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No resistance to IDX184 was detected in 3-day and 14-day clinical studies of IDX184 in genotype 1-infected HCV subjects

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BACKGROUND

- IDX184 is a liver-targeted 2'-C-methylguanosine nucleotide prodrug which inhibits the HCV NS5B polymerase of all genotypes *in vitro*. This work summarizes the resistance profile of IDX184 derived from early clinical studies.

METHODS

Clinical Trials

- Two double-blind studies were conducted in genotype 1 HCV-infected, treatment-naïve subjects
- IDX-08C-003 was a 3-day proof-of-concept study with IDX184 administered as monotherapy to HCV-infected subjects.
  - 33 subjects received once-daily doses of 25 mg, 50 mg, 75 mg or 100 mg IDX184; 8 subjects received matching placebo.
  - Samples were taken at baseline, at end of treatment (EOT) and 14 days post-treatment for NS5B population sequencing.
  - Two subjects with relatively poor virological response were selected for further examination by NS5B clonal sequencing—these subjects showed a modest on-treatment increase in viral load from nadir (0.5 to 0.7 $\log_{10}$ IU/mL) and had the weakest EOT responses to IDX184 in their respective cohorts (-0.23 and +0.1 $\log_{10}$ IU/mL compared to baseline).
  - Approximately 50 clones were obtained from samples from both subjects at baseline and after IDX184 dosing to determine if IDX184-resistant variants were present at levels too low to be detected by population sequencing.
- IDX-08C-004 was a 14-day phase II study of IDX184 administered in combination with pegylated interferon and ribavirin to HCV-infected subjects.
  - 65 subjects were assigned to receive 50 mg QD, 50 mg BID, 100 mg QD, 150 mg QD, 100 mg BID or 200 mg QD IDX184; 16 subjects received matching placebo. Two subjects dropped out of the trial after providing baseline samples.
  - Samples were taken at baseline, at EOT and 14 days post-treatment for NS5B population sequencing. None of the subjects experienced virologic breakthrough during treatment with IDX184, defined as a confirmed 1 $\log_{10}$ increase in HCV RNA from nadir or detectable HCV RNA in a subject with previously undetectable levels.

Clinical Analysis

- RNA was isolated from all clinical samples with viral load ≥ 1,000 IU/mL using the Total Nucleic Acid isolation kit (Roche Applied Science). The NS5B domain was converted into cDNA by the Roche Transcriptor kit and amplified by nested PCR using the Expand High Fidelity kit.
- For clonal sequencing, cDNA was ligated into a TOPO-TA based vector (Invitrogen) which was then used to transform bacterial cells. Positive clones were selected to inoculate LB cultures, and DNA plasmids were extracted for sequencing.
- PCR products for population sequencing and plasmids for clonal sequencing were sequenced from both directions covering the entire NS5B region. For population sequencing, a mixed base was called if the lowest signal exceeded 25% of the highest signal.
- Subject sequences were compared to consensus genotype 1a and 1b NS5B sequences derived from the Los Alamos National Laboratory (LANL) data base to assess the degree of baseline variability in subject sequences and to identify polymorphic sites within NS5B. NS5B positions were identified as polymorphic if they had ≥ 5% amino acid variability at that locus among the LANL sequences used to derive the consensus sequences.

In Vitro Analysis

- Four cell lines containing genotype 1b HCV replicons were treated with IDX184 at concentrations ranging from 9X EC_{50} up to 50X EC_{50}. Replicons were periodically sequenced to determine mutations that arose during exposure to IDX184.
- The S282T mutation was introduced into the genotype 1b replicon using standard site-directed mutagenesis techniques.
- *In vitro* resistance was determined by treating replicon-bearing cells with increasing concentrations of IDX184. EC_{50} values were calculated using XLfit© to fit dose-response curves.

