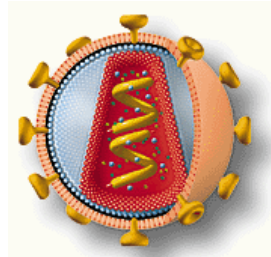


AZT Resistance Related Connection Mutations in HIV-1 RT Cause Selective Dissociation from RNase H-Competent Complexes



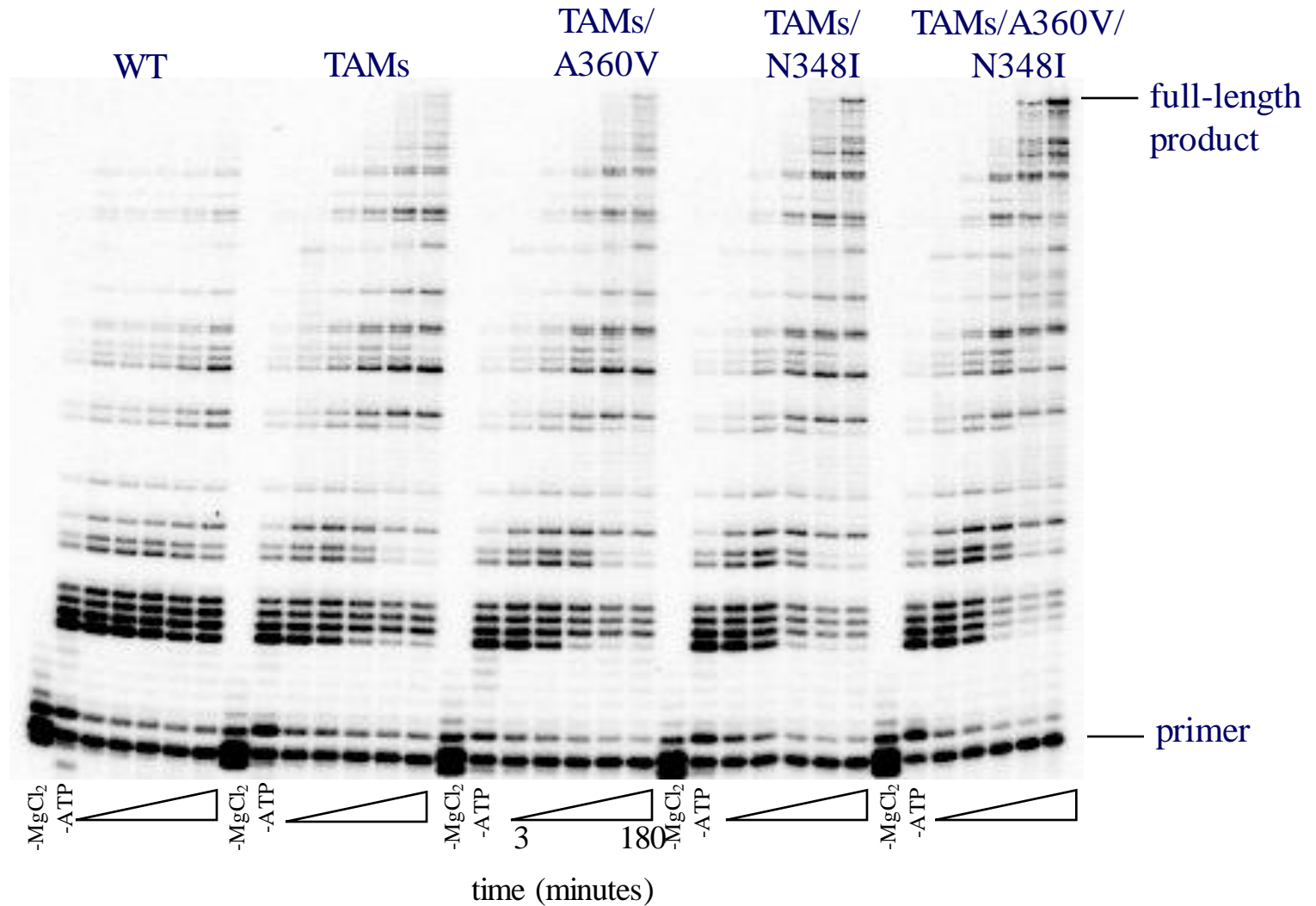
**Greg L. Beilhartz^{1‡}, Maryam Ehteshami^{1‡}, Brian J. Scarth^{1‡},
Egor P. Tchesnokov¹, Suzanne McCormick¹, Brian Wynhoven²,
P. Richard Harrigan² and Matthias Götte¹**

