原　著

HIV 脳症 5 例の臨床的特徴と経過

橋本　里奈[1)]　向井栄一郎[1)]　横幕　能行[2)]　間宮　均人[2)]　濱口　元洋[3)]

要旨：HIV 脳症 5 症例を報告した．1996 年から 2005 年 11 月の間に名古屋医療センターを受診した HIV 感染症 458 症例（うち AIDS は 127 症例）を対象とした．HIV 脳症と診断した症例はいずれも高度の免疫不全状態にあり，他の日和見感染症を 3 症例にみとめた．4 症例は HIV 感染症が判明したのとほぼ同時期に HIV 脳症と診断された．5 症例とも HIV に対して抗ウイルス療法は未施行であった．HAART を施行することで全例で症状の改善をみとめ，死亡はみとめなかった．精神科介入を要したり 1 例を除いて社会復帰できないなど，行動障害を呈した HIV 脳症の機能予後は不良であり，HAART のみの治療効果は不十分と考えられた．

（臨床神経，48：173―178, 2008）
Key words：HIV，AIDS，認知障害，行動異常，予後

はじめに

HIV 感染症は病期が進行するにつれ日和見感染症など各種疾患を合併する．なかでも HIV 脳症は AIDS 指標疾患の 1 つであり，中枢神経領域における重要な合併症として挙げられる．亜急性から慢性に進行する記銘力低下，注意や意欲の低下，思考緩慢といった認知障害と，動作緩慢や失調性歩行などの運動障害を呈し，頭部 MRI T_2 強調画像や FLAIR 画像にて大脳白質から基底核にかけてびまん性の高信号を生じ皮質下は保たれることを特徴とする[1)]．しかし我が国では HIV 脳症の臨床報告は非常に少ない．そこでわれわれは，HIV 東海北陸ブロック拠点病院である当院で経験した HIV 脳症の自験 5 症例について，その臨床的特徴と経過について検討した．

対　象

1996 年から 2005 年 11 月に名古屋医療センター（以下当院）内科を受診した HIV 感染症のうち，神経内科に紹介された症例のうち HIV 脳症と診断されたものを対象とした．

方　法

当院内科より神経内科を紹介受診した HIV 感染症の症例に対し，著者の神経内科医 2 名によって神経学的診察，髄液検査，頭部 MRI を施行した．認知障害と運動障害の双方をみとめ，血液検査，髄液検査，各種画像検査にて代謝異常や日和見感染症，悪性腫瘍等が除外されたものを HIV 脳症と診断し，神経学的所見，長谷川式簡易痴呆スケール（以下 HDS-R）もしくは Mini-mental State Examinaton（以下 MMSE），CD4 陽性細胞数（以下 CD4）および血清 HIV ウイルス量，頭部 MRI にて経過を追跡した．

結　果

上記期間に累計 458 症例の HIV 患者が受診し，そのうち AIDS 発症者は 127 症例であった．AIDS のうち 25 例に中枢神経合併症をみとめた．中枢神経合併症の内訳は Table 1 にまとめた．HIV 脳症は 5 例にみとめた．HIV 感染症の感染経路は 5 例とも同性間性行為と推定された．全例で HAART を施行し，CD4 の改善と HIV ウイルス量の抑制をみとめた．以下，症例を提示する．

症例 1　37 歳男性

職業はデザイン関係．2003 年 8 月から微熱と歩行障害が出現し，同年 9 月に動けなくなり前医に入院した．10 月に尿閉が出現．頭部 MRI では大脳，脳幹にびまん性病変があり，ADEM もしくはウェルニッケ脳症と診断され，ステロイドパルス療法とビタミン B 大量を投与されるも効果なし．その後，HIV 抗体陽性と判明したため，12 月に当院に転院した．体温 38.6℃，臥床状態で，四肢の関節腫脹があった．自発的に開眼し，寡動．発語は「イタイ」など限られた単語のみであった．知能は HDS-R は 1 点（場所について「病院」を選択できた），WAIS-R は判定不能．脳神経はほぼ正常であり，運動は指示にしたがえず評価不能，両側に強制把握をみとめた．上肢

[1)]国立病院機構名古屋医療センター神経内科〔〒460-0001　名古屋市中区三の丸 4―1―1〕
[2)]同　感染症内科
[3)]同　臨床研究センター
（受付日：2007 年 5 月 11 日）

Table 1　Complications of HIV infection in central nervous system

	-July 2000	August 2000-April 2003	May 2003-November 2005
Toxoplasmic encephalitis	3	1	2
PML	2		1
Cryptoccocal meningitis	1	1	1
Primary CNS lymphoma	1	1	
HIV encephalopathy			5
Tuberculous meningitis			1
Cytomegaloviral encephalitis			1
Other		Unknown 1 Amebic encephalitis 1	Viral meningitis 1 Hydrocephalus 1
Total	7	5	13
HIV infectious cases (AIDS cases)	106 (19)	231 (56)	363 (127)
Rate of CNS complications with AIDS	36.8%	8.9%	9.4%

PML: Progressive multifocal leukoencephalopathy

Table 2　Counts of CD4+ lymphocytes and HIV viral load before and after HAART

	Case 1	2	3	4	5
CD4+ lymphocytes (before → after HAART) (/μl)	71 → 276	11 → 281	8 → 74	17 → 244	5 → 259
HIV viral load (before → after HAART) (copies/ml)	2.1×10^5 →< 50	5.3×10^5 →< 50	5.3×10^5 →< 50	1.2×10^7 → 1.2×10^3	1.3×10^6 →< 50

Table 3　Findings of cerebrospinal fluid at time of HIV encephalopathy diagnosis

	Case 1	2	3	4	5
Cell counts (/μl)	2	5	7	3	47
Protein (mg/dl)	45	46	50	25	21
Glucose (blood glucose) (mg/dl)	29 (80)	48 (100)	48 (126)	48 (96)	85 (244)
β-2 microglobulin (μg/ml)	7.1	—	—	3.5	3.7
HIV viral load (copies/ml)	5.9×10^4	—	1.7×10^4	1.8×10^3	9.3×10

—: not examined

に振戦があり，四肢に筋強剛をみとめた．腱反射は全体に減弱し，バビンスキー徴候は両側陽性，尿閉のため尿道カテーテルが留置されており便失禁状態であった．血液検査（Table 2）ではCD4 71/μl，HIVウイルス量2.1×10^5copies/ml，HBs抗原陽性であり，HCV抗体，梅毒，β-D-グルカン，サイトメガロウイルスC10/C11抗原，トキソプラズマIgM/IgG抗体，クリプトコッカス抗原はいずれも陰性であった．髄液検査（Table 3）では細胞数2/μl，蛋白45mg/dl，糖29mg/dl，HIVウイルス量5.9×10^4copies/ml，β-2ミクログロブリン7.1μg/ml．墨汁染色陰性，結核菌，非定型抗酸菌，サイトメガロウイルス，JCウイルスのPCRはすべて陰性であった．また，一般細菌，抗酸菌，真菌培養はいずれも陰性で，細胞診も陰性であった．脳波は6～7Hzの全般性徐波をみとめた．神経伝導速度では上肢は筋電図混入が強く判定不能で，下肢はF波をふくめ正常であった．入院時頭部MRI（Fig. 1）では，脳幹および大脳白質にびまん性に広がる高信号域をみとめた．

以上から，HIV脳症と診断し，発熱の原因はカテーテル留置にともなう尿路感染症と思われた．2004年1月よりHAARTを施行し，その約1カ月後より発動性と運動障害は改善したが，下肢関節は拘縮変形のため立位歩行はできなかった．6カ月後のHDS-R 17点，WAIS-Rは言語性IQ 88，動作性IQ 69，全体IQ 77．20カ月後にはHDS-R 22点と，認知機能障害は不完全ながらも徐々に改善傾向を示した．HAART開始1カ月後においてCD4 201/μl，HIVウイルス量2.3×10^3copies/mlと改善し，22カ月後ではCD4 226/μl，ウイルス量は検出感度以下とさらに改善した．しかし，人格変化がいちじるしく，周囲に対して攻撃的言動をとったり，夜間大声で叫ぶなどの精神症状が強かったために精神科介入による投薬をおこない，約1年後に施設入所となった．頭部MRIの経時変化をFLAIR画像（Fig. 1）にて検討すると，両側左右対称性の大脳前頭葉から基底核にかけて白質の萎縮が進行していた．

症例2　35歳男性．

27歳時に梅毒の既往がある．2004年6月，乾性咳嗽，労作

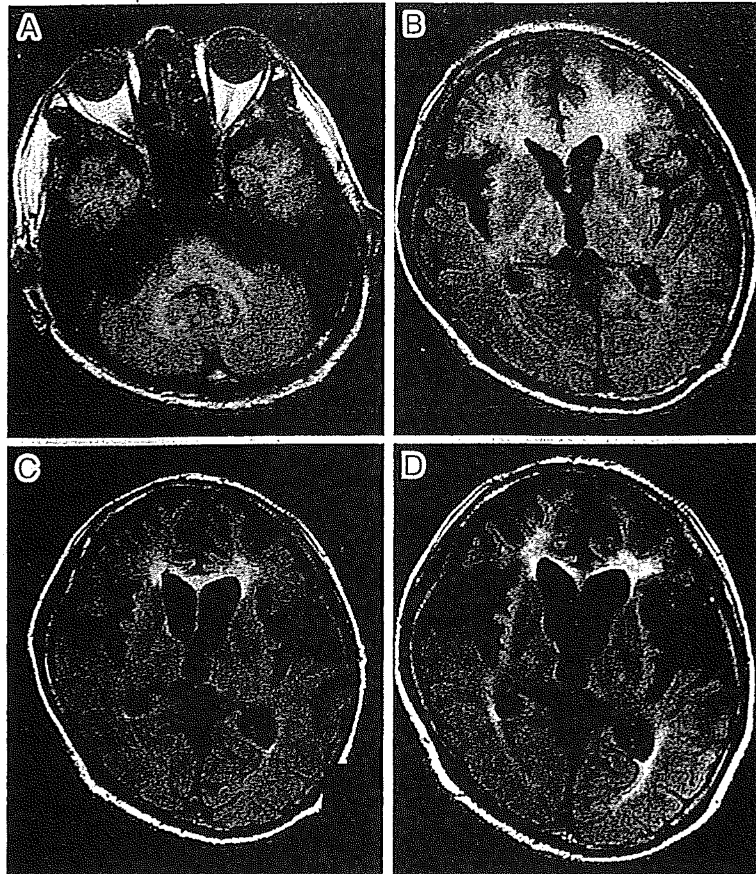

Fig. 1　Brain MRI (FLAIR) of case 1
A and B which were done at admission, revealed a diffuse lesion with high signal in the brain stem, cerebellar peduncles and cerebral white matter, atrophy of the basal ganglia and frontal lobes. C and D, which were done at 6 and 31 months respectively after HAART, showed that diffuse cerebral atrophy in the white matter had remarkably progressed.

