Drug Susceptibility Profile of OBP-601, a novel NRTI, Using a Comprehensive Panel of NRTI and/or NNRTI Resistant Viruses

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Poster # 726b

1. Abstract

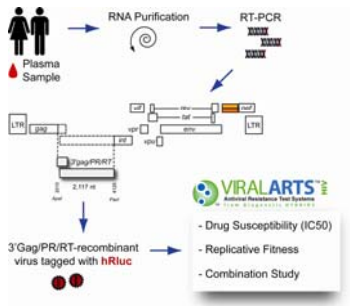
Background: Despite the relative success of antiretroviral therapy, emergence of drug resistant HIV-1 variants continues to be the major cause for treatment failure. OBP-601 (4'-Ed4T) is a novel nucleoside analog with potent anti-HIV-1 activity and limited cellular toxicity, which has a unique in vitro resistance profile. Here we evaluated (i) the ability of OBP-601 to inhibit the replication of multi-drug resistant viruses and (ii) its potential use in combination therapy.

Methods: A panel of 40 3'Gag/PR/RT-recombinant viruses were generated using PCR products from clinical samples harboring selected sets of NRTI resistance mutations (e.g., NAM, TAM41, TAM67, M41l+V118I, K65R, L74V, and Q151M, plus K103N + M184V). These viruses were employed in drug susceptibility assays to test the activity of OBP-601 alone or in combination with AZT, d4T, ABC, TDF, 3TC, EFV, NVP, LPV, ATV and darunavir. Viral replication was quantified using luciferase expression and/or an in-house RT assay, and 50% (IC50) inhibition concentration were determined. Antiviral isobolograms were calculated for the combination studies.

Results: OBP-601 susceptibility of recombinant viruses carrying wild type 3'Gag/PR/RT sequences ranged from 0.76 to 5.80 µM in our in vitro system, slightly lower than the parental compound d4T (1.57 to 6.06 µM). The anti-HIV-1 activity of OBP-601 was reduced in most viruses carrying NAM (5- to 10-fold), TAM41 (0.3- to 4.3-fold), and TAM67 (1.6- to 7.8-fold) resistance mutations, together with K103N + M184V. Interestingly, viruses carrying the Q151M substitution were hypersusceptible to OBP-601 (0.1- to 0.2-fold), even in the presence of K65R (0.3- to 1.3-fold). More important, OBP-601 showed strong (AZT, EFV) to moderate (ABC, TDF, NVP) synergistic interaction with different antiretroviral drugs in wild type viruses. Similar results were obtained with 3TC, EFV, and ABC in viruses carrying M184V, K103N, and Q151M, respectively.

Conclusions: The unique resistance profile of OBP-601, its effectiveness against drug-resistant viruses (e.g., carrying the Q151M complex), and the synergistic effect with other RTI (in both wild type and drug resistant viruses) warrant further investigation for its potential use in combination therapy against HIV-1.

3. Methods



2. Plan of study

A) Cross resistance study

Virus Study #	HIV-1 mutation in RT			IC50 (µM)						
		103N	184V	OBP-601	AZT	d4T	ABC	TDF	3TC	EFV
1	Wild type	-	-							
2	NAM	+	+							
3	TAM41 Pathway	+	+/-							
4	TAM67 Pathway	+	+/-							
5	M41L + V118I	+	+							
6	L74V	+	+							
7	K65R	+	+/-							
8	TAM + K65R	-	-							
9	Q151M	-	-							

B) Combination study with OBP-601

No.	Virus	AZT	ABC	TDF	3TC	EFV	NVP	LPV	ATV	DRV
1	wt									
2	184									
3	65									
4	103									
5	151									

