

Overcoming HIV Drug Resistance – The Quest for Novel Reverse Transcriptase and Protease Inhibitors

a report by

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Luis Menéndez-Arias is a Senior Scientist and Group Leader at the Severo Ochoa Molecular Biology Centre (CSIC-UAM), which is a joint venture of the Spanish Council for Scientific Research and the Autonomous University of Madrid. His research is mainly devoted to understanding the molecular basis of nucleotide specificity of HIV-1 reverse transcriptase (RT), as well as the mechanisms involved in the acquisition of resistance to RT inhibitors. Dr Menéndez-Arias graduated in biology from the Complutense University of Madrid in 1984, and obtained his PhD in 1989 at the Department of Biochemistry and Molecular Biology of the Faculties of Biology and Chemistry of the same university. Between 1990 and 1994, he was trained as a postdoctoral fellow in the Frederick Cancer Research and Development Center, Frederick, Maryland, USA, where he conducted studies on the biochemical properties of retroviral proteases and their implications in virus maturation and antiviral therapy.

For almost 10 years, zidovudine (azidothymidine (AZT)) remained the only drug available for treatment of infections caused by HIV. In the late 1980s and early 1990s, resistance was emerging as a serious problem that compromised the future of patients treated with AZT and other nucleoside analogue inhibitors of HIV reverse transcriptase (RT). However, in 1995, the approval of saquinavir as the first drug targeting HIV protease (PR) sparked new hopes with the introduction of potent combination therapies, which involved the use of two nucleoside RT inhibitors and one PR inhibitor. Since then, combination regimens have dramatically decreased morbidity and mortality among HIV-infected patients. However, there are important drawbacks related to drug adherence, tolerability and long-term toxicity that limit the efficacy of these treatments. In addition, the high mutation rate of the virus and its high frequency of recombination, together with its rapid turnover in an infected individual, promote the emergence of drug-resistant variants that can eventually lead to antiretroviral failure in patients adherent to treatment.

Currently used antiretroviral drugs target the viral life-cycle at three different levels. RT inhibitors block the conversion of the viral genomic single-stranded RNA into double-stranded DNA (proviral DNA), which is a key event in HIV replication. PR inhibitors act on the viral protease, which is the enzyme responsible for the processing of viral polyprotein precursors into mature viral proteins, an event that leads to the conversion of an immature virion into a mature retrovirus. Fusion inhibitors block the process that brings together the membranes of the virus and the target cell, an event required for delivering the viral capsid into the cytoplasm of the host cell.

At present, there are 21 anti-HIV drugs that have been licensed for treatment of HIV infection: seven nucleoside RT inhibitors (zidovudine, didanosine, zalcitabine, stavudine, lamivudine, abacavir and emtricitabine), one nucleotide RT inhibitor (tenofovir disoproxil fumarate), three non-nucleoside RT inhibitors (nevirapine, delavirdine and efavirenz), nine PR inhibitors (saquinavir,

ritonavir, indinavir, nelfinavir, amprenavir, lopinavir, atazanavir, fosamprenavir and tipranavir, which was formally approved by the US Food and Drug Administration (FDA) in June 2005), and one fusion inhibitor (enfuvirtide).

Resistance to HIV RT Inhibitors

The HIV RT is an asymmetric heterodimer composed of two subunits of 560 and 440 amino acids, respectively. It has two distinct enzymatic activities: an RNA- and DNA-dependent DNA polymerase activity and a ribonuclease H (RNase H) activity. Inside the host cell, nucleoside and nucleotide RT inhibitors are phosphorylated to their triphosphate form to act as competitive inhibitors (or alternative substrates) of HIV RT. In contrast, non-nucleoside analogues bind to a hydrophobic pocket located 8–15Å away from the DNA polymerase catalytic site. Resistance to drugs targeting HIV RT can be achieved through the acquisition of one or more mutations that lead to amino acid substitutions that decrease inhibitor binding. For example, the mutation M184V confers lamivudine resistance, while K103N is sufficient to confer high-level resistance to nevirapine, delavirdine or efavirenz. In contrast, the accumulation of several mutations usually leading to multidrug resistance, as in the case of A62V, V75I, F77L, F116Y and Q151M, is usually forced by combination therapies. Interestingly, HIV strains showing resistance to thymidine analogues such as zidovudine or stavudine have acquired the ability to excise the inhibitor from the blocked 3' end of the primer, through an ATP-dependent phospholytic reaction. Amino acid changes involved in the acquisition of resistance through this mechanism include thymidine analogue resistance mutations (i.e. M41L, L210W, T215Y, etc.) and unusual mutational patterns, such as dipeptide insertions at positions 69 and 70 of the viral RT.