時呼吸苦が出現．同年 8 月，発熱，呼吸苦にて前医入院した際に HIV 抗体陽性と判明し，カリニ肺炎と診断され治療を受けた．その頃からものわすれ，ふらつきを自覚．同年 9 月，当院に転院した．転院時，発動性の低下，動作緩慢，起居動作や歩行時にふらつきがあり，つぎ足歩行は不可能であり，その他の脳神経や筋力，感覚，腱反射には異常をみとめなかった．神経心理検査では，HDS-R 24 点，WAIS-R は言語性 IQ 86，動作性 IQ 65，全体 IQ 74 と認知機能障害をみとめた．また，50 音表を書くのに 80 秒かかり，立方体模写はできないなど，動作速度の低下や空間能力低下をみとめた．カリニ肺炎治療終了の 1 カ月後より HAART を開始したが，ふらつきの悪化，部屋をまちがえたり，前日のでき事を忘れたりといったことがしだいにめだつようになった．入院 1 カ月後に施行した頭部 MRI にて T_2 強調画像で両側前頭葉白質に左右対称性にびまん性に広がる高信号域をみとめ，HIV 脳症と診断した．HAART 開始 5 カ月後の神経学的診察では異常をみとめなかったが，MMSE は 28 点と認知機能障害の残存をみとめた．診断から約 1 年後，不安焦燥感を執拗に訴えて本人の強い希望で緊急入院したが，入院翌日に院内備品を持ち出したところを地下鉄職員に発見され，窃盗のうたがいで警察の事情聴取を受けた．その後，外来を不定期受診している．

症例 3　51 歳男性

職業は会社経営．2004 年 5 月に全身倦怠感と発熱が出現．同年 6 月に異常言動と失見当識が加わりしだいに傾眠となったため前医に入院し，HIV 抗体陽性と判明したため 7 月，当院に転院した．転院時 MRI では T_2 強調画像にて両側基底核に小病変をみとめた．当初，意識障害の原因としてクリプトコッカス髄膜炎，トキソプラズマ脳炎をうたがい治療を開始したが，血清および髄液中のクリプトコッカス抗原ならびに血清トキプラズマ抗体は陰性であった．血液培養から非定型抗酸菌が検出され，発熱は非定型抗酸菌敗血症によるものと診断し抗菌剤にて改善した．また，CD4 4μl と低値であったため HAART を施行した．その後，意識障害は改善し，尿失禁を残すも他の日常生活動作は自立となり約 4 カ月後に退院した．退院前後よりしだいに躁状態となり，入院中に高価な身の回りの品を換金して無断外出をしたり，退院後は妻が外出している隙に知人と旅行に行くといった行動障害が出現した．服薬アドヒアランスも不良となり，ふらつきが悪化し歩行困

難となるなどの運動機能障害が悪化したため再入院となった．再入院時，動作緩慢，失調性歩行で両側バビンスキー徴候陽性，尿失禁をみとめ，HDS-R 19点であった．血液検査はCD4 171/μl，HIVウイルス量は感度以下であった．髄液検査および頭部MRIでは日和見感染症を示唆する所見はみとめず，経過からHIV脳症を当初より合併していたと考えHAARTを継続した．深夜に家族と偽って知人を病室に招き入れるなど病棟のルールを守れずに強制退院となった．その後は精神科外来にて抗躁薬と抗精神病薬を投与し，徐々に落ち着きを取りもどしたが，HDS-Rは20点前後で推移している．

症例4　28歳男性

職業は代用教員．18歳時にバーキットリンパ腫に対し自己末梢血幹細胞移植を受け治癒している．2005年5月ころからものわすれを自覚した．同年6月，バーキットリンパ腫の経過観察のため施行した血液検査で汎血球減少を指摘され，前医に入院しHIV抗体を測定したところ，陽性と判明した．同年7月，職場で倒れているのを発見され救急車にて当院を受診した．体温37.7℃，朦朧状態で物品呼称および理解は比較的保たれているが復唱はできず，上肢の観念運動失行，右同名半盲，右注視麻痺，構音障害，右不全片麻痺，バビンスキー徴候右陽性をみとめた．入院時頭部MRIではT_2強調画像にて左右対称性びまん性の白質病変をみとめた(Fig. 2)．入院3日目より右片麻痺，失語は急速に改善し，入院1週間後の診察では失見当識をみとめるが失語や麻痺は消失していた．動作緩慢であり，50音表の書き取りに105秒かかった．MMSEは22点，立方体は模写できなかった．WAIS-Rは言語性IQ 84，動作性IQ 79，全体IQ 80と低下しており，空間能力低下，短期記憶障害などの認知機能障害をみとめた．SPECTでは両側前頭葉の血流低下に加え，左頭頂葉付近の血流増加をみとめ，脳波では左前頭部に棘波をみとめた．運動機能障害と認知機能障害をみとめ，頭部MRIでも白質病変をみとめることから，亜急性にHIV脳症を生じており，今回の入院契機であった一過性の大脳左半球症状はてんかん様発作であった可能性が考えられた．その後外来にてHAARTを施行した．発症より約6カ月後，見当識は良好だが時に単語がすっと出てこないことがある．運動障害はなく，HDS-Rは27点，MMSEは25点，50音表の書き取りは35秒で可能だがラ行が抜けていた．立方体模写は可能となった．HAART前後での頭部MRIを比較すると，わずかに病変は縮小しており，画像検査上もHIV脳症の改善をみとめた．転職し社会復帰を果たしている．

症例5　63歳男性

職業は会社員．2005年8月に微熱と全身倦怠感，体重減少を自覚した．10月初旬より上記に加えて湿性咳嗽，見当識障害，夜間せん妄が出現した．10月中旬に体重減少と呼吸苦の精査にて前医入院し，胸部CTにて間質性肺炎，胃内視鏡下生検にてサイトメガロウイルス胃炎と判明し，HIV抗体陽性であったため当院に転院した．転院時，呼吸不全をみとめ，神経学的診察では，軽度意識障害（Japan Coma Scale-2），自発性低下，動作緩慢をみとめ，指鼻試験および膝踵試験は拙劣で，起居動作や歩行時にはふらつきがあり，つぎ足歩行はできなかった．立方体模写は不可能で，1から26の数唱に26秒，同じく書き取りに36秒かかった．50音表の書き取りは途中で止まってしまい遂行できなかった．全身状態が安定した後に施行したHDS-Rは12点，WAIS-Rは言語性IQ 62，動作性IQ 52，全体IQ 55と著明な低下をみとめた．頭部MRIにて，橋と両側大脳前頭葉白質から脳梁にT_2強調画像でびまん性の高信号域をみとめた．当院転院後，ST合剤およびステロイドによるカリニ肺炎の治療を施行し呼吸状態は改善したが，神経学的には変化がなかった．11月よりHAARTを施行したところ直後に一過性の譫妄をきたしたが，開始1週間後より病室で小説を読み，徐々に他の症状も改善していった．HAART開始1カ月後の診察では意識清明，歩行は自立しているがつぎ足歩行は不可能であった．HDS-R 29点，MMSE 30点と改善をみとめた．50音表書き取りは「な」行でとまってしまった．2カ月後に自宅退院され，現在も外来にてHAARTを施行している．

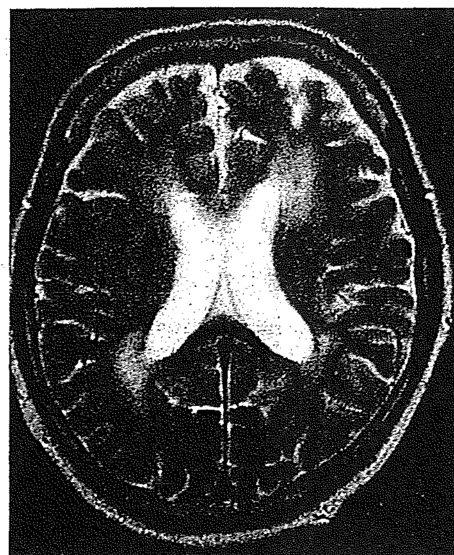

Fig. 2　Brain MRI (T2 weighted image) of case 4
It revealed that the diffuse high intensity area in cerebral white matter spared the subcortex.

考　察

HIV感染症は近年，HAARTをはじめとする治療法の進歩によって当初恐れられていた日和見感染症や悪性腫瘍は減少傾向を辿っており[2)3)]，中枢神経合併症も同様の傾向を呈している[4)～7)]．しかし，HIV脳症はHAARTによっても発症頻度が減少しないとされ[6)8)]，その理由としてはHAARTによってHIV脳症をふくめたAIDS症例全体の生命予後が改善することが指摘されている[9)]．

今回のHIV脳症5例について，診断時のCD4とHIVウイルス量をTable 2にまとめた．いずれもCD4は200/μl以下

で平均 22.4/μl ときわめて低値であり，諸外国での HAART 導入以前と同様の傾向を示している[8]．他の日和見感染症を合併しており，高度の免疫不全状態であったと思われた．

次に，HIV 脳症診断時の髄液検査所見については，Table 3 に示すように症例 5 を除いていずれも細胞数は正常（症例 5 についても 1 週間後の再検査時には正常），蛋白は正常から微増であった．測定しえた症例では，髄液中 β-2 ミクログロブリンはいずれも 2μg/ml を超えていた．髄液中の HIV ウイルス量はばらつきが多いものの症例 1，3，4 では血液中のウイルス量と比較しても高値であった．髄液中の糖は全症例とも低値を示した．HIV 脳症において髄液中の細胞数増多や蛋白の上昇がときにみとめられることは知られているが，髄液中の糖についてはあまり検討がなされておらず，Navia らが HIV 脳症 41 症例中 1 例のみ糖が低値であったと報告している[1]．われわれが経験した 5 症例において，頭蓋内の細菌感染症は髄液培養検査が陰性であったことや経過から否定的であり，髄液中の糖が低値であった理由は不明であった．

HIV 脳症の症状は運動，認知，行動の 3 つに大別される[1]．今回の 5 症例において，運動障害と認知障害は程度の差異はあるものの全症例にみとめられたが，行動異常の有無については症例差がいちじるしかった．運動機能障害については動作緩慢は全症例とも改善をみとめた．失調は完全消失にはいたらないものの改善傾向であり，結果として関節炎による関節拘縮をきたした症例 1 以外を除いては日常生活動作が自立となっており，運動機能予後は良好と思われた．

認知機能について，経過中に適宜施行した HDS-R もしくは MMSE の結果からはいずれの症例も追跡しえた範囲では改善傾向にあり，症例 1 は 31 カ月を経過した時点でもなお改善傾向にあるが，依然障害は残存している．

認知障害とならんで，行動障害は服薬アドヒアランスを大きく低下させ療養を困難とする要因となった．症例 1，2，4 では経過中に顕著な行動異常が出現し，今回の症例で唯一症例 4 のみが就労を果たした．他の症例と比較すると認知機能障害の残存はみとめていたものの，診断当初より無気力をはじめとする行動障害をともなっていなかったことがその要因と思われた．このことからは，HIV 脳症に特徴とされる運動機能障害，認知機能障害，行動障害のうち，行動障害が強いばあいには就労は困難となりうることが示唆された．

HIV 脳症の治療として，全症例ともできるだけ早期に HAART を導入した[10][11]．今回追跡しえた期間内は死亡をみとめず，他の中枢神経疾患が多くのばあいに致死性である[7][12]ことを考えると，HIV 脳症の短期間の生命予後は良好であると思われた．その一方で機能予後は不良と考えられ，HAART のみでは治療効果は不十分であると思われた．今後，HAART に加えてあらたな治療の確立が望まれる[13][14][15]．

謝辞：名古屋医療センター神経内科の諸先生方ならびにエイズ予防財団の小塚女史の協力に感謝いたします．

文 献

1) Navia BA, Jordan BD, Price RW: The AIDS dementia complex: I. clinical features. Ann Neurol 1986; 19: 517—524
2) Palella FJ, Delaney KM, Moorman AC, et al: Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. N Engl J Med 1998; 338: 853—860
3) Mocroft A, Katlama C, Johnson AM, et al: AIDS across Europe, 1994-98: the EuroSIDA Study. Lancet 2000; 356: 291—296
4) Dore GJ, Li Y, McDonald A, et al: Impact of highly active antiretroviral therapy on individual AIDS-defining illness incidence and survival in Australia. J Acquir Immune Defic Syndr 2002; 29: 388—395
5) Sacktor N, Lyles RH, Skolasky R, et al: HIV-associated neurologic disease incidence changes: Multicenter AIDS Cohort Study, 1990-1998. Neurology 2001; 56: 257—260
6) Neuenburg JK, Brodt HR, Herndier BG, et al: HIV-related neuropathology, 1985 to 1999: rising prevalence of HIV encephalopathy in the era of highly active antiretroviral therapy. J Acquir Immune Defic Syndr 2002; 31: 171—177
7) Monforte A, Duca PG, Vago L, et al: Dcreasing incidence of CNS AIDS-defining events associated with antiretroviral therapy. Neurology 2000; 54: 1856—1859
8) Dore GJ, Correll PK, Li Y, et al: Changes to AIDS dementia complex in the era of highly active antiretroviral therapy. AIDS 1999; 13: 1249—1253
9) Dore GJ, McDonald A, Li Y, et al: Marked improvement in survival following AIDS dementia complex in the era of highly active antiretroviral therapy. AIDS 2003; 17: 1539—1545
10) Husstedt IW, Frohne L, Bockenholt S, et al: Impact of highly active antiretroviral therapy on cognitive processing in HIV infection: cross-sectional and longitudinal studies of event-related potentials. AIDS Res Hum Retroviruses 2002; 18: 485—490
11) Sacktor N, Nakasujja N, Skolasky R, et al: Antiretroviral therapy improves cognitive impairment in HIV+ individuals in sub-Saharan Africa. Neurology 2006; 67: 311—314
12) Berenguer J, Miralles P, Arrizabalaga J, et al: Clinical course and prognostic factors of progressive multifocal leukoencephalopathy in patients treated with highly active antiretroviral therapy. Clin Infect Dis 2003; 36: 1047—1052
13) Tha Dana Consortium on the Therapy of HIV Dementia and Related Cognitive Disorders: A randomized, double-blind, placebo-controlled trial of deprenyl and thioctic acid in human immunodeficiency virus-associated cognitive impairment. Neurology 1998; 50: 645—651

14) Sacktor N, Schifitto G, McDermott MP, et al: Transdermal selegiline in HIV-associated cognitive impairment: pilot, placebo-controlled study. Neurology 2000; 54: 233—235
15) 岸田修二：HIV 感染症：とくに脳症の臨床像と今後の問題点．臨床神経　2004；44：852—854

Abstract

Clinical features and courses of 5 cases with HIV encephalopathy

Rina Hashimoto, M.D.[1], Eiichiro Mukai, M.D.[1], Yoshiyuki Yokomaku, M.D.[2], Naoto Mamiya, M.D.[2] and Motohiro Hamaguchi, M.D.[3]
[1]Department of Neurology, National Hospital Organization Nagoya Medical Center
[2]Department of Infectious Disease, National Hospital Organization Nagoya Medical Center
[3]Department of Clinical Reseach Center, National Hospital Organization Nagoya Medical Center

Clinical features and courses of 5 cases with HIV encephalopathy were reported. The subjects were among the patients registered as HIV patients at the Nagoya Medical Center, between 1996 and 2005. There were 458 patients with HIV infection including 127 cases of AIDS. All patients suffered from severe immunological deficiency when HIV encephalopathy developed. Other opportunistic infections had also occurred in three patients. HIV encephalopathy was one of the presenting manifestations of HIV infection in four patients, and no patients had received antiretroviral therapy. HAART improved motor disturbance and their ADL became independent except for one case. Improvements in neuropsychological examination scores were noted in all cases. Recovery from psychiatric symptoms, however, was incomplete. Four patients could not work, and 3 needed psychological treatment due to behavioral abnormalities. HIV encephalopathy is not a lethal disease but the functional prognosis was very poor. New therapy is needed for HIV encephalopathy.

