

Discovery of MK-8527, a long-acting HIV nucleoside reverse transcriptase translocation inhibitor

Izzat T. Raheem; Kerry Fillgrove; Gregory O'Donnell; Jonathan Patteson; Shih Lin Goh; Carolyn Bahnck-Teets; Qian Huang; Ernest Asante-Appiah; Min Xu; Steve S. Carroll; Jay A. Grobler; Jeffrey Hale; Ming-Tain Lai; Vinay Girijavallabhan; Tracy L. Diamond

Merck & Co., Inc., Rahway, NJ, USA

Introduction

Nucleoside reverse transcriptase translocation inhibitors (NRTTI) are potent inhibitors of HIV replication. We have invented a novel NRTTI, MK-8527, with antiviral potency and pharmacokinetics (PK) suitable for long-acting, oral dosing, and an attractive profile for HIV pre-exposure prophylaxis (PrEP). MK-8527 is a 7-deaza-deoxyadenosine analog that is phosphorylated intracellularly to its active triphosphate (TP) form, which inhibits reverse transcription.

Methods

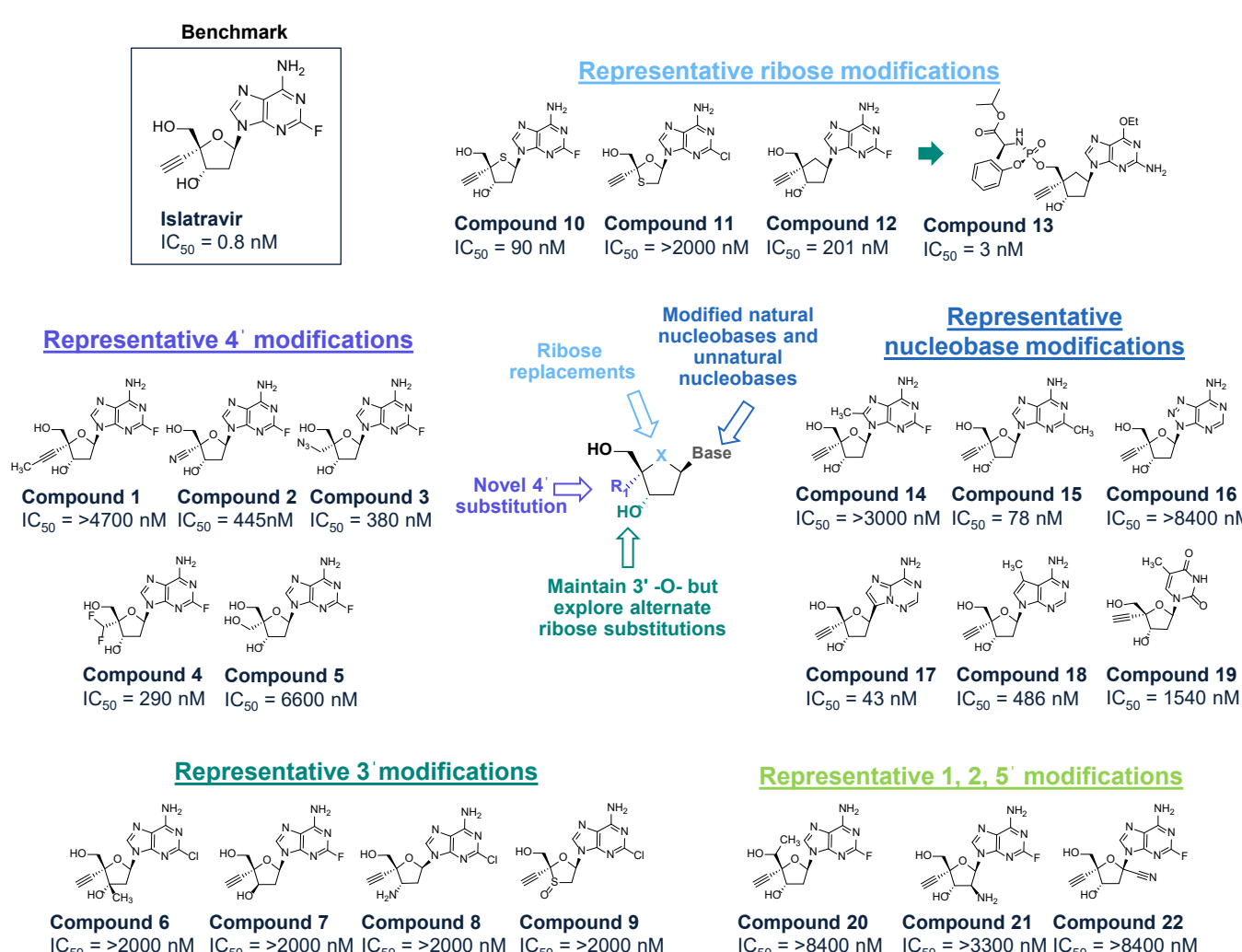
MK-8527 was invented through a lead optimization campaign focused on identifying structurally and functionally novel NRTTIs with the potential for extended-duration dosing. Off-target activity was assessed against human DNA polymerases and in a panel of 114 enzyme/receptor binding assays. PK parameters were evaluated in rats and rhesus monkeys. Persistence of RT inhibition by MK-8527-TP after washout was measured in cell-based assays, and the mechanism of MK-8527-TP was evaluated in gel-based primer extension and footprinting assays.

