Eur J Med Res (2004) 9: 273-278

PRIMARY DRUG-RESISTANCE IN HIV-POSITIVE PATIENTS ON INITIATION OF FIRST-LINE ANTIRETROVIRAL THERAPY IN GERMANY

M. Oette[1], R. Kaiser[2], M. Däumer[2], D. Akbari[1], G. Fätkenheuer[3], J. Kurt Rockstroh[4], J. Stechel[5], A. Rieke[6], S. Mauss[7], D. Schmalöer[8], K. Göbels[1], C. Vogt[1], M. Wettstein[1], D. Häussinger[1]

[1]Clinic for Gastroenterology, Hepatology and Infectious Diseases, University Clinic Düsseldorf; [2]Institute for Virology, University of Köln; [3]Clinic I for Internal Medicine, University of Köln; [4]Clinic for Internal Medicine, University of Bonn; [5]Private Practice, Köln; [6]Kemperhof-Clinic; Koblenz; [7]Private Practice, Düsseldorf; [8]Westfalendamm Clinic, Dortmund, Germany

Abstract:
Background: Resistance against antiretroviral drugs in previously untreated HIV-infected persons is of growing relevance. The aim of the study is to determine the prevalence of resistance-associated mutations in this patient group.
Methods: In a prospective multicenter-study in Nordrhein-Westfalen, Germany, genotypic resistance testing was performed in untreated HIV-positive patients before administration of first-line highly active antiretroviral therapy (HAART).
Results: Between January 2001 and August 2002 resistance testing was performed in 184 therapy-naive individuals. HAART was initiated in 143 patients, who were included into the study. 70.6% were males, mean age was 39 years, mean duration of diagnosis of HIV-infection was 1.5 years. The proportion of cases at CDC stage C was 45.4%, mean CD4-cell count was 199 /ml, mean viral load was 206,855 copies/ml. Resistance-associated mutations were detected in 20 patients (14.0%). 10.5% showed mutations indicating nucleoside reverse transcriptase inhibitor- (NRTI) resistance (M41L, E44D, D67N, T69D/N, L74V, V118I, M184V, L210W, K219Q), 2.8% showed non-nucleoside reverse transcriptase inhibitor (NNRTI) resistance (K103N, V108I, Y181C), and 2.1% showed protease-inhibitor- (PI) associated resistance (V82A, L90M), respectively. Multi-class-resistance was found in 2.1%, mutations indicating revertant variants of resistant strains were found in 4.2% (T215C/E/L/S). 86.7% of the isolates showed secondary mutations in the protease gene. No significant difference in the distribution of the parameters age, sex, duration of HIV diagnosis, CDC stage, CD4-cell count, and viral load, between groups with and without resistance was identified.
Conclusion: The prevalence of primary resistant virus strains can be estimated at 14% in chronically infected HAART-naïve HIV-patients in Germany. The majority of these cases show NRTI-associated resistance. Resistance against NNRTI or PI as well as multi-class-resistance is of low prevalence. No risk factor of predictive value can be identified for the diagnosis of resistance mutations in the individual. In conclusion, routine genotypic resistance testing in untreated HIV-positive patients should be performed before administration of first-line HAART in this region.

Key words: HIV, AIDS, drug resistance, epidemiology, Germany, HAART, subtype

INTRODUCTION

Despite documented efficacy of highly active antiretroviral therapy (HAART) of HIV infection, viral replication cannot be suppressed successfully in a substantial proportion of patients [5]. One major reason for this is the development of resistance-associated mutations. These tend to be common in treated HIV patients, as demonstrated in the "HIV Health Cost and Utilization Study in the US". Phenotypical resistance was found in half of the analyzed population and in almost 80% of persons with inefficient HAART [27].

