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1 **Hepatitis C virus infection and tight junction proteins:**
2 **the ties that bind**

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25 junction

1 **Abstract**

2

3 The hepatitis C virus (HCV) is a major cause of liver diseases ranging from liver inflammation
4 to advanced liver diseases like cirrhosis and hepatocellular carcinoma (HCC). HCV infection
5 is restricted to the liver, and more specifically to hepatocytes, which represent around 80% of
6 liver cells. The mechanism of HCV entry in human hepatocytes has been extensively
7 investigated since the discovery of the virus 30 years ago. The entry mechanism is a multi-step
8 process relying on several host factors including heparan sulfate proteoglycan (HSPG), low
9 density lipoprotein receptor (LDLR), tetraspanin CD81, Scavenger Receptor class B type I (SR-
10 BI), Epidermal Growth Factor Receptor (EGFR) and Niemann-Pick C1-like 1 (NPC1L1).
11 Moreover, in order to establish a persistent infection, HCV entry is dependent on the presence
12 of tight junction (TJ) proteins Claudin-1 (CLDN1) and Occludin (OCLN). In the liver, tight
13 junction proteins play a role in architecture and homeostasis including sealing the apical pole
14 of adjacent cells to form bile canaliculi and separating the basolateral domain drained by
15 sinusoidal blood flow. In this review, we will highlight the role of liver tight junction proteins
16 in HCV infection, and we will discuss the potential targeted therapeutic approaches to improve
17 virus eradication.

18

1 **1. Introduction**

2
3 Discovered in the late 80's [1], the hepatitis C virus (HCV) is an enveloped virus with
4 a ~9.6kb single stranded positive sense RNA genome belonging to the *Hepacivirus* genus under
5 the *Flaviviridae* family [2]. HCV is one of the main causative agents of liver disease worldwide,
6 including cirrhosis and hepatocellular carcinoma. In some regions of the globe, the prevalence
7 reaches 2.3% making HCV infection a major problem of public health with approximately 71
8 million chronically infected people and more than 399,000 deaths each year according to the
9 World Health Organization (WHO). Moreover, WHO estimates that around 2 million people
10 were newly infected in 2015, and that the global incidence rate is 23.7 for 100,000. So far, no
11 vaccine is available. The new therapies based on direct-acting antivirals (DAAs) developed
12 within the last decade have significantly improved the treatment of chronic HCV infection and
13 disease outcome [3, 4]. However, those treatments can result in the selection of DAA-resistant
14 viral variants [5, 6], and the patients cured from the virus remain at risk of hepatocellular
15 carcinoma (HCC) development [7]. Thus, in spite of improvement of care and treatment, HCV
16 infection remains a global health problem. Research efforts still continue in order to deeper
17 understand the fine interactions of the virus with its host to eventually propose alternative or
18 complementary therapeutic approaches to fully eradicate the virus.

19 HCV-host encounter begins by the interaction of the virions and membrane-anchored
20 cell entry factors (figure 1). Several of these entry factors have been identified within the last
21 20 years and participate to a multistep process allowing virus attachment to the cell surface and
22 translocation to the cytoplasm. So far, more than 30 different proteins have been reported as
23 facilitating HCV entry [8, 9]. This number reflects the high complexity of the process and
24 highlights the different possibilities of anti-HCV therapeutic options. Among those different
25 entry factors CD81 [10], Scavenger Receptor class B type I (SR-BI) [11], claudin-1 (CLDN1)
26 [12], occludin (OCLN) [13] and Epidermal Growth Factor Receptor (EGFR) [14] are arguably
27 the best known and studied entry factors (figure 1). They participate, directly or indirectly, to
28 the binding of the virus at the surface of the cell and to its entry inside the cell. Essential factors
29 of CLDN family and OCLN belong to the tight junction protein family and are crucial players
30 in HCV entry.

31 Tight junctions (TJs) are important component of the liver. They ensure the cellular
32 structure of the liver and allow the homeostasis keeping the sinusoidal blood flow away from
33 bile canaliculi-drained bile acids. TJs are formed between adjacent epithelial cells present in
34 the liver, which are hepatocytes and cholangiocytes. Hepatocytes are the main epithelial cells

1 in the liver parenchyma representing about 70% of the liver cells and playing important
2 metabolic functions [15]. The hepatocyte basolateral membrane, drained by the blood flow, is
3 physically separated from the apical membrane, forming bile canaliculi, by TJs creating the
4 blood-biliary barrier [16]. This barrier enables hepatocytes to secrete the bile into bile
5 canaliculi. The bile is then transported to the gall bladder via bile ducts formed by
6 cholangiocytes. In bile ducts, cholangiocyte intercellular TJs maintain a selective permeability
7 barrier between the liver interstitial tissue and the bile duct lumen [17]. Liver cells organization
8 and maintenance of TJs integrity are vital for liver functions, and dysfunction of TJs can have
9 pathophysiological consequences [18].

10 TJs connect adjacent cells through cytoskeletal proteins, membrane-associated
11 intracellular proteins and integral membrane proteins [19]. Apical F-actin, actin-binding
12 proteins and apical microtubules are the main component of the TJs-linked cytoskeleton
13 proteins [20, 21]. Several membrane-associated proteins are involved in TJs formation (for
14 review [20]), among them the zona-occludens (ZO) protein ZO1 was the first identified TJ-
15 associated protein [22] participating in formation of the cytosolic plaque. This cytosolic plaque
16 is a complex network of several proteins including ZO2, ZO3, ZO1-associated nucleic acid
17 binding protein (ZONAB), Cingulin. This network makes a bound between cytoskeletal
18 proteins F-actin and microtubules, and the cytoplasmic domains of integral membrane proteins
19 involved in TJs formation. Among the different integral membrane proteins forming the
20 transmembrane strands, the tetraspan proteins of CLDN family and the MARVEL domain
21 proteins occludin, tricellulin (also known as MARVEL domain-containing protein 2 or
22 MARVELD2) and MARVELD3 are the main components [23-25]. It is well documented that
23 HCV can use several TJs integral proteins for cell entry especially OCLN [13], CLDN1 [12],
24 CLDN6 and CLDN9 [26, 27]. However, given liver hepatocyte expression of CLDN6 and
25 CLDN9 is very low compared to CLDN1, this later appears to be the main CLDN family protein
26 mediating HCV entry [28-30]. A recent report also suggests HCV entry could be mediated by
27 CLDN12 [31]. However, further investigations need to be performed in order to determine the
28 relevance for entry into liver hepatocytes. In this review we will highlight the function of the
29 TJs-associated proteins involved in HCV infection of the liver, and we will provide an overview
30 of the potential therapeutic approaches currently developed to target TJs in order to improve
31 the already existing therapeutic arsenal against HCV.

