female and male participants in HTS relationships);
- Stool sample collection and storage only at w12 (First MVA.HTI/Placebo administration) following scheme detailed in Appendix 5: *ALX002.Schedule of procedures and blood volumes*;
- Fill dietary questionnaire only at w12 (First MVA.HTI/Placebo administration) (Appendix 7: *ALX002-Form12-Dietary questionnaire*);
- Provide the participant with the diary card (Appendix 2: *ALX002.Diary Card*)
- Give instructions to fill diary card;
- Schedule next visit and review trial procedures;

8.3.4 Phase A Follow-up visits (w1, w5, w9, w13, w21, w22, w24)

For Phase B follow-up visits see section 8.3.5.

At all follow-up visits of Phase A participants, the following procedures are performed:

- Review results of laboratory tests;
- Abbreviated clinical examination, including weight, vital signs, and a symptom-directed evaluation by history and/or appropriate physical exam based on participant self-reported symptoms or complaints;
- Assessment of concomitant medications;
- Assessment of any new or unresolved AEs/intercurrent illnesses;
- Plasma and PBMC storage following scheme detailed in Appendix 5: *ALX002.Schedule of procedures and blood volumes*;
- Pregnancy prevention assessment (female and male participants in HTS relationships);
- Schedule next visit and review trial procedures.
- Stool sample collection and storage only at week 22 following scheme detailed in Appendix 5: *ALX002.Schedule of procedures and blood volumes*;
- Fill dietary questionnaire only at week 22 (Appendix 7: *ALX002-Form12-Dietary questionnaire*).

At weeks 1, 5, 9, 13 and 21, for Phase A participants, a specific assessment of reactogenicity will be done in addition with the following laboratory tests:

- HIV viral load;
- Limited safety bloods (sodium, potassium, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT),
- Complete blood count (CBC): Hb, HCT, platelets, WBC, lymphocytes, & neutrophils

Participants in the Sentinel group will also perform additional follow-up visits 1 day after each IMP administration. In this visit, following procedures will be performed:

- Abbreviated clinical examination, including vital signs, and a symptom-directed evaluation by history and/or appropriate physical exam based on participant self-reported symptoms or complaints;
- Assessment of reactogenicity parameters;
- Assessment of concomitant medications;
- Assessment of any new or unresolved AEs/intercurrent illnesses AEs.
- Schedule next visit and review trial procedures.

In case a Sentinel participant would not show up to the +1 day scheduled visit, a clinical follow-up visit by telephone would be accepted to allow for next Sentinel IMP administration.

8.3.5 Phase B Follow-up visits

8.3.5.1 Phase B Remote safety visits (w1 and w5)

During Phase B, one week after 1st and 2nd DNA.HTI/placebo administration visits, a remote visit with the participant by phone contact will be performed by the physician to assess safety and tolerability. The following procedure will be performed:

- Record of any grade 3/4 adverse event. In cases where Grade ≥ 3 adverse event are reported, close monitoring (either in-person or by phone contact) will be performed by the study team to address evolution of the adverse event.

8.3.5.2 Phase B in-person visits (w9, w13, w21, w24)

During Phase B, one week after 3rd DNA.HTI/placebo administration, one week after 1st and 2nd MVA.HTI/placebo administration and at week 24, the following procedures will be performed:

- Abbreviated clinical examination, including weight, vital signs, and a symptom-directed evaluation by history and/or appropriate physical exam based on participant self-reported symptoms or complaints;
- Assessment of concomitant medications;
- Assessment of any new or unresolved AEs/intercurrent illnesses;
- Assessment of participant diary card (Appendix 2: *ALX002.Diary Card*);
- Plasma and PBMC storage following scheme detailed in Appendix 5: *ALX002.Schedule of procedures and blood volumes*;
- Pregnancy prevention assessment (female and male participants in HTS relationships);
- Schedule next visit and review trial procedures;
- Stool sample collection and storage only at week 24 following scheme detailed in Appendix 5: *ALX002.Schedule of procedures and blood volumes*;
- Fill dietary questionnaire only at week 24 (Appendix 7: *ALX002-Form12-Dietary questionnaire*).

At Phase B weeks 9, 13 and 21, a specific assessment of reactogenicity will be done in addition with the following laboratory tests:

- HIV viral load;
- Limited safety bloods (sodium, potassium, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT);
- Complete blood count (CBC): Hb, HCT, platelets, WBC, lymphocytes, & neutrophils.

8.3.6 End of Phase A/B visit (w32)

At the end of Phase A/B visit, the following procedures are performed:

- Abbreviated clinical examination, including weight, vital signs, and a symptom-directed evaluation by history and/or appropriate physical exam based on participant self-reported symptoms or complaints;
- Assessment of concomitant medications;
- Assessment of any new or unresolved AEs/intercurrent illnesses;
- Urine pregnancy test;
- HIV viral load;
- Limited safety bloods (sodium, potassium, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT);
- Complete blood count (CBC): Hb, HCT, platelets, WBC, lymphocytes, & neutrophils
- Immunology (CD4/CD8 T-cell counts);
- Plasma and PBMC storage following scheme detailed in Appendix 5: *ALX002.Schedule of procedures and blood volumes*;
- Stool sample collection and storage following scheme detailed in Appendix 5: *ALX002.Schedule of procedures and blood volumes*;
- Fill dietary questionnaire (Appendix 7: *ALX002-Form12-Dietary questionnaire*);
- Fill Expectations and worries questionnaire (Appendix 13: *ALX002-Form27- Questionnaire of Expectations and worries EoS*);
- Invite participants to participate into the Roll-over Phase. Obtain informed consent for the Roll-over Phase (Appendix 3: *ALX002.PIS and IC Phase RO*).

8.3.7 Roll-over follow-up visits (every 12 weeks)

After visit Phase A/B week 32, participants will be followed-up every 12 weeks until entering Phase C. The following procedures will be performed:

- Abbreviated clinical examination, including weight, vital signs, and a symptom-directed evaluation by history and/or appropriate physical exam based on participant self-reported symptoms or complaints;
- Assessment of concomitant medications;
- Assessment of any new or unresolved AEs/intercurrent illnesses;
- Urine pregnancy test;
- HIV viral load;
- Safety bloods (sodium, potassium, urea, creatinine, estimated glomerular filtration rate (CKD-EPI), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-GT (GGT), alkaline phosphatase (AP), total bilirubin, total protein, albumin)
- Complete blood count (CBC): Hb, HCT, platelets, WBC, lymphocytes, & neutrophils
- Immunology (CD4/CD8 T-cell counts);
- Plasma and PBMC storage following scheme detailed in Appendix 5: *ALX002.Schedule of procedures and blood volumes*.

At visits Roll-over Phase week 44 and week 68, the following additional laboratory tests will be performed:

- Fasting glucose and lipid profile (Triglycerides, Total cholesterol, HDL, LDL)
- Hepatitis C and Syphilis serologies
- Urine Multiplex PCR

During Roll-over Phase, participants will be offered to participate in Phase C. If they accept, a Phase C screening visit will be scheduled.

8.3.8 Phase C screening visit

The following procedures will be performed:

- Site personnel will obtain informed consent;
- Phase C inclusion and exclusion criteria will be reviewed;
- Data collection: antiretroviral treatment and concomitant medications;
- Comprehensive clinical examination including vital signs (temperature, pulse, and blood pressure);
- HIV transmission prevention assessment and address PrEP needs in seronegative stable sexual partners;
- Serology (hepatitis B, hepatitis C, syphilis*)
- HIV viral load*;
- Safety bloods (sodium, potassium, urea, creatinine, estimated glomerular filtration rate (CKD-EPI), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-GT (GGT), alkaline phosphatase (AP), total bilirubin, total protein, albumin)*;
- Complete blood count (CBC): Hb, HCT, platelets, WBC, lymphocytes, & neutrophils*;
- Immunology (CD4/CD8 T-cell counts)*;
- INR and PT*;
- Urine pregnancy test (female participants);
- Plasma and PBMC storage following scheme detailed in Appendix 5: *ALX002.Schedule of procedures and blood volumes*;
- Provide a calendar with all the planned visits (Appendix 6: *ALX002-Form11-Participant Calendar*);
- Pregnancy prevention assessment (female and male participants in HTS relationships);
- Schedule next visit and provide the participant with recipients, materials and instructions for stool and urine samples collection at week 0.

* All laboratory data must be obtained within a period of 12 weeks prior to enrolment. Otherwise, or if clinically indicated, these lab tests will be repeated.

8.3.9 Phase C first IMP administration (w0)

The following procedures will be performed before IMP administration:

- Review results of laboratory tests with the participant if required;
- Abbreviated clinical examination, including weight, vital signs, and a symptom-directed evaluation;
- Assessment of concomitant medications;
- Assessment of any new or unresolved AEs/intercurrent illnesses;
- Review Phase C inclusion and exclusion criteria;
- Urine pregnancy test (female participants);

Once the eligibility of the participant is confirmed, the following procedures will be performed before IMP administration:

- Body secretions collection and storage as detailed in the Study Laboratory Manual (*ALX002-Manual01-Laboratory*), following scheme detailed in Appendix 5: ALX002.Schedule of procedures and blood volumes:

  - Skin swab at each injection site
  - Saliva sample
  - Nasal swab in each nostril
  - Urine sample (1st morning void urine sample, collected by the participant at home)
  - Stool sample (allowed to be collected by the participant at home the IMP administration day or one day before IMP administration)
- Blood draw for:
  - HIV viral load following scheme detailed in Appendix 5: *ALX002.Schedule of procedures and blood volumes;*
  - Safety bloods (sodium, potassium, urea, creatinine, estimated glomerular filtration rate (CKD-EPI), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-GT (GGT), alkaline phosphatase (AP), total bilirubin, total protein, albumin);
  - Complete blood count (CBC): Hb, HCT, platelets, WBC, lymphocytes, & neutrophils;

- Plasma and PBMC storage following scheme detailed in Appendix 5: *ALX002.Schedule of procedures and blood volumes*;

Following completion of all procedures described before, IMP administration may proceed. Clinical team will confirm IMP administration to the trial pharmacist using a IMP administration prescription, IMP will be prepared (*ALX002-SOP03-IMP Preparation*, *ALX002-SOP04-IMP Masking*) and transferred to study nurse for immediate administration (*ALX002-SOP06-IMP administration*). The following procedures will be performed:

- Intramuscular administration of study product;
- Fill in the product administration form (Appendix 1: *ALX002-Form01 Product administration*);

After IMP administration, the participants will remain in the clinic for clinical monitoring. For the first 15 participants (Groups 1 and 2) vital signs will be recorded 10 minutes, 30 minutes and 2 hours post-administration. For the rest of participants (Group 3) vital signs will be recorded only 10 and 30 minutes post-administration, if no related SAE or ≥ Grade 3 AE after IMP administration are present in any of the 15 participants in Groups 1 and 2. Otherwise, same procedures as in Groups 1 and 2 will be followed (i.e., 10 minutes, 30 minutes and 2 hours post-administration).

