VOLUME 16 NO 2 PP 200–204 FEBRUARY 2011

Distribution of *HLA-B* alleles in a Ugandan HIV-infected adult population: NORA pharmacogenetic substudy of DART

Paula Munderi[1], Wendy B. Snowden[2], Ann Sarah Walker[3], Cissy Kityo[4], Michael Mosteller[5], Geoffrey Kabuye[1], Navdeep K. Thoofer[2], Francis Ssali[4], Charles F. Gilks[6] and Arlene R. Hughes[5] on behalf of the DART Trial Team*

1 *MRC/UVRI Uganda Research Unit on AIDS, Entebbe, Uganda*
2 *GlaxoSmithKline R&D, Stevenage, UK*
3 *MRC Clinical Trials Unit, London, UK*
4 *Joint Clinical Research Centre, Kampala, Uganda*
5 *GlaxoSmithKline R&D, Research Triangle Park, North Carolina, USA*
6 *Imperial College, London, UK*

Summary

OBJECTIVES To determine the frequencies of *HLA-B* alleles in Ugandan patients in the NORA substudy of the DART trial and to compare *HLA-B* allele frequencies in those with and without clinically diagnosed hypersensitivity reaction (HSR).

METHODS DNA-based *HLA-B* genotyping was used to determine HLA alleles in 247 participants who received abacavir, including all six participants ('cases') with clinically diagnosed abacavir HSR.

RESULTS The incidence of clinical abacavir HSR in this double-blinded study was 2.0% (6/300) in the abacavir group. As *HLA-B*5701* was absent throughout the entire cohort, including the six HSR 'cases', an association could not be established between *HLA-B*5701* and clinically diagnosed abacavir HSR. No other *HLA-B*57* alleles were present among the six 'cases'. *HLA-B*5703* was the most frequent *HLA-B*57* allele among the abacavir-tolerant participants.

CONCLUSION The rate of clinical HSR was low, which may reflect the expected 2–3% clinical false-positive rate seen in previous double-blind randomized studies. The presumption that these cases may be false-positive abacavir HSR is supported by the fact that no *HLA-B*5701* alleles were found in the abacavir group. Implementation of prospective *HLA-B*5701* screening must be based on benefit/risk considerations within local practice. Clinical risk management remains paramount.

keywords AIDS, pharmacogenetics, *HLA-B*5701*, abacavir, drug hypersensitivity, African Continental Ancestry Group

Introduction

Of the 4 million people in low- and middle-income countries receiving antiretroviral therapy (ART) in 2008, nearly 3 million were living in sub-Saharan Africa and 2% were receiving second-line ART (WHO 2009). Numbers on second-line therapy are expected to increase substantially as more patients fail first-line therapy. The World Health Organization (WHO)'s recommended second-line regimens are tenofovir DF + lamivudine and abacavir + didanosine, each with a boosted protease inhibitor.

Approximately, 5% of patients receiving abacavir in clinical studies in resource-rich countries develop a clinically diagnosed hypersensitivity reaction (HSR), characterized by symptoms most often including fever, rash, gastrointestinal and/or constitutional symptoms and less frequently, respiratory symptoms, lethargy or malaise and usually appearing within 6 weeks of initiating abacavir (median onset 11 days) (Brothers *et al.* 2005). Standard practice is to permanently discontinue abacavir for symptoms consistent with HSR.

In retrospective studies, the pharmacogenetic marker, *HLA-B*5701*, was associated with increased risk for abacavir HSR in European, Hispanic and Thai populations (Hughes *et al.* 2008). Furthermore, the PREDICT-1 trial demonstrated that prospective screening and exclusion of *HLA-B*5701*-positive patients from abacavir treatment significantly decreased the incidence of clinically diagnosed abacavir HSR and eliminated HSR immunologically confirmed by clinical diagnosis plus a positive abacavir skin patch test reaction (Mallal *et al.* 2008). Open-label screening and exclusion of *HLA-B*5701*-positive patients has reduced true- and false-positive abacavir HSR to very low rates in racially diverse populations (Rauch *et al.*

*DART Trial members are in Appendix.

2006; Zucman *et al.* 2007). Treatment guidelines and abacavir prescribing information recommend (USA) or require (Europe) prospective *HLA-B*5701* screening prior to abacavir initiation.

