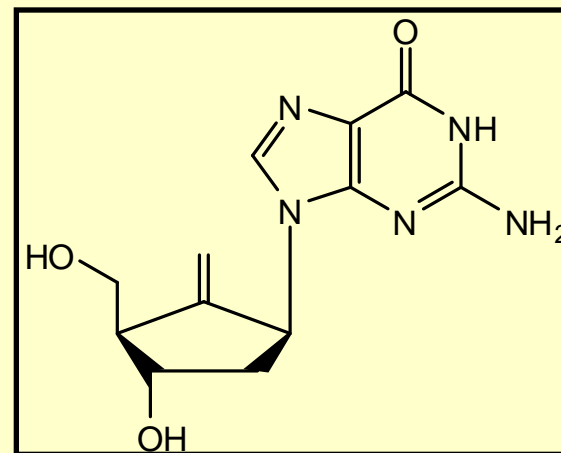


# Properties of ENTECAVIR (ETV)

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- Guanosine analogue
- Contains 3'-OH group
- Potent Anti-HBV drug
- Anti-HIV activity



Identified as potent inhibitor of hepatitis B virus replication.

**Innaimo et al. 1997**

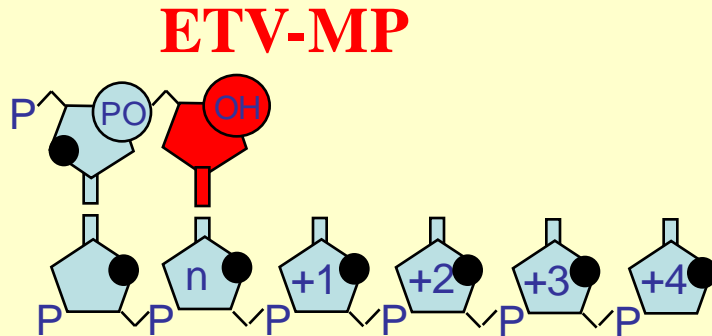
Anti HIV-1 activity and development of resistance in HIV/HBV co-infected individuals. **McMahon et al. 2007**

**ETV selects for M184V in HIV-1 RT in patients**



# ETV-TP is a Substrate for HIV-1 RT

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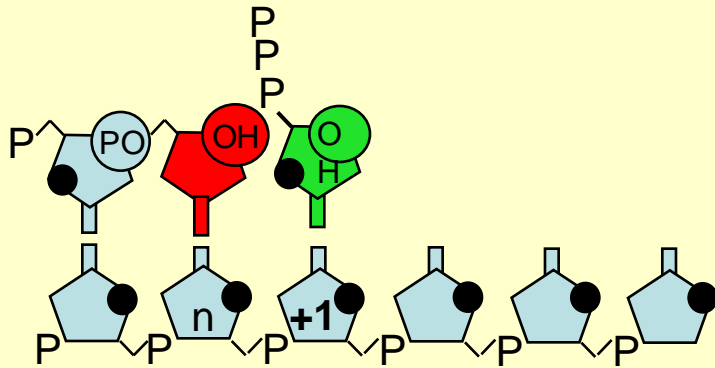


- HIV-1 RT can efficiently incorporate ETV-MP
- M184V diminishes efficiency of incorporation

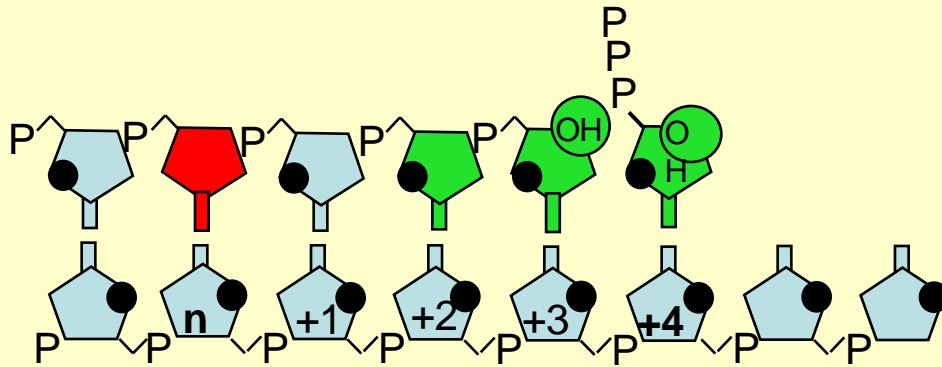
Pre-steady-state kinetic studies establish entecavir 5'-triphosphate as a substrate for HIV-1 reverse transcriptase. **Domaol et al. 2007.**



