

In Vitro Characterization of UK-453,061, a Non-Nucleoside Reverse Transcriptase Inhibitor

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Abstract

Background: UK-453,061 is an NNRTI with potent antiviral activity against HIV-1 wt and clinically relevant NNRTI-resistant viruses in vitro. Studies in healthy volunteers indicated UK-453,061 had good bioavailability, was safe and well tolerated and was efficacious when used as short-term monotherapy in HIV-1 infected patients.

Methods: The antiviral activity of UK-453,061 was tested against clinically derived viruses from treatment-naïve patients representing different HIV-1 subtypes or carrying transmitted NNRTI mutations in the PhenoSense assay. Drug combination was assessed using a cytopathic effect cell-based assay. Each combination was titrated against HIV-1 NL4-3 acutely infected SupT1 cells and virus growth determined indirectly after 5 days culture. Data were analyzed using the MacSynergy II three-dimensional model of Pritchard and Shipman.

Results: UK-453,061 inhibited a panel of 45 pseudotyped subtype B and non-subtype B viruses representing subtypes A, BF, C, D, F and G). All viruses were susceptible to UK-453,061 with IC₅₀ fold-change to the reference virus ≤ 10 (43 viruses within 2-fold). UK-453,061 was active against 61 of 62 viruses with transmitted NNRTI resistance (IC₅₀ fold-change vs reference virus ≤ 10). The remaining virus gave an IC₅₀ fold-change vs reference virus > 10 and carried a triple NNRTI mutation K101E+V106I/M+Y188F/L. NNRTI resistance mutations at K103 (48%) were the most prevalent in this virus panel, followed by Y181 (11%), K101 (11%) and Y188 (11%). Additive antiviral interactions were observed when UK-453,061 was tested in combination with PI, and the fusion inhibitor enfuvirtide, while synergistic interactions were frequently obtained with examples of the NRTI and integrase classes.

Conclusion: The in vitro data indicate UK-453,061 to be active against viruses from different origins and with transmitted NNRTI drug resistance and supports the continued development of UK-453,061 in combination with clinically approved HIV therapies.

Introduction

Non-nucleoside inhibitors of HIV-1 reverse transcriptase (RT) are key components of highly active antiretroviral therapy (HAART). There are three approved non-nucleoside reverse transcriptase inhibitors (NNRTIs) – efavirenz, nevirapine and delavirdine – but all are associated with single-point mutations in HIV-1 that can lead to decreased susceptibility and class resistance, as well as significant adverse effects. Therefore, an unmet need exists for a second-generation NNRTI with activity against clinically significant drug-resistant HIV-1 mutants and an improved safety and tolerability profile.

UK-453,061 has shown activity against clinically relevant HIV-1 RT single- and double-point mutations in in vitro assays, retaining potency similar to that observed against the wild-type RT enzyme.¹ Short term monotherapy with UK-453,061 reduces viral load in HIV infected patients.²

The data presented below summarize the in vitro antiviral activity of UK-453,061 against viruses representing different HIV-1 subtypes and viruses from treatment-naïve patients carrying transmitted clinically relevant NNRTI-resistant mutations. The antiviral effects of UK-453,061 in combination with a number of approved antiretrovirals are also presented.

Methods

In vitro antiviral activity: Drug susceptibility testing was carried out at Monogram Biosciences to determine:

- Spectrum of activity:** the antiviral activity of UK-453,061 was assessed against a panel of 45 HIV-1 isolates representing subtypes A, B, BF, C, C/H, D, F and G. Isolates carried no NNRTI resistance-associated mutations, were susceptible to efavirenz [< 3 fold-change]³ with respect to reference (wild-type) virus and represented different geographical origins.
- Transmitted NNRTI resistance:** the antiviral activity of UK-453,061 was assessed against a panel of 62 HIV-1 isolates, from treatment-naïve patients, containing NNRTI resistance-associated mutations. Efavirenz, nevirapine and delavirdine were included as controls.

