Novel Resistance Profile of the Potent NRTI 3’-Azido-2’,3’-Dideoxyguanosine (AZG)

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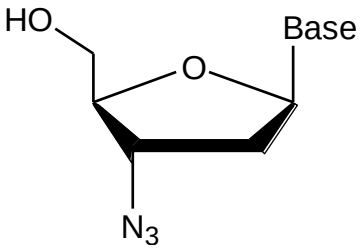
Introduction

- Well known that 3’-azidothymidine (AZT) selects TAMs that confer high-level resistance to AZT
  - Low-level cross-resistance to other NRTI
- Reason for relative specificity of resistance?
  - 3’-azido moiety?

  →**Test other 3’-azido nucleoside analogs**

Activity of 3'-Azido-2',3'-ddN Against AZT-Resistant HIV-1

Nucleoside	Base	EC_{50}, μM		Fold Resistance
		WT	41L/210W/215Y	
3'-azido-2',3'-dideoxy 	A	7.9 ± 3.4	8.6 ± 5.4	1.1
	C	15.6 ± 10.7	160.8 ± 24.1	10.7
	G	7.90 ± 4.63	10.3 ± 8.3	1.3
	T	0.031 ± 0.02	0.36 ± 0.2	11.7

Sluis-Cremer et al. 2005. J. Biol. Chem. 280: 29047

Base not 3'-azido is major determinant of activity

Anti-HIV activity of ADPs and FDA-approved NRTI in different cell lines

NRTI	EC_{50}, μM		
	P4/R5	MT-2	PBMC
3'-azido-ddG	**2.1 ± 0.9**	**1.73 ± 1.06**	**0.19 ± 0.19**
3'-azido-ddA	**10 ± 4.9**	**6.43 ± 3.10**	**0.36 ± 0.14**
ABC	6.21 ± 1.66	4.76 ± 1.63	0.91-0.98
TNV	4.67 ± 2.31	0.54 ± 0.61	N/A
ddI	2.32 ± 0.98	0.49 ± 0.32	0.1
3TC	0.78 ± 0.48	0.60±0.46	0.03-0.15
FTC	0.17 ± 0.07	0.044 ± 0.036	0.001-0.69
ddC	1.35 ± 0.70	0.13 ± 0.18	0.2
AZT	0.19 ± 0.11	0.031 ±0.024	0.01-0.09
d4T	5.72±3.34	1.87±0.76	0.1

Activity of ADP-TPs against HIV-1 containing multiple TAMs

RT	AZT EC_{50}, μM	Fold-R	3'-azido-ddG EC_{50}, μM	Fold-R
WT	0.19 ± 0.11	-	2.1 ± 0.9	-
[a]AZT2	10.4 ± 8.9	54.4	5.2 ± 2.3	2.5
[b]AZT3	11.9 ± 11.6	62.7	3.7 ± 1.4	1.8
[c]AZT7	96.7 ± 29.3	507.4	7.6 ± 2.2	3.5
[d]AZT9	58.6 ± 9.2	307.4	7.9 ± 4.9	3.7

a, M41L/L210W/T215Y
b, D67N/K70R/T215F/K219Q
c, M41L/D67N/K70R/T215F/K219Q
d, M41L/D67N/K70R/L210W/T215Y/K219Q

AZG cyotoxicity and intracellular pharmacology

- 3’-azido-ddG does not exhibit significant cytotoxicity at concentrations less than 100 μM in 4 different cell lines
- 3’-azido-ddG does not exhibit mitochondrial toxicity
- 3’-azido-ddG is rapidly taken up in cells and converted to the active triphosphate form (levels comparable to other purine analogs)
- Intracellular half life of 3’-azido-ddGTP was ~ 9 hrs (half life for AZT-TP is 3-4 hours).

