Neutralization of Several Adult and Paediatric HIV-1 Subtype C Isolates using a Shortened Synthetic Derivative of gp120 Binding Aptamer Called UCLA1

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INTRODUCTION

Previously, a gp120 binding RNA aptamer called B40 was isolated by Khati and colleagues (Khati, *et al.*, 2003). The parental B40 aptamer was then truncated to B40t77, which possessed the minimal essential region for binding to gp120 (Dey *et al.*, 2005a). In this study, a chemically synthesised derivative of the B40 parental aptamer, called UCLA1 (Cohen *et al.*, 2008), was used for neutralization of endemic subtype C clinical isolates of HIV-1 from adult and paediatric patients and subtype B lab adapted strains.

METHODS

The UCLA1 RNA aptamer was used for neutralization of a panel of HIV-1 subtype C and HIV-1 subtype B Env-pseudotyped viruses. Neutralization of HIV-1 was measured in duplicate as a reduction in luciferase gene expression after a single-round infection of TZM-bl cells with the Env-pseudotyped viruses as previously described (Gray *et al.*, 2006). The binding affinity and dissociation constant of the aptamer were measured against HIV-1 subtype C Du151 gp120 glycoprotein using the BIAcore™ Surface Plasmon Resonance Technology (SPR) technology. Cytotoxicity of the aptamer was measured overnight in TZM-bl cells using an ATP-based luminescent assay. The therapeutic index (TI) of the aptamer was determined at a CC_{50} of 500nM for all the viruses tested.

RESULTS

The UCLA1 RNA aptamer was found to be non-toxic to the TZM-bl cells (Figure 1) and exhibited high therapeutic indices for the tested viruses (Table 1). The aptamer exhibited high binding affinity for the subtype C gp120 with a very low K_D value of 5.8nM (Figure 2) compared to B40 aptamer (21nM), or B40t77 (31nM), (Dey *et al.*, 2005). The aptamer neutralized 27/39 viruses within a range of 61-100% neutralization (Figure 3), with low IC_{50} values in the nanomolar range (Table 1). 24/27 were R5 and two were X4 subtype C viruses. The aptamer also neutralized an X4 subtype B virus (HXB2) (Table 1). Representative neutralization graphs are shown in figure 4.

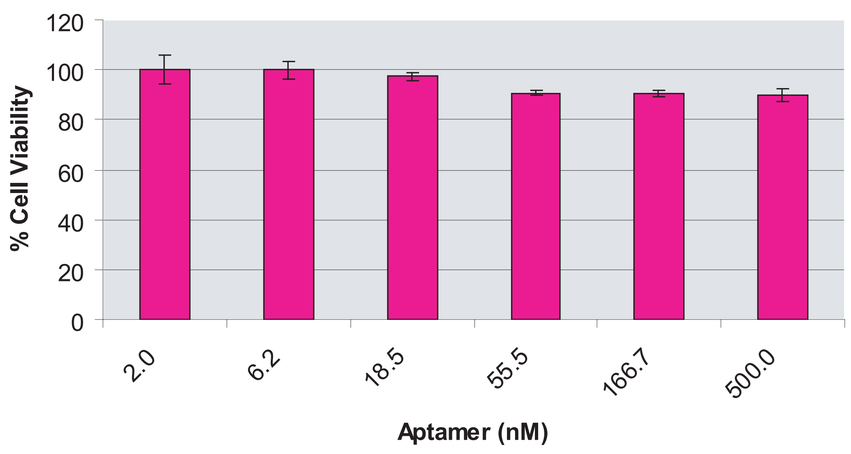

Figure 1: Viability of TZM-bl cells in the presence of UCLA1 RNA aptamer

Table 1. Percent neutralization of Env-pseudotyped viruses. IC_{50} values and therapeutic index values are reflected for each virus that was inhibited with the UCLA1 RNA aptamer by more than 50%.