MK-8527: Discovery and Preclinical Profile

Medicinal chemistry strategy

- Nucleoside periphery explored broadly with single-point modifications initially
- Leveraged traditional nucleoside modifications alongside nontraditional structural diversity
- Evaluated nucleoside cell-based antiviral potency [in MT4-GFP cells (MT4 cells which express green fluorescent protein) in 10% normal human serum (NHS); IC₅₀ shown below compound #s)] alongside nucleoside triphosphate biochemical potency
- Evaluated prodrugs opportunistically

Figure 2. MK-8527 was invented through a comprehensive chemistry campaign navigating steep structure-activity relationships (SAR)



Comprehensive chemistry campaign highlighted extremely tight SAR

- Most structural modifications evaluated resulted in >1000-fold loss in potency
- Only limited core and nucleobase modifications tolerated
- Of >600 compounds evaluated, ~25 with IC₅₀ <50 nM and good antiviral persistence

Figure 1. NRTTIs inhibit RT through multiple unique mechanisms, including inhibition of translocation

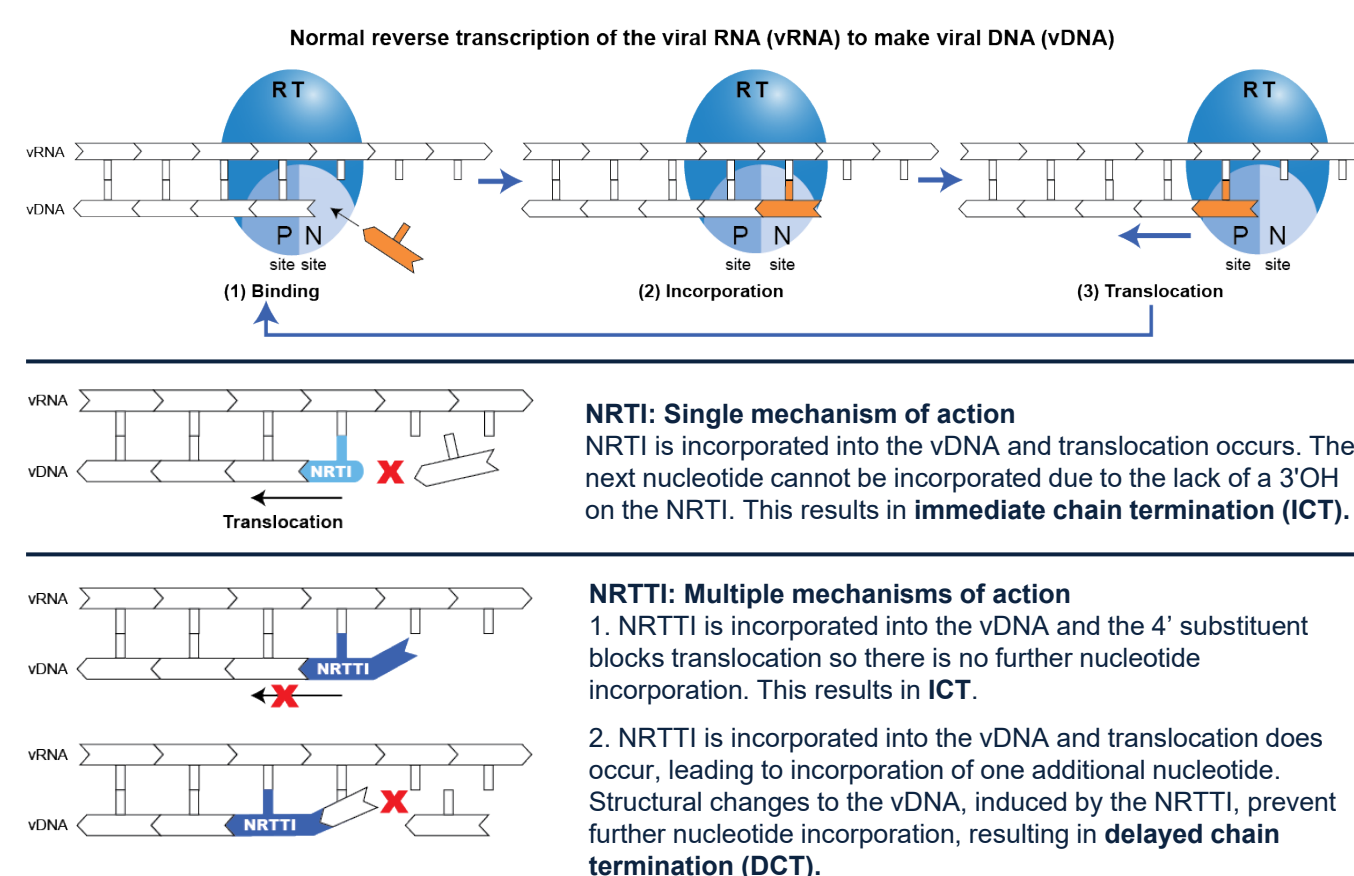
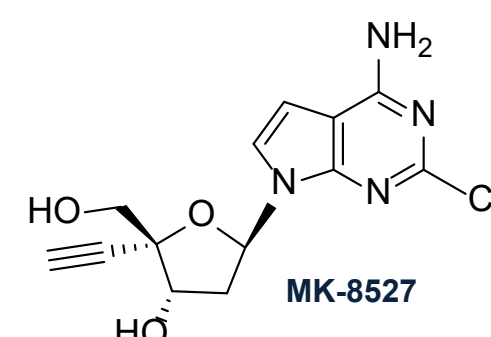