Local reactogenicity will be assessed 30 minutes after IMP administration, with an acceptable range of 25-60 minutes, as well as at the end of the visit. Before leaving the clinic, participants will be given a participant diary card and will be instructed on how to fill it in. A list of allowed medications to treat reactogenicity symptoms is provided in the participant diary card (Appendix 2: *ALX002.Diary Card*).

The following procedures will be performed at all IMP administration visits. These procedures may be performed before or after IMP administration:

- Educate participant to follow precautionary measures detailed in the diary card (see section 6.2.2) during the study, and provide with band-aids and surgical masks.
- Provide the participant with the diary card (Appendix 2: *ALX002.Diary Card*) with reminder of the precautionary measures to be followed. Give instructions to fill diary card.
- If the participant does not have it from Phase A/B, provide the Participant Identification card (Appendix 8: *ALX002.ID Card*);
- Give instructions to fill diary card;
- Pregnancy prevention assessment (female and male participant in HTS relationships);
- Provide a thermometer and a measuring tape;
- Stool sample collection and storage following scheme detailed in Appendix 5: ALX002.Schedule of procedures and blood volumes (will be performed after IMP administration if not possible to be performed before IMP administration);

- Fill dietary questionnaire (Appendix 7: *ALX002-Form12-Dietary questionnaire*);
- Schedule next visit and provide the participant with recipients, materials and instructions for stool and urine samples collection at day 1.

8.3.10 Phase C subsequent IMP administration Visits (w12 and w24)

The following procedures will be performed before IMP administration:

- Review results of laboratory tests, and provide post-test counselling as appropriate;
- Abbreviated clinical examination, including weight, vital signs, and a symptom-directed evaluation;
- Assessment of concomitant medications;
- Assessment of any new or unresolved AEs/intercurrent illnesses;
- Urine pregnancy test (female participants);
- Blood draw for HIV viral load following scheme detailed in Appendix 5: *ALX002.Schedule of procedures and blood volumes*;
- Only at visit Phase C week 24, blood draw for Safety bloods (sodium, potassium, urea, creatinine, estimated glomerular filtration rate (CKD-EPI), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-GT (GGT), alkaline phosphatase (AP), total bilirubin, total protein, albumin) and complete blood count (CBC): Hb, HCT, platelets, WBC, lymphocytes, & neutrophils;
- Only at visit Phase C week 12, body secretions collection and storage as detailed in the Study Laboratory Manual (*ALX002-Manual01-Laboratory*), following scheme detailed in Appendix 5: ALX002.Schedule of procedures and blood volumes:
    - Skin swab at each injection site
    - Saliva sample
    - Nasal swab in each nostril
    - Urine sample (1st morning void urine sample, collected by the participant at home)
    - Stool sample (allowed to be collected by the participant at home the IMP administration day or one day before IMP administration)
- Plasma and PBMC storage following scheme detailed in Appendix 5: *ALX002.Schedule of procedures and blood volumes;*

At every IMP administration visit, following completion of all procedures described before, IMP administration may proceed. Clinical team will confirm IMP administration to the trial pharmacist using a IMP administration prescription, IMP will be prepared (*ALX002-SOP03-IMP Preparation*, *ALX002-SOP04-IMP Masking*) and transferred to study nurse for immediate administration (*ALX002-SOP06-IMP administration*). The following procedures will be performed:

- Intramuscular administration of study product;
- Fill in the product administration form (Appendix 1: *ALX002-Form01 Product administration Phase C*).

After IMP administration, the participants will remain in the clinic for clinical monitoring. For the first 15 participants (Groups 1 and 2) vital signs will be recorded 10 minutes, 30 minutes and 2 hours post-administration. For the rest of participants (Group 3) vital signs will be recorded only 10 and 30 minutes post-administration, if no related SAE or ≥ Grade 3 AE after IMP administration are present in any of the 15 participants in Groups 1 and 2. Otherwise, same procedures as in Groups 1 and 2 will be followed (i.e., 10 minutes, 30 minutes and 2 hours post-administration).

Local reactogenicity will be assessed 30 minutes after IMP administration, with an acceptable range of 25-60 minutes, as well as at the end of the visit. Before leaving the clinic, participants will be given a

participant diary card and will be instructed on how to fill it in. A list of allowed medications to treat reactogenicity symptoms is provided in the participant diary card (Appendix 2: *ALX002.Diary Card*).

The following procedures will be performed at all IMP administration visits. These procedures may be performed before or after IMP administration:

- Pregnancy prevention assessment (female and male participant in HTS relationships);
- Provide the participant with the diary card (Appendix 2: *ALX002.Diary Card*) with reminder of the precautionary measures to be followed. Give instructions to fill diary card;
- Stool sample collection and storage following scheme detailed in Appendix 5: *ALX002.Schedule of procedures and blood volumes* is allowed to be performed before or after IMP administration at Phase C week 24. At Phase C week 12 it is necessary to perform stool collection before IMP administration;
- Fill dietary questionnaire, only at visit Phase C week 24 (Appendix 7: *ALX002-Form12-Dietary questionnaire*);
- Schedule next visit and review trial procedures and at week 12 provide the participant with band-aids and surgical masks to follow precautionary measures, and recipients, materials and instructions for stool and urine samples collection at day 1;

8.3.11 Phase C Follow-up visits (w1, w4, w8, w13, w16, w20, w25 and w28)

At all follow-up visits of Phase C, the following procedures will be performed:

- Review results of laboratory tests;
- Abbreviated clinical examination, including weight, vital signs, and a symptom-directed evaluation;
- Assessment of concomitant medications;
- Assessment of any new or unresolved AEs/intercurrent illnesses;
- HIV viral load following scheme detailed in Appendix 5: *ALX002.Schedule of procedures and blood volumes*
- At visits Phase C weeks 1 and 13, body secretions collection and storage as detailed in the Study Laboratory Manual (*ALX002-Manual01-Laboratory*), following scheme detailed in Appendix 5: *ALX002.Schedule of procedures and blood volumes*:
    - Skin swab at each injection site
    - Saliva sample
    - Nasal swab in each nostril
    - Urine sample (1st morning void urine sample, collected by the participant at home)
    - Stool sample (allowed to be collected by the participant at home the visit day or the day before visit)
- Plasma and PBMC storage, except for visits Phase C week 8 and week 20, following scheme detailed in Appendix 5: *ALX002.Schedule of procedures and blood volumes*;
- Pregnancy prevention assessment (female and male participants in HTS relationships);
- Reminder of the precautionary measures to be followed;
- Schedule next visit and review trial procedures.
- At visits Phase C week 16 and week 28, stool sample collection and storage following scheme detailed in Appendix 5: *ALX002.Schedule of procedures and blood volumes*;
- At visits Phase C week 16 and week 28, fill dietary questionnaire (Appendix 7: *ALX002-Form12-Dietary questionnaire*).

At visits Phase C weeks 1, 13 and 25, a specific assessment of reactogenicity will be done in addition with the following laboratory tests:

- Limited safety bloods (sodium, potassium, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT));
- Complete blood count (CBC): Hb, HCT, platelets, WBC, lymphocytes, & neutrophils
- Immunology (CD4/CD8 T-cell counts)

At visit Phase C week 28, the following specific procedures will be done:

- Serology (hepatitis B, hepatitis C and syphilis).
- HIV transmission prevention assessment and address PrEP needs in seronegative stable sexual partners
- Psychological assessment of the impact of ATI in the emotional and sexual sphere (Appendix 18: *ALX002-Form31-Questionnaire of the impact of the ATI in the psychological status Pre-ATI*).

8.3.12 Phase C day 1 after IMP administration visits (w0+1d, w12+1d, w24+1d)

All participants (Groups 1, 2 and 3) will perform a visit 1 day after each ChAdOx1.HTI/Placebo administration (Phase C week 0+1d, Phase C week 12+1d) and only Sentinel participants (Group 1) will perform an additional visit 1 day after MVA.HTI/Placebo administration (Phase C week 24+1d).

In visits Phase C week 0+1d and Phase C week 12+1d (1 day after each ChAdOx1.HTI/Placebo administration), the following procedures will be performed to all participants:

- Body secretions will be collected and stored as detailed in the Study Laboratory Manual, (*ALX002-Manual01-Laboratory*) following scheme detailed in Appendix 5: ALX002.Schedule of procedures and blood volumes:
    - Skin swab at each injection site
    - Saliva sample
    - Nasal swab in each nostril
    - Urine sample (1st morning void urine sample, collected by the participant at home)
    - Stool sample (allowed to be collected by the participant at home the visit day)
- Schedule next visit and review trial procedures and provide the participant with recipients, materials and instructions for stool and urine samples collection at day 7;

In visits Phase C week 0+1d, Phase C week 12+1d and Phase C week 24+1d the following procedures will be performed only to Sentinel participants (Group 1):

- Abbreviated clinical examination, including vital signs, and a symptom-directed evaluation;
- Assessment of reactogenicity parameters;
- Assessment of concomitant medications;
- Assessment of any new or unresolved AEs/intercurrent illnesses AEs;

In case a Sentinel participant would not show up to the +1 day scheduled visit, a clinical follow-up visit by telephone would be accepted in order to allow for next Sentinel IMP administration.

8.3.13 Phase C pre-ATI eligibility review remote visit (w29)

Four weeks after the 3rd MVA.HTI/placebo administration (visit Phase C week 28), participants will be assessed to start an ATI. All of the following criteria must be met for a participant to begin ATI:

- Viral load <50 pVL
- CD4 >400 cells/mm3

- No active syphilis
- No active Hepatitis B or C
- Participant has received all doses of CCM

Participants with active syphilis must be treated for 2 weeks before starting ATI. ATI may be postponed for up to 4 weeks after the week 32 for participants to meet the above requirements. Participants with any other acute process that may require treatment before starting ATI should consult the Investigator and may delay starting ATI for up to 4 weeks.

Participants positive for active Hepatitis B or C, or who otherwise do not meet the requirements for ATI, will be discontinued from the study and should undergo early termination assessments.

