

Supplementary Materials

Effects of Injection Volume and Route of Administration on Dolutegravir In Situ Forming Implant Pharmacokinetics

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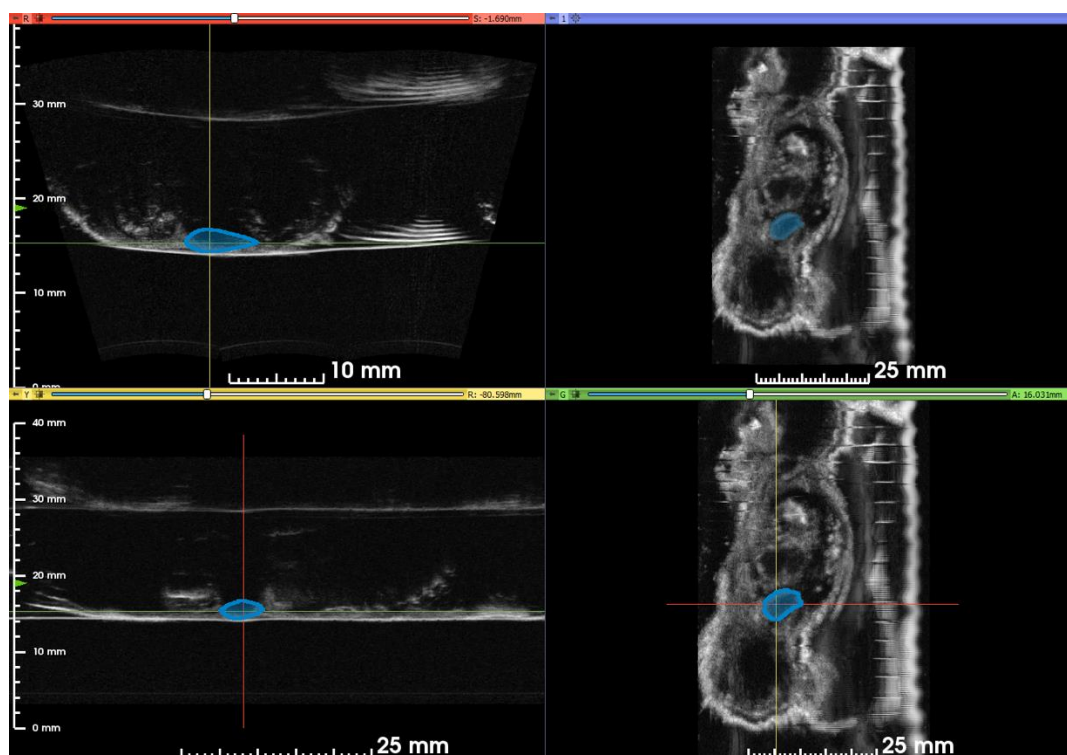


Figure S1. Representative image of the B mode ultrasound image segmentation process in SonoEQ software for a $1 \times 80 \mu\text{L}$ DTG-loaded ISFI (1:2 w/w PLGA/NMP) implant injected into female NSG mouse. The red line indicates the coronal slice location, the yellow line indicates the sagittal slice location, and the green line indicates the axial slice location. (A) Interpolated ISFI segmentation in the coronal slice (B) 3-D view of the interpolated ISFI segmentation (C) Interpolated ISFI segmentation in the sagittal slice (D) Interpolated ISFI segmentation in the axial slice.

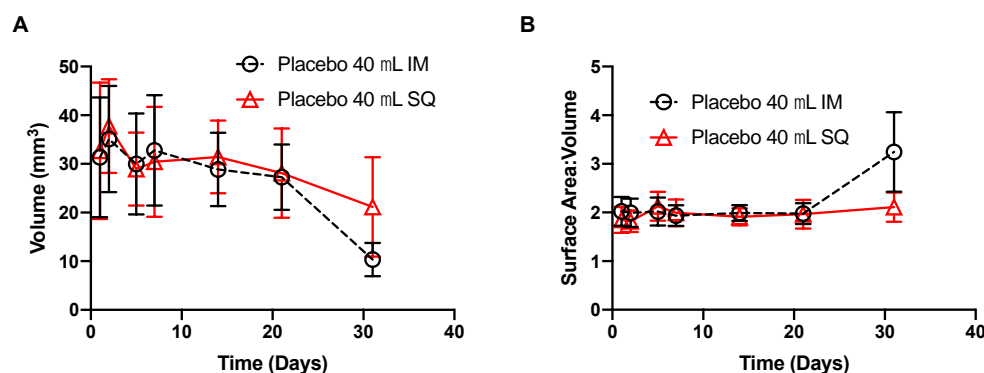


Figure S2. Comparison of $40 \mu\text{L}$ (SQ: $n = 5$ mice/group; $n = 2$ implants/mouse; IM: $n = 10$ mice/group; $n = 1$ implant/mouse) placebo ISFI (1:2 w/w PLGA/NMP) implants injected into female NSG mice.