4. Cross resistance virus list

Virus Study #	Virus ID	List of RT resistance mutations *	Virus Study #	Virus ID	List of RT resistance mutations *
1	1	none	4	19	41L, 67N, 70R, 75M, 103N, 184V, 215F, 219Q
	2	none		20	41L, 67N, 69D, 70R, 74I, 75T, 103N, 179I, 181C, 184V, 210W, 215F, 219Q
	3	none	5	21	41L, 67N, 74V, 103N, 118I, 184V, 210W, 215Y
	4	none		22	41L, 44D, 69N, 74I, 98S, 103N, 118I, 184V, 210F, 215Y, 219R
2	5	20R, 41L, 67N, 70G, 75M, 77L, 103N, 184V, 210W, 215F, 219Q		23	41L, 44D, 67N, 74V, 103N, 118I, 181C, 184V, 210W, 215Y
	6	20R, 41L, 67N, 74I, 75T, 101Q, 103N, 181C, 184V, 190A, 210W, 215F, 219Q		24	41L, 74V, 101P, 103N, 118I, 184V, 215Y
	7	41L, 67N, 69AD, 75AV, 103N, 108V, 118I, 184VM, 210W, 215Y, 219EK		25	41L, 44D, 67N, 69D, 74I, 103N, 118I, 181C, 184V, 210W, 215Y, 219N
3	8	41L, 103N, 210WL, 215C, 219Q	6	26	74V, 103N, 115F, 184V
	9	41L, 44D, 69N, 100I, 103N, 210W, 215Y		27	41L, 74V, 98S, 101R, 103N, 184V, 190A, 215Y
	10	41L, 103N, 188L, 210W, 215Y		28	74V, 100I, 103N, 115F, 184V
	11	41L, 67N, 74V, 103N, 118I, 184V, 210W, 215Y	7	29	65R, 70R, 75I, 77L, 103N, 116Y, 151M, 184V
	12	20R, 41L, 103N, 108I, 184V, 215Y, 227L		30	65R, 70R, 75I, 77L, 103N, 115F, 116Y, 151M, 219E
	13	41L, 44D, 67N, 74I, 75AV, 103N, 106M, 184V, 210W, 215Y		31	65R, 101P, 103N
	14	41L, 44AE, 67N, 69D, 98SA, 103N, 118IV, 184V, 210W, 215Y, 219NK	8	32	41L, 62V, 65R, 75I, 101Q, 116Y, 118I, 151M, 190S, 210W, 215D
4	15	67N, 70R, 103N, 116Y, 151M, 190A, 215F, 219Q		33	41L, 62V, 65R, 67N, 75I, 77L, 101Q, 116Y, 118I, 151M, 190S, 210W, 215DY
	16	41L, 67N, 70R, 98GA, 103N, 179VDE, 215F, 219Q, 225H		34	41L, 62V, 65R, 67N, 75L, 116Y, 151M, 181I, 210W, 215Y
	17	41L, 67N, 70R, 103N, 215F, 219Q	9	35	69TADN, 151M
	18	67N, 70R, 98G, 103N, 184V, 215F, 219Q		36	62V, 75I, 77L, 116Y, 151M

Shown only positions with mutations associated with drug resistance (Stanford HIV Drug Resistance Database, February)

5. IC50, fold and replicative fitness

Virus study #	Virus ID	IC50 [µM] (fold)							Fitness [% of wt control]
		OBP-601	AZT	d4T	ABC	TDF	3TC	EFV	
1	1	3.46 (1.0)	0.15 (0.9)	3.49 (0.8)	0.61 (0.9)	0.18 (0.2)	0.19 (1.0)	0.00072 (0.7)	101
	2	0.76 (0.2)	0.1 (0.6)	1.57 (0.4)	1.15 (1.7)	0.52 (0.5)	0.1 (0.5)	0.00067 (0.7)	50
	3	5.80 (1.8)	0.22 (1.3)	3.60 (0.8)	0.85 (1.3)	1.57 (1.5)	0.11 (0.6)	0.0005 (0.5)	67
	4	1.37 (0.4)	0.05 (0.3)	6.06 (1.4)	0.58 (0.9)	0.76 (0.7)	0.13 (0.7)	0.00023 (0.2)	49
2	5	19.1 (5.8)	1.17 (6.9)	14.5 (3.3)	4.03 (6.0)	2.33 (2.3)	10.0 (53)	0.032 (32)	66
	6	34.3 (10)	3.41 (20)	11.1 (2.5)	8.76 (13)	4.41 (4.3)	>100 (>500)	0.440 (440)	92
	7	14.4 (4.4)	4.81 (28)	8.45 (1.9)	16.9 (25)	7.82 (7.7)	3.03 (16)	0.540 (540)	31
3	8	1.13 (0.3)	0.09 (0.5)	1.35 (0.3)	0.58 (0.9)	0.57 (0.6)	0.26 (1.4)	0.018 (18)	31
	9	11.5 (3.5)	1.70 (10)	15.1 (3.4)	1.51 (2.3)	2.26 (2.2)	0.81 (4.3)	3.14 (3140)	35
	10	12.9 (3.9)	0.93 (5.5)	19.2 (4.4)	2.66 (4.0)	3.13 (3.1)	0.36 (1.9)	0.96 (960)	30
	11	14.3 (4.3)	2.48 (15)	16.8 (3.8)	3.73 (5.6)	1.71 (1.7)	12.1 (64)	0.025 (25)	38
	12	11.1 (3.4)	0.57 (3.3)	6.65 (1.5)	1.68 (2.5)	1.76 (1.7)	7.00 (37)	~1.9 (>1900)	29
	13	6.68 (2.0)	0.38 (2.3)	7.96 (1.8)	5.68 (8.5)	0.89 (0.9)	9.86 (52)	0.92 (920)	34
	14	4.92 (1.5)	1.54 (9.0)	10.8 (2.4)	7.12 (11)	5.30 (5.2)	0.39 (2.0)	0.020 (20)	40
4	15	9.43 (2.9)	1.61 (9.5)	15.3 (3.5)	3.78 (5.6)	2.61 (2.6)	0.50 (2.7)	0.39 (390)	22
	16	25.9 (7.8)	5.50 (32)	16.9 (3.8)	4.14 (6.2)	3.64 (3.6)	0.70 (3.7)	1.85 (1850)	43
	17	17.0 (5.2)	4.15 (24)	12.2 (2.8)	2.47 (3.7)	6.85 (6.7)	0.59 (3.1)	0.086 (86)	64
	18	10.7 (3.2)	1.83 (11)	6.00 (1.4)	10.8 (16)	0.83 (0.8)	>100 (>500)	1.67 (1670)	104
	19	7.98 (2.4)	1.89 (11.1)	9.79 (2.2)	4.26 (6.4)	3.29 (3.2)	6.76 (36)	0.012 (12)	38
	20	5.26 (1.6)	1.04 (6.1)	11.9 (2.7)	6.94 (10)	1.97 (1.9)	0.44 (50)	0.028 (28)	33