Drug combination studies: UK-453,061, in combination with a second antiretroviral from the nucleoside reverse transcriptase inhibitor (NRTI), protease inhibitor (PI), integrase inhibitor and fusion inhibitor drug classes, was titrated on to HIV-1 strain NL4-3-infected MT-2 cells. After 5 days, virus growth was determined indirectly by measuring metabolic activity of viable cells. Data were analyzed using the MacSynergyTM II three-dimensional model of Pritchard and Shipman.⁴ Volumes of synergy ($\mu\text{M}^2\%$) were calculated at 95% confidence from 3-4 replicates per assay. Virus [positive values] or antagonism [negative values] was defined as drug combinations yielding mean volumes in excess of 25 $\mu\text{M}^2\%$; additive drug interactions were defined by mean volumes of 0-25 $\mu\text{M}^2\%$.

Results

In vitro antiviral activity

Spectrum of activity

- Viruses of all subtypes, A, B, BF, C, C/H, D, F and G, were susceptible to UK-453,061 with a geometric mean EC₅₀ fold-change < 2 versus the reference virus EC₅₀ (Table 1).

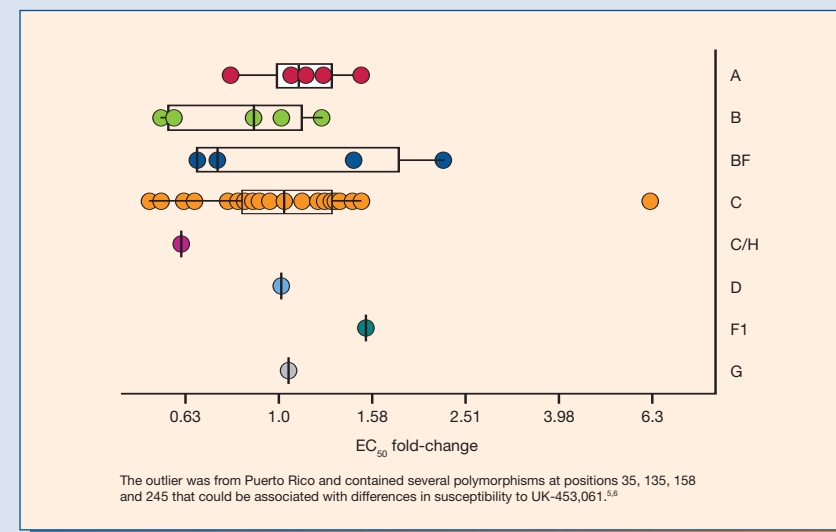
Table 1. Activity of UK-453,061 against different HIV-1 subtypes, EC₅₀ fold-change with respect to wild-type virus.

Subtype	n	Geographical origin	UK-453,061	
			EC ₅₀ fold-change ^a	95% CI
All	45		1.03	0.91-1.18
A (A1, AE, AG)	6	Australia Canada Netherlands Poland South Africa United Kingdom	1.12	0.89-1.40
B	5	Australia (n=2) Canada Poland United States	0.82	0.54-1.26
BF	5	Argentina	1.02	0.51-2.02
C	25	South Africa (n=20) Australia Canada Puerto Rico United Kingdom United States	1.07	0.88-1.30
C/H	1	South Africa	0.62	na
D	1	United Kingdom	1.02	na
F1	1	Argentina	1.54	na
G	1	United Kingdom	1.05	na

^aGeometric mean.
CI, confidence interval; na, not available.

- Of 45 viruses tested, 43 had an EC₅₀ fold-change within 2-fold of the reference virus EC₅₀. One subtype BF had an EC₅₀ fold-change of 2.3 and one subtype C had an EC₅₀ fold-change of 6.3 (this virus contained mutations in the RT gene that may be associated with differences in susceptibility) (Figure 1).