Resistance against antiretroviral drugs in previously untreated HIV-positive patients is of growing relevance in the last years. It is defined as primary resistance and was demonstrated for seroconverters in a number of countries in North America and Europe [8, 13, 18, 22, 25, 26]. These data are strong evidence for the fact that resistant virus is transmissible [14]. Although many virus strains develop reversion to wild type in the absence of drug selective pressure [31], it has been shown that resistance may persist for years even without the presence of drugs [6, 23]. Accordingly, mutated virus could be isolated in chronically HIV-infected patients years after seroconversion in different countries with varying prevalence between 2 and 29% [4, 9, 15-17, 19, 29, 30]. As routine resistance testing before first application of HAART has not been implemented in clinical routine [2], antiretroviral treatment may have reduced potency in patients having contracted resistant virus. To avoid this, clinicians should know the prevalence of mutated HIV in their own region. Up to now,

there is insufficient data on HIV primary resistance in chronically infected HIV-patients in Germany. Thus, the aim of this study was to characterize the prevalence of drug resistant HIV in this population at the time point of first HAART administration.

METHODS

In a prospective multicenter study in Nordrhein-Westfalen, Germany, genotypic resistance testing was performed in HIV-1-infected patients before first application of HAART. In this region HIV-incidence accounts for about 21% of the cases of the whole country [3]. The study received approval by the local institutional review board. Patients were treated in 3 out-patient-units at university clinics, 2 ambulances of regional hospitals, and in 5 private practices. All study centers are specialized for the treatment of HIV-patients.

Inclusion criteria were documented HIV-1-infection, both physician´s and patient´s decision for initiation of HAART, and informed consent. Exclusion criteria were previous intake of antiretroviral drugs and non-willingness to participate. The following baseline parameters were documented: Age, sex, route of HIV-transmission, duration of HIV-diagnosis, CDC-stage of disease, CD4-cell count, and viral load.

Genotypic resistance testing was performed in the institute of Virology, University of Köln, Germany. Viral RNA was isolated from patient plasma using QIAamp viral RNA mini kit (Qiagen, Hilden, Germany) according to the manufacturer´s protocol. Reverse Transcription and polymerase chain reaction (PCR) were carried out using OneStep RT-PCR kit (Qiagen, Hilden, Germany) and primers 1RES, 5'-GAAGAAATGATGACAGCATGTCAGGG-3' (nt 1,819-1,844) and 2RES, 5'-TAATTTATTACTT GTTCATTTCCTCCAAT-3' (nt 4,173-4,202). Nested PCR was carried out with HotStarTaq (Qiagen, Hilden, Germany) and the following inner primer pair: RES3, 5'-AGACAGGCTAATTTTTTAGGGA-3' (nt 2,074-2,095) and RES4, 5'-ATGGYTCTTGATAAA TTTGATATGTCC-3' (nt 3,559-3,585). The 1.5 kbp PCR product was purified by using the QIAquick spin PCR purification kit (Qiagen, Hilden, Germany). Population based sequencing of the HIV-1 pol region was done by using the ViroSeq HIV-1 Genotyping System sequencing module (Applied Biosystems, Foster City, CA, USA). Extension products were purified using MultiScreen purification plates (Millipore, Bedford, MA, USA) and Sephadex G-50 superfine (Amersham Biosciences, Upsala, Sweden) and were run on an ABI Prism 310 capillary sequencer. Sequence data were generated from raw data files by using Sequencing Analysis v3.4 (Applied Biosystems, Foster City, CA, USA). The obtained sequences were assembled and edited by using the ViroSeq HIV-1 Genotyping software v2.5 (Applied Biosystems, Foster City, CA, USA). Interpretation and classification for clinical significance of novel variants was performed according to current guidelines [1, 10]. Resistance-associated mutations were subdivided by their biological relevance: Mutations resulting in resistance against nucleoside reverse transcriptase inhibitors (NRTI), non-nucleoside reverse transcriptase inhibitors (NNRTI), protease-inhibitors (PI), and multi-class-resistance in cases with involvement of at least 2 of these groups. Furthermore, mutations indicating revertant variants of resistant strains were regarded as significant. These isolates (T215C/E/L/S) have been associated with reduced virological efficacy of HAART, although phenotypically sensitive [11, 20]. Secondary PI-mutations were recorded, but not regarded as significant.

