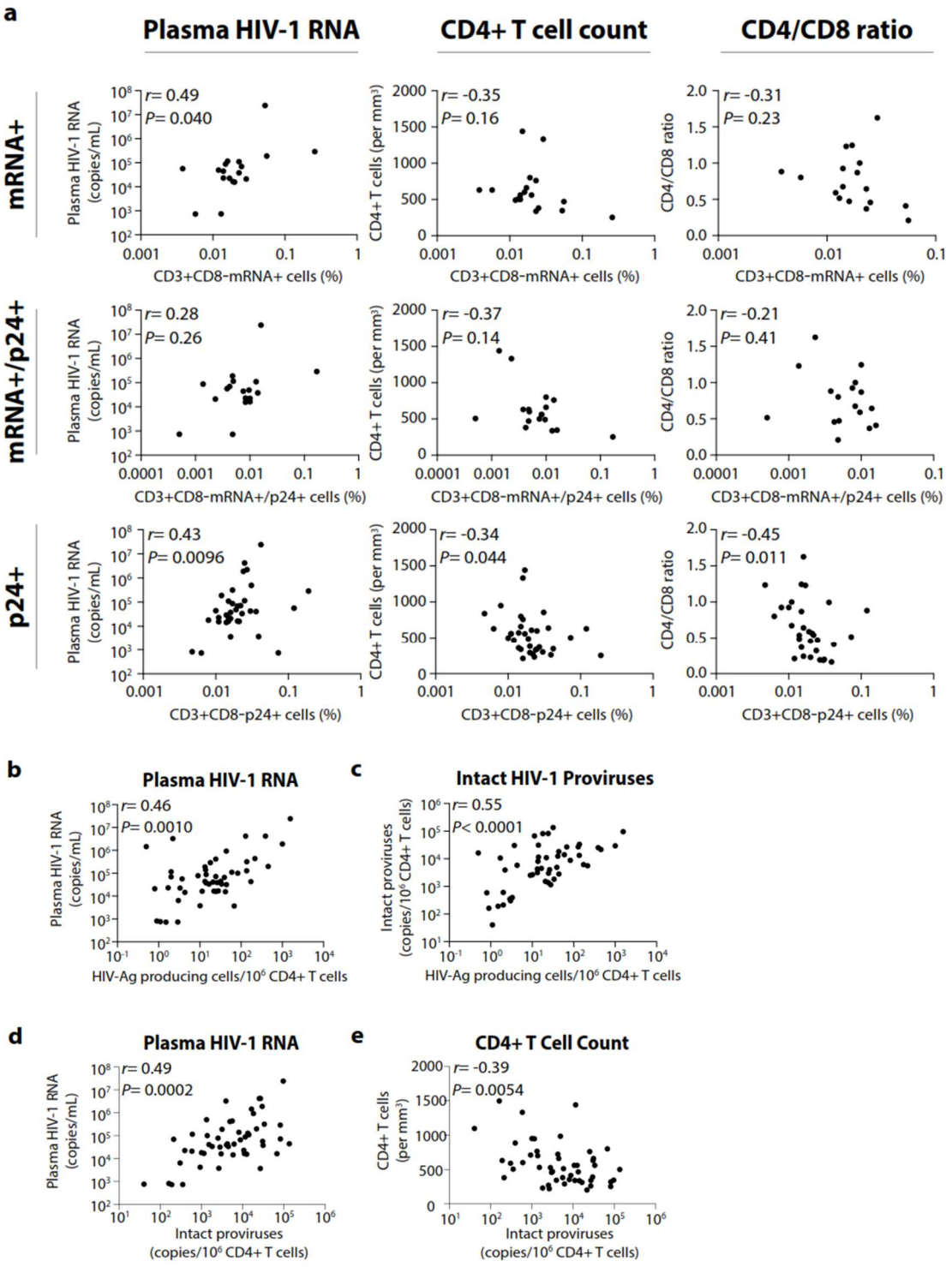
SUPPLEMENTARY MATERIALS

Early intervention with 3BNC117 and romidepsin at antiretroviral treatment initiation in people with HIV-1: a phase 1b/2a, randomized trial.

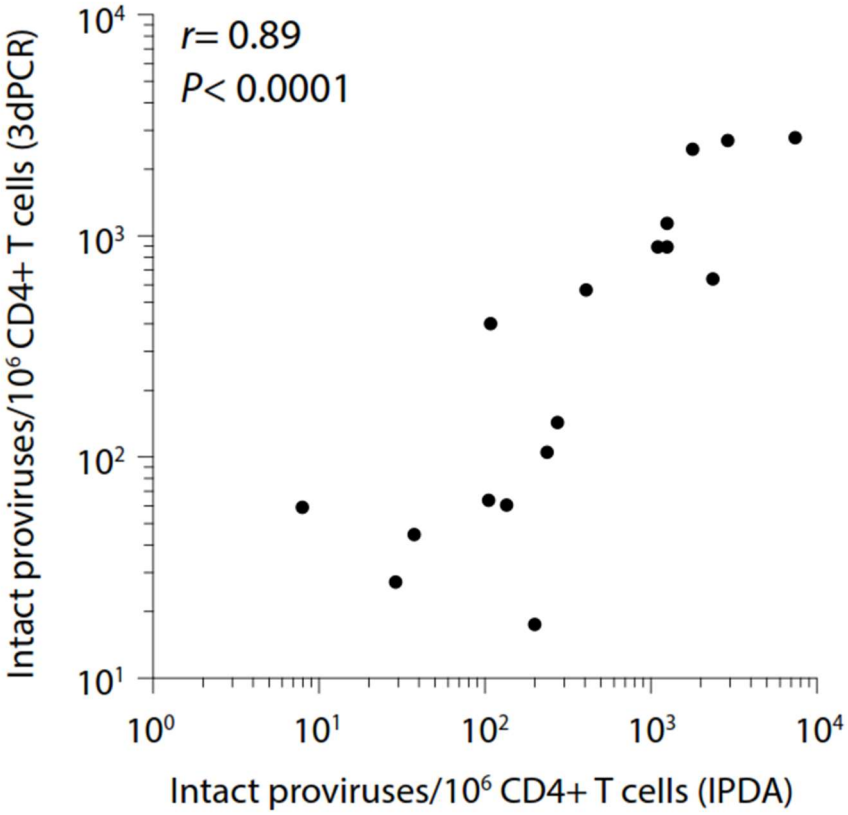
SUPPLEMENTARY FIGURES AND TABLES



Supplementary Figure S1. Correlations between assays and virological/immunological parameters.

The FISH-flow assay (**a**): Top row is CD3+CD8-mRNA+ T cells (transcriptionally active, n=18), middle row is CD3+CD8-mRNA+p24+ T cells (transcriptionally and translationally active, n=18), and bottom row is CD3+CD8-p24+ T cells (translationally active, n=35). First column is plasma HIV-1 RNA level, second column is CD4+ T cell count, and third column is CD4/CD8 ratio. The

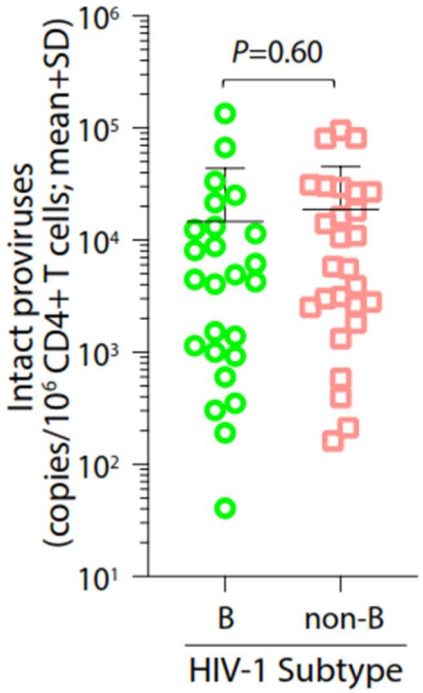
VIP-SPOT assay (**b-c**): The frequency of HIV antigen-producing CD4+ T cells (n=49) and plasma HIV-1 RNA level (**b**) and intact proviruses (**c**), respectively. The intact HIV-1 reservoir (**d-e**): The level of intact proviruses (n=52) and plasma HIV-1 RNA level (**d**) and CD4+ T cell count (**e**), respectively. *P* values calculated using Spearman's correlation coefficient.



Supplementary Figure S2. Correlation between IPDA and 3dPCR for individuals with HIV-1 subtype B.

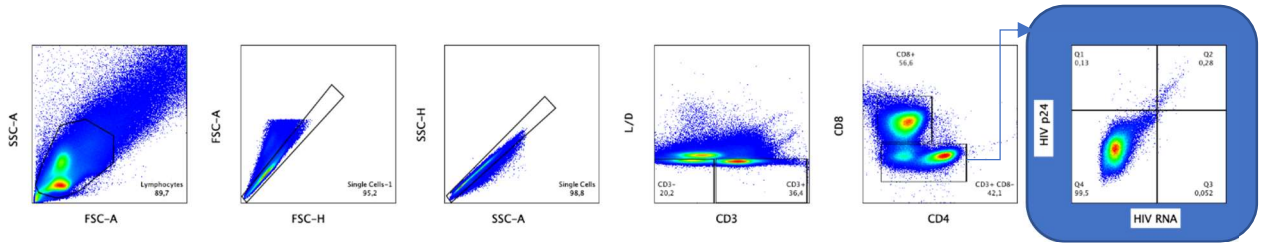
For 23 individuals who harbored HIV-1 B subtype and for whom sufficient sample was available, the level intact proviruses using IPDA was quantified (n=17). IPDA detection failure occurred in 6 (26%) of these 23 individuals, where polymorphism(s) were observed in the probe(s) and/or primer(s) in all cases (Supplemental Material, Supplementary Table S3). *P* values calculated using Spearman's correlation coefficient.

IPDA, The Intact Proviral DNA Assay; 3dPCR, duplexed ddPCR.



Supplementary Figure S3. Level of intact proviruses according to HIV-1 B or non-B subtypes. The size of the intact HIV-1 reservoir at ART initiation (day 0) among individuals with HIV-1 B (n=25) versus non-B (n=27) subtypes (lines at mean+SD). P values were calculated using unpaired two-tailed t test.

SD, standard deviation.



Supplementary Figure S4. Gating strategy for the FISH-flow assay.

		At baseline (as in Fig. 2a)				At viral rebound			
		Monogram PhenoSense		*env* sequencing		Monogram PhenoSense		*env* sequencing	
	Participant ID	IC90	MPI	Sensitive/ total	Assessment	IC90	MPI	Sensitive/ total	Assessment
ART+3BNC117 (n=5)	109	-	-	33/33	Sensitive	-	-	35/35	Sensitive
	703	0.30	99.9	-	Sensitive	0.23	99.9	-	Sensitive
	125	1.63	97.2	-	Resistant	-	-	-	NA ¥
	126	2.10	97.8	-	Resistant	-	-	36/36	Sensitive
	704	>50	44.2	-	Resistant	>50	37.1	-	Resistant
ART+3BNC117+RMD (n=6)	103	0.96	99.2	-	Sensitive	-	-	36/36	Sensitive
	107	0.25	99.9	-	Sensitive	-	-	-	Not rebounded
	304	0.74	99.9	-	Sensitive	0.69	99.9	-	Sensitive
	112	>50	08.6	-	Resistant	>50	08.6	-	Resistant
	402	-	-	7/35	Resistant	0.81	99.0		Sensitive
	709	7.44	96.3	-	Resistant	>50	86.0	-	Resistant

Supplementary Figure S5. 3BNC117-sensitivity at baseline and viral rebound.

¥ One individual (id. 125) resumed ART prior to fulfilling the restarting criteria due to national restrictions during the COVID-19 pandemic and research sampling was not done.

ART, antiretroviral therapy; COVID-19, coronavirus disease 2019; *env*, HIV-1 envelope gene; IC90, concentration of 3BNC117 required to inhibit viral replication by 90%; MPI, % inhibition observed at the highest concentration of 3BNC117 tested; NA, not available; RMD, romidepsin.

	3BNC117 sensitive (n=18)	3BNC117 resistant (n=13)	*P* values
Age - yr	43 (29-54)	37 (27-37)	0.2968
Female sex	00 (00)	00 (00)	1.000
Race			
Asian	02 (11)	01 (08)	1.000
White or Caucasian	15 (83)	12 (92)	
Other	01 (06)	00 (00)	
Time from infection to study enrollment §			
Recent (<6 months)	09 (50)	04 (31)	0.276
Long-term (>6 months)	07 (39)	08 (62)	
Unknown	02 (11)	00 (00)	
CD4+ T cell count - cells/mm^3	470 (345-630)	560 (340-800)	0.2673
HIV-1 RNA level - copies/ml	67,550 (22,400-317,431)	41,000 (18,100-71,900)	0.4712
Intact HIV-1 proviruses - copies/10^6 CD4+ T cells			
Baseline	17,796 (±24,090)	19,841 (±27,556)	0.8360
Pre-ATI	1,797 (±2,208)	2,041 (±2,257)	0.7844
HIV-1 Gag-specific CD8+ T cell responses - %			
Baseline	1.26 (1.07-3.15)	0.93 (0.23-1.90)	0.4037
Pre-ATI	1.02 (0.55-4.47)	0.70 (0.11-1-41)	0.1111
HIV-1 subtype			
B	08 (44)	07 (54)	0.722
Non-B	10 (56)	06 (46)	
Human leukocyte antigen class I alleles			
Risk: B*07, B*35	05 (28)	01 (08)	0.283
Protective: B*27, B*57, B*58	03 (17)	03 (23)	

Supplementary Table S1. Characteristics based on 3BNC117-sensitivity among the ART+3BNC117 and ART+3BNC117+RMD groups.

Data are median (IQR), n (%) or mean (±SD).

§, Time from infection to study enrollment was self-reported.

P values are calculated using between groups using two-tailed Mann-Whitney test, Fischer's exact test, or unpaired t-test.

IQR, interquartile range; SD, standard deviation.

participantID	ParticipantSequence_PsiFwdPrimer	Assay_PsiFwdPrimer	ParticipantSequence_PsiProbe	Assay_PsiProbe	ParticipantSequence_PsiRevPrimer	Assay_PsiRevPrimer
default_oligo	CAGGACTCGGCTTGCTGAAG	CAGGACTCGGCTTGCTGAAG	TTTTGGCGTACTCACCAGT	TTTTGGCGTACTCACCAGT	GCACCCATCTCTCTCCTTCTAGC	GCACCCATCTCTCTCCTTCTAGC
103	- - - - - - - - - - - - - - - - - - -	def aul t	- - - - - - - - - - - - - - - - - - -	def aul t	- -	def aul t
104	- - - - - - - - - - - - - - - - - - -	def aul t	- - - - - - - - - - - - - - - - - - -	def aul t	- -	def aul t
105	- - - - - - - - - - - - - - - - - - -	def aul t	AAA- - - - - - - - - - - - - - - - -	def aul t	- -	def aul t
107	- - - - - - - - - - - - - - - - - - G-	def aul t	- - - - - - - - - - - - - - - - - - -	def aul t	- -	def aul t
108	- - - - - - - - - - - - - - - - - - G-	def aul t	A- - - - - - - - - - - - - - - - - -	def aul t	- -	def aul t
109	- - - - - - - - - - - - - - - - - - GC	- - - - - - - - - - - - - - - - - - GC	- - - - (C) A- - - - - - - - - - - GT-	- - - - (C) A- - - - - - - - - - - GT-	- - - - - - - - - - - - - - - - - C- - - - -	- - - - - - - - - - - - - - - - - C- - - - -
110	- - - - - - - - - - - - - - - - - - GC	- - - - - - - - - - - - - - - - - - GC	- - - - (C) A- - - - - - - - - - : - -	- - - - (C) A- - - - - - - - - - : - -	- - - - - - - - - - - - - - - - - C- - - - -	- - - - - - - - - - - - - - - - - C- - - - -
112	- - - - - - - - - - - - - - - - - - GC	- - - - - - - - - - - - - - - - - - GC	- - - - (C) A- - - - - - - - - - - - -	- - - - (C) A- - - - - - - - - - - - -	- -	def aul t
116	- - - - - - - - - - - - - - - - - - -	def aul t	AA- - - - - - - - - - - - - - - - C	AA- - - - - - - - - - - - - - - - C	- - - - - - - - - - - - - - T- - - - - - - -	- - - - - - - - - - - - - - T- - - - - - - -
117	- - - - - - - - - - - - - - - - - - -	def aul t	- - - C- - - - - - - - - - - - - - -	def aul t	- -	def aul t
120	- - - - - - - - - - - - - - - - - - -	def aul t	- - - - - - - - - - - - - - - - - - -	def aul t	- -	def aul t
125	- - - - - - - - - - - - - - - - - - -	def aul t	AAA- - - - - - - - - - - - - - - - -	AAA- - - - - - - - - - - - - - - - -	- -	def aul t
126	- - - - - - - - - - - - - - - - - - GT	- - - - - - - - - - - - - - - - - - GT	AA- - - - - - - - - - - - - - - - - -	AA- - - - - - - - - - - - - - - - - -	- -	def aul t
130	- - - - - - - - - - - - - - - - - - G-	def aul t	AA- - - - - - - - - - - - - - - - - -	def aul t	- -	def aul t
131	- - - - - - - - - - - - - - - - - - -	def aul t	A- - A- - - - - - - - - - - - - C	A- - A- - - - - - - - - - - - - C	- -	def aul t
135	- - - - - - - - - - - - - - - - - - G-	def aul t	A- - - - - - - - - - - - - - - - - -	def aul t	- -	def aul t
201	- - - - - - - - - - - - - - - - - - -	def aul t	AAA- - - - - - - - - - - - - - GT-	AAA- - - - - - - - - - - - - - GT-	- -	def aul t
202	- - - - - - - - - - - - - - - - - - -	def aul t	A- - - - - - - - - - - - - - GT-	A- - - - - - - - - - - - - - GT-	- -	def aul t
203	- - - - - - - - - - - - - - - - - - G-	def aul t	A- - - - - - - - - - - - - - - - - -	def aul t	- -	def aul t
204	- - - - - - - - - - - - - - - - - - -	def aul t	AAA- - - - - - - - - - - - - GT-	AAA- - - - - - - - - - - - - GT-	- -	def aul t
205	- - - - - - - - - - - - - - - - - - -	def aul t	- - A- - - - - - - - - - - - - - C	- - A- - - - - - - - - - - - - - C	- -	def aul t
206	- - - - - - - - - - - - - - - - - - G-	def aul t	- - - - - - - - - - - - - - - - - - -	def aul t	- -	def aul t
207	- - - - - - - - - - - - - - - - - - -	def aul t	- - - - - - - - - - - - - - - - G- -	- - - - - - - - - - - - - - - - G- -	- -	def aul t
208	- - - - - - - - - - - - - - - - - - -	def aul t	- - - - - - - - - - - - - - - - - - -	def aul t	- -	def aul t
209	- - - - - - - - - - - - - - - - - - -	def aul t	- - A- - - - - - - - - - - - - - - C	- - A- - - - - - - - - - - - - - - C	- -	def aul t
210	- - - - - - - - - - - - - - - - - - -	def aul t	AAAA- - - - - - - - - - - - GT-	AAAA- - - - - - - - - - - - GT-	- -	def aul t
211	- - - - - - - - - - - - - - - - - - GC	- - - - - - - - - - - - - - - - - - GC	G- - C- - - - - - - - - - - - - - -	- - - C- - - - - - - - - - - - - - -	- -	def aul t
212	- - - - - - - - - - - - - - - - - - A	- - - - - - - - - - - - - - - - - - A	- - - - - - - - - - - - - - - - - - -	def aul t	- -	def aul t
301	- - - - - - - - - - - - - - - - - - -	def aul t	- - - C- - - - - - - - - - - - - - -	def aul t	- -	def aul t
302	- - - - - - - - - - - - - - - - - - -	def aul t	A- A- - - - - - - - - - - - : - -	A- A- - - - - - - - - - - - : - -	- -	def aul t
304	- - - - - - - - - - - - - - - - - - -	def aul t	AAA- - - - - - - - - - - - : - -	AAA- - - - - - - - - - - - : - -	- -	def aul t
305	- - - - - - - - - - - - - - - - - - -	def aul t	- - - - - - - - - - - - - - - - - - -	def aul t	- -	def aul t
306	- - - - - - - - - - - - - - - - - - -	def aul t	- - - - - - - - - - - - - - - - - - -	def aul t	- -	def aul t
307	- - - - - - - - - - - - - - - - - - G-	def aul t	AA- - - - - - - - - - - - - - - - -	def aul t	- -	def aul t
308	- - - - - - - - - - - - - - - - - - G-	- - - - - - - - - - - - - - - - - - G-	AA- - - - - - - - - - - - - GTC	AA- - - - - - - - - - - - - GTC	- -	def aul t
401	- - - - - - - - - - - - - - - - - - -	def aul t	G- - - - - - - - - - - - - - - - -	def aul t	- - - - - - - - - G- - - - - - - - - - - -	def aul t
402	- - - - - - - - - - - - - - - - - - A	- - - - - - - - - - - - - - - - - - A	- - - - - - - - - - - - - - - - - - -	def aul t	- -	def aul t
403	- - - - - - - - - - - - - - - - - - -	def aul t	AAA- - - - - - - - - - - - - - - - -	AAA- - - - - - - - - - - - - - - - -	- -	def aul t
404	- - - - - - - - - - - - - - - - - - GT	- - - - - - - - - - - - - - - - - - GT	- - - A- - - - - - - - - - - - - - -	- - - A- - - - - - - - - - - - - - -	- -	def aul t
405	- - - - - - - - - - - - - - - - - - -	def aul t	- - - - - - - - - - - - - - - - - - -	def aul t	- -	def aul t
408	- - - - - - - - - - - - - - - - - - -	def aul t	AA- - - - - - - - - - - - - G- -	AA- - - - - - - - - - - - - G- -	- -	def aul t
409	- - - - - - - - - - - - - - - - - - -	def aul t	AAA- - - - - - - - - - - - - - - - -	AAA- - - - - - - - - - - - - - - - -	- -	def aul t
410	- - - - - - - - - - - - - - - - - - GC	- - - - - - - - - - - - - - - - - - GC	AA- - - - - - - - - - - - - - - - -	AA- - - - - - - - - - - - - - - - -	- -	def aul t
411	A- - - - - - - - - - - - - - - - - -	def aul t	- - - - - - - - - - - - - - - - - - -	def aul t	- -	def aul t
412	- - - - - - - - - - - - - - - - - - -	def aul t	- - - C- - - - - - - - - - - - - - -	def aul t	- -	def aul t
701	- - - - - - - - - - - - - - - - - - -	def aul t	AA- - - - - - - - - - - - - - - - -	def aul t	- -	def aul t
702	- - - - - - - - - - - - - - - - - - -	def aul t	- - - - (C) A- - - - - - - - - - GT-	- - - - (C) A- - - - - - - - - - GT-	- -	def aul t
703	A- - - - - - - - - - - - - - - - GT	A- - - - - - - - - - - - - - - - GT	AA- - - - - - - - - - - - - - - - -	AA- - - - - - - - - - - - - - - - -	- -	def aul t
704	- - - - - - - - - - - - - - - - - - G-	def aul t	A- - - - - - - - - - - - - - - - - -	def aul t	- -	def aul t
707	- - - - - - - - - - - - - - - - - - G-	def aul t	A- - - - - - - - - - - - - - - - - -	def aul t	- -	def aul t
708	G- - - - - - - - - - - - - - - - - G-	def aul t	AA- - - - - - - - - - - - - - - - -	def aul t	- -	def aul t
709	- - - - - - - - - - - - - - - - - - -	def aul t	- - - - - - - - - - - - - - - - - - -	def aul t	- -	def aul t

ParticipantSequence_SecondaryEnvFwdPrimer	Assay_SecondaryEnvFwdPrimer	ParticipantSequence_SecondaryEnvProbe	Assay_SecondaryEnvProbe	ParticipantSequence_SecondaryEnvRevPrimer	Assay_SecondaryEnvRevPrimer
ACTATGGGCGCAGCGTC	ACTATGGGCGCAGCGTC	CTGGCCTGTACCGTCAG	CTGGCCTGTACCGTCAG	CCCCAGACTGTGAGTTGCA	CCCCAGACTGTGAGTTGCA
----------	def aul t	-----y----------	def aul t	-------Y----------	def aul t
----------	def aul t	-----y----------	def aul t	----------	def aul t
----------	def aul t	---------G------	----------G------	--------C---------	--------C---------
----------G----	def aul t	-------r--------	def aul t	----------	def aul t
----------R-y---	----------R----	----------	def aul t	----------	def aul t
--G----------A--	--G----------A--	----------	def aul t	----------	def aul t
--G----------A--	--G----------A--	----------	def aul t	----------	def aul t
----------G----	----------G----	---A-------	---A-------	----------	def aul t
----------A--	def aul t	----------	def aul t	--------C---------	def aul t
----------	def aul t	y---y-------	def aul t	----------	def aul t
G----------	def aul t	----------	def aul t	----------	def aul t
----------R----	def aul t	----------	def aul t	----------	def aul t
----------R----	----------R----	----------	def aul t	----------	def aul t
----------G----	def aul t	----------	def aul t	--------Y---------	def aul t
----------R--	def aul t	-----y----------	def aul t	----------	def aul t
----------G----	def aul t	----------	def aul t	----------	def aul t
----------A--	def aul t	----------	def aul t	----------	def aul t
----------	def aul t	----------	def aul t	----------C---	def aul t
----------	def aul t	-----A----------	-----A----------	----------	def aul t
----------A--	def aul t	----------	def aul t	--------Y---------	def aul t
----------	def aul t	----------	def aul t	----------	def aul t
----------	def aul t	-----A----------	-----A----------	----------	def aul t
----------	def aul t	-----T----------	-----T----------	----------	def aul t
----------	def aul t	----------	def aul t	----------	def aul t
----------	def aul t	-----y----------	def aul t	----------	def aul t
----------	def aul t	---------r-------	def aul t	----------C---	def aul t
----------	def aul t	----------	def aul t	--------y------YT--	--------C------YT--
----------	def aul t	----------	def aul t	----------	def aul t
----------	def aul t	----------Y-----	def aul t	----------	def aul t
----------G----	----------G----	----y----------	def aul t	----------Y-	def aul t
----------	def aul t	---------G------	----------G------	----------	def aul t
----------	def aul t	-----y----------	def aul t	----------	def aul t
----------	def aul t	----------	def aul t	----------	def aul t
----------R--	def aul t	----------	def aul t	----------	def aul t
----------G----	def aul t	----------	def aul t	----------	def aul t
----------AG	----------AG	----------	def aul t	-Y----------	def aul t
----------Y---	----------Y---	---------R------	----------R------	-----K----------	def aul t
----------G----	----------G----	----------	def aul t	--------C------CT--	--------C------CT--
------r----r-----	----------G----	----------	def aul t	--------C----C-T--	--------C----C-T--
----------	def aul t	----------	def aul t	----------C----	def aul t
----------K-	def aul t	----y----------	def aul t	--------C---------	def aul t
----------	def aul t	----------	def aul t	----------	def aul t
----------	def aul t	-----T----------	-----T----------	----------	def aul t
----------R----	def aul t	--A----------	--A----------	----------	def aul t
----------	def aul t	----------	def aul t	----------	def aul t
----------R--	def aul t	--R------r-------	--R----------	--------Y---------	def aul t
--A----------	def aul t	----------	def aul t	--------Y---------	def aul t
----------G----	----------G----	y----T----------	-----T----------	--------C------CT--	--------C------CT--
----------G----	----------G----	-----T----------	-----T----------	----------	def aul t
----------G----	----------G----	----------	def aul t	----------	def aul t
----------G----	----------G----	----------y-----	def aul t	----------CT--	----------CT--
----------	def aul t	----------	def aul t	----------Y----	def aul t

Columns beginning with "Participant sequence" show the participant's sequence in the stated oligonucleotide region	
Columns beginning with "Assay" show whether the default or a custom oligonucleotide was used	
In participants with polymorphisms at critical residue(s) in any oligo in a given assay region, we designed custom primers/probes to exatly match their autologous sequence in that region	
Symbol	**Explanation**
-	same as default primer/probe sequence
ACTGYR (upper case lettering)	mutation relative to default primer/probe sequence
actgyr (lower case lettering)	mutation relative to default primer/probe sequence that occurred in less than 10% of within-host sequences (and was not incorporated into autologous primer/probe design)
:	deletion with respect to default primer/probe sequence
(X)	insertion (of nucleotide X) relative to default primer/probe sequence

Supplementary Table S2. Individual primer/probe sets for the 3dPCR assay.