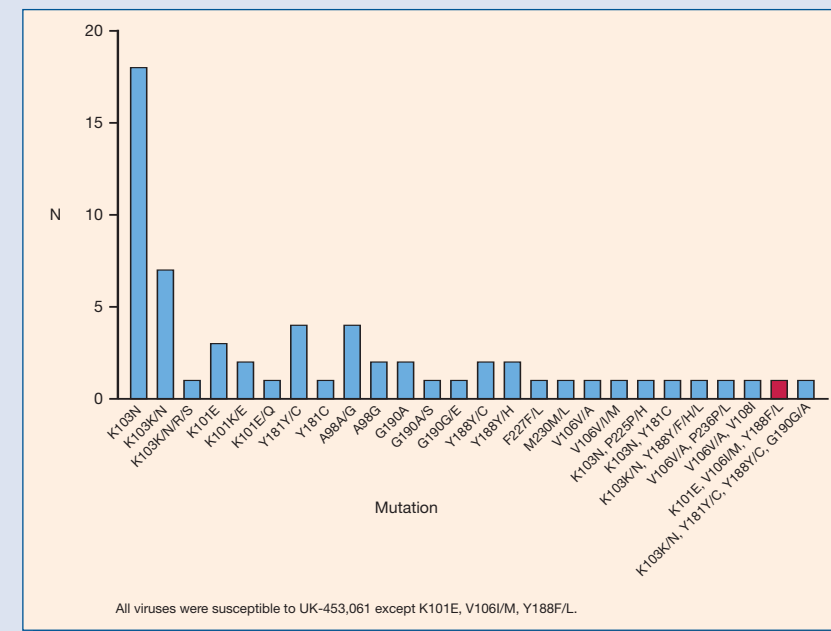
Figure 1. Box and whisker plots showing susceptibility (EC₅₀ fold-change vs wt) of HIV-1 subtypes to UK-453,061.



Transmitted NNRTI resistance

- A total of 26 different resistance mutation patterns were present in the viruses tested (Figure 2). The majority of transmitted NNRTI resistance-associated mutations in this panel were K103 (48%), followed by Y181, K101 and Y188 (all 11%).

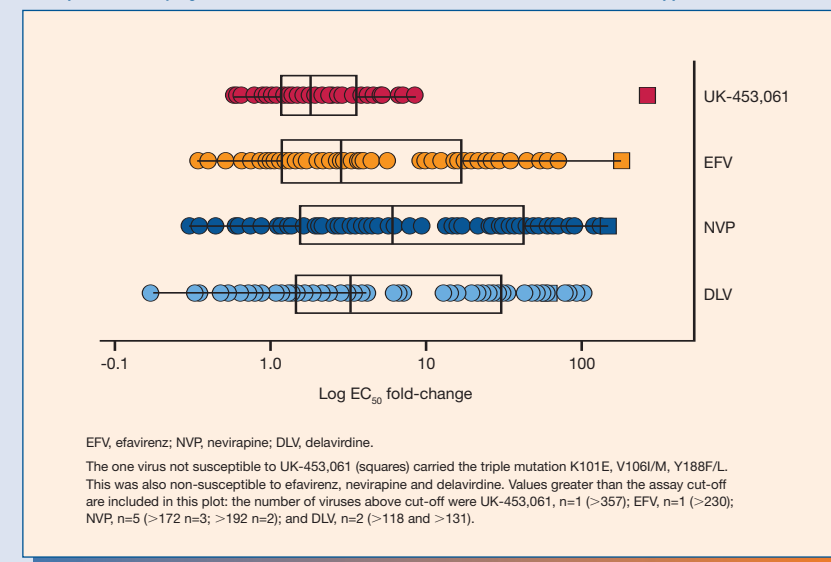
Figure 2. Prevalence of NNRTI resistance-associated mutations in the panel of 62 viruses from treatment-naïve patients tested.



All viruses were susceptible to UK-453,061 except K101E, V106I/M, Y188F/L.