The statistical analysis was performed with the help of SPSS, release 8.0. Subgroups with and without resistance were compared. For the parameters age, duration of HIV-diagnosis, CD4-cell count, and viral load Wilcoxon rank sum test was used. For the parameters sex and CDC-stage two-sided Fisher´s exact test was used. The comparison of resistance frequency between the study population and excluded patients was done with Fisher´s exact test. P-values of <0.05 were considered as significant.

RESULTS

Between January 2001 and August 2002 a genotypic resistance test was performed in 184 HIV-positive HAART-naive patients. 143 individuals entered the study by initiation of therapy, the baseline characteristics of these are listed in Table 1. The 41 remaining patients did not start a HAART regimen after resistance testing because of individual wish or physicians advice, so that they were excluded from further evaluation.

The study consisted of a predominantly male population of a mean age of 39 years with mostly homosexual and heterosexual transmission mode and manifest immunosuppression as well as high median viral load. 24.5% of the individuals carried a non-B-subtype of HIV-1. In 20 patients resistant isolates were identified (14.0%). The specification of the individual mutations including association to substance groups is shown in Table 2. The majority of resistance-associated mutations was found for NRTI, resistant strains regarding the other substance groups and multi-class resistance were uncommon.

To test for differences between the subgroups with and without resistance, the distribution of baseline characteristics was compared. The result is demonstrated in Table 3. There was no significant difference between the two groups in terms of age, sex, duration of diagnosis, CDC-stage, CD4-cell count, and viral load, respectively. Moreover, the frequency of CDC-stage C versus non-C was compared between the 2 groups. Corresponding to the previous results, no significant difference was identified ($p=0.86$). The comparison of frequencies of resistance between the study population and excluded patients was also analyzed. Although the proportion of overall resistance was higher in the study population (14.0% versus 4.9%), this difference did not reach statistical significance ($p = 0.171$).

Table 1. Patients´ characteristics.

			%	SD*
Patients	all	143	100	
Age	mean	39		9.6
Sex	male	101	70.6	
	female	42	29.4	
Duration of HIV diagnosis	mean years	1.5		3.4
Transmission route	homosexual	66	46.2	
	heterosexual	32	22.4	
	endemic region	16	11.2	
	i.v.-drug use	5	3.5	
	blood products	3	2.1	
	unknown	21	14.6	
CDC stage	CDC A	34	23.8	
	CDC B	44	30.8	
	CDC C	65	45.4	
CD4-cell count	mean	199		190
Viral load	mean	206,855		219,664

* SD: Standard deviation

Table 2. distribution of mutations.

	n	%	mutations
All patients	20	14.0	
NRTI-mutations	15	10.5	M41L, E44D, D67N, T69D/N, L74V, V118I, M184V, L210W, K219Q
NNRTI-mutations	4	2.8	K103N, V108I, Y181C
PI-primary mutations	3	2.1	V82A, L90M
Revertants	6	4.2	T215C/E/L/S
Multi-class resistance	3	2.1	mutations in at least 2 classes
PI-secondary mutations	124	86.7	L10F/I/V, G16E, K20M/R, L33F, M36I, L63P, H69Y, A71T/V, V77I, I93L

Table 3. Comparison of subgroups.

		Resistance +	Resistance -	p-value
Patients		123 (86 %)	20 (14 %)	
Age (mean)		40.5	40.0	0.47
Sex	male	15 (75.0 %)	86 (69.9 %)	0.79
	female	5 (25.0 %)	37 (30.1 %)	
Duration of HIV diagnosis (mean years)		1.3	1.6	0.99
CDC stage	A	5 (25.0 %)	29 (23.6 %)	0.86
	B	5 (25.0 %)	39 (31.7 %)	
	C	10 (50.0 %)	55 (44.7 %)	
CD4-cell count (mean /µl)		180	202	0.75
Viral load (mean c/ml)		201,597	207,711	0.81

