blood mononuclear cells (PBMCs) and lymphoid cell lines. d4T had additive and synergistic activity in combination with didanosine (ddI) and zalcitabine (ddC), respectively, in vitro. d4T combined with zidovudine (ZDV) had additive or antagonistic activity in vitro depending upon the molar ratios of the agents tested.

HIV-1 isolates with reduced susceptibility to d4T have been selected in vitro (HXB2 strain-specific) and were also obtained from patients treated with d4T. Genotypic analysis showed that d4T resistant HIV-1 strain selected in vitro contained a V75T (valine to threonine) substitution in HIV-1 reverse transcriptase (Lacey and Larder 1994; Lin et al.,1994). In another study, d4T resistant HXB2 variants selected in vitro contained an I50T (isoleucine to threonine) substitution in the reverse transcriptase (Gu et al., 1993; Lin et al.,1994). However, mutation V75T and/or I50T were very infrequent in HIV-1 isolates from patients receiving d4T therapy (Colonno, 2002). Although, recombinant viruses containing a V75T or I50T mutation exhibited reduced susceptibility to d4T, clinical HIV-1 isolates with I50T or V75T mutation exhibited very little reduced susceptibility to d4T in vitro. Results from several studies demonstrated that HIV-1 isolates from patients receiving prolonged treatment with d4T alone, or in combination with ddI contained ZDV-resistance-associated mutations and/or multi-nucleoside resistance mutations (Coakely et al., 2000; Holguin et al., 1998; Lin et al., 1994,1999; Pellegrin et al., 1999; Picard et al., 2001; Ross et al., 2001). Mutations M41L, D67N, K70R, L210W, T215Y/F and K219Q/E have been shown to confer reduced susceptibility to ZDV in vitro (Larder and Kemp, 1989; Stanford resistance data bank). These mutations have been identified in clinical HIV-1 isolates from patients receiving ZDV therapy and their role in conferring ZDV-resistance has been amply demonstrated. The multi-nucleoside resistance mutations A62V, V75I, F77L, F116Y and Q151M confer resistance to ZDV, d4T, ddI and ddC (Stanford resistance data bank).

The overall objective of AI 455099 study was to assess the antiviral efficacy and safety of the d4T ER formulations given QD relative to the d4T IR formulation given BID in combination with lamivudine (3TC) 150 mg BID and efavirenz (EFV) 600 mg QD with efficacy evaluated through 48 weeks of dosing. For efficacy results, please see reviews of the Medical Officer and Statistician. BMS has provided data on the genotypic and phenotypic analysis of HIV-1 isolates from patients receiving stavudine containing regimen from studies AI455099. The pertinent microbiology data from the above mentioned study are summarized below.

Title
Assessment of Genotypic and Phenotypic Profiles of Patient Isolates from Studies AI455099 and AI455096 following Stavudine Treatment.

Study AI455099 was a randomized 1:1 comparison of d4T ER (100 mg QD; 75 mg QD for those < 60 kg) and d4T IR (40 mg BID; 30 mg BID for those <60 kg) capsules in combination with 3TC and EFV in antiretroviral naïve HIV-1 infected subjects. Both 3TC and EFV were administered open-label. Resistance analysis data for a large number of patients were not available due to technical difficulties. The sponsor stated that results

from paired specimens (pre-and post-treatment) were available from only 20 subjects (all from AI45509 study; Table 1).

Table 1: Phenotypic and genotypic analysis of HIV-1 isolates from treatment failures or breakthroughs patients in study AI455099

Patient ID	Treatment weeks	Fold-change in susceptibility vs control			Mutation	
		D4T	3TC	EFV	K103N	M184V
11-224	0.1	0.9	0.9	0.5		
(ER)	59	0.7	>120	1.7	√	√
16-362	0.1	4.2	>124	95		√
(ER)	56	4.0	> 125	105		√
16-897	0.1	1.1	>134	0.5		√
(ER)	20	1.4	>125	0.6		√
23-510	0.1	0.8	1.1	1.1		
(ER)	24	0.7	1.0	0.5		
52-1008	-0.3	0.8	1.2	0.6		
(ER)	49	0.7	>119	180*		√
62-66	0.1	0.8	0.7	0.6		
(ER)	56	0.8	0.7	0.5		
62-89	0.1	1.0	0.7	1.0		
(ER)	56	0.9	0.8	0.8		
63-28	0.1	0.9	1.3	0.5		
(ER)	24	1.0	0.9	0.5		