With the extensive use of RT inhibitors as part of highly active antiretroviral therapies (HAART), the emergence of highly mutated viral strains that show resistance to multiple nucleoside analogues is relatively frequent. In this context, novel RT

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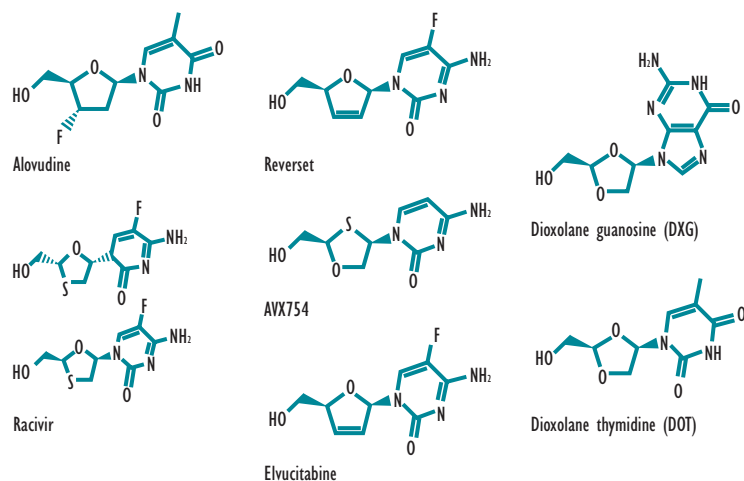
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Figure 1: Structures of Nucleoside RT Inhibitors in Clinical Development



inhibitors should display a good antiviral profile against wild-type HIV and variants resistant to currently used drugs. In addition, novel drugs should have high oral bioavailability (allowing a once-daily administration), minimal adverse effects and be easy to synthesize and formulate.

At present, there are several nucleoside RT inhibitors at different stages of clinical development (see Figure 1). Alovudine (3'-deoxy-3'-fluorothymidine, MIV-310, FLT) is a pyrimidine nucleoside analogue similar in some respects to zidovudine and stavudine. However, it has potent activity against nucleoside RT inhibitor-resistant strains of HIV-1, including zidovudine-resistant viruses. Initial clinical trials raised some concerns about its toxicity when administered at doses above 10mg/day but, in a recent report, it was shown that at a lower dose (7.5mg/day), alovudine decreased viral load 10-fold in patients failing multiple antiretroviral therapy and infected with HIV variants having at least two thymidine analogue resistance mutations.

Another promising drug is Reverset™, a fluorosubstituted derivative of β -D-2',3'-dideoxy-2',3'-dideoxycytidine (D-d4FC, RVT or DPC-817). This compound has a good pharmacokinetic profile and shows a strong antiviral effect in HIV-infected patients following a single oral dose. Reverset was effective on lamivudine- and zidovudine-resistant strains, although it showed decreased efficacy on HIV strains having the mutation K65R.

Other cytosine analogues under development are Racivir® ((\pm)- β -2',3'-dideoxy-5-fluoro-3'-thiacytidine; (\pm)-FTC), AVX754 ((-)-2'-deoxy-3'-oxa-4'-thiacytidine; (-)dOTC; SPD754) and elvucitabine (β -L-2',3'-dideoxy-5-fluorocytidine; β -L-Fd4C; ACH-126443). These compounds also show good pharmacokinetic profiles, although in the case of elvucitabine bone

marrow suppression was observed at doses above 100mg/day. The efficiency of AVX754 and elvucitabine on lamivudine- and zidovudine-resistant strains has been demonstrated *in vitro*. However, a major limitation of AVX754 is that its intracellular levels are reduced significantly when combined with lamivudine.

Dioxolane derivatives are compounds where the carbon at the 4' position of the ribose ring has been replaced by an oxygen atom. Amdoxovir (a diaminopurine dioxolane) was found to be active *in vitro* against HIV-1 and hepatitis B virus. It is deaminated intracellularly by adenosine deaminase to β -D-2,6-dioxolan guanosine (DXG), a compound that is five to 20 times more active when assayed in cultures infected with HIV. Although clinical studies of amdoxovir have been suspended due to safety concerns, DXG is still under consideration, particularly since it is active against HIV-1 mutants resistant to zidovudine (M41L/D67N/K70R/T215Y/K219Q), lamivudine (M184V), tenofovir (K65R) and didanosine (L74V), and also against multidrug-resistant strains having dipeptide insertions at codons 69–70. In contrast, DXG may not be equally effective against viral strains containing mutation Q151M. DXG resistance appears to be mostly dependent on nucleotide discrimination, since DXG-terminated primers are poorly excised through the ATP-mediated phosphorylytic reaction.

A related compound with excellent pharmacological properties is 1-(β -D-dioxolane)thymidine (DOT), which has shown efficacy on several clinically relevant HIV strains, including multidrug-resistant mutants containing M184V, thymidine analogue resistance mutations and dipeptide insertions at positions 69–70. There are other nucleoside/nucleotide derivatives whose efficiency on multidrug-resistant strains has been shown *in vitro*, for example, stampidine (a stavudine derivative), 4'-ethynyl nucleoside analogues, or α -borano- or α -thio-phosphate derivatives of zidovudine or stavudine. However, important questions related to their pharmacological properties, toxicity and clinical efficiency have not yet been addressed. ■

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