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In Vitro Tenofovir Sensitivity of HBV Populations from Clinical Specimens Containing rtA181T/V and/or rtN236T

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Introduction

- Tenofovir disoproxil fumarate (TDF) is approved for the treatment of HIV-1 and HBV infections, however the resistance profile in HBV has not been determined[1]
  - Currently no evidence of clinically significant HBV mutation(s)
- The rtA181V and rtN236T adefovir-associated resistance mutations (ADV-R) exhibit some cross resistance to tenofovir (TFV) *in vitro*[2,3]
  - The clinical significance of these mutations on TDF efficacy is unknown
- There are limited data to determine how changes in TFV *in vitro* EC_{50} (50% effective concentration) values will impact TDF clinical efficacy

Objectives

- To determine the impact of ADV-R mutations on tenofovir susceptibility *in vitro*
- To evaluate the potential correlation between changes in tenofovir susceptibility *in vitro* and clinical response

Methods

- Twenty-six clinical isolates obtained from 24 ADV-treated patients enrolled in GS-US-174-0103 (n=5) and GS-US-174-0106 (n=19) were analyzed
  - All ADV-R patients (detected by population sequencing) with replication competent virus that were treated with TDF in GS-US-174-0106 were included
- Population di-deoxy sequencing of serum HBV pol/RT (LOD 400 copies/mL) was performed on all 26 isolates
  - Covers amino acids (AA) 1-344 of HBV RT
  - Detects mutations ≥25% of the population
- 20 isolates were further analyzed using the INNO-LiPA HBV DR V3 assay
  - Detects mutations at rtL80, rtV173, rtL180, rtA181, rtT184, rtA194, rtS202, rtM204, rtI233, rtN236, and rtL250 of HBV RT
  - Detects mutations ≥5% of the population
- Phenotypic analysis of patient-derived HBV DNA (pools and clones) was conducted in HepG2 cells transiently transfected with a pool of recombinant HBV plasmid DNA derived from patient serum HBV[4]
  - PCR product containing entire HBV RT (AA 1-344) is cloned into plasmid vector expressing genotype A laboratory strain lacking corresponding RT region for transfection into HepG2 cells followed by drug treatment for 7 days
  - EC_{50} values were determined by qPCR analysis of intracellular HBV DNA; fold change values ≤ 2 are not significant
  - Patients 1003, 2003, and 1022 were selected for clonal analysis due to a high percentage of mutant subpopulations
- HBV DNA levels determined by the Roche COBAS TaqMan 48 HBV assay (lower limit of quantitation is 169 copies/mL)

Results

Table 1. Isolate Characteristics and Sequence Results

Isolate	HBV DNA (log_{10} copies/mL)	Genotype	Prior ADV Exposure (Days)	Population Sequencing[a]	INNO LiPA Sequencing[a]
2013	3.41 (BL)	A	289	WT	WT
1401	9.77 (w48)	A	335	WT	Not Done
7852-1	10.28 (w48)	A	336	WT	Not Done
3006	7.12 (BL)	B	240	WT	WT
1041	5.80 (BL)	C	266	WT	WT
3025	6.43 (BL)	C	307	WT	WT
3027	6.48 (BL)	C	520	WT	WT
3030	7.36 (BL)	C	653	WT	WT
3015	6.57 (BL)	C	686	WT	WT
3960	10.11 (w48)	D	349	WT	Not Done
2952	6.12 (w48)	D	336	WT	Not Done
7952	9.74 (w48)	D	336	WT	Not Done
2012	5.63 (BL)	D	393	WT	WT
3018	7.57 (BL)	D	602	WT	WT
1002	8.83 (BL)	E	238	WT	WT
1024	9.21 (BL)	E	725	WT	WT
3003[b]	5.55 (BL)	A	541	WT	rtA181A/T
1039[b]	5.35 (BL)	D	140	WT	rtA181A/T
4011-1[b]	6.63 (BL)	D	574	WT	rtN236N/T
1017[b]	7.75 (BL)	D	838	WT	rtA181A/T
7852-2	3.18 (w96)	A	336	rtN236N/T	Not Done
1003[b]	6.65 (BL)	B	517	rtN236N/T	rtN236N/T
1022[b]	5.30 (BL)	C	580	rtA181A/T	rtA181A/T, rtN236N/T
2003[b]	6.12 (BL)	D	489	rtA181V/A, rtN236N/T	rtA181A/V, rtN236N/T
4011-2	2.82 (w48)	D	574	rtN236T	WT
4010[b]	6.14 (BL)	D	695	rtN236N/T	rtA181A/T/V, rtN236N/T

