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Supplementary Materials for

An Intravaginal Ring That Releases the NNRTI MIV-150 Reduces SHIV Transmission in Macaques

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The PDF file includes:

SUPPLEMENTARY METHODS

MIV-150 IVR stability testing

Degradation of MIV-150 from silicone IVRs cured under different conditions was monitored by HPLC. Three cures were tested: RT, normal (90°C for 90min), and high heat (120°C for 48h). After curing, IVR fragments were cut away, and MIV-150 was extracted with DCM. The MIV-150 samples were evaporated and reconstituted in acetonitrile for injection onto HPLC. MIV-150 content in whole IVRs was extrapolated from data on the fragments. Extractions were performed in triplicate for each cure type.

RT gene sequencing

The HIV-1 RT gene of SHIV-RT was sequenced from plasma viral RNA with the following minor modifications to the recently described method (*14*): the Ultra Sens Viral RNA kit (Qiagen) was used for extraction of viral RNA from plasma and the Blunt TOPO TA cloning kit (Invitrogen) was used to clone the RT genes.

SUPPLEMENTARY FIGURE

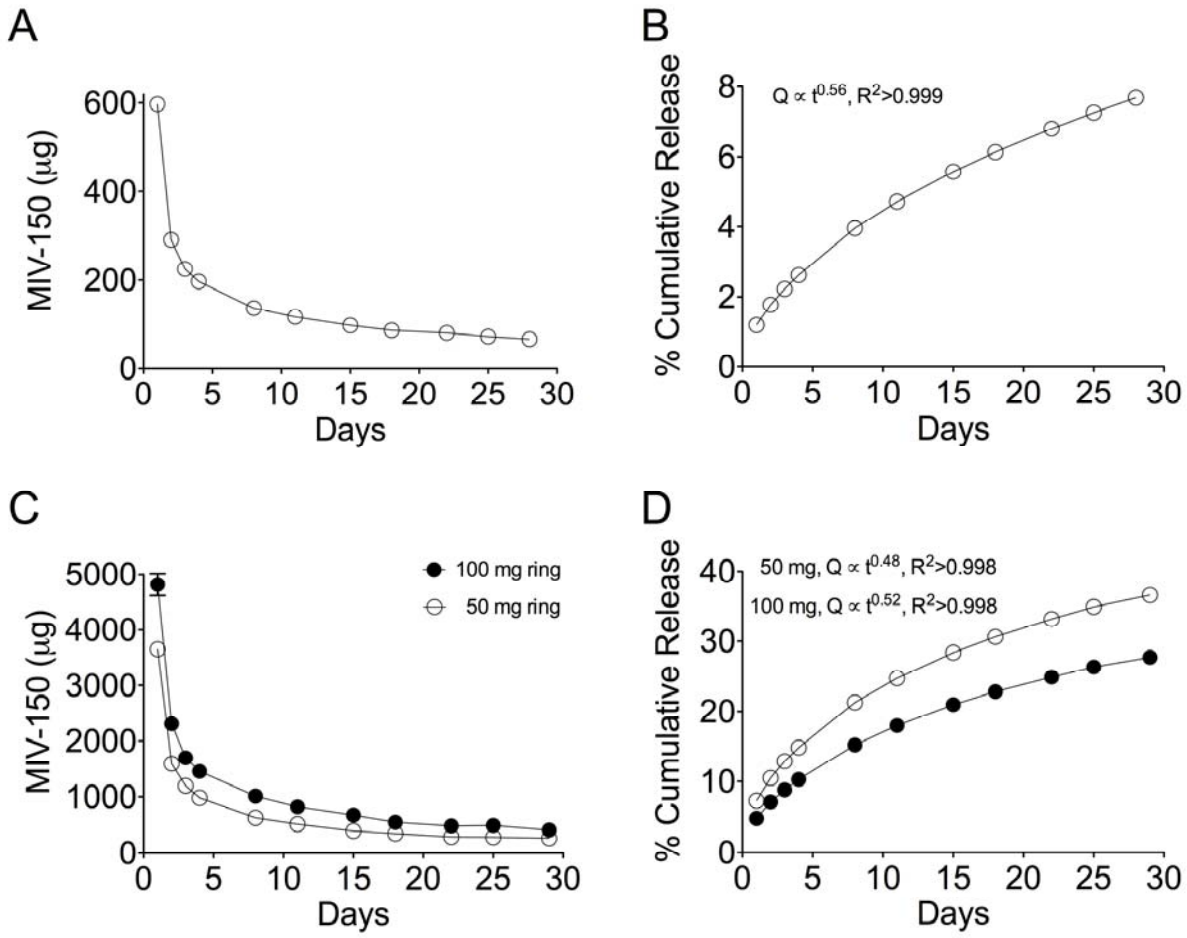

Figure S1. In vitro release of MIV-150 into an aqueous ethanol sink. Daily and percent cumulative release of MIV-150 from 50mg silicone (A and B) and 50mg and 100mg EVA (C and C) IVRs over 29 days in 50% aqueous ethanol (sink conditions).