63-592	0.1	0.9	0.7	0.4		
(ER)	56	0.8	0.7	7.7	√	
73-769	-0.1	0.6	>128	0.6		√
(ER)	24	0.6	>128 #	>280	√	√
73-784	0.1	1	1.0	1.0		
(ER)	24	1.0	0.7	0.7		
4-164	0.1	0.8	1.0	0.5		
(IR)	56	0.8	>129	0.4		√
18-132	0.1	1.0	0.9	1.3		
(IR)	59	1.0	1.0	1.2		
27-616	0.1	0.9	1.0	0.7		
(IR)	25	0.9	0.7	0.6		
48-554	0.1	1.2	1.4	34		
(IR)	56	1.7	>113•	>289		√
48-557	0.1	1.2	0.7	0.7		
(IR)	57	0.9	0.7	0.6		
62-85	0.1	1.0	0.8	0.8		
(IR)	24	0.7	0.7	0.8		
69-421[a]	0.1	0.7	0.8	1.1		
	24	0.6	>134	>261□	√	√

62-425 (IR)	0.1 24	0.9 0.9	1.0 0.8	0.5 21◊	 √	
84-789 (IR)	0.1 26	0.9 0.7	0.8 0.6	0.6 10	 √	

[a] = d4T formulation for the patient 69-421 was not reported.
*: D67D/N, K101E, G190S
#:T7T/A, D67D/G, L74L/V, K101K/P/Q/T, E224E/K, P225P/H
•: M41L, K122K/E, I135I/T, F214F/L
□: K49K/R, I142I/V, G190A
◊: E6E/A, K101K/N, G190G/A

Table 1 shows the phenotypes and genotypes of pre-and post-therapy HIV-1 isolates from patients receiving d4T ER, or d4T IR with virologic failure and/or viral RNA breakthroughs. Results presented in Table 1 are summarized below.

1. Both baseline and post-therapy isolates from 19 patients were sensitive to d4T (fold-change in IC_{50} compared to wild type HIV-1 strain ranged from 0.7 to 1.7). However, HIV-1 isolates from one patient showed a 4-fold reduced susceptibility to d4T at both time points. Post-therapy isolates from 2 patients (52-1008, 48-554) contained the ZDV resistance-associated mutations D67N and M41L.
2. Both baseline and post-therapy HIV-1 isolates from 9 patients were sensitive to all three drugs tested, i.e. d4T, 3TC and EFV.
3. Post-therapy isolates from 5 of 20 patients exhibited a >100-fold reduced susceptibility to 3TC in vitro. These post-therapy isolates contained the 3TC resistance-associated mutation M184V. Post-therapy isolates from 12 patients were sensitive to 3TC. Both baseline and post-therapy HIV-1 isolates from 3 other patients were resistant (reduced susceptibility) to 3TC (patients # 16-362, 16-897, 73-769).
4. Post-therapy isolates from 12 patients were sensitive to EFV. However, one of these isolates contained K103N mutation (patient 11-224). Post-therapy isolates from 6 patients showed a 7.7- to >261-fold reduced susceptibility to EFV in vitro. Post-therapy HIV-1 isolates from 5 of these patients contained treatment emergent K103N mutation. Post-therapy isolates from one of these 6 patients (52-1008) contained mutations K101E and G190S, but the mutation K103N was absent. Both baseline and post-therapy HIV-1 isolates from 2 patients (# 16-362, 48-554) were resistant to EFV and did not contain a K103N mutation.
5. Post-therapy isolates from 2 patients (patients 52-1008 and 69-421) were resistant to both 3TC and EFV.