2. HCV infection: attachment steps and TJ proteins engagement.

2.1 HCV attachment to hepatocytes

HCV entry is a complex multistep process beginning by a non-specific binding of the virus to heparan sulphate proteoglycan (HSPG), especially Syndecan 1 and 4, and LDLR at the surface of hepatocytes (figure 1 and [32-35]). Indeed, the blood-circulating form of the viral particles is associated with lipids, apolipoproteins (ApoA1, ApoB, ApoC1/2/3, ApoE) and cholesterol, and is known as lipoviral particles or LVPs (for review [36]). Because of their particular composition, LVPs efficiently bind to HSPG, through interaction with ApoE and ApoC1, and LDLR *via* LVPs-associated ApoE and ApoB, even though this latter is not essential to LVPs formation and infectivity [33, 37-40]. Following this first attachment step, a virus-specific binding step occurs through a physical interaction between the HCV E2 glycoprotein and SR-BI [11]. To this regards, SR-BI is considered a true HCV receptor (figure 1). The interaction of LVPs with SR-BI also occurs through ApoE and ApoB binding, but the interaction with E2 is crucial for the subsequent SR-BI function at a post-attachment step [41, 42]. Through the SR-BI-mediated function of cholesterol transfer activity, the physical interaction with E2 is thought to be involved in lipoproteins dissociation from the LVPs in order to expose the CD81-binding domain of E2 [43, 44]. Following SR-BI engagement and LVPs lipoproteins content modification, the virus subsequently binds to CD81 through direct interaction with E2 [45-48]. Therefore, CD81 is considered like SR-BI as a “classical” HCV receptor (figure 1). Moreover, it was demonstrated that three amino-acid residues within the HCV E1 glycoprotein, forming the E1-E2 heterodimer located in the viral envelop, are important for the physical interaction of this heterodimer with both SR-BI and CD81 [49]. CD81 engagement by HCV E1-E2 activates EGFR [50], which participates to the formation of CD81-CLDN1 co-receptor complex at the basolateral membrane [14]. It was recently suggested, using a polarized hepatoma cell line organoid model, that the HCV-SR-BI-CD81-EGFR (HSCE) complex migrates to close proximity to TJs to associate with CLDN1 [51]. Whether the virus indeed interacts with TJs-forming CLDN1 as suggested for hepatoma cell lines or usurps basolateral CLDN1 for cell entry as shown for human hepatocytes [30, 52, 53] is not yet clearly defined (figure 1). Even though its role is not completely deciphered, EGFR activation, along with the activation the small GTPase H-Ras and Rho GTPases Rac, Rho and CDC42 [54, 55], is believed to participate to actin remodelling facilitating the lateral movement of the HSCE complex and to further HCV internalization (figure 1 and [51]).

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2.2 HCV post-attachment and lateral diffusion to TJs

2.2.1 CLDN1 as an HCV co-receptor

CDLN1 is a 22.7 kDa tetraspan integral membrane protein expressed by many different organs including the liver where it participates to the blood-bile barrier formation and serves as an essential factor for HCV entry. The CLDN1 protein is made of one short N-terminal intracellular region of 7 residues, 4 transmembrane domains, a short intracellular loop encompassing 13 amino-acids (aa), one large and one small extracellular loops (EL) 1 and 2 (EL1, EL2) comprising 53 and 27 residues respectively, and a short C-terminal intracellular region of 27 aa (figure 2). CLDN1 was the first integral membrane TJs-associated protein identified as an HCV entry factor [12]. It was described as a late entry factor, to the opposite of HSPG, LDLR, SR-BI and CD81 that play a role in early attachment of the virus to the cell surface (for review [56]). Although widely described to be associated to TJs, CLDN1 also exists as a TJ-free protein on baso-lateral side of hepatocytes [57]. Indeed, a recent study demonstrated that some members of the CLDN family, including CLDN1, when newly synthesized in the cell, are not directly added to the junction strand network but are rather delivered to the lateral membrane and then traffic to enter the TJs through the basal side of the junction strand network [58].

Given the current knowledge on HCV entry and on the localization of CLDN1 at the cell surface, it appears that the lateral movement of the HSCE complex leads to the physical rapprochement with baso-lateral membrane-bound CLDN1. The close proximity between HCV and CLDN1 is indeed an absolute necessity for the subsequent HCV entry into the cell [52, 59]. More importantly, the physical interaction of CD81 [52] and of HCV E1-E2 heterodimer with CLDN1 [49], is a pre-requisite to the establishment of viral infection. CLDN1 is not a species-specific host factor since rat, mouse, hamster and tupaia CLDN1 are able to support HCV infection [60]. The CLDN1 region important for HCV entry has been mapped within EL1 (figure 2). Residues I₃₂ and E₄₈ have been shown to be involved in infection efficiency by facilitating the association with CD81 [12, 52]. Whether these residues are involved in HCV-CLDN1 interaction is not known. Moreover, 9 residues localized in the EL1 (i.e W₃₀-I₃₂-D₃₈-EGLW₅₁-C₅₄-C₆₄) were shown to mediate the receptor activity of CLDN1 [12, 49, 61]. Those residues belong to a hydrophobic motif highly conserved among members of the CLDN family. Among those 9 aa, the residues W₃₀-I₃₂-D₃₈-EG₄₉-W₅₁ promoting HCV entry through CD81 interaction were identified using an *in silico*-based mutagenesis approach [62]. The mutation

1 of residues L₅₀, C₅₄ and C₆₄ does not disrupt CD81-CLDN1 interaction but abrogate HCV entry,
2 possibly by modifying the CD81-CLDN1 interface region or by impeding the interaction with
3 HCV E1-E2 heterodimer. Moreover, residues C₅₄ and C₆₄ were shown to form a disulfide bridge
4 in CLDN1 EL1 [63]. This disulfide bridge is crucial for the proper structure of CLDN1 and
5 could be essential for efficient HCV-CLDN1 interaction. EL1 is involved in the CLDN1 *cis*
6 (intracellular) and *trans* (intercellular) association essential for the formation of TJs [63, 64].
7 Although EL2 is believed to mediate cell-cell contact, and EL1 controls the overall resistance
8 and charge selectivity between cells (for review [65]), EL1-mediated *trans*-association between
9 CLDN1 of neighbouring cells has been shown to be crucial for paracellular tightening with
10 strong EL1-EL1 interaction reaching values in the nanomolar range [63]. This can explain that
11 polarized cells with an increased localization of CLDN1 in TJs are less susceptible to HCV
12 infection [66]. Therefore, the model of HCV entry describing an interaction with CLDN1
13 localized on the baso-lateral membrane followed by a lateral diffusion towards TJs is much
14 more likely to occur than a direct interaction with TJs-associated CLDN1. It is not yet clear
15 whether HCV can directly bind CLDN1 or if the physical interaction is mediated through the
16 binding of HCV to CD81 [67-69]. This first attachment step to CD81 could expose some key
17 residues of the HCV E1-E2 heterodimer allowing CLDN1 engagement, or the virus could
18 recognize the CD81-CLDN1 co-receptor complex leading to physical interaction with CLDN1.
19 However, once the contact of the HSCE complex with CLDN1 is established, the lateral
20 diffusion of this complex continues along the lateral membrane until it reaches the OCLN
21 protein localized close to the TJs. The virus is then co-endocytosed with CD81 and CLDN1
22 through clathrin-mediated endocytosis (figure 1 and [51, 70, 71]). While HCV endocytosis
23 involves clathrin positive and Rab5 positive endosomes [51], it was recently shown that TJs-
24 associated CLDNs are cross-over endocytosed, from 2 adjacent cells, in a double membrane
25 vesicle into one of the adjacent cells without disruption of the *trans*-association [72]. These
26 observations are in agreement with the aforementioned model of HCV engagement with the
27 non-junctional pool of CLDN1. However, it was shown that the abrogation of cell-cell contact
28 and, therefore, the abrogation of TJs formation, renders the cells less susceptible to HCV
29 infection [73].