(Clin Neurol, 48: 173—178, 2008)

Key words: HIV, AIDS, cognitive impairment, behavioral change, prognosis

AIDS RESEARCH AND HUMAN RETROVIRUSES
Volume 24, Number 3, 2008
© Mary Ann Liebert, Inc.
DOI: 10.1089/aid.2007.0150

Short Communication

Effects of Low HIV Type 1 Load and Antiretroviral Treatment on IgG-Capture BED-Enzyme Immunoassay

TSUNEFUSA HAYASHIDA, HIROYUKI GATANAGA, JUNKO TANUMA, and SHINICHI OKA

ABSTRACT

The IgG-capture BED-enzyme immunoassay (BED-CEIA) is used widely at present to detect recent HIV-1 seroconversion. However, antibody levels and antibody kinetics are impacted by HIV-1 load and antiretroviral treatment, which may have a significant effect on the assay results. In this study, we analyzed serial samples from 11 patients with recent infection, including four patients treated by structured treatment interruption (STI), and compared the results with those of 10 untreated and 7 treated patients with chronic infection. The BED-CEIA missidentified one long-term nonprogressor hemophiliac with an extremely low HIV-1 load and five patients with chronic infection who received antiretroviral treatment. We also found that the ODn values increased slowly in patients with recent infection and low HIV-1 loads and that the ODn values fluctuated in parallel with HIV-1 load during STI. Our data indicate that the results of BED-CEIA are influenced by HIV-1 load and antiretroviral treatment. Care should be taken when interpreting the results of BED-CEIA, especially in individuals with low HIV-1 loads. Those on antiretroviral treatment should be excluded from BED-CEIA testing to improve the predictive value of detecting recent infections.

According to the Committee on HIV/AIDS Trends (the Ministry of Health, Labor, and Welfare of the Japanese government), the number of newly diagnosed cases of HIV-1 infection in Japan is continuously increasing and the most frequent mode of transmission is homosexual contact among men who have sex with men.[1] Assessing the incidence of recent infection is important to monitor the current HIV-1 epidemic, although the diagnosis of recent infections usually requires longitudinal follow-up. A new immunoglobulin G (IgG)-capture BED-enzyme immunoassay (BED-CEIA) (Calypte Biomedical Corp., Rockville, MD) was developed recently to identify recent HIV-1 infections.[2,3] BED-CEIA measures the proportion of HIV-specific IgG in serum or plasma samples, which increases after seroconversion. In brief, plates coated with goat antihuman IgG are used to capture both HIV-specific and non-HIV-IgG in test samples. The HIV-specific IgG is detected by a branched multisubtype gp41 peptide labeled with biotin. Incubation with streptavidin-peroxidase followed by tetramethylbenzidine (TMB) substrate allows colorimetric detection of HIV-IgG. The optical density (OD) values of test specimens are normalized (ODn) relative to the value of a calibrator (specimen OD/calibrator OD) to minimize interrun variations. According to the instructions provided by the manufacturer, an ODn of 0.8 corresponds to a mean seroconversion duration of 153 days and the samples with an ODn of <0.8 are considered to be from individuals with recent infection.[4] To assess the reliability of BED-CEIA, we used multiple samples from 28 HIV-1 subtype B-infected patients after obtaining written informed consent.[1]

First, we analyzed the samples of 11 patients with recent infection, 10 untreated patients with chronic infection, and 7 treated patients with chronic infection. The diagnosis of recent infection was made based on the increasing bands of Western blotting against HIV-1 antigens and the used samples were taken at the first visit. The BED-CEIA ODn values of all the 11 samples of recent infection were <0.8 (mean 0.118, SD 0.124) and were correctly identified as recent infection (Fig. 1). Blood samples were also taken from 10 antiretroviral treatment-naive patients with chronic infection (more than 2 years after the first visit). The ODn values of nine of these samples were

AIDS Clinical Center, International Medical Center of Japan, Tokyo, Japan.

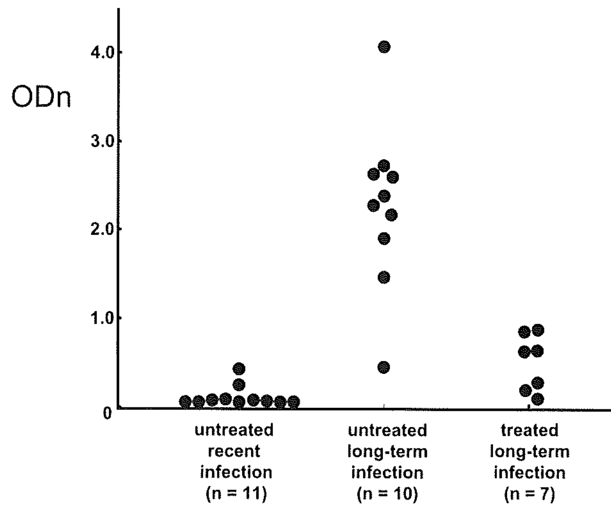

FIG. 1. ODn values of BED-CEIA in three groups of cases. Shown are the ODn values of untreated patients with recent HIV-1 infection and untreated and treated patients with chronic HIV-1 infection.

>1.4 and were correctly identified as long-term infection (mean 2.462, SD 0.718). One sample, however, was missidentified as recently infected (ODn = 0.447), which was taken from a long-term nonprogressor hemophiliac carrying an undetectable HIV-1 load (<50 copies/ml) who had not received antiretroviral treatment and who had acquired his HIV-1 infection before 1985.[5] Blood samples were also taken from seven patients with long-term infection who had received antiretroviral treatment and whose viral load had been persistently suppressed below the detection limit for more than 2 years. Their BED-CEIA ODn values (mean 0.508, SD 0.320) were lower than those of untreated patients with chronic infection ($p = 0.0003$), and five of the seven were incorrectly labeled as recently infected, indicating that antiretroviral treatment negatively alters the reliability of BED-CEIA.

Next, we analyzed serial samples from the same patients to determine the longitudinal changes in ODn. Four of the 11 patients described above with recent infection were subsequently treated with structured treatment interruption (STI), which involves repeated cycles of treatment and interruption intended to evoke a host immune response against HIV-1.[6] We compared the ODn values of these patients with those of the other seven patients who did not receive any treatment to define the natural change after recent infection (Fig. 2). In four patients with logarithmic averages of an HIV-1 load of $\geq 2.0 \times 10^4$ copies/ml, the ODn values gradually increased and all the samples taken more than 153 days after the first visit were correctly identified as long-term infection. However, in the other three patients whose logarithmic averages of HIV-1 load were $\leq 2.8 \times 10^3$ copies/ml (low viral load), the ODn values increased more slowly and many samples were misslabeled as recent infection although they were taken more than 1 year after the first visit. We also analyzed serial samples taken from the treatment-naive long-term nonprogressor hemophiliac described above, and, surprisingly, found a slow increase in the ODn value (Fig. 3). The ODn value of a sample taken in 2005, more than 20 years

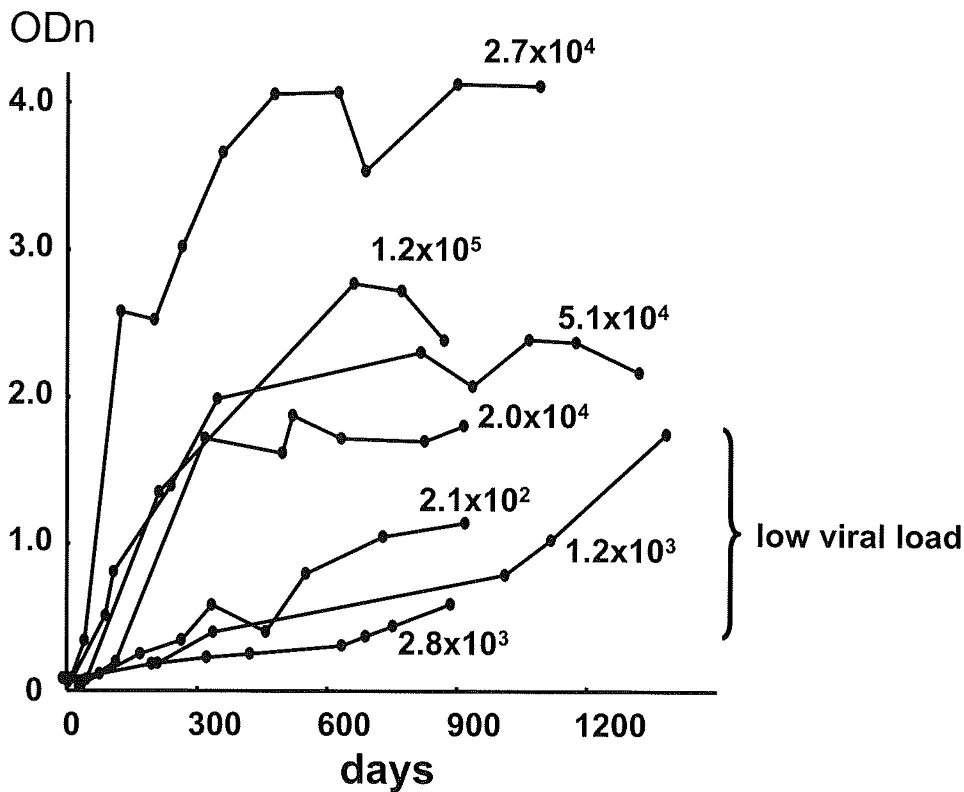

FIG. 2. Serial changes in ODn values of untreated patients with HIV-1 infection. Lines indicate serial changes in ODn values of seven patients with recent infection including three cases with low viral loads.

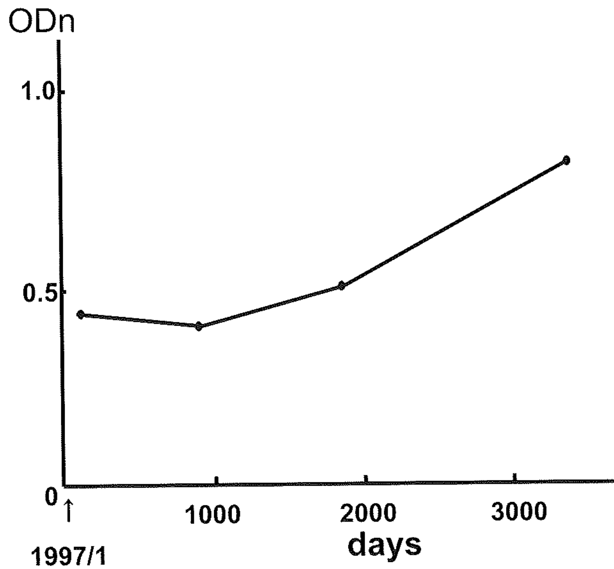

FIG. 3. Serial change in ODn values of one long-term nonprogressor hemophiliac. The line indicates serial changes in ODn values of a long-term nonprogressor with persistently undetectable HIV-1 load who acquired infection before 1985 and had not received antiretroviral treatment. Day 0 is the date of his first visit in January 1997.

after acquiring HIV-1 infection, was 0.865. These data indicate that the ODn values of patients with a low HIV-1 load increase slowly and false-positive recent infection can occur in such cases.