(A) Volume of 40 μ l IM and SQ placebo ISFIs over 30 days. (B) Surface area to volume ratio of 40 μ l IM and SQ placebo ISFIs over 30 days.

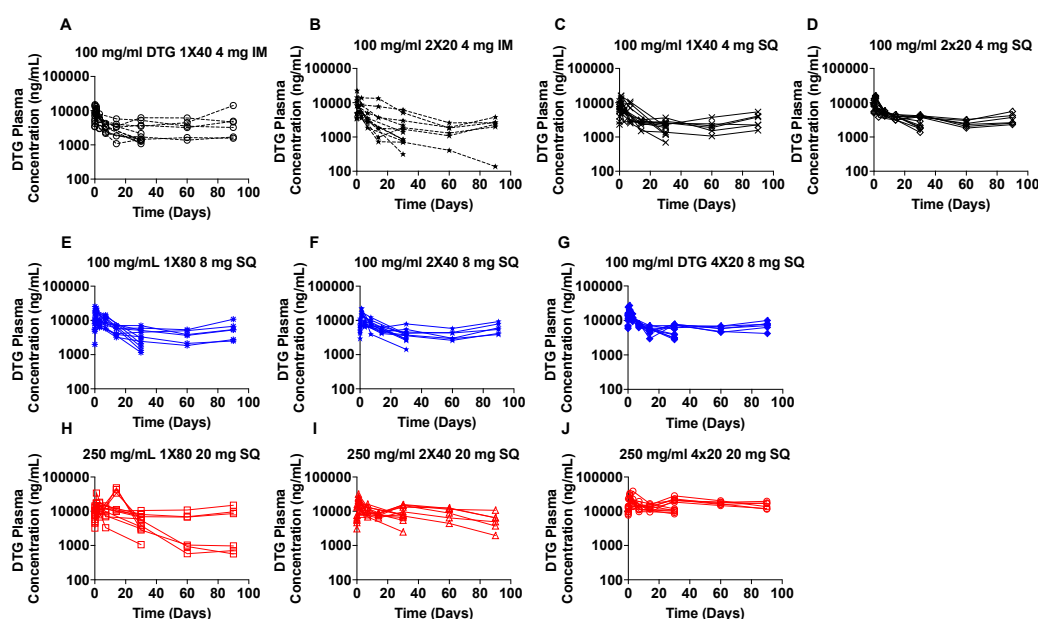


Figure S3. In vivo pharmacokinetics of DTG ISFIs (1:2 w/w PLGA/NMP) from individual female NSG mice ($n = 10$ mice/group). (A) 100 mg/ml 1×40 μ l IM ISFIs (B) 100 mg/ml 2×20 μ l IM ISFIs (C) 100 mg/ml 1×40 μ l SQ ISFIs (D) 100 mg/ml 2×20 μ l SQ ISFIs (E) 100 mg/ml 1×80 μ l SQ ISFIs (F) 100 mg/ml 2×40 μ l SQ ISFIs (G) 100 mg/ml 4×20 μ l SQ ISFIs (H) 250 mg/ml 1×80 μ l SQ ISFIs (I) 250 mg/ml 2×40 μ l SQ ISFIs (J) 250 mg/ml 4×20 μ l SQ ISFIs.

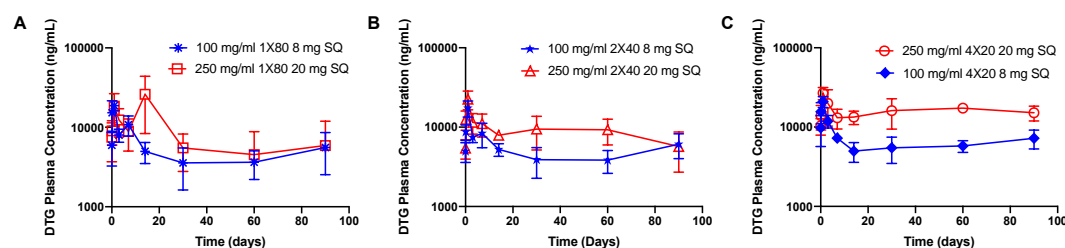


Figure S4. PK of 100 mg/ml and 250 mg/ml DTG-loaded ISFIs (1:2 w/w PLGA/NMP) injected into female NSG mice ($n = 10$ mice/group). Each graph compares the effect of injection volume when varying drug loading from 100 mg/ml to 250 mg/ml DTG injected SQ. (A) PK of 1×80 μ l ISFI injections. (B) PK of 2×40 μ l ISFI injections. (C) PK of 4×20 μ l ISFI injections.

Table S1. Summary table of partial AUC analysis for individual rodents through day 30.