Discussion

Several studies addressed the problem of resistance against antiretroviral drugs in previously untreated HIV-positive patients. Interpretation of the results is conflicting because of heterogeneity. Different time periods, resistance assays and patient populations make comparisons difficult. Especially the period of inclusion is of relevance, because of the rapid evolution of antiretroviral treatment. Many compounds have been introduced into clinical routine during the last 5 years. Thus, historical analyses may not reflect today´s reality. Moreover, contemporary data are rare with only few studies on patients who were included after the year 2000 [12, 15, 18, 25, 32, 34]. In this investigation we studied the prevalence of primary drug resistance in 2001 and 2002. Moreover, in the discussion about primary resistance it is important to define the clinical situation of data acquisition. We included chronically HIV-infected patients at the time point of HAART initiation. These patients, usually presenting with advanced stage of disease, are much more common in daily practice than HIV-seroconverters, who were studied in the majority of cited investigations.

The population of this study was characterized by a mean age of 39 years, it consisted of predominantly male persons with homosexual transmission route. Mean CD4-cell count and viral load as well as clinical staging indicated mostly advanced stage of disease. Thus, the analyzed patient group may be regarded as representative with a distribution of baseline characteristics well-known from daily clinical practice and epidemiological data [3]. The mean duration of HIV-diagnosis was 1.5 years. This is a small period in the light of the advanced stage of HIV-infection in the majority of patients, probably having progressed to immunosuppression without knowledge of own serostatus. Prescription of HAART is indicated in these patients according to current guidelines [35].

We found a prevalence of resistance-associated mutations of 14% in treatment naïve HIV-patients before initiation of first-line HAART. The comparison of groups with and without resistance revealed similar baseline characteristics. Thus, no individual risk factor for primary resistance could be identified.

The results are consistent with older data from Italy [25], Switzerland [36], and Spain [24]. Contemporary epidemiological facts from different European countries seem to be comparable to the German situation. For example, the prevalence of resistance against NRTI in seroconverters is 14.7% in Italy [32] and 12.3% in Germany [13]. The prevalence of resistance against NRTI in chronically infected patients is 7.8% in Italy [25], 14.0% in Belgium [12], 10.5% in this study, and 7.6% in several European countries [34]. The prevalence of resistance against PI in chronically infected patients is 2.5% in Belgium [12], 2.1% in this study, and 1.6% in the other European countries [34]. On the one hand, despite differing parameters like sampling and investigational period the results indicate comparability. On the other hand, it is known that in these countries the proportion of transmission types and the prevalence of non-B-subtypes vary significantly. This makes interpretation of the data difficult, as baseline characteristics in the regions are different.

It should be emphasized that also in one of the Italian studies patient inclusion was defined for the time point of initiation of HAART [25]. This is a well-defined clinical situation, but was used for inclusion into epidemiological studies only rarely. Thus, the design of this trial makes comparability for future investigations more feasible.

The majority of mutations were identified in the NRTI-encoding regions with resistance especially against d4T, AZT, and 3TC. These NRTI were the first to be used for therapy in a large subset of patients. As they still play an important role in nowaday´s HAART, these results are of substantial clinical relevance, as prescription of standard combination therapy may be ineffective in these individuals. It has been demonstrated in a study in the USA, that primary resistance may have impact on the rate of virological success of first-line treatment [22]. Thus, the prevalence of resistance-associated mutations in this study has to be regarded as similarly relevant. Only a small proportion of subjects had virus resistant against NNRTI or PI. This is a hint for HIV-acquisition in the period before widespread use of these substance groups. The latter is emphasized by the high proportion of advanced CDC-stage in the study population.

It is known that resistance-associated mutations for PI and to a lesser extent for NRTI and NNRTI may revert to wild-type in the absence of drug pressure. One of the reasons is a reduced replicative capacity of these virus strains in comparison to wild-type HI-virus. This makes interpretation more complicated because resistant variants may not disappear completely but persist as proviral DNA in sanctuary sites [28]. In a situation of drug re-exposure, these minor variants may have evolutionary advantage and emerge to become the predominant variant again. Thus, resistance attributable to these groups may be under-estimated by our data. In contrast to this, subgroup analysis revealed no significant difference of time of HIV-diagnosis, CDC-stages, CD4-cell count, viral load, as well as other baseline characteristics between the groups with and without resistance. These facts make substantial reversion to wild-type virus after transmission unlikely, because in this case further progressed clinical stages should be over-represented in the group without primary resistance.

