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NOTE

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Fungal Phenalenones Inhibit HIV-1 Integrase

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Abstract A phenalenone compound, atrovenetinone methyl acetal, was isolated from a culture broth of *Penicillium* sp. FKI-1463 as an HIV-1 integrase inhibitor, and it showed anti-HIV activity *in vitro*. HIV-1 integrase inhibition and anti-HIV activity of two other natural phenalenones were also studied. Among the tested compounds, funalenone inhibited HIV-1 integrase with an IC_{50} value of 10 μM and showed the best selectivity (anti-HIV, IC_{50}=1.7 μM; cytotoxicity, IC_{50}=87 μM).

Keywords: enzyme inhibitor, HIV interase, AIDS, phenalenone

Combined therapeutic regimens with reverse transcriptase inhibitors and protease inhibitors lead to a suppression of human immunodeficiency virus-1 (HIV-1) replication, reduction of viral load, and decline in morbidity and mortality [1, 2]. However, the therapy sometimes fails due to the emergence of mutant viruses that are resistant to these drugs [3]. Thus, it is critical to discover more effective and less toxic anti-HIV agents with different molecular targets in the viral replication cycle. We have previously screened microbial metabolites for new anti-HIV antibiotics that inhibit entry of HIV-1 into the susceptive cells, and found isochromophilones and chloropeptins by a gp120-sCD4 binding assay [4, 5] and actinohivin by a syncytium formation assay [6]. There are three viral enzymes essential for HIV-1 replication, reverse transcriptase, protease, and integrase. Of these, only integrase has not been the target of a clinically used inhibitor. HIV DNA is inserted into the host genome by a specialized DNA recombination reaction in which the viral integrase is the key player [7, 8]. The integration reaction is composed of three steps, 3′-processing, strand transfer, and gap filling, and integrase catalyses the first and second steps. The third step is thought to be catalyzed by cellular enzymes. Many natural and synthetic integrase inhibitors have been reported [8~12] but only a few compounds show high selectivity. Therefore, we screened microbial metabolites for HIV-1 integrase inhibitors, and found that a culture broth of *Penicillium* sp. FKI-1463 has the inhibitory

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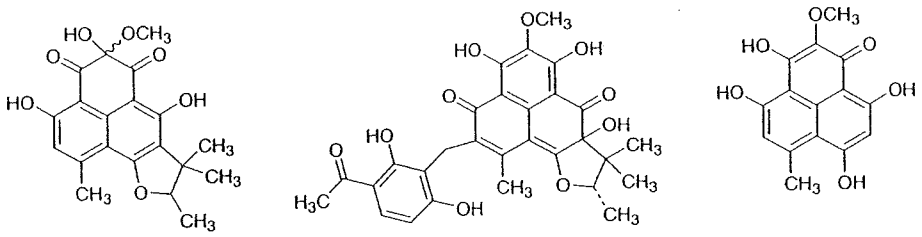
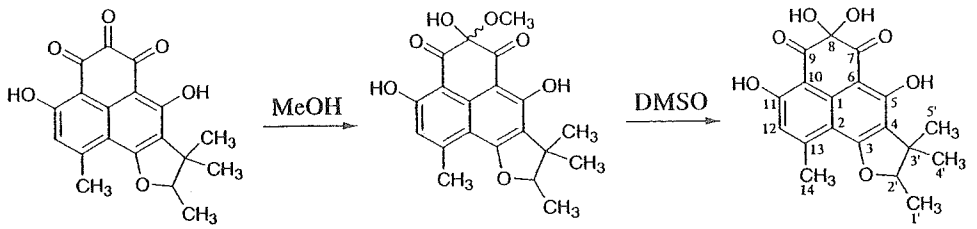


Atrovenetinone methyl acetal (1) Erabulenol B (4) Funalenone (5)

Fig. 1 Natural phenalenones.



2 1 3

Fig. 2 Conversion of atrovenetinone.

activity. The active compound was identified as a phenalenone compound, atrovenetinone methyl acetal (1, Fig. 1) [13]. This paper presents integrase-inhibiting and anti-HIV activities of 1 and other natural phenalenones.

A slant culture of the strain FKI-1463 grown on YpSs agar was inoculated into a 500-ml Erlenmeyer flask containing 100 ml of a seed medium consisting of glucose 2.0%, Polypepton (Nihon Pharmaceutical Co.) 0.5%, yeast extract 0.2% (Oriental Yeast Co.), KH_2PO_4 0.1%, $MgSO_4 \cdot 7H_2O$ 0.05%, and agar 0.1%, pH 6.0. It was cultured on a reciprocal shaker at 27°C for 3 days. One milliliter of the seed culture was transferred into each of twenty 500-ml Erlenmeyer flasks containing 100 ml of a production medium consisting of glycerol 3.0%, oatmeal (Nihon Shokuhin Seizo Co.) 2.0%, dry yeast (Gist-brocades) 1.0%, KH_2PO_4 1.0%, Na_2HPO_4 1.0%, $MgSO_4 \cdot 7H_2O$ 0.5%, pH not adjusted. The fermentation was carried out on a reciprocal shaker at 27°C for 7 days. The cultured broth (2.0 liters) was centrifuged and the mycelia were extracted with methanol, which was then removed from the extract by evaporation. The aqueous extract was partitioned with ethyl acetate at pH 3.0, and the organic layer was concentrated to dryness *in vacuo* to afford brown oil (644 mg). This was chromatographed over a silica gel column. Active fractions, eluted with $CHCl_3$-methanol (100 : 1) and $CHCl_3$-methanol (20 : 1), were concentrated to yield a crude material (284 mg). It was applied on a ODS silica gel column and eluted with aqueous CH_3CN. The 50% CH_3CN eluates were concentrated (95.5 mg) and chromatographed over Sephadex LH-20 to yield green oil (86.8 mg). It was further purified by reverse phase (Pegasil ODS, Senshu Scientific Co.) and normal phase (Pegasil Silica, Senshu Scientific Co.) HPLC to yield 50.5 mg of green oil.

The purified compound was implicated as 1 by comparison of the NMR data in $CDCl_3$ with the reported data by Nakanishi *et al.* [13]. Atrovenetinone (2) is easily converted into an acetal in alcohol (Fig. 2) [14], and the acetal is a mixture of diastereomers [13]. So, the NMR spectra of 1 are complicated. Since 2 exists as the hydrate (3) in DMSO [14], we observed the NMR spectra of the isolated compound in DMSO-d_6. The spectra were simplified, and each signal was assigned as follows: ^{1}H NMR (600 MHz) δ 13.67 (1H, s, 5-OH), 12.92 (1H, s, 11-OH), 6.86 (1H, s, 12-H), 4.70 (1H, q, J=6.5 Hz, 2′-H), 4.04 (1H, br s, 8-OH), 2.72 (3H, s, 14-H_3), 1.45 (3H, s, 5′-H_3), 1.22 (3H, s, 4′-H_3), 1.41 (3H, d, J=6.5 Hz, 1′-H_3); ^{13}C NMR (150 MHz) δ 197.7 (C-7), 196.2 (C-9), 165.1 (C-11), 164.8 (C-3), 164.5 (C-5), 147.9 (C-13), 136.7 (C-1), 118.1 (C-4), 117.6 (C-12), 109.0 (C-2), 104.9 (C-10), 101.9 (C-6), 91.1 (C-2′), 88.0 (C-8), 42.8 (C-3′), 25.2 (C-5′), 23.5 (C-14), 20.4 (C-4′), 14.3 (C-1′). The NMR data suggested that 1 was converted into 3 in DMSO solution (Fig. 2), and released methanol signals (δ_H 3.15 and δ_C 48.6) were also

Table 1 Biological activities of phenalenones

	IC_{50} (μM)			Selectivity (B/A)
	HIV-1 integrase inhibition	Anti-HIV activity (A)	Cytotoxicity (HPB-M(a)[a]) (B)	
Atrovenetinone methyl acetal (**1**)	19	6.7	13	1.9
Erabulenol B (**4**)	7.9	17	230	14
Funalenone (**5**)	10	1.7	87	51

[a] HPB-M(a) cells are human peripheral blood cells transformed by murine leukemia virus. Anti-HIV activity was measured using HPB-M(a) cells with LTR driven luciferase.

observed. Thus, the isolated compound was identified as **1**. It has been reported as a myosin light chain kinase inhibitor isolated from a culture broth of *Penicillium* sp. It may be derived from **2** during purification. Compound **2** is a phenalenone compound originally obtained by the oxidation of atrovenetin produced by *Penicillium* sp., and **2** was lately isolated from a culture broth of *Gremmeniella abietina* [14, 15].