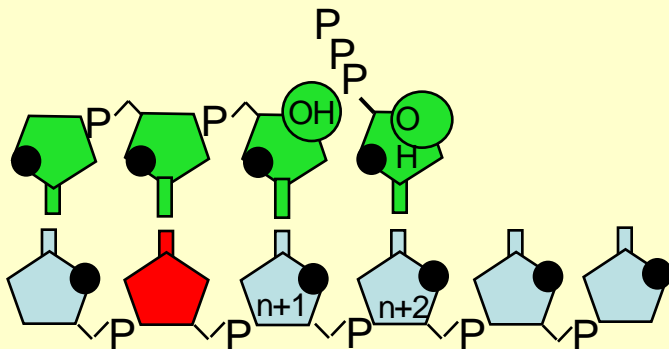
# Mechanism of Action?



*De facto* Chain-Termination



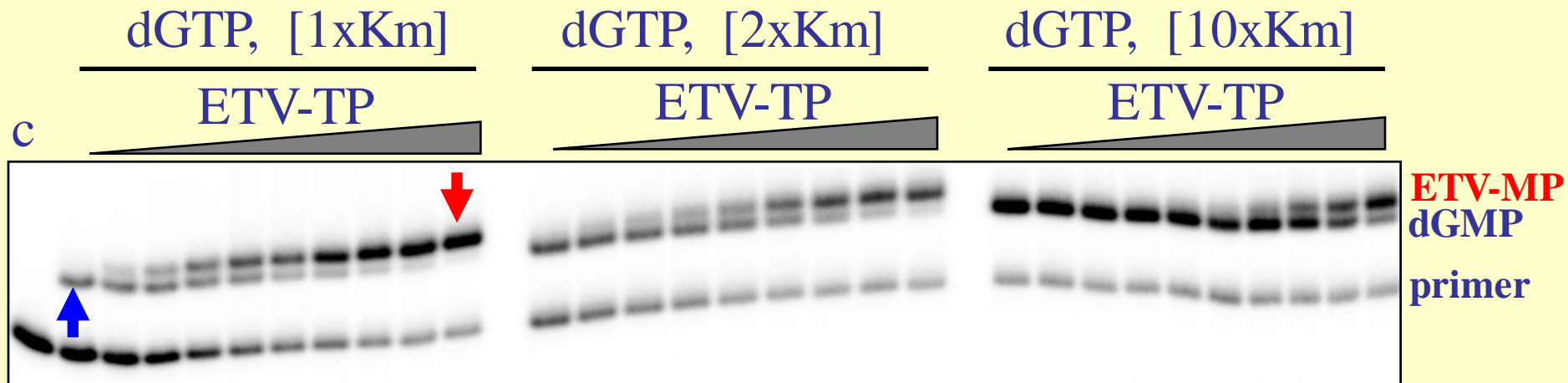
Delayed Chain-Termination



Base-Pair Confounder



# ETV-TP Competes with dGTP for Binding to HIV-RT

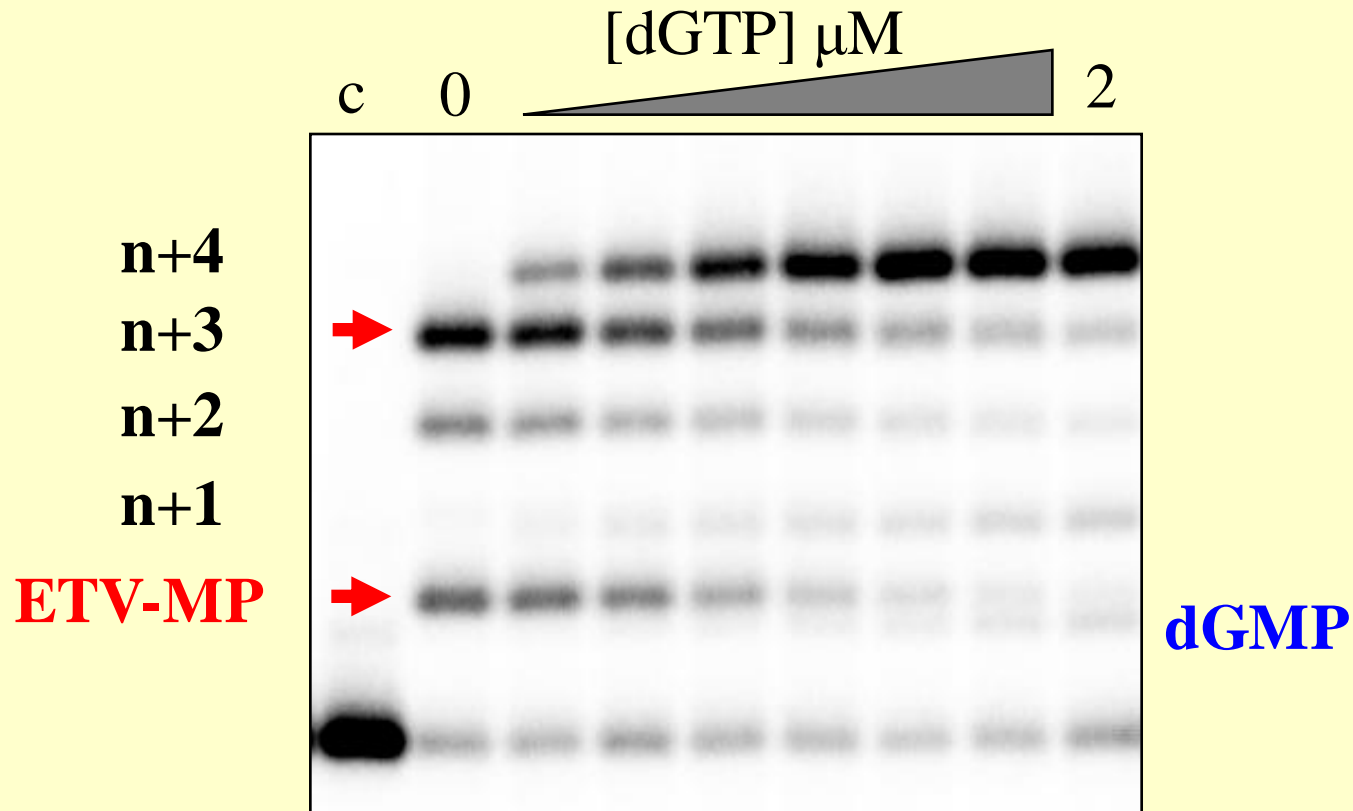


ETV-TP IC50	dGTP Km