**‡The authors have contributed equally to this work
Montreal, QC, Canada**

British Columbia, Canada



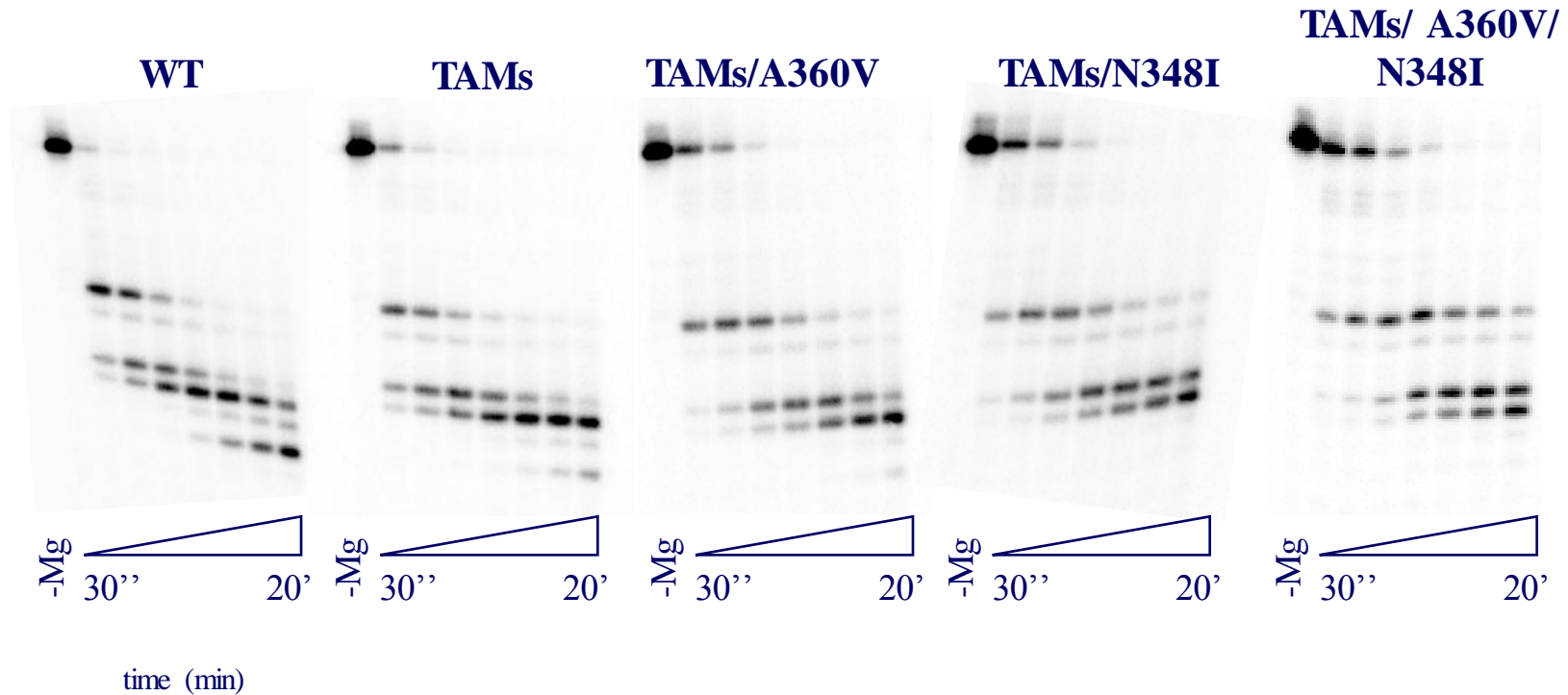
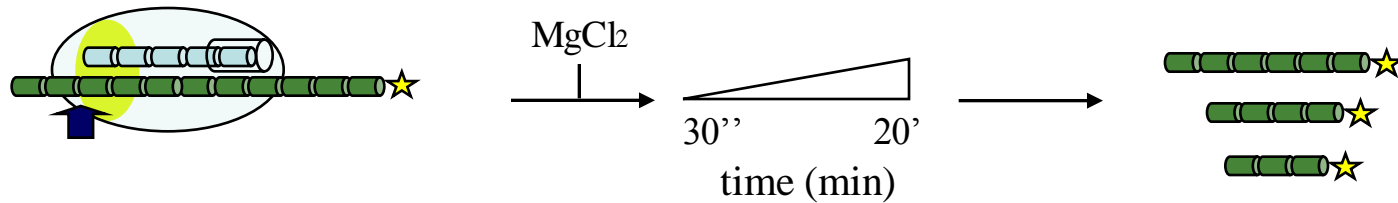
ATP-mediated AZT-MP Excision on RNA/DNA Substrate