SUPPLEMENTARY TABLES

Table S1. Sampling details of animals in PK studies

PK study	IVR insertion	Swabs (time PRI)	# of animals	Biopsies (time PRI)	# of animals**
Silicone	3wks post-depo	14 d	13	14 d	6
		28 d	12	28 d	6
EVA-40	3wks post-depo (n=12*)	0.5 h	11		
		1 d	11		
		2 d	11		
		3 d	11		
		14 d	11	14 d	6
		28 d	5	28 d	5
	5wks post-depo (n=12*)	1 h	11		
		1 d	11	1 d	6
		14 d	5	14 d	5

*One animal in each of the EVA-40 depo groups had no IVR present upon examination at the conclusion of the study and was excluded from the analysis. After initially detecting MIV-150, no MIV-150 was detected in swabs after a certain point in these animals, indicating when the IVRs came out.

**No swabs were collected following biopsy to eliminate changes in swab MIV-150 concentration or levels of innate immune mediators that might result from biopsy.

Table S2. Infection and antibody status for macaques that received silicone IVRs.

IVR duration in vagina		**IVR**	**Animal ID**	**Infection**	**Ab response**
Pre challenge	Post challenge				
2wk	2wk	Placebo	HL63	-	-
			HL66	+	+
			HL67	-	-
			HM25	+	+
			HM32	+*	-
		MIV-150	HL69	-	-
			HL70	-	-
			HL71	-	-
			HL72	+	+
			HM34	-	-
			HM35	+	+
			HM38	-	-

*Infection of HM32 was confirmed by repeated plasma viral RNA testing, nested PCR for PBMC SIV DNA, and quantitative SIV DNA PCR on PBMC-CEMx174 co-cultures at wk3.

Table S3. No selection of NNRTI-resistant variants by MIV-150 silicone IVRs.

RING	Animal ID	L100I, K101P, K103N, V108I, I178L, V179I, Y181C, Y188L, G190E, P225H
placebo	HM25	0 (9)
	HL66	0 (7)
MIV-150	HM35	0 (7)
	HL72	0 (9)

The table lists the number of clones in which amino acid mutations conferring NNRTI resistance were detected. Parentheses indicate the total number of clones sequenced per animal.

Table S4. Infection and antibody status for macaques that received EVA IVRs.

IVR duration in vagina		**IVR**	**Animal ID**	**Infection**	**Ab response**
Pre challenge	Post challenge				
2wk	2wk	Placebo	DP69	+	+
			GR96	-	-
			BR72	-	-
24h			BG88	+	+
			DJ37**	+	+
			DH73	+	+
			FL97	+	+
			$DG31_2$	+	+
			GN40	-	-
24h	0wk		$AN57_2$	+	+
			GK98	+	+
2wk	2wk	MIV-150	$AN57_1$	-	-
			$CB12_1$*	-	-
			$DA69_1$	-	-
			$DG31_1$	-	-
			DH66*	-	-
			DJ98	+	+
			$EK86_1$	-	-
			DD56	-	-
			DG41	-	-
24h			$BI34_1$	-	-
			$DJ19_1$*	-	-
			DH69	+	+
			DV94	-	-
			$EK63_1$	-	-
			FR02	-	-
			$GV94_1$	-	-
			FM70	-	-
24h	0wk		$BI34_2$	+	+
			$CB12_2$	+	+
			$DA69_2$	+	-
			$EK63_2$	+	+
			$EK86_2$	-	-

			GV93	-	-
			$GV94_2$	-	-

*No ring was detected 2wks post-challenge. Since these animals remained uninfected, we included them in the analysis.

**Euthanized on week 12 (positive two time points earlier)

Subscript numbers indicate animals that were recycled within the study with the number indicating the challenge from which the samples were taken.

Table S5. No selection of NNRTI-resistant variants by MIV-150 EVA IVRs.

RING	**Animal ID**	**L100I, K101P, K103N, V108I, I178L, V179I, Y181C, Y188L, G190E, P225H**
placebo	BG88	0 (21)
	DJ37	0 (21)
	DP69	0 (11)
MIV-150	DH69	0 (18)
	DJ98	0 (16)

The table lists the number of clones in which amino acid mutations conferring NNRTI resistance were detected. Parentheses indicate the total number of clones sequenced per animal.