In clinical studies, clinically diagnosed abacavir HSR has been reported in fewer people of black race possibly because of reduced *HLA-B*5701* carriage frequency compared to caucasians (Cao *et al.* 2001, 2004; Prins *et al.* 2008) and/or a higher proportion of false- to true-positive abacavir HSR in blacks. An earlier study concluded *HLA-B*5701* was not associated with clinically diagnosed abacavir HSR in self-reported African Americans, and *HLA-B*5701* was only present in 8–16% of clinically diagnosed cases (Hughes *et al.* 2004). However, in the SHAPE study, when clinical diagnosis of abacavir HSR was augmented with skin patch testing to identify cases, the sensitivity of *HLA-B*5701* increased to 100% in both self-reported European and African-American patients in the USA (Saag *et al.* 2008). Together, PREDICT-1, SHAPE and earlier retrospective studies suggest that prospective *HLA-B*5701* screening may have clinical utility across diverse racial populations.

Information on genetic variation in black populations in Africa is limited, particularly with respect to the highly variable major histocompatibility complex where the *HLA-B* gene is located, and there are no reported studies in African patients on associations between clinically diagnosed abacavir HSR and genetic variations in the *HLA-B* region. We conducted a pharmacogenetic substudy within the DART trial in Uganda to evaluate associations between *HLA-B*5701* carriage and clinically diagnosed abacavir HSR.

Methods

Within the DART trial (DART Trial Team 2010), the NORA (Nevirapine OR Abacavir) substudy was designed to evaluate the safety of abacavir compared with nevirapine in previously untreated Ugandan adults with symptomatic HIV disease and CD4 <200 cells/mm^3 initiating ART in a setting where all patients were under close clinical supervision. After providing informed consent, participants at the Joint Clinical Research Centre, Kampala, or the MRC/UVRI Uganda Research Unit on AIDS, Entebbe, were randomized to either zidovudine and lamivudine (co-formulated as Combivir) plus either 300 mg abacavir and nevirapine placebo or abacavir placebo and 200 mg nevirapine twice daily for 24 weeks (double dummy design). Participants were then switched to open-label active drug and continued follow-up. Safety and tolerability data from NORA have been reported elsewhere, and the six patients who experienced clinically diagnosed abacavir HSR have been previously described (DART Trial Team 2008).

Additional ethics approval was obtained for pharmacogenetic research. Of 300 participants randomized to receive abacavir in NORA, 38 (13%) died or were lost to follow-up before the pharmacogenetic research was conducted, 9 (3%) were not approached for consent in error and 6 (2%) declined to participate. The remaining 247 (82%) participants provided written informed consent and a 10 ml blood sample for the determination of *HLA-B* genotypes (Quest Diagnostics, Chantilly, VA, USA). All six patients with clinically diagnosed abacavir HSR ('cases') and 241 patients who received abacavir without evidence of HSR ('controls') were included. *HLA-B* genotypes (four-digit resolution) were determined for the six 'cases' using high-resolution DNA-based assay methods to precisely determine the *HLA* alleles present (http://hla.alleles.org). For the 241 controls, DNA samples were first genotyped using medium-resolution, 2-digit DNA-based methods to identify the serological antigens present (e.g. to discriminate *HLA-B57* from *HLA-B58*) followed by high-resolution, 4-digit assay for the subset who were *HLA-B57* positive.

Chi-square tests were used to evaluate the association between abacavir HSR and 2-digit *HLA-B* alleles. For the *HLA-B* 2-digit alleles that showed evidence of over representation among 'cases,' Fisher's exact test was used to assess the association between the presence or absence of the allele and abacavir HSR case status. No adjustments for multiple testing were made.

Results

In NORA, the incidence of clinically diagnosed HSR was 2.0% in the abacavir group (6/300) (DART Trial Team 2008). The objective of this pharmacogenetic study was to determine whether *HLA-B*5701* was associated with clinically diagnosed abacavir HSR in this Ugandan population. Among the 247 participants tested, none were *HLA-B*5701* positive (Table 1). This observation was not unexpected among the six clinically diagnosed 'cases'; in a previous study, only 8–16% of self-reported African Americans with clinically diagnosed abacavir HSR were *HLA-B*5701* positive (Hughes *et al.* 2004; Saag *et al.* 2008). However, it was unexpected that *HLA-B*5701* was not detected at all, as an *HLA-B*5701* allele frequency of 3.1% (carriage frequency ~6.2%) has previously been reported in an unrelated population from Kampala, Uganda (Cao *et al.* 2004). Thus, because of the absence of *HLA-B*5701* throughout the cohort, it was not possible to establish an association between *HLA-B*5701* and clinically diagnosed abacavir HSR.