30 CLDN1-mediated HCV entry has also been suggested to be dependent on the
31 intracellular C-terminal tail. Indeed, the extremity of this latter is composed of four residues
32 (i.e K₂₀₈-D₂₀₉-Y₂₁₀-V₂₁₁) forming a PSD95-Dgll1-ZO1 (PDZ) binding domain (figure 2), which
33 has been shown to interact with the Sec24C protein, a component of the coat protein complex
34 II (COPII) vesicular transport system [74]. As shown in this report, the knock-down of Sec24C,

1 as well as the mutation of the last two aa Y₂₁₀-V₂₁₁ of CLDN1 C-terminal region into alanine
2 residues, seem to have an impact on the proper localization of CLDN1 at the cell surface and
3 on HCV entry in Huh7.5.1 hepatoma cells [74]. However, Sec24C knock-down and YV
4 residues mutation do not drastically change the cell surface concentration of CLDN1, as shown
5 by flow cytometry analysis using an antibody specifically binding surface CLDN1. Moreover,
6 some studies have reported that a C-terminal deletion mutant of CLDN1 does not prevent
7 membrane localization of CLDN1 or HCV entry and infection [12, 75]. Therefore, the role of
8 the CLDN1 C-terminal tail and PDZ binding domain for CLDN1 trafficking to the plasma
9 membrane and the impact on HCV entry have still to be investigated to determine the relevance
10 of those findings regarding infection of the liver.

11 CLDN1 has also been demonstrated as an important factor for the spreading of the
12 infection. Indeed, the virus is able to enter in a non-infected cell as a cell-free virus, so-called
13 cell-free infection. But the infection can also be transmitted from an infected cell to an adjacent
14 non-infected cell through the so-called cell-cell transmission process. Like CD81 and SR-BI
15 [43, 76-78], CLDN1 is able to mediate HCV cell-cell transmission [30, 79-81], a process which
16 has been shown to be resistant to neutralizing antibodies targeting the HCV E1-E2
17 glycoproteins [82, 83]. Therefore, given its implication in the HCV infection process, from cell
18 entry to spreading, CLDN1 is considered as an indispensable infection factor for HCV.

19 *2.2.2 OCLN as an HCV co-receptor*

20 OCLN is a ~ 60 kDa MARVEL domain-containing integral membrane protein
21 expressed by many organs and was described as an essential factor for HCV entry [13]. The
22 OCLN protein comprise a N-terminal intracellular region of 66 aa, 4 transmembrane domains
23 corresponding to the MARVEL domain, a short intracellular loop comprised of 10 aa, 2
24 extracellular loops EL1 and EL2 comprising 46 and 48 residues respectively, and a long C-
25 terminal intracellular region of 257 aa (figure 3 and [84]). Like CLDN1, OCLN is mainly
26 associated with TJs [24], and can also be found at the baso-lateral membrane of epithelia [85].
27 However, OCLN does not show the same pattern of localization as CLDN1 in TJs. Indeed,
28 while CLDN1, and other CLDN proteins, have a continuous localization responsible for the TJ
29 strands formation, OCLN associates with CLDN-formed strands in a discontinuous and patchy
30 manner [86, 87]. Moreover, OCLN shows a much faster turnover than CLDNs [87, 88], and
31 newly synthesized OCLN appears first associated with TJs and later with lateral membrane
32 during recycling [58]. OCLN has been demonstrated as non-essential for proper formation of
33 morphologically intact TJs but seems to have a crucial role in barrier function and stability of

1 TJs [89]. OCLN was also demonstrated to have a high degree of *cis*-interaction with CLDN1
2 and to impact on the CLDN1 strands formation, making more longitudinal, more parallel and
3 less continuous meshes [90]. Moreover, the same study revealed that OCLN molecules are able
4 to *trans*-interact when expressed on 2 adjacent cells, participating in the locking of TJs [90].
5 However, the knockdown of OCLN does not seem sufficient to allow the transepithelial passage
6 of macromolecules like 10 kDa Dextran [91]. In the whole TJs organization, OCLN can
7 therefore be seen as a reinforcing and stiffening agent.

8 OCLN was the second TJ-associated protein described as an entry factor for HCV [13].
9 At least 8 splicing forms of human OCLN and 2 distinct promoters have been described in the
10 literature [92-97]. The expression of these isoforms in normal liver samples, and their relevance
11 for HCV infection have been explored [97]. The authors of the study showed that among those
12 8 isoforms 6 were detected by both RT-qPCR and western-blot and that only the 2 isoforms
13 localizing to the plasma membrane were able to mediate HCV entry in an *in vitro trans*-
14 complementation assay. Moreover, they demonstrated that the concomitant expression of
15 permissive forms of OCLN with non-permissive forms does not alter HCV entry efficiency
16 [97]. In permissive Huh7.5.1 hepatoma cell line, OCLN knockout completely abrogate HCV
17 infection [80]. To the opposite of CLDN1, OCLN has to be from human origin to enable HCV
18 infection. Especially, cells overexpressing the mouse version of OCLN (mOCLN), instead of
19 the human version (hOCLN), were shown to be resistant to HCV infection [13]. Several regions
20 of OCLN were mapped to facilitate HCV entry (figure 3). First, the hOCLN EL2 was shown to
21 enable HCV infection [13, 80, 98]. Indeed, the replacement of human EL2 in hOCLN by mouse
22 EL2 drastically decreases the infectious efficiency of the virus [13, 98]. Residues AA₂₂₄ in
23 hOCLN were identified to be of great importance for the efficacy of infection [99]. As well,
24 residues C₂₁₆ and C₂₃₇, which are thought to form a disulfide bridge stabilizing the EL2 and to
25 be involved in OCLN *cis*- and *trans*-interaction [100], were also shown to participate in the
26 HCV entry process [101]. However, whether the virus physically interacts with OCLN is not
27 yet clearly defined. Although a direct binding of the HCV E1-E2 heterodimer on OCLN has
28 never been demonstrated, a study suggests a direct interaction of OCLN with the virion using
29 isolates specific dependency on OCLN [101]. Moreover, another study demonstrated that an
30 OCLN mimic protein containing EL1 and EL2 docked on the α -helical bundle of HIV1 gp41
31 could impair HCV infection by interacting directly with the virions, abolishing their capacity
32 to bind the cells [102]. This strongly suggest a direct interaction of HCV viral particles with
33 OCLN. Importantly, the authors also showed that both EL1 and EL2 are required for OCLN-
34 HCV interaction given the mimic proteins harbouring only EL1 or EL2 were much less efficient

