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Fatores Genéticos e Terapia Anti-HIV-1: resistência a antirretrovirais, efeitos adversos, resposta imunológica e estratégias vacinais

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“Tudo flui”

-Heráclito

RESUMO

A infecção pelo HIV-1 ainda é um importante desafio de saúde-pública. Para contribuir na luta contra o vírus, foram abordados vários aspectos da história natural do HIV-1 por meio de análises genéticas com diferentes desenhos de estudo: (1) uma revisão sistemática com meta-análises e modelagem por séries temporais da prevalência de resistência primária aos antirretrovirais na América Latina e Caribe, que revelou que ainda aparenta estar baixa, mas devido à qualidade da evidência, os resultados podem estar subestimados em alguns casos; (2) um polimorfismo no gene *ITPA* foi associado à ocorrência de efeitos adversos sistêmicos decorrentes do uso de zidovudina, através de um estudo de caso-controle (p=0,03); (3) um polimorfismo no gene *CNOT1* foi associado com resposta desfavorável a uma vacina terapêutica anti-HIV-1 baseada em células dendríticas (p=0,0031) testada em um ensaio clínico de fase I com indivíduos brasileiros não-tratados com antirretrovirais, (4) uma revisão sistemática com meta-análise de protocolos de vacinas anti-HIV-1 terapêuticas baseadas em células dendríticas, que revelou onde esses protocolos podem melhorar e (5) um estudo de associação genética acerca da falha imunológica da terapia anti-HIV-1 explorando polimorfismos em genes envolvidos em vias de farmacodinâmica de antirretrovirais e na homeostasia do sistema imune não encontrou associações significativas, suscitando novos estudos. Assim, a genética é uma ferramenta importante no acompanhamento de pacientes HIV-1 positivos, podendo guiar a otimização dos tratamentos disponíveis o desenvolvimento de novas vacinas terapêuticas.

Palavras-chave: Genética. Terapia antirretroviral. Farmacogenética. Falha imunológica. Célula dendrítica.

ABSTRACT

The HIV-1 infection still is an important public health challenge. To contribute in the fight against the virus, we approached several aspects of HIV-1 infection natural history through genetic analyses, with different study designs: (1) A systematic review with meta-analysis and time series modeling of the prevalence of primary antiretroviral drug resistance in Latin America and the Caribbean, which revealed that it seems to be low, but it may be underestimated; due to the available evidence quality, (2) a polymorphism at *ITPA* gene was associated with AZT-related systemic adverse effects through a case-control study (p=0.03), (3) a polymorphism at *CNOT1* gene was associated with poor response to a dendritic cell-based therapeutic vaccine (p=0.0031) resulting from a phase I clinical trial on untreated Brazilian patients, (4) a systematic review with meta-analysis of experimental anti-HIV-1 therapeutic vaccines protocols, which appointed improvements and (5) a genetic association study between polymorphisms in genes involved in antiretroviral drugs pharmacodynamic pathways and immune system homeostasis and antiretroviral therapy immunologic failure did not find significant associations during multivariate analysis, suggesting the need of further studies. Therefore, genetic analysis is a strong ally to be used in the follow-up of HIV-1 patients, having the potential to guide treatment optimization, as well to help the development of new therapeutic vaccines.

Keywords: Genetics. Antiretroviral therapy. Pharmacogenetics. Immunological nonresponse. Dendritic cell.

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LISTA DE ABREVIATURAS, SIGLAS E SÍMBOLOS

Item	Definição
µL	Microlitro
3TC	Lamivudina
ABC	Abacavir
ABC	Cassete ligante de ATP
AIDS	Síndrome da imunodeficiência adquirida
Ala	Alanina
Arg	Arginina
Asn	Asparagina
Asp	Ácido aspártico
ATP	Adenosina trifosfato
AZT	Zidovudina
CVp	Carga viral plasmática
CYP	Citocromos P450
Cys	Cisteína
ddI	Didadnosina
dIMP	Deoxi-inosina monofosfato
dITP	Deoxi-ITP
DNA	Ácido desoxirribonucleico
EFZ	Efavirenz
Gln	Glutamina
Glu	Ácido glutâmico
Gly	Glicina
His	Histidina
HIV-1	Vírus da imunodeficiência humana tipo 1
HSH	Homens que fazem sexo com homens
IC	Intervalo de confiança
IL	Interleucina
Ile	Isoleucina
IMIP	Instituto de Medicina Integral Professor Fernando Figueira
IMP	Inosina monofosfato
INNTR	Inibidores não-análogos a nucleosídeos/nucleotídeos da transcriptase reversa
INTR	Inibidores análogos a nucleosídeos/nucleotídeos da transcriptase reversa
IP	Inibidores de protease
ITP	Inosina trifosfato
ITPA	Pirofosfatase de inosina trifosfato
Leu	Leucina
LTR	Repetições terminais longas
Lys	Lisina
Met	Metionina
mL	Mililitro
NVP	Nevirapina
OR	Razão de chances
PAMP	Padrões moleculares associados a patógenos
PCR	Reação em cadeia da polimerase
Phe	Fenilalanina
Pro	Prolina
PRR	Proteínas receptoras de reconhecimento de padrões
RNA	Ácido ribonucleico
Ser	Serina
SLC	Carreadores de solutos
SNP	Polimorfismos de único nucleotídeo
TAM	Mutações selecionadas pelo uso inadequado dos análogos de timidina
TARV	Terapia antirretroviral
TDF	Tenofovir
Thr	Treonina
Trp	Triptofano
Tyr	Tirosina
UNAIDS	Programa Conjunto das Nações Unidas voltado para o combate ao HIV/AIDS
Val	Valina

SUMÁRIO

1 INTRODUÇÃO

A humanidade está adentrando a quarta década de luta contra a epidemia do vírus da imunodeficiência humana tipo 1 (HIV-1, *human immunodeficiency virus type 1* na sigla em inglês), o agente causador da síndrome da imunodeficiência adquirida (AIDS, *acquired immunodeficiency syndrome*, na sigla em inglês)

Assim, o Programa Conjunto das Nações Unidas voltado para o combate ao HIV/AIDS (UNAIDS), lançou um audacioso plano de metas para direcionar ações governamentais nos próximos 5 a 15 anos. Esse plano foi nomeado de “90-90-90”, o qual consiste em:

- Até 2020, 90% de todas as pessoas vivendo com o HIV saibam seu status sorológico (isto é, sejam diagnosticas);
- Até 2020, 90% dos diagnosticados recebam tratamento antirretroviral adequado;
- Até 2020, 90% das pessoas em tratamento obtenham supressão viral e permaneçam inseridos nos serviços de saúde.

Caso essas metas sejam atingidas, modelos epidemiológicos preveem que até 2030 a epidemia de HIV/AIDS seria erradicada. Pode se observar que a UNAIDS pôs uma grande ênfase no tratamento anti-HIV como ferramenta de prevenção de novas infecções (tratamento como prevenção, ou *treatment-as-prevention*).

Atualmente os guias de tratamento ao redor do mundo, inclusive do Brasil, recomendam o oferecimento do tratamento o mais breve possível após o diagnóstico (“testar e tratar”, ou “*test and treat*”), devido a inúmeros estudos demonstrando que se a carga viral de um indivíduo em tratamento antirretroviral estiver suprimida, a transmissibilidade do vírus é enormemente diminuída em caso de ausência ou falha de métodos de prevenção, como o preservativo masculino (camisinha). Em outras palavras, pacientes em tratamento adequado virtualmente não transmitem o vírus a outras pessoas.

Alguns obstáculos precisam ser transpostos para atingir essa meta de aceleração na cobertura de tratamento anti-HIV às pessoas que precisam. A UNAIDS defende que o combate à epidemia deva ser pautado na defesa dos direitos humanos, inclusão e livre-escolha dos indivíduos. No entanto, alguns segmentos populacionais, como por exemplo, homens que fazem sexo com homens (HSH), travestis,

transexuais, profissionais do sexo e usuários de drogas injetáveis, ainda são bastante estigmatizados (ou até mesmo criminalizados em certos países), fazendo com que seu nível de acesso aos sistemas de saúde esteja abaixo do adequado.

Além dos aspectos sociais da epidemia, aspectos relacionados à biologia e história natural da infecção e tratamento também são importantes obstáculos para as metas 90-90-90. Por exemplo, a emergência de variantes genéticas resistentes aos antirretrovirais complica o tratamento do indivíduo, exigindo a troca do esquema terapêutico. Além disso, essas variantes podem ser transmitidas a outras pessoas (resistência primária), complicando a escolha do primeiro esquema antirretroviral.

Apesar dos avanços no desenvolvimento de novos antirretrovirais, gerando remédios mais seguros e potentes, efeitos adversos aos medicamentos ainda posam um desafio para uma parcela dos pacientes, pois afetam sua qualidade de vida e até mesmo sua adesão ao tratamento e consultas médicas. Isso pode dificultar a supressão viral nesses pacientes, comprometendo o alcance da terceira parte da meta 90-90-90.

Há ainda a possibilidade da supressão da carga viral (“sucesso virológico”) acontecer como desejado, porém não vir acompanhada de uma recuperação adequada das contagens de linfócitos T CD4+, o que é denominado de “falha imunológica”. Ainda não se sabe todos os fatores de risco para a ocorrência dessa falha. Indivíduos em falha imunológica tem um prognóstico pior quando comparado aos com sucesso imunológico, estando em maior risco de desenvolver comorbidades não relacionadas ao HIV/AIDS, como por exemplo, síndromes metabólicas, doenças cardiovasculares, envelhecimento precoce, dentre outras.

Apesar desses detalhes negativos acerca do tratamento antirretroviral, o seu advento sem dúvidas foi um dos maiores sucessos da medicina do século XX, pois ajudou a salvar milhões de vidas ao redor do mundo ao transformar a infecção pelo HIV de uma sentença de morte a uma doença crônica manejável, apesar de ter um impacto importante na qualidade de vida dos pacientes.

Com o sucesso estabelecido do tratamento antirretroviral, a comunidade científica passou então a sonhar com o desenvolvimento de uma cura para a infecção pelo HIV-1. Vários ensaios clínicos com diferentes desenhos experimentais e metodológicos, testaram vacinas anti-HIV-1 (tanto profiláticas quanto terapêuticas), ainda que com limitados sucessos. Sem dúvida, outros estudos serão necessários

para compreender a variabilidade interindividual na resposta a esse tipo de intervenção.

Dessa forma, o objetivo associado à Tese de Doutorado em andamento foi abordar diferentes aspectos da história natural da infecção pelo HIV-1 e seu tratamento (resistência primária aos antirretrovirais, efeitos adversos ao tratamento, falha imunológica do tratamento e vacinas terapêuticas), por meio da genética, de modo a contribuir para o entendimento das variabilidades interindividuais relacionadas a esses quatro aspectos.

2 REVISÃO DA LITERATURA

2.1 O HIV-1

2.1.1 Epidemiologia

Atualmente estima-se que existam cerca de 35 milhões de pessoas vivendo com o HIV-1 no mundo. Estima-se que no Brasil existem cerca de 734 mil pessoas vivendo com o HIV-1, com prevalência de 0,4% da população geral (BRASIL, 2014; UNAIDS, 2014).

Com relação à região Nordeste, no ano de 2013 registrou-se uma incidência de 16 casos de HIV/AIDS para cada 100.000 habitantes. Considerando o total de registros feitos entre 1980 e 2014 no Brasil, a região abrigou 14,3% do total. Pernambuco atualmente possui o maior número de casos detectados (26.030), quase 24% do total de casos detectados na região Nordeste no mesmo período (108.599) (BRASIL, 2014).

Contextualizando esses dados da prevalência geral da epidemia de HIV-1 no Brasil em referência a dados de diagnóstico e tratamento, obtém-se panoramas promissores. O Brasil foi um dos primeiros países em desenvolvimento a adotar o paradigma do "*test and treat*" nos seus guias de tratamento antirretroviral, em dezembro de 2013. Além disso, estima-se que 80% das pessoas (equivalendo a 589 mil indivíduos) que vivem com HIV-1 no país já possuem diagnóstico de seu *status* sorológico. Por fim, dados do ano de 2013 mostram que, entre as 355 mil pessoas em tratamento, de 72% a 83% delas já obtiveram supressão viral. Outras estatísticas mostram que o diagnóstico tardio da infecção vem caindo nos últimos anos no país.

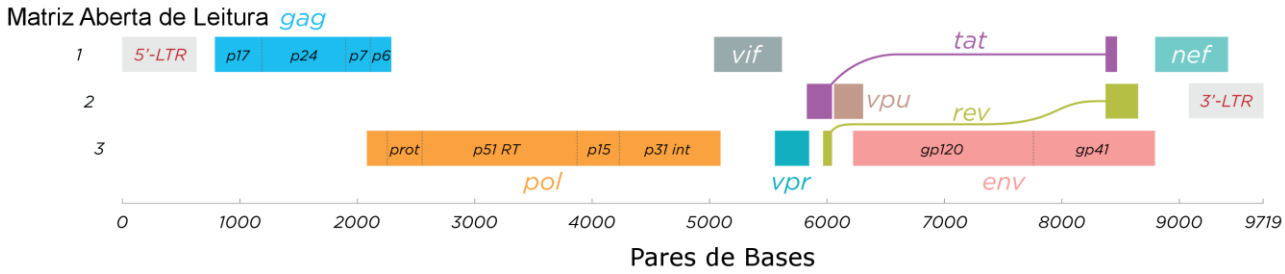
Dessa forma, o país encontra-se numa situação promissora para o cumprimento das metas 90-90-90 até 2020.

2.1.2 Estrutura genética do HIV-1

O HIV-1 é um lentivírus envelopado com um genoma composto por RNA de fita simples. Cada vírion geralmente contém duas cópias do genoma, com cerca de 9700 nucleotídeos cada uma, contando com nove matrizes abertas de leitura (*open reading frames*, ORF na sigla em inglês) que codificam 14 proteínas e sequências regulatórias da expressão das ORF, como as repetições terminais longas (*long terminal repeats*,

LTR na sigla em inglês) nas extremidades 5’ e 3’ (Figura 1). A ORF *gag* (*group-specific antigen*) codifica o polipeptídeo gag, que é clivado pela protease viral para originar proteínas estruturais, como a proteína de matriz (p17), do capsídeo (p24), nucleocapsídeo (p7) e a proteína p6. A ORF *env* (*envelope*) codifica o polipetídeo

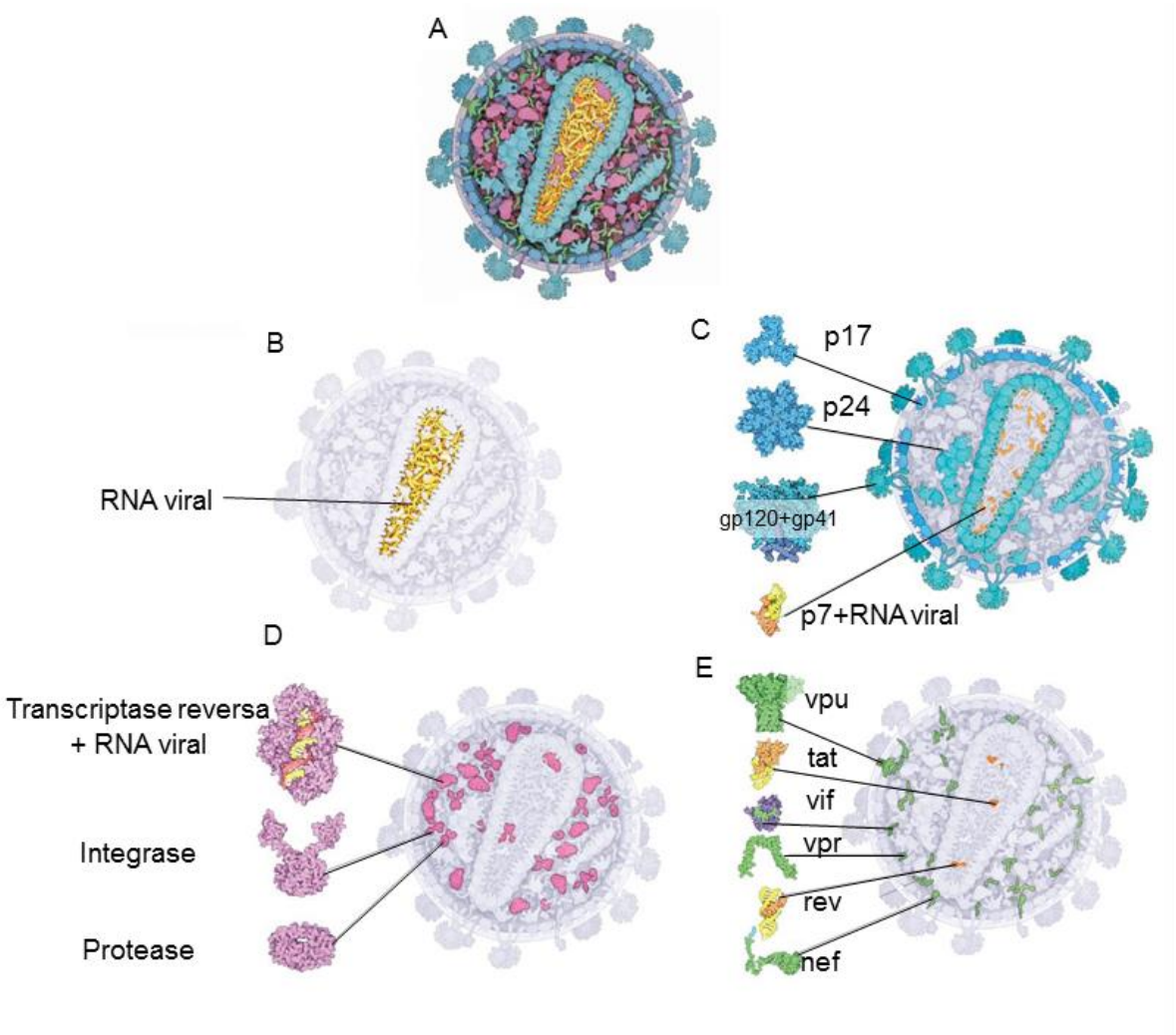
Figura 1. Esquema do genoma do HIV-1, onde são representados os genes que codificam as 14 proteínas virais, algumas estruturais (p17, p24, p7, p6, gp120 e gp41), outras envolvidas na replicação viral e infectivade viral (transcriptase reversa e protease) e acessórias envolvidas na patogênese (vif, vpr, tat, rev,nef e vpu). Flanqueando esses genes, existem repetições terminais longas (LTR), envolvidas na regulação do genoma. Figura modificada a partir do trabalho de Thomas Splettstoesser (www.scistyle.com), licença Creative Commons (CC BY-SA 3.0, https://commons.wikimedia.org/w/index.php?curid=33943759).



gp160, que origina as proteínas gp120 e gp41, envolvidas na formação do envelope viral lipoproteico (WATTS et al., 2009).

A ORF *pol* (*polimerase*) gera um polipeptídeo que contém a transcriptase reversa, a protease e a integrase, envolvidas na replicação, maturação e capacidade infecciosa da partícula viral. Seis ORF acessórias, envolvidos na infecção e patogênese (*vif*, *vpr*, *tat*, *rev*, *nef* e *vpu*) também estão presentes no genoma do vírus (WATTS et al., 2009; HIV SEQUENCE COMPENDIUM 2014, 2013). A Figura 2 representa a partícula viral e destaca suas proteínas.

Figura 2. (A) representação esquemática do HIV em corte transversal. (B) Destaque para o material genético viral compactado – duas fitas simples de RNA viral, representadas em amarelo. (C) Destaque para as proteínas estruturais, representadas em tons de azul (com exceção da p7 em tons de laranja). De cima para baixo, respectivamente: proteína de matriz (p17), proteína do capsídeo (p24), complexo proteico de gp120 com gp41 e inseridas no envelope lipoproteico e proteína do nucleocapsídeo (p7) ligado ao RNA viral. (D) Enzimas virais, representadas em cor-de-rosa. De cima para baixo, respectivamente: transcriptase reversa (representação da interação com o RNA viral no detalhe), integrase e protease. (E) Proteínas acessórias. De cima para baixo, respectivamente: vpu, tat (ligado ao RNA viral), vif (ligada a uma proteína proveniente de uma célula previamente infectada – representada em roxo), vpr, rev (representada apenas uma porção dela ligada ao RNA viral) e nef. Imagens adaptadas do Research Collaboratory for Structural Bioinformatics Protein Data Base (RCSB PDB – acessado pelo endereço www.rcsb.org) (BERMAN et al., 2000) a partir dos PDB ID (códigos de acesso a estruturas proteicas depositadas no PDB) 1HPV (KIM et al., 1995); 1BIV (YE et al., 1995); 1ETF (BATTISTE et al., 1996); 1HIW (HILL et al., 1996); 1AVV (AROLD et al., 1997); 1VPU (WILLBOLD et al., 1997); 1A1T (DE GUZMAN et al., 1998); 1QA5 (GEYER et al., 1999); 1EX4 (CHEN et al., 2000); 1JFW (PELOPONESE et al., 2000); 1HYS (SARAFIANOS et al., 2001); 1ESX (WECKER et al., 2002); 1PI7 (PARK et al., 2003); 3DCG (STANLEY et al., 2008); 3H47 (PORNILLOS et al., 2009) e 4NCO (JULIEN et al., 2013).



2.1.3 Patogênese do HIV-1

O HIV-1 infecta principalmente células T CD4+ auxiliares. As células infectadas liberam partículas virais maduras no plasma sanguíneo e em certas secreções corporais (sêmen, fluido vaginal, leite materno), as quais, quando entram em contato com a circulação sanguínea de outro indivíduo, transmitem o vírus. Isso pode ocorrer através do contato sexual sem preservativo, amamentação ou uso de seringas contaminadas (KILMARX, 2009).

Durante os dias iniciais do contato com o vírus, as células infectadas migram para tecidos linfoides, onde o contato intercelular favorece a disseminação viral. O HIV-1 explora as células, levando a uma intensa replicação. Uma grande quantidade de vírus é produzida (fase aguda da infecção), em níveis que podem ultrapassar um milhão de cópias do vírus por mililitro de plasma (SLEASMAN & GOODENOW, 2003).

Após a fase aguda da infecção, o sistema imune do hospedeiro começa a criar a resposta imune contra a presença do vírus, fazendo com que a viremia seja estabilizada. No entanto, o HIV-1 apresenta vários mecanismos de escape da resposta imune, formando “reservatórios” virais. Transientemente, ocorrem picos de replicação virais, causando doença crônica e assintomática nos tecidos linfoides (FANALES-BELASIO et al., 2010).

Ao longo dos anos de doença crônica pelo HIV-1, a capacidade de restauração do sistema imune vai sendo exaurida e não mais consegue equilibrar as perdas causadas pelo HIV-1, resultando num crescente déficit de células T CD4+ (SLEASMAN & GOODENOW, 2003).

Com isso, 10 anos após à exposição ao HIV-1 (em média), níveis de CD4+ atingem valores abaixo de 200 células/µL de sangue, o que caracteriza o estágio clínico de AIDS, visto que nesse momento, o indivíduo passa a estar em grande risco de desenvolver doença constitucional (sinais e sintomas com duração maior que um mês; febre, diarreia e perda de massa corporal) e cânceres, bem como contrair infecções oportunistas (HUTCHINSON, 2001). Portanto, a infecção por HIV-1 é uma doença crônica e fatal na grande maioria dos casos, se não for adequadamente tratada com antirretrovirais.

2.2 A TERAPIA ANTIRRETROVIRAL

A terapia antirretroviral (TARV) padrão atualmente consiste na ação combinada de três ou mais compostos anti-HIV-1, os quais atuam inibindo diferentes etapas do ciclo replicativo do HIV-1 (LEDERGERBER et al., 1999; CRESSEY & LALLEMANT, 2007), principalmente a replicação do genoma viral e o processamento proteolítico de maturação das proteínas virais (BARTLETT et al., 2001).

Mais especificamente, existem três classes de antirretrovirais em esquemas de primeira linha (ou seja, esquemas oferecidos a pessoas ainda não previamente tratadas): os inibidores análogos a nucleosídeos/nucleotídeos da transcriptase reversa (INTR), os inibidores não-análogos a nucleosídeos/nucleotídeos da transcriptase reversa (INNTR) e os inibidores de protease (IP).

Os INTR interrompem a síntese da cadeia de DNA complementar ao RNA viral, enquanto os INNTR inibem a transcriptase reversa de modo alostérico, ao se ligar em sítios que modificam a conformação tridimensional da transcriptase reversa, impedindo seu funcionamento ideal. Em resumo, ambas as classes de inibidores de transcriptase reversa são capazes de impedir a replicação do genoma viral (FURMAN et al., 1986; CHENG et al., 1987).

Os IP atuam em uma segunda via de ataque, inibindo a protease viral, resultando no bloqueio na maturação das proteínas virais recém-produzidas, abolindo a infectividade das novas partículas virais (THAISRIVONGS et al., 1991).

Diretrizes nacionais estabelecem como regra que a primeira linha de esquema terapêutico inclua dois INTR (tenofovir, TDF e lamivudina, 3TC) mais um INNTR (efavirenz, EFZ), totalizando, portanto, três medicamentos (Tabela 1). Em casos particulares, como por exemplo contraindicação a alguns componentes do esquema, os guias preveem opções de substituir o TDF por outros INTR, como a zidovudina (AZT), abacavir (ABC) ou didanosina (ddI), por exemplo, ou ainda o EFZ pela nevirapina (NVP), outro INNTR (BRASIL, 2015).

Os IP e outras classes de antirretrovirais atualmente são reservados para os casos de falha da primeira linha da TARV, ou seja, quando o paciente não consegue obter a supressão viral. Isso pode ocorrer devido à baixa adesão ao tratamento ou fatores genéticos virais (BRASIL, 2015), que serão explorados nos tópicos a seguir.

Tabela 1. Resumo da recomendação para a primeira linha de um esquema de terapia antirretroviral (TARV).

Componentes da primeira linha da TARV	Antirretroviral
1º INTR	Tenofovir (TDF) **OU** Zidovudina (AZT) - 1ª opção para substituir o TDF **OU** Abacavir (ABC) - 2ª opção para substituir o TDF **OU** Didanosina (ddI) - 3ª opção para substituir o TDF
	+
2º INTR	Lamivudina (3TC)
	+
INNTR	Efavirenz (EFZ) **OU** Nevirapina (NVP) - opção para substituir o EFZ

INTR – inibidor análogo a nucleosídeo/nucleotídeo da transcriptase reversa
INNTR – inibidor não-análogo a nucleosídeo/nucleotídeo da transcriptase reversa

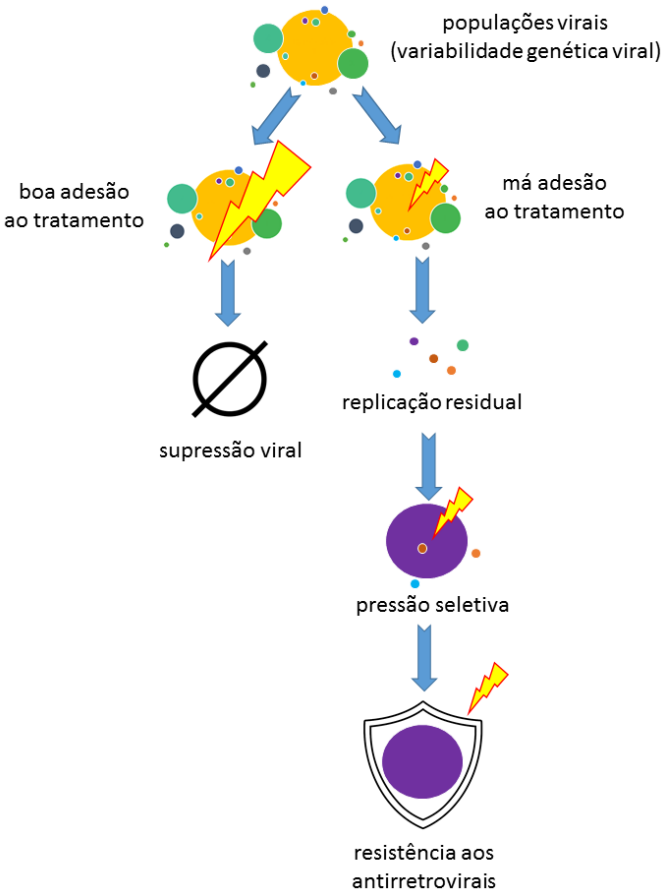
2.3 RESISTÊNCIA AOS ANTIRRETROVIRAIS E RESISTÊNCIA PRIMÁRIA

A alta variabilidade genética do HIV-1 resulta das características próprias da transcriptase reversa, uma polimerase susceptível a erros (introduz uma mutação a cada 10 mil nucleotídeos em média) e sem capacidade revisora. Como bilhões de vírus são produzidos diariamente nas células de um indivíduo infectado, uma variante com uma mutação que confira resistência ao medicamento pode emergir espontaneamente e, caso haja uma adesão inadequada ao tratamento, ser selecionada devido à pressão seletiva imposta pela presença do antirretroviral em concentrações abaixo do ideal no plasma e nos tecidos (Figura 3). Assim, essa variante se tornará cada vez mais frequente, dado que o vírus selvagem ainda assim será eliminado, mesmo com as concentrações inadequadas, e o tratamento perderá sua eficácia. Com isso, o vírus continua replicando no organismo, fenômeno chamado de falha virológica da TARV (TANG & SHAFER, 2012).

Os casos de resistência mais frequentes são resultado de mutações selecionadas pelo uso inadequado de INTR e INNTR. A seleção de apenas uma ou duas mutações no gene da transcriptase reversa são suficientes para causar resistência substancial a vários antirretrovirais da mesma classe simultaneamente (resistência múltipla). Devido a isso, diz-se que esses medicamentos tem uma baixa “barreira genética”. Já a resistência aos IP ocorre em menos frequência, pois são necessárias várias mutações selecionadas em conjunto no gene da protease para haver resistência significativa (alta “barreira genética”) (TANG & SHAFER, 2012).

Com os esforços de pesquisa sobre a ação dos antirretrovirais, atualmente estão disponíveis bancos de dados de sequências genômicas do HIV-1, os quais disponibilizam listas de mutações. Esses dados são usados por algoritmos computacionais atualizados constantemente a partir de resultados obtidos em pesquisas clínicas, de maneira a auxiliar na detecção de mutações de resistência em sequências da população viral infectando cada indivíduo (LIU & SHAFER, 2006). Dessa forma, os médicos que acompanham indivíduos vivendo com o HIV-1 podem usar essas ferramentas de forma comparativa, enviando sequências dos vírus circulantes em pacientes com suspeita de falha virológica para determinar quais variantes genéticas virais foram selecionadas e escolher o melhor “esquema de resgate” a ser adotado (RHEE et al., 2003; LIU & SHAFER, 2006).

Figura 3. Devido à alta variabilidade genética introduzida pela transcriptase reversa do HIV-1, populações geneticamente diversas em frequência variável do vírus circulam simultaneamente no indivíduo infectado (representadas por círculos coloridos de diferentes tamanhos). Na presença de tratamento adequado com boa adesão (representado por um raio grande, lado esquerdo), a replicação viral será suprimida. No entanto, na presença de má adesão (raio menor), ainda que populações virais suscetíveis sejam eliminadas, a replicação residual pode originar populações com algum grau de resistência, que então ficam livres de competição. Elas então aumentam em frequência devido à pressão seletiva dos antirretrovirais, acumulando mutações de resistência, resultando em vírus resistentes aos antirretrovirais (representada por um escudo).



Já se conhecem várias mutações que conferem redução severa na eficácia dos antirretrovirais. Entre as mutações na transcriptase reversa, estão as que são selecionadas pelo uso inadequado dos análogos de timidina (*thymidine analog mutations*, TAM na sigla em inglês), como a AZT, por exemplo. As TAM são trocas específicas de aminoácidos na transcriptase reversa[1]: Met41Leu, Asp67Asn, Lys70Arg, Thr210Trp, Thr215Phe/Tyr e Lys219Gln/Glu que conferem resistência não só à AZT, mas também ao TDF e ao ABC, justamente as opções de INTR de primeira linha. Geralmente, várias TAM ocorrem em conjunto, ilustrando o conceito de baixa barreira genética dos INTR (maior facilidade de seleção de mutações de resistência em conjunto) (RHEE et al., 2004).

Além das TAM, outras mutações comuns da transcriptase reversa de grande impacto na suscetibilidade aos INTR incluem: Lys65Arg, Lys70Glu, Leu74Val/Ile, Tyr115Phe, selecionadas pelo uso inadequado de TDF e ABC e Met184Val/Ile, selecionada pelo uso inadequado de 3TC (RHEE et al., 2004).

Existem ainda outras mutações mais raras na transcriptase reversa que conferem alto grau de resistência aos INTR, como por exemplo mutações na posição 151 ou inserções de aminoácidos ao redor da posição 69, as quais conferem resistência a basicamente todos os INTR disponíveis (GARCIA-LERMA et al., 2000; VAN VAERENBERGH et al., 2000). De um modo geral, essas mutações fazem com que a transcriptase reversa adquira uma certa atividade revisora, impedindo o bloqueio da transcrição reversa que seria promovido pelos antirretrovirais (TANG & SHAFER, 2012).

Mutações na transcriptase reversa que conferem resistência aos INNTR incluem: Leu100Ile, Lys101Glu/Pro, Lys103Asn/Ser, Val106Ala/Met, Glu138Ala/Gly/Lys/Gln, Tyr181Cys/Ile/Val, Tyr188Leu/Cys/His, Gly190Ala/Ser/Glu e Met230Leu, todas selecionadas pela baixa adesão a EFZ e NVP (GERETTI et al., 2014). De um modo geral, essas mutações diminuem a afinidade de ligação dos INNTR aos sítios alostéricos da transcriptase reversa (HSIOU et al., 2001).

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[1]Legenda: sigla do aminoácido original – posição na transcriptase reversa – sigla(s) do(s) aminoácido(s) novo(s) possíveis. Exemplo: Met184Val/Ile – troca de uma metionina na posição 184 da transcriptase reversa para uma valina ou isoleucina (nesse caso, duas mutações possíveis para essa posição).

Mutações que ocorrem no polipeptídeo da protease que promovem resistência aos IP incluem Aps30Asn, Val32Ile, Met46Ile/Leu, Ile47Val/Ala, Gly48Val/Met, Ile50Leu/Val, Ile54Val/Thr/Ala/Leu/Met, Leu76Val, Val82Ala/Thr/Phe/Ser/Leu, Ile84Val, Asn88Ser/Asp e Leu90Met (RHEE et al., 2010). Essas mutações diminuem a capacidade de ligação dos IP no sítio ativo da protease, porém dificulta o processamento das proteínas do próprio vírus, diminuindo seu *fitness* replicativo. Curiosamente, se a exposição inadequada aos IP for prolongada, mutações secundárias são selecionadas, as quais restauram um pouco da capacidade replicativa, porém mantendo a resistência aos IP. Dessa forma, a resistência aos IP ocorre de maneira mais complexa, por etapas, necessitando várias mutações em conjunto para que seja clinicamente significativa.

O vírus com as mutações de resistência descritas acima pode então ser transmitido a outras pessoas. Esse fenômeno é chamado de “resistência primária aos antirretrovirais”, pois o indivíduo apresenta um grau de resistência já ao diagnóstico da infecção pelo HIV-1, o que posa como obstáculo ao tratamento de primeira linha. No Brasil e em outros países em desenvolvimento, o teste de genotipagem viral pré-tratamento ainda não é recomendado em todas as situações. Assim, uma pessoa em resistência primária precisa trocar para esquemas de segunda linha mais precocemente, os quais são mais caros e seu uso vem acompanhado de mais efeitos adversos (BRASIL, 2015).

Portanto, o monitoramento da prevalência populacional da resistência primária aos antirretrovirais nos países em desenvolvimento é um ponto chave para a garantia da continuidade do sucesso dos esquemas de primeira linha da TARV, especialmente no momento atual, devido aos novos paradigmas de tratamento (*test and treat*) e com o estabelecimento das metas 90-90-90. Por essa razão, foi produzida uma revisão sistemática suportada por meta-análises e análise de séries temporais para avaliar o atual *status* da prevalência da resistência primária aos antirretrovirais em países da América Latina e do Caribe (COELHO et al., 2015), como abordado no Capítulo I adiante.

2.4 EFEITOS ADVERSOS DA TERAPIA ANTIRRETROVIRAL

Uma reação adversa é definida como um efeito desagradável e não-intencional, que ocorre com doses normalmente usadas durante um tratamento ou que cause

alterações na funções de certos órgãos, e que requer manejo, como por exemplo, alteração do regime de dosagem ou até mesmo descontinuação do tratamento (EDWARDS & ARONSON, 2000).

No caso da TARV, desde a sua introdução em meados da década de 1990, já estava claro que reações adversas e a toxicidade dos fármacos seriam um problema importante, dado que é um tratamento vitalício, visto que a TARV não elimina o vírus do organismo, sendo apenas capaz de suprimir sua replicação (ZAHRAN, 2004).

Entre os efeitos adversos relacionados aos antirretrovirais, estão: síndrome metabólica, caracterizada por obesidade abdominal, hiperlipidemia, elevação na glicemia, hipertensão arterial, e redução nos níveis de colesterol de alta densidade e resistência à insulina (RIBAUDO et al., 2010); lipodistrofia, caracterizada por anormalidades na distribuição de gordura corporal, ocorrendo ganho de gordura subcutânea em algumas regiões (abdome, seios, na região cervical posterior) e/ou perda em outras (bochechas, glúteos) (GRINSPOON & CARR, 2005; VILLARROYA et al., 2010); erupções cutâneas (*rash*) (CARR & COOPER, 2000); efeitos neuropsiquiátricos, como distúrbios do sono (sonhos vívidos, insônia, sonolência), tontura, dificuldade de concentração, confusão, irritabilidade e dor de cabeça (CLIFFORD et al., 2005); distúrbios gastrointestinais e sistêmicos, como a febre (JACOBSON et al., 1989; VELLA et al., 1994).

O metabolismo dos antirretrovirais é um fenômeno complexo e, portanto, pode variar entre os indivíduos. Acredita-se que as variações genéticas entre as pessoas representem uma parte importante desta variabilidade (TOZZI, 2009).

O avanço da biologia molecular e sequenciamento do genoma humano permitiu o desenvolvimento da farmacogenética, o estudo de como variantes genéticas localizadas em genes codificantes de proteínas envolvidas em vias metabólicas de medicamentos influenciam no resultado final da terapia. Dessa maneira, a farmacogenética possui um grande potencial para otimizar o uso das mais diversas drogas, avaliar o uso clínico de fármacos recém descobertos, bem como servir de diretriz para o desenvolvimento de novos medicamentos (KALOW, 2002; SHAH, 2005).

Um eventual acompanhamento dos pacientes HIV-1 positivos baseado na avaliação do perfil farmacogenético para a prescrição da TARV possuiria uma grande vantagem em potencial de manejo do uso desses medicamentos, como por exemplo,

ajustamento de doses, diminuindo a possibilidade de ocorrência ou severidade de efeitos adversos (TELENTI & ZANGER, 2008).

2.5 FARMACOGENÉTICA

Três importantes superfamílias gênicas que codificam proteínas importantes nas vias farmacológicas de antirretrovirais: a família caracterizada pelo cassete ligante de adenosina trifosfato (ATP), ou ABC (do inglês *ATP-binding cassette*), a de carreadores de solutos, SLC (do inglês *solute carrier*) e a dos citocromos P450, ou CYP, do inglês *cytochrome P450* (INGELMAN-SUNDBERG et al., 2007; KIS et al., 2010).

Fisiologicamente, ou seja, na ausência de medicamentos, essas proteínas exercem funções celulares, como por exemplo, transporte de nutrientes e catabolismo de hormônios. Os antirretrovirais possuem semelhança molecular aos seus substratos fisiológicos e, portanto, essas proteínas acabam interagindo com os medicamentos e assim estabelecendo suas vias farmacológicas.

2.5.1 Superfamília cassete ligante de adenosina-trifosfato (ABC)

Essa superfamília é composta por mais de 40 genes humanos que codificam para proteínas transmembrana, as quais possuem domínios na porção citoplasmática onde a adenosina-trifosfato (ATP) se liga, por isso a razão de seu nome (ATP-*binding cassette*). Essas proteínas usam a hidrólise de ATP para fornecer a energia necessária para transportar moléculas grandes e hidrofóbicas para o meio extracelular (transporte ativo). A função dessas proteínas parece ser a de proteção contra substâncias estranhas e potencialmente tóxicas às células, medicamentos inclusive (BORST & ELFERINK, 2002).

No contexto da farmacologia de antirretrovirais, proteínas das subfamílias B, C e G (ABCB, ABCC e ABCG, respectivamente) são importantes constituintes de vias de transporte de antirretrovirais. A glicoproteína de permeabilidade (p-gp ou ABCB1, codificada pelo gene *ABCB1*), a proteína membro 1 da subfamília de proteínas de múltipla resistência a drogas (MRP1 ou ABCC1, codificada pelo gene *ABCC1*) e a proteína membro 2 da subfamília G (ABCG2, codificada pelo gene *ABCG2*) são envolvidas na expulsão de vários IP e da AZT do ambiente celular (HOCHMAN et al., 2001; COLOMBO et al., 2005; SHAIK et al., 2007; FUJIMOTO et al., 2009; JANNEH

et al., 2009; FRANKE et al., 2010; GIRAUD et al., 2010; KÖNIG et al., 2010; MOSS et al., 2011).

Os genes da superfamília ABC são expressos em diversos tecidos de importância na farmacologia dos antirretrovirais, tais como os intestinos, na barreira hematoencefálica, fígado e nos rins, visto que a expressão nesses órgãos limitam a absorção, presença dos antirretrovirais em reservatórios do HIV-1 (sistema nervoso central, testículos) e contribuem para sua excreção na bile ou urina. Além disso, ABCC1 é expresso também em linfócitos, contribuindo para a expulsão dos antirretrovirais das células alvo do vírus (FRANKE et al., 2010; KIS et al., 2010).

2.5.2 Superfamília carreadora de solutos (SLC)

A superfamília SLC é composta por mais de 300 proteínas estruturalmente diversas. São proteínas de membrana que promovem o transporte facilitado (ou passivo) de nutrientes, neurotransmissores e medicamentos (URBAN et al., 2006; HOGLUND et al., 2011).

Entre as diversas famílias de transportadores, as famílias de transportadores de cátions orgânicos (SLC22) participam do transporte facilitado de INTR para o interior das células, sendo a proteína SLC22A6 (membro 6 da subfamília A de transportadores de cátions orgânicos) uma das participantes nesse processo; esse gene é expresso principalmente nos rins, contribuindo para a excreção do TDF pelos túbulos renais (HAAS & TARR, 2015).

2.5.3 Superfamília citocromo P450 (CYP)

Os citocromos P450 envolvem mais de 50 enzimas cujos sítios ativos contêm um grupamento prostético heme. Eles promovem reações de oxidação de vários compostos endógenos, como ácidos graxos, esteroides e prostaglandinas e exógenos, como vários medicamentos usados na prática clínica. Essas reações fazem com que as drogas sejam biotransformadas em compostos oxidados mais solúveis em água e então passíveis de excreção na urina (ZANGER et al., 2008).

Os citocromos P450 provêm de genes muito polimórficos, resultando em várias isoformas proteicas. De uma forma geral, são expressos principalmente no fígado, oxidando os INNTR mais usados na TARV, como por exemplo o efavirenz (EFZ) e a nevirapina (NVP), desativando-os e permitindo sua excreção do organismo (KENT et

al., 2002; WARD et al., 2003). Algumas isoformas de destaque no metabolismo de INNTR incluem a CYP1A2, a CYP3A4, a CYP2A6 e a CYP2B6 (WARD et al., 2003; OGBURN et al., 2010; RIBAUDO et al., 2010).

2.5.4 Pirofosfatase de inosina-trifosfato (ITPA)

Além das superfamílias gênicas descritas acima, potenciais novos genes poderiam ser investigados para ajudar a desvendar a variação interindividual na ocorrência de efeitos adversos. Entre esses novos candidatos estaria o *ITPA*, gene da pirofosfatase da inosina trifosfato ou ITPA (*inosine triphosphatase pyrophosphatase*, em inglês).