Finally, we analyzed serial samples of four patients with recent infection who received antiretroviral STI therapy to determine the effect of such treatment on ODn values. Figure 4 shows the changes in HIV-1 load and BED-CEIA ODn values of one patient, in whom conventional continuous antiretroviral treatment was administered 462 days after the completion of STI. After the introduction of the first course of antiretroviral treatment, the HIV-1 load sharply decreased and ODn values were persistently low (<0.20). Following a drop in HIV-1 load to below the detection limit (<50 copies/ml), treatment was interrupted for 26 days, during which the HIV-1 load rebounded accompanied by an increase in ODn to 0.760. Then the second course of treatment was introduced, which resulted in a fall of HIV-1 load (400 copies/ml) and ODn value (0.482). The fluctuation in ODn value paralleled the HIV-1 load during STI. Similar data were obtained from the other three cases treated with STI. Considered together, these findings indicate that introduction of antiretroviral treatment resulted in a rapid fall in BED-CEIA ODn values, probably due to antiretroviral treatment-induced suppression of HIV-1 load, suggesting that ODn value are sensitive to changes in HIV-1 load.

Recently, BED-CEIA has been used in a number of cross-sectional populations to estimate incidence and showed excellent results.[3,7] There was plausible agreement between the observed and BED-CEIA-estimated incidence with specimens obtained from a longitudinal cohort study[8] and there was no missclassification of 70 pregnant women with known long-term infection.[3] However, missidentification of long-term infection as recent infection can happen in some cases.[4] Our study indicates that such missidentification is associated with low HIV-1 load and use of antiretroviral treatment, which is consistent with a previous report.[4]

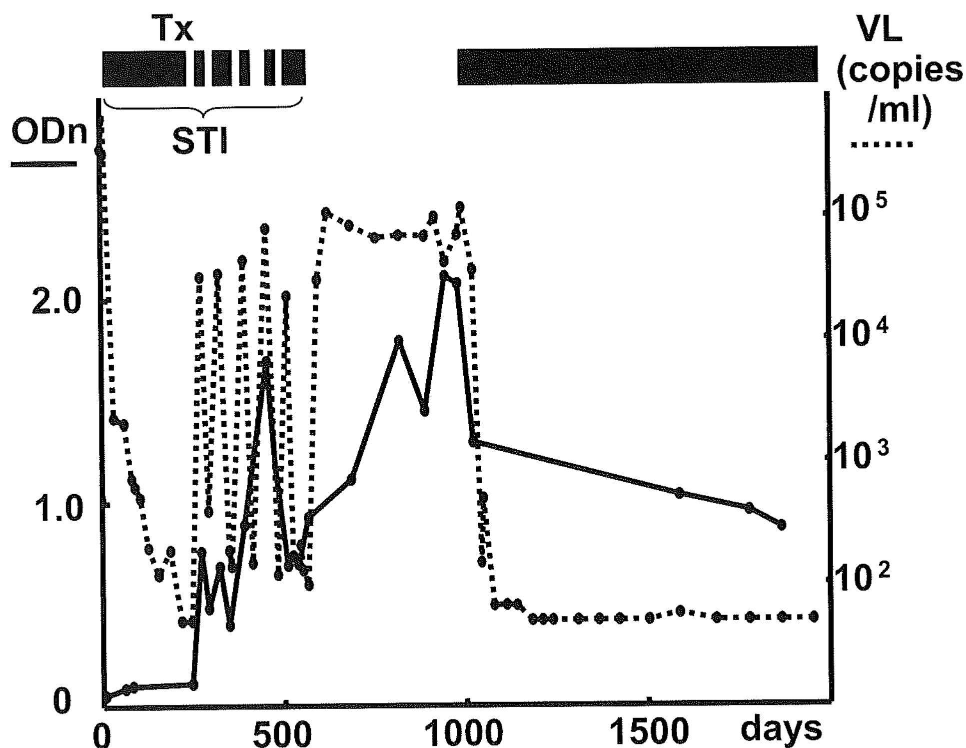

FIG. 4. Effect of antiretroviral treatment on ODn values. Bold and dotted lines show changes in ODn values and HIV-1 load, respectively, in a patient who received STI followed by conventional antiretroviral treatment.

Therefore, whenever possible, those on antiretroviral treatment should be excluded from BED-CEIA testing to improve the predictive value of detecting recent infections.

REFERENCES

1. Gatanaga H, Ibe S, Matsuda M, Yoshida S, Asagi T, Kondo M, Sadamasu K, Tsukada H, Masakane A, Mori H, Takata N, Minami R, Tateyama M, Koike T, Itoh T, Imai M, Nagashima M, Gejyo F, Ueda M, Hamaguchi M, Kojima Y, Shirasaka T, Kimura A, Yamamoto M, Fujita J, Oka S, and Sugiura W: Drug-resistant HIV-1 prevalence in patients newly diagnosed with HIV/AIDS in Japan. Antiviral Res 2007;75:75–82.
2. Parekh BS, Kennedy MS, Dobbs T, Pau CP, Byers R, Green T, Hu DJ, Vanichseni S, Young NL, Choopanya K, Mastro TD, and McDougal JS: Quantitative detection of increasing HIV type 1 antibodies after seroconversion: A simple assay for detecting recent HIV infection and estimating incidence. AIDS Res Hum Retroviruses 2002;18:295–307.
3. Nesheim S, Parekh B, Sullivan K, Bulterys M, Dobbs T, Lindsay M, Cashat-cruz C, Byers B, and Lee F: Temporal trends in HIV type 1 incidence among inner-city childbearing women in Atlanta: Use of the IgG-capture BED enzyme immunoassay. AIDS Res Hum Retroviruses 2005;21:537–544.
4. Karita E, Price M, Hunter E, Chomba E, Allen S, Fei L, Kamali A, Sanders EJ, Anzala O, Katende M, Ketter N, and the IAVI Collaborative Seroprevalence and Incidence Study Team: Investigating the utility of the HIV-1 BED capture enzyme immunoassay using cross-sectional and longitudinal seroconverter specimens from Africa. AIDS 2007;21:403–408.
5. Munkanta M, Terunuma H, Takahashi M, Hanabusa H, Miura T, Ikeda S, Sakai M, Fujii T, Takahashi Y, Oka S, Matsuda J, Ishikawa M, Taki M, Takashima Y, Mimaya J, Kimura A, and Yasunami M: HLA-B polymorphism in Japanese HIV-1-infected long-term surviving hemophiliacs. Viral Immunol 2005;18:500–505.
6. Lori F and Lisziewicz J: Structured treatment interruptions for the management of HIV infection. JAMA 2001;286:2981–2987.
7. Rehle T, Shisana O, Pillay V, Zuma K, Puren A, and Parker W: National HIV incidence measures—new insights into the South African epidemic. S Afr Med J 2007;97:194–199.
8. McDougal JS, Parekh BS, Peterson ML, Branson BM, Dobbs T, Ackers M, and Gurwith M: Comparison of HIV type 1 incidence observed during longitudinal follow-up with incidence estimated by cross-sectional analysis using the BED capture enzyme immunoassay. AIDS Res Hum Retroviruses 2006;22:945–952.

Address reprint request to:
Hiroyuki Gatanaga
AIDS Clinical Center
International Medical Center of Japan
1-21-1 Toyama
Shinjuku-ku
Tokyo 162-8655, Japan

E-mail: higatana@imcj.acc.go.jp

For reprint orders, please contact:
reprints@futuremedicine.com

Pharmacogenetic information derived from analysis of *HLA* alleles

Hiroyuki Gatanaga†, Haruhito Honda & Shinichi Oka

†Author for correspondence
AIDS Clinical Center, International Medical Center of Japan, 1-21-1 Toyama, Shinjuku-ku, Tokyo 162-8655, Japan
Tel.: +81 332 027 181;
Fax: +81 352 736 483;
E-mail: higatana@imcj.acc.go.jp

A large amount of pharmacogenetic information has, in particular, accumulated on the association between human leukocyte antigen (*HLA*) alleles and hypersensitivity to certain drugs. Prospective *HLA* typing has dramatically reduced the risk of abacavir hypersensitivity because of its strong association with *HLA-B*5701*. Significant predisposition to nevirapine hypersensitivity has been reported in Caucasian Australians harboring *HLA-DRB1*0101* with high CD4+ T-cell counts, and Sardinians and Japanese harboring *HLA-Cw8*. A strong association between carbamazepine hypersensitivity and *HLA-B*1502* has been reported in Han Chinese. Most Han Chinese individuals with allopurinol-induced severe cutaneous adverse reactions are positive for *HLA-B*5801*. *HLA* typing can stratify risk of hypersensitivity to certain drugs and allow personalized treatment, although the patients should be monitored closely even if they are negative for *HLA* alleles associated with hypersensitivity.

Hypersensitivity reactions can occur with most drugs, although their frequency, severity and clinical manifestations vary. They commonly involve the skin and mucosal surfaces, and in severe cases can result in Stevens–Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN). Other severe hypersensitivity reactions can affect other organs such as the liver (hepatitis), lung (pneumonitis) and digestive system (gastrointestinal bleeding), and show more generalized symptoms [1]. Rechallenge with the same drugs usually induces more severe reactions, even fatal reactions in some cases, suggesting that hypersensitivity reactions are immunological memory responses after sensitization. These reactions affect only a minority of patients taking the drug. However, hereditary forms of severe drug hypersensitivity and cases occurring in identical twins have been reported, implying the involvement of certain genetic factors in predisposing individuals to such hypersensitivity reactions [2,3]. Given the immunological basis of their mechanisms, it is not surprising that the associations between human leukocyte antigen (*HLA*) alleles and hypersensitivity to some drugs have been reported during the past decade. HLA is a key molecule in T-cell-mediated immune reactions. It presents antigens (usually eight or nine peptide residues) to T-cell receptors (TCRs), thereby selecting antigen-specific T cells and initiating immune responses. Such reactions usually occur in viral and bacterial infections, and microbe-derived peptides restricted by host HLA are targeted by antigen-specific immune responses [4]. Since drugs and their metabolites are small chemical compounds, they do not usually trigger immune reactions by themselves. However, they may conjugate or bind to intracellular proteins, where they are presented as antigens or haptens by MHC class I or class II molecules to $CD8^+$ or $CD4^+$ T cells, resulting in activation of drug-specific T cells [5,6].

We will review in this article the recent literature on the association between *HLA* allele and hypersensitivity reactions to abacavir, nevirapine, carbamazepine and allopurinol. We will also discuss the clinical implications of such associations, with a special focus on the association of *HLA-B*5701* with hypersensitivity to abacavir, an anti-HIV-1 agent, because it is the most well analyzed and reported. Widespread genetic screening of such association in HIV-1-infected individuals can be used to prevent hypersensitivity reactions.

Abacavir hypersensitivity & *HLA-B*5701*

The currently recommended anti-HIV-1 treatment is the use of a combination regimen. The initial regimen for treatment-naive infected individuals should contain two nucleoside/nucleotide reverse transcriptase inhibitors (NRTI) and either a non-nucleoside reverse transcriptase (NNRTI) or an HIV protease inhibitor [7,101]. The action of the NRTI drug class is to inhibit viral replication through competitive inhibition of viral RNA-dependent DNA polymerase (reverse transcriptase) that allows the creation of a nascent DNA sequence from its own RNA template, whereas NNRTI drugs function by direct binding and inactivation of the polymerase. HIV protease

Keywords: abacavir, allopurinol, carbamazepine, HIV, hypersensitivity, nevirapine

future medicine part of fsg

10.2217/14622416.9.2.207 © 2008 Future Medicine Ltd ISSN 1462-2416

inhibitors prevent the cleavage of the Gag protein and Gag–Pol protein precursors, thus inhibiting viral replication at a later stage in the replication cycle [8]. NRTIs have been prescribed since the late 1980s, and their advantages and disadvantages are well recognized. A major adverse effect of NRTI is mitochondrial toxicity, which can result in life-threatening lactic acidosis [9–11]. Two recently developed NRTIs, tenofovir disoproxil fumarate (TDF) and abacavir, have low mitochondrial toxicity and both can be prescribed with once-daily dosing [12,13]. However, only TDF is listed as a preferred NRTI in the guideline of the Department of Health and Human Services. On the other hand, abacavir is listed as an alternative NRTI because of its potential for serious hypersensitivity reactions in 5–8% of Caucasians [14,101].