Table 1 Comparison of high-resolution *HLA-B*57* allele frequencies in three samples of patients of African ancestry

	DART study *n* = 247	Ugandan sample (Cao *et al.* 2004) *n* = 161	African-American sample (Cao *et al.* 2001) *n* = 251
*B*5701*			
# of alleles	0	10	12
Allele frequency	0.000	0.031	0.024
Exact 95% CI	0–0.007*	0.015–0.056	0.012–0.041
*B*5702*			
# of alleles	8	0	7
Allele frequency	0.016	0.000	0.014
Exact 95% CI	0.007–0.032	0.000–0.011*	0.006–0.029
*B*5703*			
# of alleles	14	4	2
Allele frequency	0.028	0.012	0.004
Exact 95% CI	0.016–0.047	0.003–0.031	0.000–0.014

*Where 0 alleles, one-sided 97.5% confidence intervals are given.

Table 2 *HLA-B* allele counts and frequencies among abacavir-treated NORA participants

HLA-B allele	Cases (*n* = 6) Alleles (proportion)	Controls (*n* = 241) Alleles (proportion)	Total (*n* = 247) Alleles (proportion)	Allele proportions from 163 Ugandans (Cao *et al.* 2004)
07	1 (0.083)	23 (0.048)	24 (0.049)	0.059
08	1 (0.083)	17 (0.035)	18 (0.036)	0.062
13		10 (0.021)	10 (0.020)	0.028
14		15 (0.031)	15 (0.030)	0.053
15	2 (0.167)	64 (0.133)	66 (0.134)	0.130
18	1 (0.083)	33 (0.069)	34 (0.069)	0.053
27		4 (0.008)	4 (0.008)	0.019
35		9 (0.019)	9 (0.018)	0.065
37		1 (0.002)	1 (0.002)	0.003
38				0.006
39	1 (0.083)	4 (0.008)	5 (0.010)	0.016
40		4 (0.008)	4 (0.008)	0.031
41		9 (0.019)	9 (0.018)	0.012
42		39 (0.081)	39 (0.079)	0.022
44		28 (0.058)	28 (0.057)	0.081
45	2 (0.167)	35 (0.073)	37 (0.075)	0.028
47	1 (0.083)	6 (0.012)	7 (0.014)	0.006
49		17 (0.035)	17 (0.034)	0.037
50		1 (0.002)	1 (0.002)	0
51		10 (0.021)	10 (0.020)	0.053
52				0.016
53		46 (0.095)	46 (0.093)	0.050
55				0.012
56				0.003
57		22 (0.046)	22 (0.045)	0.044
58	1 (0.083)	66 (0.137)	67 (0.136)	0.084
81	2 (0.167)	14 (0.029)	16 (0.032)	0.009
82		5 (0.010)	5 (0.010)	0.003

Furthermore, no other *HLA-B*57* alleles were present among the six clinically diagnosed 'cases'. Among abacavir-tolerant participants, *HLA-B*5703* was the most frequent *HLA-B57* allele, followed by *HLA-B*5702* which was absent in the previously published Ugandan dataset (Cao *et al.* 2004).

No other *HLA-B* marker was strongly associated with clinically diagnosed abacavir HSR (Table 2). The only possible trend identified was with *HLA-B81* with allele frequencies of 0.167 in 'cases' and 0.029 in controls (Fisher's exact test $P = 0.054$). This was based on only two alleles among the 'cases', does not reflect any adjustment for multiple tests and it is likely to represent a chance finding.

Finally, as the *HLA-B57* allele frequencies within the NORA population were so different from previously published estimates for Ugandans, we investigated whether frequencies for common *HLA-B* alleles differed as well. The most frequently occurring *HLA-B* alleles present ('cases' and controls combined) were *HLA-B58* (0.136) and *HLA-B15* (0.134). These allele frequencies are roughly comparable with published frequencies in a Ugandan population of 8.4% and 13.0%, respectively (Cao *et al.* 2004).

Discussion

Abacavir is being used successfully in Africa, predominantly in second-line therapy, partly because of a lower incidence of abacavir HSR. However, as an abacavir HSR is heralded by the onset of relatively non-specific symptoms and signs, it has the potential to be mistaken for an acute intercurrent infection (particularly malaria). High rates of possible HSR that cannot be confirmed or may be confused with malaria could render abacavir challenging to use.