We have previously isolated the other fungal phenalenones, erabulenol B (**4**) which inhibits cholesteryl ester transfer protein and funalenone (**5**) which inhibits collagenase [16, 17]. Funalenone was also reported to inhibit bacterial cell wall synthesis enzymes MraY and MurG [18]. We evaluated integrase inhibition and anti-HIV activity of **1** together with those phenalenones. HIV-1 integrase activity was measured by strand transfer assay according to Craigie *et al.* [7]. *In vitro* anti-HIV activities of the test compounds were measured by originally established reporter human T cell line with LTR driven luciferase. The cells were infected with wild type HIV-1, and the compounds were added at different concentrations ranging from 0.0016 to 125 μg/ml. Luciferase activities of the cells, which appeared to correlate with the level of HIV-1 replication, were measured at day 7, and anti-HIV IC_{50}s of the compounds were evaluated. The IC_{50} value of **1** against integrase was 19 μM, and it also showed anti-HIV activity at 6.7 μM (Table 1). However, its cytotoxicity was relatively high. Compounds **4** and **5** showed more potent inhibition against integrase than **1**, and also exhibited anti-HIV activity. The anti-HIV activity of **5** was the most potent (1.7 μM), and its cytotoxicity (87 μM) was lower than **1**. Though **5** was reported to inhibit collagenase and bacterial cell wall synthesis enzymes [17, 18], those inhibitions were less potent than the integrase inhibition and anti-HIV activity. Therefore, **5** may be a good candidate lead compound for anti-HIV agent. Inhibition of DNA polymerases by the other phenalenones have been reported, but they did not inhibit HIV reverse transcriptase [19]. A plant metabolite, hypericin [20], is the only *ortho*- and *peri*-fused aromatic compound reported to show integrase inhibition [21].

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Discordances between Interpretation Algorithms for Genotypic Resistance to Protease and Reverse Transcriptase Inhibitors of Human Immunodeficiency Virus Are Subtype Dependent

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The major limitation of drug resistance genotyping for human immunodeficiency virus remains the interpretation of the results. We evaluated the concordance in predicting therapy response between four different interpretation algorithms (Rega 6.3, HIVDB-08/04, ANRS [07/04], and VGI 8.0). Sequences were gathered through a worldwide effort to establish a database of non-B subtype sequences, and demographic and clinical information about the patients was gathered. The most concordant results were found for nonnucleoside reverse transcriptase (RT) inhibitors (93%), followed by protease inhibitors (84%) and nucleoside RT inhibitor (NRTIs) (76%). For therapy-naive patients, for nelfinavir, especially for subtypes C and G, the discordances were driven mainly by the protease (PRO) mutational pattern 82I/V + 63P + 36I/V for subtype C and 82I + 63P + 36I + 20I for subtype G. Subtype F displayed more discordances for ritonavir in untreated patients due to the combined presence of PRO 20R and 10I/V. In therapy-experienced patients, subtype G displayed a lot of discordances for saquinavir and indinavir due to mutational patterns involving PRO 90 M and 82I. Subtype F had more discordance for nelfinavir attributable to the presence of PRO 88S and 82A + 54V. For the NRTIs lamivudine and emtricitabine, CRF01_AE had more discordances than subtype B due to the presence of RT mutational patterns 65R + 115 M and 118I + 215Y, respectively. Overall, the different algorithms agreed well on the level of resistance scored, but some of the discordances could be attributed to specific (subtype-dependent) combinations of mutations. It is not yet known whether therapy response is subtype dependent, but the advice given to clinicians based on a genotypic interpretation algorithm differs according to the subtype.

Genotyping for the assessment of anti-human immunodeficiency virus (HIV) drug resistance is often used in the management of individual patient therapy. Currently, it is recommended in European as well as American guidelines (17, 38). In several retrospective and prospective studies, genotyping proved beneficial in optimizing treatment for individual patients (5, 10, 16, 23, 25, 31, 37).

Although genotyping is commonly used, there are still many uncertainties with respect to the value of genotype in the assignment of a new regimen. The current genotypic assays are not always able to report all drug resistance mutations among non-B subtypes (11, 18, 19, 24). Regardless of subtype, genotyping is not sensitive to mutations that are present as a minor variant in the population (22, 40). Genotyping results also differ depending on the laboratory where they are performed. Quality control studies indicate that mutations, even present as a pure variant, are often underestimated (32).

However, separate from the quality and sensitivity issues, the interpretation of genotypic results is still not standardized.

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Several interpretation algorithms have been designed to aid in this, but they may differ in the prediction of therapy response and/or drug susceptibility. Studies were performed mainly on subtype B viruses, and even within this subtype, differences have been detected (6, 21, 29, 34, 35, 36).

Non-B subtypes are a challenge for these systems, since algorithms for these subtypes were designed using genotype, phenotype, and therapy response information that was largely derived from experience with subtype B. Recent analyses suggest that non-B viruses can develop specific mutations that differ from those identified in subtype B under the same treatment pressure (1, 20). For example, in CRF01_AE but not in subtype B viruses, V75M seems to be significantly associated with stavudine treatment (2) and, in subtype C but not in subtype B, V106M is a signature substitution of patients treated with efavirenz (4). There is a continuing controversy about the impact of secondary protease mutations (positions 36, 71, 77, etc.) which evolve in subtype B following protease exposure and are relatively frequent in untreated patients with non-B subtypes. It has been suggested that some of these can affect the susceptibility to certain protease inhibitor (PI) therapies in B and non-B subtypes (14, 28).

Although some short-term studies suggest little difference in therapy response in patients carrying non-B subtypes from that of patients infected with subtype B (12), other studies showed a significant difference in responses to treatment for different subtypes (8, 13). However, current studies have included a limited number of subjects. Potential differences can be due to differences in drug resistance. It is therefore important to know how the current drug resistance interpretation systems perform on different subtypes, and first of all, we need to know what the subtype-dependant discrepancies between the systems are.

Comparisons between these interpretation systems have already been made for subtype B strains; however, the subtype dependency of resistance assessment by these interpretations systems has not yet been determined (6, 21, 29, 34, 35, 36). In this study, we investigated four frequently used interpretation systems across a large number of non-B sequences to determine whether discordance between the systems was dependent on the viral subtype.

MATERIALS AND METHODS

Sequences. Sequences of HIV-1 protease (positions 1 to 99) and reverse transcriptase (RT) (positions 1 to 240) were collected from the published literature and from 14 laboratories in 12 countries through the non-B workgroup, a worldwide effort to establish a database of non-subtype B sequences (20). Three separate analyses were performed based on the treatment history of the patient at the time of sequencing: PI analysis, nucleoside RT inhibitor (NRTI) analysis, and nonnucleoside RT inhibitor (NNRTI) analysis. A sequence was included in the respective analysis either if the patient was reported to have had no previous exposure to a drug in that class or if the patient was being treated with a drug in that class at the time of sequencing, thus separating the analyses according to drug class exposure. In this way, sequences from patients that had drug exposure from a particular class in the past but were not at the time of sequencing taking a drug from that class were excluded. The treatment data gathered for this database were therapy history, with start and stop dates for a treatment, the regimens in the therapy, and the doses of the separate antivirals. Sequences were excluded when there was no therapy history.

Subtyping. Subtyping was performed by phylogenetic analysis using the subtyping tool developed by de Oliveira et al. separately for protease and reverse transcriptase sequences (7). Briefly, sequences are first analyzed using pure subtypes as a reference; in a second step, known circulating recombinant forms are added to the alignment. To detect recombination, bootscanning was performed using a sliding window of 400 nucleotides that was advanced 20 nucleotides at a time. Recombinants were included only if they were CRF01_AE or CRF02_AG since we had sufficient data for only these two circulating recombinant forms.

Algorithms. Four publicly available algorithms were applied on each of the sequences: Agence Nationale de Recherche sur le SIDA (ANRS) July 2004 (http://www.sante.gouv.fr/htm/actu/36_vih_2.htm) (25), HIV RT and Protease Sequence Database (HIVDB) August 2004 (http://hivdb.stanford.edu) (33), Rega Institute (Rega) version 6.3 (http://www.kuleuven.be/rega/cev/pdf/ResistanceAlgorithm6_3.pdf) (39), and Bayer Health Care-Diagnostics (VGI) version 8 (30) (formerly Visible Genetics).

Mutations considered. In all statistical analyses (see below), we scored all mutations that are included in one of the algorithms we used in the analyses: 18 NRTI resistance positions, i.e., 41, 44, 62, 65, 67, 69, 70, 74, 75, 77, 115, 116, 118, 151, 184, 210, 215, and 219; 16 NNRTI resistance positions, i.e., 98, 100, 101, 103, 106, 108, 179, 181, 188, 190, 225, 227, 230, 234, 236, and 318; and 23 PI resistance positions, i.e., 10, 20, 24, 30, 32, 33, 36, 46, 47, 48, 50, 53, 54, 60, 63, 71, 73, 77, 82, 84, 88, 90, and 93. For most positions, more than one mutant amino acid can be scored. All mixtures at resistance positions were scored as mutants.