The safety data for abacavir are well described and based on approximately 200,000 patients who received abacavir in clinical trials. The most important limitation to continuous use of this drug is hypersensitivity reactions [15,16]. Such reactions are multi-organ clinical syndromes, which generally occur within the first 6 weeks of abacavir treatment, and typically present with fever, skin rash, malaise/fatigue, gastrointestinal symptoms (e.g., nausea, vomiting and diarrhea) and/or respiratory symptoms (e.g., dyspnea, cough and pharyngitis) [15]. It is important to make a correct diagnosis of abacavir-related hypersensitivity reactions, since a rechallenge with abacavir after an initial reaction can evoke a more rapid reappearance of more severe symptoms within hours of re-exposure, which could result in death in some cases [17–19]. Unfortunately, abacavir hypersensitivity reactions are sometimes difficult to distinguish from systemic viral illness or similar drug reactions caused by other concurrently administered antiretroviral drugs or antibiotics [20].

Meta-analysis of clinical trials indicating a low risk of abacavir hypersensitivity reactions in black people, as well as a case report of familial hypersensitivity, are strong indicators of a genetic basis of this idiosyncratic syndrome [21,22]. Two independent studies identified a strong association between abacavir hypersensitivity and *HLA-B*5701*, which can assist clinicians in predicting those individuals who could develop hypersensitivity reactions and to make a correct diagnosis of hypersensitivity reactions in abacavir-treated individuals, although the association was observed only in Caucasians but not in the black people originally (Table 1) [23,24]. In addition to *HLA-B*5701*, the possession of *HLA-DR7* and *HLA-DQ3*, which are markers of the 57.1 ancestral haplotype, is associated with an increase in the odds ratio of hypersensitivity risk, suggesting that another causative genetic region is linked to *HLA-B*5701* [23]. Fine recombinant genetic mapping has identified a significant linkage disequilibrium of the haplotypic M493T polymorphism of heat shock protein-Hom (Hsp70-Hom; Hsp1AL) and *HLA-B*5701* in abacavir hypersensitive cases, which simplified and enhanced the discrimination of hypersensitive subjects from tolerant controls when compared with the *HLA-B*5701* test alone (Table 1) [25]. The Hsp70-Hom M493T polymorphism may facilitate loading of abacavir- or its metabolite-haptenated endogenous peptides onto *HLA-B*5701* [26]. High intracellular and extracellular levels of TNF are

Table 1. Drug hypersensitivity and associated *HLA* alleles.

Study	Drug	HLA	Population	OR	Pc	Ref.
Mallal *et al.* (2002)	Abacavir	*B*5701*	Australian	117	$<10^{-4}$	[23]
Hetherington *et al.* (2002)	Abacavir	*B*5701*	British	24	$<10^{-4}$	[24]
Martin *et al.* (2004)	Abacavir	*B*5701*	Australian	960	$<10^{-4}$	[25]
Martin *et al.* (2005)	Nevirapine	*DRB1*0101* and high CD4	Caucasian Australian	18	0.0006	[58]
Littera *et al.* (2006)	Nevirapine	*Cw8-B14(65)*‡	Sardinian	15	0.05	[59]
Gatanaga *et al.* (2007)	Nevirapine	*Cw8*	Japanese	6.2	0.03	[60]
Chung *et al.* (2004)	Carbamazepine	*B*1502*	Han Chinese	2504	$<10^{-4}$	[68]
Hung *et al.* (2006)	Carbamazepine	*B*1502*	Han Chinese	1357	$<10^{-4}$	[69]
Hung *et al.* (2005)	Allopurinol	*B*5801*	Han Chinese	580	$<10^{-4}$	[75]

‡Cw*0802 *and* B*1402 *are in strong linkage equilibrium in Sardinians.*

present in abacavir-stimulated peripheral blood mononuclear cells (PBMCs) of abacavir-hypersensitive patients, relative to those of abacavir-tolerant individuals, and depletion of $CD8^+$ T cells results in reduction of TNF levels [25]. Considering that marked infiltration of $CD8^+$ T cells is observed in cutaneous abacavir patch testing of hypersensitive patients and that higher $CD8^+$ T-cell count is a risk factor of hypersensitivity reactions, *HLA-B*5701*-restricted $CD8^+$ T cells must play a major pathogenic role in abacavir hypersensitivity reactions [27–29].

Prospective *HLA-B*5701* genetic screening has been instituted in clinical practice in Western Australia, the UK and Paris for abacavir-naive patients, and this had markedly reduced the risk of developing abacavir hypersensitivity (Table 2) [30–32]. This strategy unexpectedly reduced the proportion of patients who stopped their treatment after the appearance of symptoms that were otherwise unrelated to hypersensitivity reactions, suggesting that genetic screening seems to prevent overestimation of hypersensitivity reactions with subsequent discontinuation of abacavir in *HLA-B*5701*-negative individuals [30,32]. The PREDICT-1 study randomized patients either to receive abacavir according to standard of care or to be prospectively screened for *HLA-B*5701* before starting abacavir (to exclude *HLA-B*5701* carriers) [33]. The incidence of hypersensitivity reactions was significantly lower in the prospective screening arm compared with the control arm. However, most of the screened patients described above were Caucasian, and the utility and cost–effectiveness of the genetic screening largely depends on the prevalence of *HLA-B*5701* in the targeted population [34]. The prevalence of *HLA-B*5701* among Hispanics and black people is lower than Caucasians, and the relationship between *HLA-B*5701* and abacavir hypersensitivity was described as weak in Hispanics and nonexistent in black patients [35,36]. The SHAPE study corroborated the low rate of abacavir hypersensitivity immunologically confirmed by skin patch testing in black patients, but it also reported high sensitivity of *HLA-B*5701* in immunologically validated cases in both whites and blacks, suggesting the importance of supplementing a clinical definition of abacavir hypersensitivity by immunological assessment [37]. In our study, none of the 669 Japanese HIV-1-infected patients had *HLA-B*5701*, yet hypersensitivity reactions occurred in seven (all *HLA-B*5701*-negative, not immunologically confirmed) of 536 Japanese patients exposed to abacavir [38]. Thus, genetic screening of *HLA-B*5701* does not seem cost-effective in Japanese populations. Close monitoring of patients after abacavir prescription without HLA typing may be a more reasonable approach in the populations that do not carry *HLA-B*5701*.

Interestingly, strong reponses of *HLA-B*57*-restricted cytotoxic T lymphocytes can ocur against multiple HIV-1 epitopes, which is considered to result in slow disease progression of *HLA-B*57*-positive HIV-1-infected individuals [39,40]. One of the major *HLA-B*57*-restricted epitopes is located in codons 244–252 of HIV-1 reverse transcriptase, which is routinely sequenced as a part of drug-resistance testing [7,41,101]. Furthermore, cytotoxic T lymphocytes escape mutations (wild-type V to E, M and L) are commonly observed at codon 245 in *HLA-B*57*-positive patients, which may serve as an indirect marker for the presence of *HLA-B*5701* [40,42]. In one study [43], the negative predictive value was over 99% (meaning that the presence of wild-type amino acid V at codon 245

Table 2. Reduced frequencies of abacavir hypersensitivity reactions after *HLA-B*5701* genetic screening.

Study	Country	n (%)[‡]		p-value	Ref.
		Before screening	*After screening*		
Rauch *et al.* (2006)	Australia	16/199 (8.0)	3[§]/151 (2.0)	0.01	[30]
Reeves *et al.* (2006)	UK	20/321 (6.2)	1[¶]/155 (0.6)	0.002	[31]
Zucman *et al.* (2007)	France	11[#]/49 (22.4)	0/128 (0)	$<10^{-4}$	[32]

[‡] *Number (%) of hypersensitive patients/abacavir-treated patients.*

[§] *All three individuals were* HLA-B*5701 *positive; two inadvertently exposed to abacavir because of a lack of review of HLA results, and one on the basis of his own content.*

[¶] HLA-B*5701 *negative; non-HIV-expert physician discontinued therapy because of possible hypersensitivity reactions.*

[#] *Included five* HLA-B*5701 *negative cases of possible hypersensitivity based on wide-range clinical criteria.*

excludes the possibility of *HLA-B*5701* in >99% of cases), while the positive predictive value was low (20%). These results suggest that abacavir can be safely prescribed to most HIV-1-infected patients harboring wild-type V at codon 245 in reverse transcriptase [43]. This method can save the cost of HLA typing by utilizing the HIV-1 sequence data, which are obtained from routine resistance testing approved by the public and private health insurance industries of many developed countries. However, it may result in inadequate withholding of abacavir in a significant number of *HLA-B*5701*-negative patients infected with escape HIV-1 variants, because these escape mutations are often observed and probably able to persist over long periods even in the absence of *HLA-B*5701*-restricted cytotoxic T lymphocyte pressure. Another problem is differences among HIV-1 subtypes. The wild-type amino acid at codon 245 in reverse transcriptase is V only in HIV-1 subtype B, which is most prevalent in developed countries, but is another amino acid such as Q or E in non-B subtypes. Therefore, this method is not suitable when the obtained HIV-1 sequence in phylogenetic analysis belongs to non-B subtypes, which decreases its utility in African and Asian countries where non-B subtypes are prevalent. Considering that practical and accurate HLA typing has already been implemented and is effectively identifying *HLA-B*5701* carriers [44], direct HLA typing is a more simple and better approach to stratify the risk of abacavir hypersensitivity than speculating HLA type from HIV-1 sequences.

Nevirapine hypersensitivity & associated *HLA* alleles

Nevirapine is also a well-tolerated anti-HIV-1 agent, which is listed as an alternative NNRTI in the HIV-1 treatment guideline of the Department of Health and Human Services [45,101]. The most common adverse event associated with the use of nevirapine is hypersensitive reactions (observed in 4.9% of recipients), which are characterized by a combination of rash, fever or hepatitis, and typically occurs within the first 6 weeks of initiation of treatment and can be more rapid and severe with re-challenge [46,47]. Woman with high CD4+ T-cell counts appear to be at higher risk of hypersensitivity reactions [48,49]. The HIV-1 treatment guidelines do not recommend the use of nevirapine for female patients with CD4+ T cell counts over 250 cells/mm^3 and male patients with CD4+ T-cell counts over 400 cells/mm^3 [7,50–53,101]. A higher incidence of hypersensitivity reactions was reported in non-HIV-infected individuals who received nevirapine as part of post-exposure prophylactic treatment, probably associated with a high CD4 count [54]. Usually, cutaneous diseases, including drug hypersensitivity to sulfamethoxazole, dapsone and antituberculous agents, are extremely common in patients with HIV infection, and their incidence increases as immune function deteriorates [55]. However, conversely, in the case of nevirapine hypersensitivity, normal and relatively maintained immune function is a risk factor for unknown reasons [56].

The description of nevirapine-induced SJS in a Ugandan mother and her son suggests a genetic basis for nevirapine hypersensitivity [57]. The possession of *HLA-DRB1*0101* is associated with increased risk of nevirapine hypersensitivity involving multisystemic or hepatotoxic reactions, and which was abrogated by low CD4+ T-cell counts, in the Western Australian HIV Cohort (Table 1) [58]. Littera *et al.* reported that the *HLA-Cw*0802-B*1402* haplotype is associated with nevirapine hypersensitivity in Sardinian patients [59]. We also reported a significant association between *HLA-Cw8* and nevirapine hypersensitivity in Japanese patients, suggesting that nevirapine or its metabolite coupled with *HLA-Cw8* antigen may be expressed on the cell surface and may induce hypersensitivity reactions (Table 1) [60]. In this regard, there was no significant association between *HLA-DRB1*0101* and hypersensitivity in the Sardinian and Japanese cohorts described above, implying that primarily determining HLA alleles may be different among populations. Isolated mild rash and simple hepatotoxicity often occur within 6 weeks of nevirapine treatment initiation. It is possible that this reaction is pathologically different from the severe hypersensitivity reactions, making the definition of hypersensitivity confusing and comparison of different studies difficult [58,61,62]. Establishment of a standardized definition and accurate diagnosis of hypersensitivity seems indispensable for further study of the linkage between HLA alleles and nevirapine hypersensitivity.