Connection domain mutations increase ATP-mediated excision of AZT-MP in the background of TAMs



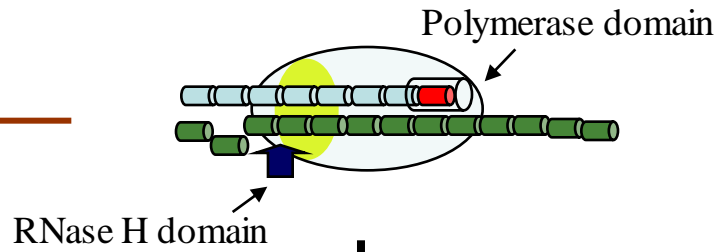
Effect of Mutations on RNase H Activity



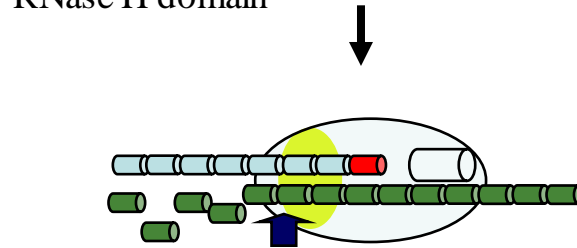
The presence of TAMs/A360V/N348I leads to the accumulation of short RNA substrates



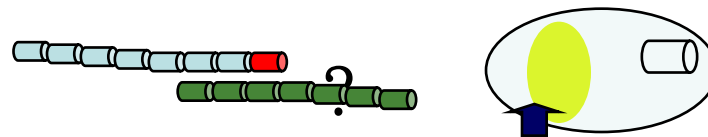
Hypothesis



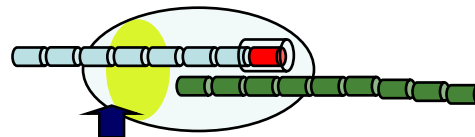
Polymerase dependent
RNase H activity



RNase H-competent
complex



RT : substrate dissociation

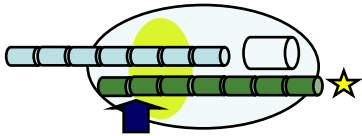


Polymerase-competent
complex



The Effect of Connection Domain Mutations on Substrate Binding... I

RNase H-Competent Complex



Equilibrium Dissociation Constants (K_d)

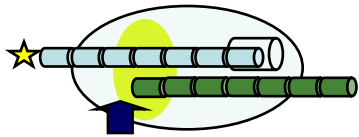
	RT enzyme	16mer substrate	
		$K_{d(\text{RNase H})}$ (nM)	Fold-Change
Substrate	WT	103.8	-
RT	TAMs	188.1	1.8
Trap + Mg ²⁺	TAMs/A360V	369.5	3.6
	TAMs/N348I	918.5	8.8
Stop	TAMs/A360V/N348I	>1000	>>>

Connection domain mutations show reduced binding to the substrate in the RNase H-competent complex