O ITPA é uma enzima de manutenção celular que remove fosfatos da inosina tri-fosfato (ITP) e da deoxi-ITP (dITP), convertendo-a em suas formas mono-fosfatadas (IMP e dIMP), portanto acredita-se que essa função sirva para manter a integridade do genoma, pois ITP pode ser erroneamente incorporada durante a replicação de DNA, o que promoveria mutações (VON AHSEN et al., 2008).

Como a ITPA é uma enzima que regula uma parte dos estoques de nucleosídeos da célula, a administração dos INTR poderia afetar a atividade da ITPA. Dessa forma, polimorfismos no *ITPA* poderiam estar presentes na população em geral e poderiam influenciar perfis de efeitos adversos relacionados com os INTR administrados em esquemas de TARV, o que gerou um estudo do tipo caso-controle em parceria com pesquisadores da Universidade de Trieste (Itália), reportado no manuscrito presente no Capítulo II.

2.5.5 Proteína membro 3, grupo I, subfamília 1 de receptores nucleares (NR1I3)

Os receptores nucleares são proteínas são “sensores” de moléculas exógenas, ativando a expressão de enzimas metabolizadoras, promovendo a eliminação dessas substâncias do organismo (WANG et al., 2003; CHEN et al., 2012). A proteína membro 3, grupo I, subfamília 1 de receptores nucleares (NR1I3), também conhecida como receptor constitutivo de androstano (do inglês *constitutive androstane receptor*, CAR), por exemplo, é reguladora da transcrição de uma grande variedade de genes, cujos produtos metabolizam uma vasta gama de fármacos (CHEN et al., 2012; PIEDADE et al., 2012).

Como mencionado anteriormente, os INNTR são metabolizados pelas proteínas do sistema CYP e por glucuronosiltransferases hepáticas. A NR1I3 induz a expressão destas proteínas na presença de EFZ, desempenhando um papel na metabolização basal desse INNTR (HARIPARSAD et al., 2004; CESPEDES & ABERG, 2006; WYEN et al., 2011).

2.6 VACINA TERAPÊUTICA ANTI-HIV-1 BASEADA EM CÉLULAS DENDRÍTICAS

A ativação da resposta imune celular durante infecções virais é mediada pelas células apresentadoras de antígenos, tais como os macrófagos, linfócitos-B e, principalmente, pelas células dendríticas, as quais atuam na captura de antígenos e ativação de linfócitos imaturos (STEINMAN, 2007; TAKEUCHI & AKIRA, 2009).

Precursoras das células dendríticas são geradas na medula óssea e depois migram para os diversos tecidos, adquirindo diferentes potenciais imunológicos, induzidas por estímulos locais específicos (BUCKWALTER & ALBERT, 2009). A capacidade fagocítica das células dendríticas permite que atuem como sentinelas nos sítios periféricos do organismo, como epitélios e mucosas genitais, que são as principais vias de entrada do HIV-1, fazendo uma primeira linha de defesa (WU & KEWALRAMANI, 2006).

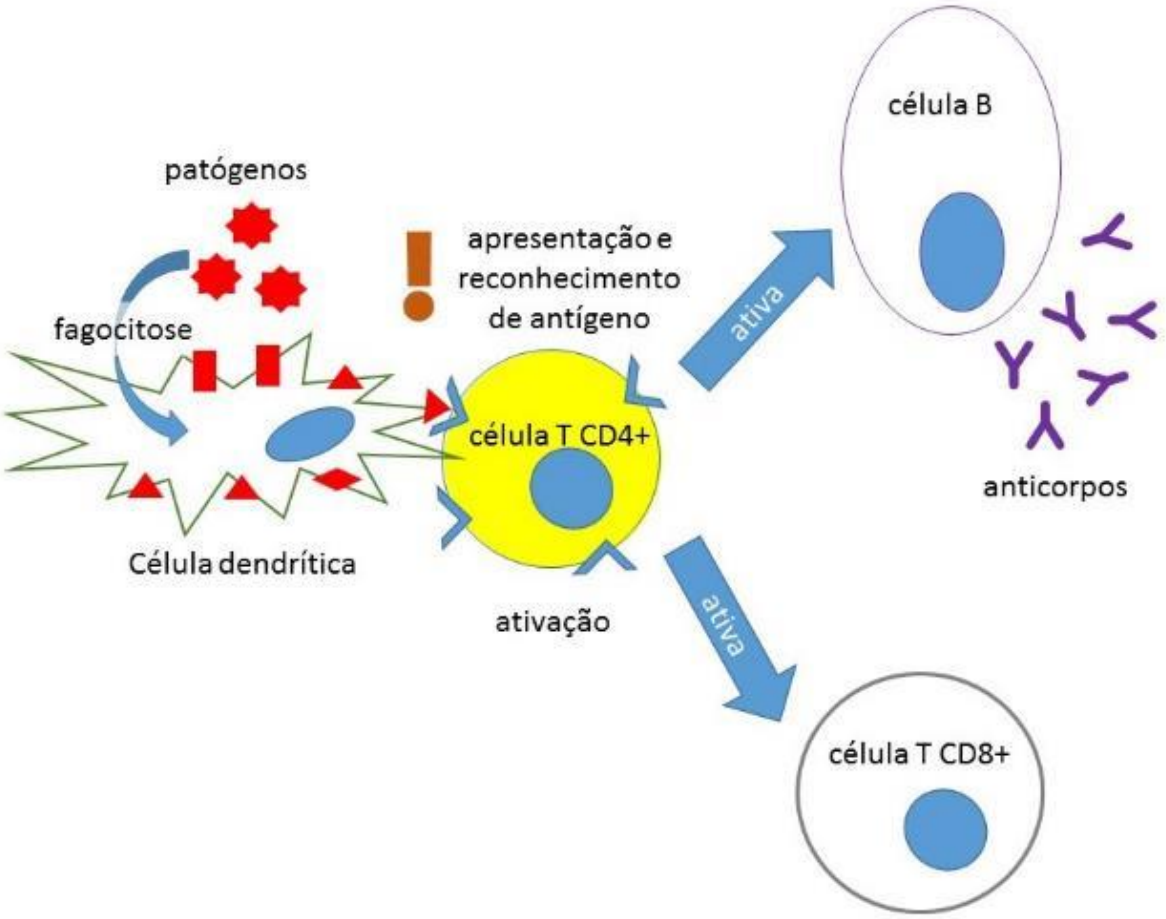
As células dendríticas possuem um grande repertório de proteínas receptoras de reconhecimento de padrões (*pattern recognition receptors,* PRR na sigla em inglês) moleculares (antígenos) provenientes de patógenos (*pathogen-associated molecular patterns*, ou PAMP na sigla em inglês). (MOGENSEN et al., 2010).

A ligação dos PAMP às PRR promove a transdução de sinais de fatores de transcrição nas células dendríticas que resultam na ativação de linfócitos (IWASAKI & MEDZHITOV, 2010; MOGENSEN et al., 2010), por meio da produção de citocinas pró-inflamatórias, como o interferon do tipo 1. Além disso, moléculas coestimulatórias também liberadas pelas células dendríticas promove maior permeabilidade dos vasos sanguíneos, facilitando a chegada de células efetoras aos sítios de infecção e ativação mais eficiente de linfócitos CD8+ e CD4+ HIV-1-específicos (BUCKWALTER & ALBERT, 2009; MANEL et al., 2010).

Devido a essa grande importância das células dendríticas na apresentação de antígenos, já que desempenham um papel central no sistema imune, indo desde a captura de antígenos, migração para os linfonodos até a ativação de células T

imaturas (Figura 4), foram feitas tentativas de estratégias vacinais terapêuticas baseadas na utilização de células dendríticas para “recuperar” a resposta do hospedeiro à infecções virais, incluindo o HIV-1 (LU et al., 2004).

Figura 4. As células dendríticas são uma das primeiras linhas de defesa contra patógenos em tecidos como as mucosas anogenitais, principais vias de infecção do HIV-1. Após fagocitarem os patógenos circulantes, elas migram para linfonodos próximos e apresentam seus antígenos na membrana para os linfócitos T CD4+ auxiliares presentes, que então são ativados. A partir de citocinas, auxiliam na ativação de linfócitos B e linfócitos T CD8+ citotóxicos, que por sua vez vão efetuar a resposta contra o patógeno em questão.



Durante a infecção pelo HIV-1, os anticorpos produzidos pelo indivíduo infectado não são capazes de neutralizar completamente os vírus circulantes. Dessa forma, boa parte do controle da replicação viral depende da ação dos linfócitos T CD8+ citotóxicos. Por essa razão, a ideia por trás do desenvolvimento de uma vacina terapêutica é “requalificar” o sistema imune para melhorar sua resposta durante infecções crônicas, como no caso do HIV-1, de forma a estabilizar a carga viral do paciente de maneira a permitir a suspensão segura ou adiamento do uso da TARV e fazer com que a concentração de HIV nos fluidos sexuais seja diminuído a ponto de

reduzir o risco de transmissão para outas pessoas. Em outras palavras, uma vacina eficaz transformaria todos os infectados pelo HIV-1 em “controladores de elite” (ANDRIEU & LU, 2007).

Um ensaio clínico de fase I de uma vacina terapêutica baseada em células dendríticas autólogas (provenientes do próprio indivíduo) foi realizado em Recife entre 2002 e 2003. Dezoito pessoas vivendo com o HIV-1 ainda virgens de tratamento foram recrutadas para o ensaio. O tempo médio de infecção era em torno de dois anos; possuíam cargas virais de 11.000 a 300.000 cópias de RNA do vírus por mililitro de plasma (mediana de 48.000) e contagem de células T CD4+ variando de 270 a 1009 células por microlitro de sangue (mediana de 523). Resumidamente, células mononononucleares do sangue periférico (PBMC) foram isoladas dos pacientes por leucoferese. Em seguida, monócitos foram isolados do total de células e postos em cultura com citocinas específicas para que se transformasse em células dendríticas imaturas, as quais foram postas em contato com vírus autólogos inativados com aldritiol-2, para que sofressem maturação. Com as células dendríticas maduras então foram preparadas três doses de vacinas injetadas por via subcutânea em intervalos de duas semanas (LU et al., 2004; ANDRIEU & LU, 2007).

Quatro meses após a primeira dose, a carga viral média foi reduzida em 80% nos 18 pacientes. Um ano após, a carga viral estava reduzida em mais de 90% em oito dos 18 pacientes, e ainda estavam virgens de tratamento. Dois anos após a vacinação, dois indivíduos dentre esses oito estavam com cargas virais abaixo das mil cópias de RNA por mililitro de plasma, enquanto as dos demais estavam voltando a aumentar. A vacina terapêutica então foi capaz de provocar a proliferação e maturação de linfócitos T CD8+ citotóxicos em pelo menos alguns dos pacientes, reduzindo bastante a replicação viral (LU et al., 2004; ANDRIEU & LU, 2007).

Pode-se observar que a vacina não funcionou da mesma forma para todos os indivíduos recrutados para o ensaio. Apesar de variáveis clínicas, imunológicas e de desenho do estudo terem sido levadas em consideração e embora todos os indivíduos estiveram sujeitos ao mesmo esquema de tratamento, o genoma dos indivíduos não foi analisado. Evidências mostram que a genética individual influencia na defesa contra o HIV-1 e progressão para doença (AN & WINKLER, 2010), então levantou-se a hipótese de que a genética dos indivíduos recrutados para o ensaio clínico modulou a resposta à vacina terapêutica. Para isso, foi realizado uma análise *genome-wide*

retrospectiva com o material genético dos indivíduos participantes (MOURA et al., 2014). Posteriormente, foi realizada uma revisão sistemática seguida de uma meta-análise de protocolos de vacinação experimentais baseados em células dendríticas de modo a reunir conhecimento a partir de experiências passadas com o intuito de melhorar futuras estratégias de cura da infecção pelo HIV-1, reportada no Capítulo III adiante.

2.7 FALHA IMUNOLÓGICA DA TERAPIA ANTIRRETROVIRAL

É consenso que desde a introdução das drogas antirretrovirais na prática clínica, milhões de vidas foram salvas. Contudo, em alguns pacientes são observadas falhas no tratamento, consistindo na incapacidade de reduzir a carga viral a níveis indetectáveis, no aparecimento de efeitos adversos severos ou na não recuperação da concentração de linfócitos T CD4+ (COELHO et al., 2013).

O restabelecimento das concentrações de linfócitos T CD4+ é tipicamente um processo bifásico que inclui um rápido crescimento nos primeiros 3-6 meses, refletindo a redistribuição das células T de memória, seguido por um crescimento lento que reflete a recuperação central de células T CD4 *naïve* pelo timo. Contudo, uma parte dos pacientes apresentam falha na recuperação dos níveis de células T CD4+, mesmo com a total supressão da replicação do HIV-1 por um longo tempo. Este fenômeno é conhecido como falha imunológica. Elucidar os mecanismos da falha imunológica é de fundamental importância, pois os indivíduos que permanecem com baixos níveis de linfócitos T CD4+ periféricos possuem um risco aumentado de desenvolverem complicações relacionadas ou não à AIDS, incluindo doenças cardiovasculares, do fígado e dos rins (LI et al., 2011).

Apesar de mais de três décadas de estudo, os mecanismos que determinam precisamente a determinação das concentrações de CD4+ durante a TARV ainda não foram elucidados. Alguns fatores de risco relacionados com a dificuldade na reconstituição imune são conhecidos, tais como idade avançada, sexo masculino, baixa contagem de células CD4+ pré-tratamento, duração da infecção pelo HIV-1 e coinfecção com outros vírus, como o da hepatite C (AIUTI & MEZZAROMA, 2006; LI et al., 2011; DOITSH et al., 2014).

No entanto, ainda não se conhece com profundidade variantes genéticas que possam estar envolvidos na falha imunológica da TARV. Possíveis genes candidatos

incluiriam (além dos genes envolvidos em vias de farmacodinâmica de antirretrovirais citados previamente) os genes envolvidos na homeostase do sistema imune. Evidências mostram que interleucinas (IL), proteínas envolvidas em diversas outras funções do sistema imune, principalmente a IL-2, IL-7, a IL-15 e seus respectivos receptores de membrana coordenam a proliferação de células T CD4+ durante a recuperação do sistema imune permitida pela presença dos antirretrovirais (LEVY, 2006).

Dessa forma, polimorfismos nesses genes influenciariam na reconstituição do número de células T CD4+, e atuariam em conjunto com polimorfismos de único nucleotídeo (*single nucleotide polymorphisms*, SNP na sigla em inglês) em genes de vias farmacológicas, de modo a contribuírem para o resultado final da terapia: sucesso (reconstituição imune) ou falha (reconstituição imune insuficiente) da TARV. Os resultados acerca dessa abordagem geraram um manuscrito reportado no Capítulo IV adiante. O estudo também foi realizado no IMIP e aprovado pelo Comitê de Ética local (parecer disponível no Anexo A).

2.8 ATIVAÇÃO E HOMEOSTASIA DO SISTEMA IMUNE

2.8.1 Citocinas e seus receptores

As citocinas são moléculas regulatórias de processos imunológicos. São produzidas por células do sistema imune, principalmente macrófagos e células apresentadoras de antígeno (monócitos, células dendríticas) em resposta a patógenos. Os invasores são capturados por essas células, destruídos e seus antígenos são processados para serem apresentados a linfócitos T auxiliares (CD4+). As citocinas secretadas no ambiente extracelular então modulam a atividade dessas células para combater as infecções, por meio do desencadeamento de vias de transdução de sinal ao se ligarem em receptores de membrana, consequentemente modulando a expressão de conjuntos de genes determinados (BORISH & STEINKE, 2003).

Dentre as citocinas, as interleucinas compõe a classe mais numerosa de citocinas, possuindo várias atividades, por vezes redundantes, na ativação e homeostasia do sistema imune. Por exemplo, a interleucina 1 beta (IL-1β) é geralmente reconhecida como uma indutora de inflamação (pró-inflamatória), enquanto as IL-4 e 10 possuem uma atividade anti-inflamatória; já a IL-2 e a IL-15 têm

importante papel na imunidade celular, atuando na proliferação de células T e B enquanto que a IL-7 está envolvida no desenvolvimento das células T no timo; o interferon gama (IFN-γ) é muito importante na imunidade mediada por células, regulando a expressão de antígenos do complexo de histocompatibilidade principal e tem aditividade antiviral (BORISH & STEINKE, 2003; NASI et al., 2005; LEVY, 2006).

Outra classe importante das citocinas são a das quimiocinas, moléculas que induzem a quimiotaxia de células do sistema imune (neutrófilos, monócitos, linfócitos, dentre outros), levando-as a sítios de infecção. Além da quimiotaxia, promovem outras funções. A quimiocina ligante 5 (assinatura C-C) (do inglês *chemokine (C-C motif) ligand 5*, CCL5), por exemplo, também auxilia na proliferação de células T (BORISH & STEINKE, 2003).

Por isso, variantes genéticas em citocinas podem ter consequências patológicas, levando a doenças crônicas ou aumentando risco de infecções (SMITH & HUMPHRIES, 2009).

2.8.2 Antígeno FAS

O antígeno FAS, codificado pelo gene *FAS*, é um receptor da família do fator de necrose tumoral presente na membrana das células. Quando o ligante fisiológico (FAS-ligante) interage com FAS, é desencadeada a via extrínseca da morte celular programada, a apoptose (STRASSER et al., 2009).

A apoptose é um fenômeno fisiológico essencial no desenvolvimento e homeostase dos tecidos no organismo, porém é mais conhecida como um mecanismo de defesa das células para tentar impedir a progressão de infecções virais. Durante a infecção pelo HIV-1, a apoptose é um dos principais mecanismos que levam à perda de células T (STRASSER et al., 2009; CUMMINS & BADLEY, 2010).

Evidências mostram que a apoptose persiste em pacientes com falha imunológica da terapia (PITRAK et al., 2015). Dessa forma, investigar variações genéticas em genes de vias de apoptose pode trazer entendimento acerca da ocorrência de falha imunológica da TARV.

3 OBJETIVOS

3.1 OBJETIVO GERAL

Abordar aspectos da história natural da infecção pelo HIV-1 e seu tratamento pela óptica da genética.

3.2 OBJETIVOS ESPECÍFICOS

- Revisar criticamente a literatura sobre a prevalência da resistência primária aos antirretrovirais em países em desenvolvimento;
- Avaliar se polimorfismo no gene *ITPA* estaria associado com a ocorrência de efeitos adversos relacionados ao uso da AZT em esquemas de primeira linha de TARV;
- Avaliar se diferentes perfis genéticos de genes envolvidos em vias farmacológicas de antirretrovirais e de genes envolvidos na homeostase do sistema imune estão relacionados com a falha imunológica da TARV;
- Descobrir variantes genéticas envolvidas na variabilidade interindividual da resposta a uma vacina terapêutica anti-HIV-1 baseada em células dendríticas retrospectiva em uma amostra de indivíduos voluntários vacinados. Paralelamente, revisar outras variáveis não-genéticas também envolvidas nessa resposta.

4 MATERIAL E MÉTODOS

4.1 POPULAÇÃO E DESENHO DE ESTUDOS

A população escolhida para os estudos reportados nos Capítulos II e IV consistiu em indivíduos vivendo com o HIV-1 em atendimento no serviço do Hospital Dia do Instituto de Medicina Integral Professor Fernando Figueira (IMIP). Os pacientes foram selecionados através de busca ativa. Após apresentação dos estudos, os pacientes que se voluntariaram assinaram um termo de consentimento livre e esclarecido, responderam a um questionário clínico e epidemiológico e tiveram amostras de sangue colhidas para proceder-se à extração de DNA genômico. O desenho metodológico do estudo foi aprovado pelo comitê de ética em pesquisa do IMIP (o parecer encontra-se no Anexo A). Os termos de consentimento livre e esclarecido e o questionário clínico e epidemiológico encontram-se nos Apêndices A e B, respectivamente.

O tipo de estudo escolhido foi o de caso-controle, que é um estudo epidemiológico observacional e retrospectivo, que procura identificar fatores de risco após o surgimento de um determinado resultado clínico, comparando-se duas amostras, uma cujos indivíduos apresentem o resultado clínico e outra cujos indivíduos não apresentem (SCHULZ & GRIMES, 2002).

Os critérios de inclusão de pacientes foram: idade superior a 18 anos e recebimento de TARV a pelo menos um ano; critérios gerais de exclusão foram: gravidez no momento do recrutamento, histórico de uso de drogas ilícitas e presença de doença autoimunes.

A classificação no grupo de casos ou no grupo de controles dependeu da questão clínica investigada: pacientes reportando efeitos adversos (anemia, dislipidemia, febre, toxicidade gastrointestinal, dor de cabeça, hepatotoxicidade, neurotoxicidade ou dermatite – *rash* cutâneo) foram classificados como casos, enquanto pacientes não reportando efeitos foram classificados como controles (Capítulo II). Pacientes em falha imunológica foram classificados como casos e pacientes em sucesso imunológico foram classificados como controles (Capítulo IV). Para essa classificação, foram considerados apenas os pacientes com supressão viral persistente, definida como carga viral plasmática (CVp) mantida indetectável (CVp<50 cópias de RNA viral/mL de sangue), ou seja, sem a ocorrência de episódios de rebote

viral (definido como duas quantificações de CVp>200 cópias/mL de sangue consecutivas ocorridas após um período de supressão viral).

Em seguida, o sucesso imunológico foi definido como a obtenção de porcentagem de células T CD4+ iguais ou maiores que 30% por duas quantificações consecutivas. A falha imunológica, portanto, foi definida como a inabilidade de obtenção desses valores, seguindo a classificação adotada pelo Ministério da Saúde (BRASIL, 2015).

Caso valores de porcentagem de células T CD4+ estivessem indisponíveis, ou o paciente já ter começado a TARV com valores superiores a 30%, uma classificação alternativa foi usada, usando os ganhos líquidos na contagem de células CD4+ T na comparação dos valores pré-tratamento com o valor após um ano de terapia. Dessa forma, sucesso imunológico foi definido como ganho igual ou superior a 200 células/µL de sangue, enquanto falha imunológica foi, portanto, definida como ganho líquido inferior a 200 células/µL quando comparado a valores obtidos antes do início da TARV.

4.2 DADOS COLETADOS

Os pacientes são acompanhados mensalmente pelos médicos do IMIP, para avaliação clínica e recebimento de prescrição dos antirretrovirais. Todo o histórico clínico dos pacientes é registrado em prontuários, os foram revisados para a coleta de resultados de exames de contagem de células T CD4+ e quantificação de carga viral, que são repetidos a cada três meses, geralmente, além de hemogramas, de urina e sorologias de coinfecções (hepatites B e C, sífilis, HTLV-1/2, citomegalovírus e toxoplasmose e interpretação de prova tuberculínica). Além disso, sexo, idade na data de início da TARV, etnia e massa corporal também foram levadas em consideração.

4.3 EXTRAÇÃO DE DNA GENÔMICO

O DNA genômico foi extraído a partir das células mononucleares presentes nas amostras de sangue periférico cedidas pelos voluntários por meio do método *salting-out*, com modificações (MILLER et al., 1988).

4.4 GENES CANDIDATOS

Os genes e polimorfismos candidatos foram selecionados através de busca na literatura e por critérios funcionais. Foram escolhidos 47 SNP em 20 genes envolvidos em vias farmacológicas dos medicamentos usados na primeira linha terapêutica anti-HIV-1 e outros relacionados com a homeostase celular e imunidade (Tabela 2).

Tabela 2. Lista de genes e polimorfismos candidatos para estudo de associação genética para explicar a ocorrência de falha imunológica da terapia anti-HIV-1.

Gene	SNP (identificador rs)	Função do gene
ABCB1	rs1128503, rs2214102, rs2235048 e rs3842	Transporte de antirretrovirais
ABCC1	rs129081, rs113264879, rs4148380, rs8056298, rs212091 e rs16967632	Transporte de antirretrovirais
ABCG2	rs115770495, rs1448784 e rs2231142	Transporte de antirretrovirais
CYP1A2	rs762551	Metabolização (excreção) de antirretrovirais
CYP2A6	rs8192726	Metabolização (excreção) de antirretrovirais
CYP2B6	rs8192709, rs28399499, rs34097093, rs28399502, rs707265 e rs1042389	Metabolização (excreção) de antirretrovirais
CYP3A4	rs4646437	Metabolização (excreção) de antirretrovirais
ITPA	rs1127354	Gene *housekeeping* (manutenção celular)
SLC22A6	rs11568628, rs11568629 e rs4149170	Transporte de antirretrovirais
NR1I3	rs3003596	Regulação do metabolismo de antirretrovirais
CCL5	rs2107538	Quimiociona imunoregulatória
FAS	rs1800682 e rs2234767	Regulação da apoptose
IFNG	rs2069709	Citocina imunoregulatória
IL10	rs1800871, rs1800890 e rs2222202	Citocina imunoregulatória
IL10RA	rs3135932 e rs9610	Receptor da IL-10
IL15	rs10519613 e rs10833	Ativação de células T
IL1B	rs16944	Citocina pró-inflamatória
IL2	rs2069762	Proliferação de células T e B
IL4	rs2243250	Citocina imunoregulatória
IL7R	rs11567762, rs1494555, rs3194051, rs3822731, rs6897932 e rs987106	Desenvolvimento de células T

4.5 GENOTIPAGEM DE POLIMORFISMOS

4.5.1 Reação em cadeia da polimerase em tempo real com sondas TaqMan

O SNP rs1127354 (94 C>A, Pro32Thr) do gene *ITPA* foi genotipado por meio de ensaio de sondas *TaqMan* (C_27465000_10), seguindo as instruções do fabricante num termociclador Applied Biosystems 7500 (Life Technologies, anteriormente Applied Biosystems, Foster City, Califórnia, EUA): desnaturação inicial a 95 ºC por 10 min, seguida por 35 ciclos de amplificação (desnaturação a 95 ºC por 15 s e anelamento/extensão a 60 ºC por 1 min). Os genótipos foram determinados através de discriminação por sinais de fluorescência alelo-específicos. Os resultados da genotipagem estão detalhados no Capítulo II.

De modo breve, as sondas *TaqMan* são uma das metodologias de reação em cadeia da polimerase (PCR) em tempo real mais utilizadas. A PCR em tempo real é uma variação da PCR convencional, que é realizada com uma molécula, que, quando excitada por um comprimento de onda apropriado, é capaz de emitir um sinal de fluorescência, a qual é captada em tempo real por um sistema óptico acoplado ao termociclador. As sondas *TaqMan* consistem em um par de primers e pelo menos um par de sondas. Cada sonda possui duas moléculas acopladas nas regiões 5’ e 3’ respectivamente: uma fluorescente (*reporter*) e outra que absorve a fluorescência da primeira (*quencher*). Durante a PCR, a sonda hibrida na região do polimorfismo; em seguida, os primers se hibridam, flanqueando a sonda. A DNA polimerase Taq estende a cadeia a partir dos primers, vai de encontro à sonda e a hidrolisa. Assim, a molécula *reporter* fica livre da *quencher* e passa a emitir fluorescência específica (HOLLAND et al., 1991) para o alelo encontrado naquela região; se houver a emissão de dois tipos de fluorescência (duas sondas hibridaram), a amostra provém de um indivíduo heterozigoto para a região analisada.

4.5.2 Tecnologia Illumina

Os demais SNP dispostos na Tabela 2 foram genotipados com a tecnologia multiplex GoldenGate® Illumina na plataforma de VeraCode® de acordo com as instruções do fabricante. De modo breve, a tecnologia GoldenGate é capaz de determinar o genótipo de vários polimorfismos simultaneamente (*multiplex*) com o auxílio de partículas paramagnéticas. Para isso, o DNA genômico precisa ser

preparado com tampões especiais para hibridização de oligonucleotídeos alelo e lócus-específicos. Os oligonucleotídeos alelo-específicos diferem na última base 3’ (para discriminação alélica) e em sequências universais na região 5’ sobressalente (que não hibridiza no DNA genômico). Os oligonucleotídeos lócus-específicos contém uma sequência de endereçamento que identifica um dado SNP em um dado ensaio, bem como uma sequência universal na região 3’ sobressalente. Depois de uma lavagem, é realizada uma etapa de extensão e ligação dos oligonucleotídeos, gerando moldes para uma PCR universal com os *primers* marcados com fluoróforos alelo-específicos. Em seguida, os produtos de PCR são finalmente hibridizados nas partículas paramagnéticas por complementariedade com a sequência de endereçamento. Um sistema computadorizado então capta a fluorescência emitida, o que permite a genotipagem instantânea e robusta de vários polimorfismos simultaneamente por amostra (ILLUMINA INC., 2010).

Os resultados dessas genotipagens estão reportados no Capítulo IV.

4.6 REVISÕES SISTEMÁTICAS, META-ANÁLISE E SÉRIE TEMPORAL

Foram realizadas duas revisões sistemáticas com meta-análises. A primeira revisão sistemática reuniu dados de estudos em prevalência da resistência primária a antirretrovirais na América Latina e Caribe com o objetivo de fazer uma estimativa geral dessa prevalência da resistência primária nesses países (Capítulo I). A segunda revisão sistemática reuniu dados de protocolos experimentais de vacinas terapêuticas anti-HIV-1 baseadas em células dendríticas com o objetivo de avaliar o que pode ser melhorado nos protocolos (Capítulo III). Resumidamente, a revisão sistemática é uma ferramenta poderosa de busca, reunião e sumarização de dados reportados por pesquisas independentes, o que auxilia os pesquisadores a se manterem atualizados acerca de um tema, a tomar decisões de saúde pública e ajudar a direcionar pesquisas futuras (LIBERATI et al., 2009).

Os dados reunidos pela revisão sistemática foram então analisados por meta-análises, para obter uma estimativa da frequência geral de vírus resistentes em pacientes nunca antes tratados na América Latina e Caribe (Capítulo I) e a proporção de pacientes vacinados com resposta imunológica, ainda que transiente, contra o vírus (Capítulo III).

Na maior parte das análises, o modelo de meta-análise escolhido foi o de efeitos aleatórios para obter uma estimativa geral das prevalências dos fenômenos investigados. A heterogeneidade entre os estudos (ou seja, a variabilidade de tamanho amostral entre os estudos reunidos) foi estimada pela medida I^2 e IC de 95% calculados para cada estimativa. Gráficos de meta-análise (*forest-plots*) também foram produzidos. Os resultados detalhados estão reportados nos Capítulos I e III.

De modo breve, uma meta-análise é uma ferramenta estatística para agregar dados e obter uma medida geral que represente a proporção de eventos de interesse numa amostra, ou ainda uma associação geral de uma variável com um resultado clínico, no que se obtém razões de chances ou risco relativos gerais (LIBERATI et al., 2009).

Adicionalmente, foi realizado um modelo de séries temporais para analisar dados de prevalência primária no Brasil com um modelo auto-regressivo integrado de média móvel, ou ARIMA na sigla em inglês (mais detalhes no Capítulo I). Concisamente, uma série temporal é uma sequência de medições de dados em pontos ordenados ao longo do tempo, e é voltada para predições de eventos futuros (BOX et al., 2013).

4.7 TESTES DE HIPÓTESES UNIVARIADOS

O Teste t de Welch foi usado para avaliar se determinados valores das variáveis numéricas (por exemplo, idade no início da terapia e massa corporal) estariam associados com efeitos adversos decorrentes do uso de AZT (Capítulo II) e com a falha imunológica (Capítulo IV).

O teste de χ^2 foi usado para testar a associação das variáveis categóricas com os *status* clínicos em investigação. Para esse teste, os pacientes foram estratificados em tabelas de contingência para dispor a estratificação dos grupos de estudo de acordo com as variáveis estudadas (por exemplo, sexo, etnia, esquemas de TARV recebidos, dentre outras – Capítulos II e IV). Adicionalmente, estimadores da probabilidade de sobrevivência de Kaplan-Meier foram obtidos para avaliar se as variáveis genéticas e clínicas exerceram influência significativa no tempo até a recuperação imunológica durante a TARV. Em seguida, os estimadores foram comparados através do teste de *log rank* de Cox-Mantel (Capítulo IV).

Após a genotipagem foi realizada a contagem manual de alelos e genótipos obtidos e a verificação da conformidade ao Princípio de Hardy-Weinberg através de um teste de qui-quadrado (χ^2). Tabelas de contingência também foram usadas para dispor a contagem de alelos e genótipos entre os dois grupos de estudo e permitir a realização do Teste Exato de Fisher, que verifica se a proporção de alelos ou genótipos se distribui igualmente entre os grupos. Para os testes envolvendo as frequências alélicas foi adicionalmente calculada uma razão de chances (*odds ratio*, OR) e um intervalo de confiança de 95% (IC 95%) associado, os quais representam a força de associação entre o alelo de risco e o resultado clínico (Capítulos II e IV).

Todos os testes estatísticos foram realizados com a ajuda do software R 3.3.2 (R CORE TEAM, 2014), a um nível de significância previamente estabelecido de 5% (α =0,05).

4.8 REGRESSÃO LOGÍSTICA MULTIVARIADA

Um modelo de regressão logística multivariado foi usado para avaliar se o SNP rs1127354 (94 C>A, Pro32Thr) do gene *ITPA* estava associado com efeitos adversos relacionados ao uso da AZT quando controlado por idade, sexo, etnia e massa corporal (Capítulo II). Resumidamente, A regressão logística é uma técnica estatística aplicável em situações em que se deseja explicar uma variável resposta do tipo dicotômica (ou binária), ou seja, com apenas dois valores possíveis. Dessa forma, é um método bastante adequado para análises de epidemiologia, pois se detém em, tentar determinar quais fatores estão relacionados ao desenvolvimento de um determinado *status* clínico específico (HOSMER & LEMESHOW, 2000; BALDING, 2006).

4.9 ANÁLISE DE SOBREVIVÊNCIA MULTIVARIADA

Variáveis genéticas com associação estatisticamente significativas com a ocorrência de falha imunológica foram incluídas num modelo de riscos proporcionais de Cox controladas por idade, sexo, etnia e esquema terapêutico, para avaliar se algumas dessas variáveis influenciaram no tempo até a recuperação imunológica durante a TARV (Capítulo IV). De modo resumido, a análise de sobrevivência é um método estatístico que, assim como a regressão logística, também é usada para explicar a ocorrência de uma variável de resposta dicotômica. Entretanto, na análise

de sobrevivência, o tempo tem um papel central. Dessa forma, o foco dessa análise é avaliar como outras variáveis influenciam no tempo necessário até um evento de interesse (DAVID W. HOSMER et al., 2008; KLEINBAUM & KLEIN, 2012). No caso, o evento de interesse seria a recuperação imunológica a ponto de o indivíduo atingir e manter 30% de células T CD4+ dentre o total de linfócitos (sucesso imunológico). Caso o indivíduo não atingisse esse valor mesmo com supressão viral, estaria em falha imunológica, como foi definido anteriormente.

5 CAPÍTULO I

Meta-Analysis and Time Series Modeling Allow a Systematic Review of Primary HIV-1 Drug-Resistant Prevalence in Latin America and Caribbean

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Abstract: Here we review the prevalence of HIV-1 primary drug resistance in Latin America and Caribbean using meta-analysis as well as time-series modeling. We also discuss whether there could be a drawback to HIV/AIDS programs due to drug resistance in Latin America and Caribbean in the next years. We observed that, although some studies report low or moderate primary drug resistance prevalence in Caribbean countries, this evidence needs to be updated. In other countries, such as Brazil and Argentina, the prevalence of drug resistance appears to be rising. Mutations conferring resistance against reverse transcriptase inhibitors were the most frequent in the analyzed populations (70% of all mutational events). HIV-1 subtype B was the most prevalent in Latin America and the Caribbean, although subtype C and B/F recombinants have significant contributions in Argentina and Brazil. Thus, we suggest that primary drug resistance in Latin America and the Caribbean could have been underestimated. Clinical monitoring should be improved to offer better therapy, reducing the risk for HIV-1 resistance emergence and spread, principally in vulnerable populations, such as men who have sex with men transmission group, sex workers and intravenous drug users.

Keywords: Drug resistance mutation, HAART, primary drug resistance, meta-analysis, systematic review, time series.

1. INTRODUCTION

Estimations indicate that around 35 million people are living with human immunodeficiency virus type 1 (HIV-1) globally. Among these, around 1.75 million live in Latin America and Caribbean. The implementation of highly active antiretroviral therapy (HAART) in 1996 saved 6.6 million lives [1]. However, the fight against HIV-1 is far to be over. One of the most challenging aspects in the management of HIV-1 infection is the emergence of strains resistant to antiretroviral drugs.

HIV-1 typically produces high levels of viral particles. As its reverse transcriptase (RT) is error-prone, it consequently generates high degree of genetic diversity. Poor adherence to HAART regimens leads to suboptimal drug levels, which are insufficient to maintain persistent virus suppression. The virus then continues to replicate, albeit at lower replication rates. Thus, if a mutation conferring resistance to drugs arises, this will turn into a selective advantage for resistant *quasispecies*. In some cases, a single mutation can cause cross-resistance against all members of an antiretroviral drug class [2, 3].

A resistant HIV-1 strain may be transmitted to other persons. This is defined as primary HIV-1 drug resistance (PDR), and it complicates the choice of which regimen a patient with PDR should receive, because it increases the risk of

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therapy failure (incomplete viral replication suppression), and consequently, disease progression [4].

Latin America and Caribbean countries have been improving HAART coverage in the past few years [1]. However, as more people are being treated, resistant strains could occur more frequently [4], and, since pre-treatment HIV-1 genotyping for resistance evaluation is generally not available in low/medium-income countries, PDR could turn into a significant threat to the HIV/AIDS programs from these countries.

Therefore, we decided to perform a systematic review of Latin America and Caribbean literature to overview PDR prevalence in the past years through meta-analysis and time-series modeling.

2. METHODS

2.1. Literature Search Strategy and Study Selection

We searched Latin America and Caribbean literature (from 1980 to June 2014) focusing HIV primary antiretro-viral resistance through PubMed database using MeSH (Medical Subject Headings) search terms. For example: ((“Country name” [Mesh]) AND “Antiretroviral Therapy, Highly Active” [Mesh]) AND “Drug Resistance” [Mesh]; ((“Country name” [Mesh]) AND “HIV/genetics” [Mesh]) AND “Drug Resistance” [Mesh] – in which “Country name” stands for each country from the geopolitical aspect defined above; or combinations of search terms with country names in PubMed search tool: “HIV DRM”; “HIV ARV resistance”.

After full text retrieval, the authors reviewed all potentially relevant studies. The inclusion criteria were (1) be an observational study, (2) include primary resistance prevalence estimation or available data so we could estimate it and (3) preferentially (though not mandatorily) include a list or description of the detected major mutations (mutations that relate to high resistance to antiretroviral drugs). We excluded studies that dealt with secondary resistance and pediatric or vertical transmission patients. For studies in which both types of resistance were reported, we included only the primary resistance data if they could be clearly distinguished from the secondary resistance information.

We extracted study characteristic data – HAART naïve HIV positive individuals sample number, number and percentage of females among the total sample size, age and demographics. We classified the demographics in six categories: pregnant women, men who have sex with men (MSM), intravenous drug users (IDU), sex workers, inmates and general population (defined as a cohort composed of people with different HIV risk behaviors, or sometimes unknown/unspecified by the authors – such as anonymous blood bank donors).

2.2. Statistical Analysis

We performed a meta-analysis in a per country basis, but only if there were at least three studies, not performed by the same research group, for each country. All meta-analysis were performed through “meta” package [5] for R software version 3.1.1 [6].

Briefly, prevalence estimates were log-transformed for meta-analysis. Heterogeneity between studies was assessed by τ^2 statistic and I^2 measure and if they were significantly different from zero as evaluated by Cochran’s Q test with n-1 degrees of freedom (in which n is the number of studies included and with significance level α=0.10 for this test). A random effects model was assumed if heterogeneity was detected (DerSimonian and Laird method [7]). A fixed effects model was chosen if otherwise [8]. Ninety-five percent confidence intervals (95% CI) were calculated for each pooled prevalence.

Additionally, we performed a time-series analysis using Brazilian studies’ data, since more data were available for this country, allowing us to model PDR prevalence change over time, as mentioned in Results and Discussion section. Concisely, a time series is a sequence of measurements taken at ordered points in time. We performed an auto-regressive moving average (ARIMA) model, as proposed by Box and Jenkins (1970, 2013) [9]. We denote the model in the form ARIMA (p, d, q), where p, d, and q are non-negative integers numbers that represents, respectively, the autoregressive, integrated and moving average orders of the model. These orders represent the number of estimated regression parameters during model fitting.

First, we sorted studies chronologically in calendar years according to sample collection starting period. We used month ranges as stated in the studies whenever possible to order studies conducted in the same years range. Thus, we considered each study as an independent time point.

After that, we used the test proposed by Dickey and Fuller (1979) [10] to check whether the PDR prevalence series was stationary (i.e. to check if data parameters such as mean and variance did not change over time). Subsequently, we used the "forecast" package [11], also from R software, to choose the ARIMA model that best fitted our original data, using Akaike Information Criterion (AIC). After choosing the model, we performed the following diagnostics tests: standardized residuals test, autocorrelation function (ACF) of residuals test and Ljung-Box statistics [12], to check whether the assumptions of the model have been satisfied. If all the assumptions were met, the model was deemed useful for describing the PDR prevalence change over time according to Brazilian data and forecast the prevalence a few years further from the most recent collection sample date found during the literature search.

We interpreted the PDR prevalence estimates in three levels: low (prevalence lower than 5.0%), moderate (between 5.0% and 15.0%) and high (higher than 15.0%) based on the World Health Organization consensus [13].

3. RESULTS AND DISCUSSION

3.1. Studies Selection

The search produced 655 unique abstracts, from which 206 were potentially eligible for our review according to our criteria. Further 123 studies were excluded due to different outcomes being investigated (study not focused on primary resistance), inappropriate populations studied (pediatric patients, secondary resistance) or because we were not able to extract suitable data for statistical analysis due to the way they were reported by the authors. Fig. (**1**) depicts the flowchart detailing studies search, inclusions and exclusions.

Thus, 83 studies were suitable for the statistical analysis, but 14 of them could not be included since had fewer studies than the threshold defined in the Methods session (three studies).

Finally, 24 studies (nine from Argentina, three from Cuba, four from Chile, four from Mexico and four from Venezuela) were meta-analyzed. Forty-five studies from Brazil were analyzed through time-series.

The 83 studies included original articles, short communications and sequence notes, reporting epidemiological and/or phylogenetical findings. The median sample number was 76 (interquartile range, IQR=44-126.5; minimum and maximum 16 and 1655, respectively). The median age of the recruited individuals (for those studies with available information) was 34 years old (IQR=30.7-35.8). In average, 39.6% of recruited individuals by the studies were women. The majority (64 studies) dealt with HIV general population; MSM samples were recruited in seven studies; six dealt with pregnant women cohorts; sex workers were sampled in two; male inmates, IDU and persons involved with occupational exposure were sampled in one study each. A single study sampled both MSM and IDU. Table **1** details the information about each report, except for Brazilian studies, which are displayed chronologically according to the sample collection period on Table **2**.

3.2. Prevalence Summaries

Sixty-nine studies (among the total 83) reported which drug resistance interpretation algorithm was used. Since the majority of them (45 studies) used Stanford University HIVdb algorithm [14], we also used this algorithm (surveillance mutation list, June 2013 version) to improve consistency between studies. We recalculated prevalences according to Stanford algorithm major mutation list, thus disregarding minor (accessory) mutations whenever possible. Thus, please note that the reported prevalences in this review may not reflect the same published by the original authors.

3.2.1. Caribbean

The literature search identified nine studies conducted in Caribbean and associated countries, published between 1999 and 2013. Due to the low number of studies, formal statistical analysis could not be performed according to our methodological criteria, with exception for three Cuban studies.