In resource-rich countries, implementation of prospective *HLA-B*5701* screening before initiating abacavir has decreased the incidence of abacavir HSR in racially mixed patient populations. Our finding that *HLA-B*5701* was not associated with clinically diagnosed abacavir HSR in patients of African race from Uganda was consistent with previous data where *HLA-B*5701* was only present in 8–16% of clinically diagnosed HSR cases in self-reported African Americans (Hughes *et al.* 2004; Saag *et al.* 2008). In contrast, the 6.2% HLA-B*5701 carriage frequency previously reported (Cao *et al.* 2004) is considerably

higher than that observed across other sub-Saharan African populations (0–1%) suggesting that the Ugandan population in the Cao publication may differ from that in the NORA substudy, from other sub-Saharan African populations (Middleton *et al.* 2003; Meyer *et al.* 2007) and even from African-American populations (2.4% carriage frequency) (http://www.ncbi.nlm.nih.gov/gv/mhc/main.fcgi?cmd=init).

Diagnosis of abacavir HSR in NORA was based on clinical presentation alone; 'cases' had symptoms consistent with standard presentation of the abacavir HSR. Clinically diagnosed abacavir HSR was not subject to immunological confirmation (i.e. abacavir skin patch testing). Clinical monitoring for abacavir HSR and timely discontinuation of abacavir when abacavir HSR symptoms are observed are critical elements for the appropriate use of abacavir. Any decision to implement prospective *HLA-B*5701* screening must be based on benefit/risk considerations by local practice – as noted in a recent study which also found 0% carriage (95% CI 0–0.7%) in 534 Koreans (Park *et al.* 2009). In resource-constrained settings where the ability to prospectively determine *HLA-B*5701* status prior to the initiation of abacavir may be limited, clinical risk management is paramount. Patients and health care professionals should have confidence that when clinical symptoms are closely monitored abacavir HSR can be readily detected; prompt discontinuation of abacavir in such instances results in resolution of symptoms.

Acknowledgements

We thank all the patients and staff from all the centres participating in the DART trial. The DART trial was funded by the UK Medical Research Council, the UK Department for International Development and the Rockefeller Foundation. First-line drugs for the NORA substudy were provided by GlaxoSmithKline and Boehringer Ingelheim. HLA-B genotyping was funded by GlaxoSmithKline. BWS, MM, NT and AH are employees of GlaxoSmithKline. There is no other conflict of interest.

References

Brothers C, Cutrell A, Zhao H *et al.* (2005) Once daily administration of abacavir is not a clinical risk factor for suspected hypersensitivity reactions in clinical trials and rash alone is not sufficient to diagnose the reaction. *12th Conference on Retroviruses and Opportunistic Infections*, Boston, Abstract 836.

Cao K, Hollenback J, Shi X, Shi W, Chopek M & Fernandez-Vina MA (2001) Analysis of the frequencies of HLA-A, B, and C alleles and haplotypes in the five major ethnic groups of the United States reveals high levels of diversity in these loci and contrasting distribution patterns in these populations. *Human Immunology* **62**, 1009–1030.

Cao K, Moormann AM, Lyke KE *et al.* (2004) Differentiation between African populations is evidenced by the diversity of alleles and haplotypes of HLA class I loci. *Tissue Antigens* **63**, 293–325.

DART Trial Team (2008) Twenty-four-week safety and tolerability of nevirapine vs. abacavir in combination with zidovudine/lamivudine as first-line antiretroviral therapy: a randomized double-blind trial (NORA). *Tropical Medicine and International Health* **13**, 6–16.

DART Trial Team (2010) Routine versus clinically driven laboratory monitoring of HIV antiretroviral therapy in Africa (DART): a randomised non-inferiority trial. *Lancet* **375**, 123–131.

Hughes AR, Mosteller M, Bansal AT *et al.* (2004) Association of genetic variations in HLA-B region with hypersensitivity to abacavir in some, but not all, populations. *Pharmacogenomics* **5**, 203–211.

Hughes AR, Spreen WR, Mosteller M *et al.* (2008) Pharmacogenetics of hypersensitivity to abacavir: from PGx hypothesis to confirmation to clinical utility. *The Pharmacogenomics Journal* **8**, 365–374.

Mallal S, Phillips E, Carosi G *et al.* (2008) HLA-B*5701 screening for hypersensitivity to abacavir. *New England Journal of Medicine* **358**, 568–579.

Meyer D, Singe RM, Mack SJ, Lancaster A, Nelson MP & Erlich H (2007) Single locus polymorphism of classical HLA genes. In: *Immunobiology of the Human MHC: Proceedings of the 13th International Histocompatibility Workshop and Conference* (ed. JA Hansen) IHWG Press, Seattle.

