

New therapeutic options for HCV infection in the monoclonal antibody era

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SUMMARY

Hepatitis C virus (HCV) infection is a leading cause of chronic liver disease and the most common indication for liver transplantation. Current therapies are ineffective in a relevant percentage of patients raising the urgent medical need to develop adequate therapies for this infection. Broadly neutralizing human monoclonal antibodies (mAbs) directed against the HCV E2 glycoprotein (HCV/E2), the major target of the neutralizing humoral immune response, are considered as a possible novel therapeutic strategy for this infection. In the last few years, several anti-HCV/E2 human mAbs have been described in literature to be possibly used for therapeutic or prophylactic purposes. In this review, we illustrate the best candidates for an anti-HCV mAb-based therapy, considering their cross-neutralization profiles and their ability to overcome possible viral escape mechanisms.

KEY WORDS: HCV, mAbs, Anti-HCV/E2 mAbs, Viral escape.

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INTRODUCTION

Hepatitis C virus (HCV) is a major cause of chronic liver disease worldwide and is estimated to infect about 3% (170 million) of the world's population (Ascione *et al.*, 2007). The available interferon (IFN)-based treatment for HCV infection is effective in about a half of infected patients and is associated with several side effects. The recently introduced HCV/NS3-protease inhibitors (*boceprevir* and *telaprevir*) increased the rate of sustained viral response (SVR), but their use is limited only to genotype 1-infected patients and it is associated with the rapid onset of drug resistance (Kwo *et al.*, 2010; McHutchison *et al.*, 2010), leaving the medical need of new therapies to be fulfilled. In addition, the high percentage of HCV-positive patients progressing to a chronic infection (80%) presents an increased risk to develop liver failure, cirrhosis, hepatocellular carcinoma

as well as autoimmune disorders (Alter *et al.*, 1992; Makris *et al.*, 1996; Perotti *et al.*, 2008a; Sautto *et al.*, 2012b).

Although the role of CD4+ and CD8+ T cells in clearing HCV infection is widely ascertained (Neumann-Haefelin *et al.*, 2005), several findings suggest an important role played by the humoral immune response, as clearly demonstrated by the fact that administration of polyclonal anti-HCV Ig preparation can prevent sexual transmission (Piazza *et al.*, 1997). Beside this, it has been found that in serum samples from a well-characterized single-source outbreak of HCV, viral clearance was associated with a rapid onset of broadly neutralizing Abs (nAbs) in the early phase of infection. In contrast, the Abs from patients progressing to chronic infection displayed a complete absence of, or a delayed induction of, or a reduced capacity to neutralize the virus in the same time point (Pestka *et al.*, 2007). In line with this, a more recent study using an acutely infected patient cohort demonstrated that high-titer nAbs peaked at the time of viral clearance in all spontaneous responders, whereas chronically evolving individuals displayed low-titer or absent nAbs during the early acute infection (Dowd *et al.*, 2009). Together with other observations (Feroy *et al.*, 1998; Bjoro

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et al., 1994), these studies provide evidence that the rapid induction of nAbs during the early phase of infection may assist in controlling HCV replication as well as in the chronic phase or in a post-transplantation setting.

Given the variability of HCV, viral escape from Ab-mediated neutralization by means of diverse strategies could arise, but the overall inefficiency of the anti-HCV Ab response can be due to the overall balance of the humoral response, as demonstrated in the previously reported studies (Burioni *et al.*, 2008b; Di Lorenzo *et al.*, 2011). Indeed, prophylaxis or therapy with a mixture of synergic broadly neutralizing monoclonal antibodies (mAbs) could reduce the viral escape phenomenon (Burioni *et al.*, 2008b; Chandra *et al.*, 2010) and provide a more favorable virus-host interplay. Moreover, the definition of conserved B-cell epitopes able to elicit protective immunity can be a key element for the design of more effective vaccines (Clementi *et al.*, 2012). In particular, the HCV envelope glycoproteins E1 and E2 (HCV/E1-E2), which are involved in HCV entry into host cells, represent the major targets of nAbs (Burioni *et al.*, 1998; Mancini *et al.*, 2009). But, giving the low immunogenicity of E1 glycoprotein, to date the majority of neutralizing mAbs against HCV infection target conserved regions on E2 (Keck *et al.*, 2004b; Meunier *et al.*, 2008). In this review we will overview some well-characterized anti-HCV/E2 human mAbs recently described in literature with a brief molecular depiction of the targeted epitopes followed by comments on their prophylactic and therapeutic potential.