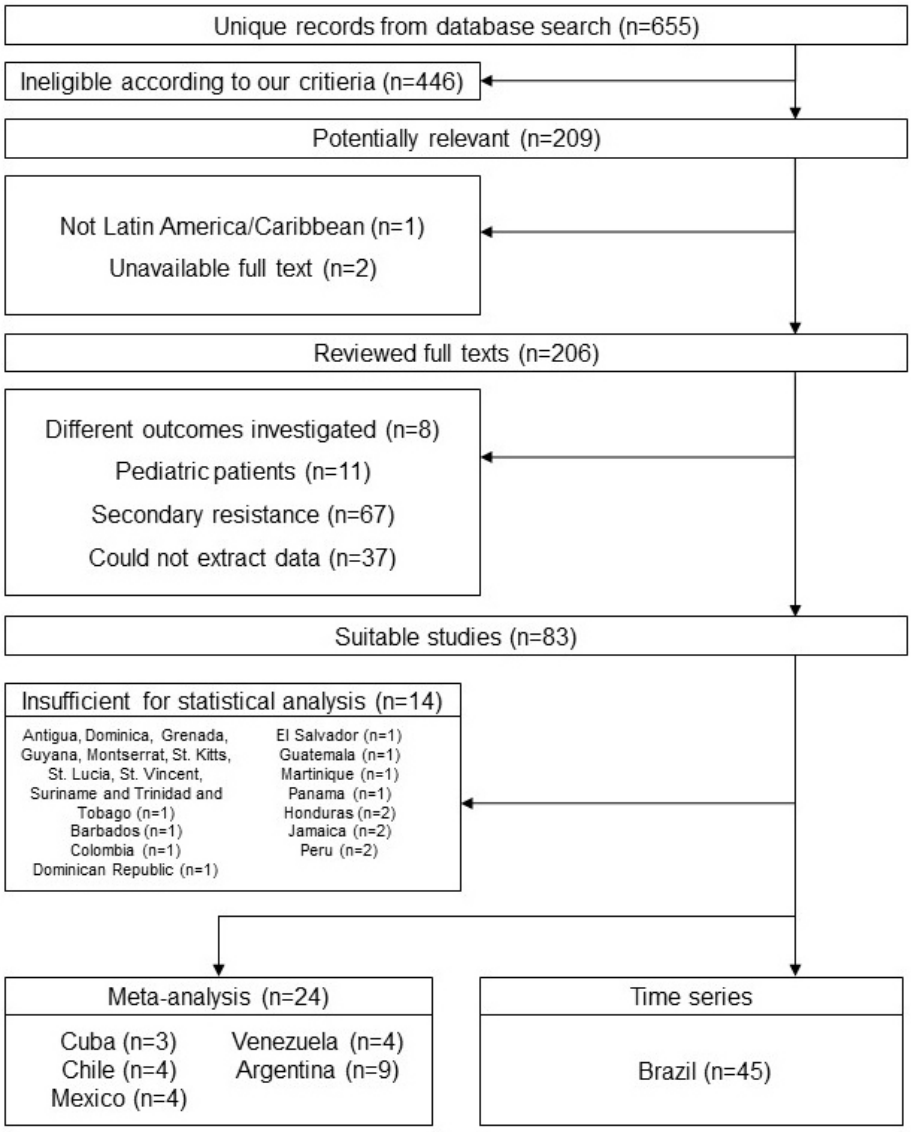

Fig. (1). Flow chart of studies selection for reviewing and inclusion on analysis.

Overall, PDR was found to be low to moderate in Caribbean countries, ranging from zero to 18.5%. A survey with samples from nine countries (Antigua and Barbuda, Dominica, Grenada, Guyana, Montserrat, St. Kitts and Nevis, St. Lucia, St. Vincent, Suriname and Trinidad and Tobago) did not report any major mutations [15].

Barbados, Dominican Republic and Martinique all had reported PDR prevalence lower than 10%: 2.8%, 7.8% and 7.2%, respectively [16-18]. Recent studies from Jamaica and Cuba reported moderate PDR prevalence. Data from Hamilton *et al.* (2012) [19] allowed us to estimate major mutations frequency of 18.5%, whereas data from Barrow *et al.* (2013) [20] yielded 12.6%, both for Jamaican populations.

Data from three Cuban studies apparently show a trend for PDR prevalence increment in the last twelve years. Ruibal-Brunet *et al.* (2001) [21] observed a prevalence of 7.4%. Later, Perez *et al.* (2007) [22] reported the close estimate of 5.2%. However, the most recent study from Machado *et al.* (2013) [23] in newly diagnosed individuals, reported a prevalence of 21.5%, the highest among Caribbean countries. According to our meta-analysis estimate, PDR pooled prevalence in Cuba is around 10.0%,

Table 1. Studies characteristics and reported primary drug resistance prevalence in Caribbean and Latin American countries.

Region, *Countries* and Studies	HIV-Infected Individuals Demographics	N Female (%)	Age (Years)	Reported Primary Drug Resistance Prevalence (%)
Caribbean				
Barbados				
[16]	General population	14/36 (38.9)	34.5 [30-41.3][a]	1/36 (2.8)
Cuba				
[21]	General population	NR	NR	2/27 (7.4)
[22]	General population	NR	NR	13/250 (5.2)
[23]	General population	30/200 (15.0)	35.3 [11.3][b]	43/200 (21.5)
Dominican Republic				
[17]	General population	56/103 (54.4)	38.6 [NR][b]	8/103 (7.8)
Jamaica				
[19]	General population	NR	NR	17/92 (18.5)
[20]	General population	64/103 (62.1)	37.3 [NR][b]	10/79 (12.6)
Martinique				
[18]	General population	23/70 (32.9)	NR	5/69 (7.2)
Nine countries*				
[15]	General population	54/94 (57.5)	22 [NR][b]	0/94 (0.0)
Continental Latin America				
Argentina				
[39]	(Transsexual) sex workers	0 (0.0)	29 [24-35][a]	12/62 (19.4)
[40]	General population	NR	NR	16/214 (7.5)
[41]	MSM, IDU	0 (0.0)	NR	2/23 (8.7)
[42]	(Female) sex workers	16/16 (100.0)	NR	3/16 (18.8)
[43]	General population	33/107 (30.8)	33 [NR][a]	4/98 (4.1)
[44]	General population	13/52 (25.0)	36 [11][b]	4/52 (7.7)
[45]	General population	33/152 (21.7)	37 [NR][b]	12/152 (7.9)
[46]	Pregnant women	78/78 (100.0)	25 [19–34][a]	7/78 (9.0)
[83]	General population	71/284 (25.0)	NR	12/284 (4.2)
Brazil				
See Table 2				
Chile				
[35]	General population	6/60 (10.0)	37.1 [23-60][a]	1/60 (1.7)
[36]	General population	NR	NR	2/79 (2.5)
[37]	MSM majority	0/25 (0.0)	35 [25-45][a]	3/25 (12.0%)
[38]	MSM majority	8/74 (10.8)	32 [18-58][a]	3/74 (4.1%)
Colombia				
[28]	General population	18/103 (17.5)	34 [18–59][a]	6/103 (5.8)
El Salvador				
[29]	General population	40/88 (45.5)	35.5 [NR][b]	5/88 (5.7)
Guatemala				
[84]	General population	64/145 (44.1)	37.3 [NR][b]	4/145 (2.8)

(Table 1) contd…..

Region, *Countries* and Studies	HIV-Infected Individuals Demographics	N Female (%)	Age (Years)	Reported Primary Drug Resistance Prevalence (%)
Honduras				
[31]	General population	NR	NR	18/239 (7.5)
[30]	General population	95/200 (47.0)	31 [15–64][a]	14/200 (7.0)
Mexico				
[25]	General population	NR	NR	41/1655 (2.5)
[51]	General population	9/96 (9.4)	NR	7/96 (7.3)
[85]	General population	4/42 (9.5)	33 [20-58][a]	1/42 (2.4)
[86]	Pregnant women majority	38/46 (82.6)	27 [6][b]	1/41 (2.4)
Panama				
[32]	General population	17/47 (36.2)	22 [20-24][a]	6/47 (12.8)
Peru				
[26]	MSM	0 (0.0)	NR	12/359 (3.3)
[27]	General population	46/112 (41.1)	NR	1/96 (1.0)
Venezuela				
[47]	General population	NR	NR	1/31 (3.2)
[48]	General population	3/20 (15.0)	NR	2/20 (10.0)
[49]	General population	14/65 (21.5)	32.6 [18-58][a]	4/62 (6.5)
[50]	General population	NR	NR	7/63 (11.1)

*Antigua and Barbuda, Dominica, Grenada, Guyana, Montserrat, St. Kitts and Nevis, St. Lucia, St. Vincent, Suriname and Trinidad and Tobago.
General population – cohort composed of people with different HIV risk behaviors, or sometimes unknown/unspecified by the authors.
MSM – men who have sex with men.
NR – not reported.
[a]Median [interquartile range].
[b]Mean [standard deviation].

and can be as high as 28.0% (95% CI=3.0-28.0). These findings are summarized in Table **3**.

The relatively low number of studies in Caribbean region is a matter of concern, because, with the exception from Dominican Republic, Cuba and Jamaica, we could not find recently published studies on PDR prevalence (five among the nine studies were published five or more years ago) in the other Caribbean countries. Thus, these PDR prevalences may be actually underestimated (maybe even unknown in other countries in the region).

In the past few years, international task forces have been created for implement and improve PDR monitoring in the Caribbean and Latin America [24]. Therefore, it is possible that in the near future this knowledge gap regarding the Caribbean HIV population will be fulfilled, and ideally stabilize potential increasing PDR rates.

3.2.2. Continental Latin America

As for Caribbean countries, PDR prevalence in continental Latin America seems to be low to moderate. Data from some countries reported prevalences under 6.0%, such as Guatemala (2.8%) [25], Peru (two estimates: 3.3% and 1.0%) [26, 27], Colombia (5.8%) [28] and El Salvador (5.7%) [29].

Honduras apparently has moderate prevalence around 7.0% and 7.5% (Murillo *et al.* (2010) [30] and Lloyd *et al.* [31], respectively). A study from Panama observed a prevalence of 12.8% [32].

Noteworthy, Lama *et al.* (2006) [26] surveyed Peruvian MSM populations, whereas Soria *et al.* (2012) [27] sampled from HIV general population also from Peru. The estimates were close, but nonetheless PDR was higher in MSM individuals. Interestingly, previous evidence in European populations showed that MSM individuals infected

Table 2. Studies' characteristics and reported primary drug resistance prevalence in Brazil. The studies are ordered chronologically according to sample collection starting period.

Study	Sample Collection Period (Calendar Years)	Study Characteristics			Reported Primary Drug Resistance Prevalence (%)
		HIV-Infected Individuals Demographics	N Women (%)	Age (Years)	
[70]	1989 to 2005	General population	124/240 (51.7)	NR	6/290 (2.1)
[87]	1996	General population	NR	NR	3/32 (9.4)
[55]	1996 to 2012	MSM	0/64 (0.0)	30.6 [19-54][a]	9/64 (14.1)
[88]	1997	General population	19/48 (40.0)	35 [NR][b]	2/48 (4.2)
[89]	1998	General population	NR	NR	0/44 (0.0)
[90]	1998 to 2002	General population	NR	NR	21/341 (6.2)
[56]	1998 to 2003	MSM	NR	NR	10/50 (20.0)
[91]	1999 to 2001	IDU	NR	NR	3/38 (7.9)
[92]	1999 to 2001	General population	24/71 (33.8)	NR	1/71 (1.4)
[93]	2000	General population	11/56 (19.6)	28.4 [NR][b]	7/50 (14)
[94]	2000 to 2001	Occupational exposure	20/44 (45.5)	35.8 [21-62][a]	2/16 (12.5)
[61]	2000 to 2001	General population	52/129 (40.3)	31 [NR][b]	3/76 (3.9)
[53]	2001	General population	155/380 (40.8)	30.7 [9.1][b]	22/409 (5.4)
[95]	2001	General population	61/112 (54.5)	31 [25-37][a]	0/112 (0.0)
[96]	2001 to 2005	General population	15/27 (55.6)	30.1 [NR]	0/27 (0.0)
[97]	2002	General population	40/85 (47.0)	35.2 [11.0][b]	2/25 (8.0)
[64]	2002 to 2003	General population	30/84 (35.7)	NR	3/84 (3.6)
[98]	2002 to 2006	General population	34/123 (27.6)	37 [NR][b]	8/123 (6.5)
[99]	2003	Pregnant women	35/35 (100.0)	24 [17–35][a]	0/35 (0.0)
[100]	2003 to 2004	General population	NR	37 [NR][b]	9/56 (16.1)
[101]	2004 to 2006	General population	81/209 (38.8)	33 [27-40][a]	18/204 (8.8)
[102]	2004 to 2006	General population	NR	NR	7/50 (14.0)
[103]	2005	General population	12/44 (27.3)	35 [30–37][a]	2/62 (3.2)
[104]	2005 to 2006	General population	NR	NR	3/32 (9.4)
[105]	2005 to 2007	General population	116/246 (47.2)	NR	39/246 (15.9)
[62]	2005 to 2008	General population	89/205 (43.4)	35.4 [11.7][b]	7/205 (3.4)
[106]	2005 to 2008	Pregnant women	197/197 (100.0)	26 [NR][b]	21/197 (10.7)
[107]	2005 to 2008	General population	25/82 (30.5)	34.1 [NR][b]	6/82 (7.3)
[108]	2006 to 2007	General population	45/99 (45.4)	35 [10.01][b]	8/99 (8.1)
[109]	2006 to 2008	General population	15/33 (45.4)	35 [NR][b]	6/33 (18.2)
[110]	2007	General population	135/400 (33.8)	36 [15–66][a]	22/387 (5.7)
[111]	2007 to 2008	General population	32/103 (31.1)	32 [15–71][a]	10/103 (9.7)
[54]	2007 to 2008	General population	122/223 (54.7)	36 [8.0][b]	17/210 (8.1)
[65]	2007 to 2009	General population	61/130 (46.9)	NR	8/130 (6.1)
[72]	2008 to 2009	General population	21/52 (40.4)	30 [14–65][a]	6/52 (11.5)
[63]	2008 to 2009	General population	42/82 (51.2)	37.8 [NR][b]	8/82 (9.8)
[112]	2008 to 2009	General population	NR	32.15 [NR][b]	17/225 (7.6)
[77]	2008 and 2010	General population	19/49 (38.8)	36 [19-64][a]	3/49 (6.1)
[57]	2008 to 2009	MSM	0/44 (0.0)	NR	10/44 (22.7)
[113]	2008 to 2009	General population	38/92 (41.3)	36 [NR][b]	5/92 (5.4)
[114]	2008 to 2010	Pregnant women	30/30 (100.0)	25 [NR][b]	4/30 (13.3)
[115]	2009	General population	28/48 (58.3)	35.1 [11.2][b]	2/48 (4.2)
[116]	2009	(Male) inmates	0/38 (0.0)	31.5 [NR][b]	4/38 (10.5)
[117]	2010 to 2011	Pregnant women	16/16 (100.0)	25 [15–38][a]	4/16 (25)
[118]	2011	MSM (majority)	11/101 (10.9)	31 [NR][b]	14/101 (13.9)

General population – cohort composed of people with different HIV risk behaviors, or sometimes unknown/unspecified by the authors.
MSM – men who have sex with men.
NR – not reported.
[a]Median [interquartile range].
[b]Mean [standard deviation].

Table 3. Meta-analysis results summary.

Country	Number of Studies (Included/ Reviewed)	Heterogeneity				Model Selection	Pooled Primary Drug Resistance Prevalence (%), [95% CI]
		Estimation (τ^2)	I^2	Q Statistic	*p-Value*		
Argentina	9/17	0.21	59.4%	19.7	0.01	random effects	8.4 [5.7-12.0]
Chile	4/5	0.01	21.8%	3.84	0.28	fixed effects	3.3 [1.1-6.2]
Cuba	3/6	1.08	91.8%	24.5	<0.001	random effects	10.0 [3.0-28.3]
Mexico	4/8	0.29	58.3%	7.20	0.066	random effects	3.5 [1.7-7.1]
Venezuela	4/7	0	0%	1.95	0.58	fixed effects	7.5 [3.8-12.2]

95% CI - 95% confidence interval.

with HIV-1 subtype B (the same population profile as in the Peruvian sample) tended to be more likely infected with a resistant HIV-1 strain than individuals reporting other types of risk behavior/transmission route [33, 34].

Studies from Chile followed a similar trend. Whereas Afani *et al*. (2005) [35] and Rios *et al*. (2007) [36] reported prevalence between 1.7% and 2.5% in the general population, Acevedo *et al*. (2007) [37] and [38] observed higher prevalence among samples with MSM majority (12.0% and 4.1%, respectively). Nonetheless, overall PDR prevalence in Chile seems to be low. Our meta-analysis estimates a prevalence of 3.3% (95% CI=1.1-6.2).

PDR prevalence also appears to be higher among Argentinian MSM. Carobene *et al.* (2014) [39] reported a prevalence of 19.4% among transsexual sex workers infected with recombinant BF and B subtypes from Buenos Aires and major Argentinian cities. Pando *et al.* (2011) [40] sampled from HIV general population infected with the same subtypes, also in Buenos Aires during approximately the same period as did Carobene *et al.* They found an overall PDR prevalence of 8.4% (7.5% if considering only major mutations as defined by Stanford University HIVdb algorithm [14]). Andreani *et al.* (2011) [41] found a similar prevalence (8.7%) in MSM and IDU men at risk to HIV-1 re-exposure. Thus, subtype B and MSM transmission route may also be risk factors for transmitted antiretroviral resistance in Latin America. Female sex workers may also be at risk, since a sample from Argentinian BF and B subtypes-infected sex workers had a relatively high PDR prevalence – 18.8% [42]. Certainly, more studies are necessary to address this issue.

Aside from these studies, other data point to a rise in PDR prevalence in Argentina in the past few years. Kijaj *et al.* (2001) [43] reported a prevalence of 4.1% between 1997 to 2000 period. Dilernia *et al.* (2007) observed a prevalence of 4.2% between 2003 and 2005, whereas Petroni *et al.* (2006) [44], Rodriguez-Rodrigues *et al.* (2013) [45] and Pando *et al.* (2011) [40] reported prevalences above 7.0% between 2003 and 2009 (7.7%, 7.9% and 7.5%, respectively). Cecchini *et al.* (2013) [46] recently reported a PDR prevalence of 9.0% in a cohort of pregnant women sampled between 2008 and 2011. Including all the Argentinian studies cited above in a meta-analysis, we estimate a pooled PDR prevalence of 8.4% (95% CI=5.7-12.0), which is considered moderate.

Venezuela also seems to have moderate PDR prevalence (pooled prevalence=7.5%; 95% CI=3.8-12.2). Some authors of the four Venezuelan studies included in our meta-analysis acknowledge that PDR prevalence has been increasing in the country. Delgado *et al.* (2001) [47] initially reported a prevalence around 3.0% (considered low). Later, other authors reported prevalences higher than 5.0%: Bouchard *et al.* (2007) [48] observed 10.0%, Castillo *et al.* (2009) [49] reported a 6.5% prevalence, and the most recent survey, by Rangel *et al.* (2009) [50], found a prevalence of 11.1%.

In contrast to Argentina and Venezuela, Mexico apparently has low PDR prevalence. Three among four studies, including a relatively recent national survey with the highest sample number among all reviewed studies [25] reported rates around 2.5%. A single study [51] reported a prevalence of 16.0%

(7.3% if considering only high-level resistance). Our meta-analysis estimates that major mutations frequency in Mexico is low (3.5%; 95% CI=1.7-7.1). This can be consequence of the delay of implementation of universal access to HAART in Mexico, which started around 2004 [25]. Thus, it is possible that effective HAART coverage was low before this period and in the few years later, resulting in low selection rates for resistant strains. All meta-analysis results are summarized in Table **3**.

3.2.3. Brazil

We included Brazilian studies in a specific session due to the extensive data published throughout the years. Among the 83 papers discussed in this review, 45 were conducted on Brazilian samples. HIV/AIDS epidemiological notification and prevention programs started already in the first decade of AIDS discovery and detection in Brazil, and in 1996 it was one of the first developing countries to provide free-of-charge HAART for all eligible patients attending the public healthcare system; this is considered as a quite successful model of program against HIV/AIDS [52].

The higher number of studies allowed us to model through time series analysis how PDR prevalence evolved during more than 20 years of research and forecast changes for the next few years.

According to Dickey-Fuller test results, our data were not stationary (p=0.13), thus requiring additional differentiation before fitting to a non-seasonal ARIMA model. The best model (AIC=284.9) was an ARIMA (3, 1, 3), and diagnostic tests results showed that the assumptions of the model were met, i.e. residual errors were randomly distributed and not auto-correlated (data not shown). Therefore, this model was suitable to describe PDR prevalence change over time in Brazil.

Thus, our model defined a rising trend in PDR prevalence in Brazil between 1989 and 2011, for which data are available. Using the model to predict three forward time points, we estimate that 2014 actual PDR prevalence is around 20.6% (95% CI=10.7-30.6). The estimates for 2012 and 2013 were 7.3% (80% CI=1.0-13.6) and 15.3% (95% CI=5.6-25.0), respectively. Note that the CI for the 2012 estimate was set at 80% because the 95% CI lower bound yielded a spurious result (a negative number; prevalence is only expressed by positive numbers); so we were less strict for the definition of CI for this estimate. We did not estimate too much forward time points to avoid excessive speculation, since it would generate more imprecision at each new forecast. Fig. (**2**) shows a graphical representation of PDR prevalence changes over time in Brazil.

Brazil is politically divided in five regions: "Central-West", North, Northeast, South and Southeast. Two previously published national surveys sampling in cities from almost all of these regions found overall intermediate levels of PDR prevalence. The 2002 survey [53] found a prevalence of 6.6% and the 2009 survey [54] observed a prevalence of 8.1%. Nonetheless, the authors acknowledge that major cities from Southeast Brazil, such as São Paulo and Rio de Janeiro, have moderate levels of PDR. It is also important to note that Brazilian MSM populations, similarly as discussed about Argentinian and Peruvian studies, seem to be more at risk to be infected with resistant strains, with reported prevalences between 14% and 22% [55-57].

Our data came predominantly from studies that sampled cities in Southeast and South Brazil (20 studies from Southeastern cities; nine from Southern cities; one with cities from both Southeast and South; one with cities from three regions: Southeast, South and Central-West; seven in Central-Western cities, two from Northeast and a single one from North Brazil. The remaining four were global national surveys). Thus, our estimate could be biased by the observations made in these regions, yielding overestimates.

Understandably, these regional "oversampling" may reflect the fact that the majority of Brazil total HIV cases since 1980 are concentrated in Southeast and South (55.5% and 20.0% of the cases, respectively) [58]. The Southeast was the region where the first Brazilian AIDS cases emerged in the 1980's decade. Thus, it is reasonable to suppose that the HIV therapy implementation in this region started earlier than in other regions, thus favoring the early selection of resistant strains among patients with suboptimal adherence during the monotherapy period or in the early HAART years.

The relatively low number of studies in other regions are a matter of concern, since previous

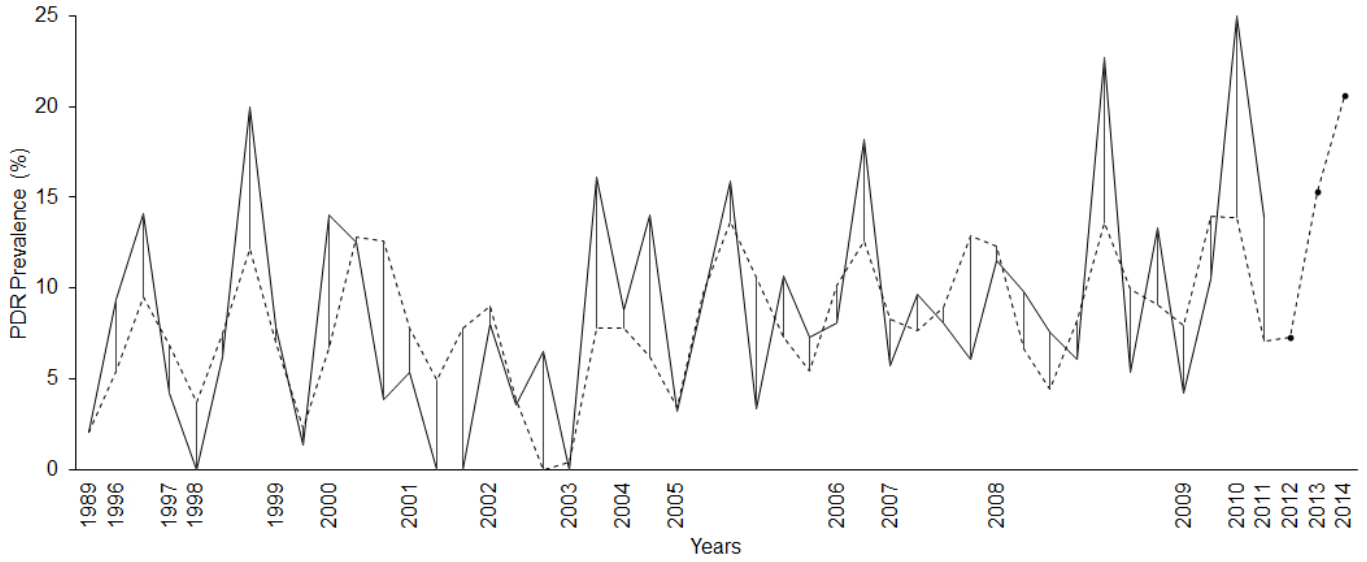

Fig. (2). Primary HIV drug resistance (PDR) prevalence time series and predicted values in Brazil. The solid line represent the prevalences at each reviewed study/time point, starting at 1989 and ending in 2011. The dashed line represents fitted predictions according to the ARIMA (3,1,3) model. The three filled circles represent three forward time points. The first circle is the 2012 estimate (7.3%, 80% CI=1.0-13.6), the second is the 2013 estimate (15.3%, 95% CI=5.6-25.0) and the third the 2014 estimate (20.6%, 95% CI=10.7-30.6).

analysis pointed out that Brazil's HIV epidemics has been in the process of "interiorization" and "pauperization" [59], meaning that HIV/AIDS cases are migrating to the countryside and affecting more people with lower socioeconomic status. Indeed, recent data show that detection rate of AIDS cases in Central-West (countryside Brazil - the only landlocked Brazilian region), Northeast and North regions (the most impoverished areas in the country) arose, whereas somewhat decreased in Southeast and South, between 2003 and 2012.

Thus, it is virtually unknown how many of these new cases in Central-West, Northeast and North regions were caused by resistant strains, which could further complicate treatment choices for populations already underserved in basic healthcare, or even favor selection of more resistant strains due to poor clinical/adherence monitoring.

To conclude, we should interpret cautiously the estimates we observed, but we may highlight that PDR prevalence is apparently rising in Brazil, and it is (at least) at moderate levels now. Moreover, the Southeast region is a possible "hotspot" of resistant strains circulation, which could influence significantly the spread of resistant strains to other regions in the future.

3.3. Subtypes and Mutations

HIV-1 B subtype was the most frequent among the individuals sampled in the 83 reviewed studies (mean percentage 69.2% of the samples), followed by subtype C (8.6%), B/F recombinants (7.5%) and subtype F (5.4%). Other subtypes and recombinant forms such as CRF or URF were 9.0% of the samples in average.

The majority of individuals from Chile, El Salvador, Guatemala, Honduras, Jamaica, Mexico, Peru and Venezuela were infected by subtype B. Presumably, subtype distribution in the other Caribbean countries reviewed here also follows this pattern, as suggested by previous evidence [60]. Maybe Cuba is an exception, where other forms may prevail over subtype B, as reported elsewhere [22, 23].

Subtypes F and B/F recombinants had a significant frequency among subjects from Argentina and Brazil. B/F recombinants were even the majority in some Argentinian samples [39, 44].

Subtype C is frequent in the South Brazil region, being the majority among some samples [61-63]. Subtype F seems to have substantial frequencies (over 20.0%) in the Northeast region [64, 65].

Some authors evidence that different HIV-1 subtypes develop drug resistance mutations in unique ways, each favoring different patterns and frequency of mutations when challenged with antiretroviral therapy pressure [66-70].

Our study design does not allow us to elaborate further on this topic, since it is retrospective. Prospective studies with individuals matched by subtype and drug regimen would be more informative. However, we acknowledge that it can be influential in the PDR prevalence over time in countries with complex epidemics, such as Argentina and Brazil. Of note, some authors even highlight the spread of subtype C from South Brazil to major cities in Brazil and regions such as Central-West, reflecting the trend of "interiorization" mentioned earlier [59, 71, 72]. Thus, the spread of different subtypes into new areas could hypothetically change the local PDR prevalence, in a founder effect-like manner. Table **4** summarizes the subtypes frequencies for those studies in which this information was available.

In relation to the types of mutation reported by authors, we observed that among 53 studies from which we could extract mutation lists with fair accuracy, we could count 922 mutation events in 567 individuals (1.6 mutations per individual, on average) infected with HIV-1 resistant strains. Seven-hundred and twelve mutations (77.2%, 1.2 RT mutations per individual) were located at RT region, whereas 210 (22.8%) were distributed in protease (PR) codons (0.4 PR mutations per individual). Further analysis showed that among the 712 mutation events on RT region, 399 were associated to nucleoside analog RT inhibitor (NRTI) resistance and 313 were related to non-nucleoside analog RT inhibitor (NNRTI) resistance.

The NRTI resistance-associated mutations frequency distribution was T215F/Y (112 events), M184V/I (84 events), M41L (62 events), K219Q/E (47 events), D67N (41 events), K70R (19 events), T210W (22 events each) and L74V/I (12 events). NNRTI resistance-associated mutations were distributed in this manner: K103N/S (145 events), G190A/S/E (46 events), Y181C/I/V (44 events), K101E/P (23 events), V106A/M (25 events), E138A/G/K/Q (six events), Y188L/C/H (14 events), M230L (seven events) and L100I (three events). Finally, for Protease inhibitor (PI) resistance-associated mutations: M46I/L (64 events), V82A/T/F/S/L (42 events), L90M (35 events), I54V/T/A/L/M (27 events), D30N (13 events), N88S/D (10 events), I84V (six events), L76V (seven events), I47V/A (two events), G48V/M (two events), V32I and I50L/V (one event each).

Interestingly, these observations seem to confirm the concept of low "genetic barrier" for reverse transcriptase inhibitors (both NRTI and NNRTI). Just one or two mutations are sufficient to induce resistance against these drug classes, values similar to the average mutation number (1.2 RT mutations, as mentioned earlier). In contrast, more mutations are needed to induce resistance against PI (high "genetic barrier"). As expected, thymidine analog mutations were the most frequent in the samples, since low/medium-income countries (as is the case of Latin America and Caribbean) tend to use thymidine analogs, such as zidovudine, more frequently, favoring the emergence of these mutations [3].

Studies dealing with RT and PR mutations were the majority of the works surveyed before final inclusion in this review. We also found some studies in Brazil [73-78], Venezuela [79] and multinational surveys including Latin America/Caribbean countries [80, 81] that investigated mutations possibly related to resistance against integrase or fusion inhibitors. However, to our knowledge, these drug classes are not commonly used in first-line drug regimens, at least in Brazil clinical setting, being preferred as salvage regimen choices [58]. Thus, we did not include these studies in the final review because, in our opinion, these mutations, even if present, would have no clinical relevance for HAART naïve individuals.

CONCLUSION

We reviewed literature data concerning PDR prevalence in Latin American and Caribbean. We observed that (1) these regions have been reporting low to moderate levels of PDR prevalence; (2) subtype B dominates the epidemics, but Argentina and Brazil have significant contributions of B/F recombinants and subtype C and (3) NRTI and NNRTI resistance-associated mutations were more frequent, corresponding to more than 70% of mutational events observed. PI resistance-

Table 4. Summary of HIV-1 subtypes detected by each study and mean mutation number per individuals with primary HIV-1 drug resistance.

Country, Study	HIV-1 Subtypes					Mean Mutation Number Per Individual	
	B	C	F	B/F Recombinant	Other Forms	Protease Gene	Reverse Transcriptase Gene
Argentina							
[39]	38.7	4.8	0.0	54.8	1.6	1.0	2.3
[40]	57.9	2.3	0.5	39.3	0.0	0.8	2.1
[43]	NR	NR	NR	NR	NR	1.3	4.5
[44]	40.0	0.0	0.0	60.0	0.0	3.0	1.0
[45]	46.0	0.0	0.0	50.6	3.4	0.7	1.3
Brazil							
[53]	NR	NR	NR	NR	NR	ND	0.7
[54]	17.0	0.0	7.0	0.0	0.0	0.3	0.4
[55]	68.8	6.3	17.2	0.0	7.8	ND	1.6
[56]	91.4	2.5	1.2	3.7	1.2	0.5	1.1
[57]	81.8	7.7	0.0	0.0	6.9	0.3	1.2
[62]	22.0	64.4	0.0	0.0	13.7	0.7	0.7
[63]	13.4	65.9	0.0	0.0	0.0	0.3	1.3
[64]	72.6	1.2	22.6	3.6	0.0	ND	1.0
[65]	56.9	3.1	37.7	2.3	0.0	0.4	1.4
[70]	72.8	0.0	27.2	0.0	0.0	2.0	ND
[72]	78.8	5.8	1.9	0.0	13.5	0.2	1.3
[77]	65.3	10.2	8.2	8.2	8.2	ND	0.3
[88]	NR	NR	NR	NR	NR	ND	1.0
[93]	70.9	1.8	5.5	0.0	21.8	ND	0.6
[98]	82.0	5.7	6.5	0.0	5.8	0.4	0.8
[100]	78.6	21.4	0.0	0.0	0.0	ND	1.8
[102]	81.0	0.0	8.4	7.4	3.2	ND	0.3
[105]	78.0	0.0	9.8	5.7	6.5	0.3	0.5
[106]	81.0	1.0	10.0	0.0	0.0	0.2	0.4
[107]	85.3	3.7	3.7	7.3	0.0	0.2	0.7
[108]	26.2	39.4	1.1	0.0	33.3	0.3	0.9
[109]	66.7	6.1	12.1	15.2	0.0	ND	1.2
[110]	66.0	12.8	0.0	0.0	21.3	0.2	1.1
[111]	82.5	3.1	6.2	7.2	1.0	0.2	1.2
[112]	76.0	7.0	6.0	0.0	11.0	0.2	1.1
[113]	71.7	5.4	3.3	0.0	19.6	ND	0.8
[114]	61.2	12.2	4.1	20.4	0.0	0.3	0.8
[115]	39.6	25.0	8.3	12.5	14.6	1.0	0.5
[116]	13.2	34.2	0.0	0.0	0.0	0.5	0.3
[118]	77.9	2.7	1.8	10.6	0.0	0.6	0.9

(Table 4) contd…..

Country, Study	HIV-1 Subtypes					Mean Mutation Number Per Individual	
	B	C	F	B/F Recombinant	Other forms	Protease Gene	Reverse Transcriptase Gene
Chile							
[35]	NR	NR	NR	NR	NR	ND	1.0
[36]	85.0	0.0	0.0	15.0	0.0	ND	5.5
[37]	NR	NR	NR	NR	NR	ND	1.3
[38]	NR	NR	NR	NR	NR	0.3	1.3
Colombia							
[28]	NR	NR	NR	NR	NR	0.2	1.5
Cuba							
[21]	77.8	3.7	0.0	0.0	18.5	ND	0.5
[22]	43.6	4.0	0.0	0.0	52.4	0.5	0.8
[23]	36.5	0.0	0.0	0.0	63.5	0.02	1.4
El Salvador							
[29]	100.0	0.0	0.0	0.0	0.0	ND	0.8
Guatemala							
[84]	96.6	0.7	0.7	0.0	2.1	ND	0.6
Honduras							
[30]	99.0	0.0	0.0	0.0	1.0	ND	1.1
[31]	99.1	0.0	0.3	0.0	0.6	0.8	2.2
Jamaica							
[19]	100.0	0.0	0.0	0.0	0.0	ND	2.4
[20]	NR	NR	NR	NR	NR	ND	0.8
Mexico							
[25]	99.9	0.0	0.0	0.0	0.1	0.5	2.7
[51]	NR	NR	NR	NR	NR	0.9	2.9
Panama							
[32]	NR	NR	NR	NR	NR	ND	2.2
Peru							
[26]	100.0	0.0	0.0	0.0	0.0	0.8	0.9
Dominican Republic							
[17]	100.0	0.0	0.0	0.0	0.0	ND	1.0
Venezuela							
[49]	100.0	0.0	0.0	0.0	0.0	ND	1.3
[50]	100.0	0.0	0.0	0.0	0.0	0.1	1.0

NR – not reported.
ND – not detected.

associated mutations were the minority, reflecting the choice of first-line drug regimens in the area, which have thymidine analogs and NNRTI, drug classes with low "genetic barrier".

Even though PDR in Latin America and Caribbean appears to be not widespread, we still consider it a challenge for HIV clinicians due to few, relatively "outdated" studies. This, associated to the delay between sample collection dates and results publishing, lead us to hypothesize that PDR prevalence could be, in principle, underestimated.

We are aware of the review's limitations, including the fact that it was not possible to distinguish between recently and chronically infected individuals in a suitable manner for statistical analysis. As far as we know, methodologies for infection period estimation were introduced around 2008 [82]. Since most data reviewed here were published before this date, no information regarding infection time is available. We suggest that future studies regarding PDR on Latin America and Caribbean should include infection time estimation to better assess resistance transmission in HIV-1 infected individuals.

Moreover, PDR is simply an aspect of the broader field of HIV-1 drug resistance. We decided not to include secondary resistance because we reasoned that most studies regarding this topic are retrospective, which tend to sample patients that already had therapy failure. However, to perform secondary resistance prevalence estimation, the studies needed to be prospective: including a sample of individuals starting therapy and then performing follow-up and subsequently observing how many of them presented resistance/treatment failure. This kind of study design is not generally present in primary resistance investigations. Therefore, we reasoned that reviewing secondary resistance prevalence would raise too many biases and we feared that we could obtain inaccurate results; thus, we focused our efforts only on PDR.

As pre-treatment HIV-1 genotyping is not generally available in low/medium-income countries, close monitoring of patient clinical history and treatment adherence, principally in vulnerable populations (MSM, IDU, sex workers), is still the best way to favor the therapy success, reducing the emergence and spread of HIV-1 resistant strains.

CONFLICT OF INTEREST

The authors declare that there has been no conflict of interest.

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Antonio Coelho designed the study, collected, analyzed data and wrote the manuscript; Ronald Moura, Ronaldo da Silva, Anselmo Kamada, Rafael Guimarães and Lucas Brandão collected and summarized the data; Hemílio Coelho analyzed the data and Sergio Crovella critically revised the manuscript. All authors reviewed and approved the final manuscript version.

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REFERENCES

[1] UNAIDS. Global report: UNAIDS report on the global AIDS epidemic 2013: UNAIDS 2013.

[2] Beerenwinkel N, Däumer M, Sing T*, et al.* Estimating HIV Evolutionary Pathways and the Genetic Barrier to Drug Resistance. Journal of Infectious Diseases 2005; 191(11): 1953-60.

[3] Shafer RW, Schapiro JM. HIV-1 drug resistance mutations: an updated framework for the second decade of HAART. AIDS reviews 2008; 10(2): 67.

[4] Vella S, Palmisano L. The Global Status of Resistance to Antiretroviral Drugs. Clinical Infectious Diseases 2005; 41(Supplement 4): S239-S46.

[5] Schwarzer G. meta: Meta-Analysis with R. R package version 3.7-1. 2014.

[6] R Core Team. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing 2014; Viena, Austria. URL http: //www.R-project.org/.

[7] DerSimonian R, Kacker R. Random-effects model for meta-analysis of clinical trials: An update. Contemporary Clinical Trials 2007; 28(2): 105-14.

[8] Mantel N, Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. 1959.

[9] Box GE, Jenkins GM, Reinsel GC. Time series analysis: forecasting and control. 4th ed. Hoboken, New Jersey: John Wiley & Sons 2013.

[10] Dickey DA, Fuller WA. Distribution of the estimators for autoregressive time series with a unit root. Journal of the American statistical association 1979; 74(366a): 427-31.

[11] Hyndman RJ, Athanasopoulos G, Razbash S*, et al.* forecast: Forecasting functions for time series and linear models. R package version 5.5. 2014.

[12] LJUNG GM, BOX GEP. On a measure of lack of fit in time series models. Biometrika 1978; 65(2): 297-303.

[13] Bennett DE, Myatt M, Bertagnolio S, Sutherland D, Gilks CF. Recommendations for surveillance of transmitted HIV drug resistance in countries scaling up antiretroviral treatment. Antiviral therapy 2008; 13 Suppl 2: 25-36.

[14] Liu TF, Shafer RW. Web resources for HIV type 1 genotypic-resistance test interpretation. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 2006; 42(11): 1608-18.

[15] Vaughan HE, Cane P, Pillay D, Tedder RS. Characterization of HIV type 1 clades in the Caribbean using pol gene sequences. AIDS research and human retroviruses 2003; 19(10): 929-32.

[16] Gittens MV, Roth WW, Roach T*, et al.* The molecular epidemiology and drug resistance determination of HIV type 1 subtype B infection in Barbados. AIDS research and human retroviruses 2003; 19(4): 313-9.

[17] Myers JE, Taylor BS, Rojas Fermin RA*, et al.* Transmitted drug resistance among antiretroviral-naive patients with established HIV type 1 infection in Santo Domingo, Dominican Republic and review of the Latin American and Caribbean literature. AIDS research and human retroviruses 2012; 28(7): 667-74.

[18] Cesaire R, Dos Santos G, Abel S*, et al.* Drug resistance mutations among HIV-1 strains from antiretroviral-naive patients in Martinique, French West Indies. Journal of acquired immune deficiency syndromes 1999; 22(4): 401-5.

[19] Hamilton CL, Eyzaguirre LM, Amarakoon, II*, et al.* Analysis of protease and reverse transcriptase genes of HIV for antiretroviral drug resistance in Jamaican adults. AIDS research and human retroviruses 2012; 28(8): 923-7.

[20] Barrow GJ, Hylton-Kong T, Rodriguez N, Yamamura Y, Figueroa JP. HIV-1 drug resistance in treatment-naive chronically infected patients in Jamaica. Antiviral therapy 2013; 18(7): 941-4.

[21] Ruibal-Brunet IJ, Cuevas MT, Diaz-Torres H*, et al.* Genotypic resistance mutations to antiretroviral drugs in HIV-1 B and non-B subtypes from Cuba. Revista panamericana de salud publica = Pan American journal of public health 2001; 10(3): 174-80.

[22] Perez L, Alvarez LP, Carmona R*, et al.* Genotypic resistance to antiretroviral drugs in patients infected with several HIV type 1 genetic forms in Cuba. AIDS research and human retroviruses 2007; 23(3): 407-14.

[23] Machado LY, Dubed M, Diaz H*, et al.* Transmitted HIV type 1 drug resistance in newly diagnosed Cuban patients. AIDS research and human retroviruses 2013; 29(2): 411-4.

[24] Jack N, Ravasi G, Schrooten W*, et al.* Implementing Early-Warning Indicators of HIV Drug Resistance in the Caribbean. Clinical Infectious Diseases 2012; 54(suppl 4): S290-S93.

[25] Avila-Rios S, Garcia-Morales C, Garrido-Rodriguez D*, et al.* National prevalence and trends of HIV transmitted drug resistance in Mexico. PloS one 2011; 6(11): e27812.

[26] Lama JR, Sanchez J, Suarez L*, et al.* Linking HIV and antiretroviral drug resistance surveillance in Peru: a model for a third-generation HIV sentinel surveillance. Journal of acquired immune deficiency syndromes 2006; 42(4): 501-5.

[27] Soria J, Bull M, Mitchell C*, et al.* Transmitted HIV resistance to first-line antiretroviral therapy in Lima, Peru. AIDS research and human retroviruses 2012; 28(4): 333-8.

[28] DiazGranados CA, Mantilla M, Lenis W. Antiretroviral drug resistance in HIV-infected patients in Colombia. International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases 2010; 14(4): e298-303.

[29] Holguin A, Yebra G, Martin L*, et al.* Transmitted drug-resistance in human immunodeficiency virus-infected adult population in El Salvador, Central America. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases 2013; 19(12): E523-32.

[30] Murillo W, Paz-Bailey G, Morales S*, et al.* Transmitted drug resistance and type of infection in newly diagnosed HIV-1 individuals in Honduras. Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology 2010; 49(4): 239-44.

[31] Lloyd B, O'Connell RJ, Michael NL*, et al.* Prevalence of resistance mutations in HIV-1-Infected Hondurans at the beginning of the National Antiretroviral Therapy Program. AIDS research and human retroviruses 2008; 24(4): 529-35.