7 to 10 days after visit Phase C week 28, the investigator will review the results of laboratory tests to confirm eligibility for ATI start. The participant will be contacted by phone to comment on the results and recommend measures for HIV transmission prevention. If his/her HIV negative stable partners are interested in receiving PrEP during ATI, they will be referred for PrEP start. (See section 9.6.3)

To prevent HIV transmission during ATI, the following measures will be recommended:

- Use of condom
- Not sharing injection drug equipment, such as needles
- HIV transmission prevention assessment and address PrEP needs in seronegative stable sexual partners. Effort will be made to start PrEP at least one week before ATI and to maintain it after participant's cART resumption, until at least one pVL<50 copies/mL

8.3.14 Phase C ATI start visit (w32)

At ATI start, visit Phase C week 32, the following procedures will be performed:

- Review results of laboratory tests;
- Abbreviated clinical examination, including weight, vital signs, and a symptom-directed evaluation;
- Assessment of concomitant medications;
- Assessment of any new or unresolved AEs/intercurrent illnesses;
- Fill dietary questionnaire (Appendix 7: *ALX002-Form12-Dietary questionnaire*).
- Plasma and PBMC storage following scheme detailed in Appendix 5: *ALX002.Schedule of procedures and blood volumes*;
- Stool sample collection and storage, following scheme detailed in Appendix 5: *ALX002.Schedule of procedures and blood volumes*;
- Urine pregnancy test (female participants);
- Blood draw for:
    - HIV viral load following scheme detailed in Appendix 5: *ALX002.Schedule of procedures and blood volumes;*
    - Safety bloods (sodium, potassium, urea, creatinine, estimated glomerular filtration rate (CKD-EPI), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-GT (GGT), alkaline phosphatase (AP), total bilirubin, total protein, albumin);
    - Complete blood count (CBC): Hb, HCT, platelets, WBC, lymphocytes, & neutrophils
    - Immunology (CD4/CD8 T-cell counts);
- ATI period explanation including: cART interruption, weekly visits at BCN-Checkpoint community center or at UPIC for clinical and analytical monitoring, and criteria for cART resumption;
- Pregnancy prevention assessment (female and male participants in HTS relationships);
- Reminder of the precautionary measures to be followed;

- HIV transmission prevention assessment and address PrEP needs in seronegative stable sexual partners
- Assure that the participant has ART medication for at least one month, to be taken only in case of cART resumption after medical indication;
- Cancel regular cART hospital prescription;
- Provide contact information of BCN-Checkpoint site if willing to perform monitoring there;
- Schedule next visit and review trial procedures.

8.3.15 ATI Follow-up visits (weekly)

Follow-up visits during ATI may be performed at BCN-Checkpoint community center or in UPIC. If visits are performed in BCN-Checkpoint community center, blood collected will be transferred the same day to the Hospital Universitari Germans Trias i Pujol laboratories.

The following procedures will be performed:

- Review results of laboratory tests;
- Reporting of symptoms suggestive of acute retroviral syndrome (including its severity grade), through the *ATI monitoring form* (appendix 14: *ALX002-form31-ATI monitoring*). Symptoms to be registered:
    - Fever of unknown origin
    - Headache
    - Pharyngitis
    - Lymphadenopathy
    - Transaminase elevation
    - Lymphopenia
    - Oral candidiasis
    - Myalgia/arthralgia
    - Night sweats
    - Fatigue
    - Nausea, vomiting
    - Hepato/splenomegaly
    - Rash
    - Diarrhea
    - Thrombocytopenia
    - Others
- Symptom-directed physical exam;
- Assessment of concomitant medications;
- Assessment of any new or unresolved AEs/intercurrent illnesses;
- Review cART resumption criteria during ATI (see section 6.2.3);
- Blood draw for HIV viral load and CD4/CD8 T-cell counts following scheme detailed in Appendix 5: *ALX002.Schedule of procedures and blood volumes;*
- Complete blood count (CBC): Hb, HCT, platelets, WBC, lymphocytes, & neutrophils following scheme detailed in Appendix 5: *ALX002.Schedule of procedures and blood volumes;*
- Pregnancy prevention assessment (female and male participants in HTS relationships);
- Reminder of the precautionary measures to be followed;
- HIV transmission prevention assessment and address PrEP needs in seronegative stable sexual partners
- Schedule next visit and review trial procedures.
- At visit Phase C week 44, stool sample collection and storage following scheme detailed in Appendix 5: *ALX002.Schedule of procedures and blood volumes*;

- At visit Phase C week 44, fill dietary questionnaire (Appendix 7: *ALX002-Form12-Dietary questionnaire*).

Every two weeks during the first month, and monthly during the rest of ATI period, and if any condition for cART resumption is not met, the additional procedures will be performed:

- Plasma and PBMC storage following scheme detailed in Appendix 5: *ALX002.Schedule of procedures and blood volumes*. When participants' viral load comes back for the first time detectable (pVL >50 copies/mL) and the next weekly visit does not includes PBMC/plasma storage , 40ml of additional blood will be drawn for PBMC/plasma isolation and storage in the next visit.

Every four weeks, and if any condition for cART resumption is not met, the following laboratory tests will be added:

- Limited safety bloods (sodium, potassium, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT));

At the patient's request or in the opinion of the psychologist, a psychological assessment of the impact of ATI in the emotional and sexual sphere, could be performed during the ATI follow-up visits. These assessments will be performed in UPIC.

8.3.16 End of ATI and cART resumption visit (Phase C week 56)

If any criterion for cART resumption is met, participant will be contacted and scheduled for the End of ATI visit (visit Phase C week 56), where the additional procedures will be performed:

- cART prescription will be reactivated and the participant will resume cART;
- Blood draw for:
  - HIV viral load;
  - Safety bloods (sodium, potassium, urea, creatinine, estimated glomerular filtration rate (CKD-EPI), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-GT (GGT), alkaline phosphatase (AP), total bilirubin, total protein, albumin);
  - Complete blood count (CBC): Hb, HCT, platelets, WBC, lymphocytes, & neutrophils;
  - Immunology (CD4/CD8 T-cell counts);
- Plasma storage for potential genotypic resistance test;
- Plasma and PBMC storage following scheme detailed in Appendix 5: *ALX002.Schedule of procedures and blood volumes*;
- Fill dietary questionnaire (Appendix 7: *ALX002-Form12-Dietary questionnaire*).
- Stool sample collection and storage, following scheme detailed in Appendix 5: *ALX002.Schedule of procedures and blood volumes*;
- Pregnancy prevention assessment (female and male participants in HTS relationships);
- Reminder of the precautionary measures to be followed;
- HIV transmission prevention assessment and address PrEP needs in seronegative stable sexual partners, if pVL is not <50 copies/mL.
- Schedule post-cART resumption safety follow-up visits (week 4 and 12) at UPIC.

For participants who maintain pVL<2,000 copies/ml at 24 weeks after ATI start (visit Phase C week 56) and willing to stay off cART will be offered to end the study and participate in a roll-over investigator initiated study out of the scope of the present protocol. For them, this visit will be considered his/her last study visit and the procedures described in section 8.3.18 (End of study visit (Phase C week 68 or visit Phase C week 56 for participants with <2,000 pVL copies /mL)) will be followed.

8.3.17 Post-cART resumption safety follow-up visit (Phase C week 60)

Participants will be followed to monitor safety clinical, virological and immune outcomes after cART resumption. Post-cART resumption visits will be performed at UPIC. The first post-cART resumption safety follow-up visit will be scheduled 4 weeks after cART resumption. The following procedures will be performed:

- Review results of laboratory tests;
- Abbreviated clinical examination, including weight, vital signs, and a symptom-directed evaluation;
- Assessment of concomitant medications;
- Assessment of any new or unresolved AEs/intercurrent illnesses;
- Plasma and PBMC storage following scheme detailed in Appendix 5: *ALX002.Schedule of procedures and blood volumes*;
- Blood draw for:
    - HIV viral load following scheme detailed in Appendix 5: *ALX002.Schedule of procedures and blood volumes;*
    - Limited safety bloods (sodium, potassium, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT));
    - Complete blood count (CBC): Hb, HCT, platelets, WBC, lymphocytes, & neutrophils;
    - Immunology (CD4/CD8 T-cell counts);
- HIV transmission prevention assessment and address PrEP needs in seronegative stable sexual partners, if pVL is not <50 copies/mL.
- Psychological assessment of the impact of ATI in the emotional and sexual sphere. (Appendix 18: *ALX002-Form32-Questionnaire of the impact of the ATI in the psychological status Post-ATI*);
- Pregnancy prevention assessment (female and male participants in HTS relationships);
- Reminder of the precautionary measures to be followed;
- Schedule next visit and review trial procedures.

8.3.18 End-of-study visit (Phase C week 68 or visit Phase C week 56 for participants with pVL < 2,000 copies/mL)

The end of study visit will be at visit Phase C week 56 for participants who maintain pVL <2,000 cop/ml at the end of ATI and decide to participate In a roll-over study, and 12 weeks after cART resumption (visit Phase C week 68) for participants who met any criterion for cART resumption.
. The following procedures will be performed:

- Review results of laboratory tests;
- Abbreviated clinical examination, including weight, vital signs, and a symptom-directed evaluation;
- Assessment of concomitant medications;
- Assessment of any new or unresolved AEs/intercurrent illnesses;
- Serology (hepatitis B, hepatitis C, syphilis);
- Urine pregnancy test;
- Plasma and PBMC storage following scheme detailed in Appendix 5: *ALX002.Schedule of procedures and blood volumes*;
- Blood draw for:
    - HIV viral load following scheme detailed in Appendix 5: *ALX002.Schedule of procedures and blood volumes*;
    - Limited safety bloods (sodium, potassium, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT));

  - Safety bloods (sodium, potassium, urea, creatinine, estimated glomerular filtration rate (CKD-EPI), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-GT (GGT), alkaline phosphatase (AP), total bilirubin, total protein, albumin);
  - Complete blood count (CBC): Hb, HCT, platelets, WBC, lymphocytes, & neutrophils;
  - Immunology (CD4/CD8 T-cell counts);
- HIV transmission prevention assessment and address PrEP needs in seronegative stable sexual partners, if pVL is not <50 copies/mL.
- Pregnancy prevention assessment (female and male participants in HTS relationships);
- Inform the end of the precautionary measures to be followed;
- Stool sample collection and storage following scheme detailed in Appendix 5: *ALX002.Schedule of procedures and blood volumes*;
- Fill dietary questionnaire (Appendix 7: *ALX002-Form12-Dietary questionnaire*);
- Fill Expectations and worries questionnaire (Appendix 13: *ALX002-Form27-Questionnaire of expectations and worries EoS*);

8.4 PROCEDURES FOR EVALUATION OF VACCINE RESPONSE

8.4.1 Clinical record and physical examination

Demographic and history of past and foreseen vaccinations, history of medical conditions and concomitant medications data will be collected in order to characterize the study population.