Comments

1. HIV-1 isolates from patient 11-224 at week 59 contained K103N and M184V mutations in the RT gene, but reduced susceptibility was observed only for 3TC. No change in susceptibility to EFV was observed at week 48 despite the presence of the K103N mutation. This is a discrepant result.
2. HIV-1 isolates from patient 16-362 at baseline and week 56 were resistant to 3TC and EFV but the mutation K103 was not detected in the RT gene of HIV-1 isolates obtained at both time points. Both baseline and post-therapy isolates were also resistant to d4T.
3. HIV-1 isolates from patient 16-897 were resistant to 3TC at baseline and on weeks 20. The mutation M184V was detected in isolates from both baseline and week 20.
4. HIV-1 isolates from patient 52-1008 at week 49 were resistant to 3TC and EFV, but the K103N mutation was not detected. K101E and G190S mutations were detected and possibly conferred phenotypic resistance to EFV.
5. HIV-1 isolates from patient 48-554 were resistant to EFV at baseline and week 56 but no K103N mutations was detected in isolates from both time points. Mutation I135I/T causes low level resistance to NNRTIs. Post therapy isolates at week 56 were also resistant to 3TC and these isolates contained the mutation M184V.

CONCLUSIONS

With respect to microbiology, this NDA is supported. The sponsor has provided data on the genotypic and phenotypic analyses of HIV-1 isolates from 20 patients receiving long term d4T (ER or IR) therapy in combination with 3TC and EFV with virologic failure and/or viral RNA breakthroughs. The pre- and post-therapy HIV-1 isolates from 19 of 20 patients were susceptible to d4T. Mutations which confer reduced susceptibility to d4T in cell culture were not detected in any of the post-therapy HIV-1 isolates. However, ZDV-resistance-associated mutations (M41L, D67N) were detected in post-therapy isolates from 2 patients. Post-therapy isolates from 5 of 20 patients exhibited a >100-fold reduced susceptibility to 3TC in vitro. These post-therapy isolates contained the 3TC-resistance-associated mutation M184V. Similarly, post-therapy isolates from 6 patients showed a 7.7- to >261-fold reduced susceptibility to EFV. Post-therapy HIV-1 isolates from 5 of these patients contained the treatment emergent K103N mutation. Post-therapy isolates from one of these 6 patients (52-1008) contained K101E and G190S mutations, but the mutation K103N was absent. The phenotypes (drug susceptibility) of post-therapy HIV-1 isolates to 3TC and EFV correlated with genotypes (mutations). The sponsor has updated the label to reflect competition of intracellular phosphorylation between stavudine and ribavirin.

METHODOLOGY

Plasma was stored and frozen at baseline and at the time of virologic failure. In addition, a sample was obtained for storage at the week 24 and week 56 visits of AI455099.

The protocol definition for virologic failure was that a previously suppressed subject (plasma HIV-1 RNA < 400 copies/mL) must rebound to an HIV-1 RNA >1000 copies/mL, documented on two occasions at least 14 days apart. Patients with plasma HIV-1 RNA levels >800 copies/mL on treatment weeks 20-24 (regardless of the week 48 efficacy response) were considered as virologic breakthroughs. The sponsor stated that the above definition of virologic "failure or breakthrough" was used for collecting samples for resistance testing, and help guide management decisions and differs from those used in the various efficacy analyses.

Plasma HIV-1 RNA levels were quantified at baseline with the AMPLICOR HIV-1 MONITOR® standard assay (lower limit of quantitation, LLQ = 400 copies/mL) and on-treatment with the AMPLICOR HIV-1 MONITOR® ultrasensitive assay (LLQ= <50 copies/mL). Samples from subjects in North America were tested with version 1.0 of the assay, and samples from subjects outside North America were tested with version 1.5.

All genotypic and phenotypic resistance testing was performed by [ ]. Phenotyping was performed using the [ ] HIV assay and genotypes were obtained using the [ ] HIV assay.