1 at inhibiting infection. Therefore, even though only OCLN EL2 was shown to be absolutely
2 required for enabling HCV entry [13, 80, 98], EL1 could have an important role in the process
3 by adjusting the conformation of EL2 during HCV infection [102].
4

5 Beyond the implication of the OCLN extracellular domains, which may be involved in
6 physical interaction with the virus, the C-terminal intracellular part also seems important for
7 HCV entry (figure 3). Indeed, a study using OCLN deletion mutants and point mutations
8 showed that the OCLN-C15, retaining only the 15 first aa of the C-terminal region, does not
9 support HCV infection because of an altered localization at the cell surface and accumulation
10 in the *trans*-Golgi network (TGN) [103]. In contrast, OCLN-C18, retaining the 18 first aa of
11 the C-terminal domain, properly localized at the plasma membrane and led to HCV infection.
12 Moreover, when mutated in the context of OCLN-C18, residues I₂₇₉ and W₂₈₁ led to the
13 accumulation in the TGN. The same mutations in the context of full-length OCLN had no
14 significant effect on plasma membrane localization of the protein but affected the ability of
15 OCLN to restore the permissivity to HCV infection in OCLN-KO Huh7 hepatoma cells. These
16 data point out the importance for HCV entry of key residues located at the beginning of the C-
17 terminal domain. The same study also demonstrated that the defect of TJs localization of
18 OCLN-C18 in polarized cells does not affect its ability to mediate HCV infection, indicating
19 that TJs localization of OCLN is not a pre-requisite for HCV entry [103]. These data combined
20 with the fact that abrogation of TJs formation renders the cells less susceptible to HCV infection
21 highlight the need for the virus for properly formed TJs. However, it also suggests HCV
22 engages with non-junctional CLDN1 and OCLN before being endocytosed at the lateral
23 membrane, near TJs.

24 So far, it is not well understood whether HCV entry is dependent on OCLN-CLDN1
25 *cis*-interaction. In a virus-free context, it was demonstrated that CLDN1 and OCLN are able to
26 interact through their respective extracellular domains [52, 104]. Interestingly the residues I₃₂
27 and E₄₈ in CLDN1 EL1, which are involved in CLDN1-CD81 interaction and in HCV entry,
28 were shown to promote CLDN1-OCLN *cis*-interaction [52]. On the other hand, a 27 aa peptide
29 mimicking the portion 53-80 of the CLDN1 EL1 was shown to associate with both CLDN1 and
30 OCLN, and to be able to interfere with the formation of functional TJs [104]. Those data suggest
31 the 53-80 portion of CLDN1 represents the natural contact region with OCLN and that the I₃₂-
32 E₄₈ residues could help stabilizing this interaction (figure 2). In addition, a study demonstrated
33 that a natural lipocyclodepsipeptide named MA026, isolated from the fermentation broth of
34 *Pseudomonas* sp. RtIB026, and able to inhibit HCV infection, could efficiently bind to

1 immobilized CLDN1-GST using SPR analyses [105]. The binding region was further
2 characterized using phage display, and a peptide containing the VFDSL sequence, conserved
3 in the CLDN1-OCN interface region of CLDN1 EL1 (aa 66-71), was identified. Those data
4 suggest that interfering with the potential region of contact between OCN and CLDN1 is
5 detrimental for HCV entry and, therefore, that a direct OCN-CLDN1 interaction may occur
6 during the infection process, after lateral diffusion near the TJs.

7 Like CD81, SR-BI and CLDN1, OCN has been shown to mediate cell-cell
8 transmission of the virus [80, 106]. Indeed, it was demonstrated that OCN-KO Huh7.5.1
9 hepatoma cells could not be infected through cell-cell transmission when co-culture with HCV-
10 producing Huh7.5.1 wild-type cells. Therefore, OCN is like CLDN1 an essential factor
11 enabling the establishment of a persistent HCV infection.

12

13 **3. Current treatments, inhibition of entry and TJ proteins as therapeutic targets for** 14 **HCV infection.**

15 *3.1 Limitations of current DAAs-based HCV treatments*

16 Since the advent of DAAs, developed in the last 10 years, approaches to treat chronically
17 HCV-infected patients have drastically changed [4, 107, 108]. However, even though the
18 success of these therapies is undeniable for most of the patients, some of them still fail to
19 achieve a sustained virological response (SVR) despite receiving the latest approved treatment
20 regimens. A reason for treatment failure lies within the emergence of DAA-resistant viral
21 variants and the occurrence of resistance-associated substitutions (RASs) in the viral genome
22 [5, 6, 109]. Several classes of molecules target different viral proteins, and so far each of them
23 have been associated with RASs for each HCV genotype [110]. The appearance of RASs is
24 mainly attributable to the high replication rate and the poor proof-reading activity of the viral
25 polymerase NS5B leading the virus to exist within the same host as a population of slightly
26 different viral-variants called quasi-species [111]. Therefore, RASs may develop during the
27 treatment, but can also naturally pre-exist before the treatment, impairing DAAs efficiency
28 [112-114]. Limitations of currently approved treatment regimens also include access to DAAs
29 in low-resource countries. Indeed, only 10% of HCV-infected patients have access to DAAs
30 [115]. Moreover, some populations of difficult-to-treat patients persist and require specific
31 attention, like patients with advanced or decompensated cirrhosis, first-line treatment-given
32 patients who failed previous DAAs regimen, people with advanced renal insufficiency or
33 kidney failure requiring renal transplantation, children and adolescents [116-118]. It was also

1 demonstrated that despite achievement of SVR and complete viral elimination, patients remain
2 at risk of developing HCC, in particular patients with advanced fibrosis. Although SVR is
3 linked to a reduced risk of *de novo* HCC development, the disease can occur more than 10 years
4 after viral clearance [7, 119, 120]. Despite the overall promising results of the current DAAs-
5 based treatment, there is still room for improvement and development of new therapeutic
6 options.