Scoring of discordances—statistical analyses and data mining. The algorithm specification interface at the web site for the Stanford HIV drug resistance database (http://hivdb.stanford.edu) was used to apply the interpretation algorithms to each sequence (3). We assigned three levels of resistance: susceptible (S), intermediate (I), and resistant (R). For HIVDB, which assigns five levels of resistance, we obtained three by pooling the two highest and two lowest categories.

Interpretations were considered concordant if each of the algorithms assigned the same level of resistance to a sequence for a particular drug. We considered the algorithms to be fully discordant if one of them scored the sequence S for a particular drug, and another one scored it as R. Interpretations were considered partially discordant when, among the scores of the different systems, both S and I or both R and I were found for the same drug. The numbers of fully discordant (counted as 1) and partially discordant (counted as 0.5) strains were added to compute the proportion of discordant strains.

Statistical analyses were performed to see whether the number of discordances were drug and subtype dependent. We performed a one-way analysis of variance (ANOVA) with Tukey's confidence intervals to check for differences between different drugs and different subtypes. Differences between only subtype B and each of the other subtypes have been analyzed in this study.

The data mining program Weka, version 3.4.4 (http://www.cs.waikato.ac.nz/~ml/weka/), was used to identify mutational patterns that were responsible for the observed discordances, thereby also identifying the algorithms that caused the discordances. We used this tool to build binary decision trees with which it tries to predict all observed discordances. To evaluate the predictive power of the decision trees, we performed a 10-fold cross-validation. In this method, the data set is split 10-fold and the predictive performance for every subset is evaluated for a decision tree trained on the other subsets.

We built a model for each drug in which we found a statistically significant effect of subtype on discordance. We included all subtypes in the model and tried to predict discordances (three levels, concordant, discordant, and partially discordant). For each leaf in the resulting tree that predicted discordance, we calculated the subtype distribution. Fisher exact tests were performed to analyze whether a rule in the decision tree explained significantly more discordances for a particular subtype.

RESULTS

Subtype distribution. We obtained protease and/or reverse transcriptase sequences from 5,030 patients. The subtype distribution for each analysis (PI, NRTI, or NNRTI) is shown in Table 1. In total, we obtained 6,916 (3,926 from naive and 2,990 from treated patients) sequences for PI analyses, 5,689 (2,331 naive and 3,358 treated) for NRTI analyses, and 5,557 (4,208 naive and 1,349 treated) for NNRTI analyses. Twelve protease and five RT sequences were filtered out due to suspected recombination or were untypable. The majority of the sequences were of a non-B subtype except for the PI-treated and NRTI-treated class, where the prevalence of subtype B was 82% and 66%, respectively. Subtypes H, J, and K were excluded because of a limited number of sequences.

TABLE 1. Subtype distribution for sequences in the analysis groups PI, NRTI, and NNRTI

Subtype	No. of sequences for[a]:					
	PI		NRTI		NNRTI	
	Naive	Treated	Naive	Treated	Naive	Treated
A	363	35	318	105	217	206
B	1,661	2,467	632	2,224	2,139	585
C	672	201	644	339	805	178
D	260	37	201	89	159	131
F	126	80	79	107	140	46
G	128	87	63	158	144	77
CRF01_AE	207	36	132	251	291	92
CRF02_AG	509	47	262	85	313	34

[a] A sequence was included in the analysis if there was no previous exposure to a drug in that class or the patient was being treated with a drug in that class at the time of sequencing.

Discordances. Overall, the different interpretation systems agreed well on the level of resistance. Eighty-four percent of the sequences had concordant results for PIs. In only 6% of the cases, the algorithms gave full discordant results; most of the observed differences were due to partial discordances (10%). For NRTIs, 76% of the sequences gave concordant results and 8% were fully discordant. The most concordant results, 93%, were found for NNRTI. Only 1% of the sequences caused full discordances. The results for each drug are shown in Fig. 1.

The concordance was significantly higher for therapy-naive patients than for treatment-experienced patients ($P < 0.0001$) for all drug classes.

Protease inhibitor analysis. The number of discordances seemed to be drug and subtype dependent for therapy-naive patients as well as treated patients (Tables 2 and 3).

In therapy-naive patients, results for nelfinavir were discordant in 1.8% of the sequences, while for lopinavir, this was 0.3% and for tipranavir, this was 0%. When considering the results for a single drug, the proportion of sequences displaying full or partial discordances was subtype dependent. Concerning specific subtypes in therapy-naive patients, discordances were observed for ritonavir (subtype F, $P < 0.01$) and nelfinavir (subtypes G and C) (Table 2).

In treated patients, the results were different. The highest level of discordance was obtained for amprenavir (50%), whereas 36% of the sequences were scored as discordant for lopinavir and 14% for nelfinavir. Tipranavir gave still the least discordant results; only 2% of the sequences were causing discordances between algorithms. Compared to subtype B, more discordances were observed for nelfinavir in subtype F and for indinavir and saquinavir in subtype G ($P < 0.01$), while less discordances were observed for amprenavir in subtypes C and D and for atazanavir in subtype C ($P < 0.01$) (Table 3).

Nonnucleoside reverse transcriptase inhibitor analysis. For therapy-naive patients, no differences could be found between drugs, while for treated patients, efavirenz scored the most discordances (11%), followed by delavirdine and nevirapine (5%).

The proportion of sequences displaying full or partial discordances was subtype dependent in this drug class except for delavirdine and nevirapine in naive patients. But no specific subtypes were found that had differences in the resistance interpretation compared to subtype B.

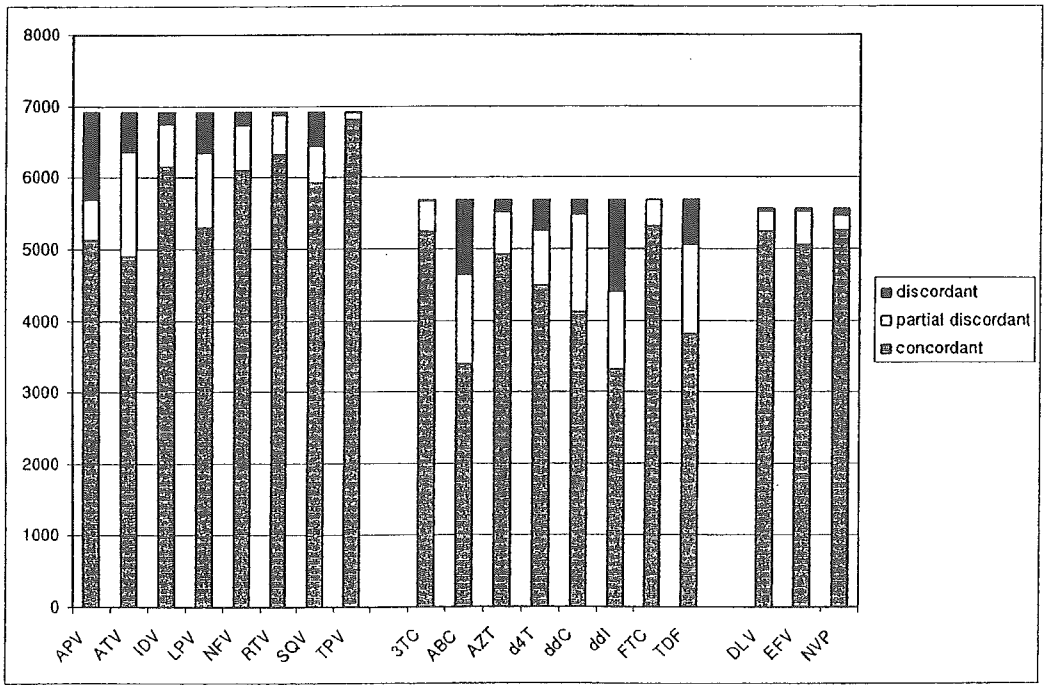

FIG. 1. Graphic representation of the number of discordant sequences per drug class. Gray bars represent the number of sequences for which concordant predictions were made by the four algorithms, white bars represent the number of sequences with partial discordance, and black bars represent sequences with discordant predictions.