HCV/E2 glycoprotein as neutralizing target of candidate therapeutic anti-HCV/E2 human mAbs

The major target cells of HCV are hepatocytes in which the virus enters by a complex and regulated manner. In particular, the HCV surface envelope type I membrane glycoproteins (E1 and E2) form non-covalent heterodimers on the surface of the HCV envelope and mediate clathrin-mediated virus endocytosis interacting consecutively with several entry cellular cofactors such as glycosaminoglycans (GAGs), low-density lipoprotein receptor (LDL-R), scavenger receptor class B type I (SR-BI), the tetraspanin CD81, the tight-junction proteins claudin-1 (CLDN-1) and oc-

cludin (OCLN) and the recently described Niemann-Pick C1-like 1 (NPC1L1) cholesterol absorption receptor (Agnello *et al.*, 1999; Barth *et al.*, 2003; Benedicto *et al.*, 2009; Evans *et al.*, 2007; Liu *et al.*, 2009; Molina *et al.*, 2007; Pileri *et al.*, 1998; Ploss *et al.*, 2009; Sainz *et al.*, 2012; Scarselli *et al.*, 2002).

After viral entry and translation of the HCV-RNA genome, the HCV/E2 glycoprotein is produced like other HCV structural proteins (core and E1) by cellular signal peptidase cleavage from the translated viral polyprotein. In particular, the C-terminal transmembrane domains of HCV/E1 and E2 have a possible role in non-covalent heterodimerization of the two glycoproteins and contain endoplasmic reticulum-retention signals that are thought to anchor the glycoproteins within lipid membranes (Dubuisson *et al.*, 1994). Instead, the N-terminal ectodomain of HCV/E2 possesses the entry determinants for infection of the host cells that are likely targeted by nAbs. A molecular model of HCV/E2 has recently been proposed, but a crystal structure of the glycoprotein is not yet available. The proposed model assigns a typical class II fusion protein structure to HCV/E2, similar to the fusion proteins of members of the alpha- and flaviviruses, consisting of three distinct domains, where the putative fusion peptide is located in domain II of the HCV/E2 protein (Krey *et al.*, 2010). Despite conserved function between clinical isolates, the HCV/E2 glycoprotein tolerates great genetic diversity. Indeed, the ectodomain contains at least three highly variable regions that are under positive selection during acute and chronic infection. Hypervariable region (HVR) 1 is located at the N-terminus of HCV/E2 and plays an important role in entry, virion interaction with HDL (which has been shown to augment entry) and viral cell-to-cell transmission (as interacting with the SR-BI coreceptor), as well as Ab binding and disease outcome (Bartosch *et al.*, 2003a; Bartosch *et al.*, 2005; Bartosch *et al.*, 2003b; Brimacombe *et al.*, 2011; Kato *et al.*, 1993; Scarselli *et al.*, 2002; Voisset *et al.*, 2005; Weiner *et al.*, 1992). In particular HVR1, has been implicated both in non-nAb induction as well as in viral neutralization (Shimizu *et al.*, 1996). Indeed, the N-terminal region of HVR1 (aminoacid residues 384-395) has been described as the target of non-nAbs, while the C-

terminal region (aminoacid residues 396-407) as the target of genotype-restricted nAbs (Hsu *et al.*, 2003; Vieyres *et al.*, 2011). The other two hypervariable regions, HVR2 and the intergenotypic variable region (igVR) are thought to be involved in HCV/E1-E2 heterodimerization and virus infectivity (Krey *et al.*, 2010).