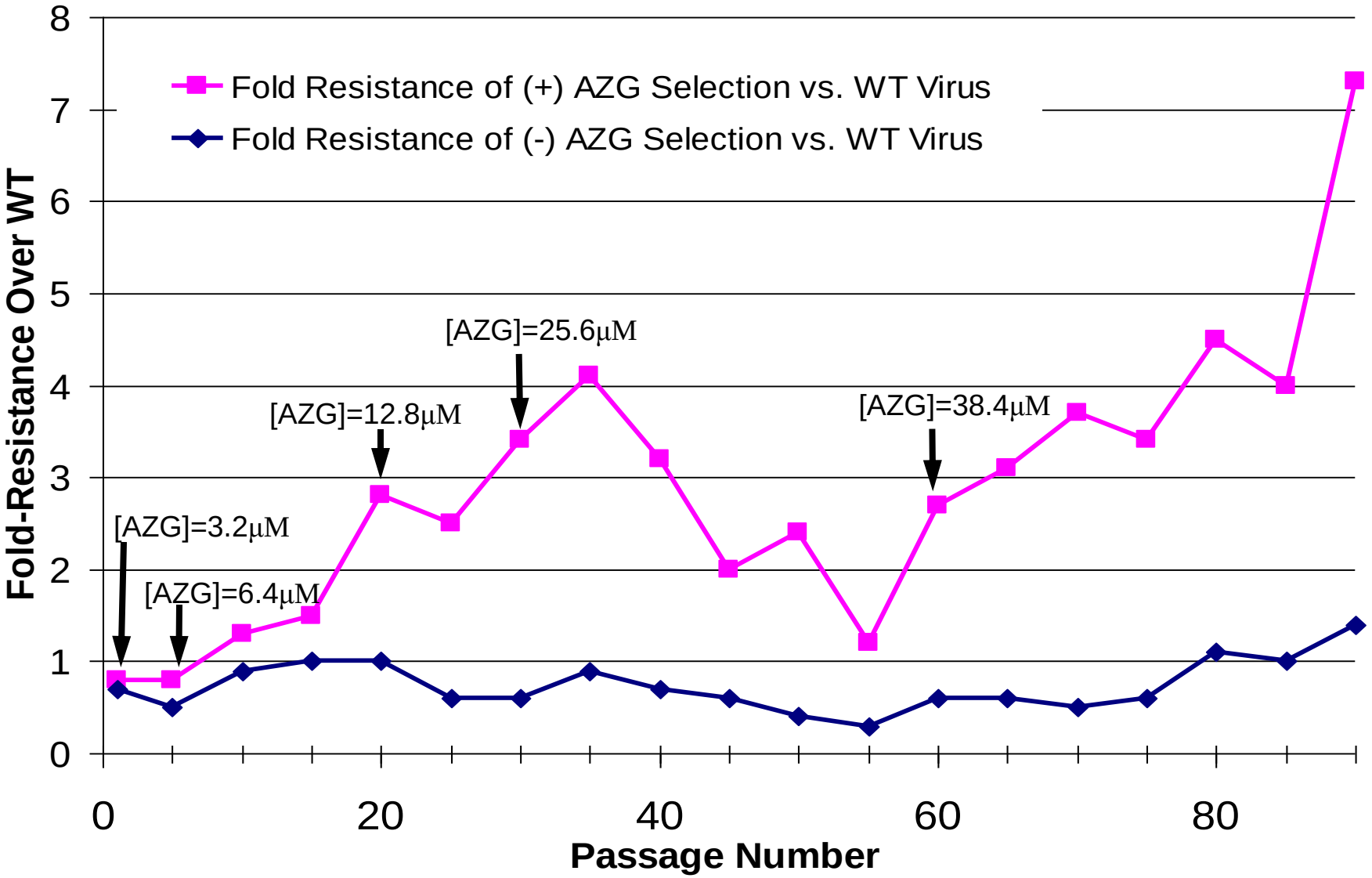
Hypotheses

- AZG will not select AZT resistant virus *in vitro*
- AZG resistance will be due to mutations other than TAMs

Methods

- *In vitro* selections
  - AZG-resistant virus was selected by serial passage of virus through increasing [AZG] in MT-2 and PBMC.
- Drug susceptibility
  - Determined using a single-cycle replication assay in P4/R5 cells with a luminescent β-Gal substrate.
- Genotypic analysis
  - Viral RNA was RT-PCR amplified and sequenced on an ABI 3100 using BigDye chemisty. Viral genomes were isolated by TOPO-cloning (Invitrogen).
- Site-directed mutagenesis
  - Performed with QuickChange® site-directed mutagenesis (Stratagene).