RESULTS

IDX-08C-003 Clinical Resistance Results—NS5B Population Sequencing

- In the 3-day study a total of 121 population NS5B sequences from the 33 IDX184-treated subjects and 8 placebo subjects in the IDX-08C-003 trial were examined (Table 1).
- Baseline sequences showed significant variability relative to consensus genotype 1a and 1b NS5B sequences derived from the LANL data base.
  - At 51 residues where subject baseline sequences differed from a consensus NS5B sequence in more than one subject, 47 were identified as polymorphic sites
- Analysis of EOT NS5B population sequences identified 53 changes in amino acid composition at 40 NS5B positions in subjects who received IDX184 (1.6 mutations/subject); 6 amino acid changes were observed in placebo subjects (0.8 mutations/subject). In all cases the observed changes involved mixed populations of amino acids.
- Of the changes at 40 NS5B residues in subjects who received IDX184, 32 were observed in only one subject each, suggesting that the observed changes were random.
  - The other 8 residues at which a change was observed in more than one subject all involved mixtures of amino acids, and 5/8 were identified as being polymorphic sites
  - Subjects with changes at the 8 residues exhibited viral load reductions comparable to those without these mutations (-0.64 $\log_{10}$ IU/mL vs. -0.66 $\log_{10}$ IU/mL)
- The difference between treatment and placebo groups in the frequency of amino acid changes from baseline to end-of-treatment was not statistically significant (p = 0.11).
- No S282T mutation was detected by population sequencing in any baseline samples in IDX-08C-003. No S282T emerged following treatment with IDX184 or placebo.

Table 1: Number of Population NS5B Sequences Available for Analysis—IDX-08C-003

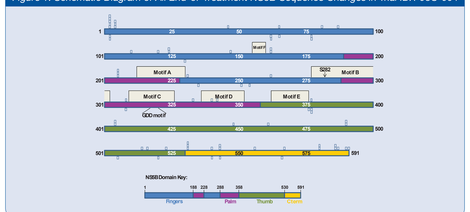
Study IDX-08C-003 3-Day Trial Population Sequences	Baseline	End of Treatment	14 Days Post-EOT
IDX184 treated[a]	32	32	33
Placebo	8	8	8

[a]One baseline sample and one EOT sample were PCR negative and failed to produce a sequence.

IDX-08C-003 Clinical Resistance Results—NS5B Clonal Sequencing

- Approximately 200 clonal NS5B sequences were examined from the two IDX-08C-003 subjects with poor viral load response during IDX184 treatment—approximately 50 sequences from each subject at baseline and at EOT (Table 2).

Figure 1: Schematic Diagram of All End-of-Treatment NS5B Sequence Changes in Trial IDX-08C-004



NS5B amino acids that changed at end of treatment relative to baseline are denoted by open boxes. The number of boxes at a given position indicates the number of changes observed. Boxes above the schematic protein represent changes in subjects receiving IDX184; boxes below the protein represent changes in placebo subjects. With only three exceptions, all changes involve mixtures of amino acids. Colored regions of the NS5B enzyme showing the fingers, palm, thumb and C-terminal domains follow O'Farrell et al. 2003[2]. Highlighted motifs A through F identify regions conserved among RNA-dependent RNA polymerases[3,4,5]. Motif B is involved in nucleotide binding and includes S282, the site of the known IDX184 resistance mutation. The location of the GDD RNA polymerase active site motif is noted[6].

- A single amino acid change was observed by population sequencing in one of the two subjects: a change from S213 at baseline to mixed S/N213 at end of treatment. However, examination of the clonal sequences showed that the relative proportions of S213 and N213 remained essentially unchanged before and after treatment. Thus, the change reported by population sequencing simply reflected minor fluctuations in a mixed population (Table 3).
- Analysis of all clonal sequences from the two selected subjects did not detect the S282T mutation before or after IDX184 treatment. No other codon could be correlated with the relatively poor virological response of the subjects.

Table 2: Number of Clonal NS5B Sequences Available for Analysis—IDX-08C-003

Study IDX-08C-003 3-Day Trial Clonal Sequences	Baseline	End of Treatment
Subject 038	50	48
Subject 001	47	50

Table 3: Clonal Analysis of an Observed S213S/N Post-Treatment Change in One IDX-08C-003 Subject

	Number of Clones with S	Number of Clones with N	Number of Clones with Other	Total Clonal Sequences
Baseline	36	9	5	50
End of treatment	36	7	5	48