The comprehensive screening visit physical examination will include height, weight, vital signs (temperature, pulse, and blood pressure), general appearance, abdominal examination and other components as indicated by participant history or symptoms.

8.4.2 Laboratory tests

The following parameters will be characterized according to the study schedule (Appendix 5: *ALX002.Schedule of procedures and blood volumes*):

- Complete blood count (CBC):
  - Hematocrit
  - Hemoglobin
  - Leucocytes
  - Lymphocyte
  - Neutrophils
  - Platelet count
  - Coagulation (INR and PT)

- Blood biochemistry:
  - Urea
  - Creatinine
  - Sodium, potassium
  - Total Bilirubin
  - Total protein
  - Albumin
  - Liver enzymes: aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-GT, alkaline phosphatase
  - Estimated glomerular filtration rate (CKD-EPI)
  - Glucose (only in Roll-over Phase week 44 and week 68 visits)

Lipid profile: Triglycerides, Total cholesterol, HDL, LDL (only in Roll-over Phase week 44 and week 68 visits)

- Immunology:
  CD4 lymphocytes count
  CD4 lymphocytes percentage
  CD8 lymphocytes count
  CD8 lymphocytes percentage
  CD4/CD8 Ratio

- Pregnancy test in women
  Urine HCG

- Microbiology:
  Hepatitis B serology (HBsAg, and HBsAb if vaccinated and not available)
  Hepatitis C serology and Hepatitis C RNA if history of cured HCV infection
  Syphilis serology
  HIV-1 RNA
  Urinary Multiplex PCR for *Chlamydia trachomatis, Neisseria gonorrhoeae, Mycoplasma genitalium, Mycoplasma hominis, Mycoplasma urealyticum, Ureoplasma parvum, Trichomonas vaginalis* (only in Roll-over Phase week 44 and week 68 visits)

- HLA type (High-Resolution HLA-A, HLA-B, HLA-C) for optimal epitope mapping

Before the beginning of the study, the contract laboratory will provide the sponsor and the investigator with a list of the reference normal values of the parameters to be assessed in the AELIX-002 study.

- Specific Lab Procedures

To be assessed throughout the study.

- De-novo T cell responses to HTI-encoded regions as determined by IFNγ ELISPOT assay.
- Breadth and magnitude of total vaccine induced HIV-specific responses measured by IFNγ ELISPOT.

PBMC, plasma and faeces will be stored as detailed in the Study Laboratory Manual (*ALX002-Manual01-Laboratory)* for other exploratory assays to further characterise vaccine-expanded T cell populations such as in-vitro viral suppressive capacity, polyfunctionality, functional avidity as well as to address potential changes in the proviral DNA, cell-associated RNA, induction of anti-vector antibodies, innate immune markers, epigenetic and microbiome studies.

- Shedding evaluation (only Phase C)

Body secretions samples collected before ChAdOx1.HTI/Placebo administration and at days 1 and 7 after each ChAdOx1.HTI/Placebo administration will be processed and stored as detailed in the Study Laboratory Manual (*ALX002-Manual01-Laboratory*) until shipment to Covance, UK laboratory for shedding assessment.

8.4.3 Electrocardiogram

ECG leads are attached to the body while the participant lies flat on a bed. Leads are attached to each extremity (four total) and to six pre-defined positions on the front of the chest. A small amount of gel

is applied to the skin, which allows the electrical impulses of the heart to be more easily transmitted to the ECG leads. The leads are attached by small suction cups, Velcro straps, or by small adhesive patches attached loosely to the skin. The test takes about five minutes and is painless. At Phase C screening visit, ECG will be performed to estimate heart rate and detect potential QT prolongation, repolarization abnormalities and/or T wave inversion.

8.4.4 Participant diary card

Data on local and systemic events will be solicited with specific diary cards (Appendix 2: *ALX002.Diary Card*) for a minimum of 7 days following each immunization.

The following parameters will be collected:

Local reactogenicity, including:

- Redness (mm)
- Induration (mm)
- Pain (0-4)

General symptoms, including:

- Temperature (ºC)
- Headache (0-4)
- Fatigue (0-4)
- Nausea (0-4)
- Vomiting (0-4)
- Diarrhea (0-4)
- Abdominal pain (0-4)
- Sweating (0-4)
- Muscular pain (0-4)
- Lack of appetite (0-4)
- Other, describe

Research nurse will provide a thermometer and a measuring tape to all participants at first IMP administration visit of Phases A/B and C.

0-4 grades intensity, according to the following definitions described at DAIDS:

- 0: No symptoms
- 1: Symptoms causing no or minimal interference with usual social & functional activities.
- 2: Symptoms causing greater than minimal interference with usual social & functional activities
- 3: Symptoms causing inability to perform usual social & functional activities
- 4: Symptoms causing inability to perform basic self-care functions OR Medical or operative intervention indicated to prevent permanent impairment, persistent disability, or death

The investigator will review the participant diary card and ensure that proper record has been performed. The severity of each AE will be graded by the investigator. Criteria for grading clinical and laboratory events will be based on DAIDS grading table. The investigator will state the relationship of each AE to the IMP administration as unrelated or related. In those cases were grading of AE do not coincide with participant's description, a discussion will be registered in the medical record.

At Phase C, the participant diary card will include a reminder of the precautionary measures to be followed, according to section 6.2.2.

9 ADVERSE EVENTS

9.1 DEFINITION

Adverse event: (AE) Medical event presented by a participant or clinical research subject administered a pharmaceutical product, and which does not necessarily have a causal relation to the treatment.

Serious adverse event: (SAE) Medical event classified as such and which, regardless of the dose involved:

- Causes participant death,
- Produces a life-threatening situation for the participant,
- Requires or prolongs in hospital admission,
- Produces important or persistent incapacitation/handicap, or constitutes a congenital defect or anomaly,
- Needs action to prevent any of above situations.
- Is considered medically significant

Examples of such events are intensive care in an Emergency Service or in the home in a participant with allergic bronchospasm; blood dyscrasias or seizures not giving rise to hospital admission, or the development of drug dependency or abuse.

Unexpected adverse event: (UAE) AE related to the product in investigation the nature or intensity of which does not coincide with the information available on the product administered (Investigator Brochure (IB).

Serious unexpected adverse reaction: (SUSAR) SAE related to the product in investigation the nature or intensity of which does not coincide with the information available on the product administered (IB or SmPC).

9.2 MONITORING, RECORDING AND REPORTING OF ADVERSE EVENTS

An AE is any noxious, unintended, or untoward medical occurrence that may appear or worsen in a subject during the course of a study. It may be a new intercurrent illness, a worsening concomitant illness, an injury, or any concomitant impairment of the subject's health, including laboratory test values (as specified by the criteria below), regardless of etiology. Any worsening (i.e., any clinically significant adverse change in the frequency or intensity of a pre-existing condition) should be considered an AE. A diagnosis or syndrome should be recorded on the AE page of the CRF rather than the individual signs or symptoms of the diagnosis or syndrome.

An overdose, accidental or intentional, whether or not it is associated with an AE, or abuse, withdrawal, sensitivity or toxicity to an investigational product should be reported as an AE. If an overdose is associated with an AE, the overdose and AE should be reported as separate terms.

All participants will be monitored for AEs during the study. Assessments may include monitoring of any or all of the following parameters: the subject's clinical symptoms, laboratory, pathological, radiological or surgical findings, physical examination findings, or other appropriate tests and procedures.

All AEs will be recorded by the Investigator from the time the subject signs informed consent until the last study visit, or to 28 days after the last dose of IMP in case of early withdrawal while participant is receiving IMP. AEs and SAEs will be recorded on the AE page of the eCRF and in the subject's source documents. All SAEs must be reported to the Sponsor (see contact details at the end of the paragraph) within 24 hours of the Investigator's knowledge of the event by facsimile, or other appropriate method,

using the SAE Report Form (*ALX002-Form05-SAE*), can be found in the Investigator's File), or approved equivalent form.

9.3 DOCUMENTATION RELATED TO AE AND SAE

Each AE and SAE to take place during the study should be documented in the medical records of the subject in accordance with standard clinical practice of the researcher, and in the eCRF. For each SAE, an independent set of SAE forms will be used. Only if there are multiple SAE at the time of the initial report and these are temporary and / or clinically interrelated can they be registered on the same set of SAE forms.

The investigator should try to make a diagnosis of the event based on the signs, symptoms and / or other clinical information. An AE diagnosis has to be recorded per line or a sign/symptom if the diagnosis is not available. If a diagnosis subsequently becomes available, this then should be entered and the sign/symptom crossed out, initialled and dated by the investigator.

SAE pages found in the investigator's file shall be completed as precisely as possible, printed and shall be signed by the investigator before being sent to the sponsor. It is very important that the investigator completing the SAE form provides their opinion in regard to the relationship of the event to the study drug.

9.4 EVALUATION OF ADVERSE EVENTS

A qualified Investigator will evaluate all AEs as to:

9.4.1 Seriousness

A SAE is any AE occurring at any dose that:

- Results in death;
- Is life-threatening (i.e., in the opinion of the Investigator, the subject is at immediate risk of death from the AE);
- Requires inpatient hospitalization or prolongation of existing hospitalization (hospitalization is defined as an inpatient admission, regardless of length of stay);
- Results in persistent or significant disability/incapacity (a substantial disruption of the subject's ability to conduct normal life functions);
- Is a congenital anomaly/birth defect;
- Constitutes an important medical event.

Important medical events are defined as those occurrences that may not be immediately life threatening or result in death, hospitalization, or disability, but may jeopardize the subject or require medical or surgical intervention to prevent one of the other outcomes listed above. Medical and scientific judgment should be exercised in deciding whether such an AE should be considered serious.

Events **not considered** to be SAEs are hospitalizations for:

- Routine treatment or monitoring of the studied indication not associated with any deterioration in condition.

- Hospitalization or prolongation of hospitalization for technical, practical, or social reasons, in absence of an AE.

- A procedure that is planned (i.e., planned prior to starting of treatment on study); must be documented in the source document and the eCRF. Hospitalization or prolonged hospitalization for a complication remains a reportable SAE.

- An elective treatment of a pre-existing condition unrelated to the studied indication.