The Effect of Connection Domain Mutations on Substrate Binding... II

Polymerase-Competent Complex



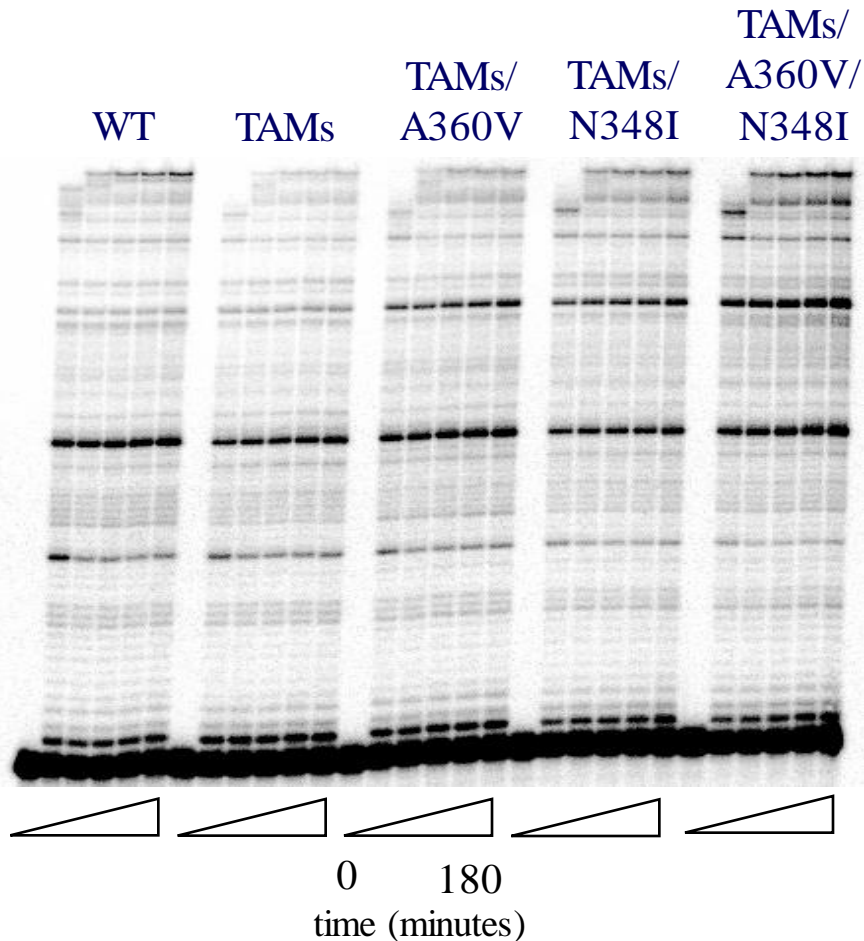
Equilibrium Dissociation Constants (K_d)

	RT enzyme	15mer substrate	
		$K_{d(\text{pol})}$ (nM)	Fold-Change
Substrate	WT	12.8	-
RT	TAMs	22.7	1.8
dNTP + Trap + Mg^{2+}	TAMs/A360V	10.5	0.8
	TAMs/N348I	21.7	1.7
Stop	TAMs/A360V/N348I	11.2	0.9

In the polymerase-competent complex, A360V, but not N348I, shows improved substrate binding in the background of TAMs