0.51 $\mu$ M	1x (0.04 $\mu$ M)
1.3 $\mu$ M	2x (0.08 $\mu$ M)
19 $\mu$ M	10x (0.4 $\mu$ M)

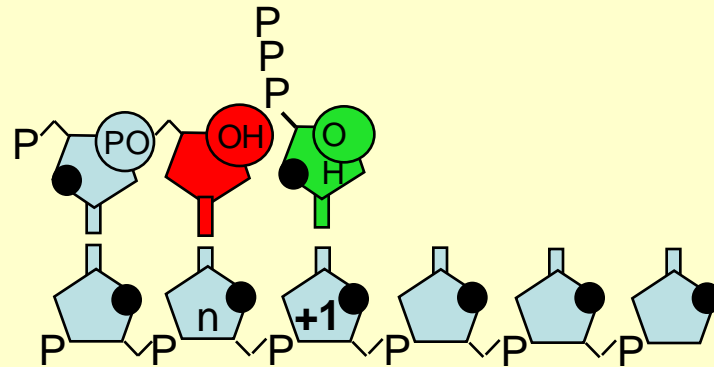


# ETV Does not Act as a Classic Chain-Terminator

ETV-MP causes strong pausing at positions  $n$ , and  $n+3$   
Data suggest inhibition of DNA synthesis at  $n+1$  and  $n+4$



