Long-acting Antiretroviral Therapy for SIV-infected Rhesus Macaques

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Study Overview

Novel long-acting antiretroviral therapy (LA-ART) offers an effective approach to prevent and treat HIV while reducing the daily pill burden and improving adherence. Development of an analogous regimen for non-human primates used in HIV cure research would reduce animal stress and costs compared with the current standard daily subcutaneous (SC) injectable ART. Here, we developed LA formulations of lenacapavir (LEN) as a solution and cabotegravir as a microsuspension (CAB-MS) for SIV suppression in vivo and directly compared this combination to the daily ART regimen containing Tenofovir Disoproxil Fumarate, Emtricitabine, and Dolutegravir (QD-ART) in rhesus macaques (RM).

Table 1. In vitro antiviral potency estimation for LEN and CAB.

Objective: Estimate SIV model in vivo target concentrations for LEN and CAB.

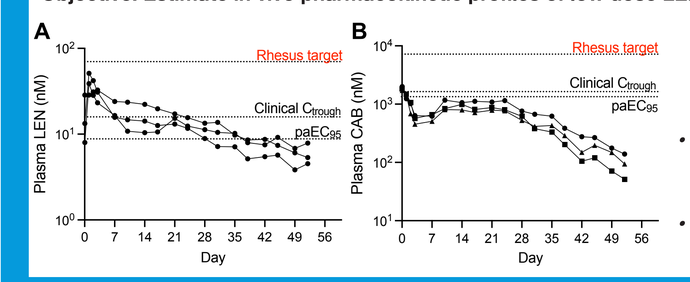
LEN antiviral potency	Rhesus PBMCs (SHIV infected)	Human PBMCs (HIV-1 infected)
EC_{50} (nM)	0.39 ± 0.16	0.05 ± 0.03
EC_{95} (nM)	0.91 ± 0.38	0.12 ± 0.07
Plasma shift value	9.7	17.4
$paEC_{95}$ (nM)	8.80 ± 3.69	2.01 ± 1.22
LEN Target: 70 nM (rh-adjusted clinical C_{trough})		

CAB antiviral potency	Rhesus PBMCs (SIVmac251 infected)	Human PBMCs (HIV-1 infected)
EC_{50} (nM)	1.31	0.33
EC_{95} (nM)	4.56	1.15
Plasma shift value	316.2	138.8
$paEC_{95}$ (nM)	1441	160
CAB Target: 7206 nM (5x $paEC_{95}$)		

EC_{50} against SHIV-SF162P3 (LEN) or SIVmac251 (CAB) in rhesus macaque PBMCs and HIV-1 in human PBMCs using a 7-day replication assay. Protein-adjusted EC_{95} ($paEC_{95}$) values derived from the EC_{50} multiplied by correspoding plasma shift value. LEN displayed 4.4-fold lower protein-adjusted potency against SHIV versus HIV-1 resulting in a rhesus-adjusted in vivo target trough concentration of 70 nM (4.4 x 16nM).

Figure 1. Low dose LEN and CAB pharmacokinetic study in RMs.

Objective: Estimate in vivo pharmacokinetic profiles of low dose LEN and CAB as benchmarks for long-acting exposure



RMs were administered a single dose of SC LEN (human commercial formulation; 100 mg; n=3) or IM CAB (formulated in N-Methylpyrrolidone; 100 mg; n=3). (A) LEN and (B) CAB concentration in plasma was determined.

- ***Need to increase LEN exposure >5-fold to reach Rhesus target.***
- ***Need to increase CAB exposure >10-fold to reach Rhesus target.***

Figure 2. Formulation development to acheive target exposure.

	%w/w	
Excipient/API	100 mg/mL	150 mg/mL
LEN	9.03	13.35
PEG300	60.88	57.39
Water for injection	28.45	26.82
P188	1.64	2.4

LEN is formulated at a concentration of 150 or 100mg/ml with a total dose of ~600 mg per animal.