The majority of detected NRTI-mutations confer high-level cross-resistance in their substance group. This fact and the high prevalence prompt the conclusion, that NRTI-resistance is the most important finding of this study. The impact of NNRTI- and PI-resistance seems to be a minor problem. The relevance of revertant isolates may also be lower than NRTI-resistance, but the presence of these mutations can lead to virological breakthrough, as shown in vivo in clinical trials [11, 20]. The frequency of these mutations is low in 2001 and 2002, as demonstrated in this study. Fortunately, with 2.1% multiclass-resistance was rare in this study. PI-secondary mutations, although common, do not play an impor-

tant role in the evaluation of primary resistance, as they do not need drug pressure for development and do not confer high-level resistance in vivo.

Future developments in primary resistance are unpredictable, as several studies found an increase of prevalence [7, 21, 30], whereas other studies demonstrated the contrary [15, 17]. Mathematical models of time trends estimate an increase of prevalence [7]. Moreover, the predominance of NRTI-mutations may be due to longer availability of this drug class. It is not clear which trend the frequency of NNRTI- and PI-mutations will show. These aspects make further epidemiological studies with longitudinal design necessary.

Resistance in untreated patients is caused by transmission of mutated virus. In order to prevent acquisition of resistant virus, it is important to reduce development of resistance in treated patients. The HCSUS-study demonstrated the enormous dimension of this problem [27]. In this context adherence is one of the major factors and should be enhanced as much as possible. By improvement of adherence, treatment failure of HAART as well as transmission of resistance can be prevented. Thus, not only the individual but also the population at risk may have benefit from improvement of HAART efficacy.

Primary HIV drug resistance is a problem of significant public health impact [33]. Based upon our observations, three conclusions should be drawn. First, the relatively high prevalence of primary resistance in the study region makes continuing surveillance of this phenomenon necessary. Second, no parameter of predictive value can be identified for the diagnosis of resistance mutations in the individual. Third, routine resistance testing should be performed before initiation of first-line HAART.

Acknowledgements: The study received a grant by the Heinz Ansmann Foundation for AIDS-research, Germany and was supported by the Deutsche Forschungsgemeinschaft (DFG-HO 1582, KA 1569)

Study centers: Oette M, Düsseldorf; Kaiser R, Köln; Fätkenheuer G, Köln; Rockstroh JK, Bonn; Stechel J, Köln; Rieke A, Koblenz; Mauss S, Schmutz G, Düsseldorf; Schmalöer D, Gehring P, Dortmund; Isernhagen K, Köln; Gantke B, Düsseldorf; Reith A, Düsseldorf.
We thank Mr. Lasse Kajala for assistance in data acquisition and handling of specimens.