7 3.2 Inhibition of HCV entry: alternative therapeutic approach

8 Host-targeting agents (HTAs) are the focus of current researches aiming at offering
9 alternative or complimentary therapeutic approaches for HCV-infected patients (for review
10 [121]). As previously described, TJ proteins CLDN1 and OCLN are host proteins absolutely
11 required for the HCV infection process. CLDN1 and OCLN indeed enable entry and spread of
12 the virus. CLDN1 and OCLN are therefore key players within the viral life cycle making them
13 very interesting targets for potential HCV therapies. During the last 10 years, in parallel of the
14 emergence of DAAs, HTAs aimed at inhibiting HCV entry have been developed. In 2010, the
15 small molecule ITX5061, an antagonist of SR-BI, was the first HTA to enter clinical trial for
16 HCV infection with limited efficacy for chronically infected patients, but with a substantial
17 effect on viral evolution in patient undergoing liver transplantation [122, 123].
18 Sylimarin/silibinin, a natural product extracted from milk thistle seeds, known to help
19 recovering from liver damage after intoxication or chronic liver disease [124, 125], has been
20 demonstrated to have an inhibitory effect on clathrin-mediated viral entry [126]. Silibinin also
21 entered clinical trial phase I and phase II but has shown conflicting results [127-130]. Other
22 compounds, like ezetimibe targeting the HCV entry factor Niemann-Pick C1 like 1 [131], or
23 the malaria drug chloroquine that affects clathrin-mediated endocytosis and virus-mediated
24 autophagy [132, 133], have been both investigated in clinical trial phase I for ezetimibe and
25 phase IV for chloroquine, with poor success [134, 135]. Since then, strategies aiming at
26 preventing HCV infection using monoclonal antibodies (mAbs) targeting HCV entry factors
27 have been set up. The first mAbs to be developed targeted CD81 and SR-BI. These anti-CD81
28 and SR-BI mAbs were shown to prevent HCV entry and spreading *in vitro* and *in vivo* in human
29 liver chimeric mice [43, 76, 136-140]. Interestingly, that class of HTAs was demonstrated to
30 efficiently synergize with DAAs both *in vitro* and *in vivo* [141].

31 3.3 TJ proteins: therapeutic targets for HCV infection

32 3.3.1 CLDN1 as a potential host target for new therapeutics

1 In parallel to the development of mAbs targeting CD81 and SR-BI, CLDN1-specific
2 mAbs were developed. In particular, our laboratory generated several CLDN1-specific mAbs
3 after immunization of rats with plasmids encoding human CLDN1 [142]. One of these CLDN1-
4 specific mAbs, namely OM-7D3-B3 (Table 1), was shown to be very potent at inhibiting HCV
5 infection in primary human hepatocytes and demonstrated its ability to cure persistent HCV
6 infection in human liver chimeric mice without toxicity towards engrafted human hepatocytes
7 [30]. Importantly, there was no appearance of viral mutants escaping the mAb-mediated
8 inhibition of infection. This CLDN1-specific mAb was also shown to impair viral spreading by
9 inhibiting cell-cell transmission. Given OM-7D3-B3 showed cross-reactivity with mouse
10 CLDN1 (mCLDN1), safety was analysed in regular Balb/C mice in which no toxicity could be
11 detected. Indeed, this antibody does not impair TJ integrity of function as shown by measuring
12 *trans*-epithelial electrical resistance in CLDN1-expressing Caco-2 cells. This is most likely due
13 to absent targeting of TJ CLDN1 as shown by immunofluorescence on polarized HepG2 cells
14 and electron microscopy on hepatocytes from human liver chimeric mice [30]. Using epitope
15 mapping, it was shown that the CLDN1 region bound by OM-7D3-B3 comprises residues W₃₀,
16 Y₃₃, Y₃₅, D₃₈, G₄₉, L₅₀, W₅₁, C₅₄ and C₆₄, which were shown as important for the proper binding
17 of the antibody (figure 2 and [142]). As described previously, this region is crucial for HCV
18 receptor function of CLDN1 by enabling CD81-CLDN1 interaction and by binding HCV
19 virions. In FRET analyses CLDN1-specific mAb prevents CD81-CLDN1 interaction
20 explaining its potency at inhibiting infection [30]. In order to enable the clinical development
21 of this mAb, a humanized version of OM-7D3-B3 was engineered on a human IgG4 S₂₂₈P
22 backbone [79]. The humanized version, named H3L3 (Table 1), was as efficient as the rat OM-
23 7D3-B3 at inhibiting HCV infection. H3L3 exhibited a pan-genotypic activity against HCV
24 entry in primary human hepatocytes, was efficient at inhibiting DAA-resistant HCV strains and
25 could synergise with current DAAs. Moreover, like the OM-7D3-B3, H3L3 cured persistent
26 infection in human liver chimeric mice in monotherapy, demonstrating the potential interest of
27 such an approach for fighting HCV chronic infection. H3L3 could be efficiently combined with
28 neutralizing antibodies targeting the HCV glycoprotein E2 in the *in vivo* model of human liver
29 chimeric mice to prevent HCV infection, providing the pre-clinical proof-of-concept for the use
30 of this combination therapy, potentially with DAAs, during organ transplant setting [143].
31 Similar to *in vivo* experiments with the rat parental mAb, no adverse effects were detected. This
32 approach could be a highly valuable strategy to prevent post-transplantation reinfection and to
33 help alleviating the urgent demand for organ transplants by allowing organ graft from HCV
34 positive donors while drastically reducing the risk of viral escape. Furthermore, given the

1 complementarity of target and mechanism of action this approach may also address resistance
2 in DAA failure.

3 Hötzel *et al.* described the production of CLDN1-specific mAbs after baculovirus
4 expression of CLDN1 and phage display library sorting (Table 1). They could obtain 6 different
5 Fab clones, further engineered as fully human IgG1, able to bind cell-expressed CLDN1, some
6 of them being able to bind mCLDN1 [144]. They demonstrated that affinity matured antibodies
7 could efficiently block *in vitro* HCV infection in human hepatoma cell line.

8 Paciello *et al.* also generated anti-CLDN1 antibodies using the phage display approach
9 [145]. They recovered 20 different scFvs clones and selected 12 clones that they converted into
10 fully human IgG4. Ten of these clones showed good apparent affinity in the low-nanomolar
11 range and 6 of them were efficient at blocking *in vitro* HCV entry in Huh7.5 hepatoma cell line
12 (Table 1). The different anti-CLDN1 mAbs were shown to bind different epitopes on CLDN1
13 ELs but the bound regions were not specifically defined. However, they showed that, combined
14 with an anti-SR-BI mAb, these CLDN1-specific mAbs could either reduce or increase *in vitro*
15 HCV infection, highlighting the fact that care must be taken when combining therapeutic
16 approaches.