Effect of N348I and A360V on Processive DNA Synthesis

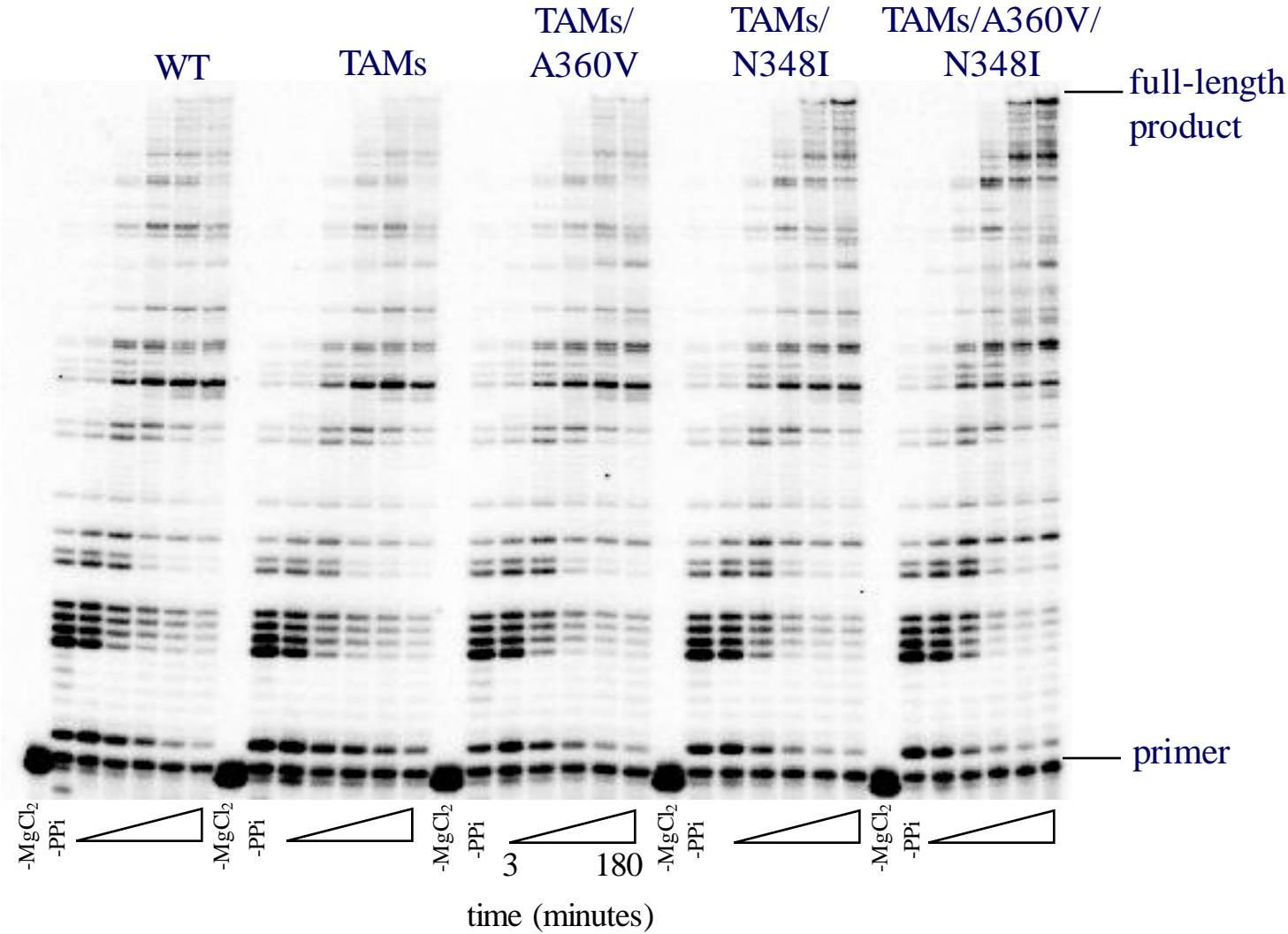


Enzyme	Processive complex (%)
WT	2.77
TAM	2.09
TAM/A360V	3.00
TAM/N348I	5.36
TAM/A360V/N348I	6.36

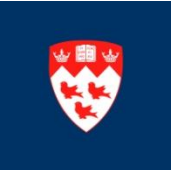
N348I and A360V increase processive DNA synthesis as compared to TAMs



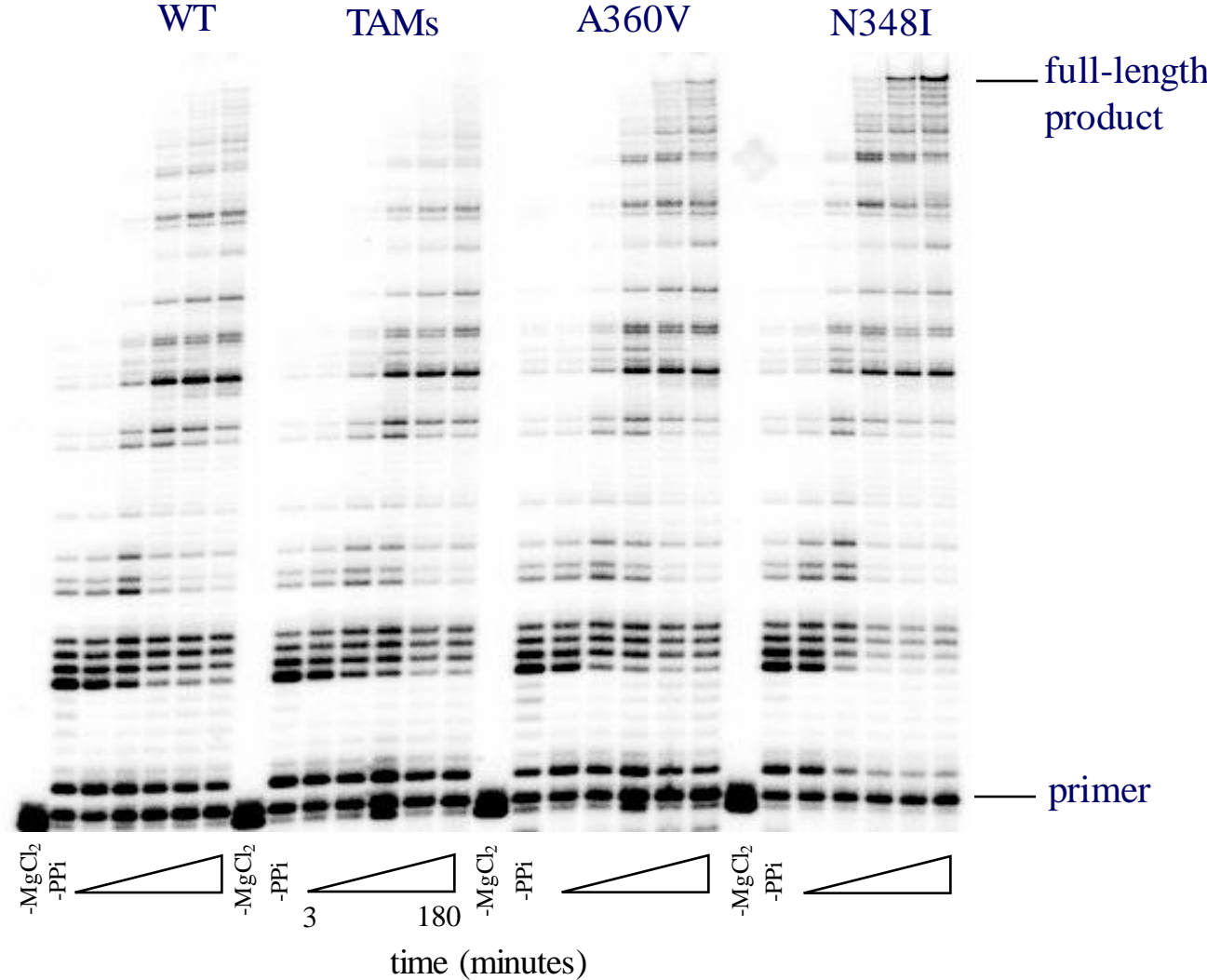
PPi-mediated AZT-MP Excision on RNA/DNA Substrate