Group	Rodent_ID	AUC 0-30d
100 mg/ml 4×20 8 mg s.c.	1.00	5209420.00
	2.00	5415020.00
	3.00	5256880.00
	4.00	5012540.00
	5.00	5816460.00
	6.00	5790730.00
	7.00	6864150.00
	8.00	5935410.00
	9.00	11523840.00
	10.00	6667800.00

	11.00	7043520.00
	12.00	6529920.00
	Median	5875935.00
	25%	5296415.00
	75%	6815062.50
100 mg/ml 2×40 8 mg s.c.	13.00	5174070.00
	14.00	3667860.00
	15.00	4642820.00
	16.00	6899390.00
	17.00	5231870.00
	18.00	5699120.00
	19.00	3595800.00
	20.00	4283025.00
	21.00	3582435.00
	22.00	3996525.00
	23.00	5467905.00
	24.00	4228005.00
	Median	4462922.50
	25%	3750026.25
	75%	5408896.25
100 mg/ml 1×80 8 mg s.c.	25.00	6173120.00
	26.00	4375730.00
	27.00	7211930.00
	28.00	5564720.00
	29.00	6188170.00
	30.00	6901030.00
	31.00	4102320.00
	32.00	3292290.00
	33.00	3509235.00
	34.00	5154390.00
	35.00	5451495.00
	36.00	4872900.00
	Median	5302942.50
	25%	4170672.50
	75%	6184407.50
100 mg/ml 2×40 4 mg s.c.	37.00	3742180.00
	38.00	3226260.00
	39.00	3358140.00
	40.00	3806360.00
	41.00	3548340.00
	42.00	3757320.00
	43.00	2878920.00
	44.00	3476655.00
	45.00	3490095.00
	46.00	3422580.00
	47.00	3479160.00
	48.00	2806050.00
	Median	3477907.50
	25%	3259230.00
	75%	3693720.00
100 mg/ml 1×40 4 mg s.c.	49.00	5633570.00
	50.00	4124750.00
	51.00	4437560.00
	52.00	2828470.00

	53.00	2180526.00
	54.00	1973695.00
	55.00	2170080.00
	56.00	2353695.00
	57.00	2571795.00
	58.00	2812590.00
	59.00	2883765.00
	60.00	2472390.00
	Median	2692192.50
	25%	2223818.25
	75%	3814503.75
250 mg/ml 4×20 20 mg s.c.	61.00	9563840.00
	62.00	9504990.00
	63.00	7988520.00
	64.00	10341550.00
	65.00	12025660.00
	66.00	8961670.00
	67.00	10467150.00
	68.00	13276800.00
	69.00	11456700.00
	70.00	11591760.00
	71.00	16205850.00
	72.00	11264700.00
	Median	10865925.00
	25%	9519702.50
	75%	11917185.00
250 mg/ml 2×40 20 mg s.c.	73.00	6306400.00
	74.00	5282100.00
	75.00	9447400.00
	76.00	8700250.00
	77.00	6502470.00
	78.00	8916600.00
	79.00	6060240.00
	80.00	5550405.00
	81.00	8455530.00
	82.00	7706550.00
	83.00	7215600.00
	84.00	8287800.00
	Median	7461075.00
	25%	6121780.00
	75%	8639070.00
250 mg/ml 1×80 20 mg s.c.	85.00	2967930.00
	86.00	6874450.00
	87.00	4889110.00
	88.00	6923920.00
	89.00	8644170.00
	90.00	5900050.00
	91.00	13552530.00
	92.00	16557450.00
	93.00	17662695.00
	94.00	8575650.00
	95.00	8430780.00
	96.00	7452900.00
	Median	7941840.00

	25%	6143650.00
	75%	12325440.00
100 mg/ml 2×20 4 mg i.m.	97.00	3038395.00
	98.00	1938817.00
	99.00	1894745.00
	100.00	1339455.00
	101.00	2299090.00
	102.00	1975879.00
	103.00	2061270.00
	104.00	1649661.00
	105.00	4026165.00
	106.00	8029950.00
	107.00	5669145.00
	108.00	1393065.00
	Median	2018574.50
	25%	1710932.00
	75%	3779222.50
100 mg/ml 1×40 4 mg i.m.	109.00	2433510.00
	110.00	3094930.00
	111.00	2144470.00
	112.00	2249010.00
	113.00	2301000.00
	114.00	2245095.00
	115.00	1714650.00
	116.00	3555510.00
	117.00	3365265.00
	118.00	3298815.00
	119.00	1456275.00
	120.00	4742055.00
	Median	2367255.00
	25%	2169626.25
	75%	3348652.50

Table S2. Pooled 90-day NCA analysis for each treatment group.