LITERATURE

1. International AIDS Society-USA resistance testing panel, HIV InSite. Antiretroviral resistance mutations. HIV Clin Trials 2001; 2: 346-355
2. The EuroGuidelines Group for HIV Resistance: Clinical and laboratory guidelines for the use of HIV-1 drug resistance testing as part of treatment management: Recommendations for the European setting. AIDS 2001; 15: 309-320
3. Robert-Koch-Institut (RKI). Epidemiol Bull 17.3.2003
4. Alexander CS, Dong W, Chan K, et al. HIV protease and reverse transcriptase variation and therapy outcome in antiretroviral-naïve individuals from a large North American cohort. AIDS 2001; 15: 601-607
5. Bartlett JA, DeMasi R, Quinn J, Moxham C, Rousseau F. Overview of the effectiveness of triple combination therapy in antiretroviral naïve HIV-1 infected adults. AIDS 2001; 15: 1369-1377
6. Birk M, Svedhem V, Sönnerborg A. Kinetics of HIV-1 RNA and resistance-associated mutations after cessation of antiretroviral combination therapy. AIDS 2001, 15: 1359-1368
7. Blower SM, Aschenbach AN, Gershengorn HB, Kahn JO. Predicting the unpredictable: Transmission of drug-resistant HIV. Nat Med 2001; 7: 1016-1020
8. Chaix ML, Descamps D, Deveau C, et al. Antiretroviral resistance, molecular epidemiology, and response to initial therapy among patients with HIV-1 primary infection in 1999-2000 in France. 11. International HIV Drug Resistance Workshop, Sevilla 2.-5.7.2002. Antiviral Ther 2002; 7 (suppl 1): 180, Abs 166
9. Clevenbergh P, Cua E, Dam E, et al. Prevalence of non-nucleoside reverse transcriptase inhibitor (NNRTI) resistance-associated mutations and polymorphisms in NNRTI-naïve HIV-infected patients. HIV Clin Trials 2002; 3: 36-44
10. D'Aquila RT, Schapiro JM, Brun-Vezinet F, et al. Drug resistance mutations in HIV-1. Top HIV Med 2002; 10: 21-25
11. de Mendoza C. Mutations at codon 215: The silent threat. AIDS Rev 2001; 3: 50-51
12. Derdelinckx I, Van Vaerenbergh K, De Wit S, et al. Evolution of primary resistance in Belgium (1995-2000). 1st European HIV Drug Resistance Workshop, Luxembourg 6.-8.3.2003, Abstr 57, P 4.6
13. Duwe S, Brunn M, Altmann D, et al. Frequency of genotypic and phenotypic drug-resistant HIV-1 among therapy-naïve patients of the German Seroconverter Study. J Acquir Immune Defic Syndr 2001; 26: 266-273
14. Erice A, Mayers DL, Strike DG, et al. Brief report: Primary infection with zidovudine-resistant human immunodeficiency virus type 1. New Engl J Med 1993; 328: 1163-1165
15. Gallego O, Ruiz L, Vallejo A, et al. Changes in the rate of genotypic resistance to antiretroviral drugs in Spain. AIDS 2001; 15: 1894-1896
16. Hanna GJ, Balaguera HU, Steger KA, Freedberg KA, Craven DE, D´Aquila RT. Drug-selected and non-clade B pol genotypes in chronically HIV-1-infected antiretroviral-naïve adults: response to antiretroviral therapy. 5. International Workshop on HIV Drug Resistance & Treatment Strategies, Scottsdale 4.-8-6.2001. Antiviral Ther 2001; 6 (suppl 1): 111, Abs 144
17. Jayaraman GC, Gleeson T, Sandstrom P, Archibald CP, for the Canadian HIV Strain and Drug Resistance Surveillance Programme. Prevalence and determinants of HIV-1 drug resistance in Canada (1997-2001). 11. International HIV Drug Resistance Workshop, Sevilla 2.-5.7.2002. Antiviral Ther 2002; 7 (suppl 1): S183, Abs 169
18. Jørgensen LB, Bøgh M, Gerstoft J, et al. Prevalence of drug resistance mutations and non-B subtypes in newly diagnosed HIV-1 positive patients in Denmark. 11. International HIV Drug Resistance Workshop, Sevilla 2.-5.7.2002. Antiviral Ther 2002; 7 (suppl 1): S191, Abs 177
19. Kijak GH, Pampuro SE, Avila MM, et al. Resistance profiles to antiretroviral drugs in HIV-1 drug-naïve patients in Argentina. Antiviral Ther 2001; 6: 71-77
20. Lanier ER, Ait-Khaled M, Craig C, Scott J, Vavro C. Effect of baseline 215D/C/S ´revertant´ mutations on virologic response to lamivudine/ zidovudine-containing regimens and emergence of 215Y upon virological failure. 11. International HIV Drug Resistance Workshop, Sevilla 2.-5.7.2002. Antiviral Ther 2002; 7 (suppl 1): S158, Abs 146