a. Results for ADV-R mutations only
b. ADV-R patients included in Figure 1 analysis

Results (cont'd)

Table 2A. TFV EC_{50} Values for Isolates without ADV-R Mutations

Isolate	Mean TFV EC_{50} (µM) ± SD[a]	Fold Change from pHY92
2013	16.6 ± 3.3	1.0
1401	12.8 ± 1.2	0.8
7852-1	11.4 ± 2.1	0.7
3006	13 ± 1.5	0.8
1041	14.4 ± 1.8	0.9
3025	10.2 ± 4.0	0.6
3027	10.8 ± 2.9	0.7
3030	16.3 ± 3.5	1.0
3015	10.1 ± 0.7	0.6
3960	11.2 ± 4.2	0.7
2952	12.3 ± 2.7	0.8
7952	7.8 ± 1.1	0.5
2012	19.5 ± 7.1	1.2
3018	14.4 ± 3.6	0.9
1002	12.2 ± 6.5	0.7
1024	7.0 ± 3.0	0.4
pHY92[b]	16.3 ± 3.8	1.0
ADV-R[b]	47.9 ± 12.4	2.9

a. Values represent average of 3-4 independent assays for isolates
b. pHY92 and ADV-R are genotype A laboratory strains used as controls

Table 2B. TFV EC_{50} Values for Isolates with ADV-R Mutations

Isolate	ADV-R Mutations[a]	Mean TFV EC_{50} (µM) ± SD[b]	Fold Change from pHY92
3003	rtA181A/T	12.6 ± 2.9	0.8
1039	rtA181A/T	8.8 ± 4.0	0.5
4011-1	rtN236N/T	9.3 ± 3.8	0.6
1017	rtA181A/T	20.6 ± 4.3	1.3
7852-2	rtN236N/T	15.3 ± 1.8	0.9
1003	rtN236N/T	14.4 ± 4.0	0.9
1022	rtA181A/T, rtN236N/T	21.4 ± 4.1	1.3
2003	rtA181V/A, rtN236N/T	44.6 ± 22.6	2.7
4011-2	rtN236T	12.2 ± 8.1	0.7
4010	rtA181A/T/V, rtN236N/T	24.6 ± 2.9	1.5
pHY92[c]	WT	16.3 ± 3.8	1.0
ADV-R[c]	rtA181V, rtN236T	47.9 ± 12.4	2.9

a. Mutations detected in either INNO-LiPA or population sequencing
b. Values represent average of 3-4 independent assays for isolates
c. pHY92 and ADV-R are genotype A laboratory strains used as controls

Figure 1. HBV DNA Change from Baseline for TDF-Treated Patients with and without ADV-R Through 24 Weeks