17 Kondoh and colleagues developed mouse monoclonal antibodies against human
18 CLDN1 (hCLDN1) after immunisation of mice with a hCLDN1 expression plasmid [146].
19 They isolated 4 different clones of mAbs, namely 2C1, 3A2, 5F2 and 7A5 (Table 1), that were
20 shown to efficiently bind hCLDN1 but not mCLDN1. They investigated the CLDN1 epitopes
21 targeted by their mAbs and determined that the binding regions were shared between EL1 and
22 EL2 (figure 2), more specifically the region encompassing residues S₇₄ in EL1 and M₁₅₂ in EL2,
23 which are different between hCLDN1 and mCLDN1 (i.e N₇₄, L₁₅₂). These mAbs were able to
24 block HCV infection *in vitro* in Huh7.5.1 hepatoma cells, without disrupting TJs function. Two
25 of these mAbs, clones 3A2 and 7A5, were also shown to prevent HCV infection in a human
26 liver chimeric mouse model, even though viral relapse could be observed for 3 mice out of 4
27 for 7A5, and 1 mouse out of 4 for 3A2. Based on these data, in order to accelerate their clinical
28 development, they further engineered 2 of these mAbs, clone 2C1 and 3A2 (Table 1), into either
29 Fab fragments or human chimeric IgG1 or IgG4 and investigated their effect on HCV infection
30 [147]. They demonstrated that only the full IgGs were able to efficiently block HCV entry *in*
31 *vitro* in Huh7.5.1 hepatoma cells, to the opposite of Fab fragments that showed only a limited
32 effect. They showed that both IgG1 and IgG4 clones had an equivalent efficacy at inhibiting
33 HCV infection *in vitro*. Moreover, they engineered these 2 clones on a mutated IgG4 backbone
34 named S₂₂₈P, known to avoid the generation of bispecific antibodies *in vivo* [148]. The mutated

1 IgG4 anti-CLDN1 mAbs were as efficient as IgG1 and wild type IgG4 to prevent HCV infection
2 *in vitro*. They also showed that mutated IgG4 and wild type IgG4 clones activated FcγRIIIa
3 100-fold less than the IgG1 counterparts, indicating the IgG4 subclass retains the inhibitory
4 activity on HCV infection with potentially reduced side effects due to the activation of FcγRIIIa,
5 thereby avoiding antibody-dependant cellular cytotoxicity (ADCC).

6 Other approaches applied CLDN1-derived peptides or recombinant proteins to prevent
7 HCV infection. A study by Si *et al.* showed that an 18-amino acid peptide derived from the
8 region 1-18 of CLDN1, named CL58 (Table 1), was very potent at inhibiting entry and spread
9 of different HCV genotypes *in vitro* in Huh7 hepatoma cells at inhibitory concentration 50
10 (IC₅₀) around 2μM [149]. Interestingly, CL58 does not retain the extracellular region of CLDN1
11 responsible for HCV entry but corresponds to the intracellular N-terminal part and half of the
12 first transmembrane domain. The authors demonstrated that CL58 did not interfere with HCV
13 replication, but rather inhibited HCV infection by interfering with the fusion process of the
14 virus with endosomes (figure 1). They also showed the peptide did not disrupt TJs integrity.
15 Interestingly, when co-overexpressed in 293T cells the peptide is co-immunoprecipitated with
16 the HCV glycoprotein E1 and E2, indicating it can interact with the virus. This could explain
17 its ability to block HCV infection at the endosomal step. Indeed, the HCV E1E2 glycoproteins
18 are believed to act as fusion proteins triggering endosomal fusion (for review [9, 150]), and
19 CL58 could interfere with this event by a mechanism that has yet to be determined.

20 Two studies by Bonander *et al.* and Tawar *et al.*, also demonstrated that fully
21 recombinant CLDN1 or a protein retaining the large EL from CD81 and EL1-EL2 from CLDN1
22 could impair HCV infection [151, 152]. Indeed, Bonander and colleagues produced a fully
23 recombinant CLDN1 protein in the yeast strain *Pichia pastoris* X33 and extracted CLDN1 to
24 form proteoliposomes (Table 1). They showed that under these conditions CLDN1 folded
25 properly and could be recognized by CLDN1-specific mAb. Moreover, these CLDN1-
26 containing proteoliposomes were shown to inhibit HCV infection at the entry level in Huh7.5
27 hepatoma cells. However, the proteoliposomes-mediated inhibition was not specific to HCV
28 given vesicular stomatitis virus pseudo-particles entry was also block using this approach [151].
29 The other study by Tawar and colleagues engineered a recombinant protein produced in
30 bacterial strain BL21 (DE3) and containing the CD81 large EL and CLDN1 EL1-EL2 (Table
31 1). Originally developed to detect anti-host cell receptor auto-antibodies in chronically HCV
32 infected people by ELISA, this recombinant protein was also shown to block HCV infection *in*
33 *vitro* in Huh7.5.1 cells in a dose dependent manner when mixed with the virus before

1 inoculation. It is believed that the recombinant protein adopts a conformation modelling the
2 CD81-CLDN1 co-receptor complex able to bind HCV virions.

3 Some other studies have shown that non-specific CLDN1 compounds were able to
4 modulate the available pool of CLDN1 at the plasma membrane by inducing a proteasome-
5 mediated degradation or a defect of recycling causing intracellular accumulation of CLDN1
6 [75, 153]. Indeed, it has been demonstrated that a pre-treatment of Huh7.5.1 hepatoma cells
7 with acetylsalicylic acid, or aspirin, is able to impair HCV entry by decreasing the total cellular
8 content of CLDN1 [153]. The authors showed the effect is reversed by exposing the cells to the
9 proteasome inhibitor MG132, indicating the mechanism of action of aspirin is mediated by
10 proteasomal degradation of CLDN1. Another study recently identified serotonin 6 receptor (5-
11 HT6) antagonists as HCV entry inhibitors [75]. Two closely related compounds, namely
12 SB258585 and SB399885, belonging to the family of piperazinylbenzenesulfonamides, have
13 been demonstrated to inhibit a late HCV entry step. Indeed, these 2 molecules have been shown
14 to alter the recycling of CLDN1 and to induce its intracellular accumulation. The authors
15 showed the effect is independent from 5-HT6, which was not detected in liver biopsies or in
16 their cell-based model. However, they showed that inhibition of PKA, which is involved in
17 CLDN1 recycling, recapitulates the effects observed with SB258585 and SB399885. Therefore,
18 they concluded these compounds could act through PKA inhibition by antagonizing one or
19 several other GPCRs associated with G α stimulatory protein.