Carbamazepine-induced SJS/TEN & *HLA-B*1502*

Carbamazepine is one of the most widely used anticonvulsants, and is also used in bipolar depression and trigeminal neuralgia. Carbamazepine is generally well tolerated but can cause dose-dependent adverse reactions such as dizziness and nystagmus [63]. It is also associated with idiosyncratic hypersensitivity reactions, most

commonly skin rashes such as SJS and TEN, accompanied with fever, lymphadenopathy, and multiorgan-sysem abnormalities [64]. A high frequency of carbamazepine-related hypersensitivity reactions was reported in South-East Asian countries compared with 0.01–0.1% in Caucasians [64–67]. Furthermore, carbamazepine hypersensitivity was reported in identical twins [3]. These studies suggest that susceptibility to such reactions may be genetically determined.

A Taiwanese study reported a strong association between carbamazepine-induced SJS/TEN and the *HLA-B*1502* allele in Han Chinese [68]. The finding was confirmed later by the same group in another study that included patients who were Han Chinese or Chinese descendants from Taiwan, Hong Kong, China and the USA (Table 1) [69]. The allele frequency of *HLA-B*1502* is 3–12% in South-East Asians and less than 0.1% in Caucasians, which may explain the higher incidence of carbamazepine-induced SJS/TEN in South-East Asia. In one European study, 15 patients with carbamazepine-induced SJS/TEN were analyzed and five patients who had a parent of Asian origin were positive for the *HLA-B*1502* allele. The remaining ten patients, who were Caucasians, were *HLA-B*1502*-negative [70]. Another European study of Caucasians did not find any *HLA-B*1502*-positive patients who were hypersensitive to carbamazepine [71]. Considered together, *HLA-B*1502* does not seem to be associated with carbamazepine hypersensitivity in the Caucasian population and ethnicity seems important. While it seems conceivable that the causative genetic region of carbamazepine hypersensitivity is linked to *HLA-B*1502*, especially in the Han Chinese population, fine recombinant genetic mapping confirmed the susceptibility gene is *HLA-B*1502* itself [69].

Allopurinol-induced severe cutaneous adverse reactions & *HLA-B*5801*

Allopurinol is widely used for hyperuricemia and recurrent urate kidney stones [72]. However, it is also one of the most frequent causes of severe cutaneous adverse reactions including SJS and TEN [73]. Familial predisposition has been reported and susceptibility to such idiosyncratic reactions is thought to be genetically determined [74]. One Taiwanese study reported a strong association between allopurinol hypersensitivity and *HLA-B*5801* in a Han Chinese population and recombinant genetic mapping further identified *HLA-B*5801* itself as the major susceptibility gene (Table 1) [75]. In support of these results, a Japanese group reported three cases with different manifestations of allopurinol hypersensitivity and all of them were positive for *HLA-B*58* [76].

Conclusion

We reviewed here the HLA association with hypersensitivity to abacavir, nevirapine, carbamazepine and allopurinol. Considering that hypersensitivity reactions to abacavir can be life-threatening and even fatal, abacavir prescription to *HLA-B*5701* should be avoided. The following prescriptions should be followed by close monitoring of the patients: nevirapine to patients positive for *HLA-DRB1*0101* or *Cw8*, carbamazepine to *HLA-B*1502* holders and allopurinol to *HLA-B*5801*-positive patients, even if the patient is from a population with no described allele association, because one cannot exclude possible association. It is noteworthy that pharmacogenetic studies are more likely to yield negative results when conducted in populations with low frequencies of the possibly associated allele [77]. More importantly, patients treated with any of these drugs should be monitored closely even if they are negative for *HLA* alleles that are known to be associated with hypersensitivity. Hypersensitivity reactions can potentially occur in any patient as they may hold *HLA* alleles that have yet unreported associations with hypersensitivity. Application of genetic screening should not substitute appropriate clinical vigilance and patient management.

Before abacavir-containing treatment is introduced for HIV-infected patients, HLA analysis should be performed to exclude *HLA-B*5701*, unless the patient is from a population which does not carry *HLA-B*5701*. Such exclusion of *HLA-B*5701* would markedly reduce the possibility of hypersensitivity reactions and prevent overestimation of hypersensitive reaction that could otherwise result in excessive discontinuation of treatment [29–31].

HLA associations with nevirapine hypersensitivity have been reported, but the odds ratios are not high [58–60]. According to the HIV-1 treatment guidelines, avoiding nevirapine prescription is reasonable for female patients with $CD4^+$ T-cell counts over 250 cells/mm^3 and male patients with $CD4^+$ T-cell counts over 400 cells/mm^3, without HLA typing [7,53,101].

Strong associations between carbamazepine hypersensitivity and *HLA-B*1502*, and between allopurinol hypersensitivity and *HLA-B*5801* have been reported in Han Chinese population [68,69,75].

Analysis of these associations in different ethnic populations is urgently needed before it is widely applied in clinical practice.

Future perspective

Current pharmacogenetic information is limited in relation to the genes of HLA, metabolizing enzymes and drug transfer proteins. Considering that the technology to identify genetic variants across the whole genome is advancing rapidly, many more significant genetic factors for drug efficacy and adverse reactions are likely to be identified in the future. Identification of such factors is important not only to discover new pharmacological mechanisms, but also to improve the currently available drugs and to develop novel drugs. In such whole-genome analysis, drug-induced phenotypes should be carefully observed in genetically variable populations, which will be feasible only through international collaboration.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending or royalties.

No writing assistance was utilized in the production of this manuscript.

Executive summary

- Human leukocyte antigen (HLA) information can help predict risk of some drug hypersensitivity.

***Abacavir hypersensitivity &* HLA-B*5701**

- Abacavir hypersensitivity is strongly associated with *HLA-B*5701*.
- Prospective HLA screening can markedly reduce the risk of abacavir hypersensitivity.

Nevirapine hypersensitivity & associated* HLA *alleles

- Significant predisposition to nevirapine hypersensitivity has been reported in Caucasian Australians harboring *HLA-DRB1*0101* with high CD4+ T-cell counts, and Sardinians and Japanese harboring *HLA-Cw8*.

***Carbamazepine-induced SJS/TEN &* HLA-B*1502**

- Carbamazepine hypersensitivity is frequent in *HLA-B*1502*-positive Han Chinese.

***Allopurinol-induced severe cutaneous adverse reactions &* HLA-B*5801**

- Most Han Chinese individuals with allopurinol-induced severe cutaneous adverse reactions are positive for *HLA-B*5801*.

Conclusion

- Prospective HLA screening can stratify the risk of hypersensitivity to abacavir, nevirapine, carbamazepine and allopurinol, and allows personalized medicine.
- Application of genetic screening should not substitute appropriate clinical vigilance and patient management.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Roujeau JC: Clinical heterogeneity of drug hypersensitivity. *Toxicology* 209, 123–129 (2005).
2. Gennis MA, Vemuri R, Burns EA, Hill JV, Miller MA, Spielberg SP: Familial occurrence of hypersensitivity to phenytoin. *Am. J. Med.* 91, 631–634 (1991).
3. Edwards SG, Hubbard V, Aylett S, Wren D: Concordance of primary generalized epilepsy and carbamazepine hypersensitivity in monozygotic twins. *Postgrad. Med. J.* 75, 680–681 (1999).
4. Rudolph MG, Stanfield RL, Wilson IA: How TCRs bind MHCs, peptides, and coreceptors. *Annu. Rev. Immunol.* 24, 419–466 (2006).
5. Pichler WL: Delayed drug hypersensitivity reactions. *Ann. Intern. Med.* 139, 683–693 (2003).
6. Roujeau JC: Immune mechanisms in drug allergy. *Allergol. Int.* 55, 27–33 (2006).
   •• **Review of the recently reported drug-specific T-cell clone and discussion of its role in drug-hypersensitivity reactions.**
7. Hammer SM, Saag MS, Schechter M *et al.*: Treatment for adult HIV infection: 2006 recommendations of the International AIDS Society – USA Panel. *JAMA* 296, 827–843 (2006).
8. Debouck C: The HIV-1 protease as a therapeutic target for AIDS. *AIDS Res. Hum. Retroviruses* 8, 153–164 (1992).
9. Boubaker K, Flepp M, Sudre P *et al.*: Hyperlactatemia and antiretroviral therapy: the Swiss HIV Cohort Study. *Clin. Infect. Dis.* 33, 1931–1937 (2001).
10. Montaner JS, Cote HC, Harris M *et al.*: Mitochondrial toxicity in the era of HAART: evaluating venous lactate and peripheral blood mitochondrial DNA in HIV-infected patients taking antiretroviral therapy. *J. Acquir. Immune Defic. Syndr.* 34, S85–S90 (2003).
11. Cossarizza A, Moyle G: Antiretroviral nucleoside and nucleotide analogues and mitochondria. *AIDS* 18, 137–151 (2004).
12. Birkus G, Hitchcock MJ, Cihlar T: Assessment of mitochondrial toxicities in human cells treated with tenofovir: comparison with other nucleoside reverse transcriptase inhibitor. *Antimicrob. Agents Chemother.* 46, 716–723 (2002).
13. McComsey GA, Paulsen DM, Lonergan JT *et al.*: Improvement in lipoatrophy, mitochondrial DNA levels and fat apoptosis after replacing stavudine with abacavir or zidovudine. *AIDS* 19, 15–23 (2005).