Group	Dose (mg)	C _{max} (ng/ml)	SE _{C_{max}} (ng/ml)	T _{max} (hr)	AUC _{0-12h} (hr*ng/ml)	SE _{AUC_{0-12h}} (hr*ng/ml)
100 mg/ml 4×20 s.c.	8	20916.67	1329.77	24.00	15501924.17	1015938.51
100 mg/ml 2×40 s.c.	8	17383.33	1618.11	24.00	10912253.33	957211.45
100 mg/ml 1×80 s.c.	8	18766.67	1022.96	24.00	10797044.17	1129956.20
250 mg/ml 4×20 s.c.	20	26883.33	1925.52	24.00	34759638.33	1650513.36
250 mg/ml 2×40 s.c.	20	22500.00	2423.22	24.00	19355618.75	1902268.03
250 mg/ml 1×80 s.c.	20	26080.00	7250.18	336.00	18438518.33	1198904.52
100 mg/ml 2×20 s.c.	4	14600.00	508.59	24.00	7434050.00	404444.56
100 mg/ml 1×40 s.c.	4	11888.33	1718.43	24.00	6214291.00	568354.27

100 mg/ml 2×20 i.m.	4	12638.33	2164.93	3.00	5721920.83	1194308.24
100 mg/ml 1×40 i.m.	4	11366.67	1268.22	3.00	7839729.17	1370045.07

^a SE denotes standard error estimate for each PK parameter

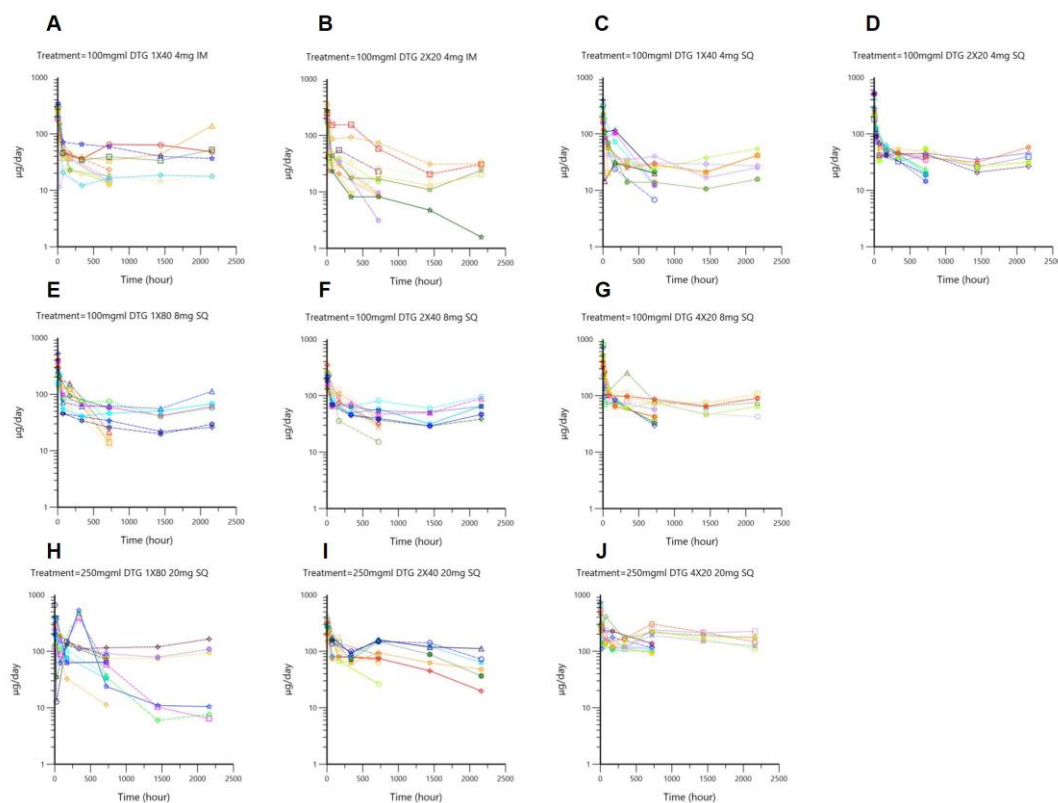


Figure S5. Release rates of DTG ISFIs (1:2 w/w PLGA/NMP) from individual female NSG mice (n = 10 mice/group) as modeled by deconvolution analysis. (A) 100 mg/ml 1 × 40 µl IM ISFIs (B) 100 mg/ml 2 × 20 µl IM ISFIs (C) 100 mg/ml 1 × 40 µl SQ ISFIs (D) 100 mg/ml 2 × 20 µl SQ ISFIs (E) 100 mg/ml 1 × 80 µl SQ ISFIs (F) 100 mg/ml 2 × 40 µl SQ ISFIs (G) 100 mg/ml 4 × 20 µl SQ ISFIs (H) 250 mg/ml 1 × 80 SQ ISFIs (I) 250 mg/ml 2 × 40 SQ ISFIs (J) 250 mg/ml 4 × 20 µl SQ ISFIs.