Participant ID	IPDA result	ParticipantSequence_PsiFwdPrimer	ParticipantSequence_PsiProbe	ParticipantSequence_PsiRevPrimer	ParticipantSequence_IPDAEnvFwdPrimer	ParticipantSequence_IPDAEnvProbe	ParticipantSequence_IPDAEnvRevPrimer
deafult_oligo_seq	n/a	CAGGACTCGGCTTGCTGAAG	TTTTGGCGTACTCACCAGT	GCACCCATCTCTCTCCTTCTAGC	AGTGGTGCAGAGAGAAAAAAGAGC	CCTTGGGTTCTTGGGA	GTCTGGCCTGTACCGTCAGC
103	PASS	- - - - - - - - - - - - - - - - - - -	- - - - - - - - - - - - - - - - - - -	- -	- -	- - - k - - - - - - - - - - -	- - - - - - - y - - - - - - - - - - -
104	PASS	- - - - - - - - - - - - - - - - - - -	- - - - - - - - - - - - - - - - - - -	- -	- -	- - - - - - - - - - - - - - - -	- - - - - - - y - - - - - - - - - - -
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127	ND (insufficient sample)	- - - - - - - - - - - - - - - - - - -	AAA- - - - - - - - - - - - : - -	- -	- -	- - - - - - - - - - - - - - - -	- - - - - - - - - - - T - - - - - - -
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137	PASS	- - - - - - - - - - - - - - - - - - -	- - - - - - - - - - - - - - - - - - -	- -	- -	- - - - - - - - - - - - - - - -	- - - - - - - - - - - - - - - - - - -
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207	PASS	- - - - - - - - - - - - - - - - - - -	- - - - - - - - - - - - - - - G - -	- -	- -	- - - - - - - - - - - - A - - C	- - - - - - - T - - - - - - - - - - -
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Columns beginning with "Participant sequence" show the participant's sequence in the stated oligonucleotide region	
Symbol	**Explanation**
-	same as default primer/probe sequence
ACTGYR (upper case lettering)	mutation relative to default primer/probe sequence
actgyr (lower case lettering)	mutation relative to default primer/probe sequence that occurred in less than 10% of within-host sequences
:	deletion with respect to default primer/probe sequence
(X)	insertion (of nucleotide X) relative to default primer/probe sequence
ND	not determined

Supplementary Table S3. The sequences in the IPDA Ψ and *env* regions.

Median change in intact proviruses / 10^6 CD4+ T cells	Δ Time (days)	0-365	IQR	*P*	0-180	IQR	*P*	180-365	IQR	*P*
	ART	2709	734-11,442	ref	1816	718-9123	ref	342	7-3765	ref
	ART+3BNC117	5032	2013-26,165	0.21	2734	1511-21,175	0.21	468	81-3038	0.86
	ART+RMD	5382	2583-8695	0.53	4622	1746-7823	0.35	723	117-872	0.35
	ART+3BNC117+RMD	10,274	355-18,846	0.36	9763	432-16,047	0.24	52	-33-1694	0.24

Supplementary Table S4. The median declines in the intact HIV-1 reservoir.

The median (interquartile ranges [IQR]) changes in intact proviruses per 10^6 CD4+ T cells for the four groups at indicated time periods. *P* values comparing between groups were calculated using two-tailed Mann-Whitney test. ART only n=14, ART+3BNC117 n=14, ART+RMD n=10, ART+3BNC117+RMD n=14.

ART, antiretroviral therapy; IQR, interquartile range; RMD, romidepsin.

	ATI participants (n=20)	non-ATI participants (n=35)	*P* values
Age - yr	37 (29-46)	36 (28-43)	0.6301
Female sex	02 (10)	03 (09)	1.000
Race			
Asian	02 (10)	02 (06)	0.654
Black or African European	02 (10)	01 (03)	
White or Caucasian	15 (75)	30 (86)	
Other	01 (05)	02 (06)	
Time from infection to study enrollment §			
Recent (<6 months)	11 (55)	14 (40)	0.572
Long-term (>6 months)	09 (45)	17 (49)	
Unknown	00 (00)	04 (11)	
CD4+ T cell count - cells/mm^3	529 (364-615)	498 (340-760)	0.9073
HIV-1 RNA level - copies/ml	43,450 (20,250-152,473)	49,400 (16,281-290,000)	0.8887
Intact HIV-1 proviruses - copies/10^6 CD4+ T cells	23,759 (±37,217)	12,495 (±18,689)	0.1534
HIV-1 Gag-specific CD8+ T cell responses - %	0.56 (0.25-1.74)	1.25 (0.60-1.92)	0.2090
HIV-1 subtype			
B	08 (40)	20 (57)	0.269
Non-B	12 (60)	15 (43)	
Human leukocyte antigen class I alleles			
Risk: B*07, B*35	05 (25)	09 (26)	1.000
Protective: B*27, B*57, B*58	04 (20)	07 (20)	

Supplementary Table S5. Characteristics of ATI versus non-ATI participants.

Data are median (IQR), n (%) or mean (±SD).

§, Time from infection to study enrollment was self-reported.

P values are calculated using between groups using two-tailed Mann-Whitney test, Fischer's exact test, or unpaired t-test.

ATI, analytical treatment interruption; IQR, interquartile range; SD, standard deviation.

	Days	0 (start)	7	14	21	28	35	42	49	56	70	84 (end)
ART (n=4)	120	<20	<20	54	4,160	9,110	3,880	1,310	979	798	636	817
	208	<20	<20	57	31,900	22,800						
	702	<20D	<20D	1,300	2,600	8,600	54,000					
	707	<20	<20	<20D	100	640	3,400	7,200	2,700	1,200	420	320
ART+3BNC117 (n=5)	109	<20	<20D	<20	40	852	1,340	1,020	1,820	4,120	4,530	4,060
	125	<20	153	16,600 ¥								
	126	<20D	1,230	31,500	161,000							
	703	<20	<20	<20D	95	16,000	280,000					
	704	<20	<20	<20D	710	19,000	520,000					
ART+RMD (n=5)	104	<20D	<20	41	4,610	5,330	6,040					
	117	<20	<20	179	5,390	157,000						
	204	<20	<20D	<20D	<20	<20D	<20D	<20	<20	<20D	<20D	1220
	307	20	58	NA	175	6,420	22,500					
	708	<20D	2,100	96,000	250,000							
ART+3BNC117 +RMD (n=6)	103	<20	<20	<20	235	7,130	2,950	484	882	1,400	2,190	1,160
	107	<20D	<20	<20	<20	<20	<20	<20	<20	<20D	<20D	<20D
	112	<20	<20	10,800	39,800							
	304	<20	182	1,600	4,620	2,260	4,350	4,410	4,250	1,980	2,180	1,570
	402	<20	<20	99,500	1,270,000							
	709	<20	<20D	<20	<20	<20	NA	90	1,100	6,900	11,000	

Supplementary Table S6. Individual plasma HIV-1 RNA levels during 12 weeks of analytical treatment interruption.

Data are plasma HIV-1 RNA copies per mL. Numbers in black indicate detectable plasma HIV-1 RNA. Grey squares indicate protocol-defined time point of viral rebound. ¥ One individual (id. 125) resumed ART prior to fulfilling the restarting criteria due to national restrictions during the COVID-19 pandemic.

ART, antiretroviral therapy; COVID-19, coronavirus disease 2019; D, detectable; NA, not available; RMD, romidepsin; <20, plasma HIV-1 RNA level below limit of quantification (20 copies/mL).

CONSORT 2010 checklist of information to include when reporting a randomized trial

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	3
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	4-5
	2b	Specific objectives or hypotheses	5
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	28
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	NA
Participants	4a	Eligibility criteria for participants	28
	4b	Settings and locations where the data were collected	28
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	29
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	29-37
	6b	Any changes to trial outcomes after the trial commenced, with reasons	37-38
Sample size	7a	How sample size was determined	37-38
	7b	When applicable, explanation of any interim analyses and stopping guidelines	NA
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	29
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	29
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	29
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	29

Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	NA
	11b	If relevant, description of the similarity of interventions	NA
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	37-38
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	NA
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	6
	13b	For each group, losses and exclusions after randomisation, together with reasons	6
Recruitment	14a	Dates defining the periods of recruitment and follow-up	6
	14b	Why the trial ended or was stopped	6
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	Tab 1
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	Ext Fig. 1
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)	7-11
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	NA
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	7-11
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	11
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	15
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	15
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	12-16
Other information			
Registration	23	Registration number and name of trial registry	3
Protocol	24	Where the full trial protocol can be accessed, if available	Supplementary
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	17

Early administration of anti-latency reversing therapy and broadly neutralizing antibodies to limit the establishment of the HIV-1 reservoir during initiation of antiretroviral treatment - a randomized controlled trial (eCLEAR)

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1.10 Guidelines

This study will be conducted in accordance with the protocol, The Helsinki Declaration (1996 version), ICH guidelines for GCP and national ethical guidelines and law.

1.11 Time plan

The study will be conducted between the first quarter of 2017 and fourth quarter of 2019. Total duration of the trial is 2 years with an expected final report in the last quarter of 2020.

The study enrolment will begin at the Department of Infectious Diseases, Aarhus University Hospital and subsequently rolled out to the collaborating sites, when the necessary regulatory approvals have been obtained.

1.12 Signature Page 1

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable EU regulations and ICH guidelines.

The Sponsor, Lead Principal Investigator and Coordinating Investigator should sign Signature Page 1. A copy of this Signature Page 1 should be filed with the holder of the Regulatory documents and a copy should be maintained at each site.

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2 INTRODUCTION

2.1 Background and Rationale

Since the introduction of combination antiretroviral therapy (ART), the morbidity and mortality among human immunodeficiency virus (HIV)-infected individuals have dramatically decreased. However, despite the successes of ART, HIV-infected individuals continue to be burdened by side effects of treatment, stigmatization, and excess morbidity and mortality [1–5]. For these reasons, a curative therapy for HIV-infection remains the ultimate but still unachieved goal in infectious diseases.

HIV latency is best understood as an accidental consequence of viral tropism for activated CD4+ T cells. In short, HIV preferentially infects activated CD4+ T cells that do not survive for more than a few days hereafter. HIV-1 infection leads to the rapid death of activated CD4+ T cells either by viral cytopathic effects (CPE) or immune effector mechanisms [6], but rarely, activated CD4+ T cells become infected as they are reverting back to a resting memory state. Alternatively, CD4+ T cells may become infected directly in the resting state [7]. The resting memory CD4+ T cells have a very long life span and may expand during homeostatic proliferation. Latently infected CD4+ T memory cells are capable of resuming viral replication upon subsequent activation [8–12].

The presence of quiescent but replication competent proviruses within resting CD4+ T cells was demonstrated as early as in 1995 [13,14]. Despite potent inhibition of viral replication with standard ART, these replication competent proviruses persist in all infected individuals and drives rapid viral rebound upon discontinuation of ART [15]. This stable, latent reservoir is widely recognized as the primary barrier preventing eradication of HIV infection [16–19]. Though ART normally results in suppression of viral production to plasma levels that are below the limits of detection by standard assays, residual low-level viremia can be demonstrated in almost all infected individuals on ART using ultra-sensitive assays [20,21]. The origin of this residual low-level viremia is still not well known.

One proposed strategy to eliminate the HIV-1 reservoir is the 'kick-and-kill' strategy [19,22,23]. This widely accepted approach involves 3 key steps:

1. Shutting down virus spread and thus preventing de novo infection by ART
2. Activation ("kick") of HIV-1 expression in latently infected cells using latency reversing agents (LRA)
3. Killing of cells that are actively producing HIV-1 through immunotherapy

Many different LRAs have been proposed but the majority of clinical interest has been focused on epigenetic modulation of the molecular mechanisms that block the transcription of integrated HIV-1 DNA from the host cell. To this end, a group of small molecule drugs called histone deacetylase inhibitors (HDACi) have shown promising results [24].

In a pilot study conducted among 6 HIV-1 patients on long-term ART with no detectable plasma viremia, we demonstrated that 3 infusions of 5 mg/m^2 of romidepsin, a very potent HDACi, resulted in significant increases in HIV-1 transcription and, more importantly, a release of HIV-1 particles into the blood stream (**Figure 1**) [25].

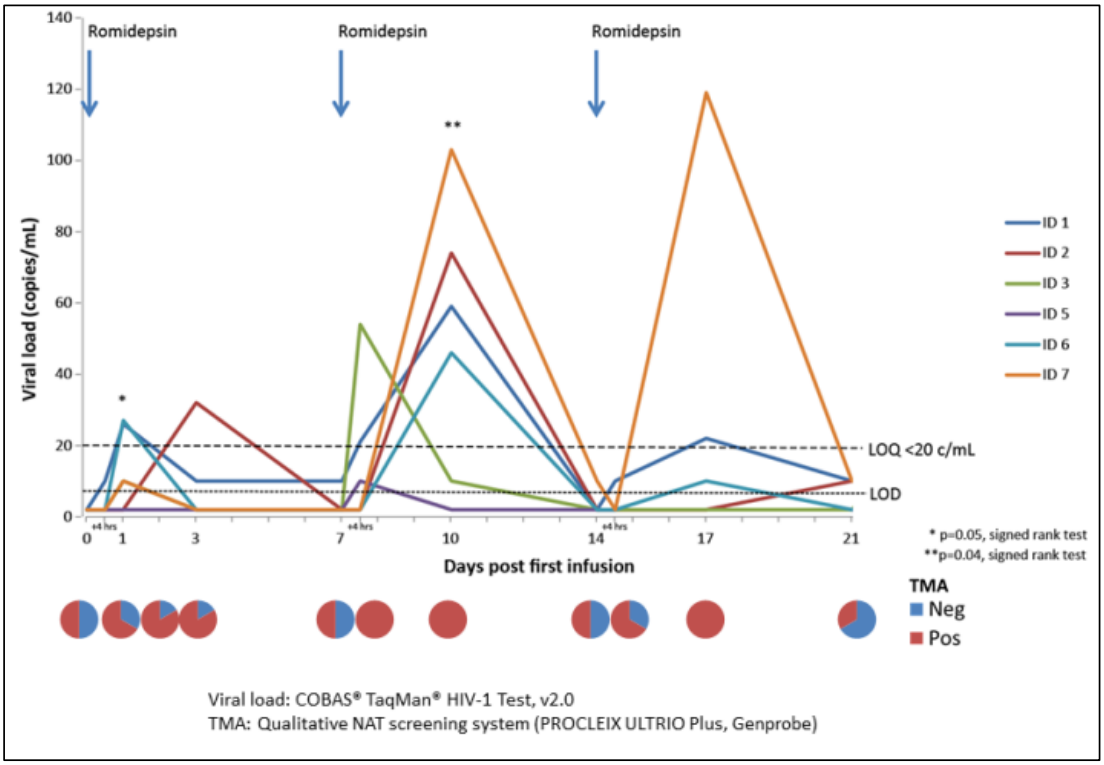


Figure 1. Induction of HIV-1 transcription upon romidepsin infusions in HIV-1 infected individuals on ART.

However, in these 6 patients the reservoir size measured as total HIV-1 DNA in CD4+ T cells did not significantly change from baseline to end of follow-up indicating that additional interventions may be needed to effectively kill virus-producing cells.

Altogether, this calls for combining LRA with immune targeted interventions such as HIV-1-specific monoclonal antibodies. Unlike ART, broadly neutralizing antibodies (bNAbs) can engage the host immune system by virtue of their Fc effector domains and thereby accelerate clearance of cell-free virus, induce antibody dependent cytotoxicity (ADCC) mediating killing of infected cells, and produce immune complexes that activate dendritic cells to become potent antigen presenting cells [26]. Further, some classes of bNAbs can prevent cell-cell transmission of HIV-1, whereas ART's activity in this regard is still debated [27,28]. In support of the idea that bNAbs and viral inducers can alter the reservoir, combinations of these agents were shown to interfere with the establishment of and disrupt the reservoir in humanized mice by a mechanism that requires engagement of antibody Fc effector functions [29,30]. - 3BNC117, which targets the CD4 binding site on the HIV-1 envelope, is one of the most potent and broad bNAbs cloned to date [31]. It has significant activity in both mouse and non-humane primates preclinical studies [29,30,32–35]. Further, in a recent phase 1 landmark study, 3BNC117 was shown to have anti-viral activity in untreated viremic HIV-1 infected individuals [36] Thirty-seven subjects have received 3BNC117 at doses ranging from 1 to 30 mg/kg with no significant adverse events to date. Moreover, 3BNC117 has favorable pharmacokinetic properties with a half-life of 17 to 18 or 9 to 10 days in uninfected and viremic HIV-1 infected individuals, respectively. Thus, 3BNC117 would be an excellent candidate for an invention aimed at killing HIV-1-producing cells.

The timing of intervening is of much debate and some researchers have questioned whether such a stable reservoir of HIV-1 DNA is also present during active infection [37], because of the high virus-driven immune activation. If the latent HIV-1 DNA pool is much more labile during active infection, then this possibility presents a potential avenue for intervention – early latency reversal [38]. This is supported by a recent animal study showing that in active simian immunodeficiency virus (SIV) infection of macaques [39] when viral loads were low, the latent reservoir had a very slow turnover/long half-life (many years). This is consistent with the low turnover of virus in patients with HIV-1 on long-term ART. However, in animals with high viral loads, the turnover/half-life of SIV DNA within resting CD4+ T cells was fast/short (days), which suggests that high virus-driven immune activation during active infection might prevent the establishment of true latency. This idea is also supported by findings from a study in patients after the initiation of ART that suggested rapid exchange between productively and latently infected cell pools during both the acute and chronic state of untreated HIV infection [40,41]. Additionally, studies in HIV-1 infected patients show a delay between the appearance of mutations in plasma and their appearance in the proviral DNA, both for immune-adaptive mutations [42] and drug-resistance mutations [43–45]. These findings suggest a new strategy to enhance the reduction or elimination of the viral reservoir. Since many agents currently under investigation aim to purge the latent reservoir through activation of latent cells, targeting the reservoir when it is already turning over rapidly/having a short half-life during active infection might be easier to achieve than when it is stable under long-term ART [46].

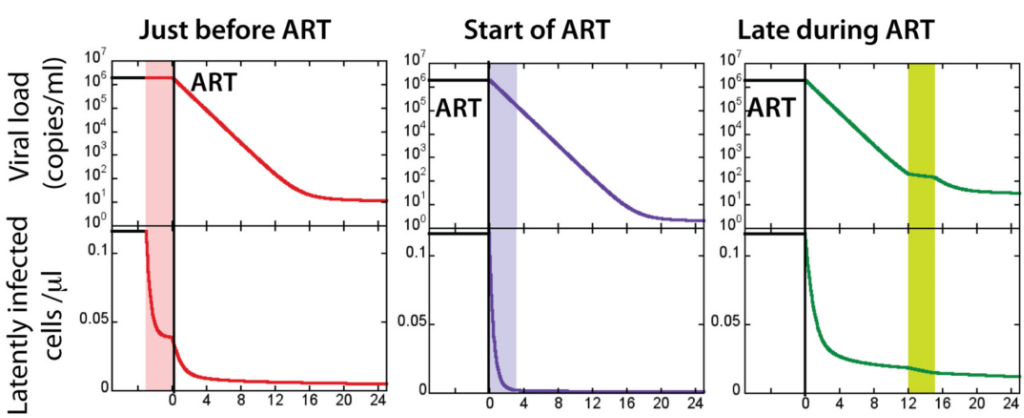


Figure 2. Effects of LRAs at different times relative to ART initiation.

The possible effects of LRAs at different times relative to ART can be investigated by use of a model of HIV infection (*See figure 2 beneath from Petravic et al. JVI 2014* [47]).The models suggested that short-term treatment with LRA would have the strongest effect on latency if administered just after commencement of ART, where it would further enhance the already high virus-driven activation rate of latent cells.

We propose to conduct a proof-of-concept study that will combine 3BNC117-mediated clearance of viral-antigen producing cells with a potent LRA, romidepsin, as a novel approach to reduce the HIV-1 reservoir in ART naïve patients initiating ART [38].

The proposed study will be conducted among treatment naïve HIV-1-infected patients randomized 1:1:1:1 to either ART alone, ART + romidepsin, ART + 3BNC117 or ART + romidepsin + 3BNC117. Subjects will continue background ART while receiving the remaining course of romidepsin and/or 3BNC117.

The proposed trial will address several gaps in the current knowledge:

1. The impact of combining ART and LRA on the size of the HIV-1 reservoir
2. The impact of combining ART and bNAbs on the size of the HIV-1 reservoir
3. The impact of combining ART, LRA and bNAbs on the size of the HIV-1 reservoir
4. The effect of adding LRA and/or bNAbs to ART on immunological preservation and recovery (e.g. CD4+ cell count recovery)
5. The effect of adding LRA and/or bNAbs to ART on time to viral suppression following ART initiation

Hence, our proposal will not only provide insights into the clinical effects of these novel interventions aimed at reducing the HIV-1 reservoir in persons initiating ART as well the potential immunological and biochemical benefits.

2.1.1 Overview of the Investigational Medicinal Products (IMPs)

2.1.1.1 Romidepsin

The basic structure of chromatin consists of the nucleosome, which comprises DNA wrapped around a core of histones. Histone proteins compact massive amounts of genomic DNA into a size and structure that can be in the nucleus. These proteins are post-translationally modified by, among others, lysine acetylation. The post-translational modifications of greatest interest in this setting are acetylation and deacetylation. These processes are regulated by two groups of enzymes with opposite activities: histone acetyltransferases (HAT) and HDACs. HDACs catalyze the removal of acetyl groups (deacetylation) from lysine residues in histone tails, leading to a closed chromatin configuration and transcriptional repression/silencing [19,48,49]. HDACs also deacetylate non-histone proteins, such as transcription factors. Epigenetics refers to the regulation of gene expression via post-translational modification of protein complexes associated with DNA, without alterations in the DNA sequence [50].

The molecular mechanisms by which HIV establishes latency are manifold and complex and include enzymatic processes that affect the chromatin organization of the HIV-promoter region, one of the key determinants of transcriptional activity [9,48,49,51,52]. There are 18 known human HDACs which are grouped into 4 classes [53]. Of these, the class I HDACs (1, 2, 3 and 8) may be particular important to maintaining HIV latency [53,54]. Moreover, HDACi have consistently shown the ability to reactivate and induce expression of HIV-1 from latently infected cells [11,53–58].

Romidepsin (FK228, FR901228, NSC630176, depsipeptide) is a potent novel HDACi used in the treatment of patients diagnosed with peripheral T cell lymphoma (PTCL) and cutaneous T cell lymphoma (CTCL). Romidepsin is an inhibitor of class I HDACs (1, 2, 3 and 8) of which HDAC 3 is of particular interest in the disruption of HIV latency [59,60]. In 2012, romidepsin was shown

to be 1000 times more potent than vorinostat in the induction of latent HIV-1, and induced HIV-1 RNA expression ex vivo in 12 of 13 patients with HIV on ART making romidepsin an attractive candidate for purging the latent HIV-1 reservoir [61]. Romidepsin is formulated as a solution for intravenous (IV) infusion and is approved by the FDA for treatment of patients diagnosed PTCL and CTCL.

2.1.1.2 3BNC117

In HIV-1 infected subjects, 10 to 25% develop sera that contain bNAbs including some that neutralize the majority of viruses from diverse genetic subtypes [62–66]. Early attempts to use passively transferred bNAbs to HIV-1 infected patients undergoing treatment interruption showed limited effect [67,68]. These trials were performed before the advent of cloning and structure based design methods that uncovered anti-HIV-1 antibodies that have a broader spectrum of activity and are orders of magnitude more potent than neutralizing antibodies previously available [31,69–72].

3BNC117 is a monoclonal antibody (mAb) of the IgG1κ isotype that targets the CD4-binding site (CD4bs) on the HIV envelope spike gp-120. 3BNC117 was isolated from an HIV-infected individual with high titers of bNAbs and who is an HIV-nonprogressor [31]. Cloning the heavy and light chain variable regions isolated from a single memory B cell generated the mAb. When compared with previously published VRC01 (a different bNAb) neutralization data, 3BNC117 is unusual in its potency and breadth, and shows much greater inhibitory potential across a broad panel of HIV-1 [31].