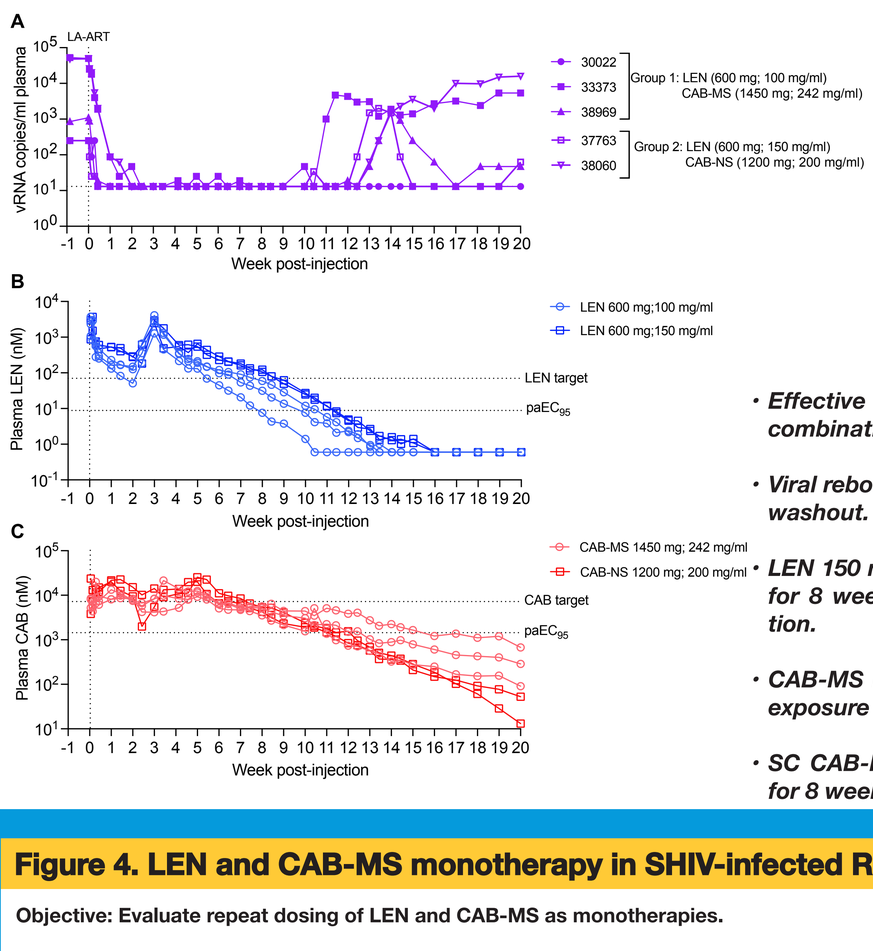
Excipient/API	%w/w
CAB	25
PEG3350	5
Water for injection	66
Tween80	4

CAB powder was milled to obtain a particle size distribution with a D90 of 14um.

CAB-MS is a 242mg/ml microsuspension with a total dose of ~1,450 mg per animal.

Figure 3. Single dose LEN and CAB test formulations in SHIV-infected RMs.

Objective: Evaluate test formulations in order to achieve target exposure after single administration.