TABLE 2. Interalgorithm discordances between genotypic drug resistance interpretation for sequences obtained from therapy-naive patients infected with HIV-1

Drug	Discordances (%)[a]	*P* value for subtype dependency[b]	Subtypes[c]
Protease inhibitors			
Nelfinavir	1.8	<0.01	G and C more than B
Atazanavir	1.1	<0.01	
Ritonavir	1.1	<0.01	F more than B
Amprenavir	0.6	NS	
Indinavir	0.5	NS	
Saquinavir	0.4	NS	
Lopinavir	0.3	NS	
Tipranavir	0	NS	
Nonnucleoside reverse transcriptase inhibitors			
Delavirdine	5	NS	
Nevirapine	5	NS	
Efavirenz	5	<0.01	
Nucleoside reverse transcriptase inhibitors			
Zidovudine	1.6	<0.01	
Zalcitabine	1.2	<0.01	
Stavudine	1	<0.01	C less than B
Abacavir	0.7	NS	
Didanosine	0.6	NS	
Tenofovir	0.4	NS	
Lamivudine	0.2	NS	
Emtricitabine	0.1	NS	

[a] Percentage of sequences that had discordant results between genotypic interpretation algorithms.
[b] One-way ANOVA was used to evaluate whether the number of discordances was subtype dependent (*P* of <0.05 was considered significant). NS, not significant.
[c] If the number of discordances was subtype dependent, Tukey's confidence intervals were used for a pairwise analysis to look for subtypes that caused significantly fewer or more discordances than subtype B. Although the percentage of discordances for some drugs was significantly subtype dependent, this did not always relate to a specific subtype that displayed significantly more or less discordances then subtype B.

TABLE 3. Interalgorithm discordances between genotypic drug resistance interpretation for sequences obtained from therapy-experienced patients infected with HIV-1

Drug	Discordances (%)[a]	*P* value for subtype dependency[b]	Subtypes[c]
Protease inhibitors			
Amprenavir	50	<0.01	C and D less than B
Atazanavir	42	<0.01	C less than B
Lopinavir	36	<0.01	
Saquinavir	24	<0.01	G more than B
Indinavir	15	<0.01	G more than B
Nelfinavir	14	<0.01	F more than B
Ritonavir	9	<0.01	
Tipranavir	2	NS	
Nonnucleoside reverse transcriptase inhibitors			
Efavirenz	11	<0.01	
Delavirdine	5	<0.01	
Nevirapine	5	<0.01	
Nucleoside reverse transcriptase inhibitors			
Didanosine	54	<0.01	C and D less than B
Abacavir	49	<0.01	
Tenofovir	37	<0.01	G, A, C, and D less than B
Zalcitabine	26	<0.01	C less than B
Stavudine	23	<0.01	
Zidovudine	13	<0.01	
Lamivudine	7	<0.01	CRF01_AE more than B
Emtricitabine	5	<0.01	CRF01_AE more than B

[a] Percentage of sequences that had discordant results between genotypic interpretation algorithms.
[b] One-way ANOVA was used to evaluate whether the number of discordances was subtype dependent (*P* value of <0.05 was considered significant). NS, not significant.
[c] If the number of discordances was subtype dependent, Tukey's confidence intervals were used for a pairwise analysis to look for subtypes that caused significantly fewer or more discordances than subtype B. Although the percentage of discordances for some drugs was significantly subtype dependent, this did not always relate to a specific subtype that displayed significantly more or fewer discordances then subtype B.

Nucleoside reverse transcriptase inhibitor analysis. In 1.6% of the sequences, zidovudine (AZT) was responsible for most of the discordances in therapy-naive patients; didanosine (ddI) was responsible for most of the discordances in treated patients (54%). The difference between drugs in this class was significant for both therapy-naive (Table 2) and therapy-experienced (Table 3) patients.

For zidovudine, zalcitabine, and stavudine in the naive population, the number of discordances was associated with subtype ($P < 0.01$). For only stavudine, subtype C was found to display less discordances than subtype B.

The number of discordances was significantly associated with subtype for all drugs in therapy-experienced patients ($P < 0.01$). For lamivudine and emtricitabine, CRF01_AE seemed to display significantly more discordances than subtype B. Subtypes C and D had fewer discordant interpretations for didanosine, and subtype C had also fewer for zalcitabine. For tenofovir, a lot of non-B subtypes had fewer discordant results than subtype B. This was the case for subtypes A, C, D, and G.

Mutational features of the subtype dependency. The results have been summarized in Table 4.

In therapy-naive patients among non-B subtype viruses, subtypes C and G showed partial discordances with respect to saquinavir susceptibility.

For subtype C, the most frequent pattern that caused partial discordances was a combination of protease (PRO) 82V/I + 63P + 36V/I. This pattern significantly explained more partial discordances for subtype C than for subtype B ($P < 0.0001$). This seemed due to the HIVDB interpretation algorithm. All subtype C sequences displaying this pattern also had the PRO 93L mutation. This mutation is taken into account for only nelfinavir by the HIVDB algorithm, which scores this pattern as intermediate, while all other algorithms score these sequences susceptible.

Two rules were discovered in the tree for subtype G that explained significantly more discordances than subtype B. One was a rule very similar to that for subtype C, PRO 82I + 63P + 36I ($P = 0.04$), and the other rule was PRO 82I + 63mt (any mutation) + 20I ($P = 0.01$). In practice, these rules cover the same sequences, as all subtype G sequences with the first pattern also harbor a mutation at position PRO 20 and all

TABLE 4. Mutations at least partially responsible for the subtype dependent behavior of genotypic interpretation algorithms for a drugs and algorithms responsible for the observed discordances

Drug[a]	Subtype	Mutation patterns (score)[b]	Algorithm responsible[c]
Naive population			
Nelfinavir	C	82I/V + 63P + 36I/V (SISS)	HIVDB (all sequences also 93L, taken into account by only HIVDB)
	G	82I + 63P + 36I (SISS) and 82I + 63mt + 20I (SISS)	HIVDB (high weight for 82I)
Ritonavir	F	20R + 10V/I (nrSIS)	Rega (all sequences also 36I, three secondary PI mutations scored as I by only Rega)
Treated population			
Saquinavir	G	90M + 82I (SRIR)	ANRS (does not score this as resistant)
Indinavir	G	90M + 82I + 54V (RRSI) and	HIVDB and ANRS (all sequences also 36I, pattern scored as R by HIVDB and ANRS)
		90M + 82I + 71T + 20I (RISI)	Rega (L90M not scored as R)
Nelfinavir	F	88S (RRSI) and 82A + 54V (IRRR)	Rega (scores this as S) ANRS (all sequences also 36I, not scored as R by ANRS)
Lamivudine	CRF01_AE	65R + 151M (IRRI)	ANRS and VGI (do not have a rule for the presence of both)
Emtricitabine	CRF01_AE	118I + 215Y (SSInr)	Rega (all sequences also 41L and 67N, 67N scored only by Rega)

[a] Only drugs for which the subtype dependence was proven and for which we found subtypes that displayed significantly more or fewer discordances than subtype B are shown. As explained in the text, the decision trees for the drugs where subtype B displayed more discordances were often too complex. Those are not included in this table.

[b] Positions at which mutations are responsible for discordances as revealed by data mining analysis. The order of the scores is shown alphabetically according to the algorithm name (ANRS, HIVDB, Rega, and VGI). Only the scoring patterns that accounted for most of the discordances (>85%) are shown. nr, no rule available for the drug.

[c] Algorithm(s) responsible for the observed discordances. Some information is provided in parentheses as to why these algorithms cause a discordance.

sequences with the second pattern also harbor a mutation at position PRO 36. Again, these discordances were due to the HIVDB algorithm, which is the only one that takes into account mutations at position PRO 20 and gives a rather high weight for the PRO 82I mutation for nelfinavir.

For ritonavir, subtype F caused more discordances than subtype B. We found a rule, PRO 20R + 10V/I, in the decision tree explaining significantly more subtype F partial discordances than those observed in subtype B. An example of the Weka decision tree with subsequent statistical analyses is shown in Fig. 2. Those subtype F sequences all had the PRO 36I mutation and thus harbored three secondary PI mutations. The Rega algorithm scores this as intermediate for ritonavir, while all other algorithms score this as susceptible.

