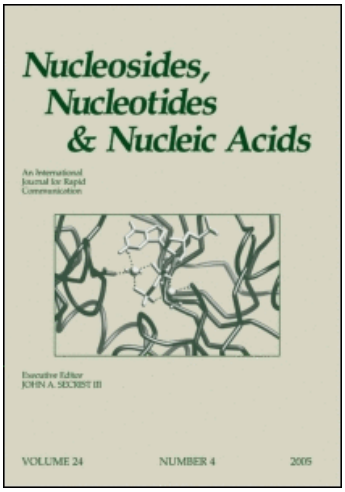

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Antiviral Activity of Steric-Block Oligonucleotides Targeting the HIV-1 *Trans*-Activation Response and Packaging Signal Stem-Loop RNAs

Douglas Brown[a]; Andrey A. Arzumanov[b]; John J. Turner[b]; Dmitry A. Stetsenko[b]; Andrew M. L. Lever[a]; Michael J. Gait[b]
[a] Department of Medicine, University of Cambridge, Addenbrooke's Hospital, Cambridge, UK [b] Medical Research Council, Laboratory of Molecular Biology, Cambridge, UK

To cite this Article Brown, Douglas , Arzumanov, Andrey A. , Turner, John J. , Stetsenko, Dmitry A. , Lever, Andrew M. L. and Gait, Michael J.(2005) 'Antiviral Activity of Steric-Block Oligonucleotides Targeting the HIV-1 *Trans*-Activation Response and Packaging Signal Stem-Loop RNAs', Nucleosides, Nucleotides and Nucleic Acids, 24: 5, 393 — 396
To link to this Article: DOI: 10.1081/NCN-200059813
URL: http://dx.doi.org/10.1081/NCN-200059813

Nucleosides, Nucleotides, and Nucleic Acids, 24 (5–7):393–396, (2005)

ISSN: 1525-7770 print/ 1532-2335 online
DOI: 10.1081/NCN-200059813

Taylor & Francis
Taylor & Francis Group

ANTIVIRAL ACTIVITY OF STERIC-BLOCK OLIGONUCLEOTIDES TARGETING THE HIV-1 *TRANS*-ACTIVATION RESPONSE AND PACKAGING SIGNAL STEM-LOOP RNAs

Douglas Brown ▫ *Department of Medicine, University of Cambridge, Addenbrooke's Hospital, Cambridge, UK*

Andrey A. Arzumanov, John J. Turner, and Dmitry A. Stetsenko ▫ *Medical Research Council, Laboratory of Molecular Biology, Cambridge, UK*

Andrew M. L. Lever ▫ *Department of Medicine, University of Cambridge, Addenbrooke's Hospital, Cambridge, UK*

Michael J. Gait ▫ *Medical Research Council, Laboratory of Molecular Biology, Cambridge, UK*

▫ *Mixmer oligonucleotides consisting of residues of both 2′-O-methylnucleosides (OMe) and locked nucleic acids (LNA) were designed targeting two stem-loops in the 5′-UTR of HIV-1 RNA, the trans-activation response region (TAR), which is the site of binding of the Tat protein, and the SL3 loop, which is the primary packaging element that binds the Gag polyprotein. These oligonucleotides were found to inhibit syncitia formation dose- and sequence-dependently when delivered to HeLa T4 LTR β-Gal cells and subsequently infected with HIV-1.*

INTRODUCTION

The human immunodeficiency virus type 1 (HIV-1) encodes a 5′-leader RNA sequence (5′-UTR) that contains several functional elements that are essential for virus production.[1] These elements include a number of RNA stem-loops that act as binding sites for virus-specific proteins. Interference with such interactions would be expected to inhibit viral replication and provides potential opportunities for the development of novel anti-HIV agents.

We thank Donna Williams and Matthew Watson for supply of oligonucleotides. This work is funded in part by a grant from EC Framework 5 (contract QLK3-CT-2002-01989).

