

# ***In vitro* resistance and cross-resistance profiles of IDX320, a potent macrocyclic HCV protease inhibitor**

**Lisa B. Lallo, Bin Li, Mary A. Soubasakos, Joshua Gillum, Ilaria Serra, Angie Bonin, John P. Bilello, Maria Seifer, and David N. Standing**

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# Introduction

- IDX320 is a novel macrocyclic HCV protease inhibitor currently in a Phase Ib/IIa clinical study.
- IDX320 bound genotype 1b protease tightly with fast association ( $2.7 \times 10^4 \text{ M}^{-1}\text{s}^{-1}$ ), very slow dissociation ( $2.1 \times 10^{-5} \text{ s}^{-1}$ ), an equilibrium constant of 0.8 nM and a dissociation half-life of over 9 hours.
- Pharmacokinetic results from a Phase I healthy volunteer study following 400 mg QD of IDX320 for three days demonstrated<sup>1</sup>:
  - a plasma half-life of 31 hours
  - mean IDX320 plasma  $C_{24h}$  concentrations approximately 40 to 150 fold above the GT1a  $EC_{50}$  and 300 to 1,000 fold above the GT1b  $EC_{50}$

<sup>1</sup> J. v.d. Wetering de Rooij et al, *IDX320, A Novel Macrocyclic HCV Protease Inhibitor: Safety, Tolerability and Pharmacokinetics (PK) in a Phase I Clinical Study*, 5<sup>th</sup> International Workshop on Clinical Pharmacology of Hepatitis Therapy, June 2010

# IDX320: Multi-Genotypic Activity *In Vitro*

HCV protease	IDX320 IC <sub>50</sub> (nM)
Genotype 1a	1.1 ± 0.1
Genotype 1b	1.2 ± 0.1
Genotype 2a	1.9 ± 0.5
Genotype 3a	23.0 ± 1.9
Genotype 4a	0.81 ± 0.03

- IDX320 did not inhibit nine cellular proteases (IC<sub>50</sub> > 10 μM).

## IDX320: Potent Activity in Cell Culture Models

Model	EC <sub>50</sub> (nM)	CC <sub>50</sub> (μM)	Selectivity Index
1b replicon	0.5 ± 0.1	25.2 ± 5.3	50,400
1a replicon	3.4 ± 1.1	> 77	> 22,647
2a virus	4.4 ± 0.6	11.3 ± 0.1	2,568

- IDX320 did not inhibit a panel of 14 DNA and RNA viruses (EC<sub>50</sub> > 3 μM).

# IDX320: Antiviral Activity Against Replicons Containing Known PI Resistance Mutations

Single mutations were introduced into the GT1b replicon.

NS3 mutation	IDX320 fold-change
T54A	1.4 ± 0.2
R155K	9.0 ± 1.7
R155Q	0.6 ± 0.1
A156S	0.5 ± 0.1
A156T	27.0 ± 9.5
<b>D168V</b>	<b>4587 ± 1003</b>

- IDX320 was fully active against replicons bearing single NS3 T54A, R155Q, or A156S mutations.
- R155K and A156T replicons were in general more susceptible to IDX320 compared to other PIs (10- to 496-fold and 41- to 1344-fold resistance, respectively; Lenz, *et al* AAC 54:1878 2010).

# Lack of Cross-Resistance Between IDX320 and Other Classes of HCV Inhibitors (NIs, NNIs, or NS5A Inhibitors)

HCV target	Mutation	IDX320 fold-change
NS5A	L31F	1.08 ± 0.07
	L31M	0.86 ± 0.03
	L31V	0.79 ± 0.03
	Y93C	0.57 ± 0.07
	Y93H	0.62 ± 0.10
NS5B	S282T	1.37 ± 0.62
	C316Y	1.71 ± 0.56
	M414T	1.59 ± 0.51
	M423T	0.85 ± 0.08
	C445F	0.94 ± 0.07
	Y448H	1.66 ± 0.43

# Resistance Selection and Characterization

- Resistance selection was performed by continuous culture of genotype 1a or 1b replicon cell lines with 20x or 50x  $EC_{50}$  of IDX320 in the presence of G418.
- Population sequencing of NS3 was performed at every passage.
- Population sequencing of the entire replicon and clonal sequencing of NS3 was performed at the end of selection.
- Phenotypic susceptibility to IDX320, as well as other HCV agents, was evaluated over time or at the end of selection.