Increased PPi-mediated excision of AZT-MP observed with connection domain mutations in the background of TAMs



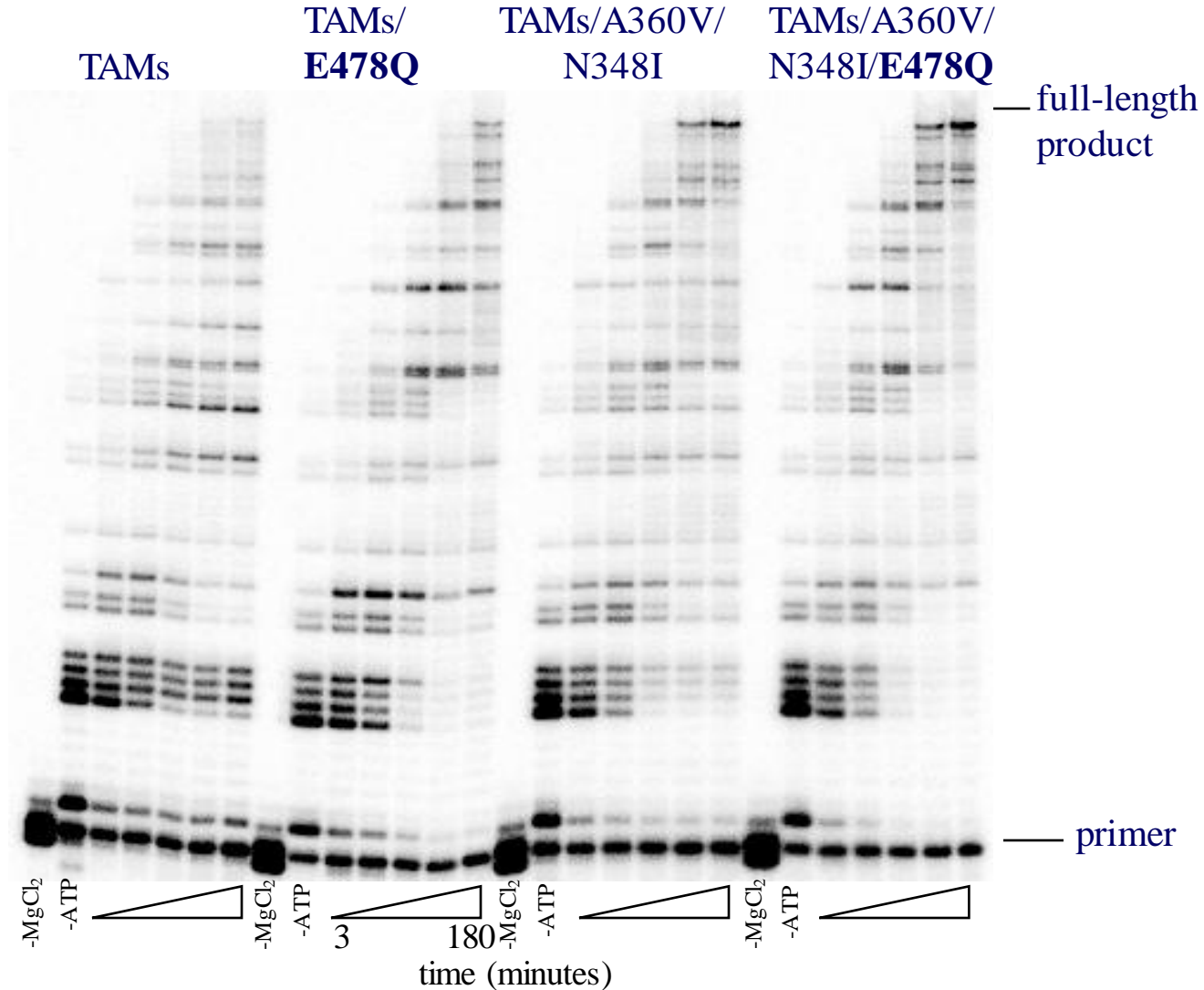
Is PPI-mediated Excision Increased Independently of TAMs?



