Evidence of hepatitis B virus resistance to both Tenofovir alafenamide (TAF) and Tenofovir Disoproxil Fumarate (TDF)

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Background

Nucleot(s)ide analogues (NA) are the most widely used antiviral treatments for chronic hepatitis B virus (HBV) infection, targeting viral reverse transcriptase (RT) to inhibit viral replication (1). However, NAs are not curative and therefore require long-term administration in most cases, with a risk of selecting resistance associated mutations (RAMs) (2). Tenofovir (TFV) is a nucleotide analogue formulated as either tenofovir disoproxil fumarate (TDF) or tenofovir alafenamide (TAF). There is a high genetic barrier to the selection of tenofovir (TFV) resistance, but the distribution and clinical significance of TFV resistance remain poorly understood. We here present assimilated evidence for the evidence for TFV RAMs with the aim of cataloguing and characterising mutations that are likely to be of most clinical significance.

Methodology

We carried out a systematic literature search in PubMed and Scopus using PRISMA criteria (fig1) to identify relevant papers describing clinical or laboratory evidence of HBV resistance to tenofovir. We identified 15 studies: seven studies were case reports, four were *in vitro* studies and four were longitudinal studies of CHB (with or without HIV coinfection). We compared HBV RT to HIV RT, in order to map the likely sites of TFV RAMs.

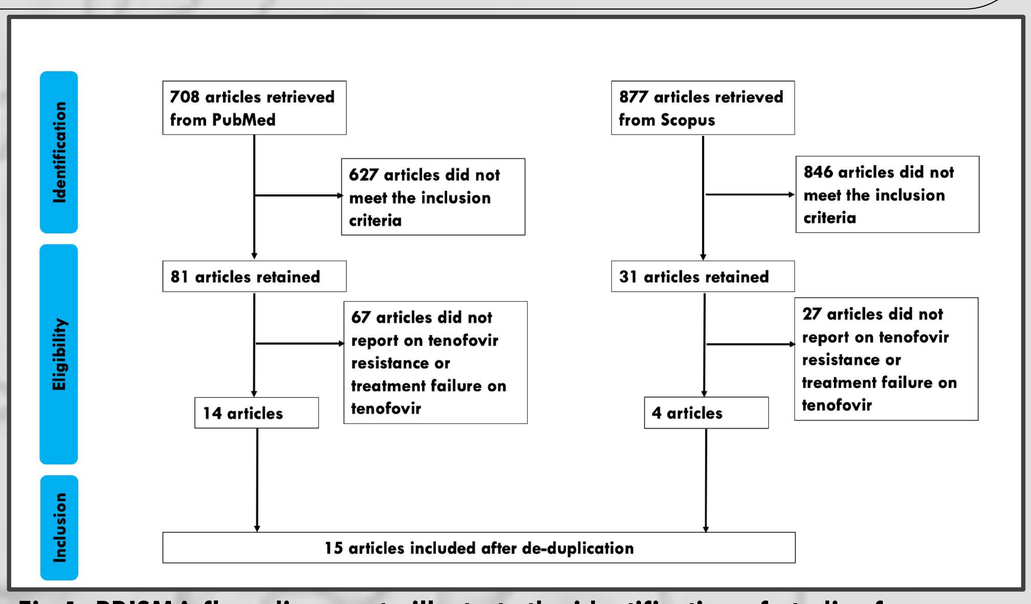
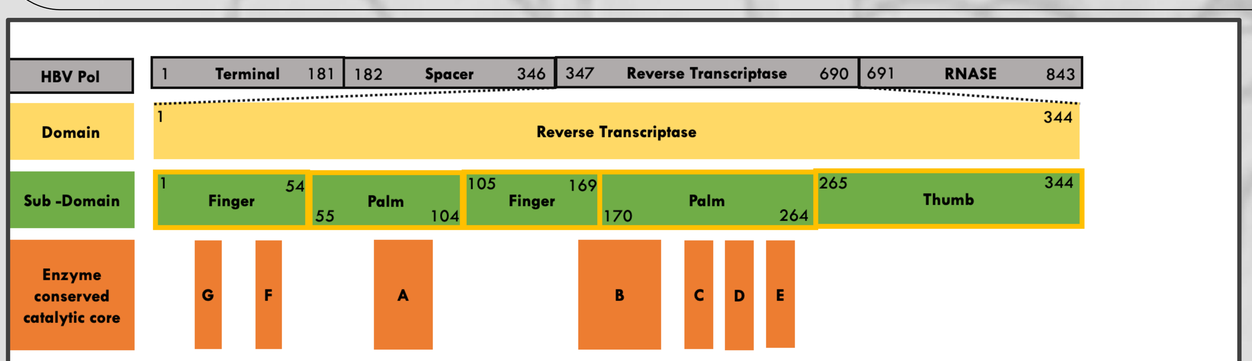


Fig 1: PRISMA flow diagram to illustrate the identification of studies for inclusion.



Enzyme conserved catalytic core	RAMs associated with TDF/TAF located within active sites
A (rt75 – rt91)	S78T; L80M;L91I
B (rt163 – rt189)	I63V; V173L; P177G; **L180M**; **A181T/V**; T184A/L
C (rt200 – rt210)	A200V; **M204I/V**; V207L
D (rt230 – rt241)	**N236T**
E (rt247 – rt257)	F249A; C256S
F(rt37 – rt47)	Nil known
G (rt26 – rt36) &	Nil known

Enzyme domains	RAMs associated with TDF/TAF located within active sites
Finger (rt1 – 54)	Y9H
Palm (rt55 – 104)	**S106C/G**
Finger (rt105 – 169)	T118C/G; F122L; H126Y; Q130S; D134E; **R153W/Q**
Palm (rt170 – 264)	V191I; R192P; **A194T**; L217R; F221Y; S223A; L229V/W; D263E
Thumb (rt265-344)	Q267L; **I269L**; V278I/V; A317S; K333Q; N337H

Fig 2: Mutations associated with tenofovir (TFV) resistance located within and outside the active sites of the HBV RT enzyme.