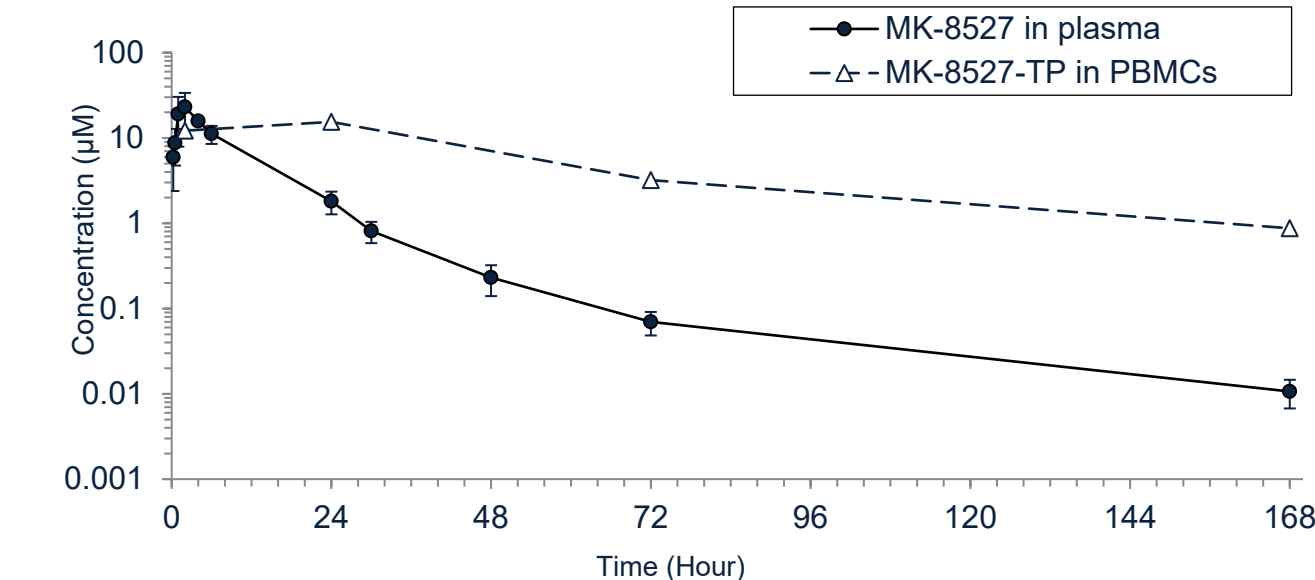
Figure 3. MK-8527 is a structurally novel, potent HIV-1 inhibitor with a favorable off-target profile and PK properties suitable for long-acting oral dosing.