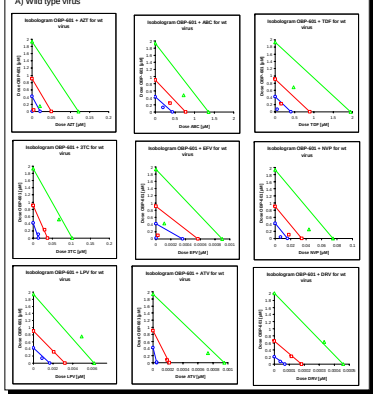
6. IC50, fold and replicative fitness (cont.)

Virus study #	Virus ID	IC50 [µM] (fold)							Fitness [% of wt control]
		OBP-601	AZT	d4T	ABC	TDF	3TC	EFV	
5	21	13.7 (4.2)	0.96 (5.7)	4.43 (1.0)	3.46 (5.2)	1.36 (1.3)	9.37 (49)	0.010 (10)	27
	22	15.7 (4.7)	2.46 (15)	2.20 (0.5)	7.33 (11)	1.55 (1.5)	17.6 (93)	0.027 (27)	77
	23	12.5 (3.8)	2.60 (15)	2.81 (0.6)	1.39 (2.1)	0.75 (0.7)	21.5 (110)	0.033 (33)	36
	24	20.7 (6.3)	0.79 (4.7)	6.78 (1.5)	4.64 (6.9)	2.07 (2.0)	9.97 (53)	>10 (>10000)	34
	25	27.4 (8.3)	3.26 (19)	2.09 (0.5)	12.1 (18)	7.27 (7.1)	>100 (>500)	0.14 (140)	52
6	26	20.0 (6.1)	0.86 (5.1)	13.4 (3.0)	5.25 (7.8)	0.94 (0.9)	14.8 (78)	2.44 (2440)	29
	27	10.2 (3.1)	0.24 (1.4)	7.37 (1.7)	9.18 (14)	0.85 (0.8)	19.4 (100)	0.022 (22)	46
	28	11.8 (3.6)	0.18 (1.1)	3.77 (0.9)	7.76 (12)	1.81 (1.8)	16.3 (86)	>10 (>10000)	50
7	29	11.4 (3.4)	5.85 (34)	13.4 (3.0)	7.06 (11)	5.87 (5.8)	- 17.0 (90)	1.02 (1020)	45
	30	3.81 (1.2)	10.5 (62)	>100 (>22)	7.98 (12)	4.81 (4.7)	4.52 (24)	0.320 (320)	41
	31	5.30 (1.6)	0.38 (2.2)	24.1 (5.5)	2.32 (3.5)	1.27 (1.2)	2.35 (12.4)	2.77 (2770)	42
8	32	4.18 (1.3)	5.23 (31)	83.7 (19)	6.72 (10)	2.26 (2.2)	1.42 (7.5)	1.60 (1600)	13
	33	0.89 (0.3)	3.37 (20)	36.0 (8.2)	4.10 (6.1)	1.72 (1.7)	3.15 (17)	0.51 (510)	77
	34	2.25 (0.7)	1.33 (7.8)	97.5 (22)	4.42 (6.6)	1.01 (1.0)	0.86 (4.5)	0.0011 (1.1)	53
9	35	0.52 (0.2)	0.43 (2.5)	20.9 (4.8)	1.97 (2.9)	1.01 (1.0)	0.29 (1.5)	0.0006 (0.6)	37
	36	0.35 (0.1)	3.64 (21)	23.6 (5.4)	1.56 (2.3)	4.33 (4.2)	1.07 (5.6)	0.0004 (0.4)	95

IC50 values were calculated in triplicate. Fold was calculated based on IC50 of NL4-3-fluc2 wild type control. Replicative fitness values are from the average of eight growth competitions and expressed as a percentage of the replicative fitness of a wild type control NL4-3-fluc2.