Fold AZG-resistance following *in vitro* selection in MT-2 Cells



Resistance and cross-resistance of wild-type (WT) and AZG selected virus passage 88

DRUG	EC_{50} (μM)		Fold Resistance
	WT	AZG P88	
3TC	1.38±0.7	19.7±8.9	14.3
AZG	1.40±0.05	10.3±1.2	7.3
AZT	0.068±0.014	0.385±0.335	5.6
ddI	6.2±0.2	26.6±1.2	4.3
d4T	6.46±0.67	22.6±0.6	3.5
TNV	3.0±0.4	5.35±0.67	1.8

Determined in P4/R5 cells

Population sequencing at passage 88

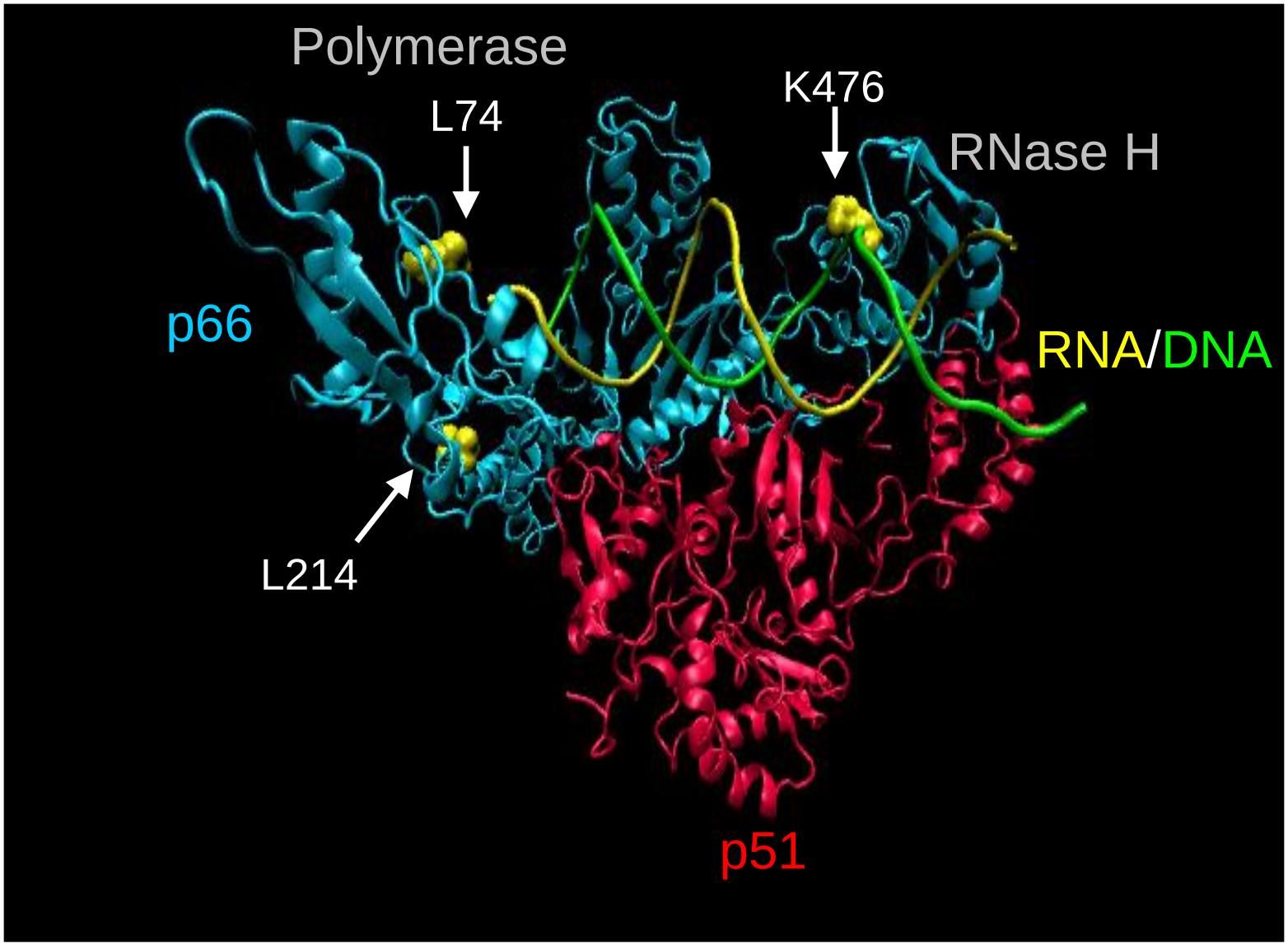
Reference	P88 #1	P88 #2	Notes
L74	V	V	**L74V**
F77	L	L	**F77L**
V106	I	I	**V106I**
L214	F	F	**L214F**
476N	N	N	***K476N***