Methodology for Phenotypic Analysis [ ] HIV):

All phenotypic data reported were generated by [ ]. Briefly, the [ ] HIV assay uses nucleic acid amplification to derive viral sequences from a patient's plasma. A resistance test vector is constructed by incorporating the patient-derived segment into a viral vector with an indicator gene, such as the firefly luciferase gene, replacing HIV-1 envelope (env) gene. The population of resistance test vectors from a given patient is tested in the laboratory against increasing concentrations of each of the approved antiretroviral drugs and the resulting data are plotted as an inhibition curve. The inhibition curve of the patient virus is compared to that of a drug-sensitive reference virus. The results from this assay are expressed as IC_{50}'s (concentration of drug which inhibits the virus by 50%). If the patient virus requires a significantly higher drug concentration to inhibit its replication, as compared to the drug sensitive reference virus, then the patient virus has reduced susceptibility to that drug (Petropolous et al., 2000). Based on the fold-increase in IC_{50} concentration relative to wild-type HIV-1, samples were described as susceptible (<2.5-fold increase), intermediate resistance (>2.5-fold and <10-fold increase) or high level resistance (>10-fold) to each study drug.
The phenotypic cutoffs (fold change) used for drug susceptible isolates in this study were: d4T 1.7-fold, 3TC 2.5-fold, and EFV 2.55-fold.

Methodology for Genotypic Analysis ([ ] HIV Assay):

[ ] HIV utilizes DNA sequence analysis to detect mutations in HIV-1 protease (PR) and reverse transcriptase (RT) associated with resistance to PR and RT inhibitors,

respectively. [ ] HIV is performed at the [ ], using blood samples (plasma or serum) containing at least 500 copies HIV-1 RNA per mL.

The [ ] HIV Assay Protocol is based on the dideoxynucleotide chain termination method of DNA sequencing. In dideoxy sequencing, DNA polymerase is used to synthesize the complementary strand of a single-stranded DNA template by the sequential addition of one of four deoxyribonucleotides (dATP, dCTP, dGTP, dTTP) to the 3'-hydroxyl end of a complementary oligonucleotide primer. During the reaction, strand elongation is periodically terminated by the random incorporation of one of four dideoxyribonucleotide analogues (ddATP, ddCTP, ddGTP, ddTTP) lacking a 3'-hydroxyl group. Each dideoxynucleotide is labeled with a distinct flurophore and the dideoxynucleotide concentrations are optimized to ensure that termination events occur fairly randomly at each nucleotide position in the DNA fragment. Thus, reaction products consist of a pool of DNA fragments comprised of every possible fragment length where the sites of termination are defined by the emission of the fluorescent-label of the terminal dideoxynucleotide. "Cycle sequencing" refers to a modification of the original reaction chemistry that employs a thermo-stable DNA polymerase. The procedure permits multiple rounds of strand elongation by ramping between temperatures that favor primer binding, strand elongation, and strand denaturation. Cycle sequencing results in a linear amplification of the sequencing reaction products resulting in increased reaction signal and improved sensitivity.

The template for DNA sequence analysis, Resistance Test Vector DNA (RTV-DNA) derived from the [ ] HIV drug susceptibility assay, is combined with specific oligonucleotide primers and [ ] (deoxy-nucleotides, dye-terminator dideoxynucleotides, and thermostable polymerase) and the resultant reaction mixtures are incubated in a thermal cycler. Sequencing reaction products are purified using sephadex resin chromatography, lyophilized, and resuspended in electrophoresis loading solution. The reaction products are separated by capillary electrophoresis using [ ] DNA Analyzer, which enables single nucleotide resolution of the DNA fragments. The instrument uses laser-induced fluorescence to create an image of the sample data from which the DNA sequence can be determined. Custom [ ] HIV software [ ] is used for analysis of the sequence files. The software program also performs the translation of assembled nucleotide sequence into the PR and RT amino acid sequence, and the comparison of patient-derived virus amino acid sequence to the sequence of a well-characterized drug- sensitive (wild-type) reference virus (NL4-3).