[32] Castillo J, Arteaga G, Mendoza Y*, et al.* HIV transmitted drug resistance in adult and pediatric populations in Panama. Revista panamericana de salud publica = Pan American journal of public health 2011; 30(6): 649-56.

[33] Vercauteren J, Wensing AMJ, van de Vijver DAMC*, et al.* Transmission of Drug-Resistant HIV-1 Is Stabilizing in Europe. Journal of Infectious Diseases 2009; 200(10): 1503-08.

[34] Booth CL, Garcia-Diaz AM, Youle MS*, et al.* Prevalence and predictors of antiretroviral drug resistance in newly diagnosed HIV-1 infection. Journal of Antimicrobial Chemotherapy 2007; 59(3): 517-24.

[35] Afani A, Ayala M, Meyer A, Cabrera R, Acevedo W. Primary resistance to antiretroviral therapy in patients with HIV/AIDS in Chile. Revista medica de Chile 2005; 133(3): 295-301.

[36] Rios M, Delgado E, Perez-Alvarez L*, et al.* Antiretroviral drug resistance and phylogenetic diversity of HIV-1 in Chile. Journal of medical virology 2007; 79(6): 647-56.

[37] Acevedo W, Gallardo AM, Galaz J, Afani A, Cortes E. Detection of primary antiretroviral resistance in Chilean patients recently infected with human immunodeficiency virus (HIV). Revista medica de Chile 2007; 135(11): 1406-13.

[38] Afani A, Beltran C, Maria Gallardo A*, et al.* Prevalence of primary antiretroviral resistance among HIV infected patients in Chile. Revista medica de Chile 2010; 138(6): 669-76.

[39] Carobene M, Bolcic F, Farias MS, Quarleri J, Avila MM. HIV, HBV, and HCV molecular epidemiology among trans (transvestites, transsexuals, and transgender) sex workers in Argentina. Journal of medical virology 2014; 86(1): 64-70.

[40] Pando MA, Gomez-Carrillo M, Vignoles M*, et al.* Incidence of HIV type 1 infection, antiretroviral drug resistance, and molecular characterization in newly diagnosed individuals in Argentina: A Global Fund Project. AIDS research and human retroviruses 2011; 27(1): 17-23.

[41] Andreani G, Espada C, Ceballos A*, et al.* Detection of HIV-1 dual infections in highly exposed treated patients. Virology journal 2011; 8: 392.

[42] Pando MA, Eyzaguirre LM, Carrion G*, et al.* High genetic variability of HIV-1 in female sex workers from Argentina. Retrovirology 2007; 4: 58.

[43] Kijak GH, Pampuro SE, Avila MM*, et al.* Resistance profiles to antiretroviral drugs in HIV-1 drug-naive patients in Argentina. Antiviral therapy 2001; 6(1): 71-7.

[44] Petroni A, Deluchi G, Pryluka D*, et al.* Update on primary HIV-1 resistance in Argentina: emergence of mutations conferring high-level resistance to nonnucleoside reverse transcriptase inhibitors in drug-naive patients. Journal of acquired immune deficiency syndromes 2006; 42(4): 506-10.

[45] Rodriguez-Rodrigues N, Duran A, Bouzas MB*, et al.* Increasing trends in primary NNRTI resistance among newly HIV-1-diagnosed individuals in Buenos Aires, Argentina. Journal of the International AIDS Society 2013; 16: 18519.

[46] Cecchini DM, Zapiola I, Fernandez Giuliano S*, et al.* Etravirine resistance mutations in HIV-infected pregnant women. HIV medicine 2013; 14(2): 125-6.

[47] Delgado E, Leon-Ponte M, Villahermosa ML*, et al.* Analysis of HIV type 1 protease and reverse transcriptase sequences from Venezuela for drug resistance-associated mutations and subtype classification: a UNAIDS study. AIDS research and human retroviruses 2001; 17(8): 753-8.

[48] Bouchard M, Masquelier B, Moreno M*, et al.* HIV type 1 drug resistance among naive patients from Venezuela. AIDS research and human retroviruses 2007; 23(3): 482-5.

[49] Castillo J, Comegna M, Quijada W*, et al.* Surveillance of HIV type 1 drug resistance among naive patients from Venezuela. AIDS research and human retroviruses 2009; 25(12): 1329-33.

[50] Rangel HR, Garzaro DJ, Torres JR*, et al.* Prevalence of antiretroviral drug resistance among treatment-naive and treated HIV-infected patients in Venezuela. Memorias do Instituto Oswaldo Cruz 2009; 104(3): 522-5.

[51] Escoto-Delgadillo M, Vazquez-Valls E, Ramirez-Rodriguez M*, et al.* Drug-resistance mutations in antiretroviral-naive patients with established HIV-1 infection in Mexico. HIV medicine 2005; 6(6): 403-9.

[52] Nunn AS, da Fonseca EM, Bastos FI, Gruskin S. AIDS Treatment In Brazil: Impacts And Challenges. Health Affairs 2009; 28(4): 1103-13.

[53] Brindeiro RM, Diaz RS, Sabino EC*, et al.* Brazilian Network for HIV Drug Resistance Surveillance (HIV-BResNet): a survey of chronically infected individuals. Aids 2003; 17(7): 1063-9.

[54] Inocencio LA, Pereira AA, Sucupira MC*, et al.* Brazilian Network for HIV Drug Resistance Surveillance: a survey of individuals recently diagnosed with HIV. Journal of the International AIDS Society 2009; 12: 20.

[55] Tupinambas U, Duani H, Martins AV, Aleixo AW, Greco DB. Transmitted human immunodeficiency virus-1 drug resistance in a cohort of men who have sex with men in Belo Horizonte, Brazil--1996-2012. Memorias do Instituto Oswaldo Cruz 2013; 108(4): 470-5.

[56] Dudley DM, Chin EN, Bimber BN*, et al.* Low-cost ultra-wide genotyping using Roche/454 pyrosequencing for surveillance of HIV drug resistance. PloS one 2012; 7(5): e36494.

[57] Bermudez-Aza EH, Kerr LR, Kendall C*, et al.* Antiretroviral drug resistance in a respondent-driven sample of HIV-infected men who have sex with men in Brazil. Journal of acquired immune deficiency syndromes 2011; 57 Suppl 3: S186-92.

[58] Brasil. Ministério da Saúde. Secretária de Vigilância em Saúde. Programa Nacional de DST e Aids. Protocolo Clínico e Diretrizes Terapêuticas para Manejo da Infecção pelo HIV em Adultos. Brasília: Ministério da Saúde: Ministério da Saúde, Secretaria de Vigilância em Saúde, Programa Nacional de DST e Aids 2013.

[59] de Brito AM, de Castilho EA, Szwarcwald CL. AIDS e infecção pelo HIV no Brasil: uma epidemia multifacetada. Revista da Sociedade Brasileira de Medicina Tropical 2000; 34(2): 207-17.

[60] Nadai Y, Eyzaguirre LM, Sill A*, et al.* HIV-1 epidemic in the Caribbean is dominated by subtype B. PloS one 2009; 4(3): e4814.

[61] Rodrigues R, Scherer LC, Oliveira CM*, et al.* Low prevalence of primary antiretroviral resistance mutations and predominance of HIV-1 clade C at polymerase gene in newly diagnosed individuals from south Brazil. Virus research 2006; 116(1-2): 201-7.

[62] Santos AF, Silveira J, Muniz CP*, et al.* Primary HIV-1 drug resistance in the C-terminal domains of viral reverse transcriptase among drug-naive patients from Southern Brazil. Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology 2011; 52(4): 373-6.

[63] Graf T, Passaes CP, Ferreira LG*, et al.* HIV-1 genetic diversity and drug resistance among treatment naive patients from Southern Brazil: an association of HIV-1 subtypes with exposure categories. Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology 2011; 51(3): 186-91.

[64] de Medeiros LB, Lacerda HR, Cavalcanti AM, de Albuquerque Mde F. Primary resistance of human immunodeficiency virus type 1 in a reference center in Recife, Pernambuco, Brazil. Memorias do Instituto Oswaldo Cruz 2006; 101(8): 845-9.

[65] Cavalcanti AM, Brito AM, Salustiano DM*, et al.* Primary resistance of HIV to antiretrovirals among individuals recently diagnosed at voluntary counselling and testing centres in the metropolitan region of Recife, Pernambuco. Memorias do Instituto Oswaldo Cruz 2012; 107(4): 450-7.

[66] Carobene MG, Rubio AE, Carrillo MG*, et al.* Differences in Frequencies of Drug Resistance–Associated Mutations in the HIV-1 pol Gene of B Subtype and BF Intersubtype Recombinant Samples. JAIDS Journal of Acquired Immune Deficiency Syndromes 2004; 35(2): 207-09.

[67] Grossman Z, Istomin V, Averbuch D*, et al.* Genetic variation at NNRTI resistance-associated positions in patients infected with HIV-1 subtype C. Aids 2004; 18(6): 909-15.

[68] Soares EA, Santos AF, Sousa TM*, et al.* Differential drug resistance acquisition in HIV-1 of subtypes B and C. PloS one 2007; 2(8): e730.

[69] Cunha RD, Abreu CM, Gonzalez LMF*, et al.* Differential *In Vitro* Kinetics of Drug Resistance Mutation Acquisition in HIV-1 RT of Subtypes B and C. PloS one 2012; 7(10): e46622.

[70] Dumans AT, Barreto CC, Santos AF*, et al.* Distinct resistance mutation and polymorphism acquisition in HIV-1 protease of subtypes B and F1 from children and adult patients under virological failure. Infection, genetics and evolution : journal of molecular epidemiology and evolutionary genetics in infectious diseases 2009; 9(1): 62-70.

[71] Brigido LF, Ferreira JL, Almeida VC*, et al.* Southern Brazil HIV type 1 C expansion into the state of Sao Paulo, Brazil. AIDS research and human retroviruses 2011; 27(3): 339-44.

[72] Carvalho BC, Cardoso LP, Damasceno S, Stefani MM. Moderate prevalence of transmitted drug resistance and interiorization of HIV type 1 subtype C in the inland North State of Tocantins, Brazil. AIDS research and human retroviruses 2011; 27(10): 1081-7.

[73] Passaes CB, Guimaraes ML, Fernandez SL*, et al.* Lack of primary mutations associated with integrase inhibitors among HIV-1 subtypes B, C, and F circulating in Brazil. Journal of acquired immune deficiency syndromes 2009; 51(1): 7-12.

[74] Arruda LB, Fonseca LA, Duarte AJ, Casseb J. Genetic diversity on the integrase region of the pol gene among HIV type 1-infected patients naive for integrase inhibitors in Sao Paulo City, Brazil. AIDS research and human retroviruses 2010; 26(1): 105-7.

[75] Teixeira C, de Sa-Filho D, Alkmim W*, et al.* Short communication: high polymorphism rates in the HR1 and HR2 gp41 and presence of primary resistance-related mutations in HIV type 1 circulating in Brazil: possible impact on enfuvirtide efficacy. AIDS research and human retroviruses 2010; 26(3): 307-11.

[76] Araujo LA, Junqueira DM, de Medeiros RM, Matte MC, Almeida SE. Naturally occurring resistance mutations to HIV-1 entry inhibitors in subtypes B, C, and CRF31_BC.

Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology 2012; 54(1): 6-10.

[77] da Silveira AA, Cardoso LP, Francisco RB, de Araujo Stefani MM. HIV type 1 molecular epidemiology in pol and gp41 genes among naive patients from Mato Grosso do Sul State, central western Brazil. AIDS research and human retroviruses 2012; 28(3): 304-7.

[78] Iamarino A, de Melo FL, Braconi CT, Zanotto PM. BF integrase genes of HIV-1 circulating in Sao Paulo, Brazil, with a recurrent recombination region. PloS one 2012; 7(4): e34324.

[79] Rangel HR, Garzaro D, Fabbro R*, et al.* Absence of primary integrase resistance mutations in HIV type 1-infected patients in Venezuela. AIDS research and human retroviruses 2010; 26(8): 923-6.

[80] Eshleman SH, Hudelson SE, Bruce R*, et al.* Analysis of HIV type 1 gp41 sequences in diverse HIV type 1 strains. AIDS research and human retroviruses 2007; 23(12): 1593-8.

[81] Eshleman SH, Hudelson SE, Smith P*, et al.* Analysis of pol integrase sequences in diverse HIV type 1 strains using a prototype genotyping assay. AIDS research and human retroviruses 2009; 25(3): 343-5.

[82] Murphy G, Parry JV. Assays for the detection of recent infections with human immunodeficiency virus type 1. Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin 2008; 13(36).

[83] Dilernia DA, Gomez AM, Lourtau L*, et al.* HIV type 1 genetic diversity surveillance among newly diagnosed individuals from 2003 to 2005 in Buenos Aires, Argentina. AIDS research and human retroviruses 2007; 23(10): 1201-7.

[84] Avila-Rios S, Mejia-Villatoro CR, Garcia-Morales C*, et al.* Prevalence and patterns of HIV transmitted drug resistance in Guatemala. Revista panamericana de salud publica = Pan American journal of public health 2011; 30(6): 641-8.

[85] Valle-Bahena OM, Ramos-Jimenez J, Ortiz-Lopez R*, et al.* Frequency of protease and reverse transcriptase drug resistance mutations in naive HIV-infected patients. Archives of medical research 2006; 37(8): 1022-7.

[86] Viani RM, Hsia K, Hubbard P*, et al.* Prevalence of primary HIV-1 drug resistance in pregnant women and in newly diagnosed adults at Tijuana General Hospital, Baja California, Mexico. International journal of STD & AIDS 2007; 18(4): 235-8.

[87] Brindeiro R, Vanderborght B, Caride E*, et al.* Sequence diversity of the reverse transcriptase of human immunodeficiency virus type 1 from untreated Brazilian individuals. Antimicrobial agents and chemotherapy 1999; 43(7): 1674-80.

[88] Pilcher CD, Perkins MD, Fiscus SA*, et al.* Genotypic resistance and the treatment of HIV-1 infection in Espirito Santo, Brazil. The Journal of infectious diseases 1999; 179(5): 1259-63.

[89] Dumans AT, Soares MA, Pieniazek D*, et al.* Prevalence of protease and reverse transcriptase drug resistance mutations over time in drug-naive human immunodeficiency virus type 1-positive individuals in Rio de Janeiro, Brazil. Antimicrobial agents and chemotherapy 2002; 46(9): 3075-9.

[90] Barreto CC, Nishyia A, Araujo LV*, et al.* Trends in antiretroviral drug resistance and clade distributions among HIV-1--infected blood donors in Sao Paulo, Brazil. Journal of acquired immune deficiency syndromes 2006; 41(3): 338-41.

[91] Maia Teixeira SL, Bastos FI, Hacker MA, Guimaraes ML, Morgado MG. Trends in drug resistance mutations in antiretroviral-naive intravenous drug users of Rio de Janeiro. Journal of medical virology 2006; 78(6): 764-9.

[92] Varella RB, Ferreira SB, de Castro MB, Zalis MG, Tavares MD. Human immunodeficiency virus type 1 protease and reverse transcriptase mutation patterns among treatment-naive patients in different stages of infection in Rio de Janeiro, Brazil. Journal of medical virology 2007; 79(8): 1033-9.

[93] Pires IL, Soares MA, Speranza FA*, et al.* Prevalence of human immunodeficiency virus drug resistance mutations and subtypes in drug-naive, infected individuals in the army health service of Rio de Janeiro, Brazil. Journal of clinical microbiology 2004; 42(1): 426-30.

[94] El-Far F, Medeiros EA, Gasparoto CT, Diaz RS. Antiretroviral drug resistance among patients with human immunodeficiency virus who act as sources or potential sources in occupational accidents involving healthcare workers. Infection control and hospital epidemiology : the official journal of the Society of Hospital Epidemiologists of America 2005; 26(9): 782-8.

[95] Soares MA, De Oliveira T, Brindeiro RM*, et al.* A specific subtype C of human immunodeficiency virus type 1 circulates in Brazil. Aids 2003; 17(1): 11-21.

[96] Eyer-Silva WA, Morgado MG. A genotyping study of human immunodeficiency virus type-1 drug resistance in a small Brazilian municipality. Memorias do Instituto Oswaldo Cruz 2005; 100(8): 869-73.

[97] Soares EA, Martinez AM, Souza TM*, et al.* HIV-1 subtype C dissemination in southern Brazil. Aids 2005; 19 Suppl 4: S81-6.

[98] Gonsalez CR, Alcalde R, Nishiya A*, et al.* Drug resistance among chronic HIV-1-infected patients naive for use of anti-retroviral therapy in Sao Paulo city. Virus research 2007; 129(2): 87-90.

[99] Cardoso LP, Pereira GA, Viegas AA, Schmaltz LE, Stefani MM. HIV-1 primary and secondary antiretroviral drug resistance and genetic diversity among pregnant women from central Brazil. Journal of medical virology 2010; 82(3): 351-7.

[100] Diaz RS, Vasconcelos L, Hayden RL*, et al.* Similar efficacy of lopinavir/ritonavir-containing regimens among clades B and F HIV-1-Infected individuals in Brazil. Journal of acquired immune deficiency syndromes 2008; 47(3): 399-401.

[101] Brigido LF, Nunes CC, Oliveira CM*, et al.* HIV type 1 subtype C and CB Pol recombinants prevail at the cities with the highest AIDS prevalence rate in Brazil. AIDS research and human retroviruses 2007; 23(12): 1579-86.

[102] Eyer-Silva WA, Couto-Fernandez JC, Silva-de-Jesus C, Morgado MG. Prevalence of HIV type 1 drug resistance mutations in treatment-naive and experienced patients from resource-limited settings with universal access to antiretroviral therapy: a survey in two small Brazilian cities. Memorias do Instituto Oswaldo Cruz 2008; 103(2): 143-9.

[103] Brigido LF, Franco HM, Custodio RM*, et al.* Molecular characteristics of HIV type 1 circulating in Sao Paulo, Brazil. AIDS research and human retroviruses 2005; 21(7): 673-82.

[104] Ferreira JL, Thomaz M, Rodrigues R*, et al.* Molecular characterisation of newly identified HIV-1 infections in Curitiba, Brazil: preponderance of clade C among males with recent infections. Memorias do Instituto Oswaldo Cruz 2008; 103(8): 800-8.

[105] Velasco-de-Castro CA, Grinsztejn B, Veloso VG*, et al.* HIV-1 diversity and drug resistance mutations among people seeking HIV diagnosis in voluntary counseling and testing sites in Rio de Janeiro, Brazil. PloS one 2014; 9(1): e87622.

[106] Pilotto JH, Grinsztejn B, Veloso VG*, et al.* Moderate prevalence of transmitted drug resistance mutations among antiretroviral-naive HIV-infected pregnant women in Rio de Janeiro, Brazil. AIDS research and human retroviruses 2013; 29(4): 681-6.

[107] Pfrimer IA, Bizinoto MC, Brandao NA*, et al.* Intermediate levels of transmitted antiretroviral drug resistance in Midwestern Brazil. AIDS research and human retroviruses 2013; 29(2): 205-6.

[108] de Medeiros RM, Junqueira DM, Matte MC*, et al.* Co-circulation HIV-1 subtypes B, C, and CRF31_BC in a drug-naive population from Southernmost Brazil: analysis of primary resistance mutations. Journal of medical virology 2011; 83(10): 1682-8.

[109] de Sa-Filho DJ, Ambar RF, Duarte NB*, et al.* HIV type 1 diversity from newly diagnosed patients in Santos metropolitan area/Brazil. AIDS research and human retroviruses 2009; 25(9): 925-9.

[110] Sprinz E, Netto EM, Patelli M*, et al.* Primary antiretroviral drug resistance among HIV type 1-infected individuals in Brazil. AIDS research and human retroviruses 2009; 25(9): 861-7.

[111] Cardoso LP, Queiroz BB, Stefani MM. HIV-1 pol phylogenetic diversity and antiretroviral resistance mutations in treatment naive patients from Central West Brazil. Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology 2009; 46(2): 134-9.

[112] Ferreira JL, Rodrigues R, Lanca AM*, et al.* Transmitted Drug Resistance among People Living with HIV/Aids at Major Cities of Sao Paulo State, Brazil. Advances in virology 2013; 2013: 878237.

[113] Ferreira AS, Cardoso LP, Stefani MM. Moderate prevalence of transmitted drug resistance and high HIV-1 genetic diversity in patients from Mato Grosso State, Central Western Brazil. Journal of medical virology 2011; 83(8): 1301-7.

[114] Reis MN, de Alcantara KC, Cardoso LP, Stefani MM. Polymorphisms in the HIV-1 gp41 env gene, natural resistance to enfuvirtide (T-20) and pol resistance among pregnant Brazilian women. Journal of medical virology 2014; 86(1): 8-17.

[115] Gaspareto KV, Mello FM, Dias JR*, et al.* Genetic diversity and primary resistance among HIV-1-positive patients from Maringa, Parana, Brazil. Revista do Instituto de Medicina Tropical de Sao Paulo 2012; 54(4): 207-13.

[116] Prellwitz IM, Alves BM, Ikeda ML*, et al.* HIV behind bars: human immunodeficiency virus cluster analysis and drug resistance in a reference correctional unit from southern Brazil. PloS one 2013; 8(7): e69033.

[117] da Costa ZB, de Lima YA, Martelli CM, Stefani MM. Transmitted HIV resistance among pregnant young women infected with HIV-1 in Brazil. AIDS patient care and STDs 2013; 27(8): 439-41.

[118] Sanabani SS, Pastena ER, da Costa AC*, et al.* Characterization of partial and near full-length genomes of HIV-1 strains sampled from recently infected individuals in Sao Paulo, Brazil. PloS one 2011; 6(10): e25869.

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6 CAPÍTULO II

Role of inosine triphosphate pyrophosphatase gene variant on fever incidence during zidovudine antiretroviral therapy

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Role of inosine triphosphate pyrophosphatase gene variant on fever incidence during zidovudine antiretroviral therapy

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ABSTRACT. Zidovudine, the antiretroviral drug used to treat HIV infection, commonly causes adverse effects, such as systemic fever and gastrointestinal alterations. In the present study, the potential role of inosine triphosphate pyrophosphatase (*ITPA*) gene variant on the incidence of adverse events during antiretroviral therapy (ART) of HIV with zidovudine was discussed. Individuals from Northeastern Brazil (N = 204) receiving treatment for HIV-1 infection were recruited. Zidovudine-related adverse effects developed during the treatment were registered. The rs1127354 polymorphism in the *ITPA* gene was

genotyped using real-time PCR to assess whether this single nucleotide polymorphism was associated with the occurrence of zidovudine-related adverse effects. We observed a significant association between the *ITPA* variant genotype and the reported systemic fever (odds ratio = 7.17, 95% confidence interval = 1.19-43.15; P = 0.032). Zidovudine use could indirectly lead to an increase in the levels of inosine monophosphate in an antimetabolite-like manner, which is converted to inosine triphosphate (ITP). The rs1127354 variant caused a decrease in ITPA activity, thereby leading to ITP accumulation. This in turn resulted in cytotoxicity, which was manifested by neutropenia and fever. Therefore, we hypothesized a pharmacogenetic model involving the ITPA variant genotype in multifactorial components that act together to determine the onset of zidovudine-related adverse effects.

Key words: Antiretroviral; Adverse effect; AZT; ITPA

INTRODUCTION

The introduction of antiretroviral therapy (ART) in clinical practice significantly reduced the number of deaths due to human immunodeficiency virus type 1 (HIV-1) infection, which is the etiological agent of acquired immunodeficiency syndrome (AIDS). Current ART regimens consist of a combination of three antiretroviral drugs that target HIV-1 proteins, hampering the viral life-cycle completion (Cressey Lallemant, 2007). Azidothymidine (AZT), also known as zidovudine, was the first antiretroviral drug developed in the early years of the HIV-1 pandemics. AZT is a nucleoside analog reverse transcriptase inhibitor (NRTI), a class of molecules that blocks the synthesis of viral genetic material by inhibiting the DNA chain growth (Alves et al., 2012). AZT is still commonly used in first-line ART regimens in Brazil, as it is a relatively cheap and effective drug directly produced in Brazil. It is used in combination with another NRTI (the two NRTIs being the "backbone" of the ART regimens) and a third drug, such as a protease inhibitor (Ministério da Saúde, 2010).

ART is a lifelong commitment; therefore, it is sometimes associated with adverse drug reactions, which can be mild and temporary or very severe and life threatening. NRTIs may cause metabolic defects, such as dyslipidemia and lipodystrophy (Montessori et al., 2004). Some reports showed that AZT use is associated with systemic (such as fever) and gastrointestinal adverse effects (Jacobson et al., 1989; Vella et al., 1994).

Since AZT is a nucleoside analog, we hypothesized that it may interfere with the intracellular nucleoside/nucleotide biosynthesis pathways, similar to the mode of action of antimetabolite drugs, such as mercaptopurine and methotrexate, which interfere with purine metabolism (Marinaki et al., 2004).

Studies on the pharmacogenetics of nucleoside purine analogs (Mira et al., 2007; Fellay et al., 2010; Stocco et al., 2010) led to the hypothesis that inosine triphosphate pyrophosphatase (ITPA) could play an indirect role on AZT metabolism, possibly influencing the occurrence of adverse effects related to the drug.

ITPA is a housekeeping enzyme that dephosphorylates inosine triphosphate (ITP) and deoxy-ITP, converting them to monophosphate forms. This may be related to the protection

of genome integrity, because the incorporation of inosine during nucleic acid synthesis may cause errors (von Ahsen et al., 2008).

A relatively common missense variant of the ITPA gene (*ITPA*), a single nucleotide polymorphism (SNP), which was identified as rs1127354 (94 C>A, Pro32Thr), is known to abolish the gene function. This missense SNP in heterozygotes was associated with the reduction in erythrocyte enzyme activity to approximately 25%, while no enzyme activity was detected in A/A homozygotes (Maeda et al., 2005). This trait is benign, but has already been described as a risk factor for the occurrence of adverse effects during antimetabolite therapy (Marinaki et al., 2004).

The discovery of novel genetic markers associated with ART response together with other markers associated with immune response against HIV-1 infection (Samie et al., 2014; Said et al., 2016) helped in the optimization of current regimens and improvement of the quality of life in patients sustaining lifelong ART treatment. In this study, we evaluated whether the *ITPA* rs1127354 polymorphism was associated with the occurrence of adverse effects of ART regimens containing AZT in HIV-1-positive patients from Northeast Brazil.

MATERIAL AND METHODS

We enrolled 204 patients from the metropolitan region of Recife (Northeast Brazil), at Instituto de Medicina Integral Professor Fernando Figueira (IMIP) for a genetic association, retrospective case-control study between May 2011 and August 2012. For inclusion in the study, each patient had to be between 18 and 50 years of age on the ART start date. In addition, patients should have been receiving ART (AZT-containing regimens) for at least 1 year with good treatment compliance, had no history of illegal drug abuse, and had no chronic diseases other than HIV-1 infection [no human T-lymphotropic virus type 1, hepatitis B or hepatitis C co-infections or autoimmune diseases, such as diabetes or systemic lupus erythematosus]. Each patient provided written consent for participation in the study and for the collection of blood samples for posterior genomic DNA extraction. They were invited and interviewed by the physicians/researchers. The IMIP Research Ethics Committee approved the study (protocol No. 2273-11).

Each patient answered a questionnaire, which was used to record the gender, age (in years) and body mass (in kg) on the ART start date. In addition, the therapy adherence status [indirectly measured through medication possession ratio of the first year of the therapy as proposed by Fairman and Matheral (2000)], and self-reported race: as “white”, “black” and “pardo” (multiracial) following (Coelho et al., 2015) the stratification rationale were also recorded. The outcome was the occurrence of any adverse effect ascribed to the use of AZT by the patient’s physician. The patients received standard AZT + lamivudine (3TC) regimens (300 + 150 mg combination pill, twice daily).

The *ITPA* rs1127354 polymorphism was genotyped using a TaqMan assay (C_27465000_10) following the manufacturer instructions in an Applied Biosystems 7500 Real-Time PCR System (Life Technologies, formerly Applied Biosystems, Foster City, CA, USA) through allele-specific fluorescence signal discrimination. Following allele assignment for each patient, the allele and genotype frequencies were determined by simple counting. The adherence to Hardy-Weinberg equilibrium was assessed using the χ^2 test. Polymorphism genotypes were considered as the primary predictors for the occurrence of adverse effects, and the remaining variables described above were considered as possible confounders. Therefore, they were included on a logistic regression model to assess whether the SNP had any influence

on the outcome. In other words, the model assessed whether the SNP was associated with the development of AZT-related adverse effects when controlled for the patients' characteristics. The odds ratios (OR) and their respective 95% confidence intervals (CI) were calculated.

All statistical analyses were performed using logistic regression through R software version 3.0.1 (R Core Team, 2013).

RESULTS

The sample consisted of 151 (74.0%) women and 53 (26%) men, with a median age of 34 years and an interquartile range 30-40 on the ART start date. The majority of the patients reported being afro-descendants (80.9%). The demographic characteristics of the patients and the adverse events developed during the first year of ART are summarized in Table 1.

Table 1. Demographic characteristics of the enrolled HIV patients receiving AZT + 3TC backbone regimens.

Characteristic	Patients (N = 204)
Gender (% women)	74.0
Race (% afro-descendant)	80.9
Body mass (kg), median (IQR)	60.2 (53.9-68.2)
Age (years old), median (IQR)	34 (30-40)
Prevalence of adverse effects (%)	
Anemia	10.3
Dyslipidemia	7.4
Fever	2.9
Gastrointestinal toxicity	25.5
Headache	10.3
Hepatotoxicity	3.4
Neurotoxicity	23.0
Cutaneous rash	16.2

AZT = zidovudine; 3TC = lamivudine; IQR = interquartile range.

Some AZT-related adverse effects were reported in the first year of ART in these patients. For example, the two most common adverse effects were gastrointestinal toxicity (reported in 25.5% of the patients in the sample) and neurotoxicity (23.0%), followed by cutaneous rash (16.2%), anemia and headaches (both 10.3%), dyslipidemia (7.4%), hepatotoxicity (3.4%) and fever (2.9%). The prevalence of each of the reported AZT-related adverse effects is also presented in Table 1.

Genotyping was successful in 190 patients. The frequency of the variant genotype (C/A) was 7.4%. The genotype counts were in accordance with the Hardy-Weinberg equilibrium. No A/A homozygous patients were found in our sample.

After the confounding factors (gender, age, and self-reported race) were included on a logistic regression model and adjusted, it was observed that the *ITPA* variant allele was associated with the occurrence of fever, but not with other adverse effects. We observed a higher frequency of *ITPA* variant genotype in patients reported with fever after AZT treatment when compared to patients with no fever reported (33.3 *vs* 6.5%, respectively; OR = 7.17, 95%CI = 1.19-43.15; P = 0.032). The allele and genotype counts as well as the genetic association test results with all the reported adverse effects (logistic regression modeling) are summarized in Table 2.

Table 2. Genetic association tests through logistic regression modeling to determine the influence of *ITPA* rs1127354 (94 C>A, Pro32Thr) variant over the occurrence of zidovudine-related (AZT) adverse effects.

Allele and genotype counts according to adverse effects	Cases N (%)	Controls N (%)	Logistic regression OR (95%CI)	P value
Anemia				
A	2 (5.3)	12 (3.5)		
C	36 (94.7)	330 (96.5)		
C/A	2 (10.5)	12 (7.0)	1.56 (0.32-7.56)	0.58
C/C	17 (89.5)	159 (93.0)	Reference	
Dyslipidemia				
A	0 (0.0)	14 (4.0)		
C	28 (100.0)	338 (96.0)		
C/A	0 (0.0)	14 (8.0)	Not calculated	0.99
C/C	14 (100.0)	162 (92.0)	Reference	
Fever				
A	2 (16.7)	12 (3.3)		
C	10 (83.3)	356 (96.7)		
C/A	2 (33.3)	12 (6.5)	7.17 (1.19-43.15)	**0.032**
C/C	4 (66.7)	172 (93.5)	Reference	
Gastrointestinal toxicity				
A	5 (5.2)	9 (3.2)		
C	91 (94.8)	275 (96.8)		
C/A	5 (10.4)	9 (6.3)	1.72 (0.55-5.41)	0.36
C/C	43 (89.6)	133 (93.7)	Reference	
Headache				
A	2 (5.3)	12 (3.5)		
C	36 (94.7)	330 (96.5)		
C/A	2 (10.5)	12 (7.0)	1.56 (0.32-7.56)	0.58
C/C	17 (89.5)	159 (93.0)	Reference	
Hepatotoxicity				
A	0 (0.0)	14 (3.8)		
C	14 (100.0)	352 (96.2)		
C/A	0 (0.0)	14 (7.7)	Not calculated	0.99
C/C	7 (100.0)	169 (92.3)	Reference	
Neurotoxicity				
A	4 (4.3)	10 (3.5)		
C	88 (95.7)	278 (96.5)		
C/A	4 (8.7)	10 (6.9)	1.28 (0.38-4.28)	0.69
C/C	42 (91.3)	134 (93.1)	Reference	
Cutaneous rash				
A	3 (5.4)	11 (3.4)		
C	53 (94.6)	313 (96.6)		
C/A	3 (10.7)	11 (6.8)	1.64 (0.43-6.32)	0.47
C/C	25 (89.3)	151 (93.2)	Reference	

OR = odds ratio; CI = confidence interval.

DISCUSSION

The findings of this study led us to hypothesize a model to explain the mechanism by which ITPA modulates the occurrence of adverse effects during AZT therapy (Figure 1). To exert an antiretroviral effect, AZT must be phosphorylated to the AZT-triphosphate form (Peter and Gambertoglio, 1998). However, AZT-monophosphate is both a substrate and an inhibitor of thymidylate kinase (DTYMK), the enzyme that produces AZT-diphosphate (Furman et al., 1986). This inhibition would decrease the deoxythymidine triphosphate (dTTP) pool, since DTYMK is also involved in the phosphorylation of thymidine nucleotides.

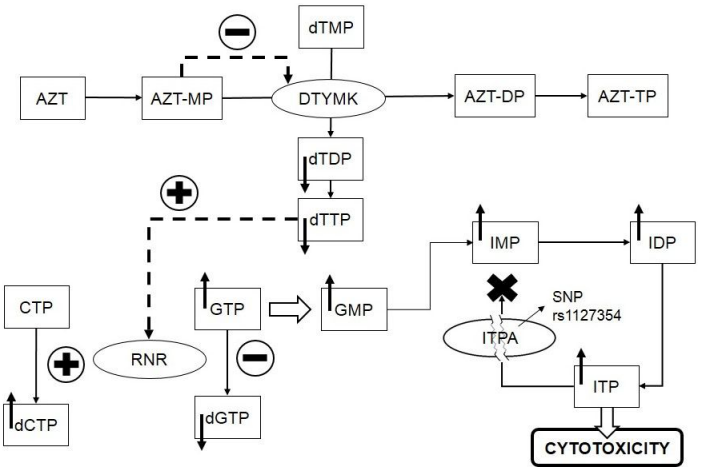

Figure 1. Relation between missense rs1127354 variant (94 C>A, Pro32Thr) on the inosine triphosphatase (ITPA) and zidovudine (AZT) adverse effects in an antimetabolite-like manner. AZT undergoes sequential steps of phosphorylation, generating AZT mono- (AZT-MP), di- (AZT-DP) and triphosphate (AZT-TP). AZT-MP is both a substrate and inhibitor of thymidylate kinase (DTYMK), interfering with phosphorylation of deoxythymidine monophosphate (dTMP) to diphosphate and triphosphate forms (dTDP and dTTP). The reduction of dTTP pools stimulates the ribonucleotide reductase (RNR) to shift from production of deoxyguanine triphosphate (dGTP) to deoxycytidine (dCTP) triphosphate instead. Consequently, guanosine nucleotide levels would rise, leading to higher synthesis of inosine monophosphate (IMP). Thus, inosine triphosphate levels (ITP) levels would also increase due to IMP phosphorylation. However, as the ITPA activity is compromised by the missense allele, ITP would not be converted to IMP, accumulating in cells, causing cytotoxicity, manifested as neutropenic fever. Some enzymes were ommitted in the depiction for simplicity.

The decrease in dTTP quantity would stimulate (by allosteric regulation) the ribonucleotide reductase enzyme to shift to the synthesis of deoxycytidine diphosphate (dCDP) instead of deoxyguanosine diphosphate (Frick et al., 1988). This would lead to two consequences, as previously reported in the cells exposed to prolonged dosages of AZT *in vitro*: 1) the dCDP and consequently dCTP pools would increase, finally leading to a higher uridine production via pyrimidine salvage pathway and 2) an imbalance of guanosine nucleotides, leading to GTP and GMP accumulation. Higher GMP quantities would result in higher levels of inosine monophosphate (IMP) via GMP reductase. The IMP pools are then converted to hypoxanthine (Agarwal et al., 1995).

Thus, AZT use would indirectly lead to increase in IMP levels in an antimetabolite-like manner, which may be converted to ITP. Since the ITPA activity is diminished owing to the rs1127354 variant in some people, the ITP levels would accumulate leading to cytotoxicity (Stocco et al., 2010), which is manifested by neutropenia and fever (Stocco et al., 2009). The occurrence of fever could be explained by an undiagnosed mild infection during the course of ART treatment, a phenomenon already described during cancer chemotherapy (Bow, 2013). This new model for analyzing the mechanism by which AZT disturbs the nucleotide pools and how ITPA variants modulate the risk for AZT-related adverse effects needs further validation by *in vitro* and clinical studies.

Being retrospective, our study has many limitations. Therefore, we proposed a novel possible marker (*ITPA* variant genotype) in the multifactorial pharmacogenetic components that act together, to determine the onset of AZT adverse effects.

Conflicts of interest

The authors declare no conflict of interest.

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REFERENCES

Agarwal RP, He J, Bansal M and Gupta V (1995). Effect of long-term zidovudine exposure on salvage and de novo purine and pyrimidine nucleotide syntheses. *Biochim. Biophys. Acta* 1266: 223-228. http://dx.doi.org/10.1016/0167-4889(95)00018-N

Alves S, D'avila S, de Freitas MA, Freitas C, et al. (2012). Progress Report on the Brazilian Response to HIV/AIDS. Departamento de DST-AIDS e Hepatites Virais, Brasil, pp. 125. Available at [http://files.unaids.org/en/dataanalysis/knowyourresponse/countryprogressreports/2012countries/UNGASS_2012_ingles_rev_08jun.pdf].

Bow EJ (2013). Infection in neutropenic patients with cancer. *Crit. Care Clin.* 29: 411-441. http://dx.doi.org/10.1016/j.ccc.2013.03.002

Coelho AV, Moura RR, Cavalcanti CA, Guimarães RL, et al. (2015). A rapid screening of ancestry for genetic association studies in an admixed population from Pernambuco, Brazil. *Genet. Mol. Res.* 14: 2876-2884. http://dx.doi.org/10.4238/2015.March.31.18

Cressey TR and Lallemant M (2007). Pharmacogenetics of antiretroviral drugs for the treatment of HIV-infected patients: an update. *Infect. Genet. Evol.* 7: 333-342. http://dx.doi.org/10.1016/j.meegid.2006.08.004

Fairman K and Matheral B (2000). Evaluating medication adherence: which measure is right for your program? *Strategies* 2: 4.

Fellay J, Thompson AJ, Ge D, Gumbs CE, et al. (2010). ITPA gene variants protect against anaemia in patients treated for chronic hepatitis C. *Nature* 464: 405-408. http://dx.doi.org/10.1038/nature08825

Frick LW, Nelson DJ, St Clair MH, Furman PA, et al. (1988). Effects of 3'-azido-3'-deoxythymidine on the deoxynucleotide triphosphate pools of cultured human cells. *Biochem. Biophys. Res. Commun.* 154: 124-129. http://dx.doi.org/10.1016/0006-291X(88)90659-6

Furman PA, Fyfe JA, St Clair MH, Weinhold K, et al. (1986). Phosphorylation of 3'-azido-3'-deoxythymidine and selective interaction of the 5'-triphosphate with human immunodeficiency virus reverse transcriptase. *Proc. Natl. Acad. Sci. USA* 83: 8333-8337. http://dx.doi.org/10.1073/pnas.83.21.8333

Jacobson MA, McGrath MS, Joseph P, Molaghan JB, et al. (1989). Zidovudine-induced fever. *J. Acquir. Immune Defic. Syndr.* 2: 382-388.

Maeda T, Sumi S, Ueta A, Ohkubo Y, et al. (2005). Genetic basis of inosine triphosphate pyrophosphohydrolase deficiency in the Japanese population. *Mol. Genet. Metab.* 85: 271-279. http://dx.doi.org/10.1016/j.ymgme.2005.03.011

Marinaki AM, Duley JA, Arenas M, Ansari A, et al. (2004). Mutation in the ITPA gene predicts intolerance to azathioprine. *Nucleosides Nucleotides Nucleic Acids* 23: 1393-1397. http://dx.doi.org/10.1081/NCN-200027639

Ministério da Saúde. (2010). Quais são os antirretrovirais? Secretária de Vigilância em Saúde. Departamento de DST, Aids e Hepatites Virais. Available at [http://www.aids.gov.br/pagina/quais-sao-os-antirretrovirais].

Mira JA, López-Cortés LF, Merino D, Arizcorreta-Yarza A, et al.; Grupo para el Estudio de las Hepatitis Viricas de la Sociedad Andaluza de Enfermedades Infecciosas (2007). Predictors of severe haematological toxicity secondary to pegylated interferon plus ribavirin treatment in HIV-HCV-coinfected patients. *Antivir. Ther. (Lond.)* 12: 1225-1235.

Montessori V, Press N, Harris M, Akagi L, et al. (2004). Adverse effects of antiretroviral therapy for HIV infection. *CMAJ* 170: 229-238.

Peter K and Gambertoglio JG (1998). Intracellular phosphorylation of zidovudine (ZDV) and other nucleoside reverse transcriptase inhibitors (RTI) used for human immunodeficiency virus (HIV) infection. *Pharm. Res.* 15: 819-825. http://dx.doi.org/10.1023/A:1011956011207

R Core Team (2013). R: A Language and Environment for Statistical Computing. *R Foundation for Statistical Computing*. Vienna, Austria. URL http://www.R-project.org/.

Said EA, Al-Yafei F, Zadjali F, Al-Balushi MS, et al. (2016). Frequency of TLR4 (1063A/G and 1363C/T) polymorphisms in healthy and HIV-infected Omani individuals and their relationship to viral load and T cell count. *Genet. Mol. Res.* 15: http://dx.doi.org/10.4238/gmr.15027671.