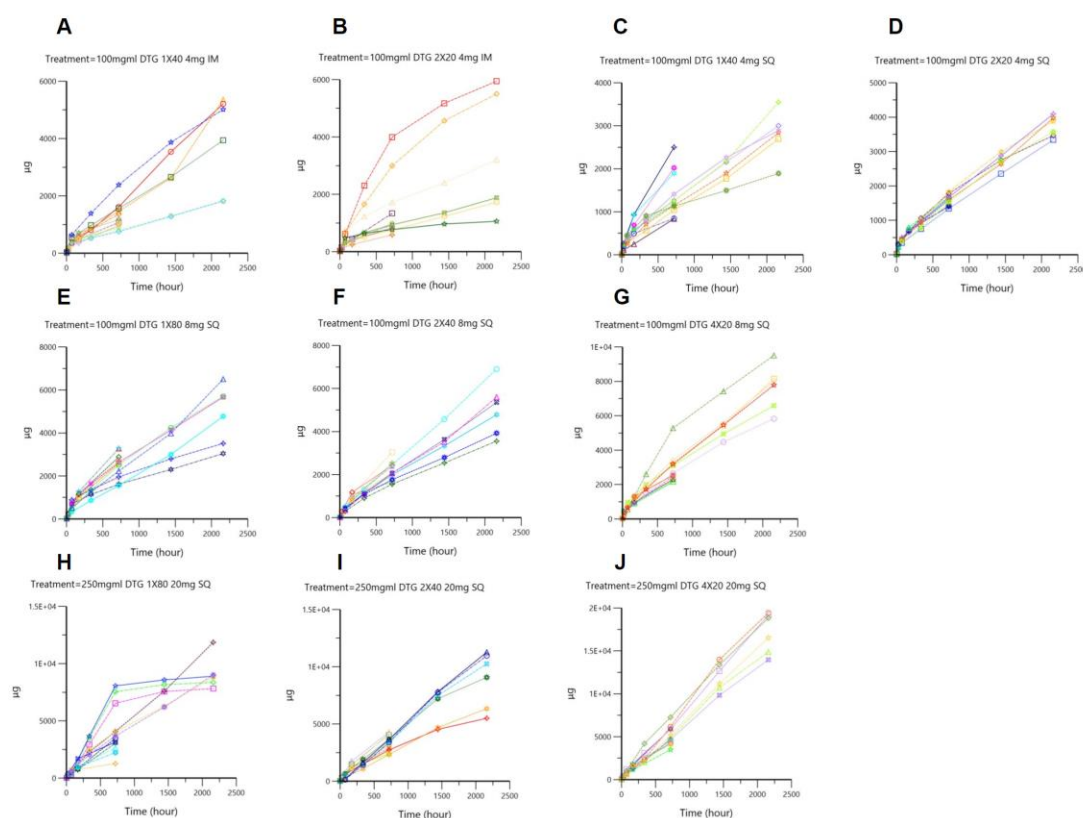


Figure S6. Cumulative release of DTG from ISFIs (1:2 w/w PLGA/NMP) from individual female NSG mice ($n = 10$ mice/group) as modeled by deconvolution analysis. (A) 100 mg/ml 1×40 μ l IM ISFIs (B) 100 mg/ml 2×20 μ l IM ISFIs (C) 100 mg/ml 1×40 μ l SQ ISFIs (D) 100 mg/ml 2×20 μ l SQ ISFIs (E) 100 mg/ml 1×80 μ l SQ ISFIs (F) 100 mg/ml 2×40 μ l SQ ISFIs (G) 100 mg/ml 4×20 μ l SQ ISFIs (H) 250 mg/ml 1×80 μ l SQ ISFIs (I) 250 mg/ml 2×40 μ l SQ ISFIs (J) 250 mg/ml 4×20 μ l SQ ISFIs.