Address correspondence to Douglas Brown, Department of Medicine, University of Cambridge, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ, UK

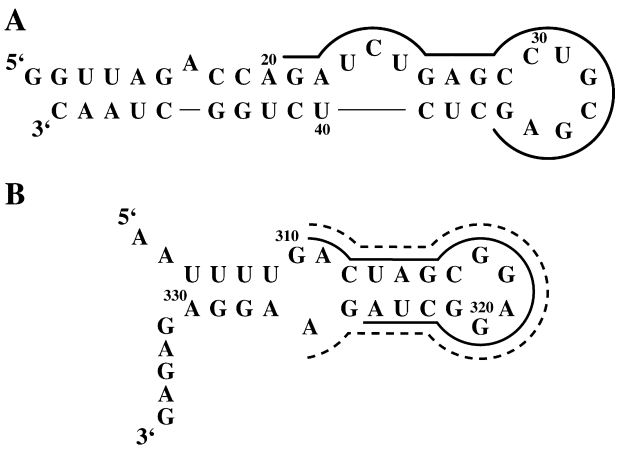

FIGURE 1 A) Secondary structure of the top part of the RNA stem-loop known as the trans-activation response element (TAR) showing (solid line) the region targeted by the 16-mer OMe/LNA oligonucleotide (*C*U*C*C*C*AGG*C*U*C*AGA*T*C, where underlined residues are LNA). Numbering refers to the residues counted from the 5′-end of HIV-1 transcripts. B) Secondary structure of the SL3 RNA stem-loop that is the binding site for HIV-1 gag protein, showing the regions targeted by 16-mer OMe/LNA (U*C*U*A*G*C*C*U*C*C*G*C*U*A*GU, dashed line, where underlined residues are LNA), and 14-mer OMe/LNA (U*A*G*C*C*U*C*C*G*C*U*A*GU, solid line).

Trans-activation of transcription is triggered by the interaction of the HIV-1 Tat protein and host cellular factors with a 59-residue stem-loop RNA known as the *trans*-activation responsive element (TAR) (Figure 1A), which occurs at the extreme 5′-end of all HIV transcripts. We have shown that steric-block mixmer oligonucleotides of 12–16 residues containing both 2′-*O*-methyl and locked nucleic acid units (OMe/LNA) complementary to TAR effectively inhibit binding of the Tat protein in vitro and inhibit Tat-dependent in vitro transcription in the presence of HeLa cell nuclear extract.[2] Such oligonucleotides were also found to inhibit Tat-dependent *trans*-activation when delivered by cationic lipids into cultured HeLa cells, as measured by reduction of expression of a firefly luciferase reporter from an integrated plasmid system.[3,4] The inhibitory activity of the OMe/LNA oligonucleotides showed sequence-dependence as well as dose-dependence with an IC_{50} for the 16-mer OMe/LNA mixmer (containing 6 LNA units) of 120 ± 30 nM.[4]

We now show that the 16-mer OMe/LNA mixmer targeted to TAR, when delivered by Lipofectamine 2000 into HeLa T4 LTR β-Gal cells for 3 h, the cells washed and subsequently infected with HIV-1, reduces cell syncitia formation caused by cell surface expression of HIV proteins (Figure 2). The oligonucleotide showed a dose-dependent reduction of syncitia formation as measured by the relative fusion index. By contrast, mismatched and scrambled oligonucleotides up to 1 μM did not show a significant reduction in the relative fusion index.

A second essential RNA-protein interaction in HIV-1 is the packaging of viral RNA by the Gag polyprotein. The principal signal for viral packaging has been localised to a stem-loop (known as SL3) within a complex region of RNA

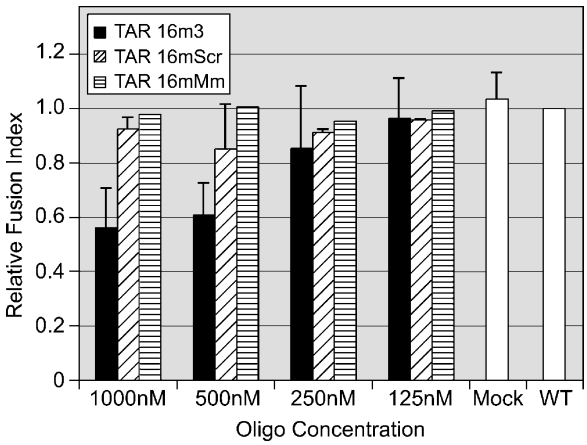

FIGURE 2 Relative fusion index values of HeLa T4 LTR β-Gal cells when transfected with TAR targeted oligonucleotides prior to infection with HIV-1. Cells were transfected with varying concentrations of TAR targeted or control oligonucleotides (mismatched and scrambled sequences) for 3 h prior to infection with HIV-1. Cells then incubated for 72 h and stained for β-Gal expression. Presence of syncitia was determined by light microscopy and were scored by size (number of nuclei per syncitia) and frequency. Relative fusion index values were calculated by (N − S)/T where N = number of β-Gal stained nuclei contained in syncitia, S = number of individual syncitia, and T = total number of stained nuclei in field of view. TAR16m3 = OMe/LNA oligonucleotide targeted to TAR (see Figure 1 legend); TAR16mScr = scrambled sequence; TAR16mMm = mismatched sequence.