21. Little SJ. Is transmitted drug resistance in HIV on the rise? It seems so. BMJ 2001; 322: 1074-1075
22. Little SJ, Holte S, Routy JP, et al. Antiretroviral drug resistance among patients recently infected with HIV. New Engl J Med 2002; 347: 385-394
23. Pellegrin I, Garrigue I, Caumont A, et al. Persistence of zidovudine-resistance mutations in HIV-1 isolates from patients removed from zidovudine therapy for at least 3 years and switched to a stavudine-containing regimen. AIDS 2001; 15: 1071-1073
24. Perez-Olmeda M, Del Romero J, Rubio A, et al. Primary HIV-1 drug resistance in Spain before and after the introduction of protease inhibitors. J Med Virol 2001; 63: 85-87
25. Perno CF, Cozzi-Lepri A, Balotta C, et al. Low prevalence or primary mutations associated with drug resistance in antiviral-naive patients at therapy initiation. AIDS 2002; 16: 619-624
26. Pillay D, Cane P, Churchill D, Fisher M, Dean G, Porter K, for the UK Register of HIV-1 Seroconverters. The impact of transmitted drug resistance on time to CD4 < 350 cells/ ml. 11. International HIV Drug Resistance Workshop, Sevilla 2.-5.7.2002. Antiviral Ther 2002; 7 (suppl 1): S192, Abs 178
27. Richman DD, Bozzette S, Morton S, et al. The prevalence of antiretroviral drug resistance in the US. 41st International Conference on Antimicrobial Agents and Chemotherapy, Chicago 16.-19.12.2001; Abs LB-17
28. Trabaud MA, Leriche-Guerin K, Regis C, et al. Prevalence of primary resistance to zidovudine and lamivudine in drug-naïve human immunodeficiency virus type-1 infected patients: high proportion of reverse transcriptase codon 215 mutant in circulating lymphocytes and free virus. J Med Virol 2000; 61: 352-359
29. Van Vaerenbergh K, Debaisieux L, De Cabooter N, et al. Prevalence of genotypic resistance among antiretroviral drug-naïve HIV-1-infected patients in Belgium. Antiviral Ther 2001; 6: 63-70
30. Verbiest W, Brown S, Cohen C, et al. Prevalence of HIV-1 drug resistance in antiretroviral-naïve patients: A prospective study. AIDS 2001; 15: 647-650
31. Verhofstede C, Wanzeele FV, Van Der Gucht B, De Cabooter N, Plum J. Interruption of reverse transcriptase inhibitors or a switch from reverse transcriptase to protease inhibitors resulted in a fast reappearance of virus strains with a reverse transcriptase inhibitor-sensitive genotype. AIDS 1999; 13: 2541-2546
32. Violin M, Forbici F, Cozzi-Lepri A, et al. Primary HIV-1 resistance in recently and chronically infected individuals of the Italian Cohort Naive for Antiretrovirals. J Biol Regul Homeost Agents 2002; 16: 37-43
33. Wainberg MA, Friedland GF. Public Health implications of antiretroviral therapy and HIV drug resistance. JAMA 1998; 279: 1977-1983
34. Wensing AMJ, van de Vijver DAMC, Hoepelman A, et al. Combined analysis of resistance transmission over time of chronically and acute infected HIV patients in Europe. 1st European HIV Drug Resistance Workshop, Luxembourg 6.-8.3.2003, Abstr 54, P 4.3
35. Yeni PG, Hammer SM, Carpenter CC, et al. Antiretroviral treatment for adult HIV infection in 2002: updated recommendations of the International AIDS Society-USA Panel. JAMA 2002; 288: 222-235
36. Yerly S, Jost S, Telenti A, et al. Transmission of drug resistance: impact of primary and chronic HIV infection. 11. International HIV Drug Resistance Workshop, Sevilla 2.-5.7.2002. Antiviral Ther 2002; 7 (suppl 1): S197, Abs 183

Received: January 19, 2004 / Accepted: January 28, 2004

Address for correspondence:
Dr. med. Mark Oette
Clinic for Gastroenterology, Hepatology
and Infectious Diseases
University Clinic Düsseldorf
Moorenstr. 5,
D-40225 Düsseldorf, Germany
Phone 0049-211/ 81-18942
Fax 0049-211/ 81-16294
e-mail oettem@med.uni-duesseldorf.de