Env Clone	Subtype	CoR	% Neutralization	IC_{50} (nM)	Therapeutic Index	Accession number
Du123.6	C	R5	24	nt	nd	DQ411850
Du151.2	C	R5	25	nt	nd	DQ411851
Du156.12	C	R5	85	0.63	794	DQ411852
Du172.17	C	R5	78	0.60	833	DQ411853
Du422.1	C	R5	73	1.34	373	DQ411854
CAP08.2.00.F6J	C	R5	46	nt	nd	EF203976
CAP45.2.00.G3	C	R5	34	nt	nd	DQ435682
CAP61.2.00.F10J	C	R5	84	0.38	1316	EF203957
CAP63.2.00.A9J	C	R5	88	0.35	1429	EF203973
CAP84.2.00.32J	C	R5	45	nt	nd	EF203963
CAP85.2.00.09J	C	R5	80	0.42	1190	EF203985
CAP88.2.00.B6J	C	R5	76	1.74	287	EF203970
CAP210.2.00.E8	C	R5	93	0.34	1471	DQ435683
CAP228.2.00.51J	C	R5	83	0.35	1429	EF203968
CAP239.2.00.G3J	C	R5	68	0.94	532	EF203983
CAP244.2.00.D3	C	R5	66	0.44	1136	DQ435684
CAP248.2.00	C	R5	67	0.93	538	FJ229824
CAP255.2.00.16J	C	R5	70	0.40	1250	EF203982
CAP256.2.00.F7J	C	R5	77	0.42	1190	EF203980
Consensus C	C	R5	78	0.43	1163	DQ401075
COT6.15	C	R5	23	nt	nd	DQ447266
COT9.6	C	R5	57	0.70	714	DQ447272
RP1.12	C	X4	82	0.60	833	DQ447271
RP4.3	C	R5	30	nt	nd	DQ447270
RP6.6	C	R5	68	0.47	1064	DQ447269
TM3.8	C	R5	77	0.13	3846	DQ447268
TM7.9	C	R5	63	0.16	3125	DQ447267
SW7.14	C	X4	77	0.73	685	AF411966
ZM53M.PB12	C	R5	61	0.95	526	AY423984
ZM109F.PB4	C	R5	34	nt	nd	AY424138
ZM135M.PL10a	C	R5	25	nt	nd	AY424079
ZM197.M.PB7	C	R5	87	0.38	1316	DQ388515
ZM214M.PL15	C	R5	76	0.23	2174	DQ388516
ZM233M.PB6	C	R5	92	0.43	1163	DQ388517
ZM249M.PL1	C	R5	74	0.55	909	DQ388514
HXB2.C8.3	B	X4	100	1.04	481	AF358142
SF162.LS	B	R5	13	nt	nd	EU123924
BaL.1	B	R5	24	nt	nd	DQ318210
BaL.26	B	R5	32	nt	nd	DQ318211

nt, no titre at IC_{50}
nd, not determined
IC_{50} is the concentration of the aptamer causing 50% reduction of RLU compared to the virus control.
Therapeutic index = $CC_{50}/IC_{50,}$ where CC_{50} is the concentration of the aptamer that inhibits cell viability by 50%. The CC_{50} was calculated at a standard of 500nM.