- Emergency outpatient treatment or observation that does not result in admission, unless fulfilling other seriousness criteria above.

If an AE is considered serious, both the AE page/screen of the eCRF and the SAE Report Form must be completed.

For each SAE, the Investigator will provide information on severity, start and end dates, relationship to IMP, action taken regarding IMP, and outcome.

9.4.2 Severity / Intensity

For both AEs and SAEs, the Investigator must assess the severity / intensity of the event.

Intensity will be assessed by the investigator using the following terms according to the Division of DAIDS table for grading the severity of adult and pediatric adverse events, Version 2.1. [March 2017]

- Grade 1 = Mild

- Grade 2 = Moderate

- Grade 3 = Severe

- Grade 4 = Life threatening

- Grade 5 = Death

The term "severe" is often used to describe the intensity of a specific event (as in mild, moderate or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache). This criterion is *not* the same as "serious" which is based on subject/event *outcome* or *action* criteria associated with events that pose a threat to a subject's life or functioning.

Seriousness, not severity, serves as a guide for defining regulatory obligations.

9.4.3 Causality

Relatedness is an assessment made by a study clinician of whether or not the event is related to the study agent. Degrees of relatedness will be categorized according to current DAIDS-approved guidelines. Per the Manual for Expedited Reporting of Adverse Events to DAIDS (Version 2.0, January 2010), the relationship categories that will be used for this study are:

Related: There is a reasonable possibility that the AE may be related to the study agent(s) suggested by:

- A plausible, reasonable time sequence exists in relation to administration of the drug or its plasma or tissue concentrations.

- The observed manifestation coincides with the known adverse reactions profile of the implicated drug.
- The event cannot be or unlikely be explained by a concurrent disease or by other drugs or chemical substances.
- Response to withdrawal is clinically plausible, i.e., the condition improves on discontinuing administration of the drug.

Not Related: There is not a reasonable possibility that the AE is related to the study agent(s).

9.4.4 Duration

For both AEs and SAEs, the Investigator will provide a record of the start and stop dates of the event.

9.4.5 Action Taken

The Investigator will report the action taken with IMP as a result of an AE or SAE, as applicable (e.g., discontinuation of IMP) and report if concomitant and/or additional treatments were given for the event.

9.4.6 Outcome

Any SAE will be followed preferably until:

- Resolution of the event;
- Stabilization of the event; or
- Resetting the baseline situation of the event, in case baseline situation is available.

Otherwise, they will continue until:

- The event can be attributed to products other than the study medication or factors unrelated to the study; or
- It is unlikely to obtain further information.

9.5 ABNORMAL LABORATORY VALUES

An abnormal laboratory value is considered to be an AE if the abnormality:

- Results in discontinuation from the study;
- Requires treatment, modification/ interruption of IMP dose, or any other therapeutic intervention; or
- Is judged to be of significant clinical importance.

Regardless of severity grade, only laboratory abnormalities that fulfil a seriousness criterion need to be documented as a serious adverse event.

If a laboratory abnormality is one component of a diagnosis or syndrome, then only the diagnosis or syndrome should be recorded on the AE page/screen of the eCRF. If the abnormality was not a part of a diagnosis or syndrome, then the laboratory abnormality should be recorded as the AE.

9.6 FEMALES OF CHILDBEARING POTENTIAL AND STABLE SERONEGATIVE SEXUAL PARTNERS

9.6.1 Females of childbearing potential

Pregnancies and suspected pregnancies (including a positive pregnancy test regardless of age or disease state) of a female subject occurring while the subject is within 28 days of the subject's last dose of IMPs, are considered immediately reportable events. IMP administrations are to be discontinued

immediately. The pregnancy, suspected pregnancy, or positive pregnancy test must be reported to the Sponsor.

The Investigator will obtain female's consent (see Appendix 12: *ALX002.Pregnancy PIS and IC*) to be followed until completion of the pregnancy, and must notify the Sponsor immediately about the outcome of the pregnancy (either normal or abnormal outcome).

If the outcome of the pregnancy was abnormal (e.g., spontaneous or therapeutic abortion), the Investigator should report the abnormal outcome as an AE. If the abnormal outcome meets any of the serious criteria, it must be reported as an SAE within 24 hours of the Investigator's knowledge of the event using the SAE Report Form, or approved equivalent form.

All neonatal deaths that occur within 28 days of birth should be reported, without regard to causality, as SAEs. In addition, any infant death after 28 days that the Investigator suspects is related to the in utero exposure to the IMP should also be reported within 24 hours of the Investigator's knowledge of the event using the SAE Report Form, or approved equivalent form.

9.6.2 Male participants

The investigator shall use their best efforts in order to ensure that male participants

- Inform him/her if their partners get pregnant while the participant is still treated with the IMP;
- Provide him/her the contact details of the healthcare provider who follows the pregnancy in question.

If a female partner of a male participant taking the IMP becomes pregnant, the investigator shall:

- Advise that the partner consults her general practitioner or gynecologist as soon as possible;
- Obtain informed consent from the partner to collect follow-up data on the pregnancy until birth (Appendix 12, pregnancy of partner informed consent);
- Provide the information collected to AELIX Therapeutics Pharmacovigilance, using the Pregnancy Reporting Form or an approved equivalent form.

9.6.3 Stable seronegative sexual partners

To prevent the potential risk of HIV transmission, the use of condom and advice of not sharing injection devices (e.g., needles) will be reinforced to all participants who enrol in Phase C. Additionally, stable HIV-negative sexual partners of study participants will be eligible for pre-exposure prophylaxis (PrEP).

PrEP consists of a combination of tenofovir disoproxil fumarate plus emtricitabine (TDF/FTC), and it has been approved by the European Medicines Agency to reduce the risk of sexually acquired HIV-1 infection in adults and adolescents at high risk [86], although its routine use has not been implemented by Spanish authorities yet.

At visit Phase C screening, week 28, week 29, during ATI period, and after cART resumption until 24 weeks after reaching pVL<50 copies/mL, PrEP needs in HIV-negative stable sexual partners of participants will be assessed. PrEP will be offered to a limited number of eligible serodiscordant couples per participant based on the risk of sexual HIV transmission and a co-signed (participant and partner) PrEP Information sheet will be asked (Appendix 16: *Co-signed Information sheet for PrEP)*. More details on PrEP implementation can be found in Appendix 15: *PrEP implementation Plan.*

9.7 PHARMACOVIGILANCE / SAFETY MONITORING COMMITTEE

A Safety Monitoring Committee (SMC) will be formed to review safety data from the study. The SMC could also be convened by the PI to address whether the study should be paused or prematurely stopped due to safety issues during the trial. The committee will be composed of the three external experts in pharmacovigilance and HIV vaccine trials, four non-voting members and a non-voting consultant, as required.

As per protocol, regular SMC meetings will be scheduled approximately every 3 months and at the end of study to review AE classification, duration, grading and relation to IMP before database lock. Following the pharmacovigilance plan (Figure 2), additional meetings will be convened:

Figure 2: Pharmacovigilance plan

Event	Causality	Severity	Site Action	SMC Action
SAE	Related	Grade 4/5	Immediate vaccination pause. Inform in <24h sponsor by phone and email/fax. SAE reporting	Immediate SMC review. Vaccinations not allowed until SMC decision available.
SAE	Not-related	Grade 4/5	Study/vaccinations continue. Inform in <24h sponsor by phone and email/fax SAE reporting	Immediate SMC review to consider study pause
AE*	Related	Grade 3 lasting >72h within first 7 days after vaccination	Immediate vaccinations pause. Inform in <24h sponsor by phone and email/fax	Immediate SMC review. Vaccinations not allowed until SMC decision available (≤72h)
AE	Related	Grade 1/2 other Grade 3	Study/vaccinations continue. Routine data submission	Routine review by SMC at regular meetings
AE	Not-related	Grade 1-3	Study /vaccinations continue. Routine data submission	Routine review by SMC at regular meetings

*In case of any related Grade 3 event lasting more than 72h occurs (confirmed by the site investigator) in any participant within the first 7 days after receiving a IMP administration, IMP administrations will be paused. The Safety Monitoring Committee (SMC) will be convened urgently to assess safety data, relationship to study product and to allow for continuing the trial. Efforts will be made to have SMC resolution within the next 72hours after notification to avoid significant deviations on schedule of procedures. Efforts will be made to notify as soon as possible the SMC (i.e. close follow-up of any Grade 3 event reported at +1d visits) SMC meetings via teleconference will be allowed to fulfil with described timelines.

Other reasons to call the SMC include withdrawal of more than 50% of study participants to consider a premature stop of the trial.

The SMC was called on March 2018 to assess the interim report, including safety data until week 22 from Phase A participants, and made recommendations on the progression to Phase B.

The SMC will be called on January 2019 to assess the interim report, including safety data until week 32 from Phase A participants and week 21 from Phase B participants, and will make recommendations on the progression to Phase C.

See the Safety monitoring committee charter (Appendix 9: *ALX002.SMC*) for details.

9.8 STRUCTURED RISK ANALYSIS

9.8.1 Risks associated to IMP administration

In the Participant Information Sheet all known and unknown risks of the IMP of the study are explained.

The study is performed in a Phase I clinical trial unit with the infrastructure, equipment, and study personnel needed in case of any emergency.

Phase A participants are closely monitored after each IMP administration (+1 day in Sentinel participants, and +1 week in all participants) to detect any adverse event occurring during the first days after each IMP administration. Safety laboratory tests are scheduled in the study to monitor lab abnormalities occurring during the trial.

To minimize any risks associated with IMP administration, in Phase A of the study a progressive inclusion of participants into the trial is performed (as detailed in the study protocol, Section 6.2.1) and a maximum of 3 individuals (2 receiving the active IMP and 1 receiving placebo) are vaccinated on the same day.

Phase B includes the same study products and regimen as in Phase A. Participants are recruited only after a favourable report from the Safety Monitoring Committee (SMC) on week 22 interim report from Phase A has been released.

During Phase B, safety and tolerability is assessed 1 week after each IMP administration. Based on favourable safety data from Phase A, safety assessment performed at 1 week after first and second DNA.HTI/placebo administrations are performed remotely by a phone contact visit. The rest of study visits are in-person visits and include laboratory assessments.

Phase C includes a new study product and a different regimen of administration than Phase A/B. Nevertheless, participants will be recruited only after a favourable report from the SMC on safety data until week 32 from Phase A participants and week 21 from Phase B participants has been released.