IDX-08C-004 Clinical Resistance Results—NS5B Population Sequencing

- In the 14-day study a total of 161 population NS5B sequences from the 65 IDX184-treated subjects and 16 placebo subjects in the IDX-08C-004 trial were examined (Table 4).
- Baseline sequences showed significant variability relative to consensus genotype 1a and 1b NS5B sequences derived from the LANL data base.
  - Every baseline sequence examined showed variation from NS5B consensus, with 4 to 27 variations per subject.
  - Genotype 1a subject baseline characteristics
    - An average of 11.7 variations from consensus were observed in genotype 1a subjects.
    - In 68 genotype 1a baseline sequences, there were 794 variations from the 1a consensus sequence at 140 NS5B amino acid positions; 607 of the variations (76%) were at 48 polymorphic loci.
  - Genotype 1b subject baseline characteristics
    - An average of 13.5 variations from consensus were observed in genotype 1b subjects.
    - In 13 genotype 1b baseline sequences, there were 175 variations from the 1b consensus sequence at 84 NS5B amino acid positions; 140 of the variations (80%) were at 55 polymorphic loci.
- At EOT 40 subjects had viral load ≥ 1,000 IU/mL, and NS5B sequences were obtained from 38. Twenty-four were from subjects receiving IDX184, and 14 were from placebo subjects. In pairwise comparisons 7/24 IDX184 subjects (29%) and 5/14 placebo subjects showed no amino acid change from baseline (36%).
- Analysis of EOT NS5B population sequences identified 52 amino acid changes at 36 NS5B positions in 24 subjects who received IDX184 (2.2 mutations/subject) versus 20 amino acid changes at 16 NS5B positions in 14 placebo subjects (1.4 mutations/subject).
- The difference between treatment and placebo groups in the frequency of amino acid changes from baseline to EOT was not statistically significant (p = 0.24).
- Changes observed in all available EOT sequences (N=24 IDX184-treated subjects; N=14 placebo subjects) compared to baseline sequences in the IDX-08C-004 trial are shown diagrammatically in Figure 1.
- Changes overwhelmingly involved mixed populations of amino acids (69/72) and were randomly distributed throughout NS5B. No changes were observed within motif B, a region of NS5B that is associated with nucleotide binding and which contains S282, the known nucleotide resistance locus.
- Only three amino acid changes in two subjects receiving IDX184 did not involve mixed amino acids (Table 5):
  - A342T in one subject
  - Y448C, A567T in a different subject
  - Both subjects showed ≥ 2.5 $\log_{10}$ IU/mL drop in viral load at EOT
  - Both subjects were infected with genotype 1a virus and had C/T IL28B genotype
  - These three changes (along with mixed population changes) observed in both subjects at EOT reverted to baseline values when examined 14 days later
- S282T was not observed as a preexisting polymorphism in any baseline sample, nor did S282T arise in any IDX184-treated or placebo subject after treatment.

Table 4: Number of Population NS5B Sequences Available for Analysis—IDX-08C-004

Study IDX-08C-003 14-Day Trial Population Sequences	Baseline	End of Treatment	14 Days Post-EOT
IDX184 treated	65	24	31
Placebo	16	14	11

Table 5: Changes Following IDX184 Treatment in Two IDX-08C-004 Subjects That Did Not Involve Mixed Amino Acids

Subject	Virus Genotype	Dosing Cohort	NS5B Position	Amino Acid at Baseline	Amino Acid at EOT	Amino Acid at 14 Days Post-EOT	Change in Viral Load at EOT ($\log_{10}$ IU/mL)	IL28B Status
101-003	1a	100 mg QD	342 231	A N/S	T N	A N/S	-2.7	C/T
107-006	1a	50 mg QD	448 567 580	Y A T/A	C T A	Y A T/A	-2.5	C/T

Correlation of Clinical Observations with *In Vitro* Results[1]

- In four cell lines cultured to select for resistance to IDX184, only the S282T mutation emerged in all cultures, with an average time to appearance of 52 days (range: 25-77 days).
- While one or two additional mutations were observed in each cell line, none were observed in more than one cell line. To date, mutations other than S282T that appear *in vitro* following exposure to IDX184 appear to be random consequences of an error-prone viral polymerase.
- Resistant cell lines showed an average 24-fold increase in IDX184 EC_{50}, comparable to the 23-fold increase in EC_{50} seen with the S282T site-directed mutant replicon; this suggests that the S282T mutation is responsible for the observed resistance *in vitro*.
- S282T replicons had reduced replication capacity—5% of wild type replicon.
- In the genotype 1b replicon the S282T mutation is the only substitution that we have identified to date to confer resistance to the 2'-C-methylnucleoside class of HCV polymerase inhibitors.

CONCLUSIONS

- In 3- and 14-day clinical trials with IDX184, no pre-existing S282T variant was detected.
- The S282T resistance mutation was not detected following IDX184 treatment in either trial.
- In the two clinical trials as well as in the preclinical studies, no other NS5B or other HCV mutation (N>1) has yet been identified as showing an association with IDX184 treatment.
- In preclinical resistance selection studies in cultured cells, the S282T mutation took an average of over 7 weeks to appear and S282T replicon-bearing cells exhibited 5% of wild-type replication capacity.
- Thus, the combined preclinical and clinical results are consistent with the concept of a high genetic barrier to resistance for nucleoside agents such as IDX184.

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Disclosures

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