For NRTIs, subtype B gave a lot of discordant interpretations. The rule predictive for this discordance in the decision tree was any mutation at RT 215, but this was not significant ($P = 0.07$). When examining the data, we found that the dis-

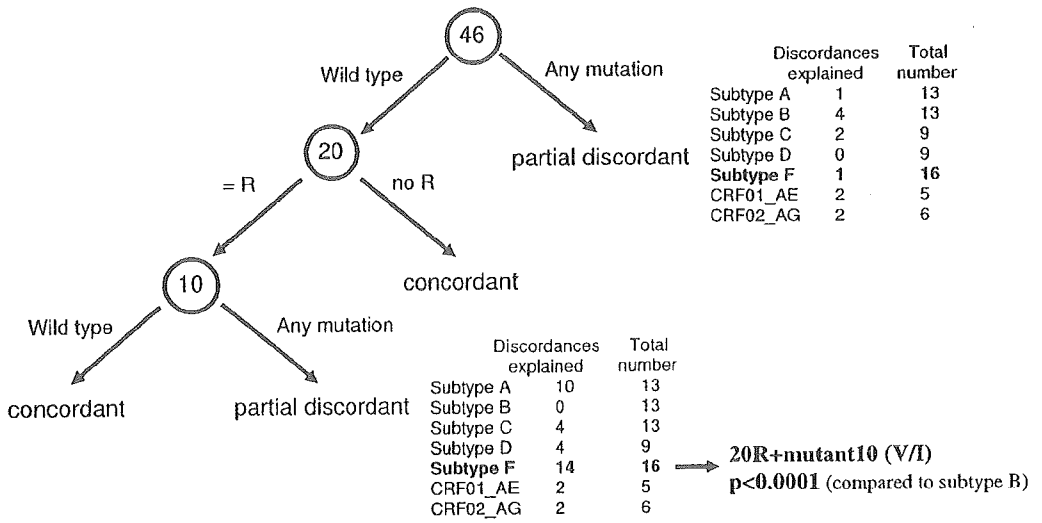

FIG. 2. Representation of the Weka decision tree for ritonavir in our untreated population. In the circles, the amino acid position is represented and, along the arrows, the mutation present is shown. R, arginine. We found that subtype F displayed more discordance. In the Weka decision tree, two rules were found, i.e., (i) any mutations at position PRO 46 and (ii) 20R + mutant 10. We calculated the number of discordances found that were explained by these rules and compared these numbers for subtype F and subtype B. Only the second rule explained significantly more discordances for subtype F than for subtype B.

cordances for stavudine were due to the ANRS system, which scores the presence of a mutation at 215 by itself as intermediately susceptible; all the other systems score this as susceptible. We found that subtype B more often had a mutation at this position than did subtype C, although this was not significant.

For the PI saquinavir in therapy-experienced patients, the full discordances observed in subtype G sequences could be attributed to mutations PRO 90 M + 82I. This was due to the ANRS interpretation system, which does not score this as resistant (as HIVDB and VGI did) if PRO 82I is present. Only PRO 82A is taken into account by ANRS.

For indinavir, subtype G also displayed more discordances than subtype B, apparently due to PRO 90 M + 82I + 54V, which was scored as resistant by HIVDB and ANRS because all these samples also had the PRO 36I mutation. Another rule predictive for discordance was PRO 90 M + 82I + 71T + 20I. The Rega system scores this pattern as susceptible, since the PRO 90 M mutation by itself is not scored as resistant by this algorithm.

Subtype F causes more discordances for nelfinavir in treated patients. The PRO 88S mutation was partially responsible for these discordances. The Rega algorithm considers these isolates to be susceptible, while the score from other algorithms was at least intermediate resistant. The partial discordances for subtype F are explained by PRO 82A + 54V. All these sequences had also PRO 36I, which is not considered resistant by ANRS relative to the other algorithms.

Subtype B displayed a lot of discordances for amprenavir. In fact, the decision tree incorporated subtype in this model. The resulting rule was PRO 90 M + 54V + 20R + 82A. All these sequences had an additional PRO 36I mutation, which is not included in the amprenavir rules of the Rega algorithm. This mutation pattern scored as intermediate for this system, while for the other algorithms, the additional PRO 36I mutation is responsible for the resistant score.

For atazanavir, subtype B caused a lot of discordances. The decision tree was very complex, and no clear rule had a high coverage and was predictive for the observed discordances in all subtypes. The atazanavir rules incorporate a number of mutations also observed for other PIs. Patients harboring a subtype B virus are probably treated with protease inhibitors more often and for a longer time, since subtype B has dominated since the beginning of the epidemic in countries where treatment was available and subsequently has been subject to drug selective pressure earlier. In these sequences, the large background of PI resistance mutations probably causes the discordances observed for atazanavir.

For lamivudine and emtricitabine (FTC), CRF01_AE scored more discordances than subtype B. For lamivudine resistance interpretation, this was caused by RT 65R + 151 M ($P < 0.05$). ANRS scores the presence of both mutations separately as intermediate but does not provide a rule for the presence of both of them, while the Rega algorithm for example scores this combination as resistant.

For emtricitabine, no clear rules were found in the tree, although it seemed that RT 41L + 67N + 118I + 215Y caused most of the partial discordances observed for CRF01_AE. The Rega algorithm is the only one that scores the RT 67N mutation for FTC. VGI does not provide rules for FTC.

For didanosine, tenofovir, and zalcitabine, subtype B had a lot more discordant interpretations than a number of non-B subtypes. The decision trees were very complex and also for these drugs, no clear rules could be deduced.

DISCUSSION

HIV genotypic information has led to an improved understanding of mutations in *pol*, which is associated with virological failure. Although resistance genotyping still has some limitations, it is often used to guide therapy start or change. One of the major problems is the interpretation of genotypic results. The knowledge on which such interpretation systems are built is based mainly on subtype B data. Considering the possible differences in therapy response in other subtypes, it would be interesting to verify whether our genotypic interpretation systems are equally valid for all subtypes. A first approach is to map discrepancies in drug resistance interpretation algorithms between subtypes and to identify which mutational patterns are responsible for such discrepancies. Such patterns can then further be investigated by, for example, in vitro mutagenesis and measuring the associated phenotype, taking into account that virus replication under drug selective pressure not only is a matter of protease and RT mutations but also is determined by the whole viral genome.

In this study, performed on sequences obtained from 5,030 patients, we investigated subtype-dependant discrepancies between four commonly used interpretation systems (Rega 6.3, HIVDB-08/04, ANRS [07/04], and VGI 8.0). The versions analyzed were the ones available to us at the time of analysis. In the meantime, updates have become available for all of these systems. None of these systems include subtype-dependant rules.

We did find drug- and subtype-dependent differences in the drug susceptibility/therapy response predictions of commonly used interpretation algorithms. We also identified mutational patterns that seemed to be partially responsible for the observed discordances.

Concordance was the lowest in the interpretation of therapy-experienced sequences, which means that it is less clear which mutations are really important for resistance development. This may explain some of the differences seen between algorithms in predicting treatment outcome (6). For lopinavir especially, the pathway towards resistance is unclear, which explains the high number of discordant results between the interpretation systems found in therapy-experienced patients (26, 27).

Our analyses revealed that the proportion of discordances between commonly used algorithms is subtype dependent for many drugs, in naive as well as in therapy-experienced patients. Concordance was higher in naive patients. However, non-B subtype sequences and subtype B sequences overall had equal numbers of resistance mutations. Both groups had mostly "wild-type" sequences. Therefore, the higher number of concordances is probably due to a larger agreement on what is a wild-type sequence.

In naive patients, discordances were found for nelfinavir (subtypes C and G). Incidentally, it is known that the pathway towards resistance for nelfinavir differs for subtypes C and G from that for subtype B. The PRO D30N mutation is not the

preferred one as in subtype B; it seems that, rather, the PRO L90M is selected (15) (P. Gomes, I. Diogo, M. F. Gonves, et al., Abstr. 9th Conf. Retrovir. Opportunistic Infect., abstr. 46, 2002). We found mutational patterns that partially explained these discordances. Those were mostly due to combinations of secondary PI mutations, which are often present as a polymorphism in non-B subtypes. Some algorithms include these mutations in their rules, while others do not. The PRO 93L mutation for example, is included by only HIVDB and not by the other systems. This mutation was present in all subtype C sequences with the pattern PRO 82I/V + 63P + 36I/V. Similarly for subtype G, the PRO 20I mutation is incorporated by only HIVDB.

For subtype F and ritonavir, the pattern PRO 20R + 10V/I also included the PRO 36I mutation. Three secondary PI mutations are scored as intermediate by only the Rega Algorithm.

For NNRTIs, we did not find any subtype-dependent discordances in resistance scoring, although some differences in resistance development have already been reported for subtype C under efavirenz treatment (2).

For NRTIs, only in naive patients did we find that the proportion of discordances is subtype dependent for stavudine. Subtype C had significantly less discordances than subtype B due to a mutation on RT 215 that occurred more frequently in subtype B sequences.

For PI resistance in treated patients, a lot of discordances are observed for subtype G in predicting resistance for saquinavir and indinavir and in subtype F for nelfinavir resistance prediction. The patterns observed here are related to a single algorithm that scores this differently. Differences often occur due to the presence of the PRO 36I mutation, which is present as a polymorphism in non-B subtypes. This mutation often triggers the switch to score an isolate as intermediate, while other systems do not take into account the substitution and consider the isolate to be susceptible. Apparently, there is no agreement on the role of some of these polymorphic resistance mutations in PI resistance.