20 3.3.2 OCLN as a potential host target for new therapeutics

21 Similar to CLDN1, OCLN is a candidate target for the development of therapeutic
22 antibodies for inhibition of HCV infection. To this regard, the group led by Masayoshi
23 Fukusawa developed anti-OCLN mAbs after immunisation of rats with plasmid encoding
24 hOCLN [154]. They isolated 4 different clones, named 1-3, 32-1, 37-5 and 44-10, which could
25 efficiently bind cells endogenously or over-expressing hOCLN (Table 1). Clone 1-3 bound
26 OCLN EL2, while the 3 others bound EL1 (figure 3). Moreover clone 1-3 was shown to be able
27 to bind mOCLN, while the 3 others were not. The 4 mAbs were demonstrated to impair HCV
28 infection *in vitro* in Huh7.5.1 cells without affecting TJs integrity or cell viability. However,
29 clone 1-3, which binds OCLN EL2, was shown to be more efficient at inhibiting HCV entry
30 compared to the 3 other clones targeting EL1. This is in agreement with the HCV entry process
31 relying essentially on OCLN EL2 (see section 2.2.2). The authors also showed that clones 1-3
32 and 37-5 were able to block HCV spreading *in vitro* and that they could prevent HCV infection

1 *in vivo* in a human liver chimeric mouse model. They further characterized the binding
2 properties of these 4 mAbs and demonstrated that they all bind crab-eating monkey OCLN,
3 while only clone 1-3 binds mouse and rat OCLN, but none could bind dog OCLN [155].
4 Binding capacity of clone 1-3 was shown to be affected by mutation of P₂₂₂, A₂₂₃, C₂₁₆ and C₂₃₇
5 (figure 3), the 3 later being important for successful HCV infection (see section 2.2.2). EL1
6 binding mAbs, clones 32-1, 37-5 and 44-10 were shown to target an epitope encompassing
7 residues Y₁₀₄, P₁₀₅, G₁₀₇, G₁₀₈, G₁₁₀ and F₁₁₁ (figure 3). Given these mAbs are less effective than
8 EL2-binding mAb clone 1-3 at inhibiting HCV infection, EL1 plays a less preponderant role in
9 HCV entry as previously suggested (see section 2.2.2). The same group further generated
10 humanized versions of clones 1-3 and 37-5 on a human IgG4 S₂₂₈P backbone [156]. These
11 humanized mAbs, named Xi 1-3 and Xi 37-5 (Table 1), retain their capacity at binding OCLN
12 EL2 and EL1 respectively and at inhibiting HCV entry *in vitro*, even though a slight increase
13 of IC₅₀ is observed. Importantly, given these mAbs are intended to be used in the frame of HCV
14 infection, they should avoid inducing antibody-dependant cell cytotoxicity (ADCC). Therefore,
15 the authors showed that their human IgG4-build mAbs were indeed impaired for FcγRIIIa
16 activation, indicating they should not lead to ADCC of mAb-bound cells.

17 Another group led by Hideki Chiba also generated anti-OCLN mAbs after immunization
18 of mice with a hOCLN EL2-derived peptide ALCNQFYTPAATGLYVD (aa 214-230) fused
19 to the keyhole limpet hemocyanin [157]. They could recover 5 different clones, named 15, 23,
20 46, 67 and 111, and assessed 3 different sub-clones of each for HCV entry inhibition. Only the
21 sub-clone 67-2 was shown to prevent HCV infection *in vitro* in a double-chamber culture
22 system as well as in 3D culture but was less efficient in a monolayer culture system (Table 1).
23 This argues in favour of the targeting of baso-lateral side-localized OCLN. Moreover, this mAb,
24 which cross-reacts with mouse OCLN, did not either affect cell viability or alter TJs barrier
25 function. Given the 67-2 mAb is not specific for hOCLN, the authors suggested this mAb could
26 bind the aa 216-222 region of OCLN encompassing de C₂₁₆, which has been shown to be
27 important for *cis*- and *trans*-interaction of OCLN (figure 3 and [100]). Given the C₂₁₆ is
28 involved in a disulphide bridge formation with C₂₃₇, which has been demonstrated to be
29 shielded and inaccessible from the outer space [85], the authors also speculated the 67-2 mAb
30 targets TJ-unengaged OCLN and eliminates this later by preventing the C₂₁₆-based OCLN
31 oligomerization irrespective of the presence or absence of HCV. They then assumed that HCV
32 infection relies on the presence of free rather than on TJ-engaged OCLN.

33 *3.3.3 TJ proteins as new therapeutic targets compared to other HCV entry factors*

1 CLDN1 and OCLN targeting compounds are promising therapeutic approaches to treat
2 HCV infection. However, as described earlier (see section 3.2), other entry factors have proven
3 to be attractive targets. The choice of the best target, and whether TJ proteins are better options
4 compared to the other entry factors must be discussed. Based on the current knowledge and the
5 available data, it remains difficult to definitely decide. However, while showing some
6 encouraging effect on HCV viral load, some entry factor inhibitors do not confer full protection
7 or enable viral eradication. For instance, a two-week pre-treatment of human liver chimeric
8 uPA-SCID mice with ezetimibe, a NPC1L1 inhibitor, before HCV inoculation only delay viral
9 emergence in the serum [131]. Moreover, discontinuation of ezetimibe treatment is
10 recommended when using DAAs (i.e daclatasvir, simeprevir, glecaprevir/pibrentasvir,
11 paritaprevir/ritonavir/ombitasvir plus dasabuvir, and sofosbuvir/velpatasvir/voxilaprevir)
12 because ezetimibe is a substrate of the OATP1B1 transporter responsible for the hepatic uptake
13 of drugs and endogenous compounds from the blood. OATP1B1 has been shown to be inhibited
14 by DAAs, which could lead to drug interaction with ezetimibe (for review [158]). Erlotinib, an
15 EGFR inhibitor, shows the same effect on HCV than ezetimibe. A one-week pre-treatment
16 followed by a three-week treatment after inoculation delay the kinetic of HCV infection [14].
17 Whether erlotinib can be used in combination therapy with DAAs or other HTAs has still to be
18 investigated. Like CLDN1- and OCLN-, CD81- and SR-BI-specific mAbs have been
19 preclinically tested in humanized liver mice and were shown to prevent HCV infection [136-
20 140]. They were not reported to induce toxicity in this mouse models, and an anti-CD81 was
21 shown to be safe in a transgenic mouse model to human CD81 [159]. However, further
22 investigations will be needed in humans to confirm the safety profile of these mAbs. So far, the
23 OM-7D3-B3 CLDN1-specific mAb was the only one to enable HCV clearance in persistently
24 infected uPA-SCID mice, and to that regard could be consider the best option for treating HCV
25 infection [30]. Nevertheless, it is currently difficult to determine which therapeutic approach is
26 the best and to which target priority should be given in any regimens for HCV infected patients.
27 It would be of interest to use combination therapies of several HTAs or DAAs/HTAs which can
28 synergized to achieve rapid and persistent HCV cure.