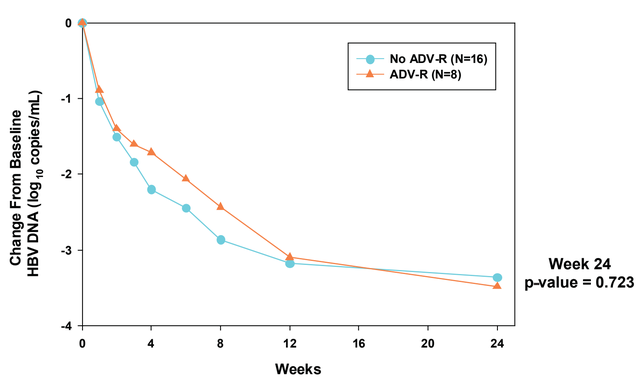
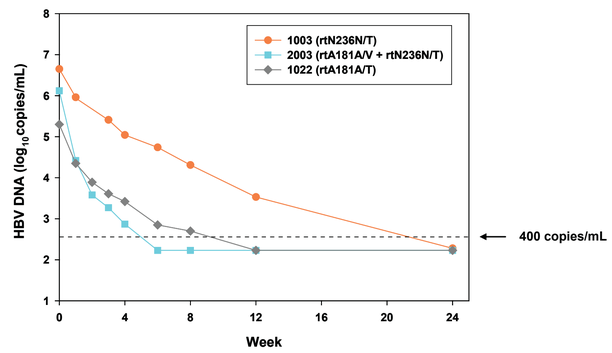


Table 3. TFV EC_{50} Values for Clones From Patients 2003, 1003, and 1022

Patient	ADV-R Mutations[a]	Mean TFV EC_{50} (µM) ± SD[b]	Fold Change from pHY92
2003_pool[c]	rtA181V/A, rtN236N/T	44.6 ± 22.6	2.7
2003_clone 2	rtA181V, rtN236T	49.3 ± 5.5	3.0
2003_clone 9	rtN236T	>200	>12.3
2003_clone 10	rtA181V	57.9 ± 22.2	3.6
2003_clone 13	WT	21.8 ± 7.7	1.3
1003_pool[d]	rtN236N/T	14.4 ± 4.0	0.9
1003_clone 2	WT	11.4 ± 1.9	0.7
1003_clone 10	rtN236T	>200	>12.3
1022_pool[e]	rtA181A/T	21.4 ± 4.1	1.3
1022_clone 8	WT	12.8 ± 0.8	0.8
1022_clone 9	rtA181T	12.0 ± 2.8	0.7

a. Mutations detected in either INNO-LiPA or population sequencing
b. Values represent average of 2-4 independent assays
c. Mutant percentages: rtA181V = 17%, rtN236T = 33%, rtA181V+rtN236T = 42%
d. Mutant percentage: rtN236T = 56%
e. Mutant percentage: rtA181T = 38%

Figure 2. HBV DNA Levels for TDF-Treated Patients 1003, 2003, and 1022 Through 24 Weeks



- 25/26 isolates had mean TFV EC_{50} values that fell within the 2-fold assay variability; 1 isolate (with rtA181V + rtN236T mutations in > 90% of the population) demonstrated 2.7-fold reduced susceptibility to TFV (Tables 2A-B)
- No significant differences observed for mean EC_{50} (p = 0.140) and fold change (p = 0.133) when comparing RT pools from isolates with and without rtA181T/V and/or rtN236T (Tables 2A-B)
- The reduction in HBV DNA over the first 24 weeks of treatment with TDF was similar between patients with and without ADV-R (Figure 1)
- Phenotypic analysis of clones with and without ADV-R from three patients showed reduced TFV susceptibility *in vitro* for rtA181V and rtN236T, but not rtA181T (Table 3)
  - All three patients had HBV DNA levels of <400 copies/mL by Week 24 (Figure 2)

Conclusions

- **The presence of HBV mutant subpopulations (≤ 50%) of rtA181T/V and/or rtN236T did not have an impact on TFV susceptibility in *in vitro* phenotyping assays**
  - **Wild-type virus in these samples likely contributes to this result**
- **The presence of subpopulations of ADV-R HBV, including one patient with >90% mutant virus, did not have an impact on clinical response to TDF through 24 weeks of treatment**

References & Acknowledgements

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