In preclinical studies carried out in humanized mice and non-human primates, 3BNC117 alone or in combination with other neutralizing antibodies led to protection from HIV-1 or SHIV infection and also to sustained suppression of (S)HIV-1 plasma viremia for prolonged periods of time [29,30,32–35,73].

2.1.2 Clinical Experience

2.1.2.1 Romidepsin

As of 31 December 2011, more than 1300 patients have been treated with romidepsin in clinical studies, and of those a total of 891 patients with at least one dose of romidepsin as monotherapy. Of the 891 patients, 447 were patients with hematologic malignancies and 444 with solid tumors. All 891 patients are described with safety data, which is included in the pooled safety analysis presented in the Investigators Brochure (IB).

2.1.2.2 3BNC117

In a recent proof-of-concept phase 1 human trial, 12 uninfected and 17 HIV-1-infected individuals were given a single dose of 3BNC117 IV and monitored for 56 days [36]. At the highest dosage level tested in the study, 30 mg/kg, all eight infected individuals treated showed rapid decrease in their amount of virus measured in their blood that varied between individuals from 0.8 to 2.5 on log10 scale. The median time to reach the lowest viral load was one week, but could be as long as three weeks. The drop in viral load depended on the individual's starting viral load and also the sensitivity of their particular strains of HIV to the antibody.

In half of the individuals receiving the highest dose, viral loads remained below starting levels even at the end of the 8-week study period and resistance to 3BNC117 did not occur. It is likely that antibodies may be able to enhance the patient's immune responses against HIV, which can in turn lead to better control of the infection. In addition, antibodies like 3BNC117 may be able to kill viruses hidden in infected cells, which serve as viral reservoirs inaccessible to current ART. A total of 41 subjects have received 3BNC117 at doses ranging from 1 to 30 mg/kg (16 HIV-uninfected, 19 viremic HIV-1-infected and 6 ART-suppressed HIV-1-infected; protocol MCA-835), and there have been no significant adverse events related to 3BNC117 to date. Fourteen individuals (3 HIV-uninfected and 11 HIV-1-infected) have been administered one dose of 30 mg/kg. Nonclinical and clinical data on 3BNC117 are collected in the IB.

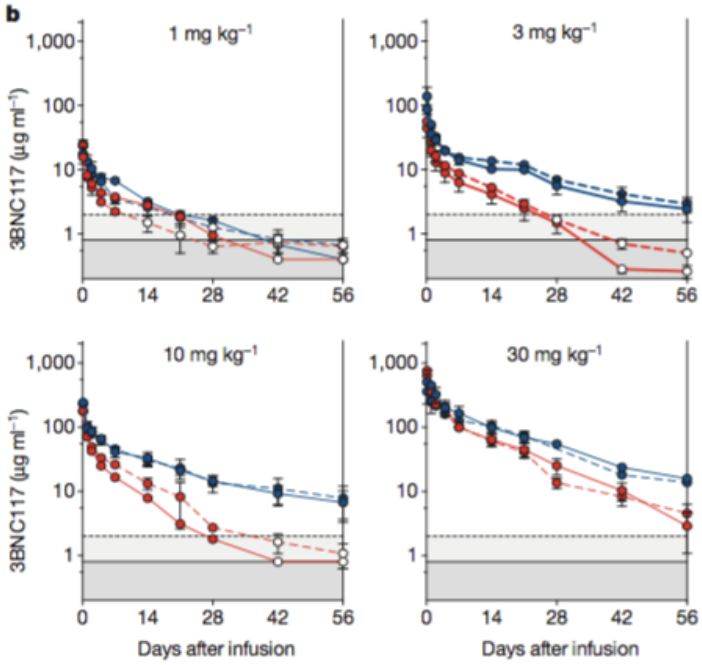
2.1.3 Pharmacokinetics (PK)

2.1.3.1 Romidepsin

The general plasma disposition profile of romidepsin after IV administration is characterized by a rapid initial distribution to virtually all organs, including the central nervous system (CNS) [74], followed by a slower terminal systemic elimination. Romidepsin exhibited dose-proportional and linear pharmacokinetics across doses ranging from 1.0 to 24.9 mg/m^2 when administered IV over 4 hours in patients with advanced cancers. In patients with T cell lymphomas who received 14 mg/m^2 of romidepsin IV over a 4-hour period on days 1, 8, and 15 of a 28-day cycle, geometric mean values of the maximum plasma concentration (Cmax) and the area under the plasma concentration versus time curve (AUC) were 377 ng/mL and 1549 ng*hr/mL, respectively. Romidepsin is highly protein bound in plasma (92 to 94%) over the concentration range of 50 to 1000 ng/mL with alpha-1-acid-glycoprotein (AAG or Orosomucoid) being the principal binding protein. In vitro, romidepsin accumulates into human hepatocytes via an unknown active uptake process. Romidepsin undergoes extensive metabolism in vitro primarily by CYP3A4 with minor contribution from CYP3A5, CYP1A1, CYP2B6, and CYP2C19. The clearance of its metabolites is through bile and is subsequently excreted in faeces. At therapeutic concentrations, romidepsin did not cause notable induction of CYP1A2, CYP2B6 and CYP3A4 in vitro. Therefore, pharmacokinetic drug-drug interactions are unlikely to occur due to CYP450 induction or inhibition by romidepsin when co-administered with CYP450 substrates. Following 4-hour IV administration of romidepsin at 14 mg/m^2 on days 1, 8, and 15 of a 28-day cycle in patients with T cell lymphomas, the terminal half-life ($T_{1/2}$) was approximately 3 hours. No accumulation of plasma concentration of romidepsin was observed after repeated dosing. The population pharmacokinetic analysis of romidepsin showed that age, gender, or race (white vs. black) did not appear to influence the pharmacokinetics [75].

2.1.3.2 3BNC117

3BNC117 has favorable pharmacokinetic properties with a $T_{1/2}$ of 17.6 days in HIV-uninfected and 9.6 days in viremic HIV-1-infected individuals (**Figure 3**) [36]. The elimination of 3BNC117 activity is thus more rapid in viremic HIV-1-infected patients. 3BNC117 has pharmacokinetic properties consistent with a typical human IgG1 in uninfected individuals and a somewhat faster decay rate in HIV-1-viraemic individuals. Similar antigen-dependent enhanced clearance has been reported with anti-cancer antibodies. Although there may be other explanations, it is conceivable that the increased rate of antibody elimination in the presence of HIV-1 is due to accelerated clearance of antigen–antibody complexes.



Dose	HIV-1-status	Subjects	Method (subjects analyzed)	Cmax (µg/ml)			$t_{1/2\ (days)}$ (1)		
				Mean	SD	Range	Mean	SD	Range
30 mg	Neg.	3	ELISA (3)	**495.9**	233.1	360.8 - 765.0	**15.0**	1.1	14.2 - 16.3
			TZM.bl (3)	**904.0**	281.7	1166.3 - 606.2	**16.4**	1.3	15.2 -17.9
30 mg	Pos.	6	ELISA (6)	**669.8**	199.7	410.2 - 976.4	**9.9**	3.3	5.8 - 13.7
			TZM.bl (3)	**840.1**	118.4	717.4 - 953.7	**8.9**	2.3	6.7 - 11.2
All	Neg.	12	ELISA (9)	-	-	-	**17.2**	5.5	10.7 - 29.0
			TZM.bl (9)	-	-	-	**17.6**	5.7	10.0 - 26.6
All	Pos.	15	ELISA (12)	-	-	-	**9.3**	2.6	5.7 - 13.7
			TZM.bl (9)	-	-	-	**9.6**	2.9	6.1 - 13.7

(1) Estimation of half-lives; SD, standard deviation.

Figure 3. 3BNC117 decay measured in TZM.bl assays (solid lines) and ELISA (dotted lines). Mean values and s.e.m. for uninfected individuals (3 per group) are shown in blue and for HIV-1-infected individuals (2-5 per group) in red. Table summaries 3BCN117 PK based on a 56-day period post infusion.

2.1.4 Interactions with other drugs:

2.1.4.1 Romidepsin

CYP3A4 inhibitors (strong)

Romidepsin is metabolized by CYP3A4. In a pharmacokinetic drug interaction trial the strong

CYP3A4 inhibitor ketoconazole increased romidepsin AUC0-∞ and Cmax by approximately 25% and 10%, respectively [76].

CYP3A4 inducers (strong)
In a pharmacokinetic drug interaction trial with rifampin (a strong CYP3A4 inducer), romidepsin exposure was surprisingly increased by approximately 80% and 60% for AUC and Cmax, respectively. Typically, co-administration of CYP3A4 inducers decreases concentrations of drugs metabolized by CYP3A4. The increase in exposure seen after administration with rifampin is likely due to rifampin' inhibition of an undetermined hepatic uptake process that is predominantly responsible for the disposition of romidepsin. Therefore, the use of potent CYP3A4 inducers should be avoided when possible.

Highly protein bound drugs
Prolongation of the prothrombin time (PT) and elevation of the international normalized ratio (INR) were observed in a patient receiving romidepsin concomitantly with warfarin. Although the interaction potential between romidepsin and warfarin or warfarin-derivatives has not been formally studied, physicians should carefully monitor PT and INR in patients concurrently administered romidepsin and warfarin or warfarin-derivatives. Use of warfarin or warfarin-derivatives will exclude participation in the study as detailed under 5.2 (exclusion criteria).

Drugs that inhibit drug transport systems
The efflux transporter P-glycoprotein (P-gp) is a transmembrane pore shown to transport a large variety of drugs. P-gp is expressed in a wide variety of tissues, including the small and large intestine, adrenal gland, liver, kidney, placenta and capillary endothelial cells of testis and brain [77]. Romidepsin is a substrate of P-gp and when co-administred with drugs that inhibit P-gp, increased concentration of romidepsin is likely, and caution should be exercised. Romidepsin is not an inhibitor of P-gp. Use of drugs that inhibit P-gp will exclude participation in the study as detailed under 5.2 (exclusion criteria).

Estrogen-containing contraceptives
In-vitro based assay determined that romidepsin compete with beta-estradiol for binding to estrogen receptors, which could reduce the effectiveness of estrogen-containing contraceptives. Alternative methods of non-estrogen containing contraceptions should be used during romidepsin treatment.

Additionally, consideration about avoiding the use of CYP3A4 inducing herbs in order to avoid therapeutic failure of the substrate should be made. Manufacturers specifically contraindicate some combinations. Package inserts should be reviewed. Monitor for decreased effects of the CYP substrate if a CYP inducer is initiated/dose increased, and increased effects if a CYP inducer is discontinued/dose decreased.

There are no known drug-drug interactions between romidepsin and human monoclonal antibodies, and we predict that the risk of such interaction between 3BNC117 and romidepsin is extremely low. Participants will be started antiviral drugs which do not inhibit CYP3A4 as described in protocol section 4.3 to avoid drug-drug interactions.

2.1.4.2 3BNC117

Small molecules are usually eliminated by non-catabolic pathways such as hepatic metabolism, renal excretion, and biliary excretion whereas mAbs are eliminated via catabolic processes. Since mAbs and small molecules do not share common or overlapping clearance mechanisms, mAbs are not predicted to affect directly the hepatic, renal, or biliary elimination of small molecules [78]. Thus, it is highly unlikely that 3BNC117 will lead to drug-drug interactions.

In addition, because the disposition of mAbs does not occur through non-catabolic pathways such as hepatic metabolism or transporters, they do not compete directly with chemically derived entities for these pathways. Thus, from a mechanistic perspective, the likelihood of direct drug-drug interaction (mAb as a victim) occurring during co-administration of other concomitant medications is unlikely to be high.

2.1.5 Safety Profile

2.1.5.1 Romidepsin

No specific fertility studies have been conducted with romidepsin. Based on non-clinical findings in repeat-dose toxicity studies, romidepsin has the potential to affect male and female fertility. In a 26-week study in rats, testicular atrophy or degeneration at repeated dosing at ≥0.33 mg/kg/week corresponding to 1.98 mg/m^2 or a steady state mean AUC0-∞ 10.3 ng*hr/mL. In dogs romidepsin caused hypospermia in the testes at doses ≥1.0 mg/kg corresponding to 20mg/m^2. In rats, at doses ≥0.1 mg/kg/week corresponding to 0.6 mg/m^2 or a mean steady state AUC0-∞ 4.9 ng*hr/mL, females exhibited atrophy in the ovary, uterus, vagina and mammary glands. Further, maturation arrest of ovarian follicles was observed in female rats at ≥0.3 mg/kg/week corresponding to 1.8 mg/m^2. Romidepsin has in non-clinical studies showed developmental toxicity. Therefore, use of contraceptive methods for both female and male study subjects is required during and 30 days after study treatment; this is specified in the exclusion criteria.

A theoretical risk of HDACi is that they will induce activation of other retroviruses, oncogenes and/or DNA viruses, including cytomegalovirus (CMV), hepatitis B virus and JC viruses. A possible association between romidepsin and reactivation of DNA viruses has been described in 3 case reports [79]. In this small case series, there was a temporal association with reactivation and administration of the HDACi but it is important to keep in mind that these patients were all patients with advanced cancer and immunosuppression. In the CLEAR study, including aviraemic adults with HIV-1 infection, the participants received oral panobinostat (20 mg) three times per week every other week for 8 weeks while maintaining ART. Here we repeatedly quantified concentrations of CMV DNA (in urine) and Epstein-Barr virus DNA (in blood) during panobinostat treatment, but recorded no evidence of unintended DNA virus reactivation [80].

In clinical settings, as of 31 December 2011, over 1300 patients have received romidepsin alone or in combination with other drugs. 891 of these patients have received at least one dose of romidepsin as monotherapy, the most common adverse events (AE) associated with romidepsin compromised the gastrointestinal system (nausea, vomiting, diarrhea/constipation), hematologic condition (thrombocytopenia, leucopenia (neutro- and lymphopenia) and anemia), asthenic conditions (asthenia, fatigue, malaise and lethargy) and finally electrolyte abnormalities (hypomagnesiamia, hypokalemia and hypocalcemia), hyperglycaemia and pyrexia. Overall, the most common grade 3/4 events reported were anaemia, thrombocytopenia, neutropenia, leukopenia and fatigue. These AE should be interpreted in the context of patients with severe haematological cancer disease. The detailed results of the pooled safety analysis are presented in the IB.

During our pilot study of 6 HIV-1 patients on long-term ART who received 3 infusions of 5 mg/m^2 of romidepsin over the course of 3 weeks, no severe adverse events (SAEs) or suspected unexpected serious adverse reactions (SUSAR) was observed. Forty-one AE were registered during follow-up of which 35 AEs were considered related to romidepsin. All drug-related AE were mild (grade 1, n=35) and resolved spontaneously within a few days. The most common romidepsin-related AE were abdominal symptoms (e.g. nausea [n=11], borborygmia [n=4], abdominal pain [n=2]) and fatigue (n=5). Modest changes in white blood cell counts (WBC) and T cell counts were observed during the study with the lowest levels generally observed after the second romidepsin infusion, but no further decline following the third infusion. Reassuringly, neutrophil counts below 1000 cells/mm^3, CD4+ cell counts below 350 cells/mm^3, or platelet counts below 100,000 cells/mm^3 were not observed. The patients included in this study will be monitored according to the Common Terminology Criteria for Adverse Events v.4.03 (CTCAE) as specified in table 6.2.

2.1.5.2 3BNC117

mAbs are a growing part of the therapeutic arsenal. While each mAb product has unique safety issues related to its mechanism of action, the major safety concern related to mAbs in general is an infusion/hypersensitivity reaction. These types of reactions are more common for mAbs that contain murine elements, than humanized or fully human mAbs.

Passive administration of antibodies is successfully used to prevent or treat several viral diseases. For example, CMV immunoglobulin is used for the prevention of transplant-associated infection. While human rabies immunoglobulin is used in conjunction with vaccine after suspected or proven exposure to rabies. Palivizumab, a humanized monoclonal antibody (IgG) directed against the fusion protein of respiratory syncytial virus (RSV), is the first monoclonal antibody approved for clinical use and it is indicated for the prevention of serious lower respiratory tract disease caused by RSV in children at high risk of RSV disease. Pavilizumab is generally safe and well-tolerated. Rare cases of severe hypersensitivity reactions (<1 per 100,000 recipients) have been described after an initial dose, as well as after re-exposure. Several other monoclonal antibodies are being developed for use in either prevention or treatment of other viral illnesses.

Passive administration of anti-HIV-1 antibodies has also been evaluated in humans. HIV Immune Globulin (HIVIG) was in clinical use in the 1990s before the advent of highly effective ART. HIVIG was also evaluated in HIV-infected pregnant females and their newborns in a phase III trial to assess whether HIVIG plus single dose nevirapine given to mothers and infants would provide additional benefit over single dose nevirapine alone for prevention of peripartum HIV transmission. Women received a single IV infusion of HIVIG 240 mL (approximately 200 mg/kg) at 36-38 weeks gestation. Infants born to these mothers received a single IV infusion of 24 mL (approximately 400 mg/kg) of HIVIG, preferably within 18 hours of birth. Infusion-related events occurred in both mothers and infants but all infusion-related events resolved with no complications. While there was no demonstrable difference in treatment efficacy, the study showed that there were no significant differences in mortality or SAE between the two arms of the trial [81].

Several mAbs that target HIV-1 gp-120 have been evaluated in clinical studies. F105 is an IgG1 (kappa) human mAb that targets a discontinuous epitope that overlaps the CD4-binding site of gp-120. It was evaluated in phase I studies in HIV-1-infected individuals at 100 or 500 mg/m^2, single IV dose, and was found to be safe and well-tolerated. Its $T_{1/2}$ was approximately 13 days [82]. 2F5 and 4E10 are IgG1 (kappa) mAbs that target the membrane-proximal ectodomain of gp-41, while 2G12 binds to a carbohydrate moiety on the silent face of gp-41. These neutralizing antibodies were evaluated in combination in HIV-1-infected individuals. The first two studies included ART-naïve individuals with CD4+ cell counts >350 cells/mm^3, and plasma viral levels $\leq 10^4$ in one study (n=7) or $\leq 10^5$ in another study (n=8). The antibodies were administered IV at 0.5 to 1 mg doses; 4 to 8 weekly infusions were given. The antibodies were safe and well-tolerated and no clinical or laboratory abnormalities were observed throughout the studies. The median elimination $T_{1/2}$ of these antibodies were 6.6, 3.2 and 14.1 days for 4E10, 2F5 and 2G12, respectively. A low-level antibody response against 2G12 was found in two patients. Anti-4E10 and anti-2F5 IgM and IgG immune responses were not detected, even after repeated infusions of high doses of the mAbs. Of note, the examination of plasma samples showed no detectable levels of immune complexes containing 2G12 and the evaluation of urine samples did not reveal elevated protein levels to suggest development of immune-complex induced renal disease [83,84].

Two other studies included HIV-1-infected subjects on ART and plasma viral levels <50 copies/mL (n=14 and n=10). The antibodies were administered IV at doses ranging from 1 to 2 mg for each antibody; from 13 to 16 infusions were given weekly. ART was interrupted following 1 or 4 infusions. Antibody infusions were well-tolerated in most subjects; mild and transient side effects were reported only occasionally. No SAE were recorded. AE included body aches, fatigue, flushed sensation, joint soreness and redness at infusion site. Grade I post-partial thromboplastin time (PTT) prolongations were noted in one of the studies. Viral rebound was observed in eight of ten subjects (28 to 73 days post-ART interruption), while the two remaining subjects were aviremic over the course of the study [68]. In seven of eight subjects with viral rebound, clear resistance to 2G12 emerged, whereas reductions in the susceptibilities of plasma-derived recombinant viruses to 2F5 and 4E10 were neither sustained nor consistently measured. Only two of eight individuals showed evidence of a delay in viral rebound during the passive immunization; whereas rebound upon cessation of ART

was observed later in antibody-treated acutely infected individuals than in a control group of twelve individuals with acute infection (median of 8 weeks versus 3.75 weeks) [67]. Though safe, the use of mAbs generally delayed, but did not prevent, viral rebound. The emergence of resistance to 2G12, however, demonstrated that the antibody exerted selective pressure on the circulating viral strains. It is important to note that the antibodies used in these studies have far lower potency and breadth than the more recently isolated neutralizing antibodies, such as 3BNC117 [67,68].

In the previously mentioned proof-of-concept human trial, uninfected and HIV-1-infected individuals were given a single dose of 3BNC117 IV and monitored for 56 days [36]. A total of 37 subjects have received 3BNC117 at doses ranging from 1 to 30 mg/kg, and there have been no significant AE related to 3BNC117 to date.

2.1.6 Rationale for dose selection

2.1.6.1 Romidepsin

In this study 5 mg/m^2 romidepsin will be administered IV over a 2-hour period on days 10, 17, and 24 in study-arm 2 and 4. This dosing corresponds to approximately 36% of the recommended dosing in cancer treatment (14 mg/m^2) currently used in the treatment of PTCL, CTCL and clinical trials. The rationale for the reduced dose is based on an *in vitro* study, where HIV-1 were induced in memory CD4+ T cells at 5 nM concentration in 12/13 HIV-1 subjects on ART. Five (5) nM romidepsin is equivalent to a maximal plasma concentration (Cmax) at 6% of dose for CTCL patients (14 mg/m^2) or 0.9 mg/m^2 [61]. Additional, an *in vitro* study showed, that romidepsin increased histone acetylation in 78.3%-80.8% of the isolated peripheral blood mononuclear cells (PBMCs) from Rhesus Macaques at 5 nM concentration measured by flow cytometry at 24-hours after 4 hour pulse treatment with romidepsin [61]. The plan to give only one cycle of three infusions of romidepsin is based on data from our pilot study on safety and efficacy.

2.1.6.2 3BNC117

At the highest dosage level tested, 30 mg/kg, eight HIV-1-infected individuals treated showed rapid decrease in their amount of virus measured in their blood that varied between individuals from 0.8 to 2.5 on log10 scale, with most reaching their lowest viral load one week after treatment [36]. The effect of 3BNC117 on viral loads was less pronounced in the lower dosing groups.

2.1.7 ECG monitoring

The cardiovascular effect has been extensively monitored in all clinical trials of romidepsin formulated as IV use. Based on initial phase I and II studies, it was confirmed, that romidepsin was not associated with functional cardiovascular changes. Additionally, the observed effects were reversible mild ST-T wave morphology and asymptomatic sinus tachycardia not associated with elevation in cardiac troponin-I or creatinine phosphokinase. As with other HDACi a reversible prolongation of the QTc, corrected using the Fridericia formula, was observed, but not to a clinically significant extent. The clinical significance of the above-mentioned changes is unknown. As a consequence, patients with congenital long QT

syndrome, a history of significant heart disease, patients taking anti-arrhythmic medicines or medicines that are known to lead to significant QT prolongation or other cardiac toxicities will exclude participation in the study as detailed under 5.2 (exclusion criteria).

3 STUDY OBJECTIVES AND ENDPOINTS

3.1 Objectives

3.1.1 Primary Objective

- To evaluate the effect of early viral reactivation by a LRA (romidepsin) and/or administration of potent bNAbs (3BNC117) on the size of the latent HIV-1 reservoir in treatment naïve HIV-1 patients initiating ART

3.1.2 Secondary Objective

- To evaluate the safety and tolerability of the Investigational Medicinal Products (IMP)s
- To evaluate the effect of the IMPs on the amount of integrated HIV-1 DNA in CD4+ T cells
- To evaluate the effect of the IMPs on the functional HIV-1 reservoir in CD4+ T cells
- To compare viral load (plasma HIV-1 RNA) kinetics between the different study arms
- To compare the levels of immune reconstitution (CD4+ T cell recovery) between the different study arms
- To evaluate the effect of early viral reactivation by IMPs on the immunological control of HIV-infection following optional ART interruption

3.1.3 Exploratory Objective

- To compare HIV-specific immunity, T cell phenotype, immune activation, and cytokine production between the investigational treatment regimens

3.2 Endpoints

3.2.1 Primary Endpoint

- Change from baseline (day 0) to day 365 in copies of total HIV-1 DNA per 10^6 CD4+ T cells as measured by digital droplet PCR
- Time from starting ART to first plasma HIV RNA<20 copies/mL

3.2.2 Secondary Endpoints

- Safety evaluation, as measured by AEs, Adverse Reactions (ARs), SAEs, Serious ARs (SARs) and (SUSAR)
- Change from baseline (day 0) to day 365 in integrated HIV-1 DNA in CD4+ T cells (copies per million cells) as measured by alu-PCR

- The frequency of CD4+ T cells latently infected with replication competent HIV at day 365 as measured by infectious units per million (IUPM) CD4+ T cells in a viral outgrowth assay
- Plasma HIV-1 RNA kinetics following ART initiation as measured by the Cobas Taqman assay
- Numbers and proportions of HIV-specific CD4+ and CD8+ T cell
- During the optional monitored analytic treatment interruption (ATI) study
    - Time to meet criteria to re-initiate ART

3.2.3 Endpoints in exploratory analyses

- Plasma cytokine and immune activation biomarker levels
- Genetic, virological, and immunological predictors of treatment response
- Plasma 3BNC117 concentration
- Phylogenetic “foot-prints” of the investigational drugs as evidenced by the the genetic make-up of viral reservoir, inducible virus and rebound virus during ATI.

4 STUDY DESIGN AND GENERAL PROCEDURES

4.1 Design

An investigator-initiated open-label randomized controlled international multicentre interventional phase IIa trial designed to evaluate the safety and efficacy of romidepsin and 3BNC117 in HIV-1-infected patients initiating ART as intervention to reduce the HIV-1 reservoir. Participants will be randomized 1:1:1:1 in a non-blinded fashion to receive (**Figure 4**):

A. ART alone (day 0 – 365)

B. ART (day 0 – 365) + romidepsin 5 mg/m^2 (day 10±3, 17±3 and 24±3)

C. ART (day 0 – 365) + 3BNC117 30 mg/kg (day 7±3 and 21±3)

D. ART (day 0 – 365) + romidepsin 5 mg/m^2 (day 10±3, 17±3 and 24±3) + 3BNC117 30 mg/kg (day 7±3 and 21±3)

Targeted enrolment is 60 study subjects – 15 in each arm. Initiation of ART regimen will be done in collaboration with the subject's HIV primary care physician, but must be an integrase strand transfer inhibitor (INSTI)-based ART-regimen (for specification see section 4.3).