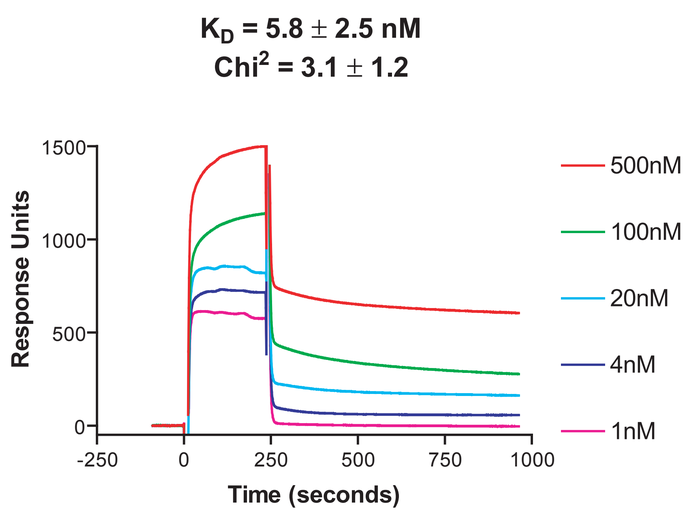

Figure 2: A sensogram showing the binding profile of 0.5 log_{10} dilutions (500 to 1.0 nM) of the UCLA1 RNA aptamer to HIV-1 subtype C Du151 gp120 glycoprotein. The aptamer exhibited an equilibrium dissociation constant (K_D) value of 5.8nM and a Chi^2 of 3.1 showing a proper fit of the data to the Langmuir binding model.

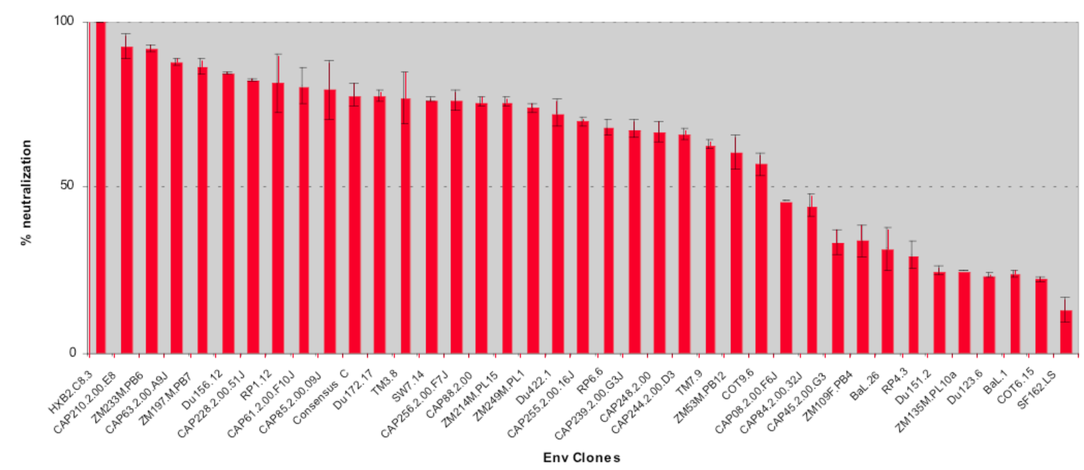

Figure 3: Neutralization of a subtype B and a panel of subtype C HIV-1 Env-pseudotyped viruses with the UCLA1 RNA aptamer. Neutralization was measured as a reduction of virus infectivity relative to the virus control (without aptamer). The IC_{50} points are indicated with the dotted line.