Samie A, Moloro GT and Nangammbi TC (2014). Interleukin-7 receptor gene polymorphism at +1237 locus and its effect on susceptibility to opportunistic infections among HIV and AIDS patients in Limpopo Province, South Africa. *Genet. Mol. Res.* 13: 8757-8766. http://dx.doi.org/10.4238/2014.October.27.17

Stocco G, Cheok MH, Crews KR, Dervieux T, et al. (2009). Genetic polymorphism of inosine triphosphate pyrophosphatase is a determinant of mercaptopurine metabolism and toxicity during treatment for acute lymphoblastic leukemia. *Clin. Pharmacol. Ther.* 85: 164-172. http://dx.doi.org/10.1038/clpt.2008.154

Stocco G, Crews KR and Evans WE (2010). Genetic polymorphism of inosine-triphosphate-pyrophosphatase influences mercaptopurine metabolism and toxicity during treatment of acute lymphoblastic leukemia individualized for thiopurine-S-methyl-transferase status. *Expert Opin. Drug Saf.* 9: 23-37. http://dx.doi.org/10.1517/14740330903426151

Vella S, Giuliano M, Dally LG, Agresti MG, et al.; The Italian Zidovudine Evaluation Group (1994). Long-term follow-up of zidovudine therapy in asymptomatic HIV infection: results of a multicenter cohort study. *J. Acquir. Immune Defic. Syndr.* 7: 31-38.

von Ahsen N, Oellerich M and Armstrong VW (2008). Characterization of the inosine triphosphatase (ITPA) gene: haplotype structure, haplotype-phenotype correlation and promoter function. *Ther. Drug Monit.* 30: 16-22. http://dx.doi.org/10.1097/FTD.0b013e318161a21a

7 CAPÍTULO III

Dendritic cell-based immunotherapies to fight HIV: how far from a success story? A systematic review and meta-analysis

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- *fundamental theoretical problems of broad interest in biology, chemistry and medicine;*
- *breakthrough experimental technical progress of broad interest in biology, chemistry and medicine; and*
- *application of the theories and novel technologies to specific experimental studies and calculations.*

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Review

Dendritic Cell-Based Immunotherapies to Fight HIV: How Far from a Success Story? A Systematic Review and Meta-Analysis

Antonio Victor Campos Coelho [1], Ronald Rodrigues de Moura [1], Anselmo Jiro Kamada [1], Ronaldo Celerino da Silva [2], Rafael Lima Guimarães [1,2], Lucas André Cavalcanti Brandão [2,3], Luiz Cláudio Arraes de Alencar [4,5] and Sergio Crovella [6,*]

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Abstract: The scientific community still faces the challenge of developing strategies to cure HIV-1. One of these pursued strategies is the development of immunotherapeutic vaccines based on dendritic cells (DCs), pulsed with the virus, that aim to boost HIV-1 specific immune response. We aimed to review DCs-based therapeutic vaccines reports and critically assess evidence to gain insights for the improvement of these strategies. We performed a systematic review, followed by meta-analysis and meta-regression, of clinical trial reports. Twelve studies were selected for meta-analysis. The experimental vaccines had low efficiency, with an overall success rate around 38% (95% confidence interval = 26.7%–51.3%). Protocols differed according to antigen choice, DC culture method, and doses, although multivariate analysis did not show an influence of any of them on overall success rate. The DC-based vaccines elicited at least some immunogenicity, that was sometimes associated with plasmatic viral load transient control. The protocols included both naïve and antiretroviral therapy (ART)-experienced individuals, and used different criteria for assessing vaccine efficacy. Although the vaccines did not work as expected, they are proof of concept that immune responses can be boosted against HIV-1. Protocol standardization and use of auxiliary approaches, such as latent HIV-1 reservoir activation and patient genomics are paramount for fine-tuning future HIV-1 cure strategies.

Keywords: human immunodeficiency virus; dendritic cell; clinical trial; vaccine; meta-regression

1. Introduction

The adaptive immune response during viral infections is mediated by antigen-presenting cells (APC), such as macrophages, B lymphocytes and dendritic cells (DCs), which capture antigens and present them to naïve lymphocytes [1,2].

Immature DCs are characterized by high endocytic capacity, with constant sampling of the surroundings of peripheral tissues (such as mucosa and epithelia) for pathogen and injury sensing [3]. DC activation, upon pathogen sensing, leads to capture of proteins, processing into peptides and antigen presentation to lymphocytes, and they are thus considered the most potent antigen-presenting cell of the immune system [4].

As the success of antiretroviral therapy (ART) enabled the scientific community to turn its efforts to the search for a definitive cure for HIV-1, several therapeutic DC-based strategies have been developed based on the rationale that providing DCs with specific antigen presentation would "boost" the recovery of host immune response against HIV-1 (Figure 1).

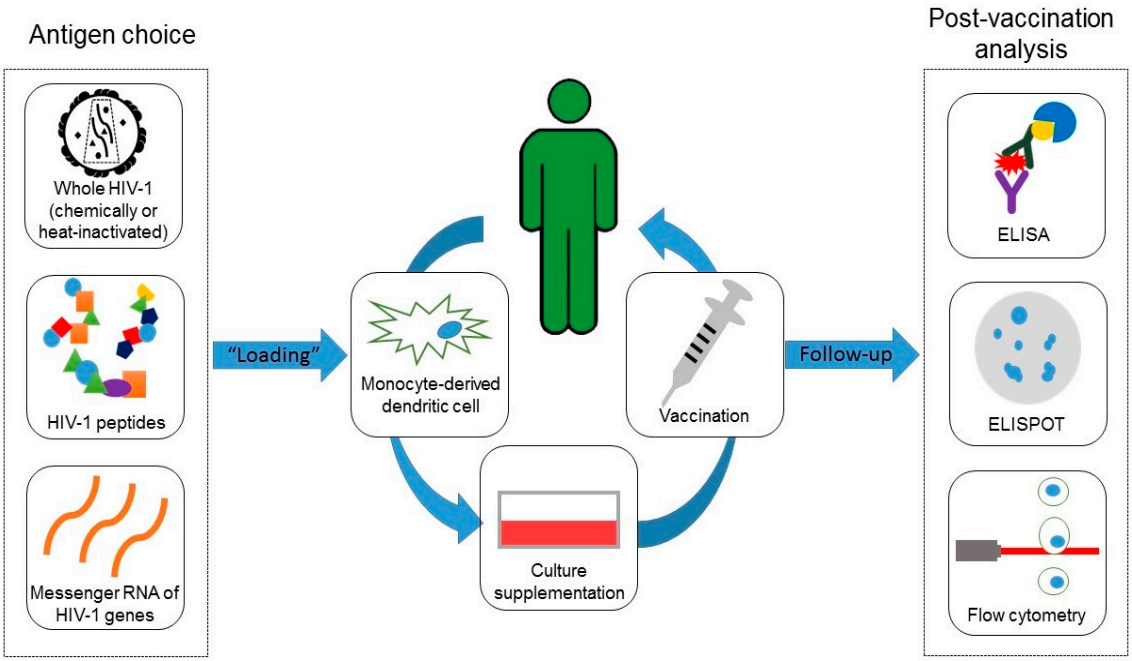

Figure 1. Rationale behind dendritic-cell based experimental anti-HIV-1 vaccines. Providing dendritic cells with specific antigen presentation will result in a reconditioning of host immune response against HIV-1, in the hopes that this will lead to a functional cure. The experimental vaccination protocols details vary, but they basically consist in choosing an antigen (**left** panel), such as autologous (taken from the volunteer) whole virus that are heat- or chemically-inactivated (**top left** panel), viral peptides such as gag or pol residues, for example (**middle left** panel) or even viral messenger RNA molecules (**bottom left** panel). Following antigen choice, dendritic cells must be obtained for vaccine preparation (**center** panel). Usually, leukocytes are collected through leukapheresis and monocytes are isolated and differentiated in vitro into immature dendritic cells. Then, dentritic cells (DCs) are "loaded" with the chosen antigen and activated with cytokine supplementation, for example with granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon alpha and gamma (IFN-α and IFN-γ), interleukins 4 and 6 (IL-4 and IL-6) and tumor necrosis alpha (TNF-α) into mature DCs. Defined amounts of mature dendritic cells are then periodically injected in the individual. Usually, immune response is assessed before, and during vaccination follow-up, through methodologies such as enzyme-linked immunosorbent assay (ELISA), enzyme-linked immunospot (ELISPOT), flow cytometry or any combination thereof (**right** panel), to evaluate (qualitatively and quantitatively) how it changed following vaccination.

Thus, we aimed to summarize reports from clinical trials with DC-based therapeutic vaccines with a systematic review supported by meta-analysis and meta-regression modeling to gain insight from past experiences to improve future therapeutic strategies against HIV-1.

2. Results

2.1. Study Screening and Characteristics

The search strategy resulted in a total of 567 unique abstracts, which were assessed for eligibility. A total of 55 abstracts reporting clinical trial findings were selected for further review. Among those, we extracted data from 12 studies and included them in the meta-analysis (Figure 2).

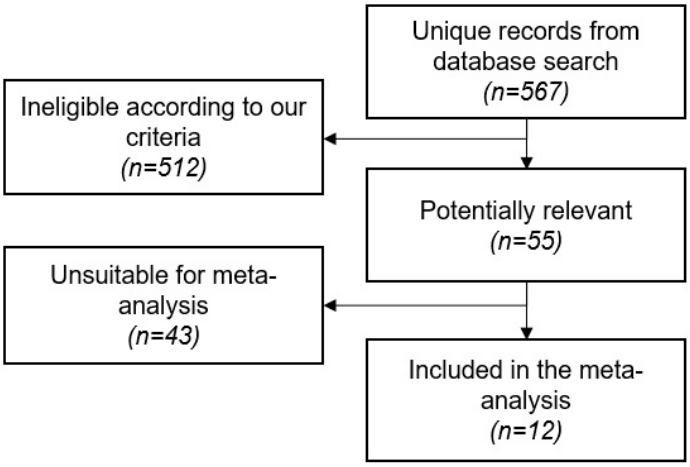

Figure 2. Summary of the number of screened, reviewed and included or excluded dendritic cell-based experimental anti-HIV-1 vaccines.

The characteristics of the 12 studies selected for inclusion in the meta-analysis are summarized in Tables 1 and 2. The Tables 1 and 2 deal with study design-related data from each protocol. Tables 3 and 4 contain information concerning technical differences among the studies.

The majority of selected studies recruited ART-experienced individuals for the vaccine trials (eight out of 12 studies). Overall, the included individuals had good immunological status, as expected by the reported inclusion criteria and judged by their pre-vaccination CD4+ T cell counts (median 632 cells/mm^3, interquartile range, IQR = 559.5–667), with only two studies reporting CD4+ T cell counts medians below 500 cells/mm^3 [5,6]. One study did not inform the CD4+ T cell counts from their recruited patients [7].

Median number of recruited individuals was 18 (IQR = 11–25, minimum and maximum: 4 and 54, respectively). Each individual received a median of four doses from the experimental DC-based vaccine (minimum of three and maximum of six doses), with a biweekly periodicity (five out of 12 studies) or every four weeks or more (seven out of 12). The form of administration of the experimental vaccine was similar in the majority of the studies, being intradermal, subcutaneous or both, mostly on axillary areas of the body. Only one study administered the vaccine intravenously in the recruited individuals [7]. No severe vaccine-associated adverse effects were reported by any of the studies. Reported events were mostly flu-like reactions and reactions at the site of vaccine injection.

There were some differences among protocols regarding DC maturation and choice of the antigen for vaccine preparation. Five out of 12 studies loaded the DCs with (heat- or chemically-) inactivated autologous whole virus [8–12]. The other five loaded HIV-1 peptides [5–7,13,14] and only two used viral mRNA, which were electroporated into DCs for antigen production [15,16]. Cytokines used in culture medium supplementation included granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon alpha and gamma (IFN-α and IFN-γ), interleukins 4 and 6 (IL-4 and IL-6) and tumor necrosis alpha (TNF-α). Other molecules for boosting DC maturation besides cytokines were: prostaglandin E2 (PGE2), lipopolysaccharide (LPS) [14], polyinosinic:polycytidylic acid [6] and CD40 ligand (CD40L) signal [16].

The median number of DCs used per vaccine dose was 7×10^6 cells (minimum 0.7×10^6 cells and maximum 15×10^6 cells), matured in culture for a median of seven days (minimum of three days and maximum of eight days). One protocol used immature DCs, which were cultured for just two days before administration into the recruited individuals [7].

2.2. Meta-Analysis and Meta-Regression Results

The total sample number polled by the meta-analysis was 173 vaccinated individuals. The experimental vaccines had low efficiency, as suggested by the meta-analysis. The overall treatment success rate was estimated at 38.2% (95% CI = 26.7–51.3) according to a random effects model (I^2 = 30.4%, moderate heterogeneity) (Figure 3).

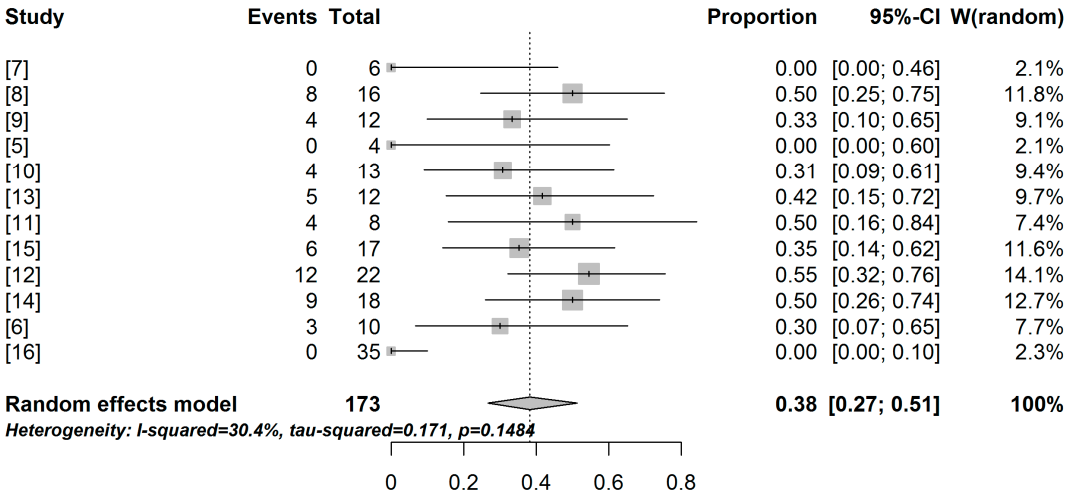

Figure 3. Forest plot of dendritic cell-based experimental anti-HIV-1 vaccines treatment success rate across studies. Each line represents a vaccination experimental protocol with the number of partial/transient responses to the vaccine (events column) among the vaccinated individuals (total column). The proportion of response to the vaccine is represented by a small vertical line inside a square. Each square is directly proportional to the sample size of each study, and the lines represent the 95% confidence interval. The proportions and confidence intervals are also represented in the column of the same name. The weight column (W(random)) represents how much each study contributed to the pooled sample size (n= 173 individuals). The pooled proportion and 95% confidence interval estimated by a random effects model meta-analysis is represented by a diamond and highlighted in bold in the lower part of the figure. Heterogeneity measures (I^2, τ^2) and the p-value of the Cochran's Q test for heterogeneity are also represented.

Table 1. Summary of the 12 studies characteristics that were included in the meta-analysis.

Ref.	Country, City, Year	Inclusion Criteria	Recruited Individuals' ART Status	Baseline CD4+ T cell count *	Vaccine Doses	Periodicity	Vaccine Administration Form	Anatomical Site (Vaccine Application)	Adverse Effects
[7]	USA, Stanford, 1998	Asymptomatic HIV-1 infection CD4+ T cell counts >350/mm^3	naive	NR	6	Monthly	intravenous injection	NR	None reported
[8]	Brazil, Recife, 2004	Age of ≥18 years; No current pregnancy; HIV-1 asymptomatic seropositivity for ≥1 year; ART-naive for at least 6 months prior to enrollment; Hemoglobin ≥10 g/dL and platelets ≥100,000	naive	554 ± 174	3	Biweekly	subcutaneous injection	left and right axillary and inguinal areas	Increase in the size of peripheral lymph nodes
[9]	Spain, Barcelona, 2005	Asymptomatic HIV-1 infection; Baseline and nadir CD4+ T cell counts >500 cells/mL; Baseline pre-ART PVL >5000 copies/mL; PVL <20 copies/mL for at least 104 weeks while on ART	experienced	754 ± 36	5	Every six weeks	subcutaneous injection	NR	Flu-like reactions
[5]	Japan, Tokyo, 2006	Undetectable viral loads (PVL <50 copies/mL) for 1 year on ART	experienced	396 (337–504)	6	Biweekly	subcutaneous injection	axillary areas	Subcutaneous bleeding or erythema at injection site General malaise
[10]	USA, Boston and New York, 2009	PVL ≤400 copies/mL and CD4+ T cell counts ≥400/mm^3 for at least 3 months prior recruitment; PVL <50 copies/mL at screening	experienced	664 (NR)	3	Weeks 3, 7 and 15	subcutaneous injection	inner aspect of the arm, 6–12 cm from the axilla	Episodes of thrombocytopenia in a patient and neutropenia in another
[13]	Denmark, Copenhagen and Hvidovre, 2009	Asymptomatic HIV-1 infection; CD4+ T cell counts ≥300/mm^3; Absence of other chronic diseases; 1000 < PVL < 100,000 copies/mL; Presence of HLA-A * 0201 allele	experienced	565 (355–982)	4	Biweekly, last dose after four weeks	subcutaneous injection	left and right axillary areas	None reported
[11]	Spain, Barcelona, 2011	Asymptomatic HIV-1 infection; ART-naive for at least two years before enrollment; Baseline CD4+ T cell counts >450 cells/mm^3; Nadir CD4+ T cell counts >350 cells/mm^3; PVL >10,000 HIV-1 copies/mL	naive	647 (532–776)	3	Biweekly	subcutaneous injection	NR	Asymptomatic enlargement of local lymph nodes Flu-like symptoms
[15]	Belgium, Brussel and Netherlands, Rotterdam, 2012	Patients on ART; PVL ≤50 copies/ml and CD4+ T cell counts ≥500/mm^3 for a period of at least 3 months prior to enrollment; Nadir CD4+ T-cell count >300/mm^3	experienced	610 (500–960)	4	Monthly	subcutaneous and intradermal injection	antero-median side of an arm or a thigh	Tonsillitis episode
[12]	Spain, Barcelona, 2013	Asymptomatic chronic HIV-1 infection; Baseline CD4+ T cell count >450 cells/mm^3; Nadir CD4+ T cell count >350 cells/mm^3; Undetectable PVL(<50 copies/mL) on ART	experienced	702 (568–900)	3	Biweekly	subcutaneous or intradermal injection	upper-inner part of both arms	Lymph node enlargement, erythema and flu-like symptoms
[14]	USA, Dallas, 2014	Asymptomatic HIV-1 infection; Baseline CD4+ T cell count >500 cells/mm^3; Baseline PVL <50 copies/mL and within the previous 3 months while on ART; Nadir CD4+ T cells count ≥300 cells/mm^3	experienced	670 (553–832)	4	Every 4 weeks	subcutaneous injection	upper and lower extremities	None reported
[6]	USA, Pittsburgh, 2015	CD4+ T cell count ≥300 cells/mm^3; 3000 < PVL < 100,000 copies/mL	naive	486 (377–881)	4 (3 doses while on ART, 1 dose after ATI)	Biweekly	subcutaneous injection	upper medial area of the arm (bilaterally)	Mild-to-moderate inflammation at the injection site; Two individuals experienced severe pruritus and pain at the injection site
[16]	USA, Philadelphia and Canada, Montreal, 2016	PVL ≤200 copies/mL for at least 3 months prior to enrollment; PVL <50 copies/mL at screening; CD4+ T cell count ≥450 cells/mm^3; Nadir CD4+ T cell count ≥200 cells/mm^3; Pre-ART (within 3 months) plasma for virus isolation availability	experienced	632 (513–765)	4	Every 4 weeks	intradermal injection	axillary lymph node	Mild local injection site reactions

ART—antiretroviral therapy; ATI—analytical treatment interruption; Baseline—period before ART start; PVL—plasmatic HIV-1 viral load; NR—not reported; * values expressed as mean ± standard deviation or median (interquartile range).

Table 2. Vaccine response criteria of the 12 studies and sample size breakdown for the estimation of global treatment success rate from dendritic cell-based experimental anti-HIV vaccines.

Ref.	Country, City, Year	Vaccine Response Criterion	Enrolled	Placebo Arm	Comparator Arm	Vaccine Arm	Removed from Analysis *n*	Responders *n*	Non-Responders *n*	Study Follow-Up Length
[7]	USA, Stanford, 1998	Any PVL decrease	6	0	0	6	0	0	6	40 weeks
[8]	Brazil, Recife, 2004	>90% PVL decrease by 1 year	20	0	0	18	2	8	10	1 year
[9]	Spain, Barcelona, 2005	PVL decrease of 0.5 log10 copies/mL 24 weeks after vaccination	18	6	0	12	0	4	8	24 weeks
[5]	Japan, Tokyo, 2006	PVL decrease of 0.5 log10 copies/mL	4	0	0	4	0	0	4	12 weeks
[10]	USA, Boston and New York, 2009	Average of the last two scheduled PVL evaluations during weeks 10–13 of ATI $\leq$5000 copies/mL	29	0	15	14	1	4	9	12 weeks
[13]	Denmark, Copenhagen and Hvidovre, 2009	A PVL decrease of 1.08 log10 copies/mL was the most pronounced change among responders	12	0	0	12	0	5	7	40 weeks
[11]	Spain, Barcelona, 2011	PVL decrease of 0.5 log10 copies/mL 24 weeks after vaccination	24	12	0	12	4	4	4	48 weeks
[15]	Belgium, Brussel and Netherlands, Rotterdam, 2012	Remaining off ART at 96 weeks following ATI	17	0	0	17	0	6	11	110 weeks
[12]	Spain, Barcelona, 2013	Post-vaccination PVL decrease $\geq$1 log	36	12	0	24	2	12	10	48 weeks
[14]	USA, Dallas, 2014	ATI maximum PVL <5 log10 copies/mL	19	0	0	19	1	9	9	48 weeks
[6]	USA, Pittsburgh, 2015	ATI PVL decrease >0.4 log10 copies/mL	11	0	0	11	1	3	7	48 weeks
[16]	USA, Philadelphia and Canada, Montreal, 2016	PVL in the vaccine arm is reduced by at least 1.1 log10 copies/mL	54	17	0	37	2	0	35	2 years

ART—antiretroviral therapy; ATI—analytical treatment interruption; PVL—plasmatic HIV-1 viral load; NR—not reported.

Table 3. Summary of technical differences among the protocols of the 12 studies that were included in the meta-analysis.

Ref.	Loaded Molecules	Loaded Molecules (Summarized)	Dendritic Cell Number	Culture Medium	Days in Culture
[7]	Gag (residues 77 to 85, SLYNTVATL motif), Env (residues 120 to 128, KLTPLCVTL motif and residues 814 to 823, SLLNATDIAV motif) and Pol (residues 956 to 964, LLWKGEGAV motif; residues 464 to 472, ILKEPVHGV motif and residues 267 to 277, VLDVGDAYFSV motif) peptides from recombinant HIV-1 MN gp160 polypeptide	peptides	2–8 × 10^6 (immature DCs)	RPMI-1640	2
[8]	AT2 (chemically)-inactivated autologous virus	whole virus	6 × 10^7	CellGro® DC Medium	7
[9]	Heat-inactivated autologous virus	whole virus	2 × 10^6	MCM	8
[5]	Gag (residues 28 to 36, KYKLKHIVW and KYRLKHIVW motifs; residues 296 to 306, RDYVDRFYKTL motif), Nef (residues 138 to 147, RYPLTFGWCF and RFPLTFGWCF motifs) and Env (residues 584 to 594, RYLRDQQLLGI and RYLQDQQLLGI motifs) peptides	peptides	0.7–1.8 ×10^6	RPMI-1640	7
[10]	Recombinant virus produced by a canarypox vector (ALVAC vCP1452)	whole virus	1.5–6 × 10^6	MCM	6
[13]	HLA A*0201-binding peptides (Gag, Pol, Env, Vpu and Vif)	peptides	1 × 10^7	X-VIVO™ 15	8
[11]	Heat-inactivated autologous virus	whole virus	8 × 10^6	X-VIVO™ 15	7
[15]	Mature DCs were electroporated with mRNA derived from pGEM-sig-Tat-DC-LAMP, pGEM-sigRev-DC-LAMP, pGEM-sig-Nef-DC-LAMP and pST1-sig-Gag-DCLAMP plasmids for peptides expression	mRNA (by electroporation)	1 × 10^7	X-VIVO™ 15	7
[12]	Heat-inactivated autologous virus	whole virus	2 × 10^6	X-VIVO™ 15	7
[14]	Viral epitopes from Gag (17 to 35, and 253 to 284 residues), Nef (66 to 97 and 116 to 145 residues) and Pol (residues 325 to 355) lipopeptides	peptides	15 × 10^6	CellGro® DC Medium	3
[6]	Autologous CD4+ T cells which had been superinfected with endogenous inactivated HIV-1 with psoralen and UVB irradiation	peptides (indirectly)	1 × 10^7	CellGro® DC Medium	6
[16]	Mature DCs were electroporated with autologous HIV-1 Gag, Nef, Rev, and Vpr mRNA for peptides expression	mRNA (by electroporation)	1.2 × 10^7	Not reported	7

AT2—aldrithiol-2; CellGro®—registered trademark from CellGenix (Freiburg im Breisgau, Germany); DCs—dendritic cells; RPMI—Roswell Park Memorial Institute medium; X-VIVO™—trademark from Lonza (Basel, Switzerland).

Table 4. Summary of cytokine supplementation of the protocols of the 12 studies that were included in the meta-analysis.

Ref.	DC Maturation Procedure								
	GM-CSF Supplementation	IFN-α Supplementation	IFN-γ Supplementation	IL-1β Supplementation	IL-4 Supplementation	IL-6 Supplementation	PGE2 Supplementation	TNF-α Supplementation	Other Molecules Supplementation
[7]	No	No	No	No	No	No	No	No	-
[8]	Yes	No	No	Yes	Yes	Yes	No	Yes	-
[9]	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	-
[5]	Yes	No	No	No	Yes	No	No	Yes	-
[10]	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	-
[13]	Yes	No	No	Yes	Yes	Yes	Yes	Yes	-
[11]	Yes	No	No	Yes	Yes	Yes	No	Yes	-
[15]	Yes	No	No	Yes	Yes	Yes	Yes	Yes	-
[12]	Yes	No	No	Yes	Yes	Yes	Yes	Yes	-
[14]	Yes	Yes	No	No	No	No	No	No	LPS
[6]	Yes	Yes	Yes	Yes	Yes	No	No	Yes	polyinosinic:polycytidylic acid
[16]	No	No	Yes	No	No	No	Yes	Yes	CD40L

GM-CSF—granulocyte-macrophage colony-stimulating factor; IFN-α—interferon alpha; IFN-γ—interferon gamma; IL-1β—interleukin 1 beta; IL-4—interleukin 4; IL-6—interleukin 6; LPS—lipopolysaccharide; MCM—monocyte conditioned medium; PGE2—prostaglandin E2; TNF-α—tumor necrosis alpha.

The univariate pre-selection meta-regression *p*-values are summarized in Table 5. The pre-selected variables were: antigen choice (whole virus, mRNA or peptides); baseline (pre-vaccination) CD4+ T cell counts; culture medium choice (RPMI-1640, CellGro®, monocyte conditioned medium or X-VIVO™ 15), presence of IL-1β and IL-6 supplementation (p = 0.26 and p = 0.21, respectively) and vaccine dose periodicity (biweekly vs. every four weeks or more, p = 0.18).

Table 5. Univariate analysis of factors and decision to include into multivariate analysis. The objective was to evaluate association with treatment success rate of dendritic cell-based experimental anti-HIV vaccines.

Variable	Linear Univariate Meta-Regression Coefficient	Coefficient Standard Error	*p*-Value	Decision
Antigen:				
whole virus	Reference	-	-	
mRNA	−0.8419	0.5442	0.1219	Pre-selected
peptides	−0.2937	0.3993	0.4621	
Baseline (pre-vaccine) CD4+ T cell counts:				
more than 700 cells/mm^3	Reference	-	-	
between 600 and 700 cells/mm^3	−0.4542	0.4405	0.3025	Pre-selected
between 500 and 600 cells/mm^3	−0.0367	0.5174	0.9434	
less than 500 cells/mm^3	−0.9804	0.7178	0.1720	
Days in culture (DCs maturation)	−0.0062	0.1118	0.9558	Not pre-selected
DC maturation culture medium:				
RPMI-1640	Reference	-	-	
CellGro®	2.8743	0.8972	0.0014	
MCM	2.2890	0.9458	0.0155	Pre-selected
X-VIVO™ 15	2.8780	0.8836	0.0011	
GM-CSF supplementation (yes or no)	3.1400	1.0378	0.0025	Pre-selected
IFN-α supplementation (yes or no)	−0.1512	0.3660	0.6795	Not pre-selected
IFN-γ supplementation (yes or no)	−1.2007	0.6481	0.0639	Pre-selected; removed due to collinearity
IL-1β supplementation (yes or no)	0.5968	0.5336	0.2633	Pre-selected
IL-4 supplementation (yes or no)	0.3529	0.5235	0.5002	Not pre-selected
IL-6 supplementation (yes or no)	0.5672	0.4479	0.2053	Pre-selected; removed due to collinearity
Number of DCs per vaccine dose	−0.0032	0.0408	0.9369	Not pre-selected
Number of vaccine doses	−0.5006	0.2386	0.0359	Pre-selected; removed due to collinearity
Periodicity of vaccine doses (biweekly or every four weeks or more)	0.4793	0.3577	0.1802	Pre-selected; removed due to collinearity
PGE2 supplementation (yes or no)	−0.1765	0.3592	0.6231	Not pre-selected
TNF-α supplementation (yes or no)	−0.1830	0.4887	0.7081	Not pre-selected

DC—dendritic cell; GM-CSF—granulocyte-macrophage colony-stimulating factor; IFNα—interferon alpha; IFNγ—interferon gamma; IL1β—interleukin 1 beta; IL4—interleukin 4; IL6—interleukin 6; PGE2—prostaglandin E2; TNFα—tumor necrosis alpha.

Table 6. Multivariate analysis for assessment of which factors were associated with treatment success rate of dendritic cell-based experimental anti-HIV vaccines.

Variable	Linear Multivariate Meta-Regression Coefficient	95% Confidence Interval	*p*-Value
(Model Intercept)	−3.4826	(−6.5475)–(−0.4176)	0.0259
Antigen:			
whole virus	Reference	-	-
mRNA	−0.6289	(−2.1234)–0.8657	0.4095
peptides	1.0689	(−3.2088)–5.3466	0.6243
Baseline (pre-vaccine) CD4+ T cell counts:			
more than 700 cells/mm^3	Reference	-	-
between 600 and 700 cells/mm^3	−0.1513	(−1.318)–1.0155	0.7994
between 500 and 600 cells/mm^3	−1.5793	(−6.0215)–2.8629	0.4859
less than 500 cells/mm^3	−3.4955	(−12.266)–5.2751	0.4347
GM-CSF supplementation (yes or no)	3.712	(−3.8567)–11.2806	0.3364
DC maturation culture medium:			
RPMI-1640	Reference	-	-
CellGro®	−1.147	(−10.2552)–7.9612	0.805
MCM	−3.4024	(−16.7094)–9.9046	0.6163
X-VIVO™ 15	−2.5523	(−15.9238)–10.8191	0.7083
IL-1β supplementation (yes or no)	2.4969	(−6.0232)–11.01	0.5657

Model deviance = 0.81; goodness-of-fit test with 11 degrees of freedom p-value = 0.99 (which indicates good fit).

Two variables were significantly associated with treatment success rate during univariate analysis: presence of GM-CSF supplementation was positively associated with treatment success rate ($p = 0.003$), whereas number of vaccine doses was negatively associated ($p = 0.04$) with success rate.

However, the multivariate meta-regression modeling using these variables failed to evidence any statistically significant differences that would explain the discrepancies on treatment success rate among protocols. Due to co-linearity with other variables in the model, IL-6, number of vaccine doses and vaccine periodicity were removed from the final model. This may be the consequence of the small number of observations (just 12 studies) relative to the number of considered variables (14 total, nine pre-selected). The removal has been necessary to avoid introducing bias in the model predictions. The final best-fit model is summarized in Table 6. No variables influenced the overall treatment success rate with statistical significance.

3. Discussion

3.1. Immune Responses Elicited by the Experimental Vaccines

The authors usually assessed the immune response in each experimental vaccine study by comparing pre-vaccination and post-vaccination peripheral blood mononuclear cells (PBMC) samples to assess cytokine expression and cytotoxic T lymphocytes (CTL) activity by standard techniques such as enzyme-linked immunosorbent assay (ELISA) [7,16], intracellular cytokine staining followed by flow cytometry analysis [6,8,13,14,16], Luminex® multiplex bead-based cytokine assay [14] or IFN-γ enzyme-linked immunospot (ELISPOT) assays [5,10–12,14,15]. Some authors additionally assessed HIV-1–specific CD4+ lymphoproliferative responses [7,9–12,15].

Vaccinated individuals' immune responses were not uniform across trials. Most, if not all studies reported that the vaccine elicited CTL responses, but only in some individuals [5,7,10,13]. Some studies showed that monocyte-derived DC from ART patients produced lower levels of IL-12 (a potent Th-1 response cytokine) after CD40L induction [17], while IL-12 reduced levels are also associated with no viral load control after DC-vaccination [6]. DCs derived from patients under ART induce higher IL-12 production under the IFN-γ and CD40L combination [17] while CD8+ cells produced more IFN-γ after DC treatment with TLR-3 ligand poly(I:C) and CD40L [18], which highlights the importance of pre-vaccination conditions in regulation of DC function.

Some authors acknowledge that these CTL and lymphoproliferative responses were in general weak and/or transient [9,11,13] or even not significant [10], but were associated with partial viral load control [8,9,11–13]. Other authors observed significant post-vaccination ELISPOT responses or a significant increase of anti-HIV-1 specific CD8+ T-cell activity as assessed by flow cytometry, these variants being associated with lower plasmatic viral loads levels during ATI [10,14]. Otherwise, other studies showed that memory CTLs with boosted effector activity were not associated with viral load control [6]. Regarding humoral immune response, some authors did not observe any significant post-vaccination change in total anti-HIV-1 antibody titers [8], nor in serum neutralizing activity [11].

This response heterogeneity possibly reflects the variability among the experimental vaccine protocols and individuals' characteristics. However, the authors in general agree that the DC-based experimental vaccines had at least some immunogenicity [12,15], as showcased by the Lu et al. [8] protocol, which promoted a prolonged partial viral load control (reduction of 90% of median plasmatic viral load over the first year post-vaccination) in eight subjects among 18 chronically-infected ART-naïve recruited Brazilian individuals. This was a non-controlled, non-randomized study, but had nevertheless promising results. We chose to not include immunogenicity as an outcome in the meta-regression analysis because we were not sure how this would be performed, since the studies used several methodologies for immunogenicity assessment; thus, they cannot be compared directly. Therefore, we must regard immunogenicity only on descriptive terms, which is unfortunate, but we would introduce bias if we attempted to include them as outcomes in the meta-regression.

Overall treatment success rate was suboptimal, and the observations showed that the vaccines provide insufficient immune boosting. Some authors noted that the vaccines need to generate a specific immune response that is effective against HIV and at the same time do not generate "counter-effective" immune activation that favor virus replication instead [13].

3.2. The Role of Host Genomic and Trascriptomic Background

An important aspect that was not taken in consideration in all protocols was the host genetic background. Among the 12 studies included in the meta-analysis, only five reported human leukocyte antigens (HLA) alleles from the vaccinated individuals. Two studies recruited only HLA-A*02:01-positive individuals [7,13] and they were the majority in the Lu et al. study [8] (10 patients among the 18 recruited were HLA-A*02:01-positive). The four Japanese patients in the Ide et al. study [5] were A*24:02-positive and Levy et al. [14] did not specify their recruited individuals' exact genotypes, only reporting that they were B27, B57 negative. Overall, no studies reported detailed HLA-B genotypes.

Since the majority of HLA-A*02:01 carriers exert selective pressure in epitope diversity due to the higher affinity of CTLs to dominant epitope, some authors suggested that focusing research on subdominant HIV-1 epitopes may help the development of simultaneous targeting of multiple of epitopes [19]. Therefore, HLA-typing in prospective volunteers may even help "personalization" of HIV-1 therapeutic vaccines for each HLA make-up across worldwide populations (some collaborative initiatives may help in this endeavor, such as HLA allele frequency databases [20]), or guide peptide optimization to improve antigenicity [13,21], since some HLA alleles confer protection against HIV disease progression [22], and thus possibly influence CTL-specific/vaccine response.

Variations in genes involved in immune modulation could be an additional reason for the efficacy variability of the reviewed experimental procedures. The first study to screen the host genetic background under DC immunotherapy analyzed 768 tag and coding single nucleotide polymorphisms (SNPs) of 146 innate immunity genes and showed an association between *MBL2* rs10824792 with a weak response and *NOS1* rs693534 SNPs with a durable response to DC immunotherapy [23]; the second study considered 22 polymorphisms in 13 HIV-1 host restriction factor genes (*APOBEC3G*, *CCL4*, *CCL5*, *CCR5*, *CUL5*, *CXCR6*, *HLA-C*, *IFNG*, *PARD3B*, *Prox1*, *SDF-1*, *TRIM5*, *ZNRD1*), finding the *PARD3B* rs11884476 SNP was associated with good response DC immunotherapy [24]. Finally, a more recent genome-wide analysis evidenced that a SNP in *CNOT1* gene (rs7188697, A→G), which codes for a protein involved in the regulation of inflammatory responses and is part of a protein complex that interferes with HIV-1 replication, was associated with poor response to the experimental immunotherapy. Although these findings were limited to the 18 analyzed subjects, host genomics should be taken in consideration during HIV-1 experimental vaccine protocols, DC-based or not, to help understand its efficacy and possibly discover new therapeutic approaches [25].

A recent study indicated that the host genome contributes to the success of DC immunotherapy from two different clinical trials performed in Brazil [8] and Spain [12]. The restriction factor *TRIM22* rs7935564 G allele was associated with better response to DC-based immunotherapy in patients in both clinical trials and was also more frequent in long term non-progressors (LTNPs), thus leading to hypothesize a role for the G allele in the control of virus replication [26]. This intriguing result, although preliminary, will prompt future analyses on the contribution of host genomics in the modulation of the multifactorial response to DC-based immune therapy.

Besides genotyping, other molecular biology techniques are being employed in HIV-1 DC-based therapy research. A recent analysis evaluated gene expression profiles from PBMCs obtained from individuals vaccinated in the Allard et al. phase I/II clinical trial [15] and compared their transcriptome profile with HIV-negative controls, melanoma-affected individuals receiving melanoma DC-based immunotherapy and individuals who received seasonal influenza vaccination, showing that the vaccinated individuals transcriptome shifted to a higher expression of genes involved in cellular stress and innate immune response, which was sustained for at least 40 weeks after ATI [27]. This result

highlights the importance of genomics-era analysis for the discovery of how individuals' organisms respond to vaccines, which could lead to insights for the improvement of current strategies or the development of new ones.

3.3. Recommendations for Future Protocols

A difficulty encountered during our analysis was that several aspects of the protocols were not standardized, principally vaccine response criteria. Therefore, future clinical trials would benefit from standardization. The DC-based immune therapy community is actively debating protocols and elaborated a tentative consensus published elsewhere [28], which accounts for vaccine preparation methods, virus inactivation, patient follow-up and many more inherent protocol variables.

For example, the included protocols with both naïve and ART-experienced individuals, and in the case of ART-experienced individuals, each protocol evaluated vaccine efficacy differently during ATI. The non-uniformity of efficacy criteria during ATI in HIV-1 experimental trials is an issue recently reviewed [29], and a unified criterion for vaccine efficacy is warranted for future protocols and should be followed. Moreover, future protocols could also benefit by individuals selection standardization, since the protocols recruited chronically-infected individuals; moreover, individuals with advanced HIV-infection and consequently low CD4+ T cell nadirs may not benefit as much as individuals with less advanced infection, as observed by some authors [5,15]. Thus, clinical trials with individuals with early HIV-1 infection may be relevant since in this condition, HIV-1 does not undergo extensive immunologic pressure (and consequently immunologic escape mutations have not been selected), reservoir size is still small and immune function is not yet compromised [30,31]. DC-based therapeutic vaccines may work better in this situation and possibly provide invaluable data about early HIV-1 infection immunology.

Regarding antigen-choice, possibly future trials should use autologous whole virus. This is strategy is time consuming, but it could stimulate a more complete and efficient response involving all possible viral epitopes. Recombinant viral proteins may be more straightforward to use, but they are costly [32], and this must be taken in consideration if the objective is mass production of DC-based immune therapy.

Laboratory methodologies could also be updated. For example, although ELISPOT is commonly used to evaluate cytotoxicity in vaccine trials [33], it simply measures cytokine production, and usually only IFN-γ, being an indirect marker of CTL activity at best. Future DC trials may benefit from the use of multiparameter flow cytometry to obtain a more accurate panel of CTL- specific response [34].

Finally, perhaps the vaccine alone may not be sufficient to cure HIV-1 infection. As there is intense research going on viral reservoirs, it may need to be combined with other strategies for latent HIV-1 activation and induce and potent and efficient CTL activity to kill HIV-1–infected cells [6,16], since current strategies were not able to reduce viral reservoir levels, at least during the available follow-up observations [35]. To this end, the pharmacological reactivation of HIV-1 has been gaining visibility in the last years as a promising strategy, defined as "kick-and-kill" or "shock-and-kill" to eradicate the virus. The depletion of HIV-1 reservoirs, the main objective of this strategy, employs pharmacological modulation of signaling pathways involved in HIV-1 replication. A recent study [36] performed in Denmark described the use of a combined vaccine-pharmacological strategy to deplete HIV reservoirs: in this study, patients were immunized with six doses of Vacc-4x over 12 weeks, followed by romidepsin administration. In this context, the DC-based immune therapy could play a role, as the authors observed an increase in the activation and enhancement of antigen-specific T-cell immune responses, prior to romidepsin treatment. Other initiatives, such as the Research In Viral Eradication of HIV Reservoirs (RIVER) Protocol are actively inquiring the feasibility of a therapeutic vaccine (although not DC-based) and kick-and-kill combination [37], and their results may provide valuable insights for DC-based protocols.

4. Material and Methods

4.1. Literature Search Strategy and Study Selection

We searched PubMed and MeSH (Medical Subject Headings) databases. Keywords included "Dendritic Cells" [Mesh] AND "Vaccines" [Mesh] AND "HIV" [Mesh]; therapeutic vaccine hiv dendritic cell; AIDS Vaccines dendritic cells; dendritic cells Follow-Up Studies hiv; dendritic cells Follow-Up Studies hiv vaccine. We also searched clinicaltrials.gov database using the following keywords: hiv dendritic and vaccine dendritic | Exclude Unknown | HIV infections.

We reviewed the retrieved texts to assess if they were reports of clinical trials of therapeutic DC-based vaccines against HIV infection which recruited HIV-infected individuals, naïve or treatment-experienced, of any age and any follow-up length. We included both uncontrolled (no control group) and controlled trials (vaccine against placebo, vaccine versus different immunizations strategies or vaccine against no vaccine). Thus, the trials should have included analytical treatment interruptions (ATI) if they enrolled ART-experienced individuals.

If the study was eligible according to these criteria, we recorded the primary outcome, defined as partial or at least transient response to the vaccine (plasmatic HIV-1 viral load decrease). We also recorded adverse events of the experimental vaccinations, safety and tolerability as secondary outcomes.

Additionally, we extracted details from the protocols followed by the authors—number of enrolled individuals and distribution across experimental groups; number of any individuals removed from analysis; cell isolation and DC maturation protocols; time of DC culture; choice of HIV-1 antigen for vaccine preparation; DC count per vaccine dose; types of culture media and cytokine supplementation; forms and routes of experimental vaccine administration; study follow-up length and chosen methodologies for assessment of immunological response to the vaccine.

Two researchers independently reviewed each study and any disagreements were resolved by discussion between them, until a consensus was reached. The collected data were registered in a standard form containing questions regarding the variables defined above.

4.2. Statistical Analysis

We performed a meta-analysis to summarize treatment success rate (proportion of individuals in the treatment group with the event of interest—viral load decrease during ATI) among individuals vaccinated during experimental protocols. The treatment success rate was chosen as effect measure because most protocols were uncontrolled (i.e., no placebo arm was enrolled).

Briefly, treatment success rates (described as proportions), were logit-transformed for obtaining the polled treatment success rate, which was back-transformed to a proportion representing the overall quantity of (partial) positive response to the experimental vaccines when considering the total number of vaccinated individuals. Heterogeneity among studies was assessed by I^2 measure. We chose a random effects meta-analysis model [38] because we considered reasonable that the treatment success rate (the "effect size" in our case) varied across studies, since they were performed in different populations, with different DC collection and maturation procedures, and so on. Thus, following initial meta-analysis, we conducted a meta-regression to assess if these protocols characteristics would explain differences in treatment success rate among them.

To this end, we first conducted univariate regression models to pre-select variables for inclusion on a final, best-fit multivariate meta-regression model with mixed effects. Any variable whose estimated linear meta-regression coefficient had an uncorrected p-value <0.30 was pre-selected (the p-value for each coefficient tests the null hypothesis that it's coefficient is equal to zero—no influence over the overall treatment success rate). The level of significance (α) was set at 0.05 for the variables included in the final model. The model fit was assessed by a χ^2 goodness-of-fit test using the test deviance as an approximation to the χ^2 distribution with n-1 degrees of freedom, in which n is the number of

studies included in the analysis. All analyses were performed through R software version 3.2.3 (R core team, Vienna, Austria), using "meta" and "metafor" packages.