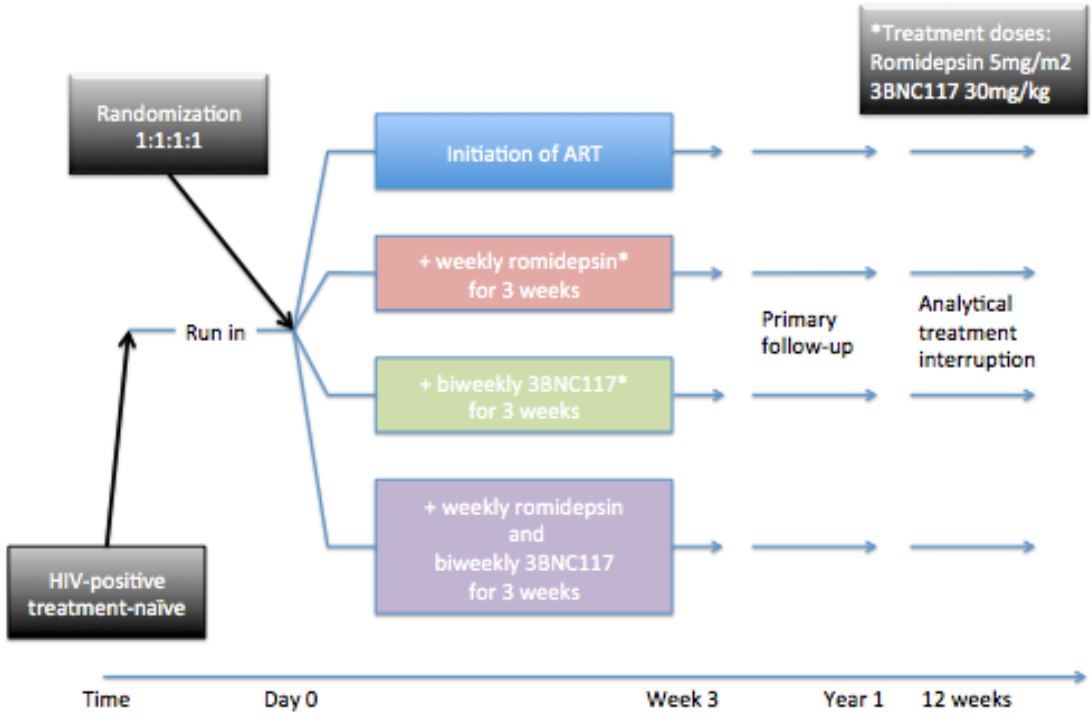


Figure 4. eCLEAR flowchart.

4.2 Health Facilities

The trial will be conducted as an international multicentre study involving institutions in Denmark (including but not limited to the Department of Infectious Diseases at Aarhus University Hospital (AUH), and the Department of Infectious Diseases at Hvidovre Hospital and Copenhagen, the Department of Internal Medicine at Regional Hospital Herning, Aalborg

and Odense University Hospital); Sweden (including but not limited to the Department of Infectious Diseases at Karolinska Hospital), Germany (including but not limited to the First Department of Internal Medicine, University Hospital of Cologne), United Kingdom (including but not limited to Department of Infectious Diseases, Guy's Hospital and Mary's Hospital).

The involved institutions in Denmark besides AUH may choose to screen and enroll individuals on-site, refer participants to AUH for investigational treatment, and continue follow-up visits at their site.

Study site	**Site number**
The Department of Infectious Diseases at AUH	101
The Department of Infectious Diseases at Hvidovre Hospital	201
The Department of Infectious Diseases at Odense University Hospital	301
The Department of Infectious Diseases at Karolinska Hospital	401
The First Department of Internal Medicine, University Hospital of Cologne	501
The Department of Internal Medicine, Regional Hospital Herning	601
The Department of Infectious Diseases at Aalborg University Hospital	701
The Department of Infectious Diseases at Rigshospitalet, Copenhagen	801
The Department of Infectious Diseases at Guy's Hospital, London	901
The Department of Infectious Diseases at St Mary's Hospital, London	1001

4.3 Initial ART regimen

Upon meeting eligibility criteria, study individuals will be started on an integrase strand transfer inhibitor (INSTI) regimen (elvitegravir, raltegravir or dolutegravir). The initial ART regimen prescribed must be chosen from the current treatment guidelines. Thus, the study ART regimen should consist of two components: a) a dual combination of two nucleoside or nucleotide analogue reverse transcriptase inhibitors (NRTIs: tenofovir/emtricitabine, tenofovir alafenamide/emtricitabine, zidovudine/lamivudine, or if genotype HLAB5701 negative; abacavir/lamivudine); b) INSTI. Study investigators will discuss initial ART regimen with the primary care physician. In cases of drug intolerance, toxicity, or case of virological failure any available ART is permitted. All ART used in the study must have approval or tentative approval from the European Medicines Agency (EMA). The study arms should have comparable initial ART regimens in order to compare decays in initial viral load kinetics. INSTI-based regimens lowers the initial plasma HIV-1 RNA significantly after initiation of ART compared to regimens including non-nucleoside reverse transcriptase inhibitor (NNRTI) [85]. However, if a department/caretaker cannot prescribe INSTI (e.g. due to financial restrictions, known resistance mutations, or other reasons) INSTI can be substituted with another antiretroviral as long as the chosen drug is not a known moderate-strong CYP3A4 inhibitor.

The initial ART regimen may be changed during the study if deemed necessary by the investigator or threating physician for reasons such as unacceptable side effects or lack of virological suppression.

4.4 Randomization

Randomization of subjects to one of the four regimens will proceed through the use of a computerized system that is accessible 24 hours a day, 365 days a year. To ensure correct randomization, the aim is for include equal number of subjects to each of the four regimens. Randomization will therefore occur in randomly permuted blocks of 4 or 8 by country in an unknown fashion. To maintain unpredictable randomization there will be a total of 80 randomization numbers. No stratification will occur. The site personnel will then be provided with a subject randomization assignment. The randomization assignment and the date of randomization will be recorded on the CRF. Once subject numbers and randomizations have been assigned, they cannot be reassigned. The computerized system will provide confirmation of the randomization assignment to the site personnel. Site personnel will retain the confirmation reports. On the subject identification list the site personnel will register subject number, randomization assignment, randomization date, gender, last and first name, medical records ID (if applicable), and date of birth.

4.5 Discontinuation of Study

Study subjects can withdraw from the study or the IMP(s) in accordance with the conditions and procedures described in 5.3 and 5.4.
Premature discontinuation of the study may occur because of a regulatory decision, change in opinion of The Ethics Committee or drug safety problems. Besides, the sponsor has the right to temporarily suspension and/or discontinuation of the study due to, but not limited to, the safety of study subjects, ethical reasons, or serious problems of recruitment.

4.6 Duration of Study

We plan to enrol patients between the third quarter of 2016 and 2018. The last planned visit date will be in the third quarter of 2019. Expected final report in the last quarter 2019.

4.7 Source Data

The following documents are defined as source data:

- Informed consent and power of attorney
- Prints of files
    - Note of each visit in patient file
    - Biochemical, immunological and virological measurements
- SAE reports

For the following information, the CRF will be the source data

- Demographics: Name, date of birth age, sex (male, female), and race/ethnicity, study ID number
- HIV status: Presumed date of infection, acquisition of infection
- ART regime: Start date, type of regimens

- Medical history: Brief medical history, including list of medical conditions with year, site and sequelae
- Concomitant medicine: List of current medical treatment
- Visit date
- Anthropometric data: Weight, height, blood pressure, pulse and temperature
- Physical exam: Signs and symptoms noted by medical examination
- Pregnancy status:
- Contraception/pregnancy counselling: type of contraception and start date
- ECG
- Info on infusion: start and stop time
- AE, AR, SAE, SAR and SUSAR reported
- Phone contact
- Extra visit

Information on demographics, HIV status, ART regime, medical history and concomitant medicine will be passed on from the patient journal.

5 SELECTION OF STUDY SUBJECTS

5.1 Inclusion Criteria

- Documented HIV-1 infection
- Age >18 years
- CD4+ T cell count >200/µL on last visit prior to study entry
- ART naïve
- Able to give informed consent

5.2 Exclusion Criteria

- Any significant acute medical illness (not including primary HIV infection) in the past 8 weeks
- Any evidence of an active AIDS-defining opportunistic infection
- Active alcohol or substance use that, in the Investigator's opinion, will prevent adequate compliance with study therapy
- The following laboratory values at screening, but the values can be repeated within the screening period, but test results must be available before baseline (day 0) and checked for eligibility:
    - Hepatic transaminases (AST or ALT) ≥3 x upper limit of normal (ULN)
    - Serum total bilirubin ≥3 ULN
    - Estimated glomerular filtration rate (eGFR) ≤60 mL/min (based on serum creatinine or other appropriate validated markers)
    - Platelet count ≤100 $x10^9$/L
    - Absolute neutrophil count ≤$1x10^9$/L
    - Serum potassium, magnesium, phosphorus outside ≥1.5 ULN/LLN
    - Total calcium (corrected for serum albumin) or ionized calcium ≥1.5 ULN/LLN
- Hepatitis B or C infection as indicated by the presence of hepatitis B surface antigen

(HBsAg) or hepatitis C virus RNA (HCV-RNA) in blood

- ECG at screening that shows QTc >450 ms when calculated using the Fridericia formula from either lead V3 or V4 [86]
- Use of:
    - Warfarin or warfarin-derivatives
    - HDACi
    - An agent definitely or possibly associated with effects on QT intervals within 2 weeks of screening
    - Drugs that induce or inhibit CYP3A4 or P-gp (see appendix A-D)
- History of:
    - Clinically significant cardiac disease, symptomatic or asymptomatic arrhythmias, syncopal episodes, or additional risk factors for Torsades de pointes (e.g. heart failure)
    - Malignancy or transplantation, including skin cancers or Kaposi sarcoma
    - Diabetes mellitus
- Receipt of strong immunosuppressive or systemic chemotherapeutic agents within 28 days prior to study entry
- Known resistance to >2 classes of ART
- Known hypersensitivity to the components of romidepsin, 3BNC117 or their analogues
- Women who are pregnant or breastfeeding, or with a positive pregnancy test during screening or Women of Child Bearing Potential (WOCBP) who are unwilling or unable to use an acceptable method of non-estrogen containing contraceptions (according to the Danish Medicines Agency guidelines) to avoid pregnancy for the 3-week study period and 4 weeks after study treatment or until undetectable plasma HIV-1 RNA using standard assays
- Males or females who are unwilling or unable to use barrier contraception during sexual intercourse for the 3-week study period, and 4 weeks after study treatment or until undetectable plasma HIV-1 RNA using standard assays

In vitro neutralization activity of 3BNC117 on autologous HIV isolates from subjects that meet eligibility criteria will be evaluated on samples collected prior to 3BNC117 infusion but will not be used as an exclusion criterion.

5.3 Subject Withdrawal from Study

Subject withdrawal from study is defined as any subject who does not complete the final follow-up visit at day 365 as defined in this protocol.
Reasons why a study subjects may be withdrawn from the study include, but are not limited to:

- Subject request (withdrawal of consent)
- Protocol violation
- AE or reactions
- Any condition, interaction, or contraindication where continued participation in the study will result in an unacceptable risk for the subject, as assessed by the Investigators or advisers

- Discontinuation of the study by the Sponsor
- Lost to follow-up

Investigator will contact subjects who fail to return for planned visits and if possible schedule a new visit. Information related to study withdrawal is documented in the CRF (End of Study) including the reason for withdrawal, date of withdrawal, and whether the subject or Investigator made this decision. Subjects withdrawn from the study will contribute with data for the statistical analyses until the date of withdrawal.
Subjects withdrawn from the study will resume routine treatment and control according to standard treatment guidelines.
Subjects withdrawn from the study after the point of randomization will not be replaced.

5.4 Subject Withdrawal from the Investigational Medicinal Product(s) (IMPs)

Withdrawal from the IMP(s) is defined as subject who discontinues study treatment but agrees to continued follow-up until completion of the study at day 365. Any subject who withdraws from the IMP(s) after having received at least one dose will be asked to return for follow-up visits 1 week after withdrawal from the IMP(s) (safety monitoring) and final follow-up visits at week 4 after withdrawal from the IMP(s). If withdrawal from the IMP(s) is due to AE, this will be followed up as detailed under 9.3.
Reasons why a study subjects may be withdrawn from the IMP(s) include, but are not limited to:

- Subject request (withdrawal of consent)
- AE or reactions
- Any condition, interaction, or contraindication where continued participation in the study will result in an unacceptable risk for the subject, as assessed by the Investigators

Information related to withdrawal from the IMP(s) is documented in the CRF (End of Study) including the reason for withdrawal, date of withdrawal, and whether the subject or Investigator made this decision. Subjects withdrawn from the IMP(s) will contribute with data for the statistical analyses until the date of withdrawal. Subjects withdrawn from the IMP(s) will resume routine treatment and control according to standard treatment guidelines.

6 STUDY TREATMENT

6.1 The Investigational Medicinal Products (IMPs)

The first IMP is defined as romidepsin, which is a highly potent class I/II HDACi belonging to the hydroxamic acid class of compounds. In this study, 5mg/m^2 romidepsin will be administered IV over a 2-hour period on days 10, 17, and 24 after initiating ART. Antiemetic prophylactic ondansetron 8 mg will be given orally approximately 30 minutes prior to romidepsin infusion. The second IMP is defined as 3BNC117, which is a bNAbs that has shown significant inhibitory potential across a broad panel of HIV-1 strains in humans. 30 mg/kg 3BNC117 will be administered IV over a 1-hour period on day 7 and 21 after initiating ART.

The study will be conducted among ART naïve HIV-1-infected patients. Subjects will continue background ART while receiving romidepsin and 3BNC117.

6.2 Dosing Modifications

6.2.1 3BNC117

3BNC117 dose modification is not allowed.

6.2.2 Romidepsin

If IMP-related toxicities are observed, treatment can be resumed only if these toxicities have resolved to the baseline level or to the CTCAE v.4.03 ≤grade 1, or as otherwise specified in the protocol.
Romidepsin may be dose-modified for a patient as per the dosing table 6.1 below.

Table 6.1. Dose reductions and escalation for re-initiation of romidepsin.

Current dosing level	Dose reduction	Dose escalation
5mg/m^2	2.5mg/m^2	No dose escalation allowed
2.5mg/m^2	No further reduction, discontinue therapy permanently	5mg/m^2

General guidelines for romidepsin dose modifications due to AE related to the IMP are provided in Table 6.2 for specific AE and in the text below for more general considerations.

Table 6.2. Criteria for romidepsin dosing delays, dose reductions and re-initiation of treatment due to IMP related toxicity (excl. QT prolongation; see 6.2.2.4)

Worst toxicity, CTCAE (v.4.03) Grade unless otherwise specified (value)		Dose Modification Guidelines
Haematological toxicities		
Thrombocytopenia (PLT)	Grade 3 (PLT <50.0 – 25.0x10^9/L), uncomplicated	No change in dosing
	Grade 4 (PLT <25.0x10^9/L) or grade 3 (PLT <50.0 – 25.0x10^9/L) with bleeding	Temporarily discontinue dosing until resolved to ≤Grade 2 or baseline, then restart at reduced dose level as per Table 6.1.
Absolut neutrophil count (ANC)	Grade 3 (<1.0-0.5 x 10^9/L), uncomplicated	No change in dosing
	Grade 3 febrile neutropenia (ANC <1.0 x 10^9/L, fever ≥38.3°C), uncomplicated	Temporarily discontinue dosing until fever resolved and ANC ≤Grade 2, then restart at reduced dose level as per Table 6.1.
	Grade 4 (ANC <0.5 x 10^9/L)	Temporarily discontinue dosing until resolved to ≤Grade 2 or baseline, then restart at reduced dose level as per Table 6.1.
Anaemia	Grade 2 (hgb <10.0 - 8.0 g/dL)	No change in dosing - consider supportive measures
	Grade 3 (hgb 8.0-6.5 g/dL) or Grade 4 (hgb <6.5 g/dL)	Temporarily discontinue dosing and use supportive measures until resolved to ≤Grade 2 or baseline, then restart at reduced dose level as per Table 6.1.
Gastrointestinal/constitutional		
Diarrhea	Grade 2 (4-6 stools/day over baseline) persisting despite the use of optimal anti-diarrheal medication	Temporarily discontinue dosing until resolved to ≤Grade 1 or baseline, then restart at unchanged dose level. Correct electrolytes prior to restart.
	Grade 3 (≥7 stools/day over baseline persisting) despite the use of optimal anti-diarrheal medication	Temporarily discontinue dosing until resolved to ≤Grade 1 or baseline, then restart at reduced dose level as per Table 6.1. Correct electrolytes prior to restart.
	Grade 4 (life threatening consequences) despite the use of optimal anti-diarrheal medication	Discontinue dosing permanently
Vomiting/nausea	Grade 1-2, not requiring treatment or controlled using standard anti-emetics	Maintain dose-level
	Grade 3-4, cannot be controlled by the use of standard anti-emetics	Temporarily discontinue dosing until resolved to ≤Grade 1 or baseline, then restart at reduced dose level as per Table 6.1. Correct electrolytes prior to restart.
Fatigue	Grade 3 (Fatigue not relieved by rest, limiting self-care ADL)	Temporarily discontinue dosing until resolved to ≤Grade 2 or baseline, then restart at an unchanged dose level if resolved within 7 days after suspending dosing; restart at reduced dose level as per Table 6.1 if resolved after more than 7 days after suspending dosing.
Hepatic		
Total bilirubin	Grade 3 (>3.0 - 10.0 x ULN) or Grade 4 (>10.0 x ULN)	Temporarily discontinue dosing until resolved to ≤Grade 2 or baseline, then restart at reduced dose level as per Table 6.1.
AST/ALAT	Grade 3 (>5.0 – 20.0 x ULN)	Temporarily discontinue dosing until resolved to ≤Grade 1 or baseline, then restart at reduced dose level as per Table 6.1.

ULN=upper limit of normal; ADL=activities of daily living; AST=aspartate aminotransferase; ALT=alanine aminotransferase.

6.2.2.1 Grade 2 Non-hematologic Toxicity

Patients experiencing CTCAE grade 2 non-hematologic AE not listed in Table 6.2, which the patient believes is/are tolerable and in the Investigator's judgment is/are acceptable, may continue treatment at the current dose and schedule. More frequent patient monitoring may be required, and patients must be informed to contact the Investigator immediately if there is any worsening of symptoms. If a patient experiences new (or treatment emergent) grade 2 non-hematologic AE considered at least possibly related to romidepsin, and which the patient finds intolerable or in the Investigator's judgment is/are not acceptable, treatment must be interrupted until the AE resolves to ≤CTCAE grade 1. Romidepsin treatment may then be restarted at the same dose and schedule. If the same intolerable grade 2 AE occurs again, romidepsin treatment must again be temporarily discontinued until the toxicity resolves to ≤CTCAE grade 1 and be restarted at one dose level lower. At the discretion of the Investigator, patients with ≥CTCAE grade 2 AE of major organs (e.g., heart, lungs, CNS) may be discontinued from further study therapy without being retreated with a dose reduction.

6.2.2.2 Grade 3 or 4 Non-hematologic Toxicity

Patients experiencing new (or treatment emergent) CTCAE grade 3 or 4 non-hematologic AE not listed in Table 6.2, must have their treatment temporarily discontinued until the AE resolves to ≤CTCAE grade 1 or baseline unless otherwise specified in Table 6.2. If the AE was considered related to romidepsin, then therapy should be restarted at one dose level lower. If the AE was considered not related to romidepsin, then therapy may be restarted (when the AE resolves to ≤ grade 1 or baseline) at the current dose.

6.2.2.3 Dose Re-escalation of Romidepsin

Patients receiving a reduced dose level of romidepsin due to toxicity may be considered for dose re-escalation if:

- Either the study treatment-related AE has reverted in severity to grade ≤1 or baseline level, and at least 1 scheduled doses at the reduced level have been administered and tolerated

or

- The AE due to which the dose was omitted/reduced is determined to be not related to romidepsin

Should this guidance be met then the patient may be dose escalated as per Table 6.1. Dose escalation should be reviewed on a case-by-case basis and should be discussed and agreed upon between Sponsor and Investigators. Prior to consideration for dose re-escalation, the clinical condition of the patient (based on performance status and laboratory data) must be determined to be such that the re-escalated dose will be tolerated.

6.2.2.4 Dose Modifications of Romidepsin for Prolonged QTcF Interval

All cardiac events should be treated as per the local standard of care and referred to a cardiologist if clinically indicated. The Fridericia formula for QT-interval correction (QTcF) will be used [86]. Any final decisions concerning dose modifications or permanently discontinuing the patient from the IMP due to QTc prolongation will be taken in discussion between the

Investigator and Sponsor. If a patient cannot be dosed due to prolonged QTc for more than 7 days since last dose, patient must be discontinued from study treatment. Dose modifications of romidepsin will be made according to Table 6.3

Table 6.3. Criteria for romidepsin dosing delays, dose reductions and re-initiation of treatment due IMP related QTc prolongation

Abnormality noted. CTCAE (v.4.03) Grade unless otherwise specified (value)	**Dose Modification Guideline**
Dose modifications are based on local readings of the average QTc of triplicate ECGs	
Grade 1 (QTc 450 - 480 ms)	Check and correct serum potassium, magnesium, phosphorus, and calcium if indicated Repeat 3 pre-dose ECG with >2 days delay If the repeat 3 pre-dose ECGs: Do not meet criteria for dosing again, discontinue subject from the study Do meet criteria for dosing, administer IMP
Grade 2 (QTc 480 - 500 ms)	Check and correct serum potassium, magnesium, phosphorus, and calcium if indicated Delay dose at least 3 days and repeat 3 pre-dose ECGs If the repeat 3 pre-dose ECGs: Do not meet criteria for dosing again, discontinue subject from the study Do meet criteria for dosing; administer IMP with dose reduction according to Table 6.1. Perform 3 repeat ECGs pre-dose and 3-hours post-dose on the next scheduled dosing day
Grade 3 (QTc ≥500 ms on at least two separate ECGs) OR Grade 4 (QTc >501 ms or >60 ms increase from baseline average and Torsade de pointes or polymorphic ventricular tachycardia or signs/symptoms of serious arrhythmia)	Check and correct serum potassium, magnesium, phosphorus, and calcium if indicated Discontinue patient from study therapy

6.3 Consent Procedure

Prior to the initiation of any study related procedures, the potential subjects will be given a copy of the most recent subject information sheet and informed consent to read. Additionally, the PI (physician) or study physician who has been designated to consent will discuss the specifics of the study including but not limited to the purpose of the research, procedures, time commitment, required tasks, IMP(s), alternative treatments, benefits, risks, confidentiality etc. in a comprehensible (non-scientific) manner, using language readily understandable by the subject. Subjects will be told that participation is voluntary and that, if they do not consent, they will not be penalized. The person consenting will assure the voluntariness of the subject. Deliberation time is minimum 24 hours.

A private, confidential setting will be provided for the potential subject to read and discuss the informed consent free from coercion, undue influence or constraints of time. All subjects

will be given a chance to ask questions and express concerns. They will be given the option to take the consent home and discuss it with family, friends, and/or health care providers. After a subject and the person conducting the consenting signs and dates the consent, the subject will be given a copy of the signed informed consent form.

By given informed consent the subject is informed, that information from the source data (see 4.7 Source Data) can be passed on to the Sponsor and PI.

6.4 Drug Supplies, Packaging and Labelling

Packaging and labelling of Romidepsin (Istodax) will be performed by Fisher Clinical Services, UK. Fisher Clinical Services is responsible for release to study sites in accordance with national regulatory requirements. The study site will request the appropriate quantity of romidepsin from the hospital pharmacy before intended use. Romidepsin will then be delivered in piggy-backs diluted in sterile saline, ready for use - with the required labelling containing project name, and manufacturing date- and time.

Packaging and labelling of 3BNC117 will be performed by Output Pharmacy Services GmbH in Germany. Output Pharmacy Services GmbH is responsible for release to study sites in accordance with national regulatory requirements. The 3BNC117 dosing back will be prepared on study site (see 6.6 Drug Preparation and Administration). Labelling will contain project name, and manufacturing date- and time.

The study is open-label so allocation will be known.

For further details on physical, chemical and pharmaceutical properties we refer to the IBs.

6.4.1 Romidepsin (Istodax ®, Celgene)

Romidepsin is manufactured by Celgene Corporation. This HDACi is a bicycle depsipeptide. At room temperature, romidepsin is a white powder and is described chemically as (1S,4S,7Z,10S,16E,21R)-7-ethylidene-4,21-bis(1-methylethyl)-2-oxa-12,13-dithia-5,8,20,23-tetraazabicyclo [8.7.6] tricos-16-ene-3,6,9,19,22-pentone. The empirical formula is $C_{24}H_{36}N_4O_6S_2$. Romidepsin is supplied as a kit containing two vials. Romidepsin for injection is a sterile lyophilized white powder and is supplied in a single-use vial containing 10 mg romidepsin and 20 mg povidone, USP. Diluent for romidepsin is a sterile clear solution and is supplied in a single-use vial containing a 2-mL deliverable volume. Diluent for romidepsin contains 80% (v/v) propylene glycol, USP and 20% (v/v) dehydrated alcohol, USP.

6.4.2 3BNC117

3BNC117 is manufactured by Celldex Therapeutics, Inc. 3BNC117 is manufactured in accordance with Good Manufacturing Practice (GMP). 3BNC117 is a recombinant fully human mAb of the IgG1κ isotype that specifically binds HIV envelope pg120. 3BNC117 is a clear liquid, provided in single-vials containing 10 mL of product at a 20 mg/mL concentration.

6.5 Drug Storage and Accountability

The hospital pharmacies at AUH, Oxford, Cologne and Stockholm are responsible for secure and correct storage of romidepsin until arrival at study site after which the Sponsor and Investigators are responsible. The Sponsor and Investigators are responsible for secure and correct storage of 3BNC117 at the study sites. The IMPs will be stored in appropriate conditions in a secure location with controlled access. The storage compartment shall be monitored regularly and the temperature shall be documented. Lists of received, used and remaining quantities of the IMPs will be kept. Any discrepancies must be solved.

