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REPLY TO PADMANABHAN AND DIXIT:

Hepatitis C virus entry inhibitors for optimally boosting direct-acting antiviral-based treatments

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We thank Padmanabhan and Dixit for their comments (1) on our paper (2). They pointed out that entry inhibitors might form potent partners for optimal drug combinations. They analyzed previously published data on 10 hepatitis C virus (HCV) entry inhibitors that are under clinical or preclinical development and found some of these HCV entry inhibitors showed high instantaneous inhibitory potentials (*IIP*s) (3) compared with *IIP*s of direct-acting antivirals (DAAs). To analyze further the utility of combining entry inhibitors with other DAAs and to extend our original results (2), we quantified the anti-HCV effect of four different classes of entry inhibitors [AR4A (anti-HCV E2 antibody) (4), BLT-1 [scavenger receptor class B type 1 (SR-BI) inhibitor] (5), erlotinib (EGF receptor inhibitor) (6), and dasatinib (EphA2 inhibitor) (6)] singly and in combination with six DAAs studied by Padmanabhan and Dixit (1) in the HCV infectious cell culture system (Fig. 1 *A* and *B*). Single treatment of these entry inhibitors exhibited a dose-dependent reduction in HCV RNA levels. Using the median effect (1–3), we estimated IC_{50}, the half-maximum inhibitory concentration, and m, the slope parameter, for each drug from its dose–response curve (Table 1), which enables us to calculate $IIP = \log[1 + (D/IC_{50})^m]$ at $D = 100 \times IC_{50}$ (i.e., IIP_{100}) (Fig. 1*C*). We found BLT-1 shows the highest IIP_{100} among the entry inhibitors, which is equivalent in value to DAAs. In addition, applying Bliss independence (7), we quantified the upper limits of anti-HCV activity for triple-drug treatments at $D = 100 \times IC_{50}$ (IIP_{100}^{Bcom}) (Fig. 1*D*). These data clearly showed that HCV entry inhibitors augmented the antiviral effect of double DAA-based treatments. Interestingly, augmentation of antiviral effects by addition of entry inhibitors largely depended on the entry inhibitor used: Triple-drug treatments, including BLT-1, showed an especially high IIP_{100}^{Bcom}, which is comparable to the IIP_{100}^{Bcom} of triple DAA-based treatments, among the tested entry inhibitors.

Entry inhibitors are primarily aimed at preventing viral infection. However, because they are effective in eliminating HCV from already established infection in human liver chimeric mice and chimpanzees, HCV entry inhibitors can be candidates for an additional choice of anti-HCV treatment (8–11). In particular, host-targeting agents such as BLT-1, erlotinib, and dasatinib show less opportunity for emergence of drug-resistant virus. The results of Padmanabhan and Dixit (1), which are further supported by the work reported here, show that entry inhibitors can be combined with other classes of DAAs to provide potentially potent anti-HCV treatment that may be especially effective for difficult-to-treat patients.