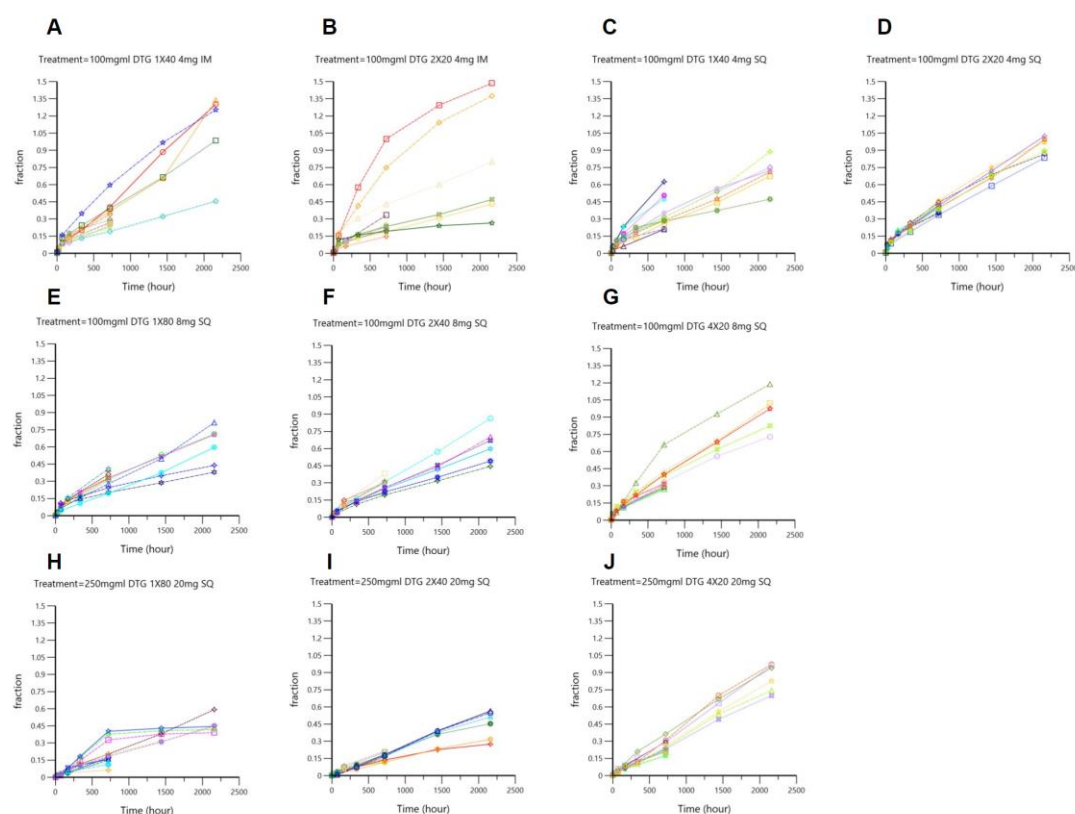


Figure S7. Fraction of DTG released from ISFIs (1:2 w/w PLGA/NMP) from individual female NSG mice ($n = 10$ mice/group) as modeled by deconvolution analysis. (A) 100 mg/ml 1×40 μ l IM ISFIs (B) 100 mg/ml 2×20 μ l IM ISFIs (C) 100 mg/ml 1×40 μ l SQ ISFIs (D) 100 mg/ml 2×20 μ l SQ ISFIs (E) 100 mg/ml 1×80 μ l SQ ISFIs (F) 100 mg/ml 2×40 μ l SQ ISFIs (G) 100 mg/ml 4×20 μ l SQ ISFIs (H) 250 mg/ml 1×80 SQ ISFIs (I) 250 mg/ml 2×40 SQ ISFIs (J) 250 mg/ml 4×20 μ l SQ ISFIs.

Table S3. Median estimated deconvolution parameters of 4×20 , 2×40 , and 1×80 μ l DTG-loaded ISFIs (1:2 w/w PLGA:NMP).

		Variable		
		Fraction Input	μ g	μ g/day
Treatment	Time (hour)	Median		
100 mg/ml DTG 1×40 4mg IM	1.00	0.00	11.69	252.86
	3.00	0.01	30.73	230.80
	24.00	0.05	185.30	77.69
	72.00	0.10	406.17	41.67
	168.00	0.12	489.22	36.72
	336.00	0.21	845.15	35.06
	720.00	0.28	1127.56	18.05
	1440.00	0.66	2641.25	36.79
	2160.00	1.12	4479.55	42.36
	Min	0.00	11.69	18.05
	Max	1.12	4479.55	252.86
100 mg/ml DTG 1×40 4mg SQ	1.00	0.00	11.55	245.03
	3.00	0.01	22.64	170.96
	24.00	0.05	182.99	91.66
	72.00	0.09	345.66	55.00
	336.00	0.20	805.36	28.98

	720.00	0.30	1213.66	21.30
	1440.00	0.51	2028.16	20.72
	2160.00	0.72	2864.04	34.28
	Min	0.00	11.55	20.72
	Max	0.72	2864.04	245.03
100 mg/ml DTG 1 × 80 8mg SQ	1.00	0.00	13.78	310.60
	3.00	0.01	42.51	321.89
	24.00	0.03	250.17	178.37
	72.00	0.08	606.46	63.46
	168.00	0.14	1107.85	126.46
	336.00	0.16	1286.80	50.86
	720.00	0.32	2552.86	33.59
	1440.00	0.44	3492.47	41.05
	2160.00	0.65	5221.91	58.92
	Min	0.00	13.78	33.59
	Max	0.65	5221.91	321.89
100 mg/ml DTG 2 × 20 4mg IM	1.00	0.00	10.66	225.89
	3.00	0.01	34.77	264.38
	24.00	0.04	143.47	44.60
	72.00	0.14	541.04	49.02
	168.00	0.10	397.55	33.79
	336.00	0.24	942.28	25.88
	720.00	0.23	931.41	15.99
	1440.00	0.47	1879.25	15.32
	2160.00	0.64	2542.03	27.39
	Min	0.00	10.66	15.32
	Max	0.64	2542.03	264.38
100 mg/ml DTG 2 × 20 4mg SQ	1.00	0.00	16.65	358.00
	3.00	0.01	29.20	220.66
	24.00	0.06	238.09	104.59
	72.00	0.11	425.83	40.94
	168.00	0.18	704.03	52.14
	336.00	0.24	941.50	43.86
	720.00	0.40	1605.80	36.25
	1440.00	0.68	2739.23	26.87
	2160.00	0.93	3724.21	35.45
	Min	0.00	16.65	26.87
	Max	0.93	3724.21	358.00
100 mg/ml DTG 2 × 40 8mg SQ	1.00	0.00	11.95	269.08
	3.00	0.00	26.56	202.16
	24.00	0.03	228.17	150.24
	72.00	0.05	418.20	70.28
	168.00	0.13	1003.98	87.63
	336.00	0.14	1116.60	56.09
	720.00	0.26	2074.43	39.25
	1440.00	0.43	3428.56	40.71
	2160.00	0.63	5070.64	64.72
	Min	0.00	11.95	39.25
	Max	0.63	5070.64	269.08
100 mg/ml DTG 4 × 20 8mg SQ	1.00	0.00	20.79	460.85
	3.00	0.01	42.47	322.96
	24.00	0.04	336.09	139.82
	72.00	0.08	644.54	116.84