# Presence of NS3 D168V Correlates with Phenotypic Resistance to IDX320 in Genotype 1b

Cell line	Dominant NS3 mutations	Minor NS3 mutations	IDX320 fold-change	Telaprevir fold-change
320R-A	D168V	D168E, E503D	1100 ± 212	0.40 ± 0.16
320R-B	D168V	A156V, D168A/E, V256A, G282A	> 1176	0.25 ± 0.05
320R-C	D168V	Q41R, Q80R, A156V, D168A/E/H/I/Y	> 374	0.26 ± 0.13
320R-D	D168V	D168A, E503D	2022 ± 415	0.23 ± 0.12
320R-E	D168V	A156P, D168A/E/Y, V256A, G282A	> 2168	0.36 ± 0.21
320R-F	D168V	D168H/Y, G282A	> 250	0.35 ± 0.08

- D168V was detected between Day 17 and 35 of selection.
- No treatment-emergent amino acid changes were observed outside of NS3.
- All 320R cell lines remained susceptible to Telaprevir.
- All genotypic changes were phenotypically evaluated using luciferase-replicons bearing a single mutation.

# Phenotypic Analysis of IDX320 Treatment-Emergent NS3 Mutations

Replicon	IDX320 fold-change	Replication capacity (% of WT)
Q41R	3.11 ± 0.27	104 ± 18
Q80R	5.67 ± 0.94	107 ± 30
A156P	ND	0.30 ± 0.05
A156V	ND	4.0 ± 0.6
D168A	575 ± 210	32 ± 10
D168E	41.4 ± 17.8	40 ± 12
D168H	1,079 ± 793	33 ± 9
D168I	>11,727	10 ± 3
<b>D168V</b>	<b>4,587 ± 1003</b>	<b>19 ± 5</b>
D168Y	1,107 ± 513	10 ± 4
V256A (helicase)	1.53 ± 0.51	6 ± 2
G282A (helicase)	1.57 ± 0.22	94 ± 8
E503D (helicase)	1.11 ± 0.14	43 ± 9

- In genotype 1b, D168V was the dominant mutation selected by IDX320 *in vitro*.

# IDX320-Resistant Genotype 1b Replicons Remain Susceptible to Other Classes of Anti-HCV Agents

- Selection was performed in a genotype 1b luciferase-replicon cell line.
- D168V again emerged as the dominant resistance mutation.
- No treatment-emergent amino acid changes were observed outside of NS3.

Cell line	Dominant mutation in NS3	Fold-change				
		IDX320	Telaprevir	IFN	IDX375	IDX184
320R-G	D168V	250 ± 66	1.0 ± 0.3	0.6 ± 0.4	0.9 ± 0.2	1.0 ± 0.2
320R-H	D168V	1,037 ± 144	0.60 ± 0.04	0.70 ± 0.06	1.3 ± 0.4	1.1 ± 0.2

- IDX320R replicon cell lines remained susceptible to Telaprevir and other classes of HCV inhibitors: IFN, nucleotide and non-nucleoside polymerase inhibitors.

# IDX320 Selected D168A in an HCV-1a Replicon Cell Line

- Some PIs select different mutants in a GT1a HCV background versus GT 1b.
- D168A emerged as the major variant in four GT1a luciferase-replicon cell lines.
  - D168V was selected as a minor variant.
- No treatment-emergent variants were observed outside of NS3.

Cell line	Mutations in NS3		Fold-change			
	Dominant	Minor	IDX320	Telaprevir	IFN	IDX184
320R-H1a A	D168A	D168V	> 3690	0.8 ± 0.3	1.4 ± 0.7	0.8 ± 0.1
320R-H1a B	D168A	D168V	589 ± 442	0.4 ± 0.1	1.0 ± 0.3	0.6 ± 0.3
320R-H1a C	D168A	D168V	> 2750	0.50 ± 0.01	0.50 ± 0.03	0.8 ± 0.2
320R-H1a D	D168A	D168V	900 ± 65	0.5 ± 0.1	1.6 ± 0.3	0.9 ± 0.2

- IDX320R 1a replicon cell lines remained susceptible to IFN and Telaprevir, as well as polymerase inhibitors.
- The NS3 R155K mutation, selected in the GT1a background by other PIs *in vitro* and *in vivo*, was not observed in these experiments.

# Conclusions

- IDX320 exhibits multi-genotypic activity against HCV protease and potent antiviral activity in HCV cell culture models.
- The primary locus for IDX320-associated resistance *in vitro* is NS3 D168.
  - D168V was selected in GT1b; D168A in GT1a
  - These variants have reduced replication capacity (eg. D168V, 19% of wild-type).
- R155K was not selected by IDX320 in either GT1a or GT1b replicons.
- IDX320 retained antiviral activity against most of the minor selected variants (D168 variants excluded).
- All IDX320-resistant cell lines remained susceptible to IFN and Telaprevir, as well as other classes of HCV direct-acting antiviral agents.
- IDX320 is currently being evaluated in a 3-Day Phase Ib/IIa monotherapy study in HCV-infected patients where the spectrum of mutations selected by this drug candidate will be further explored.

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