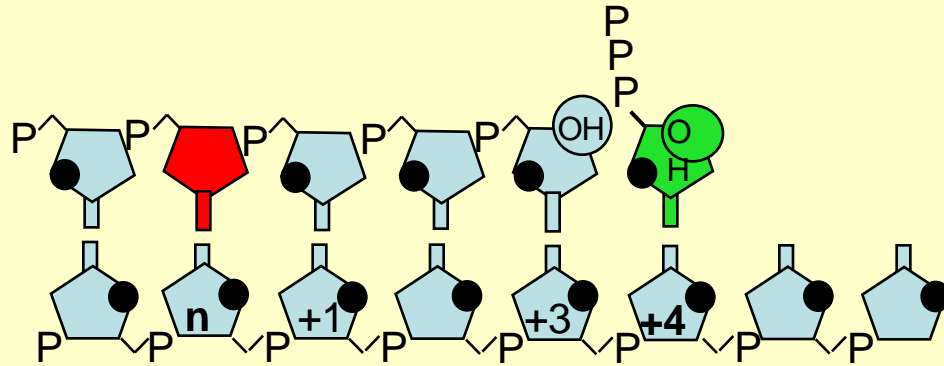
# Inhibition at Position n+1



Substrate (n+1)	Nucleotide (n)		Selectivity
	dGMP	ETV-MP	
	<b>Efficiency</b> k <sub>cat</sub> /K <sub>m</sub> (min <sup>-1</sup> *μM <sup>-1</sup> )	<b>Efficiency</b> k <sub>cat</sub> /K <sub>m</sub> (min <sup>-1</sup> *μM <sup>-1</sup> )	
dTTP	7.5	1	7.5



# Inhibition at Position n+4



Substrate  
(n+4)

Nucleotide (n)

dGMP

ETV-MP

**Efficiency**

$k_{cat}/K_m$

$(\text{min}^{-1} * \mu\text{M}^{-1})$

**Efficiency**

$k_{cat}/K_m$

$(\text{min}^{-1} * \mu\text{M}^{-1})$

**Selectivity**

dCTP

3.7

0.003

1233



# Delayed Chain-Termination is Dominant

---

$n+1$

Selectivity  
7.5

$n+4$

Selectivity  
1233

$n+4/n+1$

Selectivity  
ratio  
164

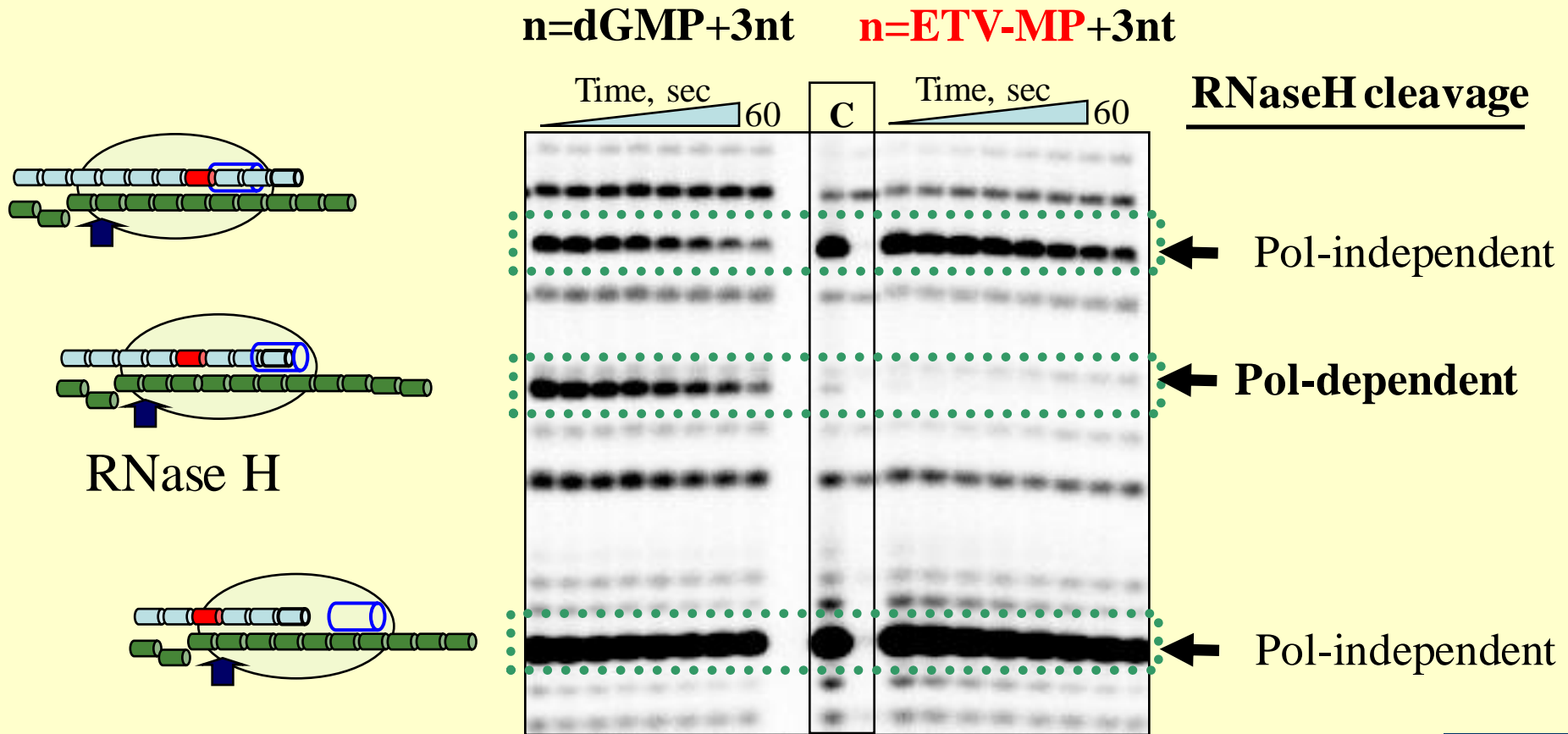
What is the underlying mechanism?