Antiviral activity	IC ₅₀ – HIV in 10% NHS (MT4-GFP cells, human PBMCs)	3.4 nM, 0.2 nM			
	[MK-8527-TP] at the IC ₅₀ in PBMCs	9.2 ± 3.5 fmole per 10 ⁶ cells			
In vitro off-target profile	IC ₅₀ – Human DNA polymerases (α, β, γ)	MK-8527-TP – 95 μM, >200 μM, >200 μM			
	IC ₅₀ – Off-target panel of 114 enzyme/receptor binding assays	MK-8527 – all >10 μM MK-8527-TP – all >10 μM			
Pharmacokinetics	Plasma clearance (Cl _p) (mL/min*kg)	Volume of distribution (Vd) (L/kg)	t _{1/2} (hr)	F (%)	
	Rat	18.1 ± 3.3	2.0 ± 0.3	2.3 ± 1.3	57
	Rhesus	12.6 ± 3.3	4.4 ± 0.8	6.7 ± 1.6	100
	Human dose prediction	<15 mg weekly			

Figure 4. Following oral dosing of MK-8527 to monkeys the intracellular half-life (t_{1/2}) of the triphosphate (MK-8527-TP) in PBMCs (~48 hr) was significantly longer than the t_{1/2} of the parent (MK-8527) in plasma (~7 hr)

Concentration-vs-time profiles of MK-8527 in plasma and MK-8527-TP in PBMCs following oral administration to rhesus monkeys at 50 mg/kg.



MK-8527: Persistence of Antiviral Activity and Mechanism of Action

Figure 5. Persistence of MK-8527 antiviral activity in vitro suggests MK-8527-TP is maintained at effective levels after washout

A persistence assay was performed in which cells were incubated with compound for 24 hr, allowing time for compound uptake and phosphorylation followed by washout. Infection was performed immediately (A) or 48 hr (B) after washout. Plates were analyzed 24 hr post-infection for the number of GFP-positive cells, IC₅₀ values were determined, and a persistence ratio was calculated for each compound. Lower persistence values suggest longer triphosphate persistence in the cells after compound washout. MK-8527-TP has similar persistence in PBMCs and MT4-GFP cells and is comparable to that of ISL-TP.

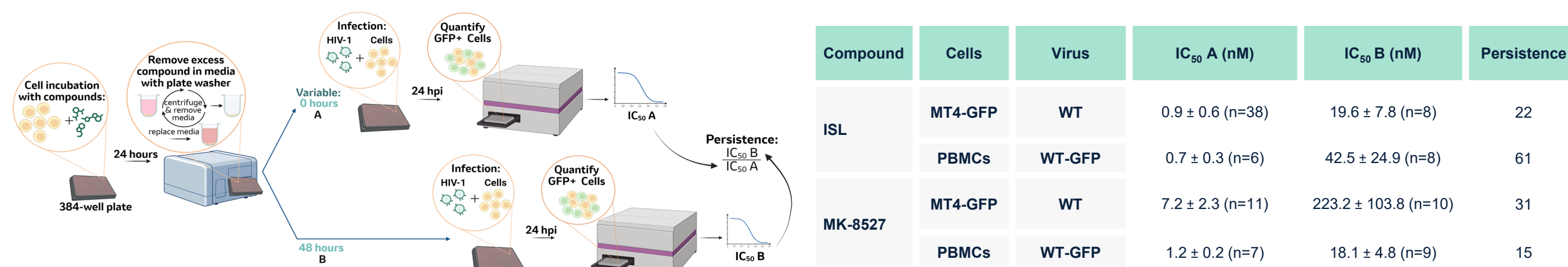


Figure 6. MK-8527-TP blocks translocation of reverse transcriptase on the primer/template

The iron footprinting assay⁴ was used to evaluate the position of reverse transcriptase on the primer/template (A) in the presence of 5 μM ddATP, an NRTI (B), 1 μM ISL-TP, an NRTTI (C), or 1 μM MK-8527-TP (D) and escalating concentrations of the next nucleotide (dTTP). MK-8527-TP locks reverse transcriptase in a pre-translocation state, similar to ISL-TP and different from ddATP.