Indeed, possible therapeutic anti-HCV/E2 mAbs not only have to be directed against conserved and protective regions but also they should not target residues within the above mentioned variable regions to avoid the onset of possible escape variants. At this regard, several strategies have been adopted in order to isolate mAbs directed against conserved regions that are crucial for the viral life cycle and avoid the selection of genotype/isolate-restricted reactive mAbs (Clementi *et al.*, 2012; Solforosi *et al.*, 2012). Thus, fine epitope definition of mAbs and determination of their cross-neutralization profiles are crucial for evaluating their clinical potential against hypervariable viruses such as HCV (Burioni *et al.*, 2010; Burioni *et al.*, 2009; Clementi *et al.*, 2011; De Marco *et al.*, 2012; Mancini *et al.*, 2012; Mancini *et al.*, 2011). The clinical relevance of this aspect has been underlined by two clinical studies that have evidenced only a modest and short-lasting reduction in viremia after infusion of the anti-HCV/E2 human mAb HCV-AB68 in preventing HCV reinfection in chronic-infected patients during and after liver transplantation (Galun *et al.*, 2007; Schiano *et al.*, 2006). Subsequent studies demonstrated that this mAb binds a conformational epitope on HCV/E2 and can efficiently neutralize HCV pseudoparticles (HCVpp) harboring genotype 1b glycoproteins, but the HCV/E2 epitope targeted by HCV-AB68 was reported to contain four residues within the C-terminal region of HVR1 (Eren *et al.*, 2006). Thus, it is possible that within the infected patients treated in these studies, variants within the quasispecies population may have existed that could escape inhibition by HCV-AB68, suggesting that frequent high dose administration of a more broadly cross-neutralizing anti-HCV/E2 mAb, or of a combination of synergic mAbs, possibly directed against well conserved regions of HCV/E2, may achieve a sustained reduction of viral load in such patients and possibly prevent graft reinfection (Burioni *et al.*, 2008b).

HCV/E2 regions targeted by broadly cross-neutralizing mAbs

Considering the multiple cellular receptors involved in HCV binding and entry into target cells, it can be speculated that several regions of HCV/E2 can be targeted by Abs interfering with HCV docking and entry processes. In particular, the majority of broadly cross-neutralizing human anti-HCV/E2 mAbs are directed against conformational epitopes encompassing the CD81 binding site (CD81bs) of HCV/E2 and neutralize HCV at a post-attachment step, as demonstrated by kinetic neutralization assays (Haberstroh *et al.*, 2008) (Table 1). Mouse and rat mAbs interfering with HCV/E2 interaction with SR-BI and with cell-to-cell transmission of HCV have been described, but their epitopes encompass HVR1, further limiting their potential employment in clinical studies (Bartosch *et al.*, 2003a; Brimacombe *et al.*, 2011; Hsu *et al.*, 2003). It is indeed important to develop human mAbs that are able to limit this route of infection, especially in a post-transplantation setting, as in this case cell-to-cell transmission can be implicated in viral rebound and escape from Ab-mediated neutralization (Burioni *et al.*, 1994). However, Abs targeting HCV coreceptors involved in cell-to-cell transmission have been described and it is believed that they could be used in a future mAb-based therapy against HCV, probably in association with other developed drugs or mAbs directed against viral determinants (Catanese *et al.*, 2010; Meuleman *et al.*, 2008; Molina *et al.*, 2008).

Several HCV/E2 domains, variously conserved within the envelope glycoprotein, have been described as interacting with CD81. Among them, suggested regions include those encompassing aminoacid residues 412-423 (also known as epitope I), 432-447 (that encloses epitope II, spanning aminoacid residues 434-446), 480-493, 528-535 and 544-551 (Clayton *et al.*, 2002; Flint *et al.*, 1999; Owsianka *et al.*, 2001; Owsianka *et al.*, 2006). In particular, residues W420, Y527, W529, G530 and D535, as well as the G436-WLAGLF-Y443 motif, show a high degree of conservation among the different HCV genotypes (Drummer *et al.*, 2006; Owsianka *et al.*, 2006). However, this last region falls within a more variable region (aminoacid residues 431-466), for this reason known as HVR3, that can be also implicated in the induction of non-nAbs interfering with the

TABLE 1 - Best well-characterized anti-HCV/E2 human mAbs endowed with potent cross-neutralizing activity and candidate for a possible mAb-based therapy for HCV infection. All aminoacid numbering is based on the H77 reference strain (HCV genotype 1a).

