Anticancer molecule AS1411 exhibits low nanomolar antiviral activity against HIV-1

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A B S T R A C T

During clinical trials, a number of fully characterized molecules are dropped along the way because they do not provide enough benefit for the patient. Some of them show limited side effects and might be of great use for other applications. AS1411 is a nucleolin-targeting aptamer that underwent phase II clinical trials as anticancer agent. Here, we show that AS1411 exhibits extremely potent antiviral activity and is therefore an attractive new lead as anti-HIV agent.

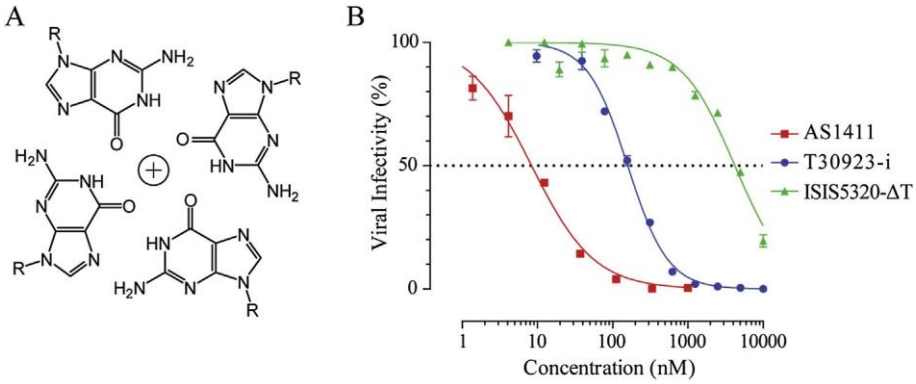
1. Introduction

Since the discovery of the human immunodeficiency virus (HIV) as the causal agent of AIDS, an armamentarium of drugs has been developed to fight this infection [1]. Unfortunately, there is still no cure nor a vaccine and emergence of resistance warrants the development of new antiviral strategies. Cancer represents another human burden that is studied by a large number of scientists. Interestingly, some pathways that are critical in cancer are also involved in HIV infection and one could expect that molecules developed in each field might have a therapeutic potential for the other application [1,2]. For example, the HIV inhibitor maraviroc acts as an antiviral by antagonism of the CCR5 cellular co-receptor while in the cell perspective, it influences cellular protein expression. Ultimately, this antiviral drug reduces cancer cell replication and dissemination [3,4]. Meanwhile, some laboratories have been focusing their research on finding molecules developed in the context of anti-cancer therapy that would exhibit antiviral properties.

Nucleolin is a ubiquitous phosphoprotein involved in various cellular processes such as transcription regulation and cell proliferation. This protein is found in the nucleus, in the cytosol and on the cell surface [5]. Nucleolin is overexpressed in numerous cancer cells and plays various roles in cancer development depending on its subcellular localization [6]. Interestingly, nucleolin has also been linked to HIV-1 replication and more precisely to the viral entry/fusion step [7,8]. Thus, molecules targeting nucleolin might present the dual advantage of being anticancer as well as antiviral agents.

AS1411 (AGRO100 or ACT-GRO-777), is a G-rich oligodeoxynucleotide with high affinity for nucleolin [9,10]. In cells, AS1411 exhibited anti-proliferative activity against cancer cells in the micromolar range without toxicity toward normal cells. Initially developed by Antisoma, AS1411 has been acquired by Advanced Cancer Therapeutics® in 2011. During phase I and II human clinical trials, evidence of anticancer activity and a favorable safety profile was reported [11].

AS1411 is a 26 base-long oligonucleotide forming G-quadruplexes [12]. These non-canonical structures, built up from the stacking of several guanine tetrads (Fig. 1A), are involved in various biological processes in human and in several pathogens [13e16]. Interestingly, several G-quadruplex forming nucleic acids have already been used to target HIV-1 viral proteins [17]. ISIS5320 and Hotoda's sequence both form G-quadruplexes that interact with the viral glycoprotein gp120, inhibiting the viral entry/fusion step at sub-micromolar concentrations [18e20]. Zintevir (AR177 or T30177) and derivatives (e.g. T30923) were shown to be potent inhibitors of HIV-1 replication in cells and of the viral integrase



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Aptamer	Sequence	HIV-1 Inhibition EC_{50} ± SD (nM)
AS1411	GGTGGTGGTGGTTGTGGTGGTGGTGG	15.4 ± 3.4
T30923-i	GIGTGGGTGGGTGGGT	83 ± 14
T30923[a]	*GGGTGGGTGGGTGGGT*	*~100 [25]*
T30177[a]	*G*TGGGTGGGTGGGTGGG*T*	*~100 [22]*
93del[a]	GGGGTGGGAGGAGGGT	20-30 [23]
Hotada's[a]	*TGGGAG*	*>1000 [19]*
ISIS5320[a]	*T*T*G*G*G*G*T*T*	*2000-6000 [20]*
ISIS5320-ΔT	TGGGGT	4200 ± 600