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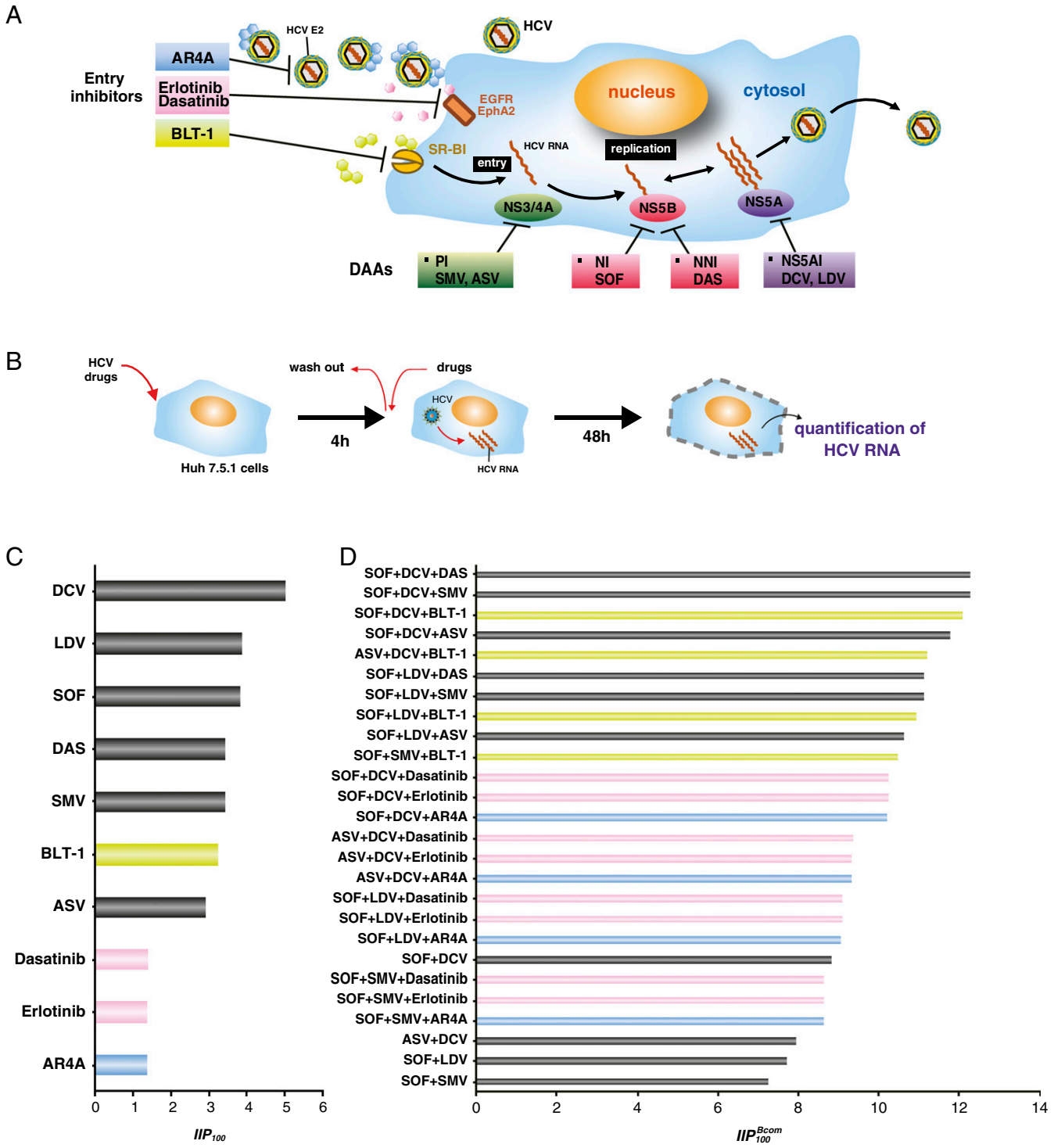

Fig. 1. Evaluation of anti-HCV drug combination with DAAs and entry inhibitors. (*A*) Schematic model of the targets of entry inhibitors and DAAs used in this study. NI, nucleoside-type polymerase inhibitor; NNI, nonnucleoside-type polymerase inhibitor; PI, protease inhibitor. (*B*) Schematic representation of the assay to evaluate the anti-HCV activity of the drugs. Upon drug treatment, Huh7.5.1 cells were inoculated with HCV JFH-1 at a multiplicity of infection of 0.5 for 4 h, and were cultured for an additional 48 h. Anti-HCV E2 antibodies were used by preincubation with HCV for 1 h and coincubation with HCV for 4 h. The infection level of HCV was quantified by measuring intracellular HCV RNA. (*C*) *IIP* values for DAAs in our study (2) and the HCV entry inhibitors (AR3A, BLT-1, and erlotinib) at a drug concentration $D = 100 \times IC_{50}$ (IIP_{100}) determined by extrapolation. (*D*) Bliss-estimated IIP_{100}^{Bcom} of triple-drug combination, including DAAs and the entry inhibitors. ASV, asunaprevir; DAS, dasabuvir; DCV, daclatasvir; LDV, ledipasvir; SOF, sofosbuvir; SMV, simeprevir.

Table 1. Parameter values for the HCV entry inhibitors in Huh 7.5.1 cells

Drug	*m*	IC_{50}	Unit
Simeprevir	1.71	20.00	nM
Asunaprevir	1.46	17.28	nM
Sofosbuvir	1.91	103.19	nM
Dasabuvir	1.72	3318.18	nM
Daclatasvir	2.51	13.37	pM
Ledipasvir	1.94	2.81	nM
Dasatinib	0.69	2.90	μM
Erlotinib	0.69	0.84	μM
BLT-1	1.62	0.96	μM
AR4A	0.68	14.72	μg/mL

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