For amprenavir and atazanavir, subtype B displayed a lot of discordances for treated patients. The decision trees for these drugs were very complex. The tree for amprenavir included subtype as a node, so a rule, PRO 90 M + 54V + 20R + 82A, could be deduced. For atazanavir, no clear rule was found. These two drugs are only recently being used in clinical practice, and the pathway towards resistance is not fully understood yet. The presence of a number of PI mutations, instead of some clear rules, is mostly used in the algorithms.

For lamivudine and emtricitabine in treated patients, CRF01_AE scored more discordances than subtype B. Although resistance for both drugs are predicted by the same rules in the algorithms, different mutation patterns are found in the decision trees. For lamivudine resistance interpretation, this was caused by RT 65R + 151 M. For emtricitabine, this was RT 41L + 67N + 118I + 215Y (although not statistically supported).

Tipranavir has a low number of discordances for naive patients as well as treated patients. This is mainly due to the limited amount of information that is available on resistance towards this drug (9). All algorithms are based on the same available information and thus predict the same level of resistance.

The four evaluated algorithms, in fact, belong to two different models. The Stanford algorithm assigns a score to each of the observed mutations and uses the sum to decide on the level of resistance, allowing complex patterns of mutations to be taken into account. The VGI, ANRS, and Rega algorithms are restrained to specific rules that describe specific mutational patterns. Therefore, the discordance for complex patterns is especially inevitable since both models use different ways to take these into account.

This study is not intended to draw conclusions on the validity of the different algorithms, but rather to identify mutation patterns that result in divergence between the algorithms, among different subtypes. The mutations and particularly the patterns of polymorphisms in non-B subtypes that are associated with viral resistance warrant further in vitro studies and ultimately need to be confirmed by clinical observation. We acknowledge, as a limitation of this study, the absence of measures of either in vitro or clinical resistance, which are phenotype and therapy outcome, respectively. However, the mutation patterns associated with discordance between the algorithms may identify the sequences of interest in larger datasets, obtained prospectively, and linked to viral load and/or CD4 data to correlate treatment outcomes.

In conclusion, the different algorithms agreed quite well on the level of resistance scored. However, where there are differences, in many cases these can be attributed to specific subtype-dependent combinations of mutations. The mutations found here should further be investigated as to whether they contribute to differences in resistance and therapy response between different subtypes. Our expertise in interpretation of genotypic resistance will increase with a scale-up of treatment to include millions of individuals with non-subtype B virus infections.

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6 HIV-1の薬剤耐性についての最近の知見

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Key words : HIV-1,anti-HIV drugs, drug resistance

Abstract
HAARTが本邦で導入されて以来HIV-1感染症治療の発展には著しいものがある。現在17種類の抗HIV薬剤が使用されAIDSによる死亡数を減少させるなど大きな効果をあげてきた。しかしその一方でHIV-1は自らの遺伝子にアミノ酸置換を誘導して薬剤耐性を獲得し，これがHAARTの効果を減弱させる原因となっている。現在新規抗HIV薬剤の開発と共にHIV-1の薬剤耐性に関する研究が盛んに行われ新しい事実が次々と明らかになった。本稿ではHIV-1薬剤耐性に関する新知見を紹介する。

はじめに

Human immunodeficiency virus type-1, HIV-1感染症の治療は，多剤併用療法：highly active antiretroviral therapy(HAART)の開発・導入と新薬開発により目覚しい進歩を遂げてきた。日本でも1997年にHAARTが開始されて以後AIDS（後天性免疫不全症候群）による患者の死亡例は減少を認めた。最近登場してきた新薬は経口吸収性と体内における薬物動態が大幅に改善され，１日１回の服用で有効血中濃度が維持できる様になってきた。このような新薬をHAARTの中核にすることにより，厳格な服薬による患者側の負担も減りQOLが改善された。今やHIV-1感染症は慢性疾患であると言う楽観的な意見すら聞かれることがある。しかし忘れてはならないのはHIV-1が感染症であることと，現代のHAARTではHIV-1感染症の治癒は望めないことである。仮に薬剤により体内におけるHIV-1の増殖を完全に押さえ込んだとしてもHIV-1を体内から完全に排除するためには約60年間の投薬が必要と試算されている。またHIV-1はその活発な増殖と低い逆転写精度（fidelity）のために高頻度で変異を起こす特徴を持ち，抗HIV薬に対しても容易に薬剤耐性を獲得する。また同じ作用機序の薬剤間では交叉耐性が顕著なことから，初回の治療に失敗してしまうと，それに代わる薬剤の選択幅が狭まってしまう。本稿ではHIV-1の薬剤耐性についての最近の知見を登場してきた薬剤を例に挙げながら紹介する。

1．抗HIV薬剤と作用機序

HIV-1の増殖には逆転写酵素(reverse transcriptase: RT)，インテグラーゼ(integrase:

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IN), そしてプロテアーゼ(protease: PR)という3つのウイルス由来の酵素が重要な役割を果たしている。その中でもRTとPRの2つが抗HIV薬剤開発の主要なターゲットとされてきた。現在使用されている抗HIV薬はRTに対する阻害剤(RT inhibitor: RTI)とプロテアーゼに対する阻害剤(PR inhibitor: PI)の2つに大別される。RTIはその阻害機序に基づき，さらにヌクレオシド系逆転写酵素阻酵害剤(nucleoside(nucleotide) analogue reverse transcriptase inhibitor: NRTI)と非ヌクレオシド(non-nucleoside reverse transcriptase: NNRTI)に分類される。

1) ヌクレオシド系逆転写酵素阻害剤(NRTI)

基本構造はヌクレオシド（ヌクレオチド）のホモログ（類似体）で，デオキシリボースの3'のOH基がH基に置換された構造を持つ。日本では現在までにzidovudine(AZT), didanosine(ddI), zalcitabine(ddC), stavudine(d4T), abacavir(ABC), tenofuvir(TDF)の計7剤が認可承認され使用されている（図1-1）。経口で摂取されたNRTIは標的細胞内で種々のヌクレオシドリン酸化酵素によって3リン酸化されて（NRTI-TP）活性型となる。NRTI-TPは逆転写酵素の基質としてdNTPの結合部位に取り込まれ，逆転写されたDNAの端に本来のdNTPの代わりに取り込まれる。しかしNRTIは3'のOH基を欠いているために次に結合する3リン酸化ヌクレオシドとの間のリン酸ジエステル結合が形成されず，NRTI-TPが取り込まれた時点でDNA鎖の伸張が止まる。このDNA伸張阻害作用によってNRTIは抗HIV効果を発揮する（図2-1）。2004年に認可されたTDFは他のNRTIsとは異なりすでにリン酸化を受けた構造を持ち，他のNRTIで律速となるヌクレオシド→ヌクレオチドの段階をスキップし速やかに2リン酸が付与されて効果を発揮する事が出来るnucleotide reverse transcriptase inhibitorである。

2)非核酸系逆転写酵素阻害剤(NNRTI)

NRTIとは異なり逆転写酵素のp66サブユニットのポリメラーゼ活性中心部位の近傍のhydrophobic pocketに結合し非拮抗阻害剤として逆転写反応を阻害する。現在nevirapine(NVP), efavirenz(EFV), delavirdine(DLV)の3薬剤が認可承認され使用されている（図1-2）。