Until dispensed to subject the IMPs should be stored as follows:

- Romidepsin: Store between 20-25°C, excursions permitted between 15-30°C
- 3BNC117: Store between 2-8**°**C

6.6 Drug Preparation and Administration

Infusions will be administered by trained study personnel with experience with IV medication and treatment of acute infusion-related reactions.

6.6.1 Romidepsin

Romidepsin will be handled in a manner consistent with recommended safe procedures for handling cytotoxic drugs.
Before IV infusion romidepsin will be reconstituted with the supplied diluent and further diluted with normal saline (NaCl 0.9%) Injection, USP. Each 10 mg single-use vial of romidepsin will be reconstituted with 2 mL of supplied diluent. With a suitable syringe, 2 mL from the supplied diluent vial are aseptically withdrawn, and slowly injected into the romidepsin for injection vial. The contents of the vial are swirled until there are no visible particles in the resulting solution. The reconstituted solution will contain romidepsin 5 mg/mL. The reconstituted solution is chemically stable for at least 8 hours at room temperature. The appropriate dose of romidepsin will be calculated (5 mg/m2) at the hospital pharmacies according to subject's surface area. The appropriate amount of romidepsin is extracted from the vial to deliver the desired dose, using proper aseptic technique. Before IV infusion, romidepsin is further diluted in 500 mL normal saline Injection, USP. The romidepsin infusion solution is chemically stable for at least 24 hours at room temperature Antiemetic prophylactic ondansetron 8 mg will be given orally approximately 30 minutes prior to infusion. Romidepsin infusion will be administered over 2 hours. Study individuals will be monitored for 1-hour post infusion at the study site.

6.6.2 3BNC117

3BNC117 will be provided in single-use vials containing 10 mL of 3BNC117 at a 20 mg/mL concentration. The appropriate dose will be calculated (30 mg/kg) at the hospital pharmacies according to subject's weight. Before IV infusion, 3BNC117 is further diluted to a volume of 250 mL normal saline Injection, USP. 3BNC117 infusion will be administered over 1 hour. 3BNC117 will be good for infusion for 3 hours after it has been diluted to its final volume of 250 mL. Study individuals will be monitored for 1-hour post infusion at the study site.

6.7 Concomitant Medication(s)

Subjects are required to continue ART regimen while receiving the IMP(s). Any changes in ART during the study will be recorded in the CRF. Any other current medical therapy either recorded at study entry or initiated during the study will be recorded in the CRF. Initiation of medical therapy specified in the exclusion criteria will result in withdrawal from the IMP(s).

7 STUDY OVERVIEW

7.1 Overview of Study Design and Follow-up

The study will comprise four phases:

1. A run-in period with pre-treatment screening and randomization phase (day –up to 28 – 0)
2. A 3-week intervention/study phase preceded by ART on day 0 (day 0 – 24)
3. A post-treatment follow-up phase during which ART is continued (day 25 – 365)
4. A 12-week optional ATI after day 365 (day 400 – 491)

7.2 Study visits

Prior to performing any study-related procedures, written informed consent must be obtained from the subject. In general, any new or previously identified signs or symptoms that the subject has experienced since the last visit will result in a targeted physical examination and if relevant appropriate diagnostic tests.

A visit (2 to 5 with no infusions and during follow-up prior to ATI) might be done over the phone if it suits the participant. Blood samples are still taken. If the participant has any clinically significant complaints, they will be referred to the department.

7.2.1 Visit 1/day -28 to 0; screening

Potential subjects will undergo screening assessment to evaluate eligibility. The following evaluation will be performed:

- Review of inclusion/exclusion criteria
- If female; pregnancy test (serum/urine)
- Instruction about pregnancy precautions
- Demographics
- Concurrent illness
- Medication history
- Medical and surgical history. Regarding HIV history, the following should be recorded, if possible:
    - HIV-1 diagnosis date
    - Presumed date of infection with HIV-1
    - The last two CD4+ T cell measurements prior screening (if two or more measurements are available)

    - The last two viral load measurements prior screening (if two or more measurements are available)
    - HIV-1 clade
    - Mode of HIV acquisition
- Vital signs (blood pressure, heart rate, temperature)
- ECG
- Physical examination (including height and weight)
- Blood samples
    - Routine biochemistry
        - Safety
        - Hepatitis
    - HIV virology
    - Immunology

7.2.2 Visit 1.5/day 0; baseline. Initiation of ART

The eligible subjects initiate ART. Subsequently the subjects will be randomized to one of the four regimens.

- Blood samples
    - HIV virology
    - Immunology
- Randomization

7.2.3 Visit 2/day 7

Participants randomized to 3BNC117 infusion (before infusion)

- If female; pregnancy test (serum/urine)
- Blood samples
    - Routine biochemistry (safety)

All participants

- Medication history
- Vital signs (blood pressure, heart rate, temperature)
- Blood samples
    - HIV virology
    - Immunology

7.2.4 Visit 2/day 7+1 hour

Only participants whom had 3BNC117 infusion (after infusion)

- Vital signs (blood pressure, heart rate, temperature)
- Evaluation of adverse events
- Blood samples
    - HIV virology

7.2.5 Visit 3/day 10

Participants randomized to romidepsin infusion (before infusion)

- If female; pregnancy test (serum/urine)
- Blood samples
    - Routine biochemistry (safety)
- Antiemetic prophylactic ondansetron 8 mg will be given orally approximately 30 minutes prior to infusion

All participants

- Medication history
- Vital signs (blood pressure, heart rate, temperature)
- Blood samples
    - HIV virology
    - Immunology

7.2.6 Visit 3/day 10+2 hours

Only participants whom had romidepsin infusion (after infusion)

- Vital signs (blood pressure, heart rate, temperature)
- Evaluation of adverse events
- Blood samples; amount
    - HIV virology

7.2.7 Visit 4/day 17

Participants randomized to romidepsin infusion (before infusion)

- If female; pregnancy test (serum/urine)
- Blood samples
    - Routine biochemistry (safety)
- Antiemetic prophylactic ondansetron 8 mg will be given orally approximately 30 minutes prior to infusion

All participants

- Medication history
- Vital signs (blood pressure, heart rate, temperature)
- Blood samples
    - HIV virology
    - Immunology

7.2.8 Visit 4/day 17+2 hours

Only participants whom had romidepsin infusion (after infusion)

- Vital signs (blood pressure, heart rate, temperature)
- Evaluation of adverse events
- Blood samples
    - HIV virology

7.2.9 Visit 5/day 21

Participants randomized to 3BNC117 infusion (before infusion)

- If female; pregnancy test (serum/urine)
- Blood samples

All participants

- Medication history
- Vital signs (blood pressure, heart rate, temperature)
- Blood samples
    - HIV virology
    - Immunology

7.2.10 Visit 5/day 21+1 hour

Only participants whom had 3BNC117 infusion (after infusion)

- Vital signs (blood pressure, heart rate, temperature)
- Evaluation of adverse events
- Blood samples
    - HIV virology

7.2.11 Visit 6/day 24

Participants randomized to romidepsin infusion (before infusion)

- If female; pregnancy test (serum/urine)
- Blood samples
    - Routine biochemistry (safety)
- Antiemetic prophylactic ondansetron 8 mg will be given orally approximately 30 minutes prior to infusion

All participants

- Medication history
- Vital signs (blood pressure, heart rate, temperature)
- Blood samples
    - HIV virology
    - Immunology

7.2.12 Visit 6/day 24+2 hours

Only participants whom had romidepsin infusion (after infusion)

- Vital signs (blood pressure, heart rate, temperature)
- Evaluation of adverse events
- Blood samples
    - HIV virology

7.2.13 Visit 7, 8, 9, 10 and 11/day 30, 60, 90, 180 and 270

All participants

- Medication history
- Vital signs (blood pressure, heart rate, temperature)
- Evaluation of adverse events
- Blood samples
    - HIV virology
    - Immunology

7.2.14 Visit 12/day 365 (pre-ATI)

All participants

- If female; pregnancy test (serum/urine)
- Medication history
- Vital signs (blood pressure, heart rate, temperature)
- Evaluation of adverse events
- Blood samples
    - Routine biochemistry (safety + hepatitis) (only for individuals participating in the ATI)
    - HIV virology
    - Immunology

7.3 The Optional Lymph Node Biopsy (separate informed consent form)

At the AUH study site a total of 8 individuals (2 individuals from each study arm) will be asked to participate.

7.3.1 Visit 1LN/between day 0 to 7

- First excisional inguinal lymph node biopsy

7.3.2 Visit 12LN/day 365

- Second excisional inguinal lymph node biopsy

7.4 The Optional Analytical Treatment Interruption (ATI)

The following visits are only for individuals participating in the optional ATI:

7.4.1 Visit 13/day 400 (day of start ATI)

- If female; pregnancy test (serum/urine)
- Medication history
- Vital signs optional (blood pressure, heart rate, temperature)
- Evaluation of adverse events
- Blood samples
    - HIV virology
    - Immunology

7.4.2 Visit 14/day 407 (ATI)

- Medication history

- Vital signs optional (blood pressure, heart rate, temperature)
- Evaluation of adverse events
- Blood samples
    - Routine biochemistry (safety)
    - HIV virology
    - Immunology

7.4.3 Visit 15/day 414 (ATI)

- If female; pregnancy test (serum/urine)
- Medication history
- Vital signs optional (blood pressure, heart rate, temperature)
- Evaluation of adverse events
- Blood samples
    - HIV virology
    - Immunology

7.4.4 Visit 16/day 421 (ATI)

- Medication history
- Vital signs optional (blood pressure, heart rate, temperature)
- Evaluation of adverse events
- Blood samples
    - HIV virology
    - Immunology

7.4.5 Visit 17/day 428 (ATI)

- If female; pregnancy test (serum/urine)
- Medication history
- Vital signs optional (blood pressure, heart rate, temperature)
- Evaluation of adverse events
- Blood samples
    - Routine biochemistry (safety)
    - HIV virology
    - Immunology

7.4.6 Visit 18/day 435 (ATI)

- Medication history
- Vital signs optional (blood pressure, heart rate, temperature)
- Evaluation of adverse events
- Blood samples
    - HIV virology
    - Immunology

7.4.7 Visit 19/day 442 (ATI)

- If female; pregnancy test (serum/urine)
- Medication history
- Vital signs optional (blood pressure, heart rate, temperature)
- Evaluation of adverse events
- Blood samples
    - HIV virology
    - Immunology

7.4.8 Visit 20/day 449 (ATI)

- Medication history
- Vital signs optional (blood pressure, heart rate, temperature)
- Evaluation of adverse events
- Blood samples
    - HIV virology
    - Immunology

7.4.9 Visit 21/day 456 (ATI)

- If female; pregnancy test (serum/urine)
- Medication history
- Vital signs optional (blood pressure, heart rate, temperature)
- Evaluation of adverse events
- Blood samples
    - Routine biochemistry (safety)
    - HIV virology
    - Immunology

7.4.10 Visit 22/day 470 (ATI)

- If female; pregnancy test (serum/urine)
- Medication history
- Vital signs optional (blood pressure, heart rate, temperature)
- Evaluation of adverse events
- Blood samples
    - HIV virology
    - Immunology

7.4.11 Visit 23/day 484 (ATI)

- If female; pregnancy test (serum/urine)
- Medication history
- Vital signs optional (blood pressure, heart rate, temperature)
- Evaluation of adverse events
- Blood samples
    - Routine biochemistry (safety)

- HIV virology
- Immunology

7.4.12 Visit 24/day 491 (end-ATI)

- Medication history
- Vital signs optional (blood pressure, heart rate, temperature)
- Evaluation of adverse events
- Blood samples
    - Routine biochemistry (safety)
    - HIV virology
    - Immunology

7.5 Resumption of ART during ATI:

7.5.1 Visit at resumption of ART (pre-end-ATI/resump-ART)

- Medication history
- Vital signs optional (blood pressure, heart rate, temperature)
- Evaluation of adverse events
- Blood samples
    - Routine biochemistry (safety)
    - HIV virology
    - Immunology

The subject will be followed every 4 weeks until the HIV-1 RNA is undetectable using standard assays on 2 consecutive measurements. Afterwards the subject will resume routine treatment and control following standard treatment guidelines.

Table 7.1. Overview of study visits, follow-up and safety monitoring (1 of 2)

	Screening	Baseline	Study period					Observation period					Pre-ATI
Visit	1	1,5	2	3	4	5	6	7	8	9	10	11	12
Week	-4 to -0		1	2	3		4		8	12	25	38	52
Day	-28 to -0	0	7[A]	10[B]	17[B]	21[A]	24[B]	30	60	90	180	270	365
Visit window			±3	±3	±3	±3	±3	±7	±15	±15	±15	±15	±15
Informed consent	X												
Inclusion/exclusion criteria	X												
Demography/medical history/concurrent illnesses	X												
Medication history	X		X	X	X	X	X	X	X	X	X	X	X
Physical examination	X												
Height/weight	X												
Vital signs (temperature, systolic/diastolic BP*, PR*, RR*)[C]	X		X	X	X	X	X	X	X	X	X	X	X
Electrocardiography	X												
Pregnancy test (serum/urine)	X		X	X	X	X	X						X
Preg. Counseling*	X		X	X	X	X	X						
Optional lymph node biopsi[F]		X											X
Blood samples[D]	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse events, unexpected adverse reactions, local/systemic reactogenicity[E]			X	X	X	X	X	X	X	X	X	X	X
3BNC117 mAb (30mg/kg) infusion			(X)			(X)							
Romidepsin (5mg/m2) infusion				(X)	(X)		(X)						

ART initiation

*) Clinical judgement.

[A] The following procedures are done 0,5-1 hour after 3BNC117 mAB infusion; C + D + E.

[B] The following procedures are done 2 hours after romidepsin infusion; C + D + E.

[F] The optional lymph node biopsi will be performed between visit 1,5 and 2 plus at visit 12.

Table 7.1. Overview of study visits, follow-up and safety monitoring (2 of 2)

Start of ATI	Analytical Treatment Interruption (ATI)										End-ATI		Pre-end-ATI/ resump-ART	+ 4 weeks →
13	14	15	16	17	18	19	20	21	22	23	24			
57	58	59	60	61	62	63	64	65	67	69	70			
400	407	414	421	428	435	442	449	456	470	484	491			
±30	±5	±5	±5	±5	±5	±5	±5	±5	±10	±10	±5			
X	X	X	X	X	X	X	X	X	X	X	X		X	X
X	X	X	X	X	X	X	X	X	X	X	X		X	X
X		X		X		X		X	X	X	X		X	X
X		X		X		X		X	X	X	X		X	X
X	X	X	X	X	X	X	X	X	X	X	X		X	X
X	X	X	X	X	X	X	X	X	X	X	X		X	X

Table 7.2. Overview of sampling plan (page 1 of 3). Screening, baseline and study period.

		Screening	Baseline	Study period									
Visit		1	1,5	2		3		4		5		6	
Week		-4 to -0		1		2		3				4	
Day		-28 to -0	0	7	+ 1 hour	10	+ 2 hours	17	+ 2 hours	21	+ 1 hour	24	+ 2 hours
Visit window			A R T	±3		±3		±3		±3		±3	
Routine biochemistry (1)	Safety	X		X		X		X		X		X	
	Hepatitis	X											
HIV virology	Plasma HIV RNA (3)	X	X	X	X	X	X	X	X	X	X	X	X
	Cell-associated HIV RNA (4)		X	X		X		X		X		X	
	Integrated HIV DNA (5)		X										
	Total HIV DNA (8)		X										
	IUPM (7)												
Immunology	CD4+ T cell count (2)	X	X	X		X		X		X		X	
	CD8+ T cell count (2)												
	3BNC117 sensitivity (6)		X										
	Soluble inflammatory markers		X	X		X		X		X		X	
Research assay			X	X		X		X		X		X	
3BNC117 mAb (30mg/kg) infusion					X						X		
Romidepsin (5mg/m2) infusion							X		X				X
* Only taken if participation in ATI													

Table 7.2. Overview of sampling plan (page 2 of 3). Observation period and Analytical Treatment Interruption (ATI).

		Observation period					Pre-ATI	Start of ATI	Analytical Treatment Interruption (ATI)										End-ATI
Visit		7	8	9	10	11	12	13	14 -	15	16	17	18	19	20	21	22	23	24
Week			8	12	25	38	52	57	58 -	59	60	61	62	63	64	65	67	69	70
Day		30	60	90	180	270	365	400	407 -	414	421	428	435	442	449	456	470	484	491
Visit window		±7	±15	±15	±15	±15	±15	±30	±5	±5	±5	±5	±5	±5	±5	±5	±10	±10	±5
Routine biochemistry (1)	Safety						X*		X			X				X		X	X
	Hepatitis						X*												
HIV virology	Plasma HIV RNA (3)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
	Cell-associated HIV RNA (4)	X		X	X		X	X											X
	Integrated HIV DNA (5)						X	X											X
	Total HIV DNA (8)						X	X											X
	IUPM (7)						X												X
Immunology	CD4+ T cell count (2)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
	CD8+ T cell count (2)																		
	3BNC117 sensitivity (6)						X												
	Soluble inflammatory markers	X		X	X		X	X											X
Research assay		X		X	X		X	X											X
3BNC117 mAb (30mg/kg) infusion																			
Romidepsin (5mg/m2) infusion																			

* Only taken if participation in ATI

Table 7.2. Overview of sampling plan (page 3 of 3). Pre-end-ATI/resumption-ART and the following 4 weeks).

			Pre-end-ATI/ resump-ART	+ 4 weeks ➔
Visit				
Week				
Day				
Visit window				
Routine biochemistry (1)	Safety		X	
	Hepatitis			
HIV virology	Plasma HIV RNA (3)		X	X
	Cell-associated HIV RNA (4)		X	
	Integrated HIV DNA (5)		X	
	Total HIV DNA (8)		X	
	IUPM (7)		X	
Immunology	CD4+ T cell count (2)		X	X
	CD8+ T cell count (2)			
	3BNC117 sensitivity (6)			
	Soluble inflammatory markers		X	
Research assay			X	
3BNC117 mAb (30mg/kg) infusion				
Romidepsin (5mg/m2) infusion				
* Only taken if participation in ATI				

7.6 Blood sampling

The blood samples (see sampling plan; table 7.2) will be analysed according to the following:

1. Routine biochemistry (safety) includes haematology parameters (haemoglobin, total and differential leukocyte count, platelet count), glucose, PTT, INR, ALAT, bilirubin, alkaline phosphatase, creatinine, sodium, potassium, phosphorus, magnesium, calcium, urea, and albumin (performed at the local reference lab). Laboratory values will be done at screening, at every visit before infusion of IMP(s)) and every fourth week during the ATI study.
2. CD4/8+ T cell count will be performed at the local reference lab
3. Plasma HIV-1 RNA will be measured using Cobas Taqman (Roche) at the local reference lab
4. Cell-associated unspliced HIV-1 RNA in total CD4+ T cells will be done using digital droplet PCR (ddPCR) (done on CD4+ T cells isolated from thawed PBMCs using a CD4+ T cell isolation kit and magnetic-activated cell sorting columns; performed in-house at the Department of Infectious Diseases, AUH)
5. Quantification of integrated HIV-1 DNA in total CD4+ T cells will be done using an alu-PCR (done on CD4+ T cells isolated from thawed PBMCs using a CD4+ T cell isolation kit and magnetic-activated cell sorting columns; performed in-house at the Department of Infectious Diseases, AUH)
6. 3BNC117 sensitivity; made as optional post-hoc analysis (performed at either University Hospital of Cologne or in-house at the Department of Infectious Diseases, AUH)
7. IUPM will be calculated in resting CD4+ T cells using limiting dilution analysis as described in section 8.2.3 (done on CD4+ T cells isolated from thawed PBMCs using a CD4+ T cell isolation kit and magnetic-activated cell sorting columns; performed in-house at the Department of Infectious Diseases, AUH)
8. Total HIV-1 DNA per 10^6 CD4+ T cells will be measured by ddPCR as described in section 8.1.1 (done on CD4+ T cells isolated from thawed PBMCs using a CD4+ T cell isolation kit and magnetic-activated cell sorting columns; performed in-house at the Department of Infectious Diseases, AUH)

7.6.1 Blood volume per analyses

1. Routine biochemistry (safety): amount 14 mL
   Hepatitis: amount 11 mL
2. CD4/8+ T cell count: amount 4 mL
3. Plasma HIV-1 RNA: amount 4 mL
4. Cell-associated unspliced HIV-1 RNA: amount 27 mL

5/8. Integrated HIV-1 DNA/total HIV-1 DNA: 45 or 90 mL

6. 3BNC117 sensitivity: amount 18 mL
7. IUPM: amount 180 mL
9. Soluble inflammatory markers: amount 18 mL

7.6.2 Total amount of blood per visit

Arranged according to time during study; amount of blood volume:
Screening: 33 mL.
Baseline (day 0): 161 mL.
Visit 2-6 (all participants): 67 mL.
Visit 2-6 (after infusion): 4 mL.
Visit 7, 9 and 10 (observation period): 53 mL.
Visit 8 and 11 (observation period): 8 mL.
Visit 12 (pre-ATI): 341 or 366 mL depending on enrolment into the ATI.
Visit 13 (start ATI): 98 mL.
Visit 14, 17, 21 and 23 (ATI): 22 mL.
Visit 15, 16, 18, 19, 20 and 22 (ATI): 8 mL.
Visit 24 (end-ATI): 292 mL.
Pre-end-ATI/resumption-ART: 292 mL.
Pre-end-ATI + 4 weeks: 8 mL.

7.7 Safety monitoring

Safety will be monitored by vital signs, clinical laboratory tests, history and physical examinations if needed and the rate and severity of AE.
If indicated in the opinion of the investigator, a physical examination will be performed prior to and after completed infusion. Routine biochemistry (safety) will be performed prior to infusion. Infusion is postponed in case of unacceptable laboratory values prior to infusion, and laboratory tests may be repeated, as clinically indicated, to obtain acceptable values before withdraw from the study:

- Hepatic transaminases (AST or ALT) ≥3 x upper limit of normal (ULN)
- Serum total bilirubin ≥3 ULN
- Estimated glomerular filtration rate (eGFR) ≤60 mL/min (based on serum creatinine or other appropriate validated markers)
- Platelet count ≤100 $x10^9$/L
- Absolute neutrophil count ≤$1x10^9$/L
- Serum potassium, magnesium, phosphorus outside ≥1.5 ULN/LLN
- Total calcium (corrected for serum albumin) or ionized calcium ≥1.5 ULN/LLN

A baseline ECG will be obtained at screening.
Upon completion of follow-up specified in this protocol at day 365, study subjects who do not enter the ATI will resume routine treatment and control following standard treatment guidelines.

7.7.1 Romidepsin

The most frequently occurring AE collected in the IB with romidepsin in monotherapy treatment are described in section 2.2.4.1. Areas of safety considerations are:

- Haematology

- The risk of reversible thrombocytopenia, neutropenia, anaemia require monitoring of haematology parameters, which will be performed as indicated in Table 7.1 and 6.2

- Cardiac
  - Asymptomatic T-wave abnormalities are the most common reported ECG abnormality. Asymptomatic sinus tachycardia has also been noted frequently. In initial phase I an II studies with IV administration of romidepsin reversible prolongation of the QT interval was noted, which is a feature of the entire class of HDACi. QTc >480 ms have not been noted in previous clinical studies, but ECG monitoring will be performed as indicated in Table 7.1 and 6.3
- Gastrointestinal system
  - Gastrointestinal side effects are common and include nausea, diarrhoea, and vomiting. Diarrhoea has been reversible and well-controlled with the use of anti-diarrhoea medications (Table 6.2 and text specify guidelines). Monitoring of gastrointestinal AE will be performed at the study visits and through monitoring of relevant electrolytes
- Hepatic and renal system
  - Periodic monitoring of hepatic and renal function will be performed as indicated in Table 7.1 and 6.2
- Reproductive system
  - In non-clinical studies romidepsin has showed the potential to affect male and female fertility. Therefore, use of contraceptive methods for both gender is required during and 4 weeks after study treatment or until undetectable plasma HIV-1 RNA using standard assays; this is specified in the exclusion criteria
- Constitutional symptoms
  - Fatigue is commonly reported with romidepsin and will be monitored through study visits as indicated in Table 7.1 and Table 6.2

7.7.2 3BNC117

3BNC117 has now been administrated to 63 individuals and was generally safe and well tolerated in all doses tested ranging from 1 to 30 mg/kg. To date, 8 ART-treated HIV-1 infected individuals have received two 3BNC117 infusions, administered 3 weeks apart. This is the first time 3BNC117 will be administered in combination with romidepsin. Areas of safety considerations are included in the following safety laboratories: Metabolic panel of sodium, potassium, chloride, glucose, urea, creatinine, AST, ALT, alkaline phosphatase, bilirubin, white blood cell count, hgb, platelet count, and coagulation (PT, INR, PTT).

- Immunologic symptoms such as listed below are possible with administration of a mAb and will be considered adverse events of interest. Potential allergic-type reactions during and immediately following the administration of 3BNC117 will be carefully monitored:
  - Constitutional symptoms, such as fever, rigors/chills
  - Injection site reaction/extravasation changes, pruritus, urticaria

- Serum sickness like syndromes as evidenced by fever, rash, arthralgia, arthritis, nephritis
- Deposition of immune complexes in the kidneys leading to renal insufficiency
- Adult Respiratory Distress Syndrome, bronchospasm/wheezing, anaphylaxis
- Cytokine release syndrome/ acute infusion reaction

- 3BNC117-resistant viral strains
  - Following administration of 3BNC117. Development of 3BNC117 resistance might limit the future use of 3BNC117 by the study subject, if this monoclonal antibody is licensed for clinical use
- Conjunctival toxicity
  - In the cross-reactivity study in human tissues, 3BNC117 was found to bind to cells in the conjunctival recesses. It is possible that this binding could lead to conjunctival toxicity. However, when rats and non-human primates were administered 3BNC117, conjunctival toxicity was not observed. Sixty-three participants have received 3BNC117 to date, and 12 participants reported mild ophthalmic complaints (such as pruritus, conjunctival erythema, increased lacrimation) during study follow-up. In all instances symptoms resolved without specific treatment and ophthalmologic evaluations 5 months after 3BNC117 administration did not show changes from baseline

7.8 Unscheduled Visits or Telephone Contacts

An unscheduled telephone contact or visit may be scheduled for further assessment of any AE. A medically qualified member of the study staff should assess the symptom that prompted the visit. Findings should be recorded in the CRF.