MAb	Epitope (aminoacid residues on HCV/E2)	Epitope class	Interaction involved	Ab-induced escape variants	Ab-mediated interference	In vivo protection	References
e20	W437, F442, W529, G530, D535	Conformation-sensitive	CD81	N.D.	No, <i>in vitro</i>	N.D.	(Mancini <i>et al.</i> , 2009; Sautto <i>et al.</i> , 2012a)
e137	W437, L438, L441, F442, T416, W420, W529, G530, D535	Conformation-sensitive	CD81	N.D.	No, <i>in vitro</i>	N.D.	(Perotti <i>et al.</i> , 2008b; Sautto <i>et al.</i> , 2012a)
HCV1	Q412-N423	Linear	CD81	Yes, <i>in vivo</i>	Yes <i>in vitro</i> and <i>in vivo</i>	Yes (chimpanzee)	(Broering <i>et al.</i> , 2009; Morin <i>et al.</i> , 2012)
AR3A	S424, G436-F447, G523, G530, D535, V538, N540	Conformation-sensitive	CD81	N.D.	N.D.	Yes, (transgenic mouse)	(Law <i>et al.</i> , 2008)
AR4A	D698, R639	Conformation-sensitive	N.D.	N.D.	N.D.	Yes, (transgenic mouse)	(Giang <i>et al.</i> , 2012)
CBH-5	C494, V497, G523, P525, G530, D535, N540, R614, H617, Y618, P619, T621, Y624	Conformation-sensitive	CD81	N.D.	N.D.	N.D.	(Iacob <i>et al.</i> , 2008; Keck <i>et al.</i> , 2008; Keck <i>et al.</i> , 2004a; Keck <i>et al.</i> , 2007; Owsianka <i>et al.</i> , 2008)
CBH-7	C494, V497, N540, W549, R614, H617, Y618, P619, T621, Y624	Conformation-sensitive	CD81	N.D.	N.D.	N.D.	
HC-84	.1 F442	Conformation-sensitive	CD81	No, <i>in vitro</i>	N.D.	N.D.	(Keck <i>et al.</i> , 2012)
	.20 C429, L441, Y613, W616	Conformation-sensitive	CD81	No, <i>in vitro</i>	N.D.	N.D.	
	.23 W420, N428, C429, W437, L441, F442, Y443, W616	Conformation-sensitive	CD81	No, <i>in vitro</i>	N.D.	N.D.	
	.24 C429, F442, Y443	Conformation-sensitive	CD81	No, <i>in vitro</i>	N.D.	N.D.	
	.25 C429, L441, F442	Conformation-sensitive	CD81	No, <i>in vitro</i>	N.D.	N.D.	
1:7	G523, W529, G530, D535	Conformation-sensitive	CD81	N.D.	N.D.	N.D.	(Johansson <i>et al.</i> , 2007)
A8	G523, W529, G530, D535	Conformation-sensitive	CD81	N.D.	N.D.	N.D.	

activity of epitope I-directed nAbs (Troesch *et al.*, 2006; Zhang *et al.*, 2009). Indeed, as speculated for HVR1, HVR3 could have multiple role both in viral neutralization as well as in induction of non-neutralizing and interfering Abs by masking a more conserved region encompassing at least the 412-423 region, as recently speculated (El Abd *et al.*, 2011; Zhang *et al.*, 2009). Secondly, an HVR3 enclosing region, spanning the 425-443 aminoacid residues, is responsible for viral escape from neutralization, with or without compromising viral fitness. On the other hand, the domain encompassing residues 529, 530 and 535 is considered as the core CD81 binding region and is the target of several broadly cross-neutralizing human mAbs (Broering *et al.*, 2009; Johansson *et al.*, 2007; Keck *et al.*, 2011; Law *et al.*, 2008; Mancini *et al.*, 2009; Perotti *et al.*, 2008b) (Figure 1A and B).

Indeed, candidate anti-HCV/E2 mAbs have not only to be highly cross-neutralizing and targeting key conserved residues, but also their activity should not be influenced by possibly present endogenous interfering Abs (Burioni *et al.*, 2001). To this end, they must either be directed against conserved neutralizing epitopes not subjected to

the mechanism of interference or must be able to prevent the binding of interfering Abs, as we recently described for two broadly cross-neutralizing anti-HCV/E2 human mAbs (e20 and e137) (Sautto *et al.*, 2012a). In particular, these mAbs were isolated by phage-display of the Ab repertoire of a chronically infected patients and firstly characterized as Fab fragments, showing a wide reactivity on HCV/E2 of different genotypes as well as a broad neutralizing activity on different HCVpp genotypes and on the authentic cell culture infectious clone (HCVcc) of genotype 2a (Bugli *et al.*, 2001; Mancini *et al.*, 2009; Perotti *et al.*, 2008b). Recently, after their conversion into whole IgG molecules, they showed a great improvement in their neutralizing activity, with IC50 values of 0.02 and 0.03 µg/mL for e20 and e137, respectively, in HCV neutralization assays using HCVpp of genotype 1a (H77 isolate) (Sautto *et al.*, 2012a).