14. DeJesus E, Herrera G, Teofilo E *et al.*: Abacavir versus zidovudine combined with lamivudine and efavirenz, for the treatment of antiretroviral-naive HIV-infected adults. *Clin. Infect. Dis.* 39, 1038–1046 (2004).
15. Hetherington S, McGuirk S, Powell G *et al.*: Hypersensitivity reactions during therapy with the nucleoside reverse transcriptase inhibitor abacavir. *Clin. Ther.* 23, 1603–1614 (2001).
16. Peyriere H, Guillemin V, Lotthe A *et al.*: Reasons for early abacavir discontinuation in HIV-infected patients. *Ann. Pharmacother.* 37, 1392–1397 (2003).
17. Walensky RP, Goldberg JH, Daily JP: Anaphylaxis after rechallenge with abacavir. *AIDS* 13, 999–1000 (1999).
18. Escaut L, Liotier JY, Albengres E, Cheminot N, Vittecoq D: Abacavir rechallenge has to be avoided in case of hypersensitivity reaction. *AIDS* 13, 1419–1420 (1999).
19. Shapiro M, Ward KM, Stern JJ: A near-fatal hypersensitivity reaction to abacavir: case report and literature review. *AIDS Read.* 11, 222–226 (2001).
20. Keiser P, Nassar N, Skiest D *et al.*: Comparison of symptoms of influenza A with abacavir-associated hypersensitivity reaction. *Int. J. STD AIDS* 14, 478–181 (2003).
21. Symonds W, Cutrell A, Edwards M *et al.*: Risk factor analysis of hypersensitivity reactions to abacavir. *Clin. Ther.* 24, 565–573 (2002).
22. Peyrieere H, Nicolas J, Siffert M, Demoly P, Hillaire-Buys D, Reynes J: Hypersensitivity related to abacavir in two members of a family. *Ann. Pharmacother.* 35, 1291–1292 (2001).
23. Mallal S, Nolan D, Witt C *et al.*: Association between presence of *HLA-B*5701*, *HLA-DR7*, and *HLA-DQ3* and hypersensitivity to HIV-1 reverse transcriptase inhibitor abacavir. *Lancet* 359, 727–732 (2002).
•• **Reports the association between *HLA-B*5701* and hypersensitivity reaction to abacavir, and the role of Hsp70 in the development of such a reaction.**
24. Hetherington S, Hughes AR, Mosteller M *et al.*: Genetic variations in *HLA-B* region and hypersensitivity reactions to abacavir. *Lancet* 359, 1121–1122 (2002).
25. Martin AM, Nolan D, Gaudieri S *et al.*: Predisposition to abacavir hypersensitivity conferred by *HLA-B*5701* and a haplotypic Hsp70-Hom variant. *Proc. Natl Acad. Sci. USA* 101, 4180–4185 (2004).
26. Martin AM, Almeida CA, Cameron P *et al.*: Immune responses to abacavir in antigen-presenting cells from hypersensitive patients. *AIDS* 21, 1233–1244 (2007).
27. Phillips EJ, Sullivan JR, Knowles SR, Shear NH: Utility of patch testing in patients with hypersensitivity syndromes associated with abacavir. *AIDS* 16, 2223–2225 (2002).
28. Phillips EJ, Wong GA, Kaul R *et al.*: Clinical and immunogenetic correlates of abacavir hypersensitivity. *AIDS* 19, 979–981 (2005).
29. Easterbrook PJ, Waters A, Murad S *et al.*: Epidemiological risk factors for hypersensitivity reactions to abacavir. *HIV Med.* 4, 321–324 (2003).
30. Rauch A, Nolan D, Martin A, McKinnon E, Almeida C, Mallal S: Prospective genetic screening decreases the incidence of abacavir hypersensitivity reactions in the Western Australian HIV Cohort Study. *Clin. Infect. Dis.* 43, 99–102 (2006).
31. Reeves I, Churchill D, Fisher M: Screening for *HLA-B*5701* reduces the frequency of abacavir hypersensitivity reactions. *Antivir. Ther.* 11, L11 (2006).
32. Zucman D, Truchis P, Majerholc C, Stegman S, Caillat-Zucman S: Prospective screening for human leukocyte antigen-*B*5701* avoids abacavir hypersensitivity reaction in the ethnically mixed French HIV population. *J. Acquir. Immune Defic. Syndr.* 45, 1–3 (2007).
33. Mallal S, Phillips E, Carosi G *et al.*: PREDICT-1: a novel randomized prospective study to determine the clinical utility of *HLA-B*5701* screening to reduce abacavir hypersensitivity in HIV-1 infected subjects (study CNA106030). Presented at: *4th International AIDS Society Conference on HIV Pathogenesis, Treatment and Prevention.* Sydney, Australia (2007) (Abstract WESS101).
34. Hughes DA, Vilar FJ, Ward CC, Alfirevic A, Park BK, Pirmohamed M: Cost–effectiveness analysis of *HLA-B*5701* genotyping in preventing abacavir hypersensitivity. *Pharmacogenetics* 14, 335–342 (2004).
35. Hughes AR, Mosteller M, Bansal T *et al.*: Association of genetic variations in HLA-B region with hypersensitivity to abacavir in some, but not all, populations. *Pharmacogenomics* 5, 203–211 (2004).
36. Phillips EJ: Genetic screening to prevent abacavir hypersensitivity reaction: are we there yet? *Clin. Infect. Dis.* 43, 103–105 (2006).
37. Saag M, Balu R, Brachman P *et al.*: High sensitivity of *HLA-B*5701* in whites and blacks in immunologically-confirmed cases of abacavir hypersensitivity (ABC HSR). *4th International AIDS Society Conference on HIV Pathogenesis, Treatment and Prevention.* Sydney, Australia (2007) (Abstract WEAB305).
38. Honda H, Tsukada K, Yazaki H *et al.*: Low incidence of abacavir-associated hypersensitivity reactions in Japanese HIV-1-infected patients. Presented at: *4th International AIDS Society Conference on HIV Pathogenesis, Treatment and Prevention.* Sydney, Australia (2007) (Abstract MOPEB005).
39. Carrington M, O'Brien SJ: The influence of *HLA* genotype on AIDS. *Annu. Rev. Med.* 54, 535–551 (2003).
•• **Discusses the association between AIDS disease progression and *HLA* genotype.**
40. Klein MR, van der Burg SH, Hovenkamp E *et al.*: Characterization of HLA-B57-restricted human immunodeficiency virus type 1 Gag- and RT-specific cytotoxic T lymphocyte responses. *J. Gen. Virol.* 79, 2191–2201 (1998).
41. Johnson VA, Brun-Vezinet F, Clotet B *et al.*: Update of the drug resistnace mutations in HIV-1: Fall 2006. *Top. HIV Med.* 14, 125–130 (2006).
42. Frater AJ, Brown H, Oxenius A *et al.*: Effective T-cell responses select human immunodeficiency virus mutants and slow disease progression. *J. Virol.* 81, 6742–6751 (2007).
43. Chui CK, Brumme ZL, Brumme CJ *et al.*: A simple screening approach to reduce *B*5701*-associated abacavir hypersensitivity on the basis of sequence variation in HIV reverse transcriptase. *Clin. Infect. Dis.* 44, 1503–1508 (2007).
44. Hammond E, Almeida CA, Mamotte C *et al.*: External quality assessment of *HLA-B*5701* reporting: an international multicentre survey. *Antivir. Ther.* 12, 1027–1032 (2007).
45. van der Valk M, Kastelein JJ, Murphy RL *et al.*: Nevirapine-containing antiretroviral therapy in HIV-1 infected patients results in an anti-atherogenic lipid profile. *AIDS* 15, 2407–2414 (2001).
46. Stern JO, Robinson PA, Love J, Lanes S, Imperiale MS, Mayers DL: A comprehensive hepatic safety analysis of nevirapine in different populations of HIV infected patients. *J. Acquir. Immune Defic. Syndr.* 34, S21–S33 (2003).

47. Dieterich DT, Robinson PA, Love J, Stern JO: Drug-induced liver injury associated with the use of nonnucleoside reverse transcriptase inhibitors. *Clin. Infect. Dis.* 38, S80–S89 (2004).
48. Sanne I, Mommeja-Marin H, Hinkle J *et al.*: Severe hepatotoxicity associated with nevirapine use in HIV-infected subjects. *J. Infect. Dis.* 191, 825–829 (2005).
49. Lyons F, Hopkins S, Kelleher B *et al.*: Maternal hepatotoxicity with nevirapine as part of combination antiretroviral therapy in pregnancy. *HIV Med.* 7, 255–260 (2006).
50. Stern JO, Robinson PA, Love J, Lanes S, Imperiale MS, Mayers DL: A comprehensive hepatic safety analysis of nevirapine in different populations of HIV infected patients. *J. Acquir. Immune Defic. Syndr.* 34, S21–S33 (2003).
51. Phanuphak N, Apornpong T, Teeratakulpisarn S *et al.*: Nevirapine-associated toxicity in HIV-infected Thai men and women, including pregnant women. *HIV Med.* 8, 357–366 (2007).
52. Bayor MS, Johann-Liang R: Hepatotoxicity associated with nevirapine use. *J. Acquir. Immune Defic. Syndr.* 35, 538–539 (2004).
53. Josephson F, Albert J, Flamholc L *et al.*: Antiretroviral treatment of HIV infection: Swedish recommendations 2007. *Scand. J. Infect. Dis.* 39, 486–507 (2007).
54. Patel SM, Johnson S, Belknap SM, Chan J, Sha BE, Bennett C: Serious adverse cutaneous and hepatic toxicities associated with nevirapine use by non-HIV-infected individuals. *J. Acquir. Immune Defic. Syndr.* 35, 120–125 (2004).
55. Coopman SA, Johnson RA, Platt R, Stern RS: Cutaneous disease and drug reactions in HIV infection. *N. Engl. J. Med.* 328, 1670–1674 (1993).
56. Phillips E, Mallal S: Drug hypersensitivity in HIV. *Curr. Opin. Allergy Clin. Immunol.* 7, 324–330 (2007).
57. Liechty C, Solberg P, Mwima G *et al.*: Nevirapine-induced Steven–Johnson syndrome in a mother and son. *AIDS* 19, 993–994 (2005)
58. Martin AM, Nolan D, James I *et al.*: Predisposition to nevirapine hypersensitivity associated with *HLA-DRB1*0101* and abrogated by low CD4 T-cell counts. *AIDS* 19, 97–99 (2005).
59. Littera R, Carcassi C, Masala A *et al.*: HLA-dependent hypersensitivity to nevirapine in Sardinian HIV patients. *AIDS* 20, 1621–1626 (2006)
60. Gatanaga H, Yazaki H, Tanuma J *et al.*: *HLA-Cw8* primarily associated with hypersensitivity to nevirapine. *AIDS* 21, 264–265 (2007)
61. Barreiro P, Soriano V, Casas E *et al.*: Prevention of nevirapine-associated exanthema using slow-dose escalation and/or corticosteroids. *AIDS* 14, 2153–2157 (2000).
62. Phillips E, Gutierrez S, Jahnke N *et al.*: Determinants of nevirapine hypersensitivity and its effect on the association between hepatitis C status and mortality in antiretroviral drug-naive HIV-positive patients. *AIDS* 21, 1561–1568 (2007).
63. Pellock JM: Carbamazepine side effects in children and adults. *Epilepsia* 28, S64–S70 (1987).
64. Vittorio CC, Muglia JJ: Anticonvulsant hypersensitivity syndrome. *Arch. Intern. Med.* 155, 2285–2290 (1995).
65. Tennis p, Stern RS: Risk of serious disorders after initiation of use of phenytoin, carbamazepine, or sodium valproate: a record linkage study. *Neurology* 49, 542–546 (1997).
66. Khoo AK, Foo CL: Toxic epidermal necrolysis in a burns centre: a 6-year review. *Burns* 22, 275–278 (1996).
67. Kamaliah MD, Zainal D, Mokhtar N, Nazmi N: Erythema multiforme, Stevens–Johonson syndrome and toxic epidermal necrolysis in northeastern Malaysia. *Int. J. Dermatol.* 37, 520–523 (1998).
68. Chung WH, Hung SI, Hong HS *et al.*: Medical genetics: a marker for Stevens–Johnson syndrome. *Nature* 428, 486 (2004).
69. Hung SI, Chung WH, Jee SH *et al.*: Genetic susceptibility to carbamazepine-induced cutaneous adverse drug reactions. *Pharmacogenet. Genomics* 16, 297–306 (2006).
70. Lonjou C, Thomas L, Borot N *et al.*: A marker for Stevens–Johnson syndrome…: ethnicity matters. *Pharmacogenomics J.* 6, 265–268 (2006).
71. Alfirevic A, Jorgensen AL, Williamson PR *et al.*: *HLA-B* locus in Caucasian patients with carbamazepine hypersensitivity. *Pharmacogenomics* 7, 813–818 (2006).
72. Wortmann RL: Gout and hyperuricemia. *Curr. Opin. Rheumatol.* 14, 281–286 (2002).
73. Roujeau JC, Kelly JP, Naldi L *et al.*: Medication use and the risk of Stevens–Johnson syndrome or toxic epidermal necrolysis. *N. Engl. J. Med.* 333, 1600–1607 (1995).
74. Melsom RD: Familial hypersensitivity to allopurinol with subsequent desensitization. *Rheumatology (Oxford)* 38, 1301 (1999).
75. Hung SI, Chung WH, Liou LB *et al.*: *HLA-B*5801* allele as a genetic marker for severe cutaneous adverse reactions caused by allopurinol. *Proc. Natl Acad. Sci. USA* 102, 4134–4139 (2005).
76. Dainichi T, Uchi H, Moroi Y, Furue M: Steven–Johnson syndrome, drug-induced hypersensitivity syndrome and toxic epidermal necrolysis caused by allopurinol in patients with a common *HLA* allele: what causes the diversity? *Dermatology* 215, 86–88 (2007).
77. Daar AS, Singer PA: Pharmacogenetics and geographical ancestry: implications for drug development and global health. *Nat. Rev. Genet.* 6, 241–246 (2005).

Website

101. Department of Health and Human Services: Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. October 10, 2006 (Accessed 18 July 2007) www.aidsinfo.nih.gov

JOURNAL OF VIROLOGY, Apr. 2008, p. 3261–3270 Vol. 82, No. 7
0022-538X/08/$08.00+0 doi:10.1128/JVI.01154-07
Copyright © 2008, American Society for Microbiology. All Rights Reserved.

Amino Acid Mutation N348I in the Connection Subdomain of Human Immunodeficiency Virus Type 1 Reverse Transcriptase Confers Multiclass Resistance to Nucleoside and Nonnucleoside Reverse Transcriptase Inhibitors[▽]†

Atsuko Hachiya,[1,2] Eiichi N. Kodama,[3]* Stefan G. Sarafianos,[4] Matthew M. Schuckmann,[4] Yasuko Sakagami,[3] Masao Matsuoka,[3] Masafumi Takiguchi,[2] Hiroyuki Gatanaga,[1] and Shinichi Oka[1]

AIDS Clinical Center, International Medical Center of Japan, Tokyo, Japan[1]; Center for AIDS Research, Kumamoto University, Kumamoto, Japan[2]; Institute for Virus Research, Kyoto University, Kyoto, Japan[3]; and Department of Molecular Microbiology and Immunology, University of Missouri School of Medicine, Bond Life Sciences Center, Columbia, Missouri[4]

Received 28 May 2007/Accepted 9 January 2008

We identified clinical isolates with phenotypic resistance to nevirapine (NVP) in the absence of known nonnucleoside reverse transcriptase inhibitor (NNRTI) mutations. This resistance is caused by N348I, a mutation at the connection subdomain of human immunodeficiency virus type 1 (HIV-1) reverse transcriptase (RT). Virologic analysis showed that N348I conferred multiclass resistance to NNRTIs (NVP and delavirdine) and to nucleoside reverse transcriptase inhibitors (zidovudine [AZT] and didanosine [ddI]). N348I impaired HIV-1 replication in a cell-type-dependent manner. Acquisition of N348I was frequently observed in AZT- and/or ddI-containing therapy (12.5%; n = 48; P < 0.0001) and was accompanied with thymidine analogue-associated mutations, e.g., T215Y (n = 5/6) and the lamivudine resistance mutation M184V (n = 1/6) in a Japanese cohort. Molecular modeling analysis shows that residue 348 is proximal to the NNRTI-binding pocket and to a flexible hinge region at the base of the p66 thumb that may be affected by the N348I mutation. Our results further highlight the role of connection subdomain residues in drug resistance.