7.9 Optional Lymph Node Biopsy and Monitored ART interruption

7.9.1 Optional Lymph Node Biopsy (between day 0 and 7 plus at day 365)

Upon study inclusion at day 0 up to day 7 and at the end-of-study at day 365, individuals at the Danish sites will be asked separately to undergo excisional inguinal lymph node biopsy in order to evaluate the effect of the IMPs on the immunological interaction in the lymphatic system. Lymph node biopsies is an optional component of the main study and participants that decline to participate in this substudy will still be eligible to be enrolled in the main study. The biopsies will be done at The Department of Plastic Surgery, AUH. An ultrasound examination will be done before the biopsy in order to locate a lateral lymph node. The biopsy will be done in local anesthetics, and take approximately 45 minutes.

7.9.2 Optional Monitored ART Interruption (day 365)

Upon completion of the final visit at day 365 subjects will be asked to interrupt ART on day 372 for 12-weeks in order to evaluate the effect of the IMPs on virological control. In the following this is referred to as the analytical treatment interruption (ATI). Enrolment into the ATI is optional and conditioned by the following criteria on individual level pertaining to the effect of the IMPs on the latent HIV-1 reservoir:

- Undetectable plasma HIV-1 RNA <50 copies/mL during the last 6 months (one 'viral blip' >50 copies/mL during the last 6 months is acceptable)
- Latest CD4+ T cell count >500 cells/mm^3

The following criteria will require resumption of ART:

- CD4+ T cell count <350 cells/mm^3
- 2 consecutive plasma HIV-1 RNA >5,000 copies/mL
- Subject request
- If ART interruption will, in the opinion of the Sponsor or Investigator contain an unacceptable risk to the subject

If a subject requires resumption of ART, the subject will be followed every 4 weeks until the HIV-1 RNA is undetectable using standard assays on 2 consecutive measurements. Afterwards the subject will resume routine treatment and control following standard treatment guidelines.
For scientific purposes, the ATI for each subject is concluded upon resumption of ART or 12-weeks after start of ATI, whichever comes first.
Subjects whom have not met criteria to re-initiate ART by the end of ATI will be asked to continue ART interruption until ART resumption criteria are met, but will after the end of the ATI be followed up at local department with monthly out-patient control visits.

7.9.2.1 Withdrawal of ART

The prolonged intracellular elimination time of tenofovir provides well-matched pharmacokinetic profiles for the most common ART regimen and would presumably not result in "functional monotherapy" after simultaneous withdrawal. However, to rule out any risk of resistance development the following principles of ART withdrawal have been decided upon:

- Patients on non-nucleoside reverse-transcriptase inhibitors will switch to un-boosted atazanavir and receive this regimen for one week prior to ART interruption
- Patients receiving INSTI can discontinue treatment regimen simultaneously

7.9.2.2 Sampling and follow-up prior, during and at the end of ATI

Prior ATI (at day 365):

- Baseline sampling (pre-ATI)

During ATI (between day 400 to 491; end-ATI):

- Blood samples including plasma HIV-1 RNA, CD4/8+ T cell count and research assays
    - Once weekly during the first 8 weeks of ART interruption
    - Once every second week hereafter

Upon viral rebound (preterm end-ATI):

- Blood samples including plasma HIV-1 RNA, CD4/8+ T cell count and research assays
    - Blood sampling is repeated within 4 days of first plasma HIV-1 RNA >1,000 copies/mL
    - Prior to re-initiating ART

7.10 Optional follow-up cohort of subjects, who are not participating in the ATI study

Subject, who are not participating in the ATI study will on day 365 be asked separately to come for an annual (year 2 to 5) control in addition to control following standard treatment guidelines in order to evaluate the effect of the IMPs on the latent HIV-1 reservoir long-term.

8 EFFICACY ASSESSMENTS

Every effort should be made to ensure that the protocol required tests and procedures are completed as described. When a protocol-required test cannot be performed as specified the Investigator will document the reason for this and any corrective or preventive actions that has been taken to ensure that protocol requirements are adhered to as soon as possible. In these cases, the Investigator will take all steps necessary to ensure the safety and well-being of the subject. Any subject who receives at least one dose of the IMP(s) will be included in the efficacy assessment.
For a detailed specification of study endpoints and related time points refer to 3.2 (End-points) in this protocol.

8.1 Primary Endpoint

8.1.1 Total HIV-1 DNA

CD4+ T cells will be isolated from thawed PBMCs using a CD4+ T cell isolation kit and magnetic-activated cell sorting (MACS) columns. Cellular DNA will be extracted using a Qiagen DNA Blood Midi Kit. Total HIV-1 DNA quantifications will be assessed using the QX100™ Droplet Digital™ PCR system (Bio-Rad).

8.2 Secondary Endpoints

8.2.1 Safety and Tolerability assessment

Subject who receives at least one dose of the IMP(s) will be included in the evaluation for safety. Safety assessment will be done by recording of all patient-reported AE and SAE. For each AE/SAE the relationship to the IMP(s) will be evaluated and the severity graded according to the CTCAE.

8.2.2 Integrated HIV-1 DNA

For quantification of integrated HIV-1 DNA, CD4+ T cells will be isolated from thawed PBMCs using a CD4+ T cell isolation kit and MACS columns. For integrated HIV-1 DNA quantification, a two-step real-time PCR Alu-LTR is used with primers and probes as previously described [87].

8.2.3 Quantification of the functional viral reservoir

A limiting dilution viral outgrowth assay will be used to quantitate the size of the latent HIV-1 reservoir at day 365 [88]. Isolated CD4+ T cells from thawed PBMCs seeded at 20,000 cells/well in round-bottom 96-well plates will be stimulated with PHA+IL2 plus irradiated

allogeneic PBMCs from HIV-negative healthy donors [89]. Additional MOLT-4/CCR5 cells will be added periodically to further amplify virus. On approximately day 14 supernatants from each well will be incubated with TZM-bl cells, a permissive HeLa cell clone that contains reporter genes for firefly luciferase, permitting sensitive and accurate measurements of infection. Luciferase activity can be quantified by luminescence and is directly proportional to the number of infectious virus particles present in the inoculum. The frequency of cell infection will be calculated using limited dilution analysis and the results will be expressed as IUPM [90–93].

8.2.4 HIV-1 Immunology

Expression of activation markers on peripheral blood NK and T cell will be performed using standard flow cytometry methods on a BD FACSVerse flow cytometer. Cryopreserved PBMC will be thawed and stained with Live-dead dye and lineage- or function-specific (e.g. NK cell activation profile: CD56, CD16, NKG2D, NKG2A, NKp46, Ki67, 2B4, CD158a; T cell activation profile: CD3, CD4, CD8, CD69, CD38, HLA-DR; and DC [lineage negative, HLA-DR positive] activation profile: CD11c, CD14, CD123, CD80, CD86, CD169). Only live, singlet cells will be included in the analysis. Lymphocytes will be identified by size and granularity. Population gating will be based upon appropriate expression of lineage specific markers. Gates for activation marker positivity will be determined using isotope control antibodies.

T cell polyfunctionality will be determined by flow cytometry following an intracellular cytokine stain (ICS). Cryo-preserved PBMCs will be stimulated with an overlapping gag-peptide pool and stained using antibodies for live-dead, CD4, CD8, CD45RA, CD27, IFN-γ, IL-2 and TNF-α.

8.2.5 Plasma HIV-1 RNA

Quantitative plasma HIV-1 RNA will be measured by the Cobas Taqman assay (Roche), which is a routine clinical assay with a sensitivity of 20 HIV-1 RNA copies/mL.

8.2.6 Other Exploratory Analyses

Plasma from patients will be evaluated for the presence of soluble markers of inflammation (e.g. IFN-γ, IFN-α, IP-10, IL-2, TNF-α, IL-6, IL-12p40 and IL-8). Plasma protein concentrations will be determined using multiplex technology (e.g. Luminex) according to manufacturer's recommendations.

8.3 Tertiary Endpoints

8.3.1 Lymph Node Biopsy

The lymph node biopsies will be used to produce lymphoid tissue and cells for: gross histopathology, in situ hybridization/immunohistochemistry, mononuclear cells for flow cytometry, cell-associated unspliced HIV-1 RNA and total HIV-1 DNA.

9 SAFETY ASSESSMENTS

9.1 Adverse Events (AE)

An AE is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding – see below), symptom, or disease temporally associated with the use of the IMP(s), whether or not causally linked to the IMP(s).
Abnormal laboratory values or test results constitute AE only if they induce clinical signs or symptoms, are considered clinically significant, or require intervention.

In addition, all cases of drug-drug interaction, pregnancy (with or without outcome), paternal exposure, lactation, lack of efficacy, overdose, drug abuse and misuse, drug maladministration or accidental exposure and dispensing errors are collected and data based even if no adverse event has been reported.
All AE will be reported in accordance with the principles of GCP and the latest requirements of the Medicines for Human Use (Clinical Trials) Regulations.

All AEs must be scored according to the CTCAE and recorded on the AE form in the CRF with the following information:

1. The severity grade (mild, moderate, severe)
2. Its relationship to the IMP(s) (suspected/not suspected)
3. Its duration (start and end dates or if continuing at final exam)
4. Whether it constitutes a serious adverse event (SAE)
5. Action taken with the IMP(s)
6. Outcome

9.2 Serious Adverse Events (SAE)

An SAE is any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening
- Results in persistent or significant disability/incapacity
- Constitutes a congenital anomaly/birth defect
- Requires inpatient hospitalization or prolongation of existing hospitalization, *unless hospitalization is for:*
    - Routine treatment or monitoring of the studied indication, not associated with any deterioration in condition
    - Elective or pre-planned treatment for a pre-existing condition that is unrelated to the indication under study and has not worsened since the start of the IMP(s)
    - Treatment on an emergency outpatient basis for an event not fulfilling any of the definitions of a SAE given above and not resulting in hospital admission
    - Social reasons and respite care in the absence of any deterioration in the patient's general condition

- Is medically significant, i.e. defined as an event that jeopardizes the patient or may require medical or surgical intervention to prevent one of the outcomes listed above

Unlike routine safety assessments, SAEs are monitored continuously and have special reporting.

9.2.1 Pregnancies

Pregnancy, although not itself a serious adverse event, will also be reported on a SAE form and be followed up to determine outcome, including spontaneous or voluntary termination, details of birth, and the presence or absence of any birth defects, congenital abnormalities or maternal and/or newborn complications. Pregnancy should be recorded on a Clinical Trial Pregnancy Form and reported by the Investigator to the local Drug Safety Department at Celgene and Rockefeller University.

9.3 Recording of Adverse Events (AE)

At each contact with the subject, the Investigator seeks information on AE by specific questioning and, as appropriate, by examination. Information on all AE should be recorded immediately in the source document, and also in the appropriate AE module of the CRF. All clearly related signs, symptoms, and abnormal diagnostic procedures results should be recorded in the source document, though they should be grouped under one diagnosis.
All AEs occurring between the day of ART initiation (baseline) to day 365 post-treatment in the observational follow-up phase must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. SAE that are still ongoing at the end of the observational follow-up phase must be followed up to determine the final outcome. Any SAE that occurs after the study period and is considered to be possibly related to the study treatment or study participation should be recorded and reported immediately.

9.4 Reporting of Serious Adverse Events (SAE)

The SAE will be noted in the CRF. Information about all SAE is collected and recorded on the SAE Report Form. The Investigator must assess the relationship to the IMP(s), complete the SAE Report Form in English, send the completed, signed form electronically to the Sponsor, and the local Drug Safety Department at Celgene and Rockefeller (**Figure 5**):

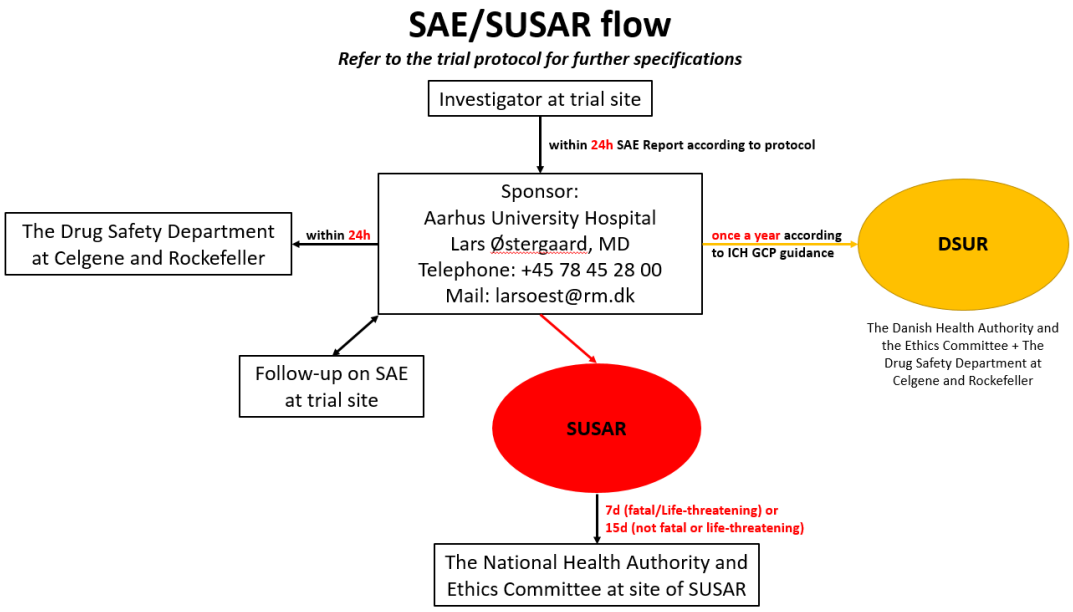


Figure 5. Work flow when reporting SAE and SUSAR.

Celgene AB
Drug Safety Nordic
Kista Science Tower
164 51 Kista, Sweden
Phone Number: +46 8 703 16 00
Fax Number: +46 8 703 16 03
Email: drugsafety-nordic@celgene.com

The Rockefeller University Hospital, New York NY
Att. Sarah Schlesinger
1230 York Ave
New York, NY 10065
Phone Number: (212) 327-7396
Fax Number: (212) 327-7234

The original copy of the SAE Report Form and a confirmation sheet must be at the study site.

9.5 SUSAR (Suspected Unexpected Serious Adverse Reaction):

If the SAE is not previously documented in the IB (new occurrence) and is thought related to the IMP(s), it qualifies as a SUSAR (**Figure 5**).

The minimum necessary information to be provided at the time of the initial report includes:

- An identifiable patient
- An identifiable medicinal and/or pharmaceutical product
- An identifiable reporter
- A SAE

9.5.1 Reporting of SUSAR to local Health and Medicines Authority and the Ethics Committee

The following describes the safety reporting requirements by timeline for reporting and associated type of event:

- ***Within 7 calendar days***
    - Any study event that is:
        - Serious and
        - Unexpected and
        - Suspected and
        - Fatal or life-threatening

- ***Within 15 calendar days***
    - Any study event that is:
        - Serious and
        - Unexpected and
        - Suspected

The Sponsor is responsible for submission of reportable SUSAR to the local Health and Medicines Authority and the Ethics Committee for their country according to regulatory requirements.

Danish Medicines Agency
Axel Heides Gade 1
2300 Copenhagen S, Denmark
Tel.: +45 7222 7400

SUSARs should be reported by filling in and submitting the Danish Medicines Agency's e-form for reporting of SUSARs (http://laegemiddelstyrelsen.dk/en/licensing/clinical-trials/adverse-reactions/reporting-of-suspected-unexpected-serious-adverse-reactions-susars-seen-in-clinical-trials-e-form).

9.6 Submission of Development Safety Update Report (DSUR)

In addition to the expedited reporting required for SUSAR, Sponsors are required to submit a safety report to the local Health Authority and the Ethics Committee according to local regulations, once a year throughout the clinical trial or on request. The Sponsor will also provide Celgene and Rockefeller with a copy of the DSUR at the time of submission to the local Health Authority and the Ethics Committee.

10 STATISTICAL ANALYSES

P-values <0.05 will be considered statistically significant. No interim analyses will be done.

10.1 Sample Size Determination

The anticipated average individual change in total HIV-1 DNA is approximately -0.85 on the log10 scale with corresponding standard deviation (SD) 0.35 from time of ART initiation to 12 months after ART initiation, when not receiving romidepsin or 3BNC117 [94]. The primary endpoint is change in total HIV-1 DNA from baseline to 1 year after treatment initiation in arm D (ART + the combined intervention) compared to arm A (ART alone). Arms B and C will be used to compare secondary endpoints on the specific effects of each of the two combinations alone, but the trial will be powered according to the primary endpoint. The primary parameters of interest are the declines in total HIV-1 DNA arms A and D on the log10 scale per 10^6 CD4+ cells from baseline (ART initiation) to 12 months, i.e. δ_A and δ_D. Based on a two sample mean comparison of the two with null-hypothesis $H_0: \delta_A = \delta_D$ and expected values of -0.85 and -1.35, respectively, and an SD of 0.35, a power of 90% is achieved with enrolment of 12 patients in each arm. Taking into account the anticipated (20%) dropouts, the aim is to enrol 15 study subjects in each arm (60 study participants in total).

10.2 Baseline Data

Continuous variables will be summarized using mean and confidence intervals or median and interquartile ranges, as appropriate. Numbers and percentage distribution will be quantified for categorical variables.

10.2.1 Safety- and Tolerability Analyses

The safety population will include all subjects who have received at least 1 dose of the IMP(s). AEs and SAEs will be recorded as described in this protocol and will be summarized according to severity assessment showing the number and percentage of subjects experiencing at least 1 event, the number of events, and the causality assessment. No formal statistical comparisons will be made – AE rates are presented for descriptive purposes.

10.2.2 ATI

Time to plasma HIV-1 RNA >1,000/mL and time to meet criteria for re-initiating ART will be presented for each subject and each group undergoing ART interruption.

10.2.3 Other secondary endpoints and exploratory analyses

Continuous variables will be summarized using mean and confidence intervals or median and interquartile ranges, as appropriate. Numbers and percentage distribution will be quantified for categorical variables. Parameters at baseline and subsequent time points will be computed for each subject. The null hypotheses of no difference from baseline to 1 year after intervention will be tested using appropriate statistical analyses. Other analytical tools may be employed in the exploratory analyses.

10.3 Stratification of the patient population

The primary analysis will be conducted both as intention-to-threat and per-protocol analyses on the total patient population.
Secondary analyses will be stratified according to characteristics that may influence either the effect of the interventions and/or the virological/immunological outcome measures. Such

characteristics include but are not limited to virus sensitivity to 3BNC117 neutralization, time from infection to enrolment, baseline CD4+ T cell count, baseline plasma HIV RNA, smoking, HLA-type, age, gender.

11 ACCESS TO SOURCE DATA

The Sponsor and PI can – based on approval from the regional Ethics Committee – have all source data (see 4.7 Source Data) passed on. During study monitoring, auditing, and/or inspection all relevant source data and study documents will be passed on for the study monitor, The Danish Medicines Agency, The Ethics Committee and their collaborators.

12 STUDY MONITORING AND QUALITY ASSURANCE

12.1 Sponsor and Investigator's Responsibility

It is Sponsor's responsibility to establish and maintain a quality assurance system that guarantees the quality of the study in all aspects. Sponsor can appoint qualified staff Investigators that may assist in the conduct of the study in accordance with the study protocol. All Investigators must be appointed and recorded on the study personnel list in due time before any study related procedures are carried out and must be supplied with the study protocol and all necessary information. Investigators are supervised by Sponsor or the Principal Investigator and act under their responsibility.
Sponsor will notify The Danish Medicines Agency and The Ethics Committee about the completion of the study within 90 days and within 1 year, but as early as possible, report the findings of the study. If the study is prematurely terminated this must be notified within 15 days and the reason for this must be clarified.
Investigator's responsibility is to conduct the clinical trial at the study site, and if the study site consists of a team of individuals, it is the Investigators responsibility to be leader of the team.

12.2 Study Monitoring

The study is monitored by the GCP-Unit at Aalborg and AUHs. The GCP-Unit and their national and international collaborators will through regular contacts, monitoring visit, telephone contacts, or written correspondence, monitor and assess the conduct of the study and contribute to high ethical, scientific, and legal standards in all aspects of the study. Sponsor, Investigators and study subjects' adherence to protocol requirements will be monitored as well as the handling of irregularities if such occur. During monitoring visits all, but not necessarily limited to, of the following issues will be discussed and assessed: informed consent, subject recruitment, follow-up, documentation, recording and reporting of AE and SAE, compliance, data quality, and data handling. Monitor will have all relevant data material passed on as specified in 11 (Access to Source Data).

12.3 Use of Case Report Forms (CRF)

Sponsor is responsible for keeping updated and accurate CRF intended to correctly register all observations and data related to the study. Recording in the CRF will usually be done during follow-up visits. Data entry into the CRF must be done comprehensively and carefully to ensure correct data interpretation. If corrections are introduced the previous text/data must continue to be readable – the correction should then be entered next to the original content alongside date and signature.

13 ETHICAL CONSIDERATIONS

13.1 Study Approvals

Sponsor must have prospective approval of the study protocol, protocol amendments, informed consent documents, recruitment advertisements, and other relevant documents from The Danish Medicines Agency, The Central Denmark Region Committee on Health Research Ethics, and The Danish Data Protection Agency before study initiation.
Subsequently the other study site must obtain approval of the same documents as mentioned above by their respective national regulatory authorities.

13.2 Ethical Conduct of the Study

It is Sponsor and Investigators' responsibility to plan and conduct the study in accordance with the protocol, The Helsinki Declaration (1996 version), guidelines for GCP; International Conference on Harmonization 1996, and national ethical guidelines and law.

13.3 Subject Recruitment, Information and Consent

Written information and advertisements approved by The Ethics Committee and Investigator databases may be used for recruitment purposes. See section 6.4 Consent Procedure for details. Eligible study subjects will be given written information upon diagnosed with HIV-1 to participate in the study unless the subject refuses. Or eligible study subjects that plan to start ART will receive a letter, which contains written information, inviting them to participate in the study unless the subject refuses. Interested subjects can contact an Investigator or study nurse to receive additional information and schedule an appointment for further information, possibly attended with a companion. If the subject then wishes more time to consider participation in the study, presence of a companion, or repetition of information, a new appointment will be scheduled for this within the time plan.
The Investigator must ensure that each subject is fully informed about the nature and objectives of the study and possible risks associated with participation.
An Investigator will obtain written informed consent from each subject before any study-specific activity is initiated using a consent form prospectively approved by The Ethics Committee. The study site will retain the original of each subject's consent form; a copy will be given the subject.

13.4 Harms, Risks and Inconveniences

There is now considerable experience with the human safety profile of romidepsin. As of 31 December 2011, more than 1300 patients have been treated with romidepsin in clinical studies, and of those a total of 891 patients with at least one dose of romidepsin as monotherapy. The experience with 3BNC177 is sparse, but both uninfected and HIV-1-infected individuals were given a single dose IV and monitored for 56 days [36]. A total of 37 subjects have received 3BNC117 at doses ranging from 1 to 30 mg/kg, and there have been no significant AE related to 3BNC117 to date.
Romidepsin is better characterized than 3BNC117, but both IMPs require monitoring. The safety profile is described in 2.2.4 (Safety profile) and safety monitoring is described in 7.2 (Safety monitoring).

Regarding the optional excisional inguinal lymph node biopsies, this procedure has been performed in 87 chronically HIV-1 infected individuals with minor complication (10%), which did not require medical attention: seroma, transient lymphedema, hematoma, and allergic reaction to surgical tape. In additional 10% had complications that needed medical attention: cellulitis, superficial infection and seroma aspiration. The conclusion from the above-mentioned study concluded, that lymph node biopsy for research purposes is generally safe in this group of patients [95].

13.5 Evaluation of Ethical and Scientific Aspects

The study treatment and procedures hold the risks, potential harms, and inconveniences mentioned above in 13.4, but several benefits to the study subjects and the scientific development counterbalance these disadvantages.
The objective of this study is to test and evaluate a specific combination treatment that addresses a fundamental problem in the treatment of HIV-infection: that despite highly effective ART it is still not possible to cure the disease. Consequently, HIV-infected patients are burdened with stigmatization and life-long treatment that may contain unpleasant side effects as well as yet unknown long-term toxicities. In addition, even well-treated HIV-infection still causes chronic immune activation and dysfunction, which is likely the reason for the increased rate of non-AIDS related mortality and morbidity seen among HIV-infected patients, even in the late ART era [3,96]. Provided, that the HIV-1 reservoir is more labile in the setting of high viremia and immune activation, our study, investigating an early strategy aiming at HIV-cure, will address an unmet medical need. Especially in light of our previous results using panobinostat and the more potent romidepsin to purge and revert HIV-1 latency [25,80]. This combination strategy will provide proof-of-concept and could be an important step towards a treatment strategy with a curing potential.

The immediate benefits for the study subjects are closely related to these considerations, but are of course dependent on the treatment efficacy. If only partial, rather than complete, eradication of the latent HIV-reservoir is achieved, this would likely result in improved immunological control of the infection, maybe to an extent that allows pausing ART for extended periods of time. Furthermore, depleting the latent HIV-reservoir and improving the immunological control of the infection may also offset some of the negative immune dysfunction events occurring in chronic HIV-infection. Previous studies have shown that larger

size of the latent HIV-reservoir negatively affects prognosis with regard to morbidity and mortality [97,98] and it is likely that a corresponding positive effect will be present by reducing the size of the reservoir.

The ultimate proof of a clinically important reservoir size reduction is to demonstrate a longer than expected time from stopping ART to viral rebound during an analytical treatment interruption (ATI). However, there are several safety issues that need to be addressed when interrupting ART. One is the increased rate of clinical events associated with treatment interruption in patients with CD4+ T cell count between 250 and 300 cells/mm^3 seen in the SMART study [99]. Of note, the absolute rate of events in the interruption arm of this study was very small and not significantly different from the continuous therapy arm in the first 3-4 months after study entry [100]. Treatment interruptions of short periods, i.e. 12 weeks, hold little risk from this perspective. To further protect from this risk, it is recommended that treatment interruptions should be performed among patients with CD4+ T cell count above 500 cells/mm^3, to monitor CD4+ T cell count frequently (i.e. every two weeks), and to re-initiate ART at CD4+ cell-counts <350/mm^3, but can be conducted safely under these circumstances [100,101]. In addition, re-suppression of virus upon treatment resumption has generally been unproblematic without emergence of drug resistance [102,103], but stepwise stopping of drugs in the 3-drug ART regimen based on their individual pharmacokinetic elimination time must be carefully executed to avoid prolonged exposure of virus to one or two drugs alone as described in section 6.3.1 [100]. Thus, in the present study we have included an optional ART interruption one year after inclusion.
The scientific value of the study is extensive as there is now an urgent need for clinical studies to support the numerous findings and hypotheses generated by *in vitro, ex vivo* and in non-clinical studies. Careful evaluation of the efficacy, safety, tolerability, and immunological function of LRA and bNAbs during initiation of ART will generate essential new knowledge regarding the possibilities and limitations of achieving cure of HIV-infection through eradication of the HIV-1 reservoir.