- UK-453,061 was active against 61 of 62 viruses carrying transmitted NNRTI resistance (Figure 3), with a geometric mean EC₅₀ fold-change of 2.1 (range 0.6-10; N=61).

Figure 3. Box and whisker plots showing susceptibility (EC₅₀ fold-change vs wt) of virus isolates from treatment-naïve patients carrying NNRTI resistance-associated mutations to UK-453,061 and approved NNRTIs.



- The majority (87%) of the viruses tested showed < 5 fold-change susceptibility to UK-453,061 (Figure 4).
- One virus gave an EC₅₀ fold-change > 357 and carried a triple NNRTI mutation K101E+V106I/M+Y188F/L.
- UK-453,061 was active against a wide range of single-point mutations (Table 2) and was active against viruses carrying multiple mutations in all but one instance (Table 3).

Figure 4. Susceptibility (EC₅₀ fold-change vs wt) of viruses from treatment-naïve patients carrying NNRTI resistance-associated mutations to UK-453,061 and approved NNRTIs.

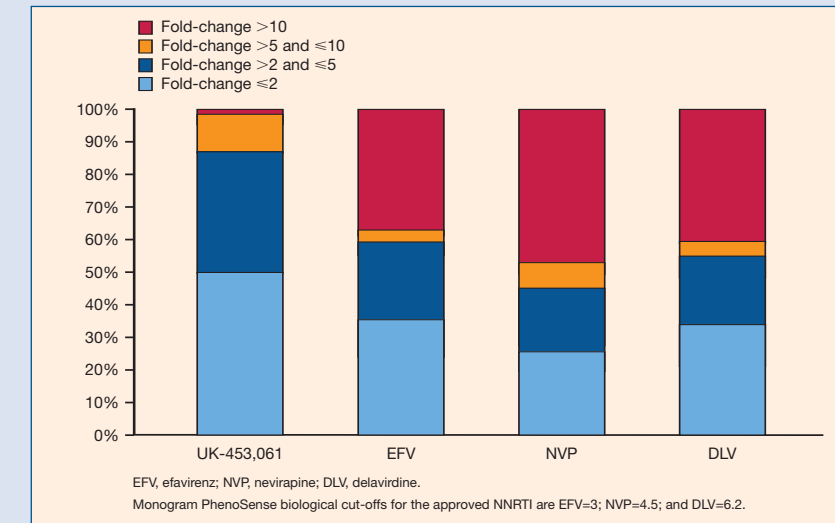


Table 2. Activity of UK-453,061 against viruses from treatment-naïve patients carrying single transmitted NNRTI resistance-associated mutations, EC₅₀ fold-change vs wt.

Mutation	n	%	UK-453,061	
			EC ₅₀ fold-change ^b	95% CI
K103	26	47	1.69	1.31-2.18
K101	6	11	3.21	1.44-7.13
A98	6	11	3.59	1.82-7.11
Y181	5	9	1.64	1.07-2.51
G190	4	7	2.35	0.66-8.36
Y188	4	7	1.64	0.99-2.71
V106	2	4	2.93	na
F227	1	2	1.78	na
M230	1	2	0.65	na

^aViruses carrying specific single-point mutation as a percentage of all viruses with single-point mutations tested (n=55).
^bGeometric mean.
CI, confidence interval; na, not available.
Many of the mutations contain wild-type polymorphism. If present at low levels, the wild-type virus may have predominated in the PhenoSense assay.

Table 3. Activity of UK-453,061 and the three approved NNRTIs against viruses from treatment-naïve patients carrying multiple transmitted NNRTI resistance-associated mutations, EC₅₀ fold-change vs wt.