Combinations of multiple drugs used for clinical treatment of human immunodeficiency virus type 1 (HIV-1) infections in highly active antiretroviral therapies (HAART) can dramatically reduce viral load, increase levels of CD4positive cells, improve survival rates, and delay the onset of AIDS. HAART typically includes two nucleoside reverse transcriptase inhibitors (NRTIs) and a nonnucleoside reverse transcriptase inhibitor (NNRTI) or a protease inhibitor (17). After prolonged therapy, however, an increasing number of treatment failures are caused by the emergence of multidrug-resistant (MDR) variants. For example, treatment with zidovudine (AZT) and dideoxynuleoside RT inhibitors such as didanosine (ddI) may result in the "Q151 complex" of clinical mutations in RT (A62V/V751/F77L/F116Y/Q151M) which causes high-level resistance to multiple NRTIs, AZT, ddI, zalcitabine (ddC), and stavudine (d4T) (21, 38). Another MDR complex of RT mutations is the "fingers insertion" complex that includes an insertion of two residues at the fingers subdomain of the p66 subunit of RT in the presence of AZT resistance mutations, e.g., M41L and T215Y (M41L/T69SSG/T215Y). This complex can emerge during combination treatment that includes NRTIs (10, 41) and confers resistance to multiple drugs by enhancing the excision reaction that causes resistance by unblocking NRTI-terminated primers (40). G333E or G333D polymorphisms with thymidine analogue-associated mutations (TAMs) and M184V have also been reported to facilitate moderate resistance to at least two NRTIs, AZT and lamivudine (3TC) (7, 22). RT mutations K103N, V106M, and Y188L are associated with resistance to multiple NNRTIs (1, 5). Since all NNRTIs bind at the same hydrophobic binding pocket, mutations in the binding pocket may result in broad cross-resistance between members of this family of drugs.

The presence of variants that are resistant to multiple drugs limits significantly the available therapeutic strategies and, even more profoundly, therapeutic options. However, so far all reports of viruses that acquire resistance to members of both families of RT inhibitors describe variants with multiple mutations at several residues that confer either NRTI or NNRTI resistance. Recently, Paolucci et al. reported that Q145M/L mutations confer cross-resistance to some NRTIs and NNRTIs (31, 32). Similarly, an NNRTI resistance mutation, Y181I, also confers resistance to d4T at the enzyme level (2). The frequency of these mutations in clinical isolates does not appear to be significant, according to the Stanford HIV resistance database (http://hivdb.stanford.edu/index.html); there is no deposition for Q145M/L, and Y181I has a prevalence of 0.02% in drug-naïve or NRTI-treated patients and 0.9% in NNRTI-treated patients.

We report here that N348I is a multiclass resistance mutation involved in resistance to both NRTIs and NNRTIs and present in a significant number of clinical isolates. Residue 348 is at the RT connection subdomain outside the region usually

* Corresponding author. Mailing address: Laboratory of Virus Immunology, Research Center for AIDS, Institute for Virus Research, Kyoto University, 53, Kawaramachi, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan. Phone and fax: 81 75 751 3986. E-mail: ekodama@virus.kyoto-u.ac.jp.

† Supplemental material for this article may be found at http://jvi.asm.org/.

▽ Published ahead of print on 23 January 2008.

sequenced as the drug resistance assay in clinical settings. The role of connection subdomain mutations in AZT resistance has been highlighted recently by Pathak and colleagues (28). The present work shows that N348I confers resistance not only to the NRTI AZT but also to another NRTI, ddI, and two NNRTIs, nevirapine (NVP) and delavirdine (DLV). Importantly, we show that the N348I variant emerges frequently during chemotherapy containing AZT and/or ddI. To our knowledge, this is the first example of a clinically significant and high-prevalence multiclass RTI resistance mutation that highlights the need for extensive phenotypic and genotypic assays to detect novel mutations with important implications on future therapeutic strategies.

MATERIALS AND METHODS

Reagents and cells. AZT, ddI, ddC, and d4T were purchased from Sigma (St. Louis, MO). 3TC, DLV, and tenofovir (TDF) were purchased from Moravek Biochemicals, Inc. (Brea, CA). NVP, abacavir (ABC), and efavirenz (EFV) were generously provided by Boehringer Ingelheim Pharmaceutics Inc. (Ridgefield, CT), GlaxoSmithKline (Philadelphia, PA), and Merck Co. Inc. (Rahway, NJ), respectively. Loviride was kindly provided by S. Shigeta, Fukushima Medical University (Fukushima, Japan). MT-2, SupT1, PM1, H9, Cos-7, and MAGIC-5 cells (CCR5-transduced HeLa-CD4/LTR-β-Gal cells) were cultured and used as described previously (14). Peripheral blood mononuclear cells (PBMCs) obtained from healthy donors were stimulated with phytohemagglutinin (PHA) for 3 days and grown in RPMI 1640 medium with 10% fetal calf serum and 10 U of interleukin-2 as described previously (15, 23).

Clinical isolates. Clinical isolates were obtained from fresh plasma of an HIV-1-infected patient attending the outpatient clinic of the AIDS Clinical Center, International Medical Center of Japan, using MAGIC-5 cells. The isolates were stored at −80°C until use, and infectivity was measured as blue cell-forming units (BFU) of MAGIC-5 cells. The Institutional Review Board approved this study (IMCJ-H13-80), and written informed consent was obtained from the patient.

Viruses and construction of recombinant HIV-1 clones. An HIV-1 infectious clone, pNL101, was kindly provided by K.-T. Jeang (NIH, Bethesda, MD) and used for generating recombinant HIV-1 clones (15). A wild-type (WT) HIV-1, designated HIV-1_{WT}, was constructed by replacing the *pol*-coding region (nucleotides [nt] 2006 of ApaI site to 5785 of SalI site of pNL101) with the HIV-1 BH10 strain. The *pol*-coding region contains a silent mutation at nt 4232 (TTTAGA to TCTAGA; mutation is italicized) for generation of an XbaI unique site. The DNA fragments amplified by reverse transcription-PCR from the primary isolates were digested with appropriate restriction enzymes and cloned into pNL-RT_{WT}. The nucleoside sequences of the PCR-amplified fragments were verified with a model 3730 automated DNA Sequencer (Applied Biosystems, Foster, CA). Viral stocks were obtained by transfection of each molecular clone into Cos-7 cells, harvested, and stored at −80°C until use.

Sequencing analysis of HIV-1 RT region. Viral RNA was extracted from plasma and/or culture supernatant of clinical isolates and subjected to reverse transcription-PCR using a OneStep RNA PCR Kit (Takara Bio, Otsu, Japan). Nested PCR was subsequently conducted for direct sequencing. Primer pairs used for amplification of the DNA fragment from nt 2574 to 3333 of pNL101 were T1 (5′-AGGGGGAATTGGAGGTTT; nt 2393 to 2410) and T4 (5′-TTCT GTTAGTGCTTTGGTT; nt 3422 to 3404) for the first PCR and T12 (5′-CCAG TAAAATTAAAGCCAG; nt 2574 to 2592) and T15 (5′-TCCCACTAACTTCT GTATGTC; nt 3335 to 3315) for the second PCR (15). Primer pairs used for amplification of DNA fragment from nt 3288 to 4316 were 3244F (5′-AT GAACTCCATCCTGACAAATG; nt 3244 to 3265) and 4428R (5′-TGTA CAATCTAATTGCCATAT; nt 4428 to 4407) for the first PCR and 3288F (5′-CCAGAAAAAGACAGCTGGACT; nt 3288 to 3308) and 4316R (5′-TG GCAGATTAAAATCACTAGCC; nt 4316 to 4295) for the second PCR (13). The nested PCR products were then subjected to the direct sequencing of the entire RT coding region, and some PCR products were further analyzed with clonal sequence determination as described previously (13, 15).

Drug susceptibility assay. HIV-1 sensitivity to various RTIs was determined in triplicate using MAGIC-5 cells as described previously (14). MAGIC-5 cells were infected with diluted virus stock (100 BFU) in the presence of increasing concentrations of RTIs, cultured for 48 h, fixed, and stained with X-Gal (5-bromo-4-chloro-3-indolyl-βD-galactopyranoside). The stained cells were counted under a light microscope. Drug concentrations reducing the cell number to 50% of that of the drug-free control (EC_{50}) were determined by referring to the dose-response curve.

Competition assay of HIV-1 replication. MT-2, SupT1, PM1, and H9 cells (2.5×10^5 cells/5 ml) and PHA-stimulated PBMCs (2.5×10^6 cells/5 ml) were infected with each virus preparation (500 BFU) for 4 h. The infected cells were then washed and cultured in a final volume of 5 ml. Culture supernatants (100 μl) were harvested from days 1 to 7 after infection, and the p24 antigen amounts were quantified (27).

Freshly prepared H9 cells (3×10^5 cells/well) were exposed to the mixture of viral preparations (300 BFU) and cultured to compare their replicative capacities, as previously described (15). On day 1 in culture, one-third of the infected H9 cells were harvested and washed twice with phosphate-buffered saline, followed by DNA extraction. Purified DNA was subjected to nested PCR to sequence the HIV-1 RT genes. The supernatant of the viral culture was transferred to uninfected H9 cells at 7-day intervals, and the cells harvested at each passage were subjected to direct DNA sequencing of the HIV-1 RT gene. Population change of the viral mixture was determined by the relative peak height on the sequencing electrogram. The persistence of the original amino acid substitution was confirmed in all infectious clones used in this assay.

Molecular modeling studies. The SYBYL and O programs were used to prepare molecular models of the complexes of WT and N348I HIV-1 RT with DNA, NVP, and the triphosphates of AZT and ddI. Starting atomic coordinates of HIV-1 RT in complex with DNA were obtained from the structures described by Tuske et al. (40), Sarafianos et al. (36), and Huang et al. (20) (Protein Data Bank [PDB] code numbers 1T05, 1N6Q, and 1RTD, respectively). Because there is no available structure of RT in complex with both NNRTI and DNA, we used structures of RT in complex with NNRTI to obtain initial coordinates of the NNRTI-binding pocket (9, 12). Specifically, we used the coordinates of the two β-sheets of the polymerase active site (β6-β9-β10 that contains the three catalytic aspartates and the YMDD motif as well as β12-β13 of the primer grip) to replace the corresponding regions in the RT-DNA complex. The N348I side chain mutation was manually modeled in the p66 subunit, and all structures were optimized using energy minimization protocols in SYBYL. The triphosphates of AZT and ddI were built based on the structures of AZT monophosphate and dTTP in PDB 1N6Q (36) and 1RTD (20) or of TDF diphosphate in the ternary complex of HIV-1 RT/DNA/TFV-DP, PDB 1T03 (40). The coordinate vector of the resulting structures was varied using a minimization procedure to minimize the potential energy by relieving short interatomic distances while maintaining structural integrity.

RESULTS

Resistance to NNRTIs observed in HIV-1 isolates. The clinical history of the patient is summarized in Fig. 1 and includes the variation of genotypic and phenotypic drug resistance profiles of sequential isolates with time (see also Table S1 and Fig. S1 in the supplemental material). In spite of the combination therapy, little immunologic and virologic response was observed; at time point 2, the CD4 count was 25/μl, and the plasma HIV-1 RNA levels were 2.1×10^6 copies/ml. However, no known drug resistance mutations associated to both NRTIs and NNRTIs were detected in the RT region at this point (Fig. 1B). Due to poor adherence, upon changing the regimen we observed only partial suppression of viral replication and limited increase in the CD4 count. TAMs with N348I accumulated during time points 3 to 6 (Fig. 1). In February 2000, the treatment was interrupted due to severe adverse effects, resulting in a rebound of viral load. In July 2000, the same therapy was resumed for approximately 1 year. No drug resistance-associated mutations were detected upon initiation of this therapy (time point 7). At time point 8, mixtures of two amino acid insertions at codon 69 with TAMs and N348I were detected, although these mutations disappeared after the treatment interruption at time point 10.

Interestingly, HIV-1 isolates at time points 5 and 6 showed resistance to NVP (44- and 25-fold, respectively) and to DLV