Middleton D, Menchaca L, Rood H & Komerofsky R (2003) New allele frequency database: http://www.allelefrequencies.net. *Tissue Antigens* **61**, 403–407.

Park WB, Choe PG, Song KH *et al.* (2009) Should HLA-B*5701 screening be performed in every ethnic group before starting abacavir? *Clinical Infectious Diseases* **48**, 365–367.

Prins JM, Rauch A, Bergin C, Ristola M, Branco T & Pearce HC (2008) CNA110329: a prospective epidemiological study to determine the prevalence of HLA-B*5701 in HIV-infected individuals in five European countries. *Journal of the International AIDS Society* **11**, 155.

Rauch A, Nolan D, Martin A, McKinnon E, Almeida C & Mallal S (2006) Prospective genetic screening decreases the incidence of abacavir hypersensitivity reactions in the Western Australian HIV cohort study. *Clinical Infectious Diseases* **43**, 99–102.

Saag M, Balu R, Phillips E *et al.* (2008) High sensitivity of human leukocyte antigen-b*5701 as a marker for immunologically confirmed abacavir hypersensitivity in white and black patients. *Clinical Infectious Diseases* **46**, 1111–1118.

WHO (2009) WHO/UNAIDS. Towards universal access: scaling up priority HIV/AIDS interventions in the health sector. Progress Report 2009. Available at http://www.data.unaids.org/pub/Report/2009/20090930_tuapr_2009_en.pdf.

Zucman D, Truchis P, Majerholc C, Stegman S & Caillat-Zucman S (2007) Prospective screening for human leukocyte antigen-B*5701 avoids abacavir hypersensitivity reaction in the ethnically mixed French HIV population. *Journal of the Acquired Immune Deficiency Syndrome* **45**, 1–3.

Corresponding Author Ann Sarah Walker, MRC Clinical Trials Unit, London, UK. E-mail: asw@ctu.mrc.ac.uk

Appendix

MRC/UVRI Uganda Research Unit on AIDS, Entebbe, Uganda: H Grosskurth, P Munderi, G Kabuye, D Nsibambi, R Kasirye, E Zalwango, M Nakazibwe, B Kikaire, G Nassuna, R Massa, K Fadhiru, M Namyalo, A Zalwango, L Generous, P Khauka, N Rutikarayo, W Nakahima, A Mugisha, J Todd, J Levin, S Muyingo, A Ruberantwari, P Kaleebu, D Yirrell, N Ndembi, F Lyagoba, P Hughes, M Aber, A Medina Lara, S Foster, J Amurwon, B Nyanzi Wakholi; Joint Clinical Research Centre, Kampala, Uganda: P Mugyenyi, C Kityo, F Ssali, D Tumukunde, T Otim, J Kabanda, H Musana, J Akao, H Kyomugisha, A Byamukama, J Sabiiti, J Komugyena, P Wavamunno, S Mukiibi, A Drasiku, R Byaruhanga, O Labeja, P Katundu, S Tugume, P Awio, A Namazzi, GT Bakeinyaga, H Katabira, D Abaine,J Tukamushaba, W Anywar, W Ojiambo, E Angweng,S Murungi, W Haguma, S Atwiine,J Kigozi; The AIDS Support Organisation (TASO), Uganda: R Ochai, D Muhweezi; Imperial College, London, UK: C Gilks, K Boocock, C Puddephatt, D Winogron, J Bohannon; MRC Clinical Trials Unit, London, UK: J Darbyshire, DM Gibb, A Burke, D Bray, A Babiker, AS Walker, H Wilkes, M Rauchenberger, S Sheehan, L Peto, K Taylor, M Spyer, A Ferrier, B Naidoo, D Dunn, R Goodall; Trial Steering Committee: I Weller (Chair), A Babiker (Trial Statistician), S Bahendeka, M Bassett, A Chogo Wapakhabulo, J Darbyshire, B Gazzard, C Gilks, H Grosskurth, J Hakim, A Latif, C Mapuchere, O Mugurungi, P Mugyenyi; Observers: C Burke, M Distel, S Jones, E Loeliger, P Naidoo, C Newland, G Pearce, S Rahim, J Rooney, M Smith, W Snowden, J-M Steens; Data and Safety Monitoring Committee: A Breckenridge (Chair), A McLaren (Chair-deceased), C Hill, J Matenga, A Pozniak, D Serwadda.