N348I alone is sufficient to increase PPI-mediated AZT-MP excision



ATP-mediated AZT-MP Excision In the Absence of RNase H Activity



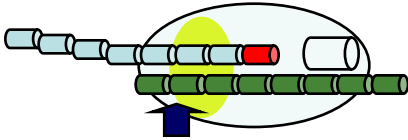
A360V and N348I can increase AZT-MP excision independently of RNase H activity



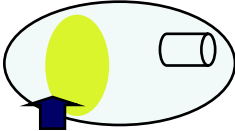
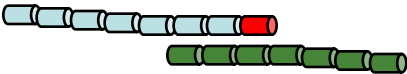
Mechanism of Resistance

RNase H-dependent
contribution to excision

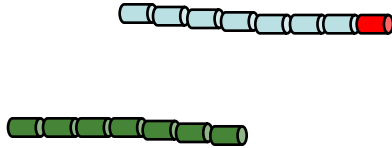
RNase H-competent complex



TAMs/N348I > TAMs/A360V > TAMs

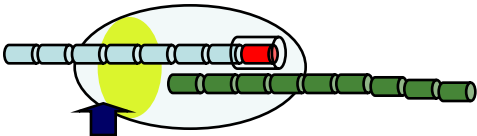


< 8mer



TAMs, TAMs/N348I
TAMs/A360V

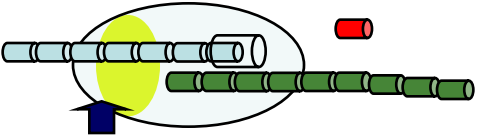
Polymerase-competent complex



RNase H-independent
contribution to excision

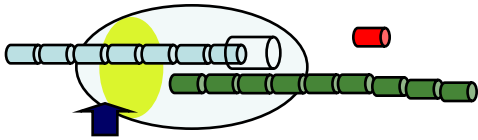
PPi

ATP
TAMs



TAMs/N348I

Processive DNA synthesis



TAMs/N348I, TAMs/A360V



Conclusions

RNase H -dependent and -independent mechanisms contribute to enhanced AZT resistance

A360V appears to compensate for TAMs-mediated deficits
- appears late following the emergence of TAMs

N348I is able to recruit PPi as a substrate for the excision reaction - appears early, independently of TAMs



Acknowledgements

McGill University

Department of Microbiology and Immunology

- Matthias Götte
- Greg Beilhartz
- Brian Scarth
- Egor Tchesnokov
- Suzanne McCormick
- Konstantin Ivanov
- Stephen Barry
- Megan Powdrill
- Colins Vasquez
- Mia Biondi
- Jean Bernatchez

BC Centre for Excellence
in HIV/AIDS

Richard Harrigan
Brian Wynhoven

Funding: Canadian Institute for Health Research

