1 Introduction

The viral enzyme reverse transcriptase (RT) is essential for viral replication. The enzyme RT is unique for retroviruses and transcribes the viral genomic RNA into a complementary DNA (cDNA) copy. Reverse transcription is a very complex process and depends on two distinct enzymatic activities of RT; a DNA polymerase that can use either RNA or DNA as template and a nuclease (Ribonuclease H or RNase H) specific for the RNA strand of RNA:DNA duplexes (1–3).

HIV-1 RT is a stable heterodimer consisting of two subunits of 66 (p66) and 51 kDa (p51) (4–6). The p51 subunit is generated by proteolytic cleavage of the p66 subunit by viral protease and lacks the C-terminal RNase H domain. Although the overall folding of the two subunits is similar, the spatial arrangement of the two subunits is completely different. The p51 subdomain adopts a closed formation and only plays a structural role, whereas the p66 subunit is organized to form a cleft into which the primer template binds and represents the polymerase active site. Crystallographic studies show that the p66 subunit resembles a right hand grasping the primer-template complex (7, 8). On the basis of this 3D structure, the enzyme has been divided into five distinct domains. These are the fingers (residues 1–90, 110–160), palm (90–110, 160–240), thumb (240–310), connection domain (310–430) and the RNAse H subdomain (430–565) at the carboxy terminus. The latter subdomain cleaves the template RNA strand and degrades the transcribed RNA. The palm domain harbors the polymerase active site, located in a cleft formed by the flanking fingers and thumb subdomain, which play a role in positioning the template. The active site of RT contains three aspartic acids at amino acids 110, 185 and 186, which are involved in metal-ion ligation and interact with the phosphates of the DNA primer and the incorporated nucleotides. These aspartates are highly conserved and required for the proper function of reverse transcription. The connection subdomain, as the name already implies, connects the polymerase and the RNAse H domain (1, 4, 9, 10).

Interestingly, unlike most polymerases, RT lacks a 3′-5′ exonuclease activity, which means that it is not able to identify and excise inappropriate nucleotides once they are incorporated in the growing DNA chain. As a consequence, RT is able to incorporate dNTP analogues in addition to the natural substrates (11, 12).

In 1987, the Food and Drug Administration (FDA) approved the first anti-HIV drug, Zidovudine (AZT), that was directed against RT. On the basis of their site of binding and mode of action, RT inhibitors can be subdivided into two classes: nucleoside and non-nucleoside inhibitors. This chapter will focus on nucleoside RT inhibitors (NRTIs) and the mechanisms of resistance to these drugs.

2 Nucleoside Reverse Transcriptase Inhibitors

The first RT inhibitor Zidovudine or AZT is a thymidine analog with an azido group at the 3′ position of the ribose (13). Subsequently, in the following years many other nucleoside analogs, deoxynucleotides, were developed and introduced in the clinic. Nucleoside analogs or nucleoside reverse transcriptase inhibitors (NRTIs) are analogs of the normal dNTP substrates of DNA polymerase with important modifications. These nucleoside analogs are administered as precursor compounds or prodrugs, which have to be tri-phosphorylated by the host cellular kinases to their active form. After binding to the polymerase active site of RT, they compete with the natural dNTPs for recognition as substrate (binding) and incorporation into the nascent DNA chain. Since nucleoside analogs lack hydroxyl moiety on the ribose group, they prevent further DNA synthesis, once they are incorporated. Thus, they inhibit viral replication via two mechanisms (14, 15).
Currently, eight NRTIs are approved by the FDA to inhibit HIV-1 reverse transcription and thereby viral replication (Fig. 1). Two thymidine analogs: Zidovudine (AZT or 3′-azido-3′-deoxythymidine) and stavudine (d4T or 2′,3′-didehydro-2′, 3′-dideoxythymidine), three cytosine analogs: zalcitabine (ddC or 2′,3′-dideoxycytidine), lamivudine [3TC or (−)-β-L-2′, 3′-dideoxy-3′-thyacytidine] and emtricitabine (FTC), the adenosine analog didanosine (ddl) or 2′,3′-dideoxynosine and the guanosine analog abacavir (ABC). Tenofovir (PMPA) is already phosphorylated once and hence is a nucleotide analog. Since both nucleoside and nucleotide RTIs act by the same mechanisms, the abbreviation NRTIs is used for both classes of compounds (14, 16, 17).

### 3 Resistance Against Nucleoside Analogs

Unfortunately, soon after the introduction of AZT monotherapy it became evident that the HIV could develop an increase up to 100-fold in IC₅₀ towards this drug in patients receiving 6 months of treatment (18). The rapid emergence and selection of virus variants harboring resistance-associated mutations is a result of several properties from the HIV-1 replication process. First, HIV-1 RT has a very high error rate. As opposed to DNA polymerases, HIV-1 RT lacks proofreading activities, making it unable to correct errors that are generated during DNA synthesis. Estimations are that HIV-1 RT generates 3 × 10⁻⁵ errors per base pair per replication cycle (19). Second, the HIV-1 genome consists of two single-stranded RNA copies. RT can jump from one to the other RNA template resulting in a high rate of recombinant viral DNA sequences (20, 21). Third, HIV has a very rapid replication rate and a high viral turnover in an HIV-infected individual (10⁷–10⁹ viral particles per day) combined with an in vivo half life of ~2 days (11, 22, 23). And fourth, host cells harbor the cytidine deaminase APOBEC3G that deaminates deoxycytidines (dCs) to deoxyuridines (dUs) in minus-strand DNA during reverse transcription. This results in a high-level G-to-A hypermutation of the proviral plus-strand cDNA and subsequent abrogation of the viral replication. The viral enzyme vif binds to APOBEC3G and suppresses its incorporation in the virion. In this way, vif can reduce the G-to-A hypermutation to a nonlethal level, but cannot prevent all substitutions (9, 24, 25).

This generates HIV-1 viral quasispecies; a pool of closely related HIV virions. Under drug pressure specific virus variants harboring mutations conferring a decrease in susceptibility will have a competitive advantage over drug-sensitive variants. Amino acid changes in the RT gene that confer nucleoside analog resistance are shown in Table 1. These mutations are all located in the palm and fingers subdomains of HIV-1 RT (5, 11, 26, 27). Some substitutions are able to confer resistance on their own. The M184V/I mutation confers resistance to 3TC, FTC, ABC and ddC, the L74V against ddI, ABC and ddC and the K65R change confers resistance to d4T, ddI, ddC, 3TC, FTC, ABC and TFV. The Q151M is a key mutation in one of the multi-drug resistance pathways and is usually accompanied by the A62V, V75I, F77L and F116Y changes (28). For some drugs, such as AZT, resistance requires two or more mutations from the group of M41L, D67N, K70R, L210W, T215Y/F and K219Q (18, 29, 30). These mutations confer resistance to d4T as well, although to a lesser extent than to AZT. Since AZT and d4T are both thymidine analogues, these mutations are referred to as thymidine analog mutations (TAMs). However, TAMs have also been shown to confer resistance against other nucleoside analogs as well.

### 4 Mechanisms of Nucleoside Drug Resistance

The mutations that are generated during nucleoside analog treatment can be subdivided into two classes based on their mode of action. One class of mutations gives HIV-1 RT the