5. Conclusions

After the systematic review of the literature regarding experimental DC-based anti-HIV-1 immunotherapeutic vaccines, we performed a meta-analysis with 12 selected reports. The vaccines did not work as the clinicians expected, since they had low success rates, but they served as a proof of concept that host immune response could be boosted against HIV-1. There are improvement opportunities for the protocols, which may involve standardization of key steps in trial design: (1) patient selection criteria (approaching their genomics, HLA-typing principally, since it likely plays a role in vaccine response); (2) antigen choice; (3) dendritic cell culture procedures and dosing; (4) unification of immune response "boosting" and success criteria.

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References

1. Steinman, R.M. Dendritic cells: Understanding immunogenicity. *Eur. J. Immunol.* **2007**, *3*, S53–S60. [CrossRef] [PubMed]
2. Takeuchi, O.; Akira, S. Innate immunity to virus infection. *Immunol. Rev.* **2009**, *227*, 75–86. [CrossRef] [PubMed]
3. Buckwalter, M.R.; Albert, M.L. Orchestration of the immune response by dendritic cells. *Curr. Biol.* **2009**, *19*, R355–R361. [CrossRef] [PubMed]
4. Wu, L.; KewalRamani, V.N. Dendritic-cell interactions with HIV: Infection and viral dissemination. *Nat. Rev. Immunol.* **2006**, *6*, 859–868. [CrossRef] [PubMed]
5. Ide, F.; Nakamura, T.; Tomizawa, M.; Kawana-Tachikawa, A.; Odawara, T.; Hosoya, N.; Iwamoto, A. Peptide-loaded dendritic-cell vaccination followed by treatment interruption for chronic HIV-1 infection: A phase 1 trial. *J. Med. Virol.* **2006**, *78*, 711–718. [CrossRef] [PubMed]
6. Macatangay, B.J.; Riddler, S.A.; Wheeler, N.D.; Spindler, J.; Lawani, M.; Hong, F.; Buffo, M.J.; Whiteside, T.L.; Kearney, M.F.; Mellors, J.W.; et al. Therapeutic vaccination with dendritic cells loaded with autologous HIV type 1-infected apoptotic cells. *J. Infect. Dis.* **2016**, *213*, 1400–1409. [CrossRef] [PubMed]
7. Kundu, S.K.; Engleman, E.; Benike, C.; Shapero, M.H.; Dupuis, M.; van Schooten, W.C.; Eibl, M.; Merigan, T.C. A pilot clinical trial of HIV antigen-pulsed allogeneic and autologous dendritic cell therapy in HIV-infected patients. *AIDS Res. Hum. Retrovir.* **1998**, *14*, 551–560. [CrossRef] [PubMed]
8. Lu, W.; Arraes, L.C.; Ferreira, W.T.; Andrieu, J.M. Therapeutic dendritic-cell vaccine for chronic HIV-1 infection. *Nat. Med.* **2004**, *10*, 1359–1365. [CrossRef] [PubMed]
9. Garcia, F.; Lejeune, M.; Climent, N.; Gil, C.; Alcami, J.; Morente, V.; Alos, L.; Ruiz, A.; Setoain, J.; Fumero, E.; et al. Therapeutic immunization with dendritic cells loaded with heat-inactivated autologous HIV-1 in patients with chronic HIV-1 infection. *J. Infect. Dis.* **2005**, *191*, 1680–1685. [CrossRef] [PubMed]
10. Gandhi, R.T.; O'Neill, D.; Bosch, R.J.; Chan, E.S.; Bucy, R.P.; Shopis, J.; Baglyos, L.; Adams, E.; Fox, L.; Purdue, L.; et al. A randomized therapeutic vaccine trial of canarypox-HIV-pulsed dendritic cells vs. canarypox-HIV alone in HIV-1-infected patients on antiretroviral therapy. *Vaccine* **2009**, *27*, 6088–6094. [CrossRef] [PubMed]

11. Garcia, F.; Climent, N.; Assoumou, L.; Gil, C.; Gonzalez, N.; Alcami, J.; Leon, A.; Romeu, J.; Dalmau, J.; Martinez-Picado, J.; et al. A therapeutic dendritic cell-based vaccine for HIV-1 infection. *J. Infect. Dis.* **2011**, *203*, 473–478. [CrossRef] [PubMed]
12. Garcia, F.; Climent, N.; Guardo, A.C.; Gil, C.; Leon, A.; Autran, B.; Lifson, J.D.; Martinez-Picado, J.; Dalmau, J.; Clotet, B.; et al. A dendritic cell-based vaccine elicits T cell responses associated with control of HIV-1 replication. *Sci. Transl. Med.* **2013**, *5*, 166ra2. [CrossRef] [PubMed]
13. Kloverpris, H.; Karlsson, I.; Bonde, J.; Thorn, M.; Vinner, L.; Pedersen, A.E.; Hentze, J.L.; Andresen, B.S.; Svane, I.M.; Gerstoft, J.; et al. Induction of novel CD8+ T-cell responses during chronic untreated HIV-1 infection by immunization with subdominant cytotoxic T-lymphocyte epitopes. *Acquir. Immune Defic. Syndr.* **2009**, *23*, 1329–1340. [CrossRef] [PubMed]
14. Levy, Y.; Thiebaut, R.; Montes, M.; Lacabaratz, C.; Sloan, L.; King, B.; Perusat, S.; Harrod, C.; Cobb, A.; Roberts, L.K.; et al. Dendritic cell-based therapeutic vaccine elicits polyfunctional HIV-specific T-cell immunity associated with control of viral load. *Eur. J. Immunol.* **2014**, *44*, 2802–2810. [CrossRef] [PubMed]
15. Allard, S.D.; de Keersmaecker, B.; de Goede, A.L.; Verschuren, E.J.; Koetsveld, J.; Reedijk, M.L.; Wylock, C.; de Bel, A.V.; Vandeloo, J.; Pistoor, F.; et al. A phase I/IIa immunotherapy trial of HIV-1-infected patients with Tat, Rev and Nef expressing dendritic cells followed by treatment interruption. *Clin. Immunol.* **2012**, *142*, 252–268. [CrossRef] [PubMed]
16. Jacobson, J.M.; Routy, J.P.; Welles, S.; DeBenedette, M.; Tcherepanova, I.; Angel, J.B.; Asmuth, D.M.; Stein, D.K.; Baril, J.G.; McKellar, M.; et al. Dendritic cell immunotherapy for HIV-1 infection using autologous HIV-1 RNA: A randomized, double-blind, placebo-controlled clinical trial. *J. Acquir. Immune Defic. Syndr.* **2016**. [CrossRef] [PubMed]
17. Fan, Z.; Huang, X.L.; Kalinski, P.; Young, S.; Rinaldo, C.R., Jr. Dendritic cell function during chronic hepatitis C virus and human immunodeficiency virus type 1 infection. *Clin. Vaccine Immunol.* **2007**, *14*, 1127–1137. [CrossRef] [PubMed]
18. Huang, X.L.; Fan, Z.; Borowski, L.; Rinaldo, C.R. Maturation of dendritic cells for enhanced activation of anti-HIV-1 $CD8^+$ T cell immunity. *J. Leukoc. Biol.* **2008**, *83*, 1530–1540. [CrossRef] [PubMed]
19. Andresen, B.S.; Vinner, L.; Tang, S.; Bragstad, K.; Kronborg, G.; Gerstoft, J.; Corbet, S.; Fomsgaard, A. Characterization of near full-length genomes of HIV type 1 strains in Denmark: Basis for a universal therapeutic vaccine. *AIDS Res. Hum. Retrovir.* **2007**, *23*, 1442–1448. [CrossRef] [PubMed]
20. Gonzalez-Galarza, F.F.; Takeshita, L.Y.; Santos, E.J.; Kempson, F.; Maia, M.H.; da Silva, A.L.; Teles e Silva, A.L.; Ghattaoraya, G.S.; Alfirevic, A.; Jones, A.R.; et al. Allele frequency net 2015 update: New features for HLA epitopes, KIR and disease and HLA adverse drug reaction associations. *Nucleic Acids Res.* **2015**, *43*, D784–D788. [CrossRef] [PubMed]
21. Corbet, S.; Nielsen, H.V.; Vinner, L.; Lauemoller, S.; Therrien, D.; Tang, S.; Kronborg, G.; Mathiesen, L.; Chaplin, P.; Brunak, S.; et al. Optimization and immune recognition of multiple novel conserved HLA-A2, human immunodeficiency virus type 1-specific CTL epitopes. *J. Gen. Virol.* **2003**, *84*, 2409–2421. [CrossRef] [PubMed]
22. Al Jabri, A.A. HLA and in vitro susceptibility to HIV infection. *Mol. Immunol.* **2002**, *38*, 959–967. [CrossRef]
23. Segat, L.; Brandao, L.A.; Guimaraes, R.L.; Pontillo, A.; Athanasakis, E.; de Oliveira, R.M.; Arraes, L.C.; de Lima Filho, J.L.; Crovella, S. Polymorphisms in innate immunity genes and patients response to dendritic cell-based HIV immuno-treatment. *Vaccine* **2010**, *28*, 2201–2206. [CrossRef] [PubMed]
24. Pontillo, A.; Da Silva, R.C.; Moura, R.; Crovella, S. Host genomic HIV restriction factors modulate the response to dendritic cell-based treatment against HIV-1. *Hum. Vaccines Immunother.* **2014**, *10*, 512–518. [CrossRef] [PubMed]
25. Moura, R.; Pontillo, A.; D'Adamo, P.; Pirastu, N.; Campos Coelho, A.; Crovella, S. Exome analysis of HIV patients submitted to dendritic cells therapeutic vaccine reveals an association of *CNOT1* gene with response to the treatment. *J. Int. AIDS Soc.* **2014**, *17*, 18938. [CrossRef] [PubMed]
26. Moura Rodrigues, R.; Plana, M.; Garcia, F.; Zupin, L.; Kuhn, L.; Crovella, S. Genome-wide scan in two groups of HIV-infected patients treated with dendritic cell-based immunotherapy. *Immunol. Res.* **2016**. [CrossRef] [PubMed]
27. De Goede, A.L.; Andeweg, A.C.; van den Ham, H.J.; Bijl, M.A.; Zaaraoui-Boutahar, F.; van IJcken, W.F.; Wilgenhof, S.; Aerts, J.L.; Gruters, R.A.; Osterhaus, A.D. DC immunotherapy in HIV-1 infection induces a major blood transcriptome shift. *Vaccine* **2015**, *33*, 2922–2929. [CrossRef] [PubMed]

28. Garcia, F.; Routy, J.P. Challenges in dendritic cells-based therapeutic vaccination in HIV-1 infection Workshop in dendritic cell-based vaccine clinical trials in HIV-1. *Vaccine* **2011**, *29*, 6454–6463. [CrossRef] [PubMed]
29. Graziani, G.M.; Angel, J.B. Evaluating the efficacy of therapeutic HIV vaccines through analytical treatment interruptions. *J. Int. AIDS Soc.* **2015**, *18*, 20497. [CrossRef] [PubMed]
30. Strain, M.C.; Little, S.J.; Daar, E.S.; Havlir, D.V.; Gunthard, H.F.; Lam, R.Y.; Daly, O.A.; Nguyen, J.; Ignacio, C.C.; Spina, C.A.; et al. Effect of treatment, during primary infection, on establishment and clearance of cellular reservoirs of HIV-1. *J. Infect. Dis.* **2005**, *191*, 1410–1418. [CrossRef] [PubMed]
31. O'Brien, M.; Markowitz, M. Should we treat acute HIV infection? *Curr. HIV/AIDS Rep.* **2012**, *9*, 101–110. [CrossRef] [PubMed]
32. Rinaldo, C.R. Dendritic cell-based human immunodeficiency virus vaccine. *J. Intern. Med.* **2009**, *265*, 138–158. [CrossRef] [PubMed]
33. Malyguine, A.M.; Strobl, S.; Dunham, K.; Shurin, M.R.; Sayers, T.J. ELISPOT assay for monitoring cytotoxic T lymphocytes (CTL) activity in cancer vaccine clinical trials. *Cells* **2012**, *1*, 111–126. [CrossRef] [PubMed]
34. Zaritskaya, L.; Shurin, M.R.; Sayers, T.J.; Malyguine, A.M. New flow cytometric assays for monitoring cell-mediated cytotoxicity. *Expert Rev. Vaccines* **2010**, *9*, 601–616. [CrossRef] [PubMed]
35. Andres, C.; Plana, M.; Guardo, A.C.; Alvarez-Fernandez, C.; Climent, N.; Gallart, T.; Leon, A.; Clotet, B.; Autran, B.; Chomont, N.; et al. HIV-1 Reservoir dynamics after vaccination and antiretroviral therapy interruption are associated with dendritic cell vaccine-induced T cell responses. *J. Virol.* **2015**, *89*, 9189–9199. [CrossRef] [PubMed]
36. Leth, S.; Schleimann, M.H.; Nissen, S.K.; Hojen, J.F.; Olesen, R.; Graversen, M.E.; Jorgensen, S.; Kjaer, A.S.; Denton, P.W.; Mork, A.; et al. Combined effect of Vacc-4x, recombinant human granulocyte macrophage colony-stimulating factor vaccination, and romidepsin on the HIV-1 reservoir (REDUC): A single-arm, phase 1B/2A trial. *Lancet HIV* **2016**, *3*, e463–e472. [CrossRef]
37. Imperial College London. Research in Viral Eradication of HIV Reservoirs (RIVER). In: ClinicalTrials.gov [Internet]. 2014-[November 2016]. Available online: http://clinicaltrials.gov/show/NCT00004451NLMIdentifier:NCT00004451 (accessed on 23 July 2016).
38. DerSimonian, R.; Kacker, R. Random-effects model for meta-analysis of clinical trials: An update. *Contemp. Clin. Trials* **2007**, *28*, 105–114. [CrossRef] [PubMed]

8 CAPÍTULO IV

Antiretroviral Therapy Immunologic Non-response in a Northeast Brazil Population: Association Study using Pharmacogenetics and Immune Markers

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Antiviral Therapy is an international, peer-reviewed journal devoted to publishing articles on the clinical development and use of antiviral agents and vaccines, and the treatment of all viral diseases.

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Antiretroviral Therapy Immunologic Non-response in a Northeast Brazil Population: Association Study using Pharmacogenetics and Immune Markers

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Running head: ART Non-response in a NE Brazil Population: Genetic Association Study

Abstract

Background: Antiretroviral therapy (ART) saved millions of lives from HIV-1 infection and AIDS, but some patients do not experience adequate CD4+ T cells gains even though viral suppression is achieved, phenomenon known as immunological non-response, which predisposes to non-AIDS systemic diseases. The genetic component of this condition is not yet completely elucidated. Thus, we aimed to discover predictive genetic markers of immune response delay through a case-control study. **Methods:** We recruited 135 patients from Recife, Northeast Brazil, among which 82 were non-responders, and genotyped a set of 94 selected single nucleotide polymorphisms (SNPs). Among those SNPs, 46 were located in genes involved in antiretroviral drugs pharmacodynamic pathways and immune system homeostasis, while the remaining 48 were ancestry informative markers (AIMs) for genetic ancestry estimation and controlling for eventual hidden population structure. **Results:** Male patients were overrepresented in non-responder group (p=0.01) and tended to have slower immune recovery than females, without reaching statistical significance (p=0.47). Non-responders also started with lower absolute CD4+ T cell counts (p<0.001). We found five SNPs significantly associated with the outcome, being three more frequent in non-responders than responders: rs3003596 (*NR1I3*) G allele (p=0.01); rs2243250 (*IL4*) A allele (p=0.02) and rs129081 (*ABCC1*) G allele (p=0.002), whereas the other two were less frequent in non-responders: rs2069762 (*IL2*) C allele (p=0.03) and rs10519613 (*IL15*) A allele (p=0.03). Patients had similar ancestry backgrounds. **Conclusions:** All significant associations were lost during multivariate survival analysis modeling. Therefore, more studies are needed to unravel the genetic basis of ART immunological non-response.

Keywords: HIV-1; antiretroviral drugs; pharmacodynamics; immunogenetics; genetic association study; survival analysis

1. Introduction

The introduction of antiretroviral therapy (ART) in the clinical practice saved millions of lives from acquired immunodeficiency syndrome (AIDS) related deaths, which is the result of chronic infection by the human immunodeficiency virus type 1 (HIV-1) [1, 2]. Current ART regimens are combinations of three drugs. The first-line regimens usually include two nucleoside analog reverse transcriptase inhibitors (NRTIs) and a non-nucleoside reverse transcriptase inhibitor (NNRTI) or a protease inhibitor (PI), and their objective is to suppress viral replication [3].

With viral suppression, reestablishment of lost CD4+ T cells typically happens in a biphasic manner: a rapid proliferation during the first three to six months of ART, caused by memory T cells redistribution, followed by a slower proliferation phase conducted by naïve T cells production by the thymus [4]. However, some patients do not present optimal CD4+ T cells gains, even with persistent viral suppression. This phenomenon is named immunological failure or immunological non-response, and it is associated with higher risk of non-AIDS cardiovascular, liver, kidney disorders and early ageing [5-8].

The immunological non-response has not yet been completely elucidated. Older age, male sex, advanced HIV-1 infection at treatment start and coinfections with other viruses are some known risk factors [4, 9, 10], but the influence of host genetic component is still debated. Promising genetic candidates include genes involved in antiretroviral drugs pharmacodynamic pathways [11, 12] and genes involved in immune functions (inflammation, apoptosis) and homeostasis, such as the interleukins IL2, IL7 and IL15, which coordinate T cell proliferation [13].

Our hypothesis was that genetic variation in these genes would affect the distribution of antiretroviral drugs, possibly favoring (undetected) residual virus replication, which in turn would drive immune activation and immune cell death, hampering CD4+ T cell recovery. Simultaneously, the SNPs in the immune system genes would favor this increased cell activation, increased apoptosis or decreased cell proliferation, leading to the suboptimal immunological response to ART. So, we aimed at identifying genetic variants associated with delay to proper CD4+ T cell counts recovery to quasi-normal or normal levels.

2. Material and methods

2.1 Subjects

We recruited 135 individuals (65 females, 48.2% and 70 males, 51.8%), coming from Recife and nearby cities, Pernambuco state, Northeast Brazil, living with HIV-1 attending healthcare at Instituto de Medicina Integral Professor Fernando Figueira (IMIP) for a case-control, observational study between 2011 and 2015.

Inclusion criteria were: age over than 18 years old, not reporting illicit drug use, and not being pregnant. Each patient provided a peripheral blood sample after giving written consent for participation in the research and authorization for review of their medical charts. IMIP Research Ethics Committee approved the study design (protocol number 3629-13).

Each patient answered a standard questionnaire about sex, age and age at ART start date, socioeconomic status, smoking and drinking habits. The data extracted from medical charts covered the whole period between the first and last plasma viral load (pVL) and CD4+ T cell absolute counts and percentage (relative to all white blood cells) measurements (retrospective follow-up).

Other extracted data included ART regimens received and their refill prescriptions and serological status for the following etiologic agents: hepatitis B and C viruses (HBV and HCV), cytomegalovirus (CMV, immunoglobulin G and M, IgG and IgM tests), human T-lymphotropic virus types 1 or 2 (HTLV-1/2), *Toxoplasma gondii* (toxoplasmosis agent, IgG and IgM tests) and *Treponema pallidum* (syphilis agent, VDRL test) and eventual AIDS-defining conditions following Center for Disease Control (CDC) 1993 Revised Classification System [14]. Table 1 displays patients' characteristics.

2.2 Patient classification

All patients achieved persistent viral suppression, which was defined as maintaining undetectable plasma viral load measurements (pVL<50 copies/mL) without viral load rebound, which was defined as two consecutive pVL>200 copies/mL at any time after initial suppression. In our setting, pVL measurements, CD4+ T cell counts and other laboratory tests are generally performed every three or four months, at the physician's discretion.

Immunologic response was defined as CD4+ T cell percentages achieving 30% or higher for two consecutive measurements during follow-up were classified as having immunological response, and immunological non-response if otherwise, following Brazilian Ministry of Health's guidelines [15]. If a patient already had pre-ART CD4+ T cell percentages>30% (early start patients, for example), we instead considered immunological response as an absolute gain of 200 cells/µL, following a previous study [4]. We preferred CD4+ T cell percentages instead of absolute counts because percentages are less variable over time [16].

Thus, 82 patients were stratified into immunological non-response and 53 into immunological response groups.

2.3 SNPs selection and genotyping

We selected genes and single nucleotide polymorphisms (SNPs) through literature search and functional criteria. We selected 46 SNPs distributed in 19 genes of antiretroviral pharmacodynamic pathways: *ABCB1* (rs1128503, rs2214102, rs2235048 and rs3842), *ABCC1* (rs129081, rs113264879, rs4148380, rs8056298, rs212091 and rs16967632), *ABCG2* (rs115770495, rs1448784 and rs2231142), *CYP1A2* (rs762551), *CYP2A6* (rs8192726), *CYP2B6* (rs8192709, rs28399499, rs34097093, rs28399502, rs707265 and rs1042389), *CYP3A4* (rs4646437) and *SLC22A6* (rs11568629, rs11568628 and rs4149170) [17-24] and immunological activation and homeostasis: *CCL5* (rs2107538), *FAS* (rs2234767 and rs1800682), *IFNG* (rs2069709), *IL10* (rs2222202, rs1800871 and rs1800890), *IL10RA* (rs3135932 and rs9610), *IL15* (rs10519613 and rs10833), *IL1B* (rs16944), *IL2* (rs2069762), *IL4* (rs2243250), *IL7R* (rs1494555, rs11567762, rs6897932, rs3822731, rs987106 and rs3194051) and *NR1I3* (rs3003596) [25-28].

Additionally, 48 SNPs that served as ancestry informative markers (AIMs) were also genotyped (all variants are listed on Supplementary Table 1). Briefly, these SNPs help estimating ancestry proportions in admixed populations such as the one enrolled in our study, controlling for population structure and reducing bias during genetic association analysis [29]. Genomic DNA was extracted through Promega® Wizard Genomic DNA Purification Kit (Fitchburg, Wisconsin, USA), following manufacturer instructions. Genotyping was performed through VeraCode® platform of GoldenGate®

Illumina Inc (San Diego, California, USA) technology, following the manufacturer's instructions.

Raw genotyping data were extracted with Illumina® Genome Studio 2.0 software and imported in an Excel® worksheet. After processing the dataset, we imported it into PLINK software, version 1.90 [30] to perform quality control (QC) filtering. Samples and variants with less than 90% global call rates were removed from analysis. Variants with significant departure from Hardy-Weinberg equilibrium were also removed, using an exact test p-value<0.001 as threshold.

2.4 Ancestry proportion estimation

We used ADMIXTURE software [31] to carry out a "supervised analysis" allowing estimation of ancestry proportions in our admixed samples, using 2000 bootstrap steps with the AIMs panel mentioned above. The calculations were made assuming three different ancestral populations (K=3; African, Amerindian and European).

2.5 Statistical analysis

Comparisons between immune non-response and immune response groups were performed through Fisher exact test or Chi-squared test for categorical variables (sex, ART regimens, genetic association tests) and Mann-Whitney test for numerical variables (age and pre-ART CD4+ T cell absolute counts). Age at ART start date was also treated as a categorical variable with four strata: *18 to 29 years*, *29 to 39 years*, *39 to 49 years* and *49 years or more*.

Additionally, univariate survival analyses were performed through Kaplan-Meier survival probability estimator. Estimators for each variable were then compared through Cox-Mantel log rank tests to assess if they exerted statistically significant influence on time to immune response.

Allele and genotypes counts and frequencies were obtained through direct counting. Compliance to Hardy-Weinberg equilibrium was also assessed through Chi-squared test. All tests were two-sided. Any variable with statistically significant association with immunologic outcome were included in a multivariate survival analysis Cox proportional hazards model along with individual African ancestry proportion (estimated by AIMs) to assess if any of these variables were associated with delayed

time to immune response (CD4+ T cell percentage reaching 30% or higher during treatment follow-up). All analyses were performed with R software, version 3.3.1 [32].

Coinfections serological status, smoking and drinking habits (data not shown) and AIDS-defining conditions were not included in further analyses due to high prevalence of missing data (over 10% of data points) in the final dataset, in order to avoid the introduction of bias into the model. Table 1 also displays univariate comparison of patients' characteristics, their respective p-values, odds ratios (OR) and 95% confidence intervals (95% CI) whenever applicable.

3. Results

3.1 Univariate analysis: patients' data

We observed that males had more than two times increased risk of presenting immunological non-response (males were 61% of non-responders and 37.7% of responders; OR=2.56; 95% CI=1.20-5.60; p=0.01). Both groups had similar ages at treatment start (median 34.5 years for non-responders and 33 years for immunologic responders; p=0.11). As expected, non-responders started treatment with less absolute CD4+ T cell counts than responders (median 187.5 cells/μL versus 375.5 cells/μL, respectively, p<0.001), and the majority of non-responders started therapy with less than 200 cells/μL (48.8% versus 9.4%; p<0.001). Patients in both groups had similar ancestry backgrounds (mean African ancestry proportion 30.2% in non-responders versus 31.3% in responders, p=0.55).

Non-responders started treatment with more advanced disease than responders; 10.9% and 29.3% of non-responders presented B (symptomatic conditions) and C (AIDS-indicator conditions) CDC system stages, respectively, versus 1.9% and 13.2% of responders, although the difference did not reach statistical significance (p=0.11).

Patients started with similar first-line ART regimens (p=0.47), with the majority (68.9%) using zidovudine as the nucleoside analog reverse transcriptase inhibitor (NRTI) alongside lamivudine, and non-nucleoside reverse transcriptase inhibitors (NNRTIs), mostly efavirenz, instead of ritonavir-"boosted" protease inhibitors (PI/r) as the third option drug (58.5%). Table 1 also details each statistical comparison between the groups.

Most patients have no available serological tests results recorded in their medical charts, but it is reasonable to say that CMV and toxoplasmosis past/latent infections were somewhat prevalent (32.6% and 25.2%, respectively positive IgG/negative IgM tests). VDRL-positive tests were 14.2% of the total. Some patients were immune to HBV due to vaccination (21.5%), a minority of them due to natural infection (6.7%) and others were susceptible (28.1%), but none presented chronic infection. We did not observe positive HCV and HTLV-1/2 serological tests within the available data. Table 2 details the numbers of reported serological tests results.

The median follow-up period extracted from medical charts was 33 months of treatment and laboratory tests history (interquartile range IQR=18-66,8; with a minimum of two months and maximum of 203 months).

The median time to achieve immunologic response was 91 months (95% CI=50-127). Men took longer to achieve immunologic response than women, needing a (median) time of 110 months (95% CI=42-127), while women needed 75 months (95% CI=42-upper bound not calculated), although it did not reach statistical significance (Cox-Mantel log rank X^2=0.50 on 1 degree of freedom; p=0.47). Age groups at ART start date also did not influence time until immunologic response (Cox-Mantel log rank X^2=1.6 on 3 degrees of freedom; p=0.67). Similarly, use of AZT instead of TDF (Cox-Mantel log rank X2=1.7 on 1 degree of freedom; p=0.19) or PIs instead of NNRTIs as third option (Cox-Mantel log rank X2=0.5 on 1 degree of freedom; p= 0.49) on ART regimens also did not influence time until response.

3.2 Genotyping quality control (QC), ancestry estimation and genetic association testing

Four candidate SNPs, rs16944 (*IL1B*), rs10833 (*IL15*) rs11568629 (*SLC22A6*) and rs16967632 (*ABCC1*) and six AIMs did not pass genotyping QC and were removed from analysis. Other variant, rs34097093 (*CYP2B6*), was also removed because all individuals in the sample had the same genotype (one of the alleles was fixed). The remaining allele and genotype frequencies were all in conformity to Hardy-Weinberg equilibrium according to PLINK software exact test. All frequencies and global call rates are displayed in Supplementary Table 2. Therefore, further analyses were performed using the remaining 41 candidate SNPs and 42 AIMs.

Five SNPs presented statistic association with immunological outcome. Three minor alleles were more frequent in non-responders than responders: rs3003596 (*NR1I3*) G allele (48.2% vs. 35.6%, p=0.01); rs2243250 (*IL4*) A allele (42.6% vs. 32.7%, p=0.02) and rs129081 (*ABCC1*) G allele (47.6% vs. 37.5%, p=0.02), whereas the other two gave inverse results: rs2069762 (*IL2*) C allele (20.1% vs. 30.2%; p=0.03) and rs10519613 (*IL15*) A allele (10.5% vs. 19.8%; p=0.03). Allele, genotype frequencies and statistical analyses for these five SNPs are displayed in Table 3 and Supplementary Table 3 displays all genetic association results.

The individuals in our sample presented a major European ancestry contribution (mean proportion 55.1%±18.2), followed by the African (32.5%±16.1) and a minor Amerindian one (12.4%±9.8), as expected, due to our previous works with other samples coming from the same general population [29, 33]. Since non-responders and responders had similar ancestry proportions (for example, mean African contribution 30.2%±15.9 vs. 31.3%±13.8, respectively; p=0.55), we believe that there is no hidden genetic structure biasing our genetic association analysis. As mentioned before, individual African genetic ancestry contributions were included in the multivariate Cox proportional hazards model for an additional “genomic control” [34]; however, all associations were lost (data not shown).

4. Discussion

ART, when followed correctly with good adherence, suppresses HIV-1 replication, decrease immune activation, favors immune recovery and protects against opportunist infections [35, 36]. However, some patients do not recover CD4+ T cell numbers to normal our quasi-normal levels (immunological failure or non-response), being at risk for non-AIDS diseases, such as cardiovascular, kidney and liver disorders [4] and early ageing [7, 8]. Thus, we performed a genetic association study through survival analysis to assess if polymorphisms in antiretroviral drugs pharmacodynamic pathways and immune system homeostasis were related with immunological failure in a sample from Recife, Northeast Brazil.

We found a high prevalence of immunological non-response (60.7%), which was higher than some estimates found in the literature, ranging between 10% and 40% [37]. However, it is important to notice that, since we focused our analysis exclusively on individuals with viral suppression to assure that viral replication is not the

responsible for impaired CD4+ T cell numbers expansion, this estimate could be inflated simply due to sample stratification.

Our sample is generally comprised of people with lower socioeconomic status with less access to sexual education and healthcare. Thus, they usually receive late or very late HIV-1 infection diagnosis and, therefore, tend to start ART with low absolute CD4+ T cells counts, advanced disease and presenting opportunistic infections symptoms. A very compromised immune system by the chronic HIV-1 infection is a predisposition factor to immunologic non-response [38], and we indeed observed that non-responders begun treatment with lower absolute CD4+ T cell counts, with almost half of them starting treatment with less than 200 cells/μL.

Previous reviews also reported that male sex, older age [37] and HCV coinfection are risk factors for immune non-response [39, 40]. We only found an association between male sex and immune failure during univariate analysis, but this association was lost during multivariate survival analysis. We also assessed HCV infection status alongside other agents (CMV, HBV, HTLV-1/2, toxoplasmosis and syphilis), but we could reach no conclusion due to the high prevalence of untested subjects for most of these infections, which hindered further analysis, since it could bias the results with sample size restriction. However, we believe that HCV and HTLV-1/2 had very low prevalence in our sample (no positive tests among those available), and therefore they are unlikely to be playing a role on immunological outcome of our sample, at least. We also did not observe any chronic HBV-infected subjects in our sample, but we detected some individuals with anti-HBV immunity due to past infections. We also found some individuals with latent CMV infection (positive IgG tests), a known causative agent of persistent immune activation [41], which can cause immune system exhaustion [42], but we cannot affirm if it was a factor favoring immune non-response in our sample, as it would be too speculative, since our serological data status is mostly lacking, as discussed above.

Sex and pre-ART CD4+ T cell counts were the only non-genetic differences between our study groups, since the individuals in our sample had similar ethnic backgrounds (as estimated by AIMs), and ART regimens types and distribution were alike between groups. Therefore, we expected to find genetic risk factors to immunologic non-response. We genotyped 46 candidate SNPs located in genes involved on antiretroviral drugs metabolism and transport and in genes involved on

immune response. We found five SNPs associated - three with susceptibility to non-response while the remaining two with favorable response.

Two of the three SNPs associated with non-response are located in genes related to drug metabolism: *NR1I3* and *ABCC1*. The former is a nuclear receptor that senses foreign substances (such as antiretroviral drugs) and upregulates expression of other proteins that metabolize these substances aiming their excretion [43] and the latter is a membrane active transport protein that ejects antiretroviral drugs from cells [44]. The third is located at *IL4* gene, which is involved in a polyfunctional immunoregulatory signal [45]. The two SNPs associated with favorable response are also located at genes related to immune system homeostasis, important for T and B cells proliferation, *IL2* [46], and T cell activation, *IL15* [47]

We expected that SNPs in these genes would work in concert to affect gene function, altering distribution of antiretroviral drugs, and having deleterious consequences on immune function, leading to the suboptimal immunological response to ART. However, all genetic associations were lost after multivariate survival analysis modeling. As other authors did not find associations focusing on the same or similar genes [48] while others did [49] (for a review of previous genetic association studies, refer to [37]), more studies are necessary to unravel the genetic component of ART immunological non-response.

5. Conclusion

We performed a genetic association study looking for genetic variants that would explain suboptimal gains CD4+ T cell counts in some individuals of a retrospective observational sample of individuals living with HIV-1 receiving ART from Northeast Brazil, but did not find any statistically significant association. Thus, more studies are necessary, so we could develop a personalized, predictive model of immunological non-response, consequently improving HIV-1 infection care, and perhaps preventing complications that came from immunological non-response, such as systemic diseases and/or early ageing.

Disclosure statement

The authors declare that they have no conflict of interest related to this manuscript.

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References

1. De Cock KM, Jaffe HW, Curran JW. Reflections on 30 years of AIDS. *Emerg Infect Dis* 2011; **17:**1044-1048.

2. World Health Organization. 2015 Progress Report on the Global Plan towards the elimination of new HIV infections among children and keeping their mothers alive. 2015.

3. Vella S, Schwartlander B, Sow SP, Eholie SP, Murphy RL. The history of antiretroviral therapy and of its implementation in resource-limited areas of the world. *AIDS* 2012; **26:**1231-1241.

4. Li T, Wu N, Dai Y*, et al.* Reduced thymic output is a major mechanism of immune reconstitution failure in HIV-infected patients after long-term antiretroviral therapy. *Clinical Infectious Diseases* 2011; **53:**944-951.

5. Teixeira L, Valdez H, McCune JM*, et al.* Poor CD4 T cell restoration after suppression of HIV-1 replication may reflect lower thymic function. *AIDS* 2001; **15:**1749-1756.

6. Tan R, Westfall AO, Willig JH*, et al.* Clinical Outcome of HIV-Infected Antiretroviral-Naive Patients With Discordant Immunologic and Virologic Responses to Highly Active Antiretroviral Therapy. *JAIDS Journal of Acquired Immune Deficiency Syndromes* 2008; **47:**553-558 510.1097/QAI.1090b1013e31816856c31816855.

7. Pathai S, Bajillan H, Landay AL, High KP. Is HIV a model of accelerated or accentuated aging? *J Gerontol A Biol Sci Med Sci* 2014; **69:**833-842.

8. Torres RA, Lewis W. Aging and HIV/AIDS: pathogenetic role of therapeutic side effects. *Lab Invest* 2014; **94:**120-128.

9. Marziali M, De Santis W, Carello R*, et al.* T-cell homeostasis alteration in HIV-1 infected subjects with low CD4 T-cell count despite undetectable virus load during HAART. *AIDS* 2006; **20:**2033-2041.

10. Doitsh G, Galloway NL, Geng X*, et al.* Cell death by pyroptosis drives CD4 T-cell depletion in HIV-1 infection. *Nature* 2014; **505:**509-514.

11. Telenti A, Zanger UM. Pharmacogenetics of anti-HIV drugs. *Annu Rev Pharmacol Toxicol* 2008; **48:**227-256.

12. Tozzi V. Pharmacogenetics of antiretrovirals. *Antiviral Res* 2009; **85:**190-200.

13. Levy Y. Cytokine-based modulation of immune function in HIV infection. *Current opinion in HIV and AIDS* 2006; **1:**69-73.

14. CDC. 1993 revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. *MMWR Recomm Rep* 1992; **41:**1-19.

15. Brasil. Ministério da Saúde. Secretária de Vigilância em Saúde. Programa Nacional de DST AeHV. *Protocolo clínico e diretrizes terapêuticas para manejo da infecção pelo HIV em adultos*. Brasília: Ministério da Saúde: Ministério da Saúde, Secretaria de Vigilância em Saúde, Programa Nacional de DST, Aids e Hepatites Virais 2015.

16. Hulgan T, Raffanti S, Kheshti A*, et al.* CD4 Lymphocyte Percentage Predicts Disease Progression in HIV-Infected Patients Initiating Highly Active Antiretroviral Therapy with CD4 Lymphocyte Counts >350 Lymphocytes/mm3. *Journal of Infectious Diseases* 2005; **192:**950-957.

17. Fellay J, Marzolini C, Meaden ER*, et al.* Response to antiretroviral treatment in HIV-1-infected individuals with allelic variants of the multidrug resistance transporter 1: a pharmacogenetics study. *Lancet* 2002; **359:**30-36.

18. Brumme ZL, Dong WW, Chan KJ*, et al.* Influence of polymorphisms within the CX3CR1 and MDR-1 genes on initial antiretroviral therapy response. *AIDS* 2003; **17:**201-208.

19. Cressey TR, Lallemant M. Pharmacogenetics of antiretroviral drugs for the treatment of HIV-infected patients: an update. *Infection, genetics and evolution : journal of molecular epidemiology and evolutionary genetics in infectious diseases* 2007; **7:**333-342.

20. Ingelman-Sundberg M, Sim SC, Gomez A, Rodriguez-Antona C. Influence of cytochrome P450 polymorphisms on drug therapies: pharmacogenetic, pharmacoepigenetic and clinical aspects. *Pharmacol Ther* 2007; **116:**496-526.

21. Gatanaga H, Hayashida T, Tsuchiya K*, et al.* Successful efavirenz dose reduction in HIV type 1-infected individuals with cytochrome P450 2B6 *6 and *26. *Clin Infect Dis* 2007; **45:**1230-1237.

22. Jung N, Lehmann C, Rubbert A*, et al.* Relevance of the Organic Cation Transporters 1 and 2 for Antiretroviral Drug Therapy in Human Immunodeficiency Virus Infection. *Drug Metabolism and Disposition* 2008; **36:**1616-1623.

23. Haas DW, Gebretsadik T, Mayo G*, et al.* Associations between CYP2B6 Polymorphisms and Pharmacokinetics after a Single Dose of Nevirapine or Efavirenz in African Americans. *Journal of Infectious Diseases* 2009; **199:**872-880.

24. Franke RM, Gardner ER, Sparreboom A. Pharmacogenetics of drug transporters. *Curr Pharm Des* 2010; **16:**220-230.

25. Nasi M, Pinti M, Bugarini R*, et al.* Genetic polymorphisms of Fas (CD95) and Fas ligand (CD178) influence the rise in CD4+ T cell count after antiretroviral therapy in drug-naive HIV-positive patients. *Immunogenetics* 2005; **57:**628-635.

26. Smith AJ, Humphries SE. Cytokine and cytokine receptor gene polymorphisms and their functionality. *Cytokine Growth Factor Rev* 2009; **20:**43-59.

27. Chew CS, Cherry CL, Kamarulzaman A, Yien TH, Aghafar Z, Price P. A longitudinal study of the effects of ART on plasma chemokine levels in Malaysian HIV patients. *Dis Markers* 2011; **31:**303-309.

28. Swart M, Whitehorn H, Ren Y, Smith P, Ramesar RS, Dandara C. PXR and CAR single nucleotide polymorphisms influence plasma efavirenz levels in South African HIV/AIDS patients. *BMC medical genetics* 2012; **13:**112.

29. Coelho AV, De Moura RR, Da Silva RC*, et al.* Meta-Analysis and Time Series Modeling Allow a Systematic Review of Primary HIV-1 Drug-Resistant Prevalence in Latin America and Caribbean. *Curr HIV Res* 2015; **13:**125-142.

30. Chang CC, Chow CC, Tellier LC, Vattikuti S, Purcell SM, Lee JJ. Second-generation PLINK: rising to the challenge of larger and richer datasets. *Gigascience* 2015; **4:**7.

31. Alexander DH, Novembre J, Lange K. Fast model-based estimation of ancestry in unrelated individuals. *Genome Res* 2009; **19:**1655-1664.

32. R Core Team. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/. Edited by Editor|. Year|; p.^pp. Pages|. City|: Publisher|.

33. Rodrigues de Moura R, Coelho AV, de Queiroz Balbino V, Crovella S, Brandao LA. Meta-analysis of Brazilian genetic admixture and comparison with other Latin America countries. *Am J Hum Biol* 2015.

34. Balding DJ. A tutorial on statistical methods for population association studies. *Nat Rev Genet* 2006; **7:**781-791.

35. Hutchinson JF. The Biology and Evolution of HIV. *Annual Review of Anthropology* 2001; **30:**85-108.

36. Bartlett JA, DeMasi R, Quinn J, Moxham C, Rousseau F. Overview of the effectiveness of triple combination therapy in antiretroviral-naive HIV-1 infected adults. *AIDS* 2001; **15:**1369-1377.

37. Peraire J, Viladés C, Pacheco YM*, et al.* Evaluation of the pharmacogenetics of immune recovery in treated HIV-infected patients. *Expert Opinion on Drug Metabolism and Toxicology* 2014; **10:**81-101.

38. Gaardbo JC, Hartling HJ, Gerstoft J, Nielsen SD. Incomplete Immune Recovery in HIV Infection: Mechanisms, Relevance for Clinical Care, and Possible Solutions. *Clinical and Developmental Immunology* 2012; **2012:**670957.

39. Miller MF, Haley C, Koziel MJ, Rowley CF. Impact of Hepatitis C Virus on Immune Restoration in HIV-Infected Patients Who Start Highly Active Antiretroviral Therapy: A Meta-analysis. *Clinical Infectious Diseases* 2005; **41:**713-720.

40. Brites-Alves C, Netto EM, Brites C. Coinfection by Hepatitis C Is Strongly Associated with Abnormal CD4/CD8 Ratio in HIV Patients under Stable ART in Salvador, Brazil. *Journal of Immunology Research* 2015; **2015:**174215.

41. Sylwester AW, Mitchell BL, Edgar JB*, et al.* Broadly targeted human cytomegalovirus-specific CD4(+) and CD8(+) T cells dominate the memory compartments of exposed subjects. *The Journal of Experimental Medicine* 2005; **202:**673-685.

42. Khaitan A, Unutmaz D. Revisiting Immune Exhaustion During HIV Infection. *Current HIV/AIDS reports* 2011; **8:**4-11.

43. Wada T, Gao J, Xie W. PXR and CAR in energy metabolism. *Trends in Endocrinology & Metabolism*; **20:**273-279.

44. Kis O, Robillard K, Chan GNY, Bendayan R. The complexities of antiretroviral drug-drug interactions: role of ABC and SLC transporters. *Trends in Pharmacological Sciences* 2010; **31:**22-35.

45. Paul WE. History of Interleukin-4. *Cytokine* 2015; **75:**3-7.

46. Liao W, Lin J-X, Leonard WJ. IL-2 Family Cytokines: New Insights into the Complex Roles of IL-2 as a Broad Regulator of T helper Cell Differentiation. *Current Opinion in Immunology* 2011; **23:**598-604.

47. Mishra A, Sullivan L, Caligiuri MA. Molecular Pathways: Interleukin-15 Signaling in Health and in Cancer. *Clinical cancer research : an official journal of the American Association for Cancer Research* 2014; **20:**2044-2050.

48. Fernandez S, Rosenow AA, James IR*, et al.* Recovery of CD4+ T Cells in HIV Patients With a Stable Virologic Response to Antiretroviral Therapy Is Associated With Polymorphisms of Interleukin-6 and Central Major Histocompatibility Complex Genes. *JAIDS Journal of Acquired Immune Deficiency Syndromes* 2006; **41:**1-5.

49. Haas DW, Geraghty DE, Andersen J*, et al.* Immunogenetics of CD4 lymphocyte count recovery during antiretroviral therapy: An AIDS Clinical Trials Group study. *J Infect Dis* 2006; **194:**1098-1107.