Variations in amino acid sequence (mutations) are identified by comparing the amino acid sequence of the patient virus to the amino acid sequence of the reference virus. Drugs that may exhibit reduced susceptibility based on the observed mutational pattern are identified using established conventions (e.g. Resistance Collaborative Group genotypic mutation Table, August 1999). [ ] HIV patient reports provide the

following information: 1) all observed mutations (amino acid substitutions) in PR and RT based on the comparison to a well characterized reference sequence (NL4-3), 2) mutations in PR and RT associated with drug resistance, and 3) the identification of drugs that may exhibit reduced susceptibility based on the observed mutational patterns.

MICROBIOLOGY LABEL

Mechanism of action

Stavudine, a nucleoside analogue of thymidine, is phosphorylated by cellular kinases to the active metabolite stavudine triphosphate. Stavudine triphosphate inhibits the activity of HIV-1 reverse transcriptase (RT) by competing with the natural substrate thymidine triphosphate (K_i= 0.0083 to 0.032 μM) and by causing DNA chain termination following its incorporation into viral DNA. Stavudine triphosphate inhibits cellular DNA polymerases β and γ and markedly reduces the synthesis of mitochondrial DNA.

Antiviral activity

The in vitro antiviral activity of stavudine was measured in peripheral blood mononuclear cells, monocytic cells, and lymphoblastoid cell lines. The concentration of drug necessary to inhibit HIV-1 replication by 50% (IC_{50}) ranged from 0.009 to 4 μM against laboratory and clinical isolates of HIV-1. Stavudine had additive and synergistic activity in combination with didanosine and zalcitabine, respectively, in vitro. Stavudine combined with zidovudine had additive or antagonistic activity in vitro depending upon the molar ratios of the agents tested. The relationship between in vitro susceptibility of HIV-1 to stavudine and the inhibition of HIV-1 replication in humans has not been established.

Drug Resistance

HIV-1 isolates with reduced susceptibility to stavudine have been selected in vitro (strain-specific) and were also obtained from patients treated with stavudine. Phenotypic analysis of HIV-1 isolates from 61 patients receiving prolonged (6-29 months) stavudine monotherapy showed that post therapy isolates from four patients exhibited IC_{50} values more than 4-fold (range 7- to 16-fold) higher than the average pretreatment susceptibility of baseline isolates. Of these, HIV-1 isolates from one patient contained the zidovudine resistance-associated mutations T215Y and K219E, and isolates from another patient contained the multiple-nucleoside-resistance-associated mutation Q151M. Mutations in the RT gene of HIV-1 isolates from the other two patients were not detected. The genetic basis for stavudine susceptibility changes has not been identified.

Cross-resistance

Several studies have demonstrated that prolonged stavudine treatment can select and/or maintain mutations associated with zidovudine resistance. HIV-1 isolates with one or more zidovudine-resistance-associated mutations (M41L, D67N, K70R, L210W, T215Y/F, K219Q/E) exhibited reduced susceptibility to stavudine in vitro.

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Phase 4 Commitments

1. Please continue to assess genotypes and phenotypes of pre-therapy and post-therapy HIV-1 isolates from a large number of patients failing stavudine therapy.
2. Please evaluate the cross-resistance of stavudine resistant HIV-1 isolates to all approved NRTIs, and the efficacy of d4T against HIV-1 resistant to all approved NRTIs.
3. Please determine the in vitro combination activity relationships of stavudine with all approved NRTIs.
4. Please determine the effect of ribavirin on anti-HIV-1 activity of stavudine in vitro.

RECOMMENDATIONS

With respect to microbiology, NDA # 21,453 is approvable.

Lalji Mishra, Ph.D.
Microbiologist

CONCURRENCES:

_____ Date_____
HFD-530 /J. Farrelly/Assoc Dir

_____ Date_____
HFD-530/J. O'Rear /TL Micro

CC:
HFD-530/NDA # 21,453
HFD-530/ Division File
HFD-530/ Micro/L. Mishra
HFD-530/CSO/S. Lynche

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Lalji Mishra
1/22/03 10:31:56 AM
MICROBIOLOGIST

Julian O Rear
1/22/03 11:07:44 AM
MICROBIOLOGIST

James Farrelly
1/27/03 07:47:02 AM
PHARMACOLOGIST