To minimize any risks associated with IMP administration during Phase C, progressive inclusion of participants will be performed, according to the same procedures that were followed in Phase A/B (as detailed in study protocol, Section 6.2.1). Safety laboratory tests are scheduled to monitor lab abnormalities occurring during the trial.

Body secretions will be collected to determine ChAdOx1.HTI vector shedding. To minimize any risks associated with vector shedding, participants will be educated to follow precautionary measures (see section 6.2.2) to reduce the risk of exposure to partners or household members or unnecessary transmission to the environment.

During Phase A, Phase B, Roll-over Phase and Phase C, clinical investigators are accessible 24/7 by an emergency cell phone and are able to monitor any adverse event requiring immediate SMC review following the pharmacovigilance plan (as detailed in Study protocol, Section 9.7). The AELIX-002 SMC incorporates investigators with expertise in pharmacovigilance of Phase I trials and will be accessible throughout the trial.

An effort is envisioned during the pre-screening and screening visits to exclude participants with pre-existing conditions that could predict a higher risk associated with IMP administrations (details described in study protocol Section 7.2).

9.8.2 Risks associated to the study procedures

In the Patient Information Sheet all risks associated with intramuscular IMP administrations, blood draws and ATI are described. Product administration and blood draws are performed by trained study nurses. Study investigators monitor any adverse event and clinical events related to the study procedures throughout the trial.

To minimize potential risks associated with cART interruption during the ATI, participants will be closely monitored, and cART will be resumed according to clinical and analytical criteria well pre-established in the study protocol (section 6.2.2). To this end, participants will be monitored weekly during the ATI period. Clinical symptoms suggestive of acute retroviral syndrome and pVL will be monitored weekly and the CD4+ count monthly.

Before ATI start, participants accept the measures to prevent HIV transmission to HIV-negative sexual partners (as detailed in study protocol Section 8.3.12).

To prevent the potential risk of HIV transmission during ATI, the use of condom and advice of not sharing injection devices (e.g., needles) will be reinforced to all participants who enroll in Phase C. Additionally, stable HIV-negative sexual partners of study participants will be eligible for pre-exposure prophylaxis (PrEP). In case that PrEP is implemented by Spanish authorities by the time of initiating ATI within this study, stable HIV-negative sexual partners of study participants will be referred for PrEP as part of routine care. Otherwise, a mechanism will be set in place to provide PrEP to stable HIV-negative sexual partners of Phase C participants.

9.9 EXPEDITED REPORTING OF ADVERSE EVENTS

9.9.1 Reporting to Regulatory Authorities

The sponsor will inform the Spanish Medicines Agency (Ministry of Health) and the competent authorities of the autonomous region providing study oversight about any important emergent safety data related to the IMPs.

The sponsor will inform the Spanish Medicines Agency (Ministry of Health) of any SUSAR which may be related to the study treatment.

The sponsor will inform competent authorities of the implicated autonomous region of any SUSAR which may be related to the study treatment, and that have been happened in participants in its autonomous region.

The Sponsor will inform relevant Regulatory Authorities:

- Of all relevant information about SUSAR that are fatal or life-threatening as soon as possible, and in any case no later than 7 days after knowledge of such a case. Relevant follow-up information for these cases will be subsequently be submitted within an additional eight days

- Of all other SUSAR as soon as possible, but within a maximum of 15 days of first knowledge by the investigator.

9.9.2 Immediate reporting by Investigator to Sponsor

The investigator will inform the Sponsor of all SAEs and AE related Grade 3 lasting >72h within first 7 days after IMP administration within 24 hours in order that the sponsor can fulfil their regulatory reporting obligations within the required timeframes.

Contact details for Sponsor
Anne Leselbaum, MD
Clinical Development Director
AELIX Therapeutics, Barcelona, Spain
T. +34 93 403 13 39 / +34 619 009 850
aleselbaum@aelixtherapeutics.com

The investigator will supply Sponsor with a copy of all SAEs which involve exposure to IMPs within 24 hours of being made aware of the event regardless of whether or not the event is listed in the reference document (e.g. IB, SmPC).

The Sponsor will provide investigators with a copy of the annual periodic safety report e.g. Development Update Safety Report (DSUR) at the time of submission to the Regulatory Authority and Ethics Committee.

9.9.3 Reporting by Sponsor to Coordinating investigator

The Sponsor will inform the Coordinating investigator of all SAEs and AE related Grade 3 lasting >72h within first 7 days after IMP administration within 24 hours in order that the Coordinating investigator can call the scientific committee to assess trial stopping rules.

Contact details for Coordinating investigator

Beatriz Mothe Pujadas
Phone: +34 934 656 374 (Ext 165), Fax: +34 934 653 968
bmothe@irsicaixa.es

10 STATISTICS

10.1 METHODS

10.1.1 Data Analysis

The analysis population (for safety, tolerability, immunogenicity and efficacy assessment) will include all participants having received at least one immunization.

10.1.2 Statistical Methods

Statistical analysis will be both descriptive and inferential.

Baseline participants characteristics will be described. Continuous variables will be described by their means, standard deviations, medians, interquartile ranges, minimums and maximums. Categorical variables will be described by number of participants and proportions or percentages. The variables that are registered as time to event will be assessed by the Kaplan–Meier estimate and plot.

The description of the number and proportion or percentage of adverse events, duration, grading, relation to IMP, and accountability for each administration of DNA.HTI, MVA.HTI and ChAdOx1.HTI will be reviewed by the Safety Monitoring Committee (SMC) at the interim analysis timepoint and at the end of study. The differences in safety parameters between groups (vaccine vs. placebo) are going to be assessed by non-paired test: t-student or Mann-Whitney for the continuous variables and Chi-square or Fisher exact test for the proportion's comparison.

For the secondary objective's analysis, the secondary endpoints of immune response, viral rebound and ATI will be evaluated:

1. Proportion of participants that develop de-novo T cell responses to HTI-encoded regions as determined by IFNγ ELISPOT assay in vaccine and placebo recipients.
2. Breadth and magnitude of total vaccine induced HIV-specific responses measured by IFNγ ELISPOT in vaccine and placebo recipients.
3. Percentage of participants with viral remission, defined as plasma viral load (pVL)<50 copies/mL 12 and 24 weeks after ATI (visits Phase C week 44 and week 56).
4. Percentage of participants with viral control, defined as a pVL<2,000 copies/mL at 12 and 24 weeks after ATI (visit Phase C week 44 and week 56).
5. Time to viral detection, defined as the time from ATI start (visit Phase C week 32) to first occurrence of detectable pVL ($\geq$50 copies/mL).
6. Time to viral rebound, defined as the time from ATI start (visit Phase C week 32) to first occurrence of pVL> 10,000 copies/mL.
7. Percentage of participants who remain off cART at 12 and 24 weeks after ATI (visits Phase C week 44 and week 56).
8. Time off cART, defined as time to cART resumption since ATI start (visit Phase C week 32).
9. Proportion of participants who develop symptoms compatible with acute retroviral syndrome (ARS).
10. Proportion of participants who develop new mutations not present in the pre-cART genotype conferring clinically-significant resistance to antiretroviral drugs (out of the individuals not reaching viral re-suppression 12 weeks after cART resumption.
11. Proportion of participants who suppress pVL to <50 copies/mL 12 weeks after cART resumption. In those participants not reaching viral re-suppression 12 weeks after cART resumption an ART genotype will be analysed from the ATI sample to address if new drug-resistance mutations have emerged.

For the exploratory objective's analysis, the exploratory endpoints of viral reservoir will be evaluated:

- Change in total proviral HIV-1 DNA per 10^6 CD4+ T cells from baseline (visit Phase A/B week 0) to ATI start (visit Phase C week 32).

In addition to the descriptive analysis, the comparisons inter and intra groups will be done regarding each study endpoint. The inter group comparison will be performed with the methods mentioned for the primary endpoint; for the intra groups comparison: paired t-tests, Wilcoxon, Chi-Square and/or McNemar tests are going to be used. The comparisons between survival curves are going to be performed by log-rang or weighted log-rank tests, as appropriate.

As informative analysis, the characteristics of participants included in Phase A and B will be compared in order to evaluate whether exists a time-effect in the inclusion.

Details of the analysis are available at statistical analysis plan (*ALX002-Manual05-Statistical*)

10.2 SAMPLE SIZE

A total of 45 participants will be included in the study.

See section 4.12 for sample size justification.

10.3 STATISTICAL SIGNIFICANCE

A two-sided alpha level of 0.05 will be taken as reference to detect statistical significance.

10.4 CRITERIA FOR STUDY COMPLETION

The study will be completed when any of these premises are met:

- Inclusion of the number of participants needed for the sample size and end of clinical monitoring
- SMC recommendation for safety reasons
- Sponsor decision

10.5 STUDY REPORTS

Interim analysis of safety (AE and laboratory abnormalities) and immunogenicity will be conducted at designated time points:

- When Phase A participants complete the visit Phase A week 22, i.e. two weeks after the second MVA.HTI vaccine or matched placebo. This report will be blinded to vaccine and placebo allocation.
- When Phase B participants complete the visit Phase B week 21, i.e. one week after the second MVA.HTI vaccine or matched placebo. This report will be blinded to vaccine and placebo allocation.

A Database freeze will be done when all participants have completed visit Phase C week 56 (i.e., End-of-ATI visit). A final report (ICH template) which will include safety/immunogenicity and efficacy data will be performed. The study blindness will be opened to elaborate this report when End-of-ATI analysis is completed. Briefly, the report will include the clinical and statistical description of the analyses performed, incorporating tables and figures, and with appendices containing the protocol, sample case report forms, investigator related information, information related to the IMPs, technical statistical documentation, related publications, participant data listings, and technical statistical details such as derivations, computations, analyses, and computer output etc.

Finally, a Follow-up safety report, which will include safety data up to 12 weeks after cART resumption (i.e., Phase C week 68: end-of-study visit) plus exploratory viral reservoir endpoints will be performed. This report will be unblinded as well.

10.6 MANAGEMENT OF MISSING DATA

In case of missing values, the Last Observation Carried forward (LOCF) method will be used.
In case of evidences showing that the missing is explained by the value that it will have (missing non at random) the characteristics of participants with and without missingness are going to be described separately. When the missingness is produced at random or completely at random the distribution of the missing values is assumed to be the same that the observed or the same than the observed values

conditioned to the value of the other covariates, respectively; for this reason any action is going to be carried out in the missingness treatment.

10.7 DEVIATIONS FROM STATISTICAL PLAN

A statistical analysis plan (*ALX002-Manual05-Statistical*) will be prepared during the course of the study and before closing the database and unblinding which will describe in detail the statistical methods to be used, the approach to be followed in case of missing values and the tables and charts to be included in the statistical report.