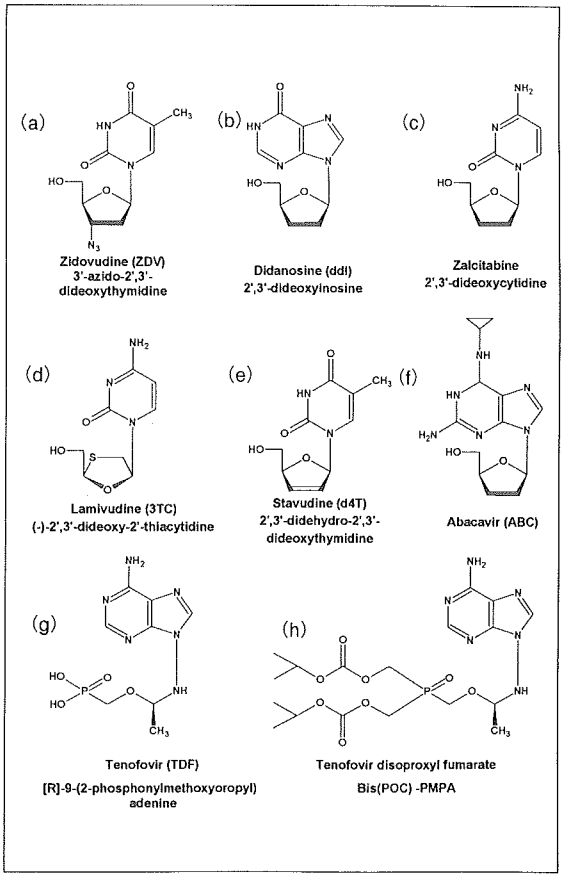


図1-1 ヌクレオシドヌクレオチド系逆転写酵素阻害剤

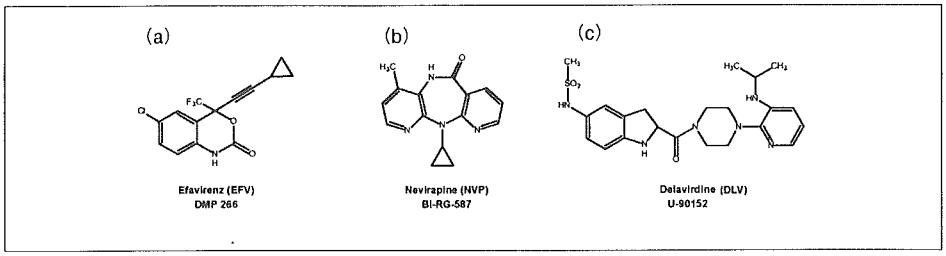


図1-2　非ヌクレオシド系逆転写酵素阻害剤

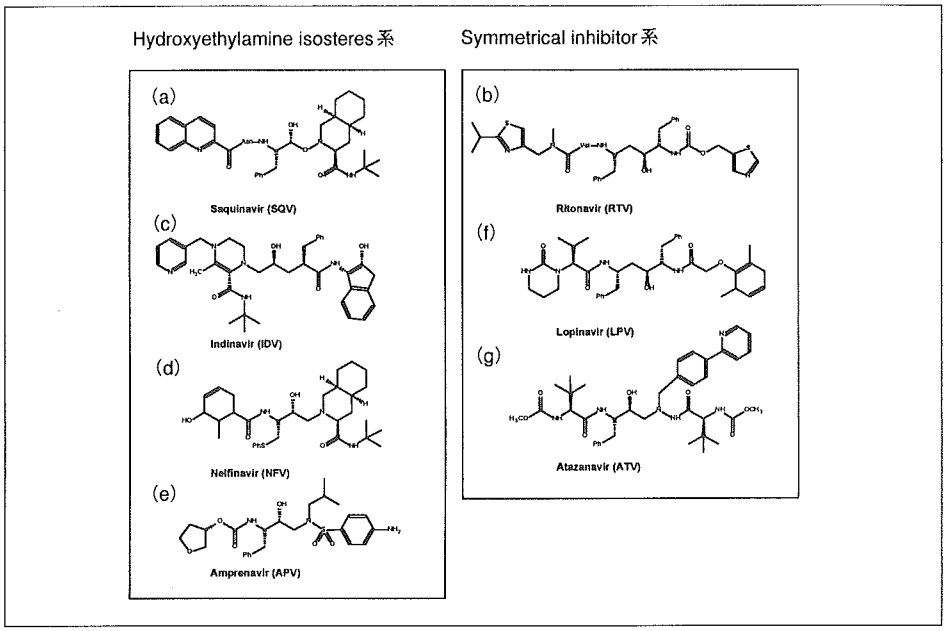


図1-3　プロテアーゼ阻害剤

3) プロテアーゼ阻害剤(PI)

HIV-1のPRはaspartic proteaseであり基質であるHIV-1 Gag前駆体の切断部分に存在するPhenilalanine-Proline配列，あるいはチロシンプロリン配列を認識し切断する活性を持っている。PIはHIV-1のPRの切断認識部位が哺乳動物のaspartic proteaseとは異なっている事を利用して設計されている。このためPIは宿主細胞のaspartic proteaseを阻害することなくHIV Gag前駆体の切断を阻害しHIVの成熟・感染性獲得を阻止する。現在nelfinavir(NFV), saquinavir(SQV), ritonavir(RTV), indinavir(IDV), lopinavir(LPV), amprenavir(APV), atazanavir(ATV)の7剤が認可使用されている（図1-3)。

2. 抗HIV薬剤に対する耐性変異とその作用機序

1) 核酸系逆転写酵素阻害剤(NRTI)

1987年に抗HIV薬として最初に開発されたAZTは当初単剤治療で用いられ，抗HIV-1効果を発揮した。しかしその効果は維持されず，程なく患者体内にはAZTに対して感受性低下したウイルスが出現してしまうことが明らかになった。このようなAZT感受性をなくしたウイルスを解析して見ると，逆転写酵素領域

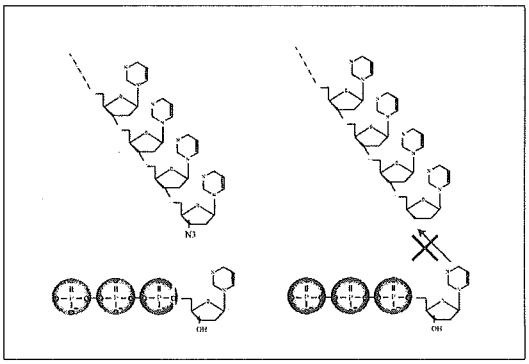


図2-1　NRTIによるDNA伸張阻害の機序
NRTIはリボースの3位の水酸基がXに置換されているため次のデオキシリボヌクレオシド3リン酸に結合しリン酸ジエステル結合を形成できないためDNAの伸張が阻害される。

に特有の変異が誘導されていることが確認された。AZTとd4Tはどちらもdeoxythymidineをベースとして開発された薬剤でAZTに対する耐性変異であるM41L，D67N，K70R，I210W，T215YF，K219QEはd4Tに対し交差耐性を示す。この6個の耐性変異をTAM(thymidine-associated mutation)と総称する。TAMは単独では高い耐性度を示さないが，長期に渡る薬剤投与によって変異が集積すると耐性度が増強する事が知られている。その耐性メカニズムはNRTIが取り込まれて伸張反応が停止したDNA鎖からNRTIを切り出す事（excision）によると考えられている[1),3)]。NRTIの多剤耐性変異(MDR)変異としては69-insertion[4)]とQ151M complex[5)]が知られている。69-insertionはT69S置換を伴うSS，SG，SA挿入変異でTAMと共存する事によりAZT，d4Tを始めとしたNRTIに対し高度耐性を示す。これはDNA鎖に取り込まれたAZT-MPあるいはNRTI-MPのexcisionを促進する事によって耐性を発揮する。Q151M complexはA65V，V75L，F77L，F116Y，Q151Mの5アミノ酸の置換が関連し，最初にQ151Mが誘導されるとHIVの増殖能(fitness)が損なわれるが，残りの4アミノ酸変異が次々と蓄積する事によってfitnessの回復が起こりNRTIへの高度耐性を獲得する。M184Vは3TCによって誘導されるがこの変異はexcision活性ではなくNRTIのDNA鎖への取り込みを直接阻害していると考えられている。M184VはTAMとは異なり単独で3TCに対して数百倍の感受性低下を誘導する。M184Vは3TCの他にddC，ddIに対して交叉耐性を示すが興味深い事にT215Yを持つウイルスにM184Vが加わるとAZTに対する感受性が回復する。またT215YにddI耐性に関連するL74Vが加わっても同様の効果が見られる。K65RはTDFに関与する耐性変異として知られるが，in vitroにおける実験とmacaque/SIVmacを用いたin vivo感染実験からM184VとK65Rは排他的変異である事が示されている[6)]。これはM184VがTDFに対する感受性を高める事やM184V/K65Rの共存がウイルスの増殖能力を損なうためと考えられる[7)]。TDFは69-insertion 多剤耐性株に対しては感受性低下を示すが，Q151M complexを持つHIVに対しては感受性が維持される[8),9)]ことからTDFはNRTI多剤耐性症例にも有効である事が期待される。NRTIの薬剤耐性獲得に関して