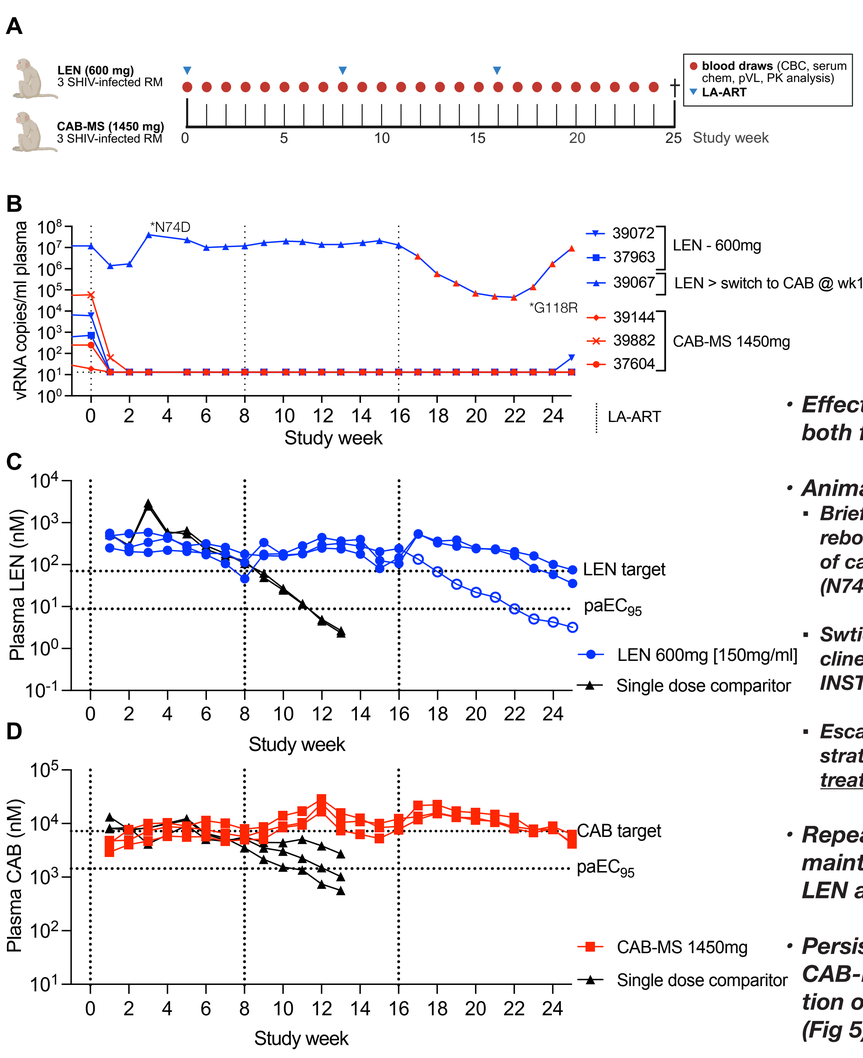


SHIV-infected RMs were given a single dose of SC LEN (600 mg; 100 mg/ml) and SC CAB-MS (1450 mg; 242 mg/ml) (n=3) or SC LEN (600 mg; 150 mg/ml) and SC CAB nanosuspension (commercially sourced as Apretude, ViiV Healthcare, CAB-NS; 1200 mg) (n=2). (A) SHIV plasma viral loads were assessed over the course of the study. Horizonal dashed line indicates limit of detection (<13 copies/ml). Vertical dashed line indicates LA-ART administration. Pharmacokinetics of (B) LEN and (C) CAB (CAB-MS and CAB-NS) in plasma.

- ***Effective SHIV suppression with both combination treatments.***
- ***Viral rebound following LEN and CAB washout.***
- ***LEN 150 mg/ml exceeds target exposure for 8 weeks; chosen for further evaluation.***
- ***CAB-MS requires higher dose to match exposure achieved with CAB-NS.***
- ***SC CAB-MS achieves near target levels for 8 weeks.***

Figure 4. LEN and CAB-MS monotherapy in SHIV-infected RMs.

Objective: Evaluate repeat dosing of LEN and CAB-MS as monotherapies.

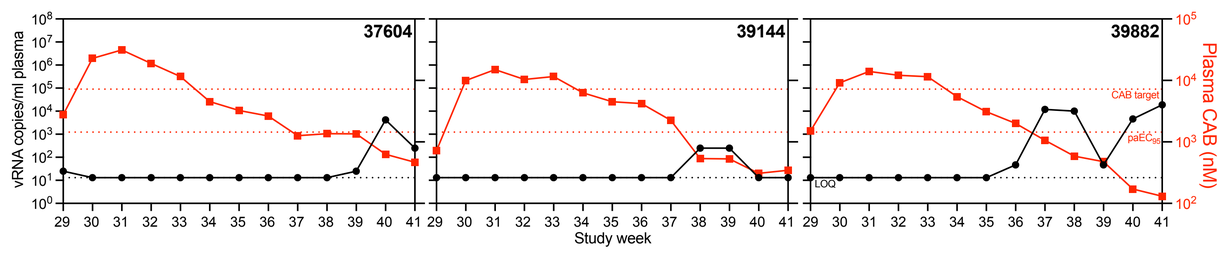


(A) Experimental outline. Two groups of 3 SHIV-infected RMs were administered either 600mg LEN SC or 1450mg CAB-MS SC at 8 week intervals. (B) SHIV plasma viral loads were assessed over the course of the study. Horizonal dashed line indicates limit of detection (<13 copies/ml). Vertical dashed line indicates LA-ART administration. Pharmacokinetics of (C) LEN and (D) CAB-MS in plasma. Note open symbols in C indicate LEN washout from 39067, which did not recieve a third LEN dose.