29

30 **4. Conclusions**

31 Despite the significant progress made to treat chronic infections, HCV remains a serious
32 concern for some populations, more than 30 years after its discovery. Indeed, development of
33 RASs and emergence of resistant virus, difficult-to-treat populations, and persisting risk of
34 HCC development are the factors that lead to promote novel therapeutic strategies. The HCV

1 life cycle is a very complex process, from virus entry to newly formed virions release.
2 Especially, the entry process is particularly orchestrated and involves more than 30 proteins,
3 highlighting as much possibility of host therapeutic targets. Among the host proteins involved
4 in HCV entry, TJs proteins CLDN1 and OCLN play a major role in mediating HCV entry into
5 the human hepatocyte. While the interactions between CLDN1 and OCLN and the virus have
6 been well characterized, the exact kinetics and trafficking are still only partially understood.
7 Targeting CLDN1 or OCLN by mAbs or peptides offer opportunities for antiviral therapies
8 addressing current unmet medical needs. DAAs could indeed be combined with CLDN1- or
9 OCLN-specific molecules to further improve efficacy and reduce treatment duration. Patients
10 with advanced disease, especially those with advanced fibrosis or comorbidity, could benefit
11 from CLDN1 or OCLN targeting compound in order to lower the HCC risk, which is a
12 limitation of current DAAs regimens. Finally, liver transplantation or transplantation of HCV-
13 positive organs could be indications for the use of CLDN1 or OCLN targeting agents that would
14 considerably reduce the risk of liver graft reinfection and might alleviate the urgent need for
15 organ transplants. There is still room for new therapies in the fight against HCV in order to
16 improve patients care. The place of TJs proteins targeting agents has still to be defined by
17 further clinical trials in comparison or in combination with currently approved DAAs regimens.
18
19

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11

12 **Conflict of interest**

13 Inserm, the University of Strasbourg and the Strasbourg University Hospitals have filed patent
14 applications for the use of anti-Claudin1 antibodies for treatment of HCV infection which have
15 been licensed to Alentis Therapeutics, Basel, Switzerland.

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2

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Figure legends:

Figure 1: Overview of the HCV entry process in human hepatocytes and highlight of strategies targeting TJ proteins. Schematic representation of the HCV entry process from cell binding to genome cytoplasmic release. After attachment to the surface of hepatocytes through interaction with HSPG, LDLR and SR-BI (1), HCV binds to CD81 and activates EGFR (2). EGFR activation, as well as H-Ras and other Rho GTPases activation, leads to the lateral diffusion of the HCV/SR-BI/CD81/EGFR (HSCE) complex towards TJs (3). During its migration the HSCE complex encounters and associates with CLDN1 and OCLN probably not involved in TJs formation (4). The virus is then internalized through clathrin-mediated endocytosis (5), before delivering its genome inside the cell *via* a pH-dependant membrane fusion mechanism (6). The blue boxes indicate the different strategies developed aiming at inhibiting HCV entry through interfering with TJ proteins. CLDN1-specific mAbs are described in [30, 79, 142-147], CLDN1-derived recombinant proteins are described in [151, 152], CLDN1-derived peptide is described in [149] and CLDN1-trafficking modulators are described in [75, 153]. OCLN-specific mAbs are described in [154-157]. Figure modified from [121].

Figure 2: Model of human claudin-1 protein structure. The TJ protein CLDN1 is composed of 4 transmembrane domains shown here as helix. Each residue of intracellular parts and extracellular loops are individually shown as a filled circle. Red-filled circles (W₃₀, I₃₂, D₃₈, EGLW₅₁) and yellow-filled circles, circled with red (C₅₄, C₆₄), depict residues involved in HCV entry, as demonstrated [12, 49, 52, 61, 62]. The yellow-filled circles (C₅₄, C₆₄) correspond to the 2 cystein residues forming a disulfide bridge in the first extracellular loop (EL1) [63]. The purple-filled circles (Y₂₁₀, V₂₁₁) at the C-terminal extremity belong to the PDZ binding domain (K₂₀₈, D₂₀₉, Y₂₁₀, V₂₁₁) suggested to be involved in CLDN1 plasma membrane localization and HCV entry [74]. The orange line indicates the peptide sequence (S₅₃-T₈₀) described as being involved in CLDN1-CLDN1 and CLDN1-OCLN interaction [104]. The red line shows the position of the CLDN1-derived peptide CL58 (M₁-W₁₈) described as being able to inhibit HCV infection [149]. The residues marked with a black star (W₃₀, Y₃₃, Y₃₅, D₃₉, GLW₅₁, C₅₄, C₆₄) correspond to the epitope recognized by the OM-7D3-B3 mAb [142]. The residues marked with a green star (S₇₄, M₁₅₂) belong to the epitope bound by the mAbs described by Fukasawa *et al.* [146].

1 **Figure 3: Model of human occludin structure.** The TJ protein OCLN is composed of 4
2 transmembrane domains shown here as helix. Each residue of intracellular parts and
3 extracellular loops are individually depicted as a filled circle. Red-filled circles (A₂₂₃, A₂₂₄, I₂₇₉,
4 W₂₈₁) and yellow-filled circles, circled with red (C₂₁₆, C₂₃₇) were shown to be involved in HCV
5 entry [99, 101, 103]. The yellow-filled circles (C₂₁₆, C₂₃₇) correspond to the 2 cystein residues
6 forming a putative disulfide bridge in the second extracellular loop (EL2) [100]. The orange
7 line correspond to the region (C₂₁₆-P₂₂₂) encompassing the epitope bond by the mAbs described
8 by Okai et al. [157]. Residues marked with a black stars in EL1 (Y₁₀₄, P₁₀₅, G₁₀₇, G₁₀₈, G₁₁₀,
9 F₁₁₁) and residues marked with a green star in EL2 (C₂₁₆, P₂₂₂, A₂₂₃, C₂₃₇) indicate the epitopes
10 bond by the different mAbs described by Shimizu et al.[155].

1 Table 1: Characteristics of compounds targeting Claudin 1 and Occludin for HCV infection in preclinical development

Target	Compound name	Type of compound	Method of production	References
<i>CLDN1</i>	OM-7D3-B3	Rat IgG2b	Rat immunization	[30]
	H3L3	Humanized IgG4	Humanization of rat mAb	[79, 143]
	5.16v2, v3, v4, v5, v6	Fully human IgG1	Phage display and mAb maturation	[144]
	A2, B9X, D10X, H9C, E9C, F7C	Fully human IgG4	Phage display	[145]
	2C1, 3A2	Mouse IgG2b	Mouse immunization	[146]
	5F2	Mouse IgG2a		
	7A5	Mouse IgG1		
	2C1, 3A2	Chimeric IgG1 and IgG4	Chimerization	[147]
	CL58	peptide	Synthetic peptide	[149]
	Full recombinant CLDN1	Recombinant protein	Pichia pastoris X33	[151]
CD81-CLDN1 EL	Recombinant protein	E. coli BL21 (DE3)	[152]	
<i>OCLN</i>	1-3, 37-5, 44-10	Rat IgG2b	Rat immunization	[154-156]
	32-1	Rat IgG2a		
	Xi1-3, Xi37-5	Humanized IgG4	Humanization of rat mAb	
	67-2	Mouse IgG1 λ	Mouse immunization	

2