Against this background and with the careful monitoring of the study subject's safety, well-being, and privileges, which at any time must receive higher priority than the interests of society and scientific development, this study is found to be ethically justified and scientifically well-balanced.

14 DATA HANDLING AND RECORD KEEPING

14.1 Access to Personal Information

All study subjects will be asked to sign a consent form at entry allowing Sponsor and Investigators, the GCP units, Ethics Committees, and regulatory authorities to get passed on the source data. All personnel must treat personal data as confidential.

14.2 Handling of Personal Information

Sponsor and Investigators including designated personnel must handle and keep all study material and documents as confidential information and take steps to prevent wrongful or premature destruction of these. All personal data are protected in accordance with the relevant national law and Data Protection Agency.
Blood samples and subject-specific documents will not contain information that directly identifies the subject, but will be supplied with a study ID unique for each subject. All study material will be treated in accordance with the national law and Data Protection Agency.
Upon completion of all study related analyses all personal data will be rendered anonymous. However, according to GCP-guidelines data will remain personally identifiable for a minimum of 5 years after completion of study procedures defined as last subject's final follow-up contact. Any retained research data will be kept for a period of 20 years.

14.3 Research Biobank

The biological material collected during the study will be used for the analyses specified in this protocol and as an integral part of a biomedical research project fulfil the criteria for a research biobank. Therefore, the following information is supplied:

- At each visit (how much and when; please refer to table 7.1 and section 7.5) blood draws will be performed
- The purpose of the research biobank is to store biological material to be used for the analyses described in this protocol (which analyses and where; please refer to section 7.4)
- Upon completion of all study-related analyses any remaining biological material will be transferred to a biobank for future research conditioned by prior approval by The Danish Data Protection Agency. At enrolment study subjects will be asked to give consent to storage of their remaining biological material in the biobank for future research (termination 01.01.2035; see approval from Data Protection Agency)
- Regarding harms, risks and inconveniences related to collection of samples, please refer to the above section on the subject (section 13.4)

15 FINANCIAL ISSUES AND INSURANCE

The total costs of the study are estimated to approximately 20,000$ per subject amounting to approximately 1.200.000$. Celgene Corporation and Rockefeller University will supply the IMPs free of charge. Subjects enrolled in Denmark are under The Danish Patient Insurance, that covers all study subjects as guaranteed by The Danish Act on the Right to Complain and Receive Compensation within the Health Service administered by The Patient Assurance Association.
Study subjects will be reimbursed for transport expenses relating to the study, but will otherwise not receive any financial compensation for participating in the study. Study subjects has the possibility of lost earnings.
Scientific and technical assisting personnel may be hired directly in the study to carry out designated task.

The trial has received 2.2 million Danish kroner from Regionernes Medicinpulje. We will apply for further financial support (e.g. amfAR, The Foundation for AIDS Research).

There will be no wage or other benefits neither for the project site or members of the project team or subjects. None of the members of the project are associated with Regionernes Medicinpulje.

16 PUBLICATION OF STUDY

16.1 Public Registration of Study

The study will be registered at https://eudract.ema.europa.eu and http://clinicaltrials.gov prior to study start upon receipt of all necessary approvals. A resume and brief outline of the study will be available according to current guidelines.

16.2 Publication of Study Results

Publication of the results of this trial will be governed by requirements for authorship promulgated by the International Committee of Medical Journal Editors (ICMJE) http://www.icmje.org. Any presentation, abstract, or manuscript will be made available for review by the pharmaceutical companies providing the IMPs at least thirty (30) days (or, for abstracts, at least five (5) working days) prior to submission.
Results from the study will be published in international peer-reviewed journals, and negative, positive as well as inconclusive test results will be attempted published.
A final list of authors cannot be specified at this time, but all contributing researchers who fulfilled the Vancouver criteria for authorship will be included in the final publication.

17 STRATEGY FOR LITERATURE REVIEW

A literature search of peer-reviewed publications on the PubMed database was conducted in May-June 2015. The following MeSH-terms were used alone or in combination: “HIV”, “HIV latency”, “HIV reservoir”, “HIV eradication”, “HIV cure”, “Romidepsin”, “HDACi”, “3BNC117”, “bNAbs”. Titles and abstracts were reviewed and studies that were judged relevant for the context of the above-mentioned clinical trial were included.

18 PROTOCOL SYNOPSIS

18.1 Title

Early administration of anti-latency reversing therapy and broadly neutralizing antibodies to limit the establishment of the HIV-1 reservoir during initiation of antiretroviral treatment - a randomized controlled trial (eCLEAR)

18.2 Background and study rationale

Despite effective highly active antiretroviral therapy (ART), HIV persists as a latent infection. Upon treatment interruption, the virus quickly replicates and viremia rebounds to pre-treatment levels. The primary barrier preventing eradication of HIV-infection by ART is a pool of long-lived latently infected memory CD4+ T cells [18]. These cells harbour integrated HIV proviral DNA capable of resuming HIV-expression upon subsequent activation [10]. In the inactive, resting state these cells are unrecognizable to the immune system and unresponsive to antiretroviral drugs. Purging and exposing the latent HIV-1 reservoir to the immune system has been sought accomplished by the use of a group of small molecule drugs called histone deacetylase inhibitors (HDACi), which have shown promising results. The concept, in a clinical setting, was initially tested studying the weak HDACi valproic acid. Reduction of the latent viral reservoir was seen in 3 of 4 patients [93], but further studies gave mixed results [91,104–106]. Following clinical trials using HDACi, plus an accumulating body of ex vivo/in vitro research, have demonstrated proof-of-concept that the state of HIV-1 latency can be disrupted safely in patients on ART by HDACi measured by an increase in HIV-1 transcription – none though with a sustained impact on the viral reservoir [25,80,92,107,108].

Some researchers have questioned whether such a stable reservoir of HIV-1 DNA is also present during active infection, due to increased immune activation [46]. If the latent HIV-1 DNA pool is indeed much more labile during active infection, then this would present a potential avenue for novel interventions. Support of this concept is provided in a recent animal study showing that in active simian immunodeficiency virus (SIV) infection of macaques when viral loads were low, the turnover of the putative latent reservoir was very slow (many years) [39]. This finding is consistent with the low turnover of virus in HIV-1 patients on ART. However, in animals with high viral loads, the turnover of SIV DNA within resting CD4+ T cells was fast (days), which suggests that high immune activation during active infection might prevent the establishment of latency. This hypothesis is also supported by findings from a study in patients after the initiation of ART that suggested rapid exchange between productively and latently infected cell pools during both chronic and acute infection [40]. Additionally, studies in HIV-1 infected patients show a delay between the appearance of mutations in plasma and their appearance in the proviral DNA, both for immune-adaptive mutations and drug-resistance mutations [42,43].

18.3 Objectives

18.3.1 Primary Objective

- To evaluate the effect of early viral reactivation by latency reversing agents (LRA) and/or administration of potent broadly neutralizing antibodies (bNAbs) on the size of the latent HIV-1 reservoir in treatment naïve HIV-1 patients initiating ART

18.3.2 Secondary Objective

- To evaluate the safety and tolerability of the Investigational Medicinal Products (IMP)s

- To evaluate the effect of the IMPs on the amount of integrated HIV-1 DNA in CD4+ T cells
- To evaluate the effect of the IMPs on the functional HIV-1 reservoir in CD4+ T cells
- To compare viral load (plasma HIV-1 RNA) kinetics between the different study arms
- To compare the levels of immune reconstitution (CD4+ T cell recovery) between the study arms
- To evaluate the effect of early viral reactivation and the IMPs on the immunological control of HIV-infection following optional ART interruption

18.3.3 Exploratory Objective

- To compare HIV-specific immunity, T cell phenotype, immune activation, and cytokine production between study arms

18.4 Design

A phase IIa multicenter non-blinded randomized controlled study with eligible participants randomized 1:1:1:1 to one of four regimens:

A. ART (day 0 – day 365)
B. ART (day 0 – day 365) + romidepsin 5 mg/m^2 (day 10±3, day 17±3, and day 24±3)
C. ART (day 0 – day 365) + 3BNC117 30 mg/kg (day 7±3 and day 21±3)
D. ART (day 0 – day 365) + romidepsin 5 mg/m^2 (day 10±3, day 17±3, and day 24±3) + 3BNC117 30 mg/kg (day 7±3 and day 21±3)

Targeted enrolment is 60 study subjects – 15 in each arm.

18.5 Endpoints

18.5.1 Primary Endpoint

- Change from baseline (day 0) to day 365 in copies of total HIV-1 DNA per 10^6 CD4+ T cells as measured by digital droplet PCR
- Time from starting ART to first plasma HIV RNA<20 copies/mL

18.5.2 Secondary Endpoints

- Safety evaluation, as measured by adverse events (AE), adverse reactions (AR), serious adverse events (SAE), serious adverse reactions (SAR) and serious unexpected serious adverse reactions (SUSAR)
- Change from baseline (day 0) to day 365 in integrated HIV-1 DNA in CD4+ T cells (copies per million cells) as measured by alu-PCR
- The frequency of CD4+ T cells latently infected with replication competent HIV at day 365 as measured by infectious units per million (IUPM) CD4+ T cells in a viral outgrowth assay
- Plasma HIV-1 RNA kinetics following ART initiation as measured by the Cobas Taqman assay
- Numbers and proportions of HIV-specific CD4+ and CD8+ T cell

- During ART-interruption study
    - Time to meet criteria to re-initiate ART

18.5.3 Endpoints in exploratory analyses

- Plasma cytokine and immune activation biomarker levels
- Genetic, virological, and immunological predictors of treatment response
- Plasma 3BNC117 concentration

19 LIST OF ABBREVIATIONS

3BNC117	Anti-HIV-1 bNAb targeting the CD4bs of gp120
AAG	Alpha-1-acid-glycoprotein or Orosomucoid
Ab	Antibody
ADL	Activities of daily living
AE	Adverse Event
AIDS	Acquired Immune Deficiency Syndrome
ALT	Alanine aminotransferase
ANC	Absolute neutrophil count
(c)ART	(combination) Antiretroviral Therapy
AR	Adverse Reactions
AST	Aspartate transaminase
ATI	Analytical treatment interruption
AUC	Area under curve
AUH	Aarhus University Hospital
bNAb	Broadly neutralizing Ab
CD	Closter of differentiation
CD4bs	CD4-binding site
CI	Confidence interval
Cmax	The maximum plasma concentration
CMV	Cytomegalovirus
CNS	Central nervous system
CPE	Cytopathic effects
CRF	Case report form
CTCAE	Common Terminology Criteria for AEs
CTCL	Cutaneous T cell lymphoma
CTL	Cytotoxic T lymphocytes
ddPCR	Digital droplet PCR
DNA	Deoxyribonucleic acid
DSUR	Development Safety Update Report
ECG	Electrocardiogram
eGFR	Estimated glomerular filtration rate
EMA	European Medicines Agency
EU	European Union
FACS	Fluorescence-activated cell sorting
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
gp120	HIV-1 envelope glycoprotein 120
HAT	Histone acetyl transferases
HBsAg	Hepatitis B surface antigen
HCV-RNA	Hepatitis C virus RNA
HDACi	HDAC inhibitors
HDACs	Histone deacetylases
HIV	Human immunodeficiency virus

IB	Investigator Brochure
ICH	The International Conference on Harmonisation
ICMJE	The International Committee of Medical Journal Editors
ICS	Intracellular cytokine stain
ID	Identification
IG	Immune Globulin
IL	Interleukin
IMP	Investigational Medicinal Product
INR	The international normalized ratio
INSTI	Integrase strand transfer inhibitor
IV	Intravenous
IUPM	Infectious units per million
LLN	Lower limit of normal
LN	Lymph node
LRA	Latency reversing agent
LTR	Long terminal repeat
mAb	Monoclonal Ab
MACS	Magnetic-activated cell sorting
NaCl	Sodium Chloride
NAT	Nucleic acid testing
NK	Natural killer
NRTI	Nucleoside or nucleotide analogue reverse transcriptase inhibitor
PBMCs	Peripheral blood mononuclear cells
PCR	Polymerase chain reaction
P-gp	Plasma-glycoprotein
PI	Principal Investigator
Pi	Protease inhibitor
PK	Pharmacokinetics
PLT	Thrombocytopenia
PTCL	Peripheral T cell lymphoma
PTT	The post-partial thromboplastin time
PT	The prothrombin time
QTcF	The Fridericia formula for QT-interval correction
QTc	QT-interval correction
RNA	Ribonucleic acid
RSV	Respiratory syncytial virus
SAE	Serious AE
SAR	Serious AR
SCA	Single copy assay
SD	Standard deviation
S.e.m.	Standard error of mean
SIV	Simian immunodeficiency virus
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMA	Transcription-mediated amplification
$T_{1/2}$	Half-time

ULN	Upper limit of normal
WBC	White blood cell counts
WOCBP	Women of Child Bearing Potential

20 LIST OF FIGURES

21 APPENDIX A. OVERVIEW OF CYP3A4 INDUCERS

From U.S. Food and Drug Administration.

Alphabetical arranged according to drug name.

Drug class	*Drug name*
Antiviral agent	Amprenavir
Gastrointestinal agent	Aprepitant
Neurologic agent	Armodafinil
Cardiac agent	Avasimibe
Cardiac agent	Bosentan
Neurologic agent	Carbamazepine
Herbal medicine	Echinacea
Antiviral agent	Efavirenz
Antiviral agent	Etravirine
Neurologic agent	Modafinil
Antimicrobial agent	Nafcillin
Neurologic agent	Phenytoin
Endocrinologic agent	Piogliazone
Rheumatologic/immunosuppressant agent	Prednisone
Antimicrobial agent	Rifampin
Neurologic agent	Rufinamide

Alphabetical arranged according to drug class.

Drug class	*Drug name*
Antimicrobial agents	Nafcillin
	Rifampin
Antiviral agents	Amprenavir
	Efavirenz
	Etravirine
Cardiac agents	Avasimibe
	Bosentan
Endocrinologic agent	Piogliazone
Gastrointestinal agent	Aprepitant
Herbal medicine	Echinacea
Neurologic agents	Armodafinil
	Carbamazepine
	Modafinil
	Phenytoin
	Rufinamide
Rheumatologic/immunosuppressant agent	Prednisone

22 APPENDIX B1. OVERVIEW OF CYP3A4 INHIBITORS

From U.S. Food and Drug Administration.

Alphabetical arranged according to drug name.

Drug class	*Drug name*
Anxiolytic agent	Alprazolam
Cardiac agent	Amiodarone
Cardiac agent	Amlodipine
Antiemetic agent	Aprepitant
Antiviral agent	Atazanavir
Cholesterol-lowering agent	Atorvastatin
Antiandrogen agent	Bicalutamide
Antimicrobial agent	Clarithromycin
Intermittent claudication agent	Cilostazol
Antihistaminic agent	Cimetidine
Antimicrobial agent	Ciprofloxacin
Cardiac agent	Conivaptan
Contraceptives (oral)	
Rheumatologic/immunosuppressant agent	Cyclosporine
Antiviral agent	Darunavir (ritonavir)
Cardiac agent	Diltiazem
Antimicrobial agent	Erythromycin
Antimicrobial agent	Fluconazole
Antidepressive agent	Fluoxetine
Antidepressive agent	Fluvoxamine
Antiviral agent	Fosamprenavir
Herbal medicine	Ginkgo
Herbal medicine	Goldenseal
Other	Grapefruit
Anticancer agent	Imatinib
Antiviral agent	Indinavir
Antimicrobial agent	Itrazonazole
Antimicrobial agent	Isoniazid
Antimicrobial agent	Ketoconazole
Antiviral agent	Lopinavir (ritonavir)
Anticancer agent	Nilotinib
Antimicrobial agent	Posaconazole
Antihistaminic agent	Ranitidine
Cardiac agent	Ranolazine
Antiviral agent	Ritonavir
Antiviral agent	Saquinavir

Antiviral agent	Tipranavir (ritonavir)
Cardiac agent	Verapamil
Antimicrobial agent	Voriconazole

Alphabetical arranged according to drug class.

Drug class	*Drug name*
Antiandrogen agent	Bicalutamide
Anticancer agents	Imatinib
	Nilotinib
Antidepressive agents	Fluoxetine
	Fluvoxamine
Antiemetic agent	Aprepitant
Antihistaminic agents	Cimetidine
	Ranitidine
Antimicrobial agents	Clarithromycin
	Erythromycin
	Itrazonazole
	Ketoconazole
	Posaconazole
	Voriconazole
	Fluconazole
	Ciprofloxacin
	Isoniazid
Antiviral agents	Atazanavir
	Indinavir
	Ritonavir
	Saquinavir
	Lopinavir (ritonavir)
	Fosamprenavir
	Darunavir (ritonavir)
	Tipranavir (ritonavir)
Anxiolytic agent	Alprazolam
Cardiac agents	Amiodarone
	Amlodipine
	Conivaptan
	Diltiazem
	Ranolazine
	Verapamil
Cholesterol-lowering agent	Atorvastatin
Contraceptives (oral)	
Herbal medicines	Ginkgo
	Goldenseal
Intermittent claudication agent	Cilostazol

Other	Grapefruit
Rheumatologic/immunosuppressant agent	Cyclosporine

23 APPENDIX C. OVERVIEW OF P-GP INDUCERS

From U.S. Food and Drug Administration.

Alphabetical arranged according to drug name.

Drug class	*Drug name*
Cardiac agent	Avasimibe
Neurologic agent	Carbamazepine
Neurologic agent	Phenytoin
Antimicrobial agent	Rifampin
Antiviral agent	Tipranavir (ritonavir)

Alphabetical arranged according to drug class.

Drug class	*Drug name*
Antimicrobial agent	Rifampin
Antiviral agent	Tipranavir (ritonavir)
Cardiac agent	Avasimibe
Neurologic agents	Carbamazepine
	Phenytoin

24 APPENDIX D1. OVERVIEW OF P-GP INHIBITORS

From U.S. Food and Drug Administration.

Alphabetical arranged according to drug name.

Drug class	*Drug name*
Cardiac agent	Amiodarone
Antimicrobial agent	Azithromycin
Cardiac agent	Captopril
Cardiac agent	Carvedilol
Antimicrobial agent	Clarithromycin
Cardiac agent	Conivaptan
Rheumatologic/immunosuppressant agent	Cyclosporine
Cardiac agent	Diltiazem
Cardiac agent	Dronedarone
Bioenhancer	Elacridar
Antimicrobial agent	Erytromycin
Cardiac agent	Felodipine
Antimicrobial agent	Itrazonazole
Antimicrobial agent	Ketoconazole
Antiviral agent	Lopinavir (ritonavir)
Cardiac agent	Quinidine
Herbal medicine	Quercetin
Cardiac agent	Ranolazine
Antiviral agent	Ritonavir
Antiviral agent	Saquinavir
Rheumatologic/immunosuppressant agent	Tacrolimus
Antiviral agent	Tipranavir (ritonavir)
Rheumatologic/immunosuppressant agent	Valspodar
Cardiac agent	Verapamil

Alphabetical arranged according to drug class.

Drug class	*Drug name*
Antimicrobial agents	Azithromycin
	Clarithromycn
	Erytromycin
	Itrazonazole
	Ketoconazole
Antiviral agents	Lopinavir (ritonavir)
	Ritonavir

	Saquinavir
	Tipranavir (ritonavir)
Bioenhancer	Elacridar
Cardiac agents	Amiodarone
	Diltiazem
	Dronedarone
	Captopril
	Carvedilol
	Conivaptan
	Felodipine
	Quinidine
	Ranolazine
	Verapamil
Herbal medicine	Quercetin
Rheumatologic/immunosuppressant agents	Cyclosporine
	Tacrolimus
	Valspodar

25 REFERENCES

1. Lohse N, et al, Hansen AB, Pedersen G, Kronborg G, Gerstoft J, et al. Survival of persons with and without HIV infection in Denmark, 1995-2005. Ann Intern Med. Department of Infectious Diseases, Odense University Hospital and University of Southern Denmark, Odense, Denmark. nl@dce.au.dk; 2007;146:87–95.
2. Sogaard OS, Lohse N, Gerstoft J, Kronborg G, Ostergaard L, Pedersen C, et al. Hospitalization for pneumonia among individuals with and without HIV infection, 1995-2007: a Danish population-based, nationwide cohort study. Clin Infect Dis [Internet]. Department of Infectious Diseases, Aarhus University Hospital, Skejby, Denmark. OKS@sks.aaa.dk, Aarhus University Hospital, Skejby, Denmark. OKS@sks.aaa.dk; 2008;47:1345–53 ST – Hospitalization for pneumonia among . Available from: http://www.ncbi.nlm.nih.gov/pubmed/18834317
3. Sogaard OS, Lohse N, Gerstoft J, Kronborg G, Ostergaard L, Pedersen C, et al. Mortality after hospitalization for pneumonia among individuals with HIV, 1995-2008: a Danish cohort study. PLoS One. Department of Infectious Diseases, Aarhus University Hospital, Skejby, Denmark. olesoega@rm.dk; 2009;4:e7022 ST – Mortality after hospitalization for pn.
4. Mahajan AP, Sayles JN, Patel V a, Remien RH, Sawires SR, Ortiz DJ, et al. Stigma in the HIV/AIDS epidemic: a review of the literature and recommendations for the way forward. AIDS [Internet]. 2008;22 Suppl 2:S67–79. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2835402&tool=pmcentrez&rendertype=abstract
5. Stutterheim SE, Pryor JB, Bos AER, Hoogendijk R, Muris P, Schaalma HP. HIV-related stigma and psychological distress: the harmful effects of specific stigma manifestations in various social settings. AIDS [Internet]. 2009;23:2353–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19741478
6. Shan L, Deng K, Shroff NS, Durand CM, Rabi SA, Yang H-CC, et al. Stimulation of HIV-1-Specific Cytolytic T Lymphocytes Facilitates Elimination of Latent Viral Reservoir after Virus Reactivation. Immunity [Internet]. Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA.: Elsevier Inc.; 2012;36:491–501. Available from: http://dx.doi.org/10.1016/j.immuni.2012.01.014
7. Anderson JL, Cheong K, Lee AKH, Saleh S, da Fonseca Pereira C, Cameron PU, et al. Entry of HIV in Primary Human Resting CD4(+) T Cells Pretreated with the Chemokine CCL19. AIDS Res. Hum. Retroviruses [Internet]. 2014;30:207–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24588576
8. Han Y, Wind-Rotolo M, Yang H-CC, Siliciano JD, Siliciano RF. Experimental approaches to the study of HIV-1 latency. Nat. Rev. Microbiol. [Internet]. Department of Medicine, Johns Hopkins University School of Medicine, 879 BRB 733 N. Broadway, Baltimore, Maryland 21205, USA.; 2007;5:95–106 ST – Experimental approaches to the study . Available from: http://www.ncbi.nlm.nih.gov/pubmed/17224919
9. Bowman MC, Archin NM, Margolis DM. Pharmaceutical approaches to eradication of persistent HIV infection. Expert Rev Mol Med. Department of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7435, USA.; 2009;11:e6 ST – Pharmaceutical approaches to eradication .
10. Finzi D, Blankson J, Siliciano JD, Margolick JB, Chadwick K, Pierson T, et al. Latent infection of CD4+ T cells provides a mechanism for lifelong persistence of HIV-1, even in patients on effective combination therapy. Nat. Med. [Internet]. Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205, USA.; 1999;5:512–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10229227
11. Archin NM, Espeseth A, Parker D, Cheema M, Hazuda D, Margolis DM. Expression of latent HIV induced by the potent HDAC inhibitor suberoylanilide hydroxamic acid. AIDS Res. Hum. Retroviruses [Internet]. 2009;25:207–12. Available from:

http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2853863&tool=pmcentrez&rendertype=abstract
12. Bosque A, Planelles V. Induction of HIV-1 latency and reactivation in primary memory CD4+ T cells. Blood [Internet]. 2009;113:58–65. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2614643&tool=pmcentrez&rendertype=abstract
13. Chun TW, Finzi D, Margolick J, Chadwick K, Schwartz D, Siliciano RF. In vivo fate of HIV-1-infected T cells: quantitative analysis of the transition to stable latency. Nat Med. Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205, USA.; 1995;1:1284–90 ST – In vivo fate of HIV – 1 – infected T cel.
14. Chun TW, Stuyver L, Mizell SB, Ehler LA, Mican J a, Baseler M, et al. Presence of an inducible HIV-1 latent reservoir during highly active antiretroviral therapy. Proc. Natl. Acad. Sci. U. S. A. Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892, USA. twchun@nih.gov; 1997;94:13193–7.
15. Davey RT, Bhat N, Yoder C, Chun TW, Metcalf JA, Dewar R, et al. HIV-1 and T cell dynamics after interruption of highly active antiretroviral therapy (HAART) in patients with a history of sustained viral suppression. Proc. Natl. Acad. Sci. U. S. A. [Internet]. National Institute of Allergy and Infectious Diseases, (NIAID), National Institutes of Health, Bethesda, MD 20892, USA. rdavey@niaid.nih.gov; 1999;96:15109–14 ST – HIV – 1 and T cell dynamics after int. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=24781&tool=pmcentrez&rendertype=abstract
16. Eisele E, Siliciano RF. Redefining the viral reservoirs that prevent HIV-1 eradication. Immunity [Internet]. Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA.; 2012;37:377–88. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22999944
17. Chomont N, El-Far M, Ancuta P, Trautmann L, Procopio FA, Yassine-Diab B, et al. HIV reservoir size and persistence are driven by T cell survival and homeostatic proliferation. Nat. Med. [Internet]. Laboratoire d'Immunologie, Centre de Recherche du Centre Hospitalier de l'Universite de Montreal Saint-Luc, Montreal, Quebec, Canada.; 2009;15:893–900. Available from: http://dx.doi.org/10.1038/nm.1972
18. Finzi D, Hermankova M, Pierson T, Carruth LM, Buck C, Chaisson RE, et al. Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. Science [Internet]. 1997;278:1295–300. Available from: http://www.sciencemag.org/cgi/doi/10.1126/science.278.5341.1295
19. Richman DD, Margolis DM, Delaney M, Greene WC, Hazuda D, Pomerantz RJ. The challenge of finding a cure for HIV infection. Science [Internet]. 2009;323:1304–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19265012
20. Palmer S, Maldarelli F, Wiegand A, Bernstein B, Hanna GJ, Brun SC, et al. Low-level viremia persists for at least 7 years in patients on suppressive antiretroviral therapy. Proc Natl Acad Sci U S A [Internet]. HIV Drug Resistance Program, National Cancer Institute, National Institutes of Health, Frederick, MD 21702-1201, USA. spalmer@ncifcrf.gov; 2008;105:3879–84 ST – Low – level viremia persists for at le. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2268833&tool=pmcentrez&rendertype=abstract
21. Sahu GK, Paar D, Frost SDW, Smith MM, Weaver S, Cloyd MW. Low-level plasma HIVs in patients on prolonged suppressive highly active antiretroviral therapy are produced mostly by cells other than CD4 T-cells. J. Med. Virol. [Internet]. 2009;81:9–15. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2912142&tool=pmcentrez&rendertype=abstract
22. Archin NM, Margolis DM. Emerging strategies to deplete the HIV reservoir. Curr. Opin.