- ***Effective SHIV suppression with both formulations.***
- ***Animal ID 39067***
  - ***Brief period of suppression, followed by rebound associated with development of capsid inhibitor resistance mutation (N74D).***
  - ***Swtich to CAB yields >2log10 SHIV decline; eventual rebound associated with INSTI resistance mutation (G118R).***
  - ***Escape from monotherapy demonstrates requirement for <u>combination treatment</u>.***
- ***Repeat SC dosing (every 8 weeks) maintains target exposure for both LEN and CAB.***
- ***Persistent edema observed with SC CAB-MS dosing, prompting evaluation of intramuscular (IM) route next (Fig 5).***

Figure 5. IM CAB-MS administration.

Objective: Evaluate SIV suppression and pharmacokinetics after IM CAB-MS administration.

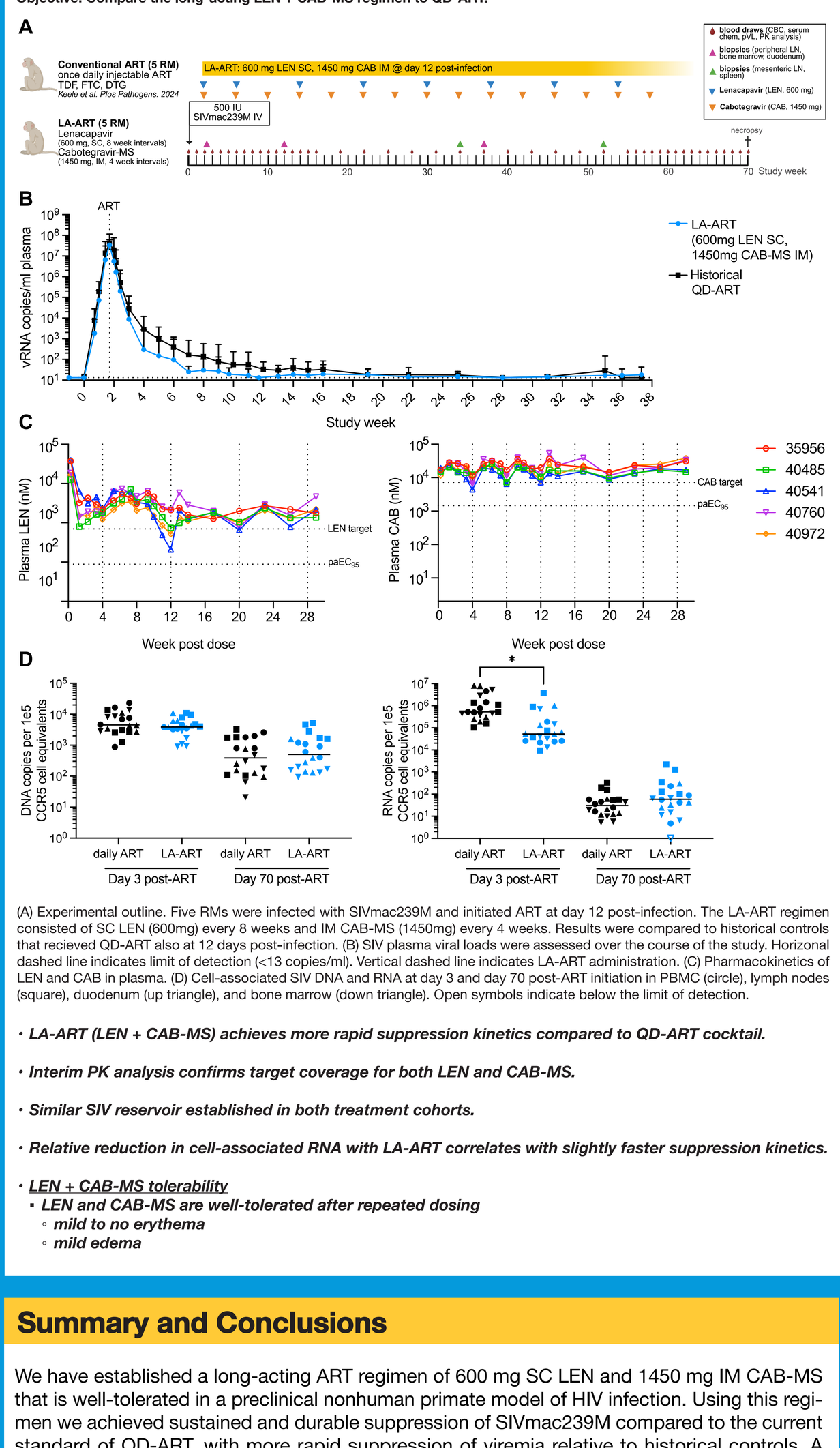


Three SHIV-infected RMs were administered a single dose of IM 1450 mg CAB-MS at study week 29. SHIV plasma viral loads were assessed longitudinally indicated in black on the left axis. Horizonal dashed line indicates limit of quantification (LOQ <13 copies/ml). Pharmacokinetics of CAB-MS in plasma indicated in red on the right axis.

- ***IM CAB-MS (1450mg) maintains target coverage for 4 weeks vs 8 weeks for SC.***
- ***Rebound correlating with drop under target exposure validates in vivo Rhesus CAB target.***
- ***Subsequent studies to continue with IM CAB-MS every 4 weeks + SC LEN every 8 weeks.***

Figure 6. LEN + CAB-MS in SIVmac239-infected RMs.

Objective: Compare the long-acting LEN + CAB-MS regimen to QD-ART.



(A) Experimental outline. Five RMs were infected with SIVmac239M and initiated ART at day 12 post-infection. The LA-ART regimen consisted of SC LEN (600mg) every 8 weeks and IM CAB-MS (1450mg) every 4 weeks. Results were compared to historical controls that recieved QD-ART also at 12 days post-infection. (B) SIV plasma viral loads were assessed over the course of the study. Horizonal dashed line indicates limit of detection (<13 copies/ml). Vertical dashed line indicates LA-ART administration. (C) Pharmacokinetics of LEN and CAB in plasma. (D) Cell-associated SIV DNA and RNA at day 3 and day 70 post-ART initiation in PBMC (circle), lymph nodes (square), duodenum (up triangle), and bone marrow (down triangle). Open symbols indicate below the limit of detection.