Mutation	EC ₅₀ fold-change			
	UK-453,061	EFV	NVP	DLV
K103K/N, Y181Y/C, Y188Y/C, G190G/A	1.90	5.11	88.00	33.00
K103K/N, Y188Y/F/H/L	6.07	37.00	158.00	53.00
K103N, P225P/H	5.37	68.00	63.00	8.00
K103N, Y181C	10.00	87.00	≥ 172.10	≥ 118.48
V106V/A, P236P/L	0.69	0.35	0.36	0.34
V106V/A, V108I	4.44	0.54	0.46	0.86
K101E, V106I/M, Y188F/L	≥ 357.09	≥ 230.81	≥ 192.23	80.00

For each mutation, n=1.
EFV, efavirenz; NVP, nevirapine; DLV, delavirdine.

Drug combination studies

- UK-453,061 was tested in combination with a selection of NRTIs, PIs, integrase inhibitors and the fusion inhibitor enfuvirtide (Table 4).
- UK-453,061 showed moderate to strongly synergistic interactions ($> 100 \mu\text{M}^2\%$) when combined with representatives of the NRTI class.

- UK-453,061 in combination with a PI generally resulted in additive interactions.
- Minor to moderate synergy (25-100 $\mu\text{M}^2\%$) was seen between UK-453,061 and integrase inhibitors. UK-453,061 combination with enfuvirtide indicated moderate synergy (minor antagonism).
- No drug cytotoxicity was observed for any of the combinations tested.

Table 4. In vitro antiviral interactions of UK-453,061 with representatives of the licensed antiretroviral classes.

Name	n	Volume ($\mu\text{M}^2\%$) ^a		Combined effect
		Synergy	Antagonism	
Nucleoside reverse transcriptase inhibitors				
Abacavir	3	90.6 \pm 26.5	-49.3 \pm 84.2	Moderate synergy (minor antagonism)
Didanosine	4	156.0 \pm 66.1	-3.7 \pm 7.3	Strong synergy
Emtricitabine	4	140.5 \pm 57.1	-5.1 \pm 8.5	Strong synergy
Lamivudine	4	175.1 \pm 81.1	-6.3 \pm 12.1	Strong synergy
Tenofovir	3	115.6 \pm 40.7	-10.8 \pm 11.9	Strong synergy
Zidovudine	3	177.5 \pm 183.8	-15.4 \pm 26.7	Strong synergy ^b
Protease inhibitors				
Atazanavir	2	6.6 \pm 9.4	-23.1 \pm 32.6	Additive
Lopinavir	2	29.1 \pm 19.4	-28.5 \pm 30.3	Minor synergy / minor antagonism ^c
Ritonavir	2	12.6 \pm 0.6	-19.1 \pm 27.1	Additive
Integrase inhibitors				
Elvitegravir	3	69.7 \pm 66.5	-20.1 \pm 34.8	Moderate synergy
Raltegravir	2	34.6 \pm 13.8	-10.7 \pm 15.1	Minor synergy
Entry inhibitors				
Enfuvirtide	3	75.1 \pm 77.8	-32.8 \pm 56.8	Moderate synergy (minor antagonism)

n, number of experiments.
^aMean \pm SD.
^bStrong synergy was seen in two experiments; an additive interaction was seen in the third experiment.
^cMinor synergy / minor antagonism was seen in one experiment; an additive interaction was seen in the second experiment.

Conclusions

- UK-453,061 is a novel and selective NNRTI that demonstrates activity against all HIV-1 subtypes (A, B, BF, C, C/H, D, F and G) tested.
- Drug susceptibility testing showed that UK-453,061 was active against 61 out of 62 viruses with transmitted NNRTI resistance-associated mutations isolated from treatment-naïve patients with HIV-1.
- UK-453,061 was active against viruses containing a wide range of the most common single NNRTI mutations commonly associated with resistance to older-generation NNRTIs.
- Strong synergy was observed with the NRTIs. Administration of UK-453,061 with NRTIs may result in enhanced antiviral activity. While in practice three or more agents may be combined, the observation of strong synergy may indicate potential clinical benefit.
- Together, these results indicate that continued clinical investigation of UK-453,061, a next-generation NNRTI, in combination with other antiretroviral agents is merited.

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