	168.00	0.12	972.13	78.12
	336.00	0.23	1876.75	94.63
	720.00	0.32	2599.09	62.61
	1440.00	0.68	5444.06	63.85
	2160.00	0.99	7908.07	81.44
	Min	0.00	20.79	62.61
	Max	0.99	7908.07	460.85
250 mg/ml DTG 1 × 80 20mg SQ	1.00	0.00	18.63	402.74
	3.00	0.00	28.53	219.79
	24.00	0.01	275.94	133.08
	72.00	0.03	503.56	128.20
	168.00	0.05	900.77	91.11
	336.00	0.14	2700.46	255.39
	720.00	0.18	3572.21	61.21
	1440.00	0.38	7580.87	42.64
	2160.00	0.44	8851.90	52.45
	Min	0.00	18.63	42.64
	Max	0.44	8851.90	402.74
250 mg/ml DTG 2 × 40 20mg SQ	1.00	0.00	14.26	325.55
	3.00	0.00	35.49	271.60
	24.00	0.01	269.01	239.59
	72.00	0.02	443.21	108.55
	168.00	0.06	1232.45	120.55
	336.00	0.07	1464.62	79.93
	720.00	0.17	3377.31	86.01
	1440.00	0.37	7352.62	103.26
	2160.00	0.48	9663.33	55.73
	Min	0.00	14.26	55.73
	Max	0.48	9663.33	325.55
250 mg/ml DTG 4 × 20 20mg SQ	1.00	0.00	23.85	542.04
	3.00	0.00	35.04	277.18
	24.00	0.02	413.79	249.53
	72.00	0.03	641.94	153.63
	168.00	0.08	1608.00	119.53
	336.00	0.11	2271.83	138.20
	720.00	0.24	4774.83	164.35
	1440.00	0.60	11912.41	180.02
	2160.00	0.88	17684.01	159.70
	Min	0.00	23.85	119.53
	Max	0.88	17684.01	542.04

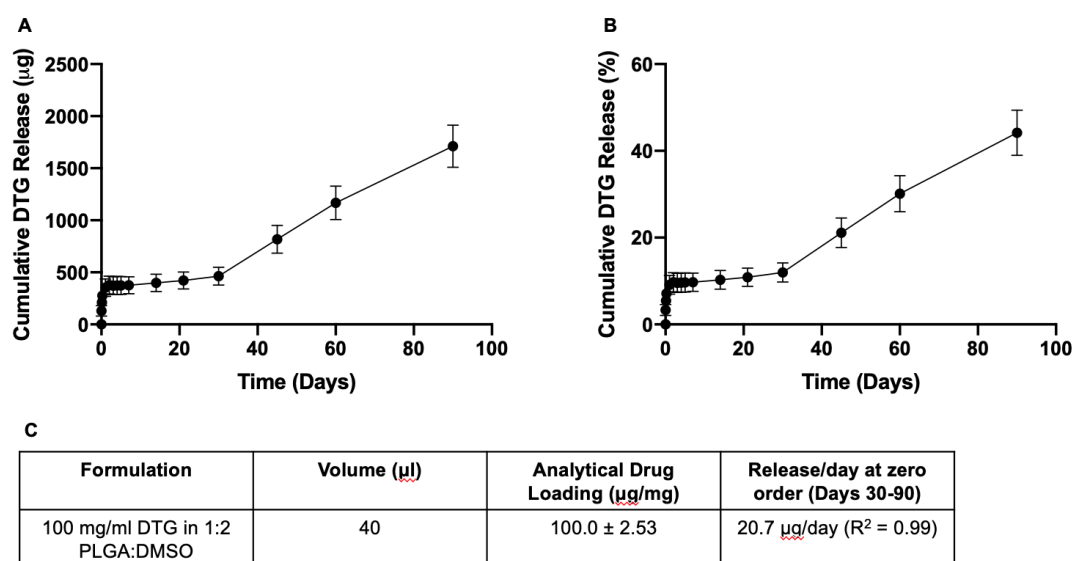


Figure S8. In vitro drug release of 40 μl 100 mg/ml DTG ISFIs ($n=4$) over 90 days in a 1:2 PLGA:DMSO polymer-solvent system shown as cumulative DTG release in μg (a) and cumulative DTG release as a percentage of initial drug loaded (b).