Clonal analysis of passage 88 RT

Reference xx_{LAI}	Clone												Total
	1	2	3	4	5	6	7	8	9	10	11	12	
L74	V	V	V	V	V	V	V	V	V	V	V	V	12
F77	L	-	L	-	L	L	L	L	L	L	-	L	9
V106	I	-	I	-	I	I	-	I	-	I	-	-	6
E122	-	-	-	-	K	-	-	K	-	-	-	-	2
L214	F	F	F	F	F	F	F	F	F	F	F	F	12
G333	-	-	E	E	-	-	-	-	-	-	-	-	2
K476	N	N	N	N	N	N	N	N	-	N	-	N	10
V518	I	-	-	-	I	I	-	I	I	-	I	-	6
	6	3	9	5	9	7	5	9	5	6	4	10	78

Note: mutations that occurred in single clones are not shown in this table

Location of L74, L214 and K476 in RT



AZG selection mutations in PBMC

Week	[AZG]	Sequence
16	0.0μM	H221H/Y
19	2.5μM	V75V/I,H221H/Y
41	3.5μM	V75I,F77L,H221Y
45	3.5μM	V75I,F77L,H221Y

AZG susceptibility of site-directed mutants

- Determined in P4/R5 cells

Mutations	EC_{50} (µM)	Fold-Resistance
P4/R5 cells		
WT	1.65	-
L74V	2.96	1.80
V75I/F77L/H221Y	2.47	1.50
L214F	?	?
K476N	?	?
L214F/K476N	?	?
L74V/L214F/K467N	?	?

AZG susceptibility of site-directed mutants

- Determined in PBMC

Mutations	EC_{50} (µM)	Fold-Resistance
WT	0.89	-
V75I	0.12	0.13
F77L	0.26	0.29
V75I/F77L	0.41	0.46
L74V	4.70	5.28
L214F	?	?
K476N	?	?
L214F/K476N	?	?
L74V/L214F/K467N	?	?

Conclusions

- AZG doesn't select for classic TAMs
  - as predicted ☺
- Unique pattern of mutations in polymerase and RNAse H domains
- Broad NRTI cross-resistance – AZT, D4T, 3TC, TNV, DDI…..
  - 74V – discrimination mechanism
  - 214F - ? excision (with TAMS)
  - 476N - ???? RNA/DNA template position or stability
  - Mutagenesis and biochemical experiments planned
- Nucleoside base matters!
- Additional base modifications in progress….

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