Any deviation from the original statistical plan must be reported and justified in the final report, if necessary.

10.8 ANALYSIS POPULATION

The analysis population (for safety, tolerability, immunogenicity and efficacy assessment) will include all participants having received at least one immunization. Those participants not receiving any immunizations will be replaced.

11 DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

Investigators and institutions will allow the monitoring, and audits by the Health Authorities or the Sponsor giving direct access to data and original source documents.

Access to personal subject information will be restricted to the Study physician / staff. To allow monitoring, audits and inspections, access to data to Health Authorities (Spanish Agency for Medicines and Health Products), the Ethics Committee and personnel authorized by the Sponsor, is guaranteed while maintaining the confidentiality thereof according to current legislation.

12 QUALITY CONTROL AND QUALITY ASSURANCE

12.1 STUDY MONITORING

In accordance with applicable regulations and Good Clinical Practice (GCP), the monitor will visit or contact the centre on a regular basis. The duration, nature and frequency of visits / contacts depend on the monitoring plan.

During these contacts, the monitor shall:

- Monitor and evaluate the progress of the study;
- Examining the data collected;
- Carry out a verification of the source documents;
- Identify any problems and find solutions;

The goal of the monitoring activity is to verify that:

- The rights and welfare of participants are respected;
- Survey data are accurate, complete and verifiable with the help of original documents;
- The study is performed according to the protocol and any amendment adopted, GCPs and regulations.

The investigator must agree to:

- Grant to monitor direct access to all relevant documentation;
- Devote part of his/her time and staff time to the monitor in order to discuss the results of the monitoring, as well as any other possible aspect.

The monitor should also contact the site before starting the study with the aim to discuss with staff the Protocol and procedures for data collection.

12.2 AUDITS AND INSPECTIONS

Sponsor can carry out an audit of quality control at its sole discretion. In this case, the investigator should agree to grant the auditor direct access to all relevant documentation and devote part of his/her time and staff time to the auditor in order to discuss the results of the monitoring, as well as any other possible aspect.

Moreover, regulatory authorities may also inspect the study. In this case, the investigator should agree to give the inspector direct access to all relevant documentation and devote part of his/her time and staff time to the inspector in order to discuss the results of the supervision, as well as any other possible aspect.

12.3 CASE REPORT FORM

Data collection will be done through an eCRF with a system of access by username and password. The application includes track changes monitoring (recording the changes that have been made and details of the user that has made these changes).

Accurate and reliable data collection is ensured by checking and cross checking the eCRF front site records conducted by the study monitor (verification of source documents). The data collected will be added to a computer database which will be reviewed for possible inconsistencies to be resolved by the research team of the study in each site.

13 ETHICS

13.1 GENERAL CONSIDERATIONS

The clinical trial will be conducted according to the principles of the Declaration of Helsinki, Fortaleza, Brasil, October 2013.

This study will be conducted according to Spanish regulations and the required documentation prior to the start will be:

- Protocol acceptance by the sponsor and the coordinating investigator
- Protocol approval by the Ethics Committee.
- Protocol authorization from the Spanish Drug Agency (Ministry of Health)

All participants will be guaranteed continued medical and nursing supervision throughout the duration of the study.

This study will conform to the standards of "Good Clinical Practice".

Also, following the "Good participatory practice guidelines" (published by the Joint United Nations Program on HIV/AIDS (UNAIDS)), representatives of the community-based HIV/STD detection centres* will participate in the review of protocol design and the Patient Information Sheet and consents, through the Community Advisory Committee (CAC)*, including all substantial modifications.

Contact details of the Community Advisory Committee (CAC)

Dr. Pep Coll,
HIV Unit, IrsiCaixa, HUGTIP, Badalona, Spain
T: +34 934 656 374
pcoll@irsicaixa.es

*Members of the following entities and NGO: Àmbit Prevenció, Gais Positius, Consorci Sanitari de Barcelona (Agència de Salut Pública), Federación de Entidades Latinoamericanas de Catalunya, Planeta Salud, Programme for the Prevention and Assistance of AIDS (from the Health Department of Health of the Generalitat de Catalunya) and the Projecte dels Noms-Hispanosida organization.

13.2 PATIENT INFORMATION SHEET AND INFORMED CONSENT

Informed consent will be obtained before including the subject in the trial. The investigator is to inform the subject of the nature, duration and purpose of the study, as well as of all the obstacles and inconveniences which – within reason – may be expected from it. Furthermore, the subject is to receive information in writing. The participants must be legally competent to give informed consent, with the possibility of taking decisions at his/her own free will. The subject has the right to leave the study at any time.

Patient information sheet (PIS), informed consent form (ICF) and summary information sheet may be found in Appendices 3: *ALX002.PIS and IC* and 4: *ALX002.Summary PIS*.

13.3 PAYMENT TO RESEARCH PARTICIPANTS

Participants will be reimbursed for their time, effort and time travels to study site due to study participation. Reimbursement amounts will be documented. Payment to participants is calculated considering the estimated time lost in transfers and visits at an estimate rate of 25€/hour.

Breakfast on IMP administration days for Group 1 and Group 2 participants will be offered.

A free taxi service for all participants will be offered during the study (also for additional unscheduled visits) and Roll-over Phase visits.

A net payment of € 1,280 (€ 1,580 for Sentinel group) for Phase A and € 840 for Phase B for meals, transfers and lost in productivity foreseen and will be set in two times:

- 50% after week 12
- 50% after week 32

A net payment of € 50 per each Roll-over visits for meals, transfers and lost in productivity is foreseen and will be paid at the end of the Roll-over Phase or after being included in Phase C.

For Phase C participants, a net payment for transfers and lost in productivity foreseen will be set in two times:

- € 1,240 for Group 1, € 1,180 for Group 2, € 1,060 for Group 3 at Phase C week 32 visit.
- € 720 at end-of-study visit (i.e., visit Phase C week 68 or visit Phase C week 56 for participants with < 2,000 pVL copies/mL).

14 DATA HANDLING AND RECORD KEEPING

14.1 DATA HANDLING

The processing of the data to be compiled by the study sponsor during the trial will be subject to current legislation as regards data protection (*Ley Orgánica 15/1999, de 13 de diciembre de protección de datos de carácter personal (LOPD), Royal Decree that develops it (RD 1720/2007) and Regulation (EU) 2016/679 of the European Parliament and of the Council of 27 April 2016 (General Data Protection Regulation*). The subject will be identified in the records by the corresponding code number only. The subject is to be guaranteed anonymity, and is to be informed that all communication will take place between him/her and the investigator and not the sponsor of the trial.

Data transmitted to third countries and other countries will in no case contain personal data. In the event that such transfer occurs, it will be for the same purposes of the study described and ensuring confidentiality at least to the level of protection of the law in Spain.

14.2 RECORD KEEPING

14.2.1 Investigator file and document retention

The investigator must keep the investigator file with the proper and accurate records to enable the study to be fully documented and data subsequently verified.

The Investigator's study file will contain the protocol and its amendments, questionnaires' forms, EC approval and authorization from the health authorities, samples of the patient's information sheet and informed consent, staff curriculum, signatures' delegation log, procedure's training log and listing of participants, as well as other appropriate documents and correspondence.

Clinical source documents from participants (usually predefined by the project to record key efficacy and safety parameters or documents that are not in the clinical record of the hospital) will be filed indicating the number of subject without personal data.

The investigator should retain these documents at least five years, according to SCO/256/2007, provided that the sponsor does not express a greater period.

14.2.2 Source documents and basic data

The information contained in the eCRF will be considered as primary data, except for subject filiation data and lab tests. Subject participation in the study will be collected on electronic health records, including assigned code number and identification of the different study visits that will take place throughout the study. At the end of the study, a copy of the eCRF will be placed on the site.

15 FINANCING AND INSURANCE

15.1 SOURCE OF FINANCING

The study will be funded by AELIX Therapeutics.

15.2 INSURANCE POLICY

In accordance with Articles 9 and 10 of the Royal Decree 1090/2015, of 4th December, the trial sponsor has a policy of liability insurance with Zurich Insurance Company PLC Branch in Spain established in Barcelona. The sponsor shall extend this policy or another with equivalent coverage until the end of the trial. The policy will cover the damages to the people that could be set as a result of the trial by an insured amount of € 500,000 per subject tested to a maximum of € 5,000,000 per year and clinical trial. This policy also covers the responsibilities of the sponsor, the principal and his/her collaborators, as well as the hospital or site where they carry out the clinical trial.

The sponsor agrees to pay the premiums to cover the liability pertaining to the trial. It is presumed, unless proven otherwise, that damage affecting the health of the person subject to testing during implementation and in the following year the completion of treatment, have occurred as a result of the trial. However, once the year ended, the test subject is required to prove the link between the trial and damage.

The site and the principal investigator undertake to inform the sponsor of any claim or legal, real or potential action if known, linkable to trial.

16 PUBLICATION POLICY

The publication of the trial results shall meet the requirements set out in Article 42 of Royal Decree 1090/2015.

17 APPENDICES

Appendix 1: Product administration form

- Participant administration form Phase A
- Participant administration form Phase B
- Participant administration form Phase C

Appendix 2: Participant diary card (attached)

Appendix 3: Patient information sheet and Informed Consent form (attached)

- Patient information sheet and Informed Consent form Phase A
- Patient information sheet and Informed Consent form Phase B
- Patient information sheet and Informed Consent form Phase RO
- Patient information sheet and Informed Consent form Phase C

Appendix 4: Summary information sheet (attached)

- Summary information sheet Phase A
- Summary information sheet Phase B

Appendix 5: Schedule of procedures and blood volumes (attached)

- Schedule of procedures Phase A Group 1 (Sentinel)
- Schedule of procedures Phase A Group 2 (Non-Sentinel)
- Blood volumes Phase A
- Schedule of procedures Phase B
- Blood volumes Phase B
- Schedule of procedures Phase C (Sentinel)
- Schedule of procedures Phase C (Non-Sentinel)
- Blood volumes Phase C

Appendix 6: Calendar of planned visits and summary of trial procedures for participants

- Calendar of visits Phase A Group 1 (Sentinel)
- Calendar of visits Phase A Group 2 (Non-Sentinel)
- Calendar of visits Phase B
- Calendar of visits Phase C (Sentinel)
- Calendar of visits Phase C (Non-Sentinel)

Appendix 7: Dietary questionnaire (attached)

Appendix 8: Participant Identification card (attached)

Appendix 9: Safety monitoring committee (attached)

Appendix 10: Serious Adverse Event (SAE) notification instructions and form

Appendix 11: List of study SOPs

- Recalls and returns SOP
- Unblinding procedures SOP
- Vaccines and Placebo preparation SOP
- Vaccines and Placebo masking SOP

- Vaccines and Placebo dispensing SOP
- Vaccines and Placebo administration and disposal SOP
- Emergency plan in case of IMP release
- IMP storage SOP
- MVA.HTI relabelling SOP

Appendix 12: Pregnancy of Partner Information Sheet and Informed Consent form (attached)

Appendix 13: Questionnaire of expectations and worries (attached)

- Questionnaire of expectations and worries screening
- Questionnaire of expectations and worries end-of-study

Appendix 14: ATI monitoring form (Phase C)

Appendix 15: ATI Patient Card

Appendix 16: PrEP implementation Plan (attached)

Appendix 17: Co-signed Information sheet PrEP (attached)

Appendix 18: Questionnaire of the impact of the ATI in the psychological status (attached)

- Questionnaire of the impact of the ATI in the psychological status Pre-ATI
- Questionnaire of the impact of the ATI in the psychological status Post-ATI

Supplementary Data S2. List of protocol amendments.