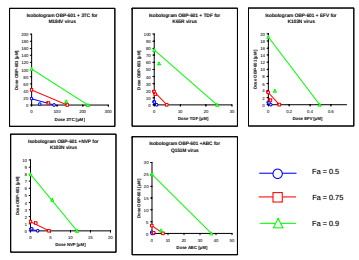
7. Combination study

A) Wild type virus



8. Combination study (cont.)

B) Viruses carrying M184V, K65R, K103N, and Q151M mutation



The combination study was performed in triplicate using constant ratio combination design. Parameters were calculated using COMPUSYN version 1.0.1 and classic isobolograms were constructed for fractional inhibition 50%, 75% and 90%.

9. Combination study summary

Virus	Drug(s)	Combination index at inhibition				Dm (µM)	m	r	Result
		ED50	ED75	ED90	ED95				
WT	OBP-601 + AZT	0.073	0.134	0.247	0.375	0.0095	0.76	0.92	strong synergism
WT	OBP-601 + ABC	0.750	0.761	0.779	0.795	0.326	1.68	0.95	moderate synergism
WT	OBP-601 + TDF	0.292	0.418	0.597	0.761	0.122	0.98	0.94	synergism
WT	OBP-601 + 3TC	1.245	1.070	0.930	0.852	0.117	1.37	0.94	nearly additive
WT	OBP-601 + EFV	0.068	0.153	0.351	0.626	0.022	0.74	0.84	strong synergism
WT	OBP-601 + NVP	0.541	0.623	0.717	0.789	0.049	1.21	0.89	moderate synergism
WT	OBP-601 + LPV	0.857	1.003	1.177	1.313	0.136	1.29	0.90	nearly additive
WT	OBP-601 + ATV	1.053	0.960	0.913	0.915	0.019	0.82	0.90	nearly additive
WT	OBP-601 + DRV	0.904	0.965	1.038	1.096	0.0745	1.03	0.98	nearly additive
M184V	OBP-601 + 3TC	0.508	0.597	0.722	0.831	34.83	1.53	0.99	synergism
K65R	OBP-601 + TDF	0.966	0.896	0.834	0.795	3.88	0.80	0.93	moderate synergism (E90>)
K103N	OBP-601 + EFV	1.018	0.581	0.334	0.231	0.451	1.00	0.95	synergism
K103N	OBP-601 + NVP	1.438	1.122	1.016	1.058	0.601	0.79	0.92	nearly additive (E90>)
Q151M	OBP-601 + ABC	0.219	0.210	0.207	0.208	0.207	0.62	0.93	strong synergism

Combination index is given for 50%, 75%, 90% and 95% inhibition effect
Dm – median-effect dose; the dose that produces 50% effect
m - slope of the median-effect plot
r - conformity parameter for goodness of fit

10. Conclusions

- OBP-601 (4'-Ed4T) is a novel nucleoside analog with potent anti-HIV-1 activity.
- OBP-601 susceptibility to recombinant wild type viruses ranged from 0.76 to 5.8 µM, slightly lower than the parental compound d4T (1.57 to 6.06 µM).
- The anti-HIV-1 activity of OBP-601 was reduced in most viruses carrying NAM (5- to 10-fold), TAM41 (0.3 to 4.3-fold), and TAM67 (1.6- to 7.8-fold) resistance mutations, together with K103N +/- M184V.
- When compared with K103N in multi-drug resistant viruses the presence of double mutants K103N + M184V only moderately increased the 50% inhibition concentrations to OBP-601 from average fold 3.2 (K103N n=9, range 0.3- to 7.8-fold) to 4.3 (K103N + M184V, n=19, range 1.5- to 10.4-fold).
- Interestingly, viruses carrying the Q151M mutation were hypersusceptible to OBP-601 (0.1- to 0.2-fold), even in the presence of K65R (0.3- to 1.3-fold)
- OBP-601 showed strong synergism in wild type virus with AZT and EFV and moderate synergism with ABC, TDF and NVP.
- Similar synergism was exhibited with 3TC, EFV, and ABC in viruses carrying M184V, K103N, and Q151M, respectively.