Table 1. General characteristics of the recruited individuals living with HIV-1 and stratification according to antiretroviral therapy immunologic response.

,	Total (n=135)	Immunologic non-responders (n=82)	Immunologic responders (n=53)	Univariate analysis
Sex, n (%)				
Females	65 (48.2)	32 (39.0)	33 (62.3)	*reference*
Males	70 (51.8)	50 (61.0)	20 (37.7)	OR=2.56 (95% CI=1.20-5.60); **p=0.01**
Age at ART start date				
Median (IQR) [min.-max.]	33 (29-39) [21-54]	34.5 (29-40) [21-54]	33 (26-37) [21-53]	W=1813.5; p=0.11
Pre-ART CD4+ T cell counts, cells/µL of peripheral whole blood				
Median (IQR) [min.-max.]	260.5 (122.8-400.5) [13-1029]	187.5 (86.8-281.5) [13-543]	375.5 (274.2-525.8) [48-1029]	W=669.5; **p<0.001**
By categories, n (%)				
Not available	39 (28.9)	8 (9.8)	7 (13.2)	*(not included in the comparison)*
Less than 200	65 (48.2)	40 (48.8)	5 (9.4)	
Between 200 and 350	25 (18.5)	23 (28.0)	15 (28.4)	X^2=34.9; df=3; **p<0.001**
Between 350 and 500	6 (4.4)	10 (12.2)	13 (24.5)	
Over 500	39 (28.9)	1 (1.2)	13 (24.5)	
Ancestry proportions estimated by AIMs, mean percentage (SD) [min.-max.]				
African	32.5 (16.1) [0.0-75.3]	30.2 (15.9) [0.0-70.2]	31.3 (13.8) [4.4-63.3]	
Amerindian	12.4 (9.8) [0.0-52.7]	13.2 (10.7) [0.0-52.7]	9.0 (9.2) [0.0-34.1]	W=1912; p=0.55
European	55.1 (18.2) [11.8-99.9]	56.2 (18.5) [11.8-99.9]	59.6 (15.7) [28.2-91.2]	
AIDS-defining conditions, CDC classification system				
Not available	93 (68.9)	49 (59.8)	44 (83.0)	*(not included in the comparison)*
A stage	1 (0.7)	0 (0.0)	1 (1.9)	
B stage	10 (7.4)	9 (10.9)	1 (1.9)	X^2=4.5; df=2; p=0.11
C stage	31 (23.0)	24 (29.3)	7 (13.2)	
First ART regimens				
Not available	9 (6.7)	5 (6.1)	4 (7.5)	*(not included in the comparison)*
Monotherapy	3 (2.2)	1 (1.2)	2 (3.8)	
Detailed regimens: [NRTI]+3TC [third option]				
ABC+3TC EFZ	1 (0.7)	1 (1.2)	0 (0.0)	
AZT+3TC ATV/r	4 (2.9)	3 (3.7)	1 (1.9)	
AZT+3TC EFZ	48 (35.7)	29 (35.4)	19 (35.8)	
AZT+3TC FPV/r	1 (0.7)	1 (1.2)	0 (0.0)	
AZT+3TC IDV/r	0 (0.0)	0 (0.0)	0 (0.0)	X^2=6.6; df=7; p=0.47
AZT+3TC LPV/r	36 (26.7)	19 (23.2)	17 (32.1)	
AZT+3TC NVP	4 (2.9)	2 (2.4)	2 (3.8)	
TDF+3TC ATV/r	3 (2.2)	1 (1.2)	2 (3.8)	
TDF+3TC EFZ	26 (19.3)	20 (24.4)	6 (11.3)	
TDF+3TC LPV/r	0 (0.0)	0 (0.0)	0 (0.0)	
ART regimens, stratified by [third option]				
NNTRI	79 (58.5)	52 (63.4)	27 (50.9)	*reference*
PI/r	44 (32.6)	24 (29.3)	20 (37.7)	OR=0.63 (95% CI=0.28-1.42); p=0.25
[NRTI] choice alongside 3TC				
ABC	1 (0.7)	1 (1.2)	0 (0.0)	*(not included in the comparison)*
AZT	93 (68.9)	54 (65.9)	39 (73.6)	*reference*
TDF	29 (21.5)	21 (25.6)	8 (15.1)	OR=1.89 (95% CI =0.71-5.46); p=0.19

3TC – lamivudine, 95% CI – 95% confidence interval, ABC – abacavir, AIDS – acquired immunodeficiency syndrome, ART – antiretroviral therapy, ATV/r – ritonavir-boosted atazanavir, CDC – Center for Disease Control (USA), df – degrees of freedom, EFZ – efavirenz, FPV/r – ritonavir-boosted fosamprenavir , IDV/r – ritonavir-boosted indinavir, IQR – interquartile range, LPV/r – ritonavir-boosted lopinavir, min.-max. – minimum and maximum values, NNRTI – non-nucleoside analog reverse transcriptase inhibitor, NRTI – nucleoside analog reverse transcriptase inhibitor, NVP – nevirapine, OR – odds ratio, p – p-value, PI – protease inhibitor, SD – standard deviation, TDF – tenofovir, W – Mann-Whitney test statistic, X2 – chi-squared test statistic.

Table 2. Coinfections serological status of the recruited patients living with HIV-1 stratification according to antiretroviral therapy immunologic response.

Etiologic agent	Total n=135 (%)	Immunologic non-responders n=82 (%)	Immunologic responders n=53 (%)
Cytomegalovirus (CMV)			
IgM test			
Untested	90 (66.7)	59 (72.0)	31 (58.5)
Negative	44 (32.6)	23 (28.0)	21 (39.6)
Positive	1 (0.7)	0 (0.0)	1 (1.9)
IgG test			
Untested	90 (66.7)	59 (72.0)	31 (58.5)
Negative	1 (0.7)	1 (1.2)	0 (0.0)
Positive	44 (32.6)	22 (26.8)	22 (41.5)
Hepatitis B virus (HBV)			
Untested	59 (43.7)	37 (45.1)	22 (41.5)
Susceptible	38 (28.1)	21 (25.6)	17 (32.1)
Chronic infection	0 (0.0)	0 (0.0)	0 (0.0)
Immune due to natural infection	9 (6.7)	6 (7.3)	3 (5.7)
Immune due to vaccination	29 (21.5)	18 (22.0)	11 (20.8)
Hepatitis C virus (HCV)			
Untested	68 (50.4)	41 (50.0)	27 (50.9)
Negative	67 (49.6)	41 (50.0)	26 (49.1)
Positive	0 (0.0)	0 (0.0)	0 (0.0)
Human T-lymphotropic virus type 1 or 2 (HTLV-1/2)			
Untested	114 (84.4)	76 (92.7)	38 (71.7)
Negative	21 (15.6)	6 (7.3)	15 (28.3)
Positive	0 (0.0)	0 (0.0)	0 (0.0)
Toxoplasma gondii			
IgM test			
Untested	90 (66.7)	59 (72.0)	31 (58.5)
Negative	45 (33.3)	23 (28.0)	22 (41.5)
Positive	0 (0.0)	0 (0.0)	0 (0.0)
IgG test			
Untested	90 (66.7)	59 (72.0)	31 (58.5)
Negative	11 (8.1)	7 (8.5)	4 (7.5)
Positive	34 (25.2)	16 (19.5)	18 (34.0)
Treponema pallidum **(VDRL test)**			
Untested	47 (34.8)	30 (36.6)	17 (32.1)
Negative	68 (50.4)	38 (46.3)	30 (56.6)
Positive	20 (14.8)	14 (17.1)	6 (11.3)

Table 3. Allele and genotype frequencies of variants showing statistically significant genetic association with immunologic outcome.

#	SNP	Type	Alleles		Gene	Gene function/pathway	Alelle and genotype frequencies								X^2	DF	p
							Immunologic non-responders				Immunologic responders						
			A1	A2			A2 (%)	A1/A1 (%)	A1/A2 (%)	A2/A2 (%)	A2 (%)	A1/A1 (%)	A1/A2 (%)	A2/A2 (%)			
5	rs3003596	Downstream gene region	A	G	*NR1I3*	Regulation of drugs and endobiotic metabolism	79 (48.2)	21 (25.6)	43 (52.4)	18 (22.0)	37 (35.6)	25 (48.1)	17 (32.7)	10 (19.2)	10.10	2	***0.01***
26	rs2069762	Upstream gene region	A	C	*IL2*	Regulates T and B lymphocytes proliferation	33 (20.1)	49 (59.8)	33 (40.2)	0 (0.0)	32 (30.2)	25 (47.2)	24 (45.3)	4 (7.5)	73.13	2	***0.03***
27	rs10519613	3' untranslated region	C	A	*IL15*	T lymphocytes activation	17 (10.5)	67 (82.7)	11 (13.6)	3 (3.7)	21 (19.8)	34 (64.2)	17 (32.1)	2 (3.8)	67.10	2	***0.03***
38	rs2243250	Upstream gene region	G	A	*IL4*	Immunoregulation	69 (42.6)	20 (24.7)	53 (65.4)	8 (9.9)	32 (32.7)	23 (46.9)	20 (40.8)	6 (12.2)	8.02	2	***0.02***
74	rs129081	3' untranslated region	C	G	*ABCC1*	Membrane transport, antiretroviral drugs efflux	78 (47.6)	24 (29.3)	38 (46.3)	20 (24.4)	39 (37.5)	16 (30.8)	33 (63.5)	3 (5.8)	82.13	2	***0.02***

- order (by chromosome and genomic position) in which the candidate and ancestry informative markers are listed on the Supplementary Tables, DF – degrees of freedom, X^2 – value of chi-squared statistic from chi-square test of independence, p – p-value.

Supplementary Table 1. List of all analyzed single nucleotide polymorphisms (SNPs) in the recruited patients.

#	SNP	Chromosome	Position	Gene (if applicable)	Official gene symbol	Function or pathway	Variant type
1	rs4908343	1	27605187	(ancestry informative marker)			
2	rs1325502	1	41894599	(ancestry informative marker)			
3	rs3737576	1	101244007	(ancestry informative marker)			
4	rs7554936	1	151150013	(ancestry informative marker)			
5	rs3003596	1	161234427	Nuclear receptor subfamily 1 group I member 3	*NR1I3*	Regulation of drugs and endobiotic metabolism	Downstream gene region
6	rs2222202	1	206772036	Interleukin 10	*IL10*	Immunoregulation	Intronic
7	rs1800871	1	206773289	Interleukin 10	*IL10*	Immunoregulation	Upstream gene region
8	rs1800890	1	206776020	Interleukin 10	*IL10*	Immunoregulation	Upstream gene region
9	rs798443	2	7828144	(ancestry informative marker)			
10	rs4666200	2	29315545	(ancestry informative marker)			
11	rs4670767	2	37714253	(ancestry informative marker)			
12	rs13400937	2	79637797	(ancestry informative marker)			
13	rs260690	2	108963282	(ancestry informative marker)			
14	rs16944	2	112837290	Interleukin 1 beta	*IL1B*	Pro-inflammation cytokine	Upstream gene region
15	rs10496971	2	145012376	(ancestry informative marker)			
16	rs9809104	3	39104938	(ancestry informative marker)			
17	rs6548616	3	79350425	(ancestry informative marker)			
18	rs12629908	3	120803869	(ancestry informative marker)			
19	rs9845457	3	136195634	(ancestry informative marker)			
20	rs1513181	3	188857208	(ancestry informative marker)			
21	rs10007810	4	41552347	(ancestry informative marker)			
22	rs115770495	4	88090508	*ATP binding cassette subfamily G member 2*	*ABCG2*	Membrane transport, antiretroviral drugs efflux	3’ untranslated region
23	rs1448784	4	88091168	ATP binding cassette subfamily G member 2	*ABCG2*	Membrane transport, antiretroviral drugs efflux	3’ untranslated region
24	rs2231142	4	88131171	ATP binding cassette subfamily G member 2	*ABCG2*	Membrane transport, antiretroviral drugs efflux	Missense (Gln141Lys)
25	rs7657799	4	104454266	(ancestry informative marker)			
26	rs2069762	4	122456825	Interleukin 12	*IL2*	Regulates T and B lymphocytes proliferation	Upstream gene region
27	rs10519613	4	141732931	Interleukin 15	*IL15*	T lymphocytes activation	3’ untranslated region
28	rs10833	4	141733394	Interleukin 15	*IL15*	T lymphocytes activation	3’ untranslated region

#	SNP	Chromosome	Position	Gene (if applicable)	Official gene symbol	Function or pathway	Variant type
29	rs316598	5	2364512	(ancestry informative marker)			
30	rs870347	5	6844922	(ancestry informative marker)			
31	rs1494555	5	35871088	Interleukin 7 receptor	*IL7R*	Signal transducer, regulates T lymphocytes development	Missense (Val138Ile)
32	rs11567762	5	35873099	Interleukin 7 receptor	*IL7R*	Signal transducer, regulates T lymphocytes development	Intronic
33	rs6897932	5	35874473	Interleukin 7 receptor	*IL7R*	Signal transducer, regulates T lymphocytes development	Missense (Thr244Ile)
34	rs3822731	5	35875138	Interleukin 7 receptor	*IL7R*	Signal transducer, regulates T lymphocytes development	Intronic
35	rs987106	5	35875491	Interleukin 7 receptor	*IL7R*	Signal transducer, regulates T lymphocytes development	Intronic
36	rs3194051	5	35876172	Interleukin 7 receptor	*IL7R*	Signal transducer, regulates T lymphocytes development	Missense (Ile356Val)
37	rs6451722	5	43711276	(ancestry informative marker)			
38	rs2243250	5	132673462	Interleukin 4	*IL4*	Immunoregulation	Upstream gene region
39	rs6422347	5	178436082	(ancestry informative marker)			
40	rs1040045	6	4746925	(ancestry informative marker)			
41	rs2504853	6	12534879	(ancestry informative marker)			
42	rs4463276	6	144734195	(ancestry informative marker)			
43	rs731257	7	12629626	(ancestry informative marker)			
44	rs3842	7	87504050	ATP binding cassette subfamily B member 1	*ABCB1*	Membrane transport, antiretroviral drugs efflux	3’ untranslated region
45	rs2235048	7	87509195	ATP binding cassette subfamily B member 1	*ABCB1*	Membrane transport, antiretroviral drugs efflux	Intronic
46	rs1128503	7	87550285	ATP binding cassette subfamily B member 1	*ABCB1*	Membrane transport, antiretroviral drugs efflux	Synonymous (Gly412Gly)
47	rs2214102	7	87600185	ATP binding cassette subfamily B member 1	*ABCB1*	Membrane transport, antiretroviral drugs efflux	5’ untranslated region
48	rs4646437	7	99767460	Cytochrome P450 family 3 subfamily A member 4	*CYP3A4*	Antiretroviral drugs metabolism	Intronic
49	rs7803075	7	131057307	(ancestry informative marker)			
50	rs10236187	7	139747578	(ancestry informative marker)			
51	rs10108270	8	4333271	(ancestry informative marker)			
52	rs3943253	8	13501991	(ancestry informative marker)			
53	rs1471939	8	29083788	(ancestry informative marker)			
54	rs4746136	10	73541236	(ancestry informative marker)			
55	rs2234767	10	88989499	Fas cell surface death receptor	*FAS*	Apoptosis	Upstream gene region
56	rs1800682	10	88990206	Fas cell surface death receptor	*FAS*	Apoptosis	Upstream gene region
57	rs4918842	10	113557053	(ancestry informative marker)			

#	SNP	Chromosome	Position	Gene (if applicable)	Official gene symbol	Function or pathway	Variant type
58	rs2946788	11	23988984	(ancestry informative marker)			
59	rs11568629	11	62984340	Solute carrier family 22 member 6	*SLC22A6*	Membrane transport, antiretroviral drugs influx/efflux	Synonymous (Pro117Pro)
60	rs11568628	11	62984439	Solute carrier family 22 member 6	*SLC22A6*	Membrane transport, antiretroviral drugs influx/efflux	Synonymous (Pro84Pro)
61	rs4149170	11	62984817	Solute carrier family 22 member 6	*SLC22A6*	Membrane transport, antiretroviral drugs influx/efflux	5' untranslated region
62	rs3135932	11	117993348	Interleukin 10 receptor subunit alpha	*IL10RA*	Signal transducer, immunoregulation	Missense (Ser159Gly)
63	rs9610	11	118001371	Interleukin 10 receptor subunit alpha	*IL10RA*	Signal transducer, immunoregulation	3' untranslated region
64	rs2416791	12	11548554	(ancestry informative marker)			
65	rs772262	12	55769950	(ancestry informative marker)			
66	rs2069709	12	68159923	Interferon Gamma	*IFNG*	Immunoregulation	Upstream gene region
67	rs9319336	13	27050219	(ancestry informative marker)			
68	rs7997709	13	34273600	(ancestry informative marker)			
69	rs9530435	13	75419751	(ancestry informative marker)			
70	rs9522149	13	111174820	(ancestry informative marker)			
71	rs1760921	14	20349972	(ancestry informative marker)			
72	rs3784230	14	105212718	(ancestry informative marker)			
73	rs762551	15	74749576	Cytochrome P450 family 1 subfamily A member 2	*CYP1A2*	Antiretroviral drugs metabolism	Intronic
74	rs129081	16	16142082	ATP binding cassette subfamily C member 1	*ABCC1*	Membrane transport, antiretroviral drugs efflux	3' untranslated region
75	rs113264879	16	16142164	ATP binding cassette subfamily C member 1	*ABCC1*	Membrane transport, antiretroviral drugs efflux	3' untranslated region
76	rs4148380	16	16142574	ATP binding cassette subfamily C member 1	*ABCC1*	Membrane transport, antiretroviral drugs efflux	3' untranslated region
77	rs8056298	16	16142666	ATP binding cassette subfamily C member 1	*ABCC1*	Membrane transport, antiretroviral drugs efflux	3' untranslated region
78	rs212091	16	16142793	ATP binding cassette subfamily C member 1	*ABCC1*	Membrane transport, antiretroviral drugs efflux	3' untranslated region
79	rs16967632	16	16142926	ATP binding cassette subfamily C member 1	*ABCC1*	Membrane transport, antiretroviral drugs efflux	3' untranslated region
80	rs2107538	17	35880776	C-C motif chemokine ligand 5	*CCL5*	Immunoregulation	Upstream gene region
81	rs11652805	17	64991033	(ancestry informative marker)			
82	rs2125345	17	75786110	(ancestry informative marker)			
83	rs4891825	18	70200427	(ancestry informative marker)			
84	rs8192726	19	40848591	Cytochrome P450 family 2 subfamily A member 6	*CYP2A6*	Antiretroviral drugs metabolism	Intronic
85	rs8192709	19	40991369	Cytochrome P450 family 2 subfamily B member 6	*CYP2B6*	Antiretroviral drugs metabolism	Missense (Arg22Cys)
86	rs28399499	19	41012316	Cytochrome P450 family 2 subfamily B member 6	*CYP2B6*	Antiretroviral drugs metabolism	Missense (Ile328Thr)

#	SNP	Chromosome	Position	Gene (if applicable)	Official gene symbol	Function or pathway	Variant type
87	rs34097093	19	41012465	Cytochrome P450 family 2 subfamily B member 6	*CYP2B6*	Antiretroviral drugs metabolism	Stop (Arg378*)
88	rs28399502	19	41016965	Cytochrome P450 family 2 subfamily B member 6	*CYP2B6*	Antiretroviral drugs metabolism	3’ untranslated region
89	rs707265	19	41018182	Cytochrome P450 family 2 subfamily B member 6	*CYP2B6*	Antiretroviral drugs metabolism	3’ untranslated region
90	rs1042389	19	41018248	Cytochrome P450 family 2 subfamily B member 6	*CYP2B6*	Antiretroviral drugs metabolism	3’ untranslated region
91	rs6104567	20	10214785	(ancestry informative marker)			
92	rs3907047	20	55384376	(ancestry informative marker)			
93	rs4821004	22	31970372	(ancestry informative marker)			
94	rs5768007	22	47812123	(ancestry informative marker)			

Supplementary Table 2. Allele and genotype frequencies of all variants analyzed.

#	SNP	Alleles		Gene (if applicable)	Immunologic non-responders					Immunologic responders					GCR	HWE p
		A1	A2		A1 (%)	A2 (%)	A1/A1 (%)	A1/A2 (%)	A2/A2 (%)	A1 (%)	A2 (%)	A1/A1 (%)	A1/A2 (%)	A2/A2 (%)		
1	rs4908343	A	G	(ancestry informative marker)	92 (56.1)	72 (43.9)	22 (26.8)	48 (58.5)	12 (14.6)	58 (55.8)	46 (44.2)	16 (30.8)	26 (50.0)	10 (19.2)	98.1	0.22
2	rs1325502	G	A	(ancestry informative marker)	104 (63.4)	60 (36.6)	33 (40.2)	38 (46.3)	11 (13.4)	69 (65.1)	37 (34.9)	23 (43.4)	23 (43.4)	7 (13.2)	100	0.85
3	rs3737576	A	G	(ancestry informative marker)	149 (90.9)	15 (9.1)	68 (82.9)	13 (15.9)	1 (1.2)	95 (89.6)	11 (10.4)	44 (83.0)	7 (13.2)	2 (3.8)	100	0.10
4	rs7554936	G	A	(ancestry informative marker)	94 (57.3)	70 (42.7)	31 (37.8)	32 (39.0)	19 (23.2)	56 (52.8)	50 (47.2)	13 (24.5)	30 (56.6)	10 (18.9)	100	0.49
5	rs3003596	A	G	*NR1I3*	85 (51.8)	79 (48.2)	21 (25.6)	43 (52.4)	18 (22.0)	67 (64.4)	37 (35.6)	25 (48.1)	17 (32.7)	10 (19.2)	98.1	0.3
6	rs2222202	G	A	*IL10*	107 (65.2)	57 (34.8)	39 (47.6)	29 (35.4)	14 (17.1)	75 (70.8)	31 (29.2)	28 (52.8)	19 (35.8)	6 (11.3)	100	0.03
7	rs1800871	G	A	*IL10*	103 (62.8)	61 (37.2)	33 (40.2)	37 (45.1)	12 (14.6)	64 (60.4)	42 (39.6)	20 (37.7)	24 (45.3)	9 (17.0)	100	0.71
8	rs1800890	T	A	*IL10*	120 (73.2)	44 (26.8)	44 (53.7)	32 (39.0)	6 (7.3)	73 (68.9)	33 (31.1)	27 (50.9)	19 (35.8)	7 (13.2)	100	0.4
9	rs798443	A	G	(ancestry informative marker)	84 (51.9)	78 (48.1)	19 (23.5)	46 (56.8)	16 (19.8)	62 (58.5)	44 (41.5)	17 (32.1)	28 (52.8)	8 (15.1)	98.8	0.23
10	rs4666200	A	G	(ancestry informative marker)	94 (57.3)	70 (42.7)	26 (31.7)	42 (51.2)	14 (17.1)	60 (56.6)	46 (43.4)	16 (30.2)	28 (52.8)	9 (17.0)	100	0.6
11	rs4670767	C	A	(ancestry informative marker)	136 (82.9)	28 (17.1)	56 (68.3)	24 (29.3)	2 (2.4)	96 (90.6)	10 (9.4)	43 (81.1)	10 (18.9)	0 (0.0)	100	1.00
12	rs13400937	C	A	(ancestry informative marker)	84 (52.5)	76 (47.5)	23 (28.8)	38 (47.5)	19 (23.8)	57 (53.8)	49 (46.2)	18 (34.0)	21 (39.6)	14 (26.4)	97.6	0.22
13	rs260690	A	C	(ancestry informative marker)	104 (63.4)	60 (36.6)	36 (43.9)	32 (39.0)	14 (17.1)	76 (71.7)	30 (28.3)	25 (47.2)	26 (49.1)	2 (3.8)	100	0.7
14	***rs16944***	***A***	***G***	***IL1B***	***79 (53.4)***	***69 (46.6)***	***22 (29.7)***	***35 (47.3)***	***17 (23.0)***	***48 (52.2)***	***44 (47.8)***	***14 (30.4)***	***20 (43.5)***	***12 (26.1)***	***77***	***0.37***
15	***rs10496971***	***A***	***C***	***(ancestry informative marker)***	***82 (89.1)***	***10 (10.9)***	***36 (78.3)***	***10 (21.7)***	***0 (0.0)***	***54 (90.0)***	***6 (10.0)***	***24 (80.0)***	***6 (20.0)***	***0 (0.0)***	***12.7***	***1.00***
16	rs9809104	A	G	(ancestry informative marker)	93 (58.1)	67 (41.9)	25 (31.3)	43 (53.8)	12 (15.0)	61 (57.5)	45 (42.5)	19 (35.8)	23 (43.4)	11 (20.8)	97.6	1.00
17	rs6548616	A	G	(ancestry informative marker)	85 (53.8)	73 (46.2)	23 (29.1)	39 (49.4)	17 (21.5)	60 (57.7)	44 (42.3)	13 (25.0)	34 (65.4)	5 (9.6)	94.4	0.21
18	rs12629908	G	A	(ancestry informative marker)	138 (84.1)	26 (15.9)	57 (69.5)	24 (29.3)	1 (1.2)	88 (86.3)	14 (13.7)	37 (72.5)	14 (27.5)	0 (0.0)	96.2	0.31
19	***rs9845457***	***NA***	***NA***	***(ancestry informative marker)***	***NA (NA)***	***NA (NA)***	***NA (NA)***	***NA (NA)***	***NA (NA)***	***NA (NA)***	***NA (NA)***	***NA (NA)***	***NA (NA)***	***NA (NA)***	***0***	***NA***
20	rs1513181	G	A	(ancestry informative marker)	121 (73.8)	43 (26.2)	45 (54.9)	31 (37.8)	6 (7.3)	82 (77.4)	24 (22.6)	33 (62.3)	16 (30.2)	4 (7.5)	100	0.49
21	rs10007810	G	A	(ancestry informative marker)	92 (56.1)	72 (43.9)	26 (31.7)	40 (48.8)	16 (19.5)	56 (52.8)	50 (47.2)	13 (24.5)	30 (56.6)	10 (18.9)	100	0.73
22	rs115770495	G	A	*ABCG2*	161 (98.2)	3 (1.8)	79 (96.3)	3 (3.7)	0 (0.0)	101 (95.3)	5 (4.7)	49 (92.5)	3 (5.7)	1 (1.9)	100	0.10
23	rs1448784	A	G	*ABCG2*	162 (98.8)	2 (1.2)	80 (97.6)	2 (2.4)	0 (0.0)	103 (97.2)	3 (2.8)	50 (94.3)	3 (5.7)	0 (0.0)	100	1.00
24	rs2231142	C	A	*ABCG2*	150 (91.5)	14 (8.5)	68 (82.9)	14 (17.1)	0 (0.0)	93 (87.7)	13 (12.3)	40 (75.5)	13 (24.5)	0 (0.0)	100	0.36
25	rs7657799	A	C	(ancestry informative marker)	115 (71.9)	45 (28.1)	43 (53.8)	29 (36.3)	8 (10.0)	78 (73.6)	28 (26.4)	28 (52.8)	22 (41.5)	3 (5.7)	97.6	0.67
26	rs2069762	A	C	*IL2*	131 (79.9)	33 (20.1)	49 (59.8)	33 (40.2)	0 (0.0)	74 (69.8)	32 (30.2)	25 (47.2)	24 (45.3)	4 (7.5)	100	0.10
27	rs10519613	C	A	*IL15*	145 (89.5)	17 (10.5)	67 (82.7)	11 (13.6)	3 (3.7)	85 (80.2)	21 (19.8)	34 (64.2)	17 (32.1)	2 (3.8)	98.8	0.14
28	***rs10833***	***G***	***A***	***IL15***	***123 (79.9)***	***31 (20.1)***	***50 (64.9)***	***23 (29.9)***	***4 (5.2)***	***77 (77.0)***	***23 (23.0)***	***29 (58.0)***	***19 (38.0)***	***2 (4.0)***	***88.2***	***0.8***

#	SNP	Alleles		Gene (if applicable)	Immunologic non-responders					Immunologic responders					GCR	HWE p
		A1	A2		A1 (%)	A2 (%)	A1/A1 (%)	A1/A2 (%)	A2/A2 (%)	A1 (%)	A2 (%)	A1/A1 (%)	A1/A2 (%)	A2/A2 (%)		
29	rs316598	G	A	(ancestry informative marker)	82 (50.6)	80 (49.4)	17 (21.0)	48 (59.3)	16 (19.8)	56 (52.8)	50 (47.2)	15 (28.3)	26 (49.1)	12 (22.6)	98.8	0.3
30	***rs870347***	***NA***	***NA***	***(ancestry informative marker)***	***NA (NA)***	***NA (NA)***	***NA (NA)***	***NA (NA)***	***NA (NA)***	***NA (NA)***	***NA (NA)***	***NA (NA)***	***NA (NA)***	***NA (NA)***	***0***	***NA***
31	rs1494555	A	G	*IL7R*	121 (73.8)	43 (26.2)	42 (51.2)	37 (45.1)	3 (3.7)	73 (68.9)	33 (31.1)	26 (49.1)	21 (39.6)	6 (11.3)	100	0.53
32	rs11567762	G	A	*IL7R*	142 (86.6)	22 (13.4)	60 (73.2)	22 (26.8)	0 (0.0)	86 (81.1)	20 (18.9)	34 (64.2)	18 (34.0)	1 (1.9)	100	0.2
33	rs6897932	G	A	*IL7R*	134 (82.7)	28 (17.3)	54 (66.7)	26 (32.1)	1 (1.2)	86 (82.7)	18 (17.3)	34 (65.4)	18 (34.6)	0 (0.0)	96.9	0.12
34	rs3822731	A	G	*IL7R*	139 (84.8)	25 (15.2)	58 (70.7)	23 (28.0)	1 (1.2)	87 (82.1)	19 (17.9)	35 (66.0)	17 (32.1)	1 (1.9)	100	0.53
35	rs987106	A	T	*IL7R*	82 (50.6)	80 (49.4)	20 (24.7)	42 (51.9)	19 (23.5)	55 (51.9)	51 (48.1)	16 (30.2)	23 (43.4)	14 (26.4)	98.8	0.73
36	rs3194051	A	G	*IL7R*	113 (69.8)	49 (30.2)	40 (49.4)	33 (40.7)	8 (9.9)	77 (74.0)	27 (26.0)	30 (57.7)	17 (32.7)	5 (9.6)	96.9	0.4
37	rs6451722	G	A	(ancestry informative marker)	87 (53.0)	77 (47.0)	24 (29.3)	39 (47.6)	19 (23.2)	61 (57.5)	45 (42.5)	17 (32.1)	27 (50.9)	9 (17.0)	100	0.86
38	rs2243250	G	A	*IL4*	93 (57.4)	69 (42.6)	20 (24.7)	53 (65.4)	8 (9.9)	66 (67.3)	32 (32.7)	23 (46.9)	20 (40.8)	6 (12.2)	91.3	0.04
39	rs6422347	A	G	(ancestry informative marker)	100 (61.0)	64 (39.0)	27 (32.9)	46 (56.1)	9 (11.0)	65 (61.3)	41 (38.7)	20 (37.7)	25 (47.2)	8 (15.1)	100	0.28
40	rs1040045	A	G	(ancestry informative marker)	87 (53.0)	77 (47.0)	25 (30.5)	37 (45.1)	20 (24.4)	64 (62.7)	38 (37.3)	18 (35.3)	28 (54.9)	5 (9.8)	96.2	1.00
41	rs2504853	G	A	(ancestry informative marker)	91 (55.5)	73 (44.5)	24 (29.3)	43 (52.4)	15 (18.3)	63 (59.4)	43 (40.6)	18 (34.0)	27 (50.9)	8 (15.1)	100	0.6
42	rs4463276	A	G	(ancestry informative marker)	85 (51.8)	79 (48.2)	24 (29.3)	37 (45.1)	21 (25.6)	60 (56.6)	46 (43.4)	18 (34.0)	24 (45.3)	11 (20.8)	100	0.3
43	rs731257	G	A	(ancestry informative marker)	140 (85.4)	24 (14.6)	61 (74.4)	18 (22.0)	3 (3.7)	90 (86.5)	14 (13.5)	38 (73.1)	14 (26.9)	0 (0.0)	98.1	0.73
44	rs3842	A	G	*ABCB1*	131 (79.9)	33 (20.1)	53 (64.6)	25 (30.5)	4 (4.9)	90 (84.9)	16 (15.1)	38 (71.7)	14 (26.4)	1 (1.9)	100	0.77
45	rs2235048	A	G	*ABCB1*	99 (60.4)	65 (39.6)	30 (36.6)	39 (47.6)	13 (15.9)	71 (68.3)	33 (31.7)	24 (46.2)	23 (44.2)	5 (9.6)	98.1	1.00
46	rs1128503	G	A	*ABCB1*	112 (68.3)	52 (31.7)	39 (47.6)	34 (41.5)	9 (11.0)	75 (70.8)	31 (29.2)	27 (50.9)	21 (39.6)	5 (9.4)	100	0.69
47	rs2214102	G	A	*ABCB1*	159 (97.0)	5 (3.0)	77 (93.9)	5 (6.1)	0 (0.0)	104 (98.1)	2 (1.9)	51 (96.2)	2 (3.8)	0 (0.0)	100	1.00
48	rs4646437	G	A	*CYP3A4*	112 (68.3)	52 (31.7)	40 (48.8)	32 (39.0)	10 (12.2)	68 (64.2)	38 (35.8)	25 (47.2)	18 (34.0)	10 (18.9)	100	0.05
49	***rs7803075***	***NA***	***NA***	***(ancestry informative marker)***	***NA (NA)***	***NA (NA)***	***NA (NA)***	***NA (NA)***	***NA (NA)***	***NA (NA)***	***NA (NA)***	***NA (NA)***	***NA (NA)***	***NA (NA)***	***0***	***NA***
50	rs10236187	A	C	(ancestry informative marker)	114 (70.4)	48 (29.6)	39 (48.1)	36 (44.4)	6 (7.4)	75 (73.5)	27 (26.5)	28 (54.9)	19 (37.3)	4 (7.8)	95	1.00
51	rs10108270	C	A	(ancestry informative marker)	90 (54.9)	74 (45.1)	29 (35.4)	32 (39.0)	21 (25.6)	54 (51.9)	50 (48.1)	14 (26.9)	26 (50.0)	12 (23.1)	98.1	0.16
52	rs3943253	A	G	(ancestry informative marker)	121 (73.8)	43 (26.2)	46 (56.1)	29 (35.4)	7 (8.5)	76 (74.5)	26 (25.5)	28 (54.9)	20 (39.2)	3 (5.9)	96.2	0.65
53	***rs1471939***	***A***	***G***	***(ancestry informative marker)***	***125 (76.2)***	***39 (23.8)***	***0 (0.0)***	***31 (88.6)***	***4 (11.4)***	***74 (71.2)***	***30 (28.8)***	***25 (48.1)***	***24 (46.2)***	***3 (5.8)***	***40.8***	***0.5***
54	rs4746136	G	A	(ancestry informative marker)	121 (74.7)	41 (25.3)	45 (55.6)	31 (38.3)	5 (6.2)	87 (82.1)	19 (17.9)	35 (66.0)	17 (32.1)	1 (1.9)	98.8	1.00
55	rs2234767	G	A	*FAS*	140 (85.4)	24 (14.6)	60 (73.2)	20 (24.4)	2 (2.4)	97 (91.5)	9 (8.5)	44 (83.0)	9 (17.0)	0 (0.0)	100	1.00
56	rs1800682	G	A	*FAS*	97 (59.1)	67 (40.9)	31 (37.8)	35 (42.7)	16 (19.5)	63 (59.4)	43 (40.6)	18 (34.0)	27 (50.9)	8 (15.1)	100	0.59
57	rs4918842	A	G	(ancestry informative marker)	134 (81.7)	30 (18.3)	53 (64.6)	28 (34.1)	1 (1.2)	87 (82.1)	19 (17.9)	36 (67.9)	15 (28.3)	2 (3.8)	100	0.57

#	SNP	Alleles		Gene (if applicable)	Immunologic non-responders					Immunologic responders					GCR	HWE p
		A1	A2		A1 (%)	A2 (%)	A1/A1 (%)	A1/A2 (%)	A2/A2 (%)	A1 (%)	A2 (%)	A1/A1 (%)	A1/A2 (%)	A2/A2 (%)		
58	rs2946788	A	C	(ancestry informative marker)	106 (64.6)	58 (35.4)	33 (40.2)	40 (48.8)	9 (11.0)	53 (51.0)	51 (49.0)	13 (25.0)	27 (51.9)	12 (23.1)	98.1	0.86
59	***rs11568629***	***A***	***G***	***SLC22A6***	***93 (98.9)***	***1 (1.1)***	***46 (97.9)***	***1 (2.1)***	***0 (0.0)***	***64 (97.0)***	***2 (3.0)***	***31 (93.9)***	***2 (6.1)***	***0 (0.0)***	***19.6***	***1.00***
60	rs11568628	C	A	*SLC22A6*	162 (98.8)	2 (1.2)	80 (97.6)	2 (2.4)	0 (0.0)	103 (97.2)	3 (2.8)	50 (94.3)	3 (5.7)	0 (0.0)	100	1.00
61	rs4149170	G	A	*SLC22A6*	128 (80.0)	32 (20.0)	52 (65.0)	24 (30.0)	4 (5.0)	82 (82.0)	18 (18.0)	35 (70.0)	12 (24.0)	3 (6.0)	91.9	0.25
62	rs3135932	A	G	*IL10RA*	151 (92.1)	13 (7.9)	69 (84.1)	13 (15.9)	0 (0.0)	100 (94.3)	6 (5.7)	47 (88.7)	6 (11.3)	0 (0.0)	100	1.00
63	rs9610	A	G	*IL10RA*	89 (54.9)	73 (45.1)	22 (27.2)	45 (55.6)	14 (17.3)	58 (54.7)	48 (45.3)	18 (34.0)	22 (41.5)	13 (24.5)	98.8	1.00
64	rs2416791	G	A	(ancestry informative marker)	97 (59.9)	65 (40.1)	28 (34.6)	41 (50.6)	12 (14.8)	63 (59.4)	43 (40.6)	15 (28.3)	33 (62.3)	5 (9.4)	98.8	0.11
65	rs772262	G	A	(ancestry informative marker)	92 (57.5)	68 (42.5)	26 (32.5)	40 (50.0)	14 (17.5)	63 (59.4)	43 (40.6)	17 (32.1)	29 (54.7)	7 (13.2)	97.6	0.48
66	rs2069709	C	A	*IFNG*	163 (99.4)	1 (0.6)	81 (98.8)	1 (1.2)	0 (0.0)	105 (99.1)	1 (0.9)	52 (98.1)	1 (1.9)	0 (0.0)	100	1.00
67	rs9319336	A	G	(ancestry informative marker)	133 (82.1)	29 (17.9)	54 (66.7)	25 (30.9)	2 (2.5)	90 (84.9)	16 (15.1)	40 (75.5)	10 (18.9)	3 (5.7)	98.8	0.53
68	rs7997709	A	G	(ancestry informative marker)	121 (73.8)	43 (26.2)	45 (54.9)	31 (37.8)	6 (7.3)	92 (86.8)	14 (13.2)	40 (75.5)	12 (22.6)	1 (1.9)	100	0.61
69	rs9530435	G	A	(ancestry informative marker)	105 (64.0)	59 (36.0)	36 (43.9)	33 (40.2)	13 (15.9)	64 (62.7)	38 (37.3)	17 (33.3)	30 (58.8)	4 (7.8)	96.2	0.85
70	rs9522149	A	G	(ancestry informative marker)	86 (54.4)	72 (45.6)	30 (38.0)	26 (32.9)	23 (29.1)	57 (55.9)	45 (44.1)	16 (31.4)	25 (49.0)	10 (19.6)	92.5	0.01
71	rs1760921	A	G	(ancestry informative marker)	110 (68.8)	50 (31.3)	36 (45.0)	38 (47.5)	6 (7.5)	79 (76.0)	25 (24.0)	29 (55.8)	21 (40.4)	2 (3.8)	95.7	0.39
72	rs3784230	G	A	(ancestry informative marker)	95 (58.6)	67 (41.4)	28 (34.6)	39 (48.1)	14 (17.3)	65 (61.3)	41 (38.7)	20 (37.7)	25 (47.2)	8 (15.1)	98.8	1.00
73	rs762551	A	C	*CYP1A2*	105 (66.5)	53 (33.5)	35 (44.3)	35 (44.3)	9 (11.4)	80 (75.5)	26 (24.5)	32 (60.4)	16 (30.2)	5 (9.4)	96.3	0.41
74	rs129081	C	G	*ABCC1*	86 (52.4)	78 (47.6)	24 (29.3)	38 (46.3)	20 (24.4)	65 (62.5)	39 (37.5)	16 (30.8)	33 (63.5)	3 (5.8)	98.1	0.48
75	rs113264879	G	A	*ABCC1*	162 (98.8)	2 (1.2)	80 (97.6)	2 (2.4)	0 (0.0)	103 (97.2)	3 (2.8)	50 (94.3)	3 (5.7)	0 (0.0)	100	1.00
76	rs4148380	G	A	*ABCC1*	158 (96.3)	6 (3.7)	76 (92.7)	6 (7.3)	0 (0.0)	98 (92.5)	8 (7.5)	45 (84.9)	8 (15.1)	0 (0.0)	100	1.00
77	rs8056298	C	A	*ABCC1*	155 (94.5)	9 (5.5)	73 (89.0)	9 (11.0)	0 (0.0)	99 (93.4)	7 (6.6)	46 (86.8)	7 (13.2)	0 (0.0)	100	1.00
78	rs212091	A	G	*ABCC1*	140 (86.4)	22 (13.6)	60 (74.1)	20 (24.7)	1 (1.2)	90 (86.5)	14 (13.5)	38 (73.1)	14 (26.9)	0 (0.0)	96.9	0.47
79	***rs16967632***	***G***	***A***	***ABCC1***	***107 (99.1)***	***1 (0.9)***	***53 (98.1)***	***1 (1.9)***	***0 (0.0)***	***59 (98.3)***	***1 (1.7)***	***29 (96.7)***	***1 (3.3)***	***0 (0.0)***	***22.5***	***1.00***
80	rs2107538	G	A	*CCL5*	124 (75.6)	40 (24.4)	47 (57.3)	30 (36.6)	5 (6.1)	81 (76.4)	25 (23.6)	32 (60.4)	17 (32.1)	4 (7.5)	100	0.64
81	***rs11652805***	***G***	***A***	***(ancestry informative marker)***	***44 (50.0)***	***44 (50.0)***	***11 (25.0)***	***22 (50.0)***	***11 (25.0)***	***35 (60.3)***	***23 (39.7)***	***9 (31.0)***	***17 (58.6)***	***3 (10.3)***	***8.4***	***0.64***
82	rs2125345	G	A	(ancestry informative marker)	87 (53.7)	75 (46.3)	23 (28.4)	41 (50.6)	17 (21.0)	67 (63.2)	39 (36.8)	21 (39.6)	25 (47.2)	7 (13.2)	98.8	0.86
83	rs4891825	A	G	(ancestry informative marker)	110 (67.1)	54 (32.9)	37 (45.1)	36 (43.9)	9 (11.0)	68 (64.2)	38 (35.8)	23 (43.4)	22 (41.5)	8 (15.1)	100	0.7
84	rs8192726	C	A	*CYP2A6*	157 (95.7)	7 (4.3)	75 (91.5)	7 (8.5)	0 (0.0)	99 (93.4)	7 (6.6)	46 (86.8)	7 (13.2)	0 (0.0)	100	1.00
85	rs8192709	G	A	*CYP2B6*	153 (96.8)	5 (3.2)	74 (93.7)	5 (6.3)	0 (0.0)	99 (95.2)	5 (4.8)	47 (90.4)	5 (9.6)	0 (0.0)	94.4	1.00
86	rs28399499	A	G	*CYP2B6*	155 (94.5)	9 (5.5)	73 (89.0)	9 (11.0)	0 (0.0)	104 (98.1)	2 (1.9)	51 (96.2)	2 (3.8)	0 (0.0)	100	1.00

#	SNP	Alleles		Gene (if applicable)	Immunologic non-responders					Immunologic responders					GCR	HWE p
		A1	A2		A1 (%)	A2 (%)	A1/A1 (%)	A1/A2 (%)	A2/A2 (%)	A1 (%)	A2 (%)	A1/A1 (%)	A1/A2 (%)	A2/A2 (%)		
87	rs34097093	G	A	*CYP2B6*	164 (100.0)	0 (0.0)	82 (100.0)	0 (0.0)	0 (0.0)	106 (100.0)	0 (0.0)	53 (100.0)	0 (0.0)	0 (0.0)	100	NA
88	rs28399502	C	A	*CYP2B6*	160 (97.6)	4 (2.4)	78 (95.1)	4 (4.9)	0 (0.0)	102 (100.0)	0 (0.0)	51 (100.0)	0 (0.0)	0 (0.0)	96.2	1.00
89	rs707265	G	A	*CYP2B6*	106 (65.4)	56 (34.6)	34 (42.0)	38 (46.9)	9 (11.1)	79 (74.5)	27 (25.5)	30 (56.6)	19 (35.8)	4 (7.5)	98.8	1.00
90	rs1042389	A	G	*CYP2B6*	129 (79.6)	33 (20.4)	53 (65.4)	23 (28.4)	5 (6.2)	86 (82.7)	18 (17.3)	35 (67.3)	16 (30.8)	1 (1.9)	96.9	0.57
91	rs6104567	A	C	(ancestry informative marker)	111 (68.5)	51 (31.5)	39 (48.1)	33 (40.7)	9 (11.1)	77 (72.6)	29 (27.4)	26 (49.1)	25 (47.2)	2 (3.8)	98.8	0.84
92	rs3907047	A	G	(ancestry informative marker)	143 (87.2)	21 (12.8)	62 (75.6)	19 (23.2)	1 (1.2)	96 (90.6)	10 (9.4)	44 (83.0)	8 (15.1)	1 (1.9)	100	0.68
93	rs4821004	A	G	(ancestry informative marker)	100 (61.0)	64 (39.0)	28 (34.1)	44 (53.7)	10 (12.2)	55 (53.9)	47 (46.1)	12 (23.5)	31 (60.8)	8 (15.7)	96.2	0.08
94	rs5768007	G	A	(ancestry informative marker)	132 (81.5)	30 (18.5)	53 (65.4)	26 (32.1)	2 (2.5)	94 (90.4)	10 (9.6)	43 (82.7)	8 (15.4)	1 (1.9)	96.9	1.00

GCR – global call rate; HWE p – p-value from the exact test to assess compliance to Hardy-Weinberg equilibrium, NA – not available.