Results

Based on review of 15 studies, we identified 37 putative TFV RAMs in HBV RT, occurring both within and outside sites of enzyme activity (fig 2). Nine of these were reported by ≥2 studies (fig 3), but only two had homologous RAMs in HIV (fig 4). The most consistently described RAMs were S78T, L180M, A181T/V, M204I/V and N236T, mostly arising as combinations of multiple mutations, (S78T resulted to TFV resistance in isolation). M204I/V mutation is a well recognised YMDD mutation associated with 3TC resistance; prior NA exposure can set the scene for development of cross-resistance to TFV. Other factors as such incomplete adherence, higher baseline HBV DNA level, positive baseline HBeAg status, HIV coinfection and NA dosage have also been associated with persistent viraemia on TFV therapy.

Mutations	Park et al 2019	Cho et al 2018	Lee et al 2014	Mikulska et al 2012	Liu et al 2014	Amini-Bavil-Olyaee et al 2009	Van Bommel et al 2012	Lada et al 2012	Sheldon et al 2005	Liu et al 2017	Total number of studies
S106C/G											2
R153W/Q											2
V173L											2
L180M											4
A181T/V											4
A194T											2
M204I/V											5
N236T											3
I269L											2

KEY:	Method used to identify TFV resistance
(i)	Sequencing HBV sequence from individuals in whom viraemia was not suppressed by TDF
(ii)	In vitro assays to measure the effect of TDF on viral replication in cell lines
(iii)	Approaches (i) and (ii) in combination

Fig 3: Mutations associated with tenofovir resistance in HBV, identified from a systematic literature review. Park E-S, et al. PMID: 30794889; Cho WH, et al. PMID: 29740207; Lee HW, et al.. PMID: 24836314.; Mikulska M, et al. PMID: 22825811; Liu Y, et al. PMID: 24118725; Amini-Bavil-Olyaee S, et al. PMID: 19263474 . ; van Bommel F, et al. PMID: 22892524. ; Lada O, et al. PMID: 22267470 ; Sheldon J, et al. PMID: 16218172; Liu Y, et al. PMID: 28017761

HBV SEQUENCE POSITION	201	202	203	204	205	206	207	208	209	210	211	212		213	214	215	216	217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232	233
HBV_Geno_A1	F	S	Y	M	D	D	V	V	L	G	A	K	-	S	V	Q	H	L	E	S	L	Y	T	A	V	T	N	F	L	L	S	L	G	I
HBV_Geno_B2	F	S	Y	M	D	D	V	V	L	G	A	K	-	S	V	Q	H	L	E	S	L	Y	A	A	V	T	N	F	L	L	S	L	G	I
HBV_Geno_C2	F	S	Y	M	D	D	V	V	L	G	A	K	-	S	V	Q	H	L	E	S	L	F	T	S	I	T	N	F	L	L	S	L	G	I
HBV_Geno_D1	F	S	Y	M	D	D	V	V	L	G	A	K	-	S	V	Q	H	L	E	S	L	F	T	A	V	T	N	F	L	L	S	L	G	I
HBV_Geno_E	F	S	Y	M	D	D	V	V	L	G	A	K	-	S	V	Q	H	L	E	S	L	Y	T	S	V	T	N	F	L	L	S	L	G	I
HBV TDF mutation hotspots				M204I/V			V207L											L217R				F221Y		S223A						L229V/W				
HIV SEQUENCE POSITION	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200	201	202	203	204	205	206	207	208	209	210	211	212	213	214
HIV_Subtype_B	Y	Q	Y	M	D	D	L	Y	V	G	S	D	L	E	I	G	Q	H	R	T	K	I	E	E	L	R	Q	H	L	L	R	W	G	L
HIV TDF mutation hotspots				M184I/V																										L210W				
Sites with same AA when comparing HIV and HBV RT after alignment			*	*	*	*				*																			*	*			*	

Fig 4: A section of the reference sequence alignment of HBV RT and HIV RT. Boxes shaded in light blue represent sites with same amino acid when comparing HIV and HBV RT after alignment and have tenofovir mutations

Discussion

There is emerging evidence for polymorphisms that reduce susceptibility to TVF; this is a concern especially in setting such as Africa where there is a widespread use of TDF and 3TC for HIV treatment. Overall, literature on TFV resistance is still sparse and heterogeneous, and more work is required to determine the relationship between genotype and phenotype. If clinically significant TFV resistance increases in prevalence, there will be a pressing need for the development of drugs that act additively or synergistically with the NAs, that inhibit a target other than DNA polymerase active site, and/or that can eradicate HBV cccDNA from hepatocytes.

References

1. Beloukas A, Geretti AM. Hepatitis B Virus Drug Resistance. http://link.springer.com/10.1007/978-3-319-47266-9_26
2. Mokaya J, et al. A systematic review of hepatitis B virus (HBV) drug and vaccine escape mutations in Africa: A call for urgent action. http://dx.plos.org/10.1371/journal.pntd.0006629

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