Clinical Trial AELIX-002

EudraCT: 2017-000532-34 **PEI:** 16-141, 16-142 and 18-204 (MVA.HTI, DNA.HTI and ChAdOx1.HTI)
Study code: Aelix-002
Title: A Phase I, Randomized, Double-Blind, Placebo-Controlled Safety, Tolerability and Immunogenicity Study of Candidate HIV-1 Vaccines DNA.HTI, MVA.HTI and ChAdOx1.HTI in Early Treated HIV-1 Positive Individuals

Content summary:	**CEIm**	**AEMPS**
Substantial amendment 01: The technical release of MVA.HTI by the QP of the main manufacturer IDT Biologika was previously obtained, but for internal reasons this manufacturer does not release to trial and a new manufacturer was added to reflect the labelling and release to trial.	-	Submission 12/06/2017 Authorization 26/07/2017
Substantial amendment 02: The changes refer principally to an increase in the shelf life of the product MVA.HTI based on new stability data and corresponding changes to the IMPD and the IB for this product. Other minor changes to the IMPD of this product were also introduced as Aelix has initiated a review of the BMR and manufacturing information with the long-term aim of updating and improving the IMPD as discussed with AEMPS in scientific advice.	Submission 17/10/2017 Approval 10/11/2017	Submission 17/10/2017 Authorization 24/11/2017
Substantial amendment 03: Main contents of this amendment include: • An increase in the study sample size, with 30 additional patients to be included during a second phase of recruitment (B), after having a favorable review by the study Safety Monitoring Committee of the interim safety report from the first 15 patients included (Phase A). • An extension sub-study (Roll-over Phase) after week 32, up to an additional year of follow-up.	Submission 18/12/2017 Approval 12/01/2018	Submission 18/12/2017 Authorization 07/03/2018

Clinical Trial AELIX-002

Content summary:	CEIm	AEMPS
Non-substantial amendment 01 (NSA01)_01-06-2018: This non-substantial amendment is to clarify exclusion criteria number 2 of the AELIX-002 Protocol version 3.0 of 15th December 2017 "Presence of resistance drug mutations in a pre-cART genotype". The design of an adaptive future intervention is still ongoing, but future plans for the AELIX-002 clinical trial have always viewed a treatment interruption phase to assess the virological outcome of the products tested, as stated in the products' development plan discussed on July 2017 with the Spanish Agency. Hence, the aim of the criterion exposed was to exclude participants with mutations in HIV genotype that would prevent the construction of a viable cART regimen post-treatment interruption. The wording of exclusion criteria number 2 is not precise and will be modified for the following: "When available, pre-cART genotypic data that demonstrates the presence of clinically significant mutations that would prevent the construction of a viable cART regimen post-treatment interruption" This modification is classified by the Sponsor as a non-substantial modification (code NSA01), and will be included in the next version of the protocol (v4.0), that is planned to be submitted to Spanish Agency and Ethics Committee by the end of 2018.	-	-
Substantial amendment 04: Two main contents are included in this amendment: - The addition of a new sequence of a booster vaccination regimen that includes a new investigational medicinal product (IMP) ChAdOx1.HTI, to be administered before an analytical treatment interruption (ATI), to assess if a booster vaccination regimen (DDDMM and CCM) is able to prevent or delay viral rebound, induce post-rebound viral control, and/or prevent or delay the need resumption of antiretroviral therapy during an analytical treatment interruption (ATI) of antiretroviral therapy in early treated HIV-1 positive individuals (Phase C). – The introduction of specific exploratory objectives regarding changes in the HIV reservoir during the study.	Submission 26/10/2018 Approval 25/01/2019	Submission 26/10/2018 Authorization 08/02/2019
Substantial amendment 05: The main contents of this amendment are: - Changes to the protocol and the new co-signed information sheet to be able to provide pre-exposure prophylaxis (PrEP) to the partners of clinical trial participants during the Analytic Treatment Interruption (ATI) phase of the trial. The proposal for PrEP was discussed with AEMPS in a scientific advice meeting held in October 2018 and informally by mail and phone in June and July 2019.	Submission 03/07/2019 Approval 12/07/2019	Submission 03/07/2019 Authorization 23/07/19

Clinical Trial AELIX-002

Content summary:	CEIm	AEMPS
Non-substantial amendment 02 (NSA02)_25-07-2019: This non-substantial amendment is to clarify that the complete blood count (CBC) will be done in all visits of ATI period. This is not contemplated in AELIX-002 Protocol version 6.0, 2nd July 2019 by mistake. During the ATI period CD4/CD8 T-cell counts will be done in each visit. In order to have these parameters, complete blood count is needed. By mistake these evaluations were not included in the protocol of the study. This modification is classified by the Sponsor as a non-substantial modification (code NSA02) and a Note-to-file has been created.	-	-
Non-substantial amendment 03 (NSA03) 17-10-2019: This non-substantial amendment is to include an additional psychological assessment at visit Phase C w60 (4 weeks after cART resumption) and the questionnaires for the psychological assessment Pre and Post Analytical treatment interruption (ATI) as appendix of the protocol. Also, we will Include an additional stool samples collection and storage and completion of the dietary questionnaire at visit Phase C w44 and at the End of ATI visit. This is not contemplated in AELIX-002 Protocol version 6.0, 2nd July 2019. This modification is classified by the Sponsor as a non-substantial modification (code NSA03) and a Note-to-file has been created. The protocol version will be modified with the following substantial amendment.	-	-
Substantial amendment 06: The main content of this amendment is: - Changes to the protocol to allow that those participants who reach 24 weeks of ATI with HIV-1 RNA plasma levels (pVL) <2,000 cop/ml to be engaged in a roll-over investigator initiated study to be maintained without cART.	Submission 05/02/2020 Approval 21/02/2020	Submission 05/02/2020 Authorization 05/03/2020

Supplementary Data S3. List of AELIX-002 Study Group members

From Fundació Lluita contra la Sida, Infectious Diseases Department, Hospital Universitari Germans Trias i Pujol, Badalona, Spain: Yovannina Alarcón-Soto, Lucía Bailón, Susana Benet, Patricia Cobarsí, Roser Escrig, Silvia Gel, Cora Loste, Miriam López, Cristina Martinez, Laura Mas, Cristina Miranda, José Moltó, Jose Muñoz, Aroa Nieto, Helena Pera, Francisco Perez, Jordi Puig Lara Teruel, Albert Tuldrà and Jessica Toro.

From IrsiCaixa AIDS Research Institute-HIVACAT, Hospital Universitari Germans Trias i Pujol, Badalona, Spain: Christian Brander, Maria Casadellà, Samandhy Cedeño, Bonaventura Clotet, Josep Coll, Tuixent Escribà, Anuska Llano, Mireia Manent, Chiara Mancuso, Beatriz Mothe, Marc Noguera-Julian, Roger Paredes, Mariona Parera, Miriam Rosás-Umbert , Marta Ruiz-Riol and Bruna Oriol-Tordera.

From Projecte dels Noms-Hispanosina, BCN Checkpoint, Barcelona, Spain: Javier Fernández, Michael Meulbroek, Félix Perez, Ferran Pujol, Angel Rivero and Jorge Saz.

From AELIX Therapeutics S.L., Barcelona, Spain: Lance Berman, Jose Luís Cabero, Margarida Garcia, Anne R. Leselbaum, Marc Mansour, Ian McGowan and Jordi Naval.

From the Jenner Institute, The Nuffield Department of Medicine, University of Oxford, UK: Tomáš Hanke and Edmund G. Wee.

From Gilead Sciences, Foster City, US: Devi Sengupta and Romas Geleziunas.

From Germans Trias i Pujol Research Institute, Badalona, Spain: Ana María Barriocanal

Supplementary Data S4. CONSORT checklist

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on Page (line)
Title and abstract			
	1a	Identification as a randomised trial in the title	1 (2)
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	2 (30-45)
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	2-3
	2b	Specific objectives or hypotheses	21-22
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	19
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	Supp. S2
Participants	4a	Eligibility criteria for participants	19
	4b	Settings and locations where the data were collected	Supp. S1(60)
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	20; Fig 1; 3 (86-95)
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	21; 23-26
	6b	Any changes to trial outcomes after the trial commenced, with reasons	Supp. S2
Sample size	7a	How sample size was determined	26
	7b	When applicable, explanation of any interim analyses and stopping guidelines	Supp. S1 (40)
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	Supp. S1 (44)
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	19
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	19
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	Supp. S1 (44)
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	3, Report

		assessing outcomes) and how	Sum
	11b	If relevant, description of the similarity of interventions	N/A
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	26 (843-869)
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	26 (843-869)
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	3-4 & Fig. 1b
	13b	For each group, losses and exclusions after randomisation, together with reasons	Fig 1b
Recruitment	14a	Dates defining the periods of recruitment and follow-up	19
	14b	Why the trial ended or was stopped	N/A
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	Table 1
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	Every Fig & Table
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	Fig. 2-3 ED table 1-4 Sup Table 1-4
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	N/A
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	8-9
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	N/A
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	11 (363-372)
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	11 (365)
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	11 (374-384)
Other information			
Registration	23	Registration number and name of trial registry	2 (34)
Protocol	24	Where the full trial protocol can be accessed, if available	Supp S1-S2
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	13 (429-437)

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

** Accessible at clinicaltrials.gov/ct2/show/record/NCT03204617