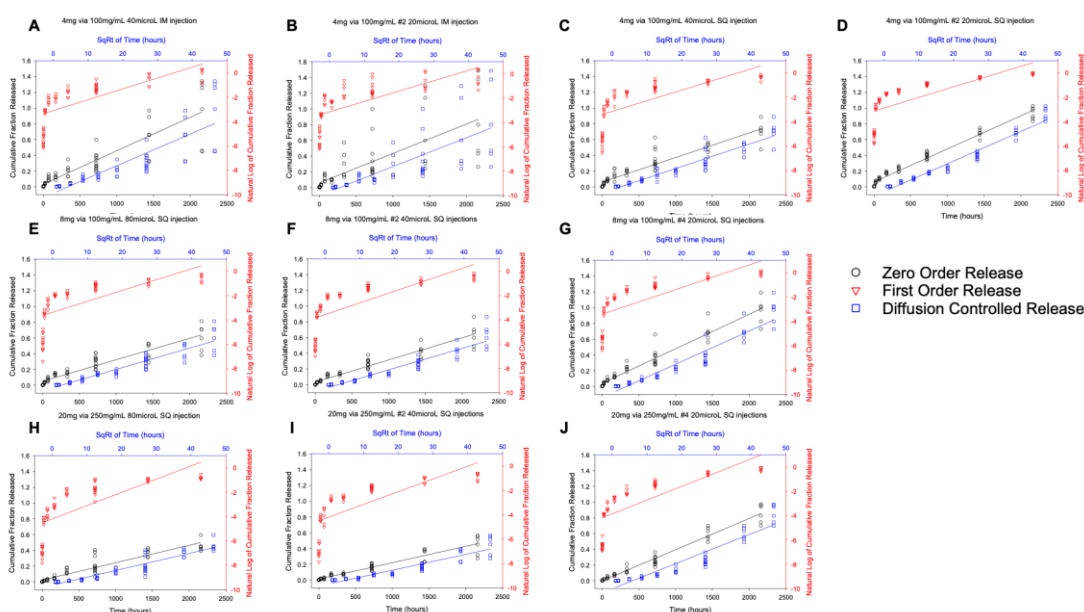


Figure S9. Fraction of DTG released from ISFIs (1:2 w/w PLGA/NMP) from individual female NSG mice ($n = 10$ mice/group) fit to zero order, first order, and diffusion-controlled release models. (A) 100 mg/ml 1 \times 40 μl IM ISFIs (B) 100 mg/ml 2 \times 20 μl IM ISFIs (C) 100 mg/ml 1 \times 40 μl SQ ISFIs (D) 100 mg/ml 2 \times 20 μl SQ ISFIs (E) 100 mg/ml 1 \times 80 μl SQ ISFIs (F) 100 mg/ml 2 \times 40 μl SQ ISFIs (G) 100 mg/ml 4 \times 20 μl SQ ISFIs (H) 250 mg/ml 1 \times 80 SQ ISFIs (I) 250 mg/ml 2 \times 40 SQ ISFIs (J) 250 mg/ml 4 \times 20 μl SQ ISFIs.

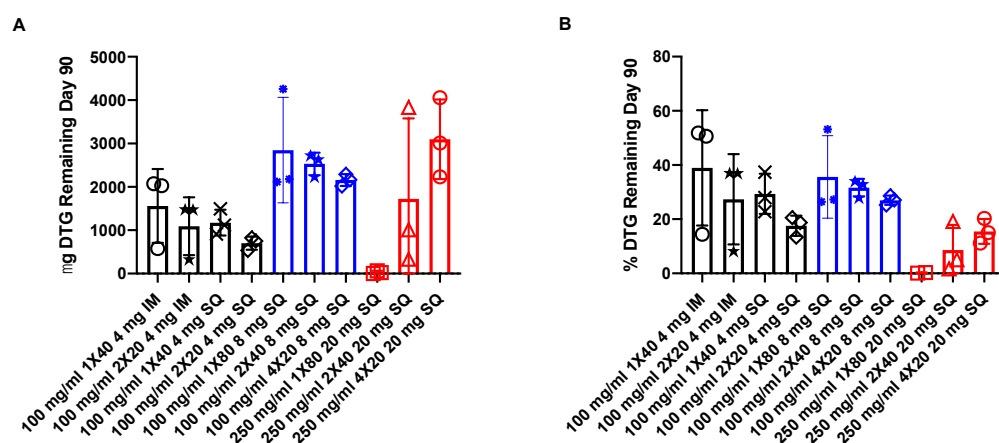


Figure S10. Residual DTG concentrations in implants injected into female NSG mice (n=3/group) and retrieved at day 90 post-implantation determined by fully dissolving implants in ACN overnight and quantifying DTG concentration by HPLC. (A) μ l of DTG remaining in ISFIs at day 90 (B) Percent DTG remaining in ISFI at day 90 based on total administered dose.