Recently, other HCV/E2 regions outside the CD81bs, have been implicated in HCV neutralization. In particular, these domains, located within the membrane proximal external region (MPER) of HCV/E2 and encompassing the extremely conserved residues D698 and R639, are

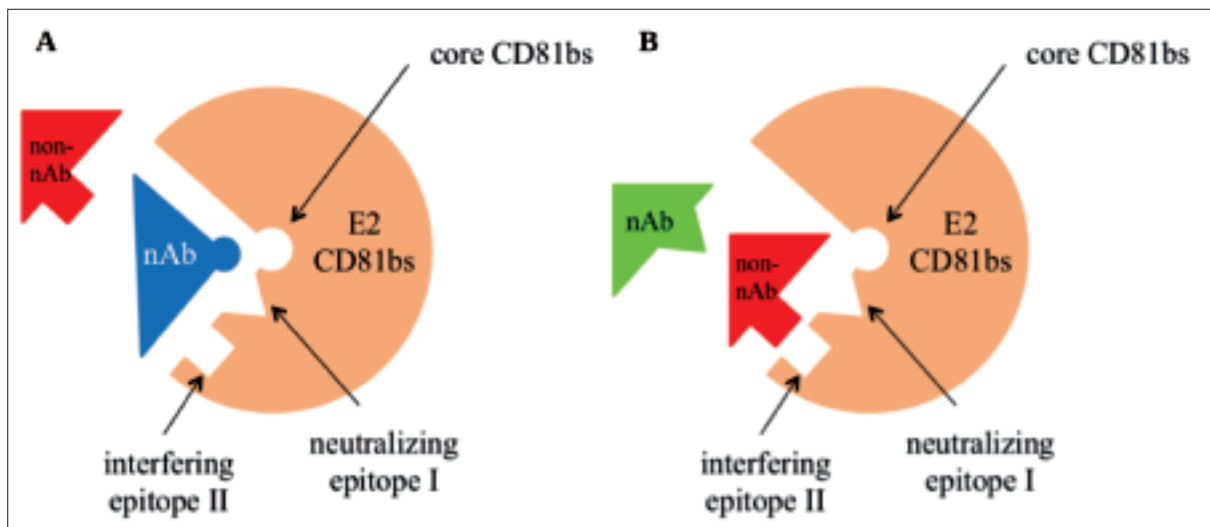


FIGURE 1 - (A) nAbs directed against the core CD81 binding region of HCV/E2 (comprising aminoacid residues 529, 530 and 535) and covering, or whose epitope encompasses, the interfering epitope II are not likely to be influenced by the presence of non-neutralizing/interfering Abs directed against epitope II. (B) The neutralizing activity of nAbs solely directed against epitope-I could be influenced by the presence of non-neutralizing/interfering Abs directed against epitope II through steric hindrance or binding competition. The E2 CD81 binding site (E2 CD81bs) is depicted in orange, nAb directed against the core CD81bs in blue, epitope-I directed nAb in green and non neutralizing/interfering nAb directed against epitope II in red. The core CD81bs, the interfering epitope II and the neutralizing epitope I are indicated by arrows.

required for the binding of two cross-neutralizing human mAbs, AR4A and AR5A, obtained by “exhaustive” panning strategy of a phage-display Ab library (Giang *et al.*, 2012). In particular, the HCV/E2 MPER is important for membrane fusion and the 687-703 aminoacid region is predicted to form an amphipathic alpha-helix partially embedded in lipid membrane (Albecka *et al.*, 2011). Indeed, supposing a helix-hinge-helix structure of this domain, similar to HIV-1 MPER, it has been speculated that AR4A mAb should inhibit the relative movement of the helices required for virus entry (Song *et al.*, 2009). Moreover, AR4A mAb protect a mouse model of infection that is rendered susceptible to HCV infection by genetic humanization and challenged with HCVcc of genotype 1b and 2a (Giang *et al.*, 2012).

Furthermore, another work describes the isolation of a series of anti-HCV/E2 human mAbs (named HC-84) whose conformational epitopes encompass aminoacid residues within the 418-446 and 611-616 regions of E2 (Keck *et al.*, 2012). In particular, the authors found that six aminoacid residues (W420, N428, L441, Y443, Y613 and W616) that are critical for HC-84 mAbs are 100% conserved in all HCV genotypes and subtypes. Indeed, they found that these residues are also critical for HCVpp infectivity and CD81 binding. Finally, these mAbs failed to generate Ab-induced HCV escape mutants using the authentic HCV infectious clone of genotype 2a (Keck *et al.*, 2012).