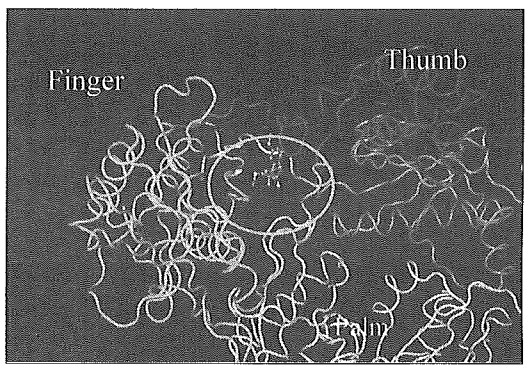
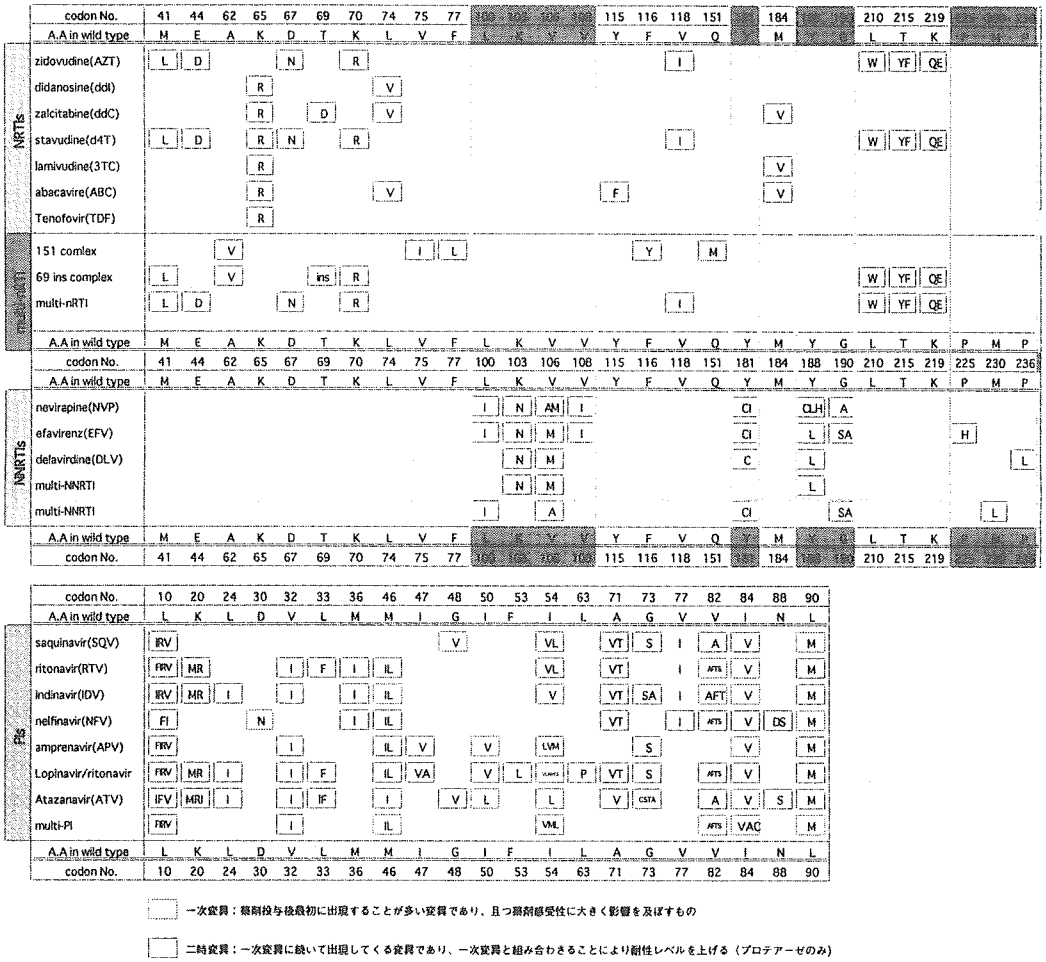


図2-2　逆転写酵素とNNRTIの結合部位
NNRTIは逆転写酵素の活性中心付近(円内）に結合し逆転写酵素とRNAプライマーとの結合を阻害する。薬剤耐性変異部位は活性中心の近傍に存在する。

表1　抗HIV-1薬剤と誘導される耐性変異のまとめ



は新たな知見も報告されている。逆転写反応ではHIV RNAテンプレートはDNA合成反応が起こった後逆転写酵素に結合したRNase Hの働きにより分解されるが，このRNase Hに活性を低下させるH539N，D549Nを導入するとAZTやd4Tに対して10倍～100倍以上の耐性が誘導され，TAMと同時に存在する事でその耐性度は1000倍以上に増強される[10]。また，このRNase Hの変異はddI，ddC，3TCに対しては耐性を呈さない。このことからRNaseHの変異はexcision活性と関連すると考えられている。

2)非核酸系逆転写酵素阻害剤(NNRTI)

NNRTIの薬剤耐性変異はNRTIとは異なり，NNRTIの結合部位であるポケットと呼ばれる部位近傍に集中して誘導される事が知られ，逆転写酵素の活性部に歪みを起こすことにより酵素活性を失活させると考えられている（図2-2）。耐性機序はRTとNNRTI間の親和性

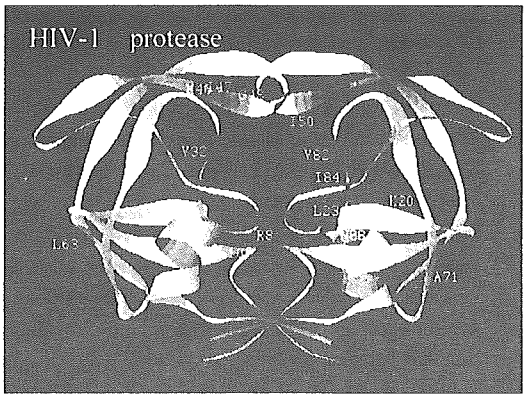


図2-3 プロテアーゼの構造と薬剤耐性変異部位
PIはプロテアーゼと基質が結合するポケットに結合しプロテアーゼの活性を阻害する。薬剤耐性変異部位（一次変異）は活性中心の近傍に存在する。

の低下であり，1つの変異が耐性に大きく影響を及ぼす。例えば181番目のチロシンがシステインに置換される事でNVPに対して100倍以上の耐性を獲得する。この耐性はDLVやEFVに対しても耐性を発揮する。NNRTIによって誘導される変異には他にL100I，K103N，V106AM，V108I，Y188CI，G190Aが知られ[11]，いずれも単一の変異で高度耐性を示し，また薬剤間の交叉耐性が顕著であるという特徴がある（図2-2）。

3)プロテアーゼ阻害剤(PI)

PIの薬剤耐性変異は一次変異(major mutation)と二次変異(minor mutation)に分類される（表1）一次変異とはPI投与後，比較的早い時期に出現する耐性変異で薬剤毎に特有なものが多い。SQVに対するG48V，IDVに対するM46I/L，NFVに対するD30N，RTVに対するV82A/F/T/S，APVに対するI50Vなどがよく知られている耐性変異である。またNFV，SQVの一次変異であるL90MはD30Nと排他的関係にあることが報告されている[12]。これらの一次変異はプロテアーゼの活性中心近傍に位置する事が多く（図2-3），プロテアーゼの構造を変化させる事でプロテアーゼとPIの結合を回避していると考えられるが，一方でこの構造変化のためプロテアーゼの活性低下を起こしウイルスの増殖能力が低下する場合も多い。この一次変異に続いて出現してくるアミノ酸置換を二次変異と称し一次変異とは区別する（表1）。二次変異はそれ自体で大きく耐性度を上昇させる事は無いが一次変異によって低下した増殖能力の回復，あるいはより高度耐性の獲得に働いていると考えられている。LPVはRTVを元に設計され2000年に認可されたPIである。当初P450による代謝を受けるためバイオアベイラビリティが低い事が問題であったが，その後RTVとの併用で効果的な血中濃度を保つ事が可能となった[13]。LPVはRTVによって誘導される一次変異V82Aを避けるような形で開発した結果，明らかな一次変異は存在せず8個以上の耐性変異が集積して高度耐性を発揮すると考えられている[14]。ATZは2003年に発売された最も新しいPIである。ATVによって誘導される一次変異はI50Lである。興味深いことにI50Lを獲得したHIV-1は他のPIに対して交叉耐性ではなく高感受性を呈すると報告されている[15]。ATVは1日1回の服薬で十分な効果が得られ副作用が従来のPIと比べ弱い点からも今後抗HIV治療で多く使用されていく事が予想される。

上述した薬剤耐性の情報はIAS-USAガイドラインで発表されるUpdate of the Drug Resistance Mutations in HIV-1で参照する事が可能である。ウェブサイトhttp://www.isausa.org/から直接ダウンロードする事も出来る[11]。2005年4月に公開されたものが2005年5月現

在最新バージョンとなっている。また抗HIV薬と薬剤耐性についての総説が刊行されているので参照されたい[16]。

おわりに

現在日本で認可されている抗HIV薬はNRTI, NNRTIそしてPIの3クラスだが，アメリカではHIV-1の宿主細胞への浸入を防ぐ融合阻害剤Enfvirtide(T-20)が新たなクラスとして登場している。またこれまでのRTやPR以外のHIV-1酵素・タンパクを標的とした新規抗HIV-1薬の開発も活発に行われているが，その中でも有望とされるのはインテグラーゼに対する阻害剤であろう。現在各国の製薬会社で開発が行われており，既存の薬剤に対して多剤耐性に陥り治療困難となった症例の福音として期待されている。しかしHIVは新規抗HIV薬剤に対しても新たな耐性変異を獲得してその抗HIV効果を掻い潜って行く事は明らかであり，HIVの薬剤耐性獲得のメカニズムの解明は今後もHIV-1研究の中で重要な鍵になると予測される。

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