Supplementary Table 3. Complete results of the genetic association tests with the immunologic outcome

#	SNP	Gene	X^2	Degrees of freedom	p-value
5	rs3003596	*NR1I3*	10.10	2	***0.01***
6	rs2222202	*IL10*	0.90	2	0.64
7	rs1800871	*IL10*	0.17	2	0.92
8	rs1800890	*IL10*	1.29	2	0.52
22	rs115770495	*ABCG2*	18.89	2	0.39
23	rs1448784	*ABCG2*	0.25	1	0.62
24	rs2231142	*ABCG2*	0.70	1	0.40
26	rs2069762	*IL2*	73.13	2	***0.03***
27	rs10519613	*IL15*	67.10	2	***0.03***
31	rs1494555	*IL7R*	30.92	2	0.21
32	rs11567762	*IL7R*	24.76	2	0.29
33	rs6897932	*IL7R*	0.71	2	0.70
34	rs3822731	*IL7R*	0.38	2	0.83
35	rs987106	*IL7R*	10.66	2	0.59
36	rs3194051	*IL7R*	0.96	2	0.62
38	rs2243250	*IL4*	8.02	2	***0.02***
44	rs3842	*ABCB1*	12.01	2	0.55
45	rs2235048	*ABCB1*	17.21	2	0.42
46	rs1128503	*ABCB1*	0.18	2	0.92
47	rs2214102	*ABCB1*	0.04	1	0.84
48	rs4646437	*CYP3A4*	12.08	2	0.55
55	rs2234767	*FAS*	25.21	2	0.28
56	rs1800682	*FAS*	0.96	2	0.62
60	rs11568628	*SLC22A6*	0.25	1	0.62
61	rs4149170	*SLC22A6*	0.57	2	0.75
62	rs3135932	*IL10RA*	0.24	1	0.63
63	rs9610	*IL10RA*	25.95	2	0.27
66	rs2069709	*IFNG*	6.22E-29	1	1.00
73	rs762551	*CYP1A2*	3.37	2	0.19
74	rs129081	*ABCC1*	82.13	2	***0.02***
75	rs113264879	*ABCC1*	0.25	1	0.62
76	rs4148380	*ABCC1*	13.42	1	0.25
77	rs8056298	*ABCC1*	0.01	1	0.91
78	rs212091	*ABCC1*	0.71	1	0.70
80	rs2107538	*CCL5*	0.34	2	0.84
84	rs8192726	*CYP2A6*	0.34	1	0.56
85	rs8192709	*CYP2B6*	0.13	1	0.72
86	rs28399499	*CYP2B6*	13.73	1	0.24
88	rs28399502	*CYP2B6*	11.65	1	0.28
89	rs707265	*CYP2B6*	27.77	2	0.25
90	rs1042389	*CYP2B6*	13.46	2	0.51

9 DISCUSSÃO GERAL

A resistência primária ainda aparenta estar baixa a moderada na América Latina e Caribe. Um estudo feito no Brasil apontou uma prevalência geral de 8,1%, porém com quatro metrópoles possuindo prevalência de resistência primária acima da média quando comparada com outras, chegando até a 15%: Belém, Brasília, São Paulo e Rio de Janeiro (INOCENCIO et al., 2009), concordando com as nossas observações. Outro ponto observado foi que a prevalência aumentou de 6,6% em 2002 para 8,1% em 2009 (INOCENCIO et al., 2009), também concordando com nosso modelo de séries temporais, que apontou uma tendência para aumento na prevalência de resistência primária.

Outro aspecto abordado foi a farmacogenética dos efeitos adversos da TARV. Um alelo do gene *ITPA* foi associado com a presença de efeitos adversos sistêmicos pelo uso de AZT, o que gerou um novo componente na determinação multifatorial da ocorrência de efeitos adversos. Um estudo recente também investigou o papel da ITPA no contexto da TARV, no qual foi reportado que a expressão do ITPA está reduzida nos linfócitos de indivíduos vivendo com o HIV-1 em tratamento, o que talvez seja consequência da própria infecção viral (PELTENBURG et al., 2016). Mais estudos são necessários, como por exemplo em modelos celulares, para melhor elucidar o papel dessa variante genética durante o andamento da infecção pelo HIV-1 e da TARV e qual sua associação com os efeitos adversos.

Além disso, foi abordada a importância do uso de análises genéticas em ensaios clínicos de vacinas terapêuticas contra o HIV-1. Assim, foi evidenciado que a genética individual deve ser levada em conta durante o desenho experimental de ensaios clínicos de estratégias para a cura do HIV-1, e uma revisão sistemática apontou semelhanças e diferenças entre esses protocolos, o que pode ajudar no desenvolvimento de futuras abordagens. Novas evidências mostram que o caminho para a cura do HIV-1 é a eliminação dos reservatórios virais (IMPERIAL COLLEGE LONDON, 2014), e como isso envolve a própria atividade do sistema imune, variantes genéticas podem influenciar nesse processo.

Elucidar a causa de diferenças interindividuais na reconstituição imune durante a TARV também foi o foco de um trabalho. Foram encontradas algumas associações genéticas de variantes localizadas em genes envolvidos em vias de farmacodinâmica de antirretrovirais, porém as associações foram perdidas durante análises

multivariadas. Uma revisão examinou os principais estudos de associação genética acerca do fenômeno da falha imunológica e concluiu que as evidências ainda são controversas, devido em parte a avaliação genética parcial (poucos genes candidatos, por exemplo) e na ausência de um critério unificado do que é a falha imunológica (PERAIRE et al., 2014), o que sinaliza que serão necessários mais trabalhos de maneira a isolar o componente genético dessa condição, de maneira a contribuir com informações que possam ajudar na otimização do tratamento e evitar complicações não relacionadas à AIDS, tais como desordens cardiovasculares e envelhecimento precoce (LI et al., 2011).

10 CONCLUSÃO

1. Os resultados descritos demonstram que a genética é útil para a investigação de vários aspectos da história natural da infecção pelo HIV-1, desde os momentos pré-tratamento, até a resposta terapêutica.
2. Dessa forma, esta prevalência pode estar, em princípio, subestimada. Portanto, o monitoramento dos pacientes em tratamento e de populações vulneráveis deve ser melhorado, para reduzir o surgimento de vírus resistente.
3. Um novo marcador genético preditivo de efeitos adversos relacionados à AZT foi proposto no gene *ITPA*
4. Revisão de protocolos de protocolos experimentais aponto que a genética deve ser levada em consideração durante a elaboração de estratégias de cura do HIV-1 usando células dendríticas autólogas
5. Mais estudos são necessários para melhor entender o componente genético da ocorrência de falha imunológica; nenhuma associação genética foi encontrada em um modelo multivariado de análise de sobrevivência. Outros fatores de risco devem ser levados em consideração, como coinfecções e *status* imunológico no momento do diagnóstico pela infecção pelo HIV-1.

REFERÊNCIAS

AIUTI, F.; MEZZAROMA, I. Failure to reconstitute CD4+ T-cells despite suppression of HIV replication under HAART. **AIDS Reviews,** v. 8, n. 2, 2006. p. 88-97.

AN, P.; WINKLER, C. A. Host genes associated with HIV/AIDS: advances in gene discovery. **Trends Genet,** v. 26, n. 3, 2010. p. 119-31.

ANDRIEU, J. M.; LU, W. A dendritic cell-based vaccine for treating HIV infection: background and preliminary results. **J Intern Med,** v. 261, n. 2, 2007. p. 123-31.

AROLD, S. et al. The crystal structure of HIV-1 Nef protein bound to the Fyn kinase SH3 domain suggests a role for this complex in altered T cell receptor signaling. **Structure,** v. 5, n. 10, 1997. p. 1361-72.

BALDING, D. J. A tutorial on statistical methods for population association studies. **Nat Rev Genet,** v. 7, n. 10, 2006. p. 781-91.

BARTLETT, J. A. et al. Overview of the effectiveness of triple combination therapy in antiretroviral-naive HIV-1 infected adults. **AIDS,** v. 15, n. 11, 2001. p. 1369-77.

BATTISTE, J. L. et al. Alpha helix-RNA major groove recognition in an HIV-1 rev peptide-RRE RNA complex. **Science,** v. 273, n. 5281, 1996. p. 1547-51.

BERMAN, H. M. et al. The Protein Data Bank. **Nucleic Acids Research,** v. 28, n. 1, 2000. p. 235-242.

BORISH, L. C.; STEINKE, J. W. 2. Cytokines and chemokines. **J Allergy Clin Immunol,** v. 111, n. 2 Suppl, 2003. p. S460-75.

BORST, P.; ELFERINK, R. O. Mammalian ABC transporters in health and disease. **Annu Rev Biochem,** v. 71, 2002. p. 537-92.

BOX, G. E.; JENKINS, G. M.; REINSEL, G. C. **Time series analysis: forecasting and control**. 4th. Hoboken, New Jersey: John Wiley & Sons, 2013. ISBN 1118619064.

BRASIL. **Boletim Epidemiológico - Aids e DST**. Brasília, DF: Ministério da Saúde, Secretaria de Vigilância em Saúde, Departamento de DST, Aids e Hepatites Virais. Ano III, nº 1, 2014. 84.

_____. **Protocolo clínico e diretrizes terapêuticas para manejo da infecção pelo HIV em adultos**. Brasília: Ministério da Saúde: Ministério da Saúde, Secretaria de Vigilância em Saúde, Programa Nacional de DST, Aids e Hepatites Virais, 2015. 227. Disponível em: < http://www.aids.gov.br/sites/default/files/anexos/publicacao/2013/55308/protocolofinal_31_7_2015_pdf_31327.pdf >.

BUCKWALTER, M. R.; ALBERT, M. L. Orchestration of the immune response by dendritic cells. **Curr Biol,** v. 19, n. 9, 2009. p. R355-61.

CARR, A.; COOPER, D. A. Adverse effects of antiretroviral therapy. **Lancet,** v. 356, n. 9239, 2000. p. 1423-30.

CESPEDES, M. S.; ABERG, J. A. Neuropsychiatric complications of antiretroviral therapy. **Drug Saf,** v. 29, n. 10, 2006. p. 865-74.

CHEN, J. C. et al. Crystal structure of the HIV-1 integrase catalytic core and C-terminal domains: a model for viral DNA binding. **Proc Natl Acad Sci U S A,** v. 97, n. 15, 2000. p. 8233-8.

CHEN, Y. et al. Nuclear receptors in the multidrug resistance through the regulation of drug-metabolizing enzymes and drug transporters. **Biochem Pharmacol,** v. 83, n. 8, 2012. p. 1112-26.

CHENG, Y. C. et al. Human immunodeficiency virus reverse transcriptase. General properties and its interactions with nucleoside triphosphate analogs. **J Biol Chem,** v. 262, n. 5, 1987. p. 2187-9.

CLIFFORD, D. B. et al. Impact of efavirenz on neuropsychological performance and symptoms in HIV-infected individuals. **Ann Intern Med,** v. 143, n. 10, 2005. p. 714-21.

COELHO, A. V. et al. Meta-Analysis and Time Series Modeling Allow a Systematic Review of Primary HIV-1 Drug-Resistant Prevalence in Latin America and Caribbean. **Curr HIV Res,** v. 13, n. 2, 2015. p. 125-42.

COELHO, A. V. et al. ABCB1 and ABCC1 variants associated with virological failure of first-line protease inhibitors antiretroviral regimens in Northeast Brazil patients. **J Clin Pharmacol,** v. 53, n. 12, 2013. p. 1286-93.

COLOMBO, S. et al. Influence of ABCB1, ABCC1, ABCC2, and ABCG2 haplotypes on the cellular exposure of nelfinavir in vivo. **Pharmacogenetics and Genomics,** v. 15, n. 9, 2005. p. 599-608.

CRESSEY, T. R.; LALLEMANT, M. Pharmacogenetics of antiretroviral drugs for the treatment of HIV-infected patients: an update. **Infect Genet Evol,** v. 7, n. 2, 2007. p. 333-42.

CUMMINS, N. W.; BADLEY, A. D. Mechanisms of HIV-associated lymphocyte apoptosis: 2010. **Cell Death Dis,** v. 1, 2010. p. e99.

DAVID W. HOSMER; STANLEY LEMESHOW; SUSANNE MAY. **Applied Survival Analysis**. 2nd edition. Hoboken, New Jersey, USA: John Wiley & Sons, 2008. 392

DE GUZMAN, R. N. et al. Structure of the HIV-1 nucleocapsid protein bound to the SL3 psi-RNA recognition element. **Science,** v. 279, n. 5349, 1998. p. 384-8.

DOITSH, G. et al. Cell death by pyroptosis drives CD4 T-cell depletion in HIV-1 infection. **Nature,** v. 505, n. 7484, 2014. p. 509-14.

EDWARDS, I. R.; ARONSON, J. K. Adverse drug reactions: definitions, diagnosis, and management. **Lancet,** v. 356, n. 9237, 2000. p. 1255-9.

FANALES-BELASIO, E. et al. HIV virology and pathogenetic mechanisms of infection: a brief overview. **Ann Ist Super Sanita,** v. 46, n. 1, 2010. p. 5-14.

FRANKE, R. M.; GARDNER, E. R.; SPARREBOOM, A. Pharmacogenetics of drug transporters. **Curr Pharm Des,** v. 16, n. 2, 2010. p. 220-30.

FUJIMOTO, H. et al. P-Glycoprotein Mediates Efflux Transport of Darunavir in Human Intestinal Caco-2 and ABCB1 Gene-Transfected Renal LLC-PK1 Cell Lines. **Biological & Pharmaceutical Bulletin,** v. 32, n. 9, 2009. p. 1588-1593.

FURMAN, P. A. et al. Phosphorylation of 3'-azido-3'-deoxythymidine and selective interaction of the 5'-triphosphate with human immunodeficiency virus reverse transcriptase. **Proc Natl Acad Sci U S A,** v. 83, n. 21, 1986. p. 8333-7.

GARCIA-LERMA, J. G. et al. Evidence of a role for the Q151L mutation and the viral background in development of multiple dideoxynucleoside-resistant human immunodeficiency virus type 1. **J Virol,** v. 74, n. 20, 2000. p. 9339-46.

GERETTI, A. M. et al. Sensitive testing of plasma HIV-1 RNA and Sanger sequencing of cellular HIV-1 DNA for the detection of drug resistance prior to starting first-line antiretroviral therapy with etravirine or efavirenz. **J Antimicrob Chemother,** v. 69, n. 4, 2014. p. 1090-7.

GEYER, M. et al. Structure of the anchor-domain of myristoylated and non-myristoylated HIV-1 Nef protein. **J Mol Biol,** v. 289, n. 1, 1999. p. 123-38.

GIRAUD, C.; MANCEAU, S.; TRELUYER, J.-M. ABC transporters in human lymphocytes: expression, activity and role, modulating factors and consequences for antiretroviral therapies. **Expert Opinion on Drug Metabolism & Toxicology,** v. 6, n. 5, 2010. p. 571-589.

GRINSPOON, S.; CARR, A. Cardiovascular risk and body-fat abnormalities in HIV-infected adults. **N Engl J Med,** v. 352, n. 1, 2005. p. 48-62.

HAAS, D. W.; TARR, P. E. Perspectives on pharmacogenomics of antiretroviral medications and HIV-associated comorbidities. **Current opinion in HIV and AIDS,** v. 10, n. 2, 2015. p. 116-122.

HARIPARSAD, N. et al. Induction of CYP3A4 by efavirenz in primary human hepatocytes: comparison with rifampin and phenobarbital. **J Clin Pharmacol,** v. 44, n. 11, 2004. p. 1273-81.

HILL, C. P. et al. Crystal structures of the trimeric human immunodeficiency virus type 1 matrix protein: implications for membrane association and assembly. **Proc Natl Acad Sci U S A,** v. 93, n. 7, 1996. p. 3099-104.

HIV SEQUENCE COMPENDIUM 2014. Los Alamos, New Mexico: Theoretical Biology and Biophysics Group, 2013. 450.

HOCHMAN, J. H. et al. P-glycoprotein-Mediated Efflux of Indinavir Metabolites in Caco-2 Cells Expressing Cytochrome P450 3A4. **Journal of Pharmacology and Experimental Therapeutics,** v. 298, n. 1, 2001. p. 323-330.

HOGLUND, P. J. et al. The solute carrier families have a remarkably long evolutionary history with the majority of the human families present before divergence of Bilaterian species. **Mol Biol Evol,** v. 28, n. 4, 2011. p. 1531-41.

HOLLAND, P. M. et al. Detection of specific polymerase chain reaction product by utilizing the 5'----3' exonuclease activity of Thermus aquaticus DNA polymerase. **Proc Natl Acad Sci U S A,** v. 88, n. 16, 1991. p. 7276-80.

HOSMER, D. W.; LEMESHOW, S. **Applied logistic regression**. 2ª ed. New York: Wiley, 2000. 373 p.

HSIOU, Y. et al. The Lys103Asn mutation of HIV-1 RT: a novel mechanism of drug resistance. **J Mol Biol,** v. 309, n. 2, 2001. p. 437-45.

HUTCHINSON, J. F. The Biology and Evolution of HIV. **Annual Review of Anthropology,** v. 30, n. ArticleType: research-article / Full publication date: 2001 / Copyright © 2001 Annual Reviews, 2001. p. 85-108.

ILLUMINA INC. GoldenGate Genotyping Assay - A flexible, pre-optimized assay. Disponível em < https://www.illumina.com/documents/products/technotes/technote_veracode_goldengate_genotyping.pdf >. Acesso em: 2 abr 2017., 2010. p.

IMPERIAL COLLEGE LONDON. Research In Viral Eradication of HIV Reservoirs (RIVER). In: ClinicalTrials.gov [Internet]. 2014-[november 2016]. Available from: http://clinicaltrials.gov/show/NCT00004451 NLM Identifier: NCT00004451., 2014. p.

INGELMAN-SUNDBERG, M. et al. Influence of cytochrome P450 polymorphisms on drug therapies: pharmacogenetic, pharmacoepigenetic and clinical aspects. **Pharmacol Ther,** v. 116, n. 3, 2007. p. 496-526.

INOCENCIO, L. A. et al. Brazilian Network for HIV Drug Resistance Surveillance: a survey of individuals recently diagnosed with HIV. **J Int AIDS Soc,** v. 12, 2009. p. 20.

IWASAKI, A.; MEDZHITOV, R. Regulation of adaptive immunity by the innate immune system. **Science,** v. 327, n. 5963, 2010. p. 291-5.

JACOBSON, M. A. et al. Zidovudine-induced fever. **J Acquir Immune Defic Syndr,** v. 2, n. 4, 1989. p. 382-8.

JANNEH, O. et al. P-glycoprotein, multidrug resistance-associated proteins and human organic anion transporting polypeptide influence the intracellular accumulation of atazanavir. **Antiviral therapy,** v. 14, n. 7, 2009. p. 965-74.

JULIEN, J. P. et al. Crystal structure of a soluble cleaved HIV-1 envelope trimer. **Science,** v. 342, n. 6165, 2013. p. 1477-83.

KALOW, W. Pharmacogenetics and personalised medicine. **Fundam Clin Pharmacol,** v. 16, n. 5, 2002. p. 337-42.

KENT, W. J. et al. The human genome browser at UCSC. **Genome Res,** v. 12, n. 6, 2002. p. 996-1006.

KILMARX, P. H. Global epidemiology of HIV. **Current opinion in HIV and AIDS,** v. 4, n. 4, 2009. p. 240-6.

KIM, E. E. et al. Crystal structure of HIV-1 protease in complex with VX-478, a potent and orally bioavailable inhibitor of the enzyme. **Journal of the American Chemical Society,** v. 117, n. 3, 1995. p. 1181-1182.

KIS, O. et al. The complexities of antiretroviral drug-drug interactions: role of ABC and SLC transporters. **Trends in Pharmacological Sciences,** v. 31, n. 1, 2010. p. 22-35.

KLEINBAUM, D. G.; KLEIN, M. **Survival Analysis: A Self-Learning Text**. 3rd edition. New York, USA: Springer, 2012. 700

KÖNIG, S. K. et al. Impact of drug transporters on cellular resistance towards saquinavir and darunavir. **Journal of Antimicrobial Chemotherapy,** v. 65, n. 11, 2010. p. 2319-2328.

LEDERGERBER, B. et al. Clinical progression and virological failure on highly active antiretroviral therapy in HIV-1 patients: a prospective cohort study. Swiss HIV Cohort Study. **Lancet,** v. 353, n. 9156, 1999. p. 863-8.

LEVY, Y. Cytokine-based modulation of immune function in HIV infection. **Current opinion in HIV and AIDS,** v. 1, n. 1, 2006. p. 69-73.

LI, T. et al. Reduced thymic output is a major mechanism of immune reconstitution failure in HIV-infected patients after long-term antiretroviral therapy. **Clinical Infectious Diseases,** v. 53, n. 9, 2011. p. 944-951.

LIBERATI, A. et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. **PLoS Med,** v. 6, n. 7, 2009. p. e1000100.

LIU, T. F.; SHAFER, R. W. Web resources for HIV type 1 genotypic-resistance test interpretation. **Clin Infect Dis,** v. 42, n. 11, 2006. p. 1608-18.

LU, W. et al. Therapeutic dendritic-cell vaccine for chronic HIV-1 infection. **Nat Med,** v. 10, n. 12, 2004. p. 1359-65.

MANEL, N. et al. A cryptic sensor for HIV-1 activates antiviral innate immunity in dendritic cells. **Nature,** v. 467, n. 7312, 2010. p. 214-7.

MILLER, S. A.; DYKES, D. D.; POLESKY, H. F. A simple salting out procedure for extracting DNA from human nucleated cells. **Nucleic Acids Res,** v. 16, n. 3, 1988. p. 1215.

MOGENSEN, T. H. et al. Innate immune recognition and activation during HIV infection. **Retrovirology,** v. 7, 2010. p. 54.

MOSS, D. M. et al. Raltegravir Is a Substrate for SLC22A6: a Putative Mechanism for the Interaction between Raltegravir and Tenofovir. **Antimicrobial Agents and Chemotherapy,** v. 55, n. 2, 2011. p. 879-887.

MOURA, R. et al. Exome analysis of HIV patients submitted to dendritic cells therapeutic vaccine reveals an association of CNOT1 gene with response to the treatment. **J Int AIDS Soc,** v. 17, 2014. p. 18938.

NASI, M. et al. Genetic polymorphisms of Fas (CD95) and Fas ligand (CD178) influence the rise in CD4+ T cell count after antiretroviral therapy in drug-naive HIV-positive patients. **Immunogenetics,** v. 57, n. 9, 2005. p. 628-35.

OGBURN, E. T. et al. Efavirenz Primary and Secondary Metabolism In Vitro and In Vivo: Identification of Novel Metabolic Pathways and Cytochrome P450 2A6 as the Principal Catalyst of Efavirenz 7-Hydroxylation. **Drug Metabolism and Disposition,** v. 38, n. 7, 2010. p. 1218-1229.

PARK, S. H. et al. Three-dimensional structure of the channel-forming trans-membrane domain of virus protein "u" (Vpu) from HIV-1. **J Mol Biol,** v. 333, n. 2, 2003. p. 409-24.

PELOPONESE, J. M., JR. et al. 1H-13C nuclear magnetic resonance assignment and structural characterization of HIV-1 Tat protein. **C R Acad Sci III,** v. 323, n. 10, 2000. p. 883-94.

PELTENBURG, N. C. et al. Inosine Triphosphate Pyrophosphohydrolase Expression: Decreased in Leukocytes of HIV-Infected Patients Using Combination Antiretroviral Therapy. **JAIDS Journal of Acquired Immune Deficiency Syndromes,** v. 73, n. 4, 2016. p. 390-395.

PERAIRE, J. et al. Evaluation of the pharmacogenetics of immune recovery in treated HIV-infected patients. **Expert Opinion on Drug Metabolism and Toxicology,** v. 10, n. 1, 2014. p. 81-101.

PIEDADE, R. et al. PXR variants and artemisinin use in Vietnamese subjects: frequency distribution and impact on the interindividual variability of CYP3A induction by artemisinin. **Antimicrob Agents Chemother,** v. 56, n. 4, 2012. p. 2153-7.

PITRAK, D. L. et al. Short communication: Apoptosis pathways in HIV-1-infected patients before and after highly active antiretroviral therapy: relevance to immune recovery. **AIDS Res Hum Retroviruses,** v. 31, n. 2, 2015. p. 208-16.

PORNILLOS, O. et al. X-ray structures of the hexameric building block of the HIV capsid. **Cell,** v. 137, n. 7, 2009. p. 1282-92.

R CORE TEAM. R: A Language and Environment for Statistical Computing. **R Foundation for Statistical Computing,** v. Viena, Austria. URL http://www.R-project.org/, 2014. p.

RHEE, S. Y. et al. Human immunodeficiency virus reverse transcriptase and protease sequence database. **Nucleic Acids Res,** v. 31, n. 1, 2003. p. 298-303.

RHEE, S. Y. et al. Distribution of human immunodeficiency virus type 1 protease and reverse transcriptase mutation patterns in 4,183 persons undergoing genotypic resistance testing. **Antimicrob Agents Chemother,** v. 48, n. 8, 2004. p. 3122-6.

RHEE, S. Y. et al. HIV-1 protease mutations and protease inhibitor cross-resistance. **Antimicrob Agents Chemother,** v. 54, n. 10, 2010. p. 4253-61.

RIBAUDO, H. J. et al. Effect of CYP2B6, ABCB1, and CYP3A5 Polymorphisms on Efavirenz Pharmacokinetics and Treatment Response: An AIDS Clinical Trials Group Study. **Journal of Infectious Diseases,** v. 202, n. 5, 2010. p. 717-722.

SARAFIANOS, S. G. et al. Crystal structure of HIV-1 reverse transcriptase in complex with a polypurine tract RNA:DNA. **EMBO J,** v. 20, n. 6, 2001. p. 1449-61.

SCHULZ, K. F.; GRIMES, D. A. Case-control studies: research in reverse. **Lancet,** v. 359, n. 9304, 2002. p. 431-434.

SHAH, R. R. Pharmacogenetics in drug regulation: promise, potential and pitfalls. **Philos Trans R Soc Lond B Biol Sci,** v. 360, n. 1460, 2005. p. 1617-38.

SHAIK, N. et al. P-glycoprotein-Mediated Active Efflux of the Anti-HIV1 Nucleoside Abacavir Limits Cellular Accumulation and Brain Distribution. **Drug Metabolism and Disposition,** v. 35, n. 11, 2007. p. 2076-2085.

SLEASMAN, J. W.; GOODENOW, M. M. 13. HIV-1 infection. **J Allergy Clin Immunol,** v. 111, n. 2 Suppl, 2003. p. S582-92.

SMITH, A. J.; HUMPHRIES, S. E. Cytokine and cytokine receptor gene polymorphisms and their functionality. **Cytokine Growth Factor Rev,** v. 20, n. 1, 2009. p. 43-59.

STANLEY, B. J. et al. Structural insight into the human immunodeficiency virus Vif SOCS box and its role in human E3 ubiquitin ligase assembly. **J Virol,** v. 82, n. 17, 2008. p. 8656-63.

STEINMAN, R. M. Dendritic cells: versatile controllers of the immune system. **Nat Med,** v. 13, n. 10, 2007. p. 1155-9.

STRASSER, A.; JOST, P. J.; NAGATA, S. The Many Roles of FAS Receptor Signaling in the Immune System. **Immunity,** v. 30, n. 2, 2009. p. 180-192.

TAKEUCHI, O.; AKIRA, S. Innate immunity to virus infection. **Immunol Rev,** v. 227, n. 1, 2009. p. 75-86.

TANG, M. W.; SHAFER, R. W. HIV-1 Antiretroviral Resistance: Scientific Principles and Clinical Applications. **Drugs,** v. 72, n. 9, 2012. p. 1-25.

TELENTI, A.; ZANGER, U. M. Pharmacogenetics of anti-HIV drugs. **Annu Rev Pharmacol Toxicol,** v. 48, 2008. p. 227-56.

THAISRIVONGS, S. et al. Inhibitors of the protease from human immunodeficiency virus: design and modeling of a compound containing a dihydroxyethylene isostere insert with high binding affinity and effective antiviral activity. **J Med Chem,** v. 34, n. 8, 1991. p. 2344-56.

TOZZI, V. Pharmacogenetics of antiretrovirals. **Antiviral Res,** v. 85, n. 1, 2009. p. 190-200.

UNAIDS. **90–90–90 - An ambitious treatment target to help end the AIDS epidemic**. 2014.

URBAN, T. J. et al. Functional genomics of membrane transporters in human populations. **Genome Res,** v. 16, n. 2, 2006. p. 223-30.

VAN VAERENBERGH, K. et al. Prevalence and characteristics of multinucleoside-resistant human immunodeficiency virus type 1 among European patients receiving combinations of nucleoside analogues. **Antimicrob Agents Chemother,** v. 44, n. 8, 2000. p. 2109-17.

VELLA, S. et al. Long-term follow-up of zidovudine therapy in asymptomatic HIV infection: results of a multicenter cohort study. The Italian Zidovudine Evaluation Group. **J Acquir Immune Defic Syndr,** v. 7, n. 1, 1994. p. 31-8.

VILLARROYA, F.; DOMINGO, P.; GIRALT, M. Drug-induced lipotoxicity: lipodystrophy associated with HIV-1 infection and antiretroviral treatment. **Biochim Biophys Acta,** v. 1801, n. 3, 2010. p. 392-9.

VON AHSEN, N.; OELLERICH, M.; ARMSTRONG, V. W. Characterization of the inosine triphosphatase (ITPA) gene: haplotype structure, haplotype-phenotype correlation and promoter function. **Ther Drug Monit,** v. 30, n. 1, 2008. p. 16-22.

WANG, X. et al. Breast Cancer Resistance Protein (BCRP/ABCG2) Induces Cellular Resistance to HIV-1 Nucleoside Reverse Transcriptase Inhibitors. **Molecular Pharmacology,** v. 63, n. 1, 2003. p. 65-72.

WARD, B. A. et al. The Cytochrome P450 2B6 (CYP2B6) Is the Main Catalyst of Efavirenz Primary and Secondary Metabolism: Implication for HIV/AIDS Therapy and Utility of Efavirenz as a Substrate Marker of CYP2B6 Catalytic Activity. **Journal of Pharmacology and Experimental Therapeutics,** v. 306, n. 1, 2003. p. 287-300.

WATTS, J. M. et al. Architecture and secondary structure of an entire HIV-1 RNA genome. **Nature,** v. 460, n. 7256, 2009. p. 711-716.

WECKER, K. et al. NMR structure of the HIV-1 regulatory protein Vpr in H2O/trifluoroethanol. Comparison with the Vpr N-terminal (1-51) and C-terminal (52-96) domains. **Eur J Biochem,** v. 269, n. 15, 2002. p. 3779-88.

WILLBOLD, D.; HOFFMANN, S.; ROSCH, P. Secondary structure and tertiary fold of the human immunodeficiency virus protein U (Vpu) cytoplasmic domain in solution. **Eur J Biochem,** v. 245, n. 3, 1997. p. 581-8.

WU, L.; KEWALRAMANI, V. N. Dendritic-cell interactions with HIV: infection and viral dissemination. **Nat Rev Immunol,** v. 6, n. 11, 2006. p. 859-68.

WYEN, C. et al. Cytochrome P450 2B6 (CYP2B6) and constitutive androstane receptor (CAR) polymorphisms are associated with early discontinuation of efavirenz-containing regimens. **J Antimicrob Chemother,** v. 66, n. 9, 2011. p. 2092-8.

YE, X.; KUMAR, R. A.; PATEL, D. J. Molecular recognition in the bovine immunodeficiency virus Tat peptide-TAR RNA complex. **Chem Biol,** v. 2, n. 12, 1995. p. 827-40.

ZAHRAN, H. E. A. Self-reported frequent mental distress among adults--United States, 1993-2001. **MMWR. Morbidity and mortality weekly report,** v. 53, n. 41, 2004. p. 963.

ZANGER, U. M. et al. Functional pharmacogenetics/genomics of human cytochromes P450 involved in drug biotransformation. **Anal Bioanal Chem,** v. 392, n. 6, 2008. p. 1093-108.

APÊNDICE A – Termo de consentimento livre e esclarecido

Título da pesquisa: Fatores Genéticos Humanos Envolvidos no Curso da Infecção pelo HIV: Transmissão Vertical, Imunidade e Resposta à Terapia Antirretroviral

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Convidamos você a participar de uma pesquisa que estamos realizando sobre diferenças genéticas envolvidas no comportamento do HIV no organismo. Algumas pessoas combatem melhor o vírus que outras durante a infecção; outras respondem melhor à terapia com os antirretrovirais. Além disso, algumas crianças adquirem o vírus durante o parto ou amamentação (transmissão vertical), enquanto outras não. Estamos fazendo essa pesquisa para tentar descobrir se diferenças genéticas expliquem essa diferença entre as pessoas convivendo com o HIV e pretendemos recrutar 400 voluntários ao todo. Essa pesquisa é importante porque poderá contribuir para que no futuro os médicos melhorem os tratamentos contra o HIV. Além disso, você poderá solicitar aos pesquisadores que comuniquem os resultados a você e a seu médico para que ele avalie se você obteria benefícios com as descobertas.

Com sua autorização, gostaríamos de realizarmos entre uma e cinco coletas de pequenas quantidades do seu sangue (no máximo 8 mL por coleta), colhidas durante os exames de rotina de acompanhamento da infecção pelo HIV. Caso sejam necessárias mais de uma coleta, elas serão feitas a cada três ou quatro meses, de acordo com a rotina de suas consultas. Solicitamos também a sua autorização para utilizar dados do prontuário, como idade que iniciou o acompanhamento médico e os resultados dos seus últimos exames de rotina para avaliar o controle do HIV. Como a Genética está em constante evolução, é possível que novas pesquisas além desta sejam realizadas com suas informações e material biológico armazenados. Caso isso ocorra, os pesquisadores entrarão em contato com o comitê de ética e com você para solicitar nova autorização.

Informamos que o material contribuído por você será armazenado no Laboratório de Imunopatologia Keizo Asami (LIKA), que fica na Universidade Federal de Pernambuco (UFPE). Seu material não será enviado a outros pesquisadores brasileiros ou estrangeiros, permanecendo apenas no LIKA. O endereço do LIKA e as formas de contato com os pesquisadores estão no começo desse documento.

Informamos que os riscos que você corre ao participar da pesquisa são apenas sintomas provocados pela coleta do sangue como: vermelhidão e dor no braço no local da coleta e enjoo. Além disso, todas as suas informações pessoais estarão seguras. Nenhuma pessoa fora da pesquisa terá acesso a elas.

A participação na pesquisa é totalmente voluntária. Não haverá nenhum gasto pela sua participação, não recebendo cobrança com o que será realizado. Você também não receberá nenhum pagamento ou benefício financeiro pela sua participação. Da mesma forma, não haverá nenhum prejuízo a você caso não queira participar ou desistir de participar desta pesquisa.

Caso você se sinta prejudicado(a) pelo andamento da pesquisa, asseguramos que você receberá todas as assistências cabíveis neste hospital, incluindo o direito de solicitar indenização aos pesquisadores por eventuais danos. Caso você possua alguma dúvida acerca dos objetivos do estudo, por favor, entre em contato com os responsáveis pela pesquisa. Além disso, se você tiver

alguma consideração ou dúvida sobre esta pesquisa, também pode entrar em contato com o comitê de Ética em Pesquisa em Seres Humanos do IMIP (CEP-IMIP), que objetiva defender os interesses dos participantes, respeitando seus direitos e contribuir para o desenvolvimento da pesquisa desde que atenda às condutas éticas.

Eu,_____**(nome completo)** compreendi as informações repassadas e autorizo que seja realizada a avaliação genética da amostra de sangue coletada, e concordo que os dados obtidos sejam utilizados para pesquisa. Declaro que fui informado(a) pela equipe do pesquisador Sergio Crovella sobre os objetivos da pesquisa e estou consciente de que:

1. Concordei em participar da pesquisa sem nenhum tipo de pressão;
2. Posso a qualquer momento entrar em contato por telefone com o pesquisador se tiver qualquer dúvida sobre os procedimentos, riscos e benefícios da pesquisa;
3. Posso a qualquer momento desistir de participar da pesquisa, sem que isso prejudique meu atendimento no hospital;
4. O pesquisador poderá ter acesso ao meu prontuário e que minhas informações pessoais serão mantidas em sigilo;
5. Recebi uma cópia deste documento.

Assinatura do Voluntário	
Assinatura da Testemunha	**Assinatura da Testemunha**
Assinatura do Pesquisador Responsável	

APÊNDICE B – Questionário clínico e epidemiológico

1. Dados pessoais (apenas para identificação, NÃO serão divulgados)

Nome do Paciente			
Data de Nascimento	**Naturalidade**	**Cidade de residência**	**Sexo**
			M ( ) F ( )

2. Informações clínicas e epidemiológicas

Etnia (segundo classificação do IBGE)		**Escolaridade (anos)**	**Renda mensal (reais)**
( ) Branca	( ) Indígena		
( ) Negra	( ) Amarela		
( ) Parda	( ) Ignorado		
Peso (kg)		**Altura (m)**	
Fumo		**Etilismo**	
SIM ( )		SIM ( )	
Se SIM, quantos cigarros por dia? _____		Se SIM, quantas un. por semana? _____	
NÃO ( )		NÃO ( )	
Se **NÃO**, parou de fumar? SIM ( ) NÃO ( )		Se **NÃO**, parou de beber? SIM ( ) NÃO ( )	

Comorbidades pré-existentes			
Desordem Psiquiátricas	SIM ( )	NÃO ( )	IGNORADO ( )
Doença autoimune	SIM ( )	NÃO ( )	IGNORADO ( )
Doença cardiovascular	SIM ( )	NÃO ( )	IGNORADO ( )
Diabetes	SIM ( )	NÃO ( )	IGNORADO ( )
Doença renal	SIM ( )	NÃO ( )	IGNORADO ( )
Doença de fígado	SIM ( )	NÃO ( )	IGNORADO ( )
Osteoporose	SIM ( )	NÃO ( )	IGNORADO ( )
Se **SIM**, houve fratura?	SIM ( )	NÃO ( )	IGNORADO ( )
	Local da fratura:		

3. Infecção pelo HIV

Modo de transmissão (marcar todos que se apliquem)	
( ) Transmissão vertical	( ) Relação sexual heterossexual
( ) Relação sexual homossexual	( ) Transfusão sanguínea
( ) Durante tratamento para hemofilia	( ) Acidente com material biológico
( ) Uso de drogas injetáveis	( ) IGNORADO
Idade de Início da vida sexual	**Data de diagnóstico de infecção pelo HIV**

Possui parceiro(a) fixo(a)?		
SIM ( )	NÃO (SOLTEIRO(A)) ( )	MÚLTIPLOS PARCEIROS(AS) ( )
O paciente é usuário de drogas?	SIM ( ) Qual? _____	NÃO ( )

3. Infecção pelo HIV (continuação)

Se a paciente for mulher, ela está atualmente grávida?				
SIM ( ) Mês de gestação:_____			NÃO ( )	
O(a) paciente tem filhos?				
Nº	**Idade**	**Sexo**	**Tipo de parto**	**Status de HIV**
1		M ( ) F ( )	normal ( ) cesariana ( )	**POS** ( ) NEG ( )
2		M ( ) F ( )	normal ( ) cesariana ( )	**POS** ( ) NEG ( )
3		M ( ) F ( )	normal ( ) cesariana ( )	**POS** ( ) NEG ( )
4		M ( ) F ( )	normal ( ) cesariana ( )	**POS** ( ) NEG ( )
5		M ( ) F ( )	normal ( ) cesariana ( )	**POS** ( ) NEG ( )

4. Coinfecções, infecções oportunistas e doenças definidoras de AIDS (ver no prontuário)

				Datas
Sorologia para Hepatite B?	POS ( )	NEG ( )	IGN ( )	
Sorologia para Hepatite C?	POS ( )	NEG ( )	IGN ( )	
Se SIM, tratamento para Hepatite C?	SIM ( )	NÃO ( )	IGN ( )	
Qual esquema?				

				Datas
Tuberculose?	POS ( )	NEG ( )	IGN ( )	
Se SIM, estado da infecção:	ATIVA ( )	LATENTE ( )	IGN ( )	
Está em tratamento?	SIM ( )	NÃO ( )	IGN ( )	
Qual esquema?				

Doenças definidora de AIDS?	
( ) Candidíase oral	( ) Candidíase do esôfago
( ) Febre ou diarreia por 1 mês ou mais	( ) Demência pela AIDS
( ) Herpes simples por 1 mês ou mais	( ) Herpes zoster
( ) Infecção pelo CMV	( ) Perda de peso acentuada
( ) Pneumonia	( ) Toxoplasmose

ANEXO A – Parecer de aprovação do comitê de ética

Instituto de Medicina Integral
Prof. Fernando Figueira
Escola de Pós-graduação em Saúde Materno Infantil
Instituição Civil Filantrópica

IMIP

DECLARAÇÃO

Declaro que o projeto de pesquisa **nº 3629 - 13** intitulado **"Fatores Genéticos humanos envolvidos no curso da Infecção pelo HIV: Transmissão vertical, imunidade e resposta à terapia antirretroviral."** apresentado pelo (a) pesquisador (a) **Antonio Victor Campos Coelhos** foi APROVADO pelo Comitê de Ética em Pesquisa em Seres Humanos do Instituto de Medicina Integral Prof. Fernando Figueira – IMIP, em reunião ordinária de 13 de novembro de 2013

Recife, 18 de novembro de 2013

Dr. José Eulálio Cabral Filho
Coordenador do Comitê de Ética
em Pesquisa em Seres Humanos do
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UTILIDADE PÚBLICA MUNICIPAL - Lei 9851 de 08/11/87
UTILIDADE PÚBLICA ESTADUAL - Lei 9813 de 14/05/84
UTILIDADE PÚBLICA FEDERAL - Dec. 86234 de 30/07/81
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