Recently, it has been described a human anti-HCV/E2 mAb, named HCV1, whose epitope overlaps with that of the well-characterized and broadly cross-neutralizing mouse mAb AP33 (aminoacid residues 412-423) (Tarr *et al.*, 2006; Owsianka *et al.*, 2005; Broering *et al.*, 2009). This human mAb has been isolated immunizing HuMAb transgenic mice (Medarex, Inc.), expressing human Ab genes, with soluble HCV/E2 envelope glycoprotein consisting of the ectodomain aminoacids 384-660. Indeed, this mAb is endowed of a broad cross-neutralizing activity against HCV isolates from at least four major genotypes.

Further studies demonstrated that L413 and W420 aminoacids, located in the hydrophobic face of the HCV1 epitope, are critical for its binding on HCV/E2 (Kong *et al.*, 2012). Additionally, passive administration of this mAb protects a

chimpanzee model from primary HCV challenge. However, a more recent study reported the onset of neutralization resistant HCV variants (carrying the N415K/D mutations) at 14 days following mAb administration into chimpanzee prior to infusion with genotype 1a (H77 isolate) HCVcc. Furthermore, the authors treated three chronically HCV-infected chimpanzees with this mAb and 21 days post-infusion one chimpanzee viral load was reduced below the detection limit with rebounding virus displaying the N417S mutation. Instead, the two other chimpanzees displayed a minor reduction in viral load without evidence of viral resistance to HCV1 (Morin *et al.*, 2012). The authors speculated that this poor-response could be explained by endogenous Abs to HCV/E2 interfering with HCV1 neutralization, as demonstrated in HCVpp-neutralization assays using HCV1 and sera from the poorly-responding chimpanzees. Thus, these data confirm that neutralization of epitope I-directed Abs could be influenced by the presence of interfering Abs that could be directed against epitope II. However, the authors reported that serum from all three chimpanzees had no appreciable Abs to these two epitopes and indeed endogenous interfering Abs are most likely directed against other regions of the envelope glycoprotein (Morin *et al.*, 2012). On the other hand, it is noteworthy that possible interfering Abs directed against epitope II could bind conformational epitopes and indeed could show no reactivity against peptides representing this epitope, as recently speculated by our group (Sautto *et al.*, 2012a). However, serum lipoproteins coating HCV virions present in sera may interfere with the neutralization of anti-HCV/E2 Abs as evidenced by different neutralization profiles exerted by different described mAbs on HCVcc compared to HCVpp (Dreux *et al.*, 2006; Voisset *et al.*, 2005).

CONCLUSIONS

In the drug development field mAbs are considered as novel useful tools against infectious as well as non-infectious diseases. Their use has allowed shedding some light on the protective mechanisms of the humoral response against hypervariable viruses. Giving this assumption, mAbs represent a potential prophylactic and therapeutic

tic tool against these viruses. Among them HCV infection is considered one of the potential candidate disease to be targeted by mAb-based therapeutic approaches as current treatment is inadequate in a post-transplant setting and are not completely effective in chronically infected patients as well as being associated with several side effects. The identification of human mAbs against broadly conserved epitopes on HCV/E2 glycoprotein, the major protective antigenic determinant of HCV, and their efficacy against different subtypes in *in vitro* and *in vivo* studies, may be an important step toward the development of new therapeutics.

The availability of different broadly protecting Abs directed against different conserved viral epitopes, also belonging to other proteins (e.g. E1), as well as cellular determinants (i.e. HCV coreceptors), may allow the development of Ab cocktails with broad protective activity against the different HCV genotypes and subtypes. Moreover, the parallel targeting of distinct crucial viral and cellular determinants on different proteins may also minimize the risk of the rapid emergence of viral escape mutants with unaltered viral fitness, which is now the case for drug-resistant escape variants. Finally, the availability of broadly cross-neutralizing anti-HCV/E1-E2 mAbs with such characteristics could also allow the design of a new generation of possible HCV immunogens capable of inducing a protective humoral immune response in vaccinated subjects (Burioni *et al.*, 2008a).

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