Fig. 1. A. Tetrad arrangement of guanosine residues. The monovalent cation that stabilizes this tetrad is represented by a plus sign. B. Representative inhibition curves obtained in the cellular-based assay. C. Antiviral activity of AS1411 and other G-quadruplex forming inhibitors. Phosphorothioate positions are shown with a star. EC_{50} and standard deviations (SD) are derived from 4 independent experiments unless reported from the literature ([a]). Data reported in gray and italic are from experimental settings that are different from those used in the present study.

in vitro [21,22]. Finally, 93del (andevir) is another example of G-quadruplex forming molecule exhibiting antiviral properties as well. The cellular inhibition of HIV-1 replication was shown to be multimodal by targeting the viral entry, reverse-transcription and integration steps [23]. Because AS1411 targets nucleolin, a potential new target for antiviral development [7,8], and forms G-quadruplex structures, we wondered if AS1411 might as well exhibit antiviral properties.

2. Results and discussion

In the present study we assessed whether AS1411 was able to inhibit HIV-1 replication in a cellular context. Interestingly, AS1411 exhibited anti-HIV-1 activity at low nanomolar concentrations with an EC_{50} of only 15.4 ± 3.4 nM (Fig. 1BeC). T30923-i is a close derivative of T30923 with a single guanosine to inosine substitution (Fig. 1C) forming a G-quadruplex as shown by NMR [24]. T30923-i was also a potent antiviral with an EC_{50} of 83 ± 14 nM (Fig. 1BeC), which is consistent with previous results obtained with T30923 [22,25,26]. Although zintevir was specifically developed as antiviral agent and was evaluated in clinical trials, AS1411 was 5- to 6-times more efficient at inhibiting viral replication than any of zintevir's derivatives tested so far (Fig. 1C). In parallel, we also tested ISIS5320-DT that derives from ISIS5320 and forms a well-defined G-quadruplex structure [27]. In the same cellular assay, ISIS5320-DT failed at inhibiting HIV-1 replication at nanomolar concentrations (EC_{50} of 4.2 ± 0.6 mM, Fig. 1BeC), which was similar to the antiviral activity of the parent molecule ISIS5320 previously reported [18]. Thus, AS1411 is the most potent antiviral molecule within the nucleic acids/G-quadruplex forming oligonucleotides family tested so far, including zintevir and andevir (Fig. 1C).

During clinical trials of AS1411 for its potential anticancer applications, it appeared that the molecule was safe but rapidly eliminated from the human body [12]. This could well also be a limiting factor for its use as antiviral drug. However, we showed here that AS1411 was a potent antiviral at 1000-fold lower concentrations than the concentrations needed to obtain an effective anticancer activity. Thus, AS1411 could, in its present form, present great therapeutic value as an anti-HIV agent. Alternatively, recent studies showed that association of AS1411 with gold particles enhances its effectiveness in *in vivo* cancer models [28,29] with an enhanced bioavailability an no increase in toxicity. Therefore, AS1411 alone or conjugated to nanoparticles represents a serious candidate for anti-HIV applications.

3. Material and methods

3.1. Oligonucleotides

Oligonucleotides were purchased from Eurogentec (Seraing, Belgium) with "Reverse-Phase Cartridge Gold purification" and dissolved in 20 mM potassium phosphate buffer pH 7.0 containing 70 mM KCl.

3.2. Antiviral activity

Infectivity of replicative HIV-1 particles was monitored as previously reported [30]. Briefly, HeLaP4 cells are reporter cells that contain a LacZ gene integrated in their genome, the expression of which is under the control of the viral LTR promoter. Antiviral activity of molecules was monitored 24 h post-infection. Fluorescence associated with the reaction product was monitored using a Cytofluor-II plate reader (Applied Biosystems, Foster City, CA) with excitation/emission filters at 360/460 nm. Data analysis (non-linear regression, IC_{50} determination and standard deviation) was performed using Prism 5.0c (GraphPad).

Authors contribution

Conceived and designed the experiments: JLM & MLA. Performed the experiments: MLA, MM & SA. Analyzed the data: MM & MLA. Wrote the paper: MM, SA, MLA & JLM.

Acknowledgments

This work was supported by the French National Agency for Research on AIDS and Viral hepatitis (AO2013) (ANRS), the Centre National de la Recherche Scientifique (CNRS), the University of Bordeaux, the Aquitaine Regional Council, and ANR Quarpdiem (ANR-12-BSV8-0008-01). MM and SA gratefully acknowledge postdoctoral fellowships from the ANRS.

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