- ***LA-ART (LEN + CAB-MS) achieves more rapid suppression kinetics compared to QD-ART cocktail.***
- ***Interim PK analysis confirms target coverage for both LEN and CAB-MS.***
- ***Similar SIV reservoir established in both treatment cohorts.***
- ***Relative reduction in cell-associated RNA with LA-ART correlates with slightly faster suppression kinetics.***
- ***<u>LEN + CAB-MS tolerability</u>***
  - ***LEN and CAB-MS are well-tolerated after repeated dosing***
    - ***mild to no erythema***
    - ***mild edema***

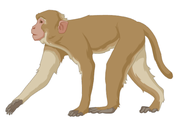
Summary and Conclusions

We have established a long-acting ART regimen of 600 mg SC LEN and 1450 mg IM CAB-MS that is well-tolerated in a preclinical nonhuman primate model of HIV infection. Using this regimen we achieved sustained and durable suppression of SIVmac239M compared to the current standard of QD-ART, with more rapid suppression of viremia relative to historical controls. A dosing strategy of every 8 weeks for LEN and every 4 weeks for CAB-MS maintained plasma concentrations above the target concentrations (70 nM and 7206 nM, respectively). Cell-associated viral RNA in tissues and blood revealed lower viral RNA immediately post-ART in animals that recieved LA-ART. No differences in viral DNA levels were observed between the groups. Post-treatment viral load kinetics are pending following analytical treatment interruption.

SC LEN + IM CAB-MS maintain durable suppression for >37 weeks.

No difference in reservoir establishment compared to QD-ART.

LA-ART reduces handling and stress for RM.



This novel, macaque-specific LA regimen of LEN + CAB robustly suppresses SIV, eliminates the need for daily injections, and better aligns the rhesus macaque model with clinical use of LA-ART.