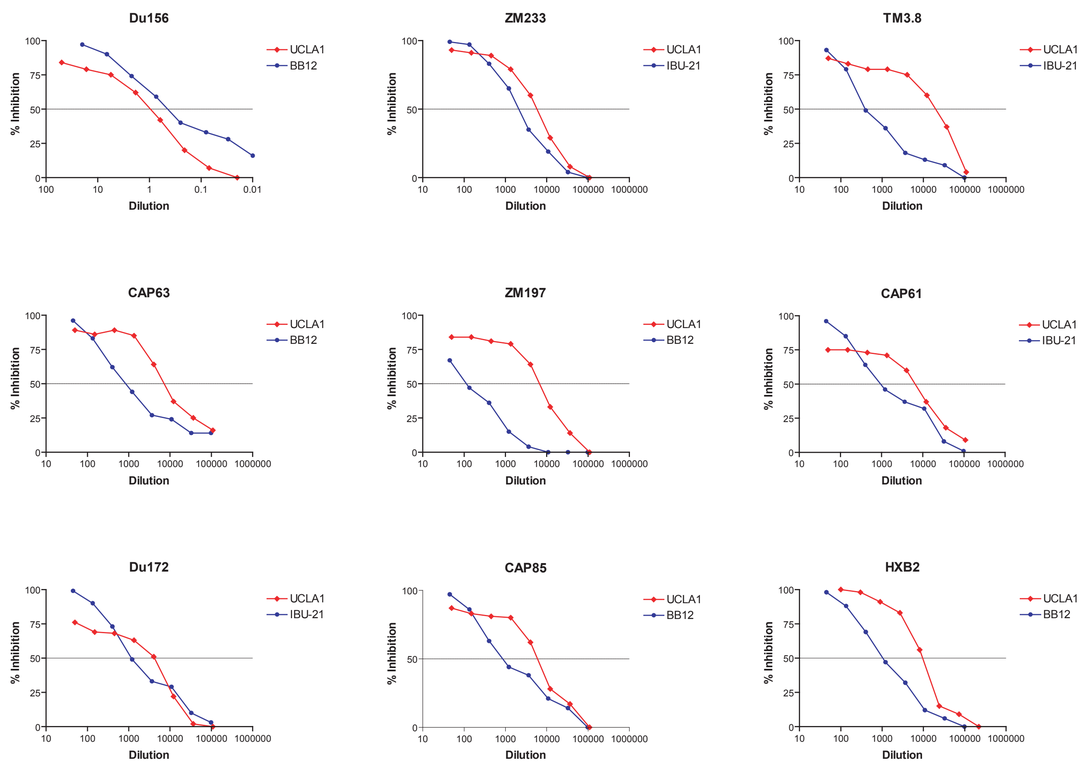

Figure 4: Representative neutralization graphs of subtype C HIV-1 Env-pseudotyped viruses with the UCLA1 RNA aptamer. The aptamer was used at starting concentrations of either 50 or 100nM equivalent to 1:50 and 1:100 dilutions, respectively. HIV-1 positive plasma pools (BB12 and IBU-21) were used as positive controls at a starting dilution of 1:45. The 50% inhibition dilution (ID_{50}) points are indicated with the dotted lines.

SUMMARY AND CONCLUSION

- The UCLA1 RNA aptamer, a derivative of B40 RNA aptamer isolated against a R5, subtype B gp120 derived from $HIV\text{-}1_{Ba\text{-}L}$ was used to neutralize a panel of subtype C HIV-1 isolates from both acute and chronically infected adult and paediatric patients.
- The aptamer potently neutralized both R5 and X4 subtype C viruses as well as a subtype B X4 virus.
- This data suggest that UCLA1 RNA aptamer binds to gp120 neutralization epitopes conserved across at least the two HIV-1 subtypes and tropism.
- The UCLA1 aptamer is potent with average IC_{50} values of less than 2nM.
- The UCLA1 aptamer is non-toxic to cells, with average CC_{50} values of more than 500 nM, resulting in high therapeutic index averaging over 1,000.
- Taken together, the data suggests that the aptamer has broad spectrum potency against several subtype C viruses and argues for development of the aptamer as a potential potent HIV-1 subtype C entry inhibitor drug, with no cytotoxicity at the estimate therapeutic dose.

ACKNOWLEDGEMENTS

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REFERENCES

[1] Cohen, C., Forzan, M., Sproat, B., Pantophlet, R., McGowan, I., Burton, D., and James, W. (2008). *Virol*, **381**:46-54.
[2] Dey, A. K., Griffiths, C., Lea, S. M. & James, W. (2005a). *Rna* **11**, 873-84.
[3] Gray, E. S., Meyers, T., Gray, G., Montefiori, D. C., & Morris, L. (2006). *PLoS Med* **3**, e255.
[4] Khati, M., Schuman, M., Ibrahim, J., Sattentau, Q., Gordon, S., & James, W. (2003). *J Virol* **77**, 12692-8.