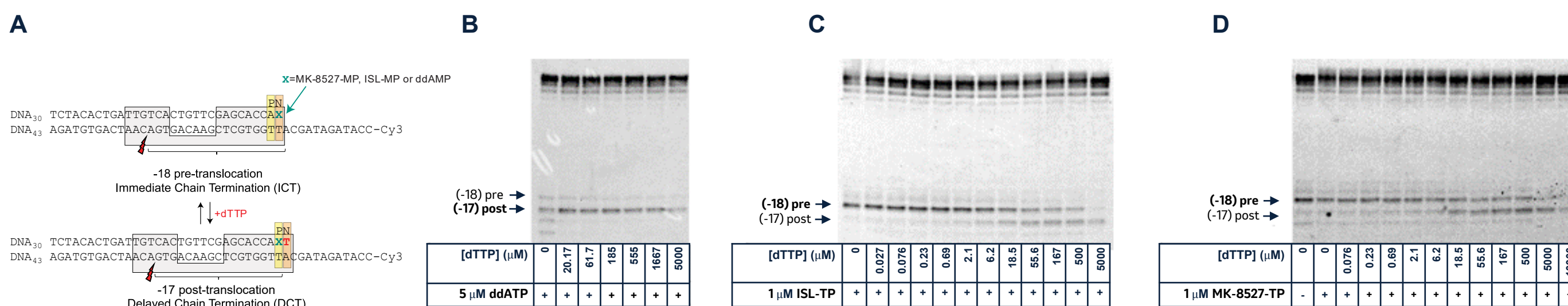
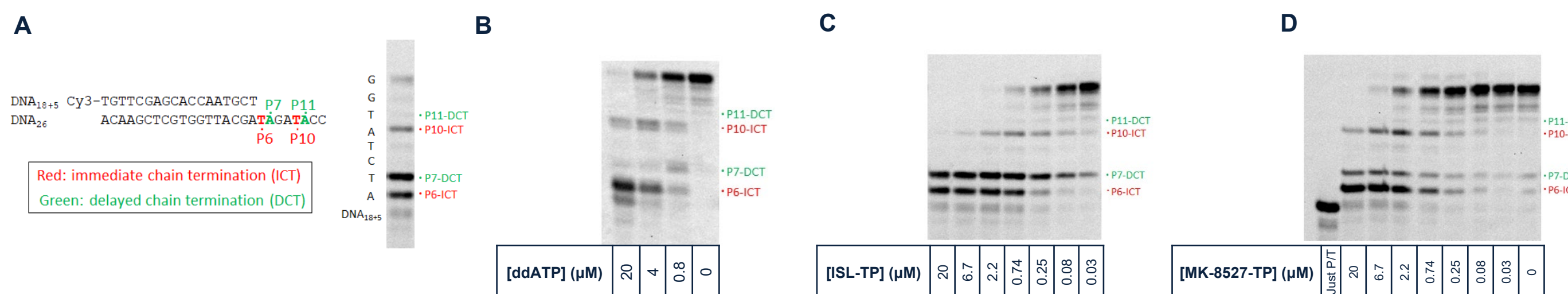


Figure 7. MK-8527-TP incorporation results in both immediate and delayed chain termination

The HIV-RT primer extension assay⁵ was used to evaluate the chain termination mechanism of MK-8527-TP in comparison to ISL-TP (an NRTTI) and ddATP (an NRTI). (A) Primer and template used along with representative gel with delayed (DCT) and immediate chain termination (ICT) sites marked. Dose dependent chain termination patterns of ddATP (B), ISL-TP (C), and MK-8527-TP (D) are shown. Incorporation of MK-8527-TP in a primer extension assay results in either ICT or DCT similar to ISL-TP and different from ddATP.



Conclusion

MK-8527 is a potent, novel NRTTI with a favorable in vitro off-target profile and PK characteristics suitable for long-acting oral dosing making it an attractive clinical candidate for HIV PrEP.

References

- Acosta-Hoyos AJ, et al. *Viruses*. 2010;2(2):
- Arts EJ, et al. *Cold Spring Harb Perspect Med*. 2012;2(4):a007161.
- Ilyodogan P, et al. *Viruses*. 2014;6(10):4095-4139. 372-394.
- Michalidis E, et al. *J Biol Chem*. 2009; 284(51):35681-91.
- Michalidis E, et al. *J Biol Chem*. 2014;289(35):24533-24548.