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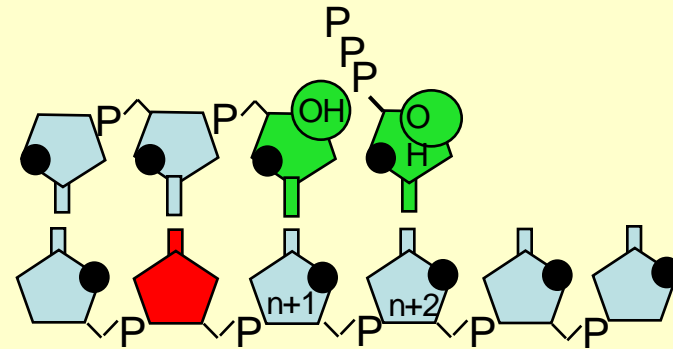
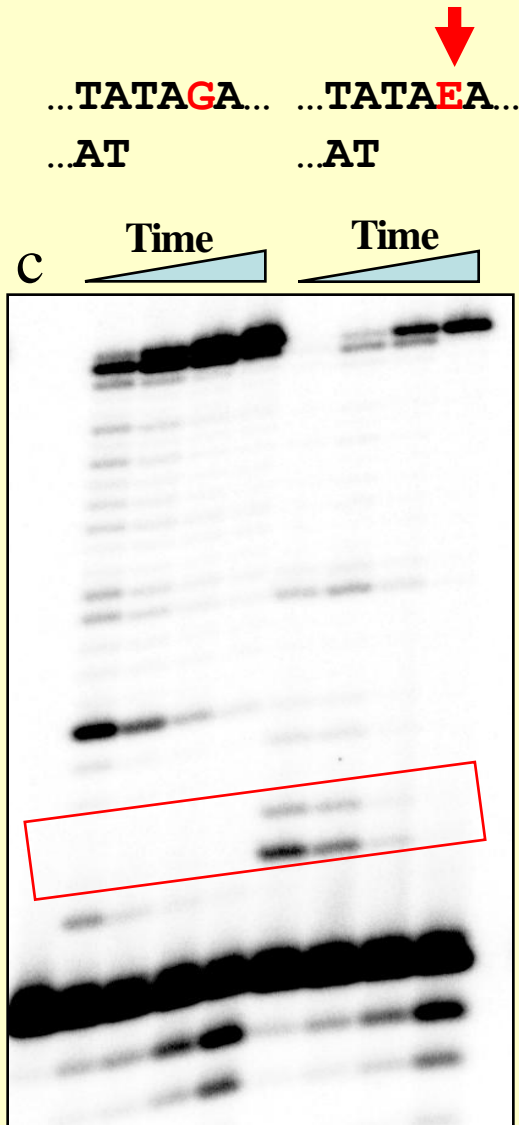
1. Increased Substrate Dissociation?
2. Unfavorable Positioning of HIV-1 RT?