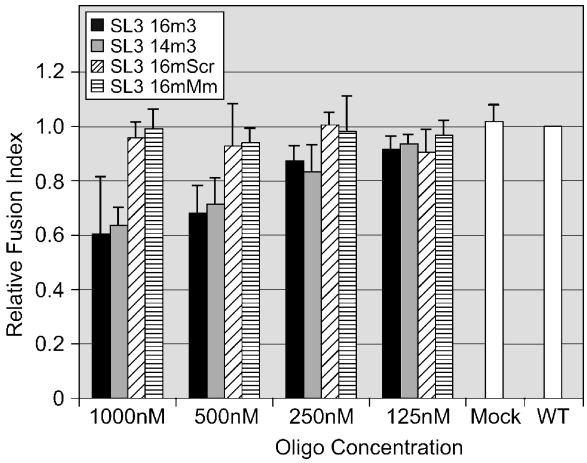

FIGURE 3 Relative fusion index values of HeLa T4 LTR β-Gal cells when transfected with SL3 targeted oligonucleotides prior to infection with HIV-1. Protocol followed as explained in Figure 2. SL316m3 and SL314m3 = OMe/LNA oligonucleotides targeted to the SL3 loop (see Figure 1 legend); SL316mScr = scrambled sequence; SL316mMm = mismatched sequence.

secondary structure that occurs just before the initiation codon of Gag (Figure 1B).[5] We have found that both 14-mer and 16-mer OMe/LNA oligonucleotides targeted to SL3 block the binding of HIV-1 Gag protein in vitro with sequence dependence.[6] Both 14-mer and 16-mer OMe/LNA oligonucleotides, when delivered by Lipofectamine 2000 into HeLa T4 LTR β-Gal cells which are subsequently infected with HIV-1, blocked syncitia formation with dose dependence (Figure 3). Neither mismatched nor scrambled 16-mer OMe/LNA controls up to 1 μM showed significant inhibition of syncitia formation.

The above results obtained for 16-mer oligonucleotides containing both OMe and LNA residues targeted to two different sites in the HIV-1 5′-UTR, the TAR and SL3 RNA stem-loops, demonstrate the potential of steric block of HIV protein interactions with viral RNA as a principle for inhibition of HIV-1 replication.

REFERENCES

1. Berkhout, B. Multiple biological roles associated with the repeat (R) region of the HIV-1 RNA genome. Adv. Pharmacol. **2000**, *48*, 29–72.
2. Arzumanov, A.; Walsh, A.P.; Liu, X.; Rajwanshi, V.K.; Wengel, J.; Gait, M.J. Oligonucleotide analogue interference with the HIV-1 Tat protein-TAR RNA interaction. Nucleosides Nucleotides Nucleic Acids **2001**, *20*, 471–480.
3. Arzumanov, A.; Walsh, A.P.; Rajwanshi, V.K.; Kumar, R.; Wengel, J.; Gait, M.J. Inhibition of HIV-1 Tat-dependent *trans*-activation by steric block chimeric 2′-O-methyl/LNA oligoribonucleotides. Biochemistry **2001**, *40*, 14645–14654.
4. Arzumanov, A.; Stetsenko, D.A.; Malakhov, A.D.; Reichelt, S.; Sørensen, M.D.; Babu, B.R.; Wengel, J.; Gait, M.J. A structure-activity study of the inhibition of HIV-1 Tat-dependent *trans*-activation by mixmer 2′-O-methyl oligoribonucleotides containing locked nucleic acid (LNA), α-LNA or 2′-thio-LNA residues. Oligonucleotides **2003**, *13*, 435–453.
5. Zeffman, A.; Hassard, S.; Varani, G.; Lever, A. The major HIV-1 packaging signal is an extended bulged stem loop whose structure is altered on interaction with the gag polyprotein. J. Mol. Biol. **2000**, *297*, 877–893.
6. Brown, D.; Arzumanov, A.A.; Gait, M.J.; Lever, A.M.L. Manuscript in preparation.