Infect. Dis. [Internet]. 2013;27:29–35. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4031321&tool=pmcentrez&rendertype=abstract
23. Deeks SG. HIV: Shock and kill. Nature. 2012;487:439–40.
24. Rasmussen TA, Tolstrup M, Søgaard OS. Reversal of Latency as Part of a Cure for HIV-1. Trends Microbiol. Elsevier Ltd; 2015;
25. Søgaard OS, Graversen ME, Leth S, Brinkmann CR, Kjær A-S, Olesen R, et al. The HDAC Inhibitor Romidepsin is Safe and Effectively Reverses HIV-1 latency in vivo as Measured by Standard Clinical Assays. AIDS 2014 Int. Meet. [Internet]. 2014; Available from: http://www.iasociety.org/Web/WebContent/File/HIV_Cure_Symposium_2014/Day_1/Abstracts/OA2-4 LB.pdf
26. West AP, Scharf L, Scheid JF, Klein F, Bjorkman PJ, Nussenzweig MC. Structural insights on the role of antibodies in HIV-1 vaccine and therapy. Cell [Internet]. Elsevier Inc.; 2014;156:633–48. Available from: http://dx.doi.org/10.1016/j.cell.2014.01.052
27. Malbec M, Porrot F, Rua R, Horwitz J, Klein F, Halper-Stromberg A, et al. Broadly neutralizing antibodies that inhibit HIV-1 cell to cell transmission. J. Exp. Med. [Internet]. 2013;210:2813–21. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3865481&tool=pmcentrez&rendertype=abstract
28. Sigal A, Kim JT, Balazs AB, Dekel E, Mayo A, Milo R, et al. Cell-to-cell spread of HIV permits ongoing replication despite antiretroviral therapy. Nature [Internet]. Nature Publishing Group; 2011;477:95–8. Available from: http://dx.doi.org/10.1038/nature10347
29. Halper-Stromberg A, Lu C-LL, Klein F, Horwitz JA, Bournazos S, Nogueira L, et al. Broadly Neutralizing Antibodies and Viral Inducers Decrease Rebound from HIV-1 Latent Reservoirs in Humanized Mice. Cell [Internet]. Elsevier Inc.; 2014;158:989–99. Available from: http://dx.doi.org/10.1016/j.cell.2014.07.043
30. Bournazos S, Klein F, Pietzsch J, Seaman MS, Nussenzweig MC, Ravetch J V. Broadly Neutralizing Anti-HIV-1 Antibodies Require Fc Effector Functions for In Vivo Activity. Cell [Internet]. Elsevier Inc.; 2014;158:1243–53. Available from: http://dx.doi.org/10.1016/j.cell.2014.08.023
31. Scheid JF, Mouquet H, Ueberheide B, Diskin R, Klein F, Oliveira TYK, et al. Sequence and structural convergence of broad and potent HIV antibodies that mimic CD4 binding. Science. 2011;333:1633–7.
32. Barouch DH, Whitney JB, Moldt B, Klein F, Oliveira TY, Liu J, et al. Therapeutic efficacy of potent neutralizing HIV-1-specific monoclonal antibodies in SHIV-infected rhesus monkeys. Nature [Internet]. Nature Publishing Group; 2013;503:224–8. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4017780&tool=pmcentrez&rendertype=abstract
33. Shingai M, Nishimura Y, Klein F, Mouquet H, Donau OK, Plishka R, et al. Antibody-mediated immunotherapy of macaques chronically infected with SHIV suppresses viraemia. Nature [Internet]. Nature Publishing Group; 2013;503:277–80. Available from: http://dx.doi.org/10.1038/nature12746
34. Horwitz J a, Halper-Stromberg A, Mouquet H, Gitlin AD, Tretiakova A, Eisenreich TR, et al. HIV-1 suppression and durable control by combining single broadly neutralizing antibodies and antiretroviral drugs in humanized mice. Proc. Natl. Acad. Sci. U. S. A. [Internet]. 2013;110:16538–43. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24043801
35. Shingai M, Donau OK, Plishka RJ, Buckler-White a, Mascola JR, Nabel GJ, et al. Passive transfer of modest titers of potent and broadly neutralizing anti-HIV monoclonal antibodies block SHIV infection in macaques. J Exp Med. 2014;211.
36. Caskey M, Klein F, Lorenzi JCC, Seaman MS, West AP, Buckley N, et al. Viraemia suppressed in HIV-1-infected humans by broadly neutralizing antibody 3BNC117. Nature [Internet]. 2015;

Available from: http://www.nature.com/doifinder/10.1038/nature14411
37. Deeks SG. HIV: How to escape treatment. Nature [Internet]. 2011;477:36–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21886150
38. Gunst JD, Tolstrup M, Rasmussen TA, Søgaard OS. The potential role for romidepsin as a component in early HIV-1 curative efforts. Expert Rev. Anti. Infect. Ther. [Internet]. 2016;7210:14787210.2016.1164031. Available from: http://www.tandfonline.com/doi/full/10.1586/14787210.2016.1164031
39. Reece J, Petravic J, Balamurali M, Loh L, Gooneratne S, de Rose R, et al. An "escape clock" for estimating the turnover of SIV DNA in resting CD4$^+$ T cells. PLoS Pathog. [Internet]. 2012;8:e1002615. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3320584&tool=pmcentrez&rendertype=abstract
40. Murray JM, McBride K, Boesecke C, Bailey M, Amin J, Suzuki K, et al. Integrated HIV DNA accumulates prior to treatment while episomal HIV DNA records ongoing transmission afterwards. Aids. 2012;26:543–50.
41. Althaus CL, Joos B, Perelson AS, Günthard HF. Quantifying the Turnover of Transcriptional Subclasses of HIV-1-Infected Cells. PLoS Comput. Biol. [Internet]. 2014;10:e1003871. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4207463&tool=pmcentrez&rendertype=abstract
42. Zhang L, Rowe L, He T, Chung C, Yu J, Yu W, et al. Compartmentalization of surface envelope glycoprotein of human immunodeficiency virus type 1 during acute and chronic infection. J. Virol. [Internet]. 2002;76:9465–73. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=136436&tool=pmcentrez&rendertype=abstract
43. Strain MC, Günthard HF, Havlir D V, Ignacio CC, Smith DM, Leigh-Brown a J, et al. Heterogeneous clearance rates of long-lived lymphocytes infected with HIV: intrinsic stability predicts lifelong persistence. Proc. Natl. Acad. Sci. U. S. A. [Internet]. 2003;100:4819–24. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=153639&tool=pmcentrez&rendertype=abstract
44. Persaud D, Palumbo P, Ziemniak C, Chen J, Ray SC, Hughes M, et al. Early archiving and predominance of nonnucleoside reverse transcriptase inhibitor-resistant HIV-1 among recently infected infants born in the United States. J. Infect. Dis. [Internet]. 2007;195:1402–10. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17436219
45. Poveda E, Briz V, Paraskevis D, Barreiro P, Hatzakis A, Soriano V. Dynamics of drug-resistant HIV-1 in plasma and peripheral blood cells in patients during and after enfuvirtide therapy. AIDS Res. Hum. Retroviruses [Internet]. 2007;23:1078–82. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17919101
46. Kent SJ, Reece JC, Petravic J, Martyushev A, Kramski M, De Rose R, et al. The search for an HIV cure: tackling latent infection. Lancet Infect. Dis. [Internet]. 2013; Available from: http://www.ncbi.nlm.nih.gov/pubmed/23481675
47. Petravic J, Martyushev A, Reece JC, Kent SJ, Davenport MP. Modeling the Timing of Antilatency Drug Administration during HIV Treatment. J. Virol. [Internet]. 2014;88:14050–6. Available from: http://jvi.asm.org/cgi/doi/10.1128/JVI.01701-14
48. Dahl V, Josefsson L, Palmer S. HIV reservoirs, latency, and reactivation: prospects for eradication. Antiviral Res. [Internet]. 2010;85:286–94. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19808057
49. Colin L, Van Lint C. Molecular control of HIV-1 postintegration latency: implications for the development of new therapeutic strategies. Retrovirology [Internet]. 2009;6:111. Available from:

http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2797771&tool=pmcentrez&rendertype=abstract
50. Inche AG, La Thangue NB, Thangue NB La. Chromatin control and cancer-drug discovery: realizing the promise. Drug Discov. Today [Internet]. 2006;11:97–109. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16533707
51. Margolis DM. Mechanisms of HIV latency: an emerging picture of complexity. Curr. HIV/AIDS Rep. [Internet]. 2010;7:37–43. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20425056
52. Mok HP, Lever A. Waking up the sleepers: HIV latency and reactivation. J. Formos. Med. Assoc. [Internet]. 2008;107:909–14. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19129050
53. Keedy KS, Archin NM, Gates AT, Espeseth A, Hazuda DJ, Margolis DM. A limited group of class I histone deacetylases acts to repress human immunodeficiency virus type 1 expression. J. Virol. [Internet]. 2009;83:4749–56. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2682072&tool=pmcentrez&rendertype=abstract
54. Archin NM, Keedy KS, Espeseth A, Dang H, Hazuda DJ, Margolis DM. Expression of latent human immunodeficiency type 1 is induced by novel and selective histone deacetylase inhibitors. AIDS [Internet]. 2009;23:1799–806. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19590405
55. Contreras X, Schweneker M, Chen C-S, McCune JM, Deeks SG, Martin J, et al. Suberoylanilide hydroxamic acid reactivates HIV from latently infected cells. J. Biol. Chem. [Internet]. 2009;284:6782–9. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2652322&tool=pmcentrez&rendertype=abstract
56. Choi B-S, Lee HS, Oh Y-T, Hyun Y-L, Ro S, Kim SS, et al. Novel histone deacetylase inhibitors CG05 and CG06 effectively reactivate latently infected HIV-1. AIDS [Internet]. 2010;24:609–11. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20154582
57. Matalon S, Palmer BE, Nold MF, Furlan A, Kassu A, Fossati G, et al. The histone deacetylase inhibitor ITF2357 decreases surface CXCR4 and CCR5 expression on CD4(+) T-cells and monocytes and is superior to valproic acid for latent HIV-1 expression in vitro. J. Acquir. Immune Defic. Syndr. [Internet]. 2010;54:1–9. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3534976&tool=pmcentrez&rendertype=abstract
58. Ylisastigui L, Archin NM, Lehrman G, Bosch RJ, Margolis DM. Coaxing HIV-1 from resting CD4 T cells : histone deacetylase inhibition allows latent viral expression. 2004;
59. Bantscheff M, Hopf C, Savitski MM, Dittmann A, Grandi P, Michon A-M, et al. Chemoproteomics profiling of HDAC inhibitors reveals selective targeting of HDAC complexes. Nat. Biotechnol. [Internet]. 2011;29:255–65. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21258344
60. Huber K, Doyon G, Plaks J, Fyne E, Mellors JW, Sluis-Cremer N. Inhibitors of histone deacetylases: correlation between isoform specificity and reactivation of HIV type 1 (HIV-1) from latently infected cells. J. Biol. Chem. [Internet]. 2011;286:22211–8. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3121366&tool=pmcentrez&rendertype=abstract
61. Geleziunas R, Stephan G, Wei G, al et. Search for small molecule activators of latent HIV. International symposium on HIV and emerging infectious diseases. http://www.isheid.com/presentations/vendredi/12-30/geleziunas/Geleziunas.pdf [Internet]. 2012; Available from: http://www.isheid.com/presentations/vendredi/12-30/geleziunas/Geleziunas.pdf
62. Stamatatos L, Morris L, Burton DR, Mascola JR. Neutralizing antibodies generated during

natural HIV-1 infection: good news for an HIV-1 vaccine? Nat. Med. [Internet]. Nature Publishing Group; 2009;15:866–70. Available from: http://dx.doi.org/10.1038/nm.1949
63. Sather DN, Armann J, Ching LK, Mavrantoni A, Sellhorn G, Caldwell Z, et al. Factors associated with the development of cross-reactive neutralizing antibodies during human immunodeficiency virus type 1 infection. J. Virol. 2009;83:757–69.
64. Simek MD, Rida W, Priddy FH, Pung P, Carrow E, Laufer DS, et al. Human immunodeficiency virus type 1 elite neutralizers: individuals with broad and potent neutralizing activity identified by using a high-throughput neutralization assay together with an analytical selection algorithm. J. Virol. 2009;83:7337–48.
65. Doria-Rose N a, Klein RM, Daniels MG, O'Dell S, Nason M, Lapedes A, et al. Breadth of human immunodeficiency virus-specific neutralizing activity in sera: clustering analysis and association with clinical variables. J. Virol. 2010;84:1631–6.
66. Li Y, Migueles S a, Welcher B, Svehla K, Phogat A, Louder MK, et al. Broad HIV-1 neutralization mediated by CD4-binding site antibodies. Nat. Med. 2007;13:1032–4.
67. Trkola A, Kuster H, Rusert P, Joos B, Fischer M, Leemann C, et al. Delay of HIV-1 rebound after cessation of antiretroviral therapy through passive transfer of human neutralizing antibodies. Nat. Med. 2005;11:615–22.
68. Mehandru S, Vcelar B, Wrin T, Stiegler G, Joos B, Mohri H, et al. Adjunctive passive immunotherapy in human immunodeficiency virus type 1-infected individuals treated with antiviral therapy during acute and early infection. J. Virol. [Internet]. 2007;81:11016–31. Available from: http://jvi.asm.org/cgi/doi/10.1128/JVI.01340-07
69. Wardemann H, Yurasov S, Schaefer A, Young JW, Meffre E, Nussenzweig MC. Predominant autoantibody production by early human B cell precursors. Science. 2003;301:1374–7.
70. Tiller T, Meffre E, Yurasov S, Tsuiji M, Nussenzweig MC, Wardemann H. Efficient generation of monoclonal antibodies from single human B cells by single cell RT-PCR and expression vector cloning. J. Immunol. Methods. 2008;329:112–24.
71. Scheid JF, Mouquet H, Feldhahn N, Walker BD, Pereyra F, Cutrell E, et al. A method for identification of HIV gp140 binding memory B cells in human blood. J. Immunol. Methods. 2009;343:65–7.
72. Scheid JF, Mouquet H, Feldhahn N, Seaman MS, Velinzon K, Pietzsch J, et al. Broad diversity of neutralizing antibodies isolated from memory B cells in HIV-infected individuals. Nature [Internet]. Nature Publishing Group; 2009;458:636–40. Available from: http://dx.doi.org/10.1038/nature07930
73. Klein F, Nogueira L, Nishimura Y, Phad G, Jr APW, Halper-stromberg A, et al. Enhanced HIV-1 immunotherapy by commonly arising antibodies that target virus escape variants. J. Exp. Med. [Internet]. 2014;211:2361–72. Available from: http://www.jem.org/cgi/doi/10.1084/jem.20141050
74. Berg SL, Stone J, Xiao JJ, Chan KK, Nuchtern J, Dauser R, et al. Plasma and cerebrospinal fluid pharmacokinetics of depsipeptide (FR901228) in nonhuman primates. Cancer Chemother. Pharmacol. [Internet]. 2004;54:85–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15042312
75. Package insert Romidepsin. http://www.istodax.com/dtc/Default.aspx [Internet]. 2013;1–13. Available from: http://www.istodax.com/dtc/Default.aspx
76. Laille E, Patel M, Jones SF, Burris H a., Infante J, Lemech C, et al. Evaluation of CYP3A-mediated drug-drug **Interactions with romidepsin in patients with advanced cancer**. J. Clin. Pharmacol. [Internet]. 2015;n/a – n/a. Available from: http://doi.wiley.com/10.1002/jcph.560
77. Leschziner GD, Andrew T, Pirmohamed M, Johnson MR. ABCB1 genotype and PGP expression, function and therapeutic drug response: a critical review and recommendations for future research. Pharmacogenomics J. [Internet]. 2007;7:154–79. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16969364
78. Zhou H, Mascelli MA. Mechanisms of monoclonal antibody-drug interactions. Annu. Rev.

Pharmacol. Toxicol. 2011;51:359–72.
79. Ritchie D, Piekarz RL, Blombery P, Karai LJ, Pittaluga S, Jaffe ES, et al. Reactivation of DNA viruses in association with histone deacetylase inhibitor therapy: a case series report. Haematologica [Internet]. 2009;94:1618–22. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2770976&tool=pmcentrez&rendertype=abstract
80. Rasmussen T a, Tolstrup M, Brinkmann CR, Olesen R, Erikstrup C, Solomon A, et al. Panobinostat, a histone deacetylase inhibitor, for latent-virus reactivation in HIV-infected patients on suppressive antiretroviral therapy: a phase 1/2, single group, clinical trial. Lancet HIV [Internet]. 2014;3018:1–9. Available from: http://linkinghub.elsevier.com/retrieve/pii/S2352301814700141
81. Onyango-Makumbi C, Omer SB, Mubiru M, Moulton LH, Nakabiito C, Musoke P, et al. Safety and Efficacy of HIV Hyperimmune Globulin for Prevention of Mother-to-Child HIV Transmission in HIV-1–Infected Pregnant Women and Their Infants in Kampala, Uganda (HIVIGLOB/NVP STUDY). JAIDS J. Acquir. Immune Defic. Syndr. 2011. p. 399–407.
82. Cavacini LA, Samore MH, Gambertoglio J, Jackson B, Duval M, Wisnewski A, et al. Phase I study of a human monoclonal antibody directed against the CD4-binding site of HIV type 1 glycoprotein 120. AIDS Res. Hum. Retroviruses [Internet]. 1998;14:545–50. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9591708
83. Armbruster C, Stiegler GM, Vcelar BA, Jäger W, Köller U, Jilch R, et al. Passive immunization with the anti-HIV-1 human monoclonal antibody (hMAb) 4E10 and the hMAb combination 4E10/2F5/2G12. J. Antimicrob. Chemother. [Internet]. 2004;54:915–20. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15456731
84. Armbruster C, Stiegler GM, Vcelar BA, Jäger W, Michael NL, Vetter N, et al. A phase I trial with two human monoclonal antibodies (hMAb 2F5, 2G12) against HIV-1. AIDS [Internet]. 2002;16:227–33. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11807307
85. Murray JM, Emery S, Kelleher AD, Law M, Chen J, Hazuda DJ, et al. Antiretroviral therapy with the integrase inhibitor raltegravir alters decay kinetics of HIV, significantly reducing the second phase. AIDS. 2007;21:2315–21.
86. Fridericia LS. The duration of systole in an electrocardiogram in normal humans and in patients with heart disease. Ann. Noninvasive Electrocardiol. 2003. p. 343–51.
87. Brussel A, Sonigo P. Analysis of Early Human Immunodeficiency Virus Type 1 DNA Synthesis by Use of a New Sensitive Assay for Quantifying Integrated Provirus Analysis of Early Human Immunodeficiency Virus Type 1 DNA Synthesis by Use of a New Sensitive Assay for Quantifying Int. J. Virol. 2003;77:10119–24.
88. Denton PW, Olesen R, Choudhary SK, Archin NM, Wahl a., Swanson MD, et al. Generation of HIV Latency in Humanized BLT Mice. J. Virol. 2012;86:630–4.
89. Laird GM, Eisele EE, Rabi SA, Lai J, Chioma S, Blankson JN, et al. Rapid Quantification of the Latent Reservoir for HIV-1 Using a Viral Outgrowth Assay. Silvestri G, editor. PLoS Pathog. [Internet]. 2013;9:e1003398. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3667757&tool=pmcentrez&rendertype=abstract
90. Archin NM, Vaidya NK, Kuruc JD, Liberty a. L, Wiegand A, Kearney MF, et al. Immediate antiviral therapy appears to restrict resting CD4+ cell HIV-1 infection without accelerating the decay of latent infection. Proc. Natl. Acad. Sci. 2012;109:9523–8.
91. Archin NM, Eron JJ, Palmer S, Hartmann-Duff A, Martinson J a, Wiegand A, et al. Valproic acid without intensified antiviral therapy has limited impact on persistent HIV infection of resting CD4+ T cells. AIDS [Internet]. 2008;22:1131–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18525258
92. Archin NM, Liberty a. L, Kashuba a. D, Choudhary SK, Kuruc JD, Crooks a. M, et al. Administration of vorinostat disrupts HIV-1 latency in patients on antiretroviral therapy.

Nature [Internet]. Nature Publishing Group; 2012;487:482–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22837004
93. Lehrman G, Hogue IB, Palmer S, Jennings C, Spina C a, Wiegand A, et al. Depletion of latent HIV-1 infection in vivo: a proof-of-concept study. Lancet [Internet]. 2005;366:549–55. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1894952&tool=pmcentrez&rendertype=abstract
94. Besson GJ, Lalama CM, Bosch RJ, Gandhi RT, Bedison M a., Aga E, et al. HIV-1 DNA Decay Dynamics in Blood During More Than a Decade of Suppressive Antiretroviral Therapy. Clin. Infect. Dis. [Internet]. 2014;59. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25073894
95. Meditz AL, Connick E, McCarter M. Safety of Excisional Inguinal Lymph Node Biopsies Performed for Research Purposes in HIV-1-Infected Women and Men. Surg. Infect. (Larchmt). [Internet]. 2014;15:399–403. Available from: http://online.liebertpub.com/doi/abs/10.1089/sur.2013.218
96. Deeks SG, Phillips AN. HIV infection, antiretroviral treatment, ageing, and non-AIDS related morbidity. BMJ [Internet]. 2009;338:a3172. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19171560
97. Tierney C, Lathey JL, Christopherson C, Bettendorf DM, D'Aquila RT, Hammer SM, et al. Prognostic value of baseline human immunodeficiency virus type 1 DNA measurement for disease progression in patients receiving nucleoside therapy. J. Infect. Dis. [Internet]. 2003;187:144–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12508159
98. Kostrikis LG, Touloumi G, Karanicolas R, Pantazis N, Anastassopoulou C, Goedert JJ, et al. Quantitation of Human Immunodeficiency Virus Type 1 DNA Forms with the Second Template Switch in Peripheral Blood Cells Predicts Disease Progression Independently of Plasma RNA Load Quantitation of Human Immunodeficiency Virus Type 1 DNA Forms with the Se. 2002;
99. El-Sadr W, Lundgren J, Neaton J, Gordin F, Abrams D, Arduino R, et al. CD4+count-guided interruption of antiretroviral treatment. N. Engl. J. Med. [Internet]. 2006;355:2283–96. Available from: http://discovery.ucl.ac.uk/6514/
100. Kutzler M a, Jacobson JM. Treatment interruption as a tool to measure changes in immunologic response to HIV-1. Curr. Opin. HIV AIDS [Internet]. 2008;3:131–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19372954
101. Paton NI. Treatment interruption strategies: how great are the risks? Curr. Opin. Infect. Dis. [Internet]. 2008;21:25–30. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18192782
102. Azzoni L, Papasavvas E, Montaner LJ. Lessons learned from HIV treatment interruption: safety, correlates of immune control, and drug sparing. Curr. HIV Res. [Internet]. 2003;1:329–42. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15046257
103. Papasavvas E, Grant RM, Sun J, Mackiewicz A, Pistilli M, Gallo C, et al. Lack of persistent drug-resistant mutations evaluated within and between treatment interruptions in chronically HIV-1-infected patients. AIDS [Internet]. 2003;17:2337–43. Available from: http://www.ncbi.nlm.nih.gov/pubmed/14571185
104. Archin NM, Cheema M, Parker D, Wiegand A, Bosch RJ, Coffin JM, et al. Antiretroviral intensification and valproic acid lack sustained effect on residual HIV-1 viremia or resting CD4+ cell infection. PLoS One [Internet]. 2010;5:e9390. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2826423&tool=pmcentrez&rendertype=abstract
105. Sagot-Lerolle N, Lamine A, Chaix M-L, Boufassa F, Aboulker J-P, Costagliola D, et al. Prolonged valproic acid treatment does not reduce the size of latent HIV reservoir. AIDS [Internet]. 2008;22:1125–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18525257

106. Siliciano JD, Lai J, Callender M, Pitt E, Zhang H, Margolick JB, et al. Stability of the latent reservoir for HIV-1 in patients receiving valproic acid. J. Infect. Dis. [Internet]. 2007;195:833–6. Available from: http://jid.oxfordjournals.org/content/195/6/833.long
107. Elliott JH, Wightman F, Solomon A, Ghneim K, Ahlers J, Cameron MJ, et al. Activation of HIV Transcription with Short-Course Vorinostat in HIV-Infected Patients on Suppressive Antiretroviral Therapy. Cullen BR, editor. PLoS Pathog. [Internet]. 2014;10:e1004473. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25393648
108. Rasmussen TA, Søgaard OS, Brinkmann C, Wightman F, Lewin SR, Melchjorsen J, et al. Comparison of HDAC inhibitors in clinical development: Effect on HIV production in latently infected cells and T-cell activation. Hum. Vaccin. Immunother. [Internet]. 2013;9:993–1001. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23370291