# Positioning of HIV-1 RT on Substrates with ETV-MP



# ETV also Exerts Inhibitory Effects when Present in the Template Strand



# Resistance Profile and Underlying Mechanisms

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- M184V confers resistance to ETV in cell-based assays.
  - TAMs do not appear to affect susceptibility to ETV
- Domaol et al. 2007**



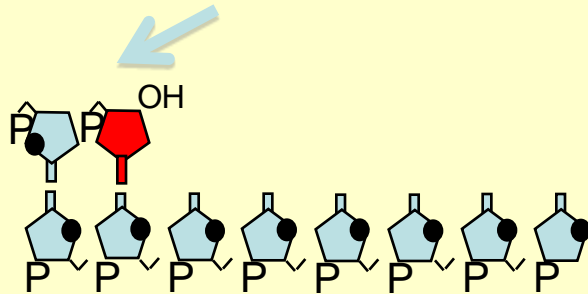
# M184V Enhances Substrate Discrimination at Position n

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	Nucleotide (n)		<b>Selectivity</b>
	dGMP	ETV-MP	
	<b>Efficiency</b> kcat/Km (min <sup>-1</sup> *μM <sup>-1</sup> )	<b>Efficiency</b> kcat/Km (min <sup>-1</sup> *μM <sup>-1</sup> )	
WT RT	13	0.7	16
TAMs	15	0.8	18
M184V	8	0.03	234



# Delayed Chain-Termination Provides Protection from Excision with TAMs



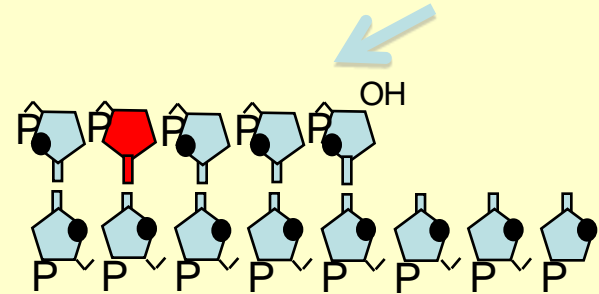
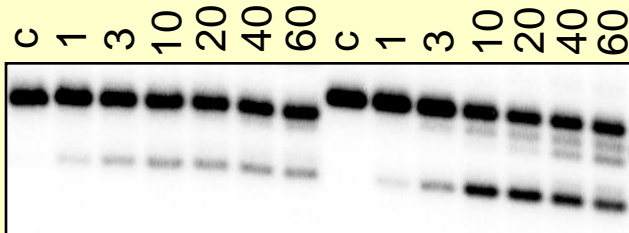
TAMs

n=dGMP

n=ETV-MP

Time, min

Time, min



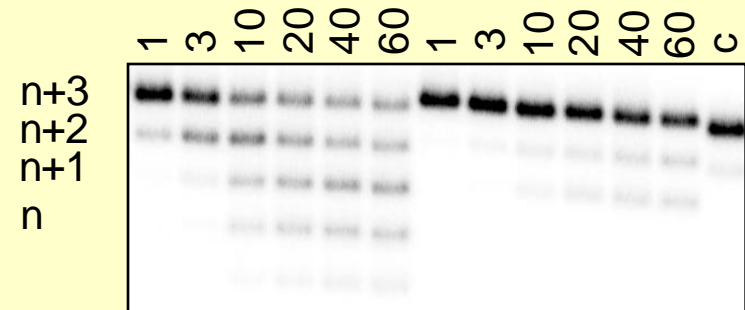
TAMs

n=dGMP

n=ETV-MP

Time, min

Time, min



# Conclusions

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- ETV-TP competes with dGTP for binding to HIV-RT.
- Delayed chain-termination at position  $n+3$  appears to be the major mechanism. Active site is not in contact with primer 3'-end.
- M184V discriminates against the inhibitor.
- ETV evades excision through delayed chain-termination.



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