Humanisation of a claudin-1-specific monoclonal antibody for clinical prevention and cure of HCV infection without escape

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ABSTRACT

Objective  HCV infection is a leading cause of chronic liver disease and a major indication for liver transplantation. Although direct-acting antivirals (DAAs) have much improved the treatment of chronic HCV infection, alternative strategies are needed for patients with treatment failure. As an essential HCV entry factor, the tight junction protein claudin-1 (CLDN1) is a promising antiviral target. However, genotype-dependent escape via CLDN6 and CLDN9 has been described in some cell lines as a possible limitation facing CLDN1-targeted therapies. Here, we evaluated the clinical potential of therapeutic strategies targeting CLDN1.

Design  We generated a humanised anti-CLDN1 monoclonal antibody (mAb) (H3L3) suitable for clinical development and characterised its anti-HCV activity using cell culture models, a large panel of primary human hepatocytes (PHH) from 12 different donors, and human liver chimeric mice.

Results  H3L3 pan-genotypically inhibited HCV pseudoparticle entry into PHH, irrespective of donor. Escape was likely precluded by low surface expression of CLDN6 and CLDN9 on PHH. Co-treatment of a panel of PHH with H3L3-specific mAb did not enhance the antiviral effect of H3L3, confirming that CLDN6 does not function as an entry factor in PHH from multiple donors. H3L3 also inhibited DAA-resistant strains of HCV and synergised with current DAAs. Finally, H3L3 cured persistent HCV infection in human-liver chimeric uPA-SCID mice in monotherapy.

Conclusions  Overall, these findings underscore the clinical potential of CLDN1-targeted therapies and describe the functional characterisation of a humanised anti-CLDN1 antibody suitable for further clinical development to complement existing therapeutic strategies for HCV.

Significance of this study

What is already known on this subject?

▸ The tight junction protein claudin-1 (CLDN1) is an essential HCV entry factor.

▸ Rodent anti-CLDN1 antibodies inhibit HCV infection by disrupting CD81-CLDN1 co-receptor complex formation.

▸ A rodent anti-CLDN1 monoclonal antibody prevented and cured chronic HCV infection in human-liver chimeric mice.

▸ Escape from CLDN1-targeted therapies via other CLDN subtypes has been described in some cell lines.

What are the new findings?

▸ We generated a humanised anti-CLDN1 monoclonal antibody (H3L3) suitable for further clinical development.

▸ H3L3 exhibited pan-genotypic activity against HCV entry into primary human hepatocytes (PHH), irrespective of donor.

▸ There was absent escape in a panel of PHH from 12 donors, indicating that CLDN6 does not function as an entry factor in PHH.

▸ Low expression of other CLDN subtypes on PHH likely precludes escape in the human liver and indicates that escape is likely not clinically relevant in the majority of patients.

How might it impact on clinical practice in the foreseeable future?

▸ These findings enable further clinical development of a CLDN1-targeted therapy to complement existing therapeutic strategies for HCV.

INTRODUCTION

Chronic HCV infection is a leading cause of liver cirrhosis and hepatocellular carcinoma worldwide. Although the approval of direct-acting antivirals (DAAs) has revolutionised HCV treatment, treatment failure can result from the selection of DAA-resistant HCV variants. 1, 2 Occult HCV infection may also persist in patients who achieve sustained virological response. 3 Furthermore, not all patient groups respond to therapy. 4, 5 For example, 10–20% of patients with decompensated cirrhosis do not respond to current DAAs, and treatment is associated with serious adverse effects (10–20%). 6 Progressive liver disease associated with chronic HCV infection remains a major indication for liver transplantation. Graft reinfection is universal in patients with detectable HCV RNA at the time of transplantation. The ability of DAAs to prevent liver graft reinfection remains to be determined. 7, 8 In one study evaluating the efficacy of sofosbuvir and ribavirin during liver
transplantation, 35% of patients experienced liver graft reinfec-
tion.9 Current DAAs act once infection is already established,
whereas inhibitors targeting entry could inhibit de novo in-
festation of the graft.8 Entry inhibitors could also protect HCV-negative
transplant recipients from infection by HCV-positive donor
organs10 and help to alleviate organ shortage.11

Host-targeting agents (HTAs) offer a promising complementa-
tory approach. HCV requires several host factors to establish
infection, including cluster of differentiation 81 (CD81),12 scav-
enger receptor BI (SR-BI),13 CLDN1,14 epidermal growth factor
receptor (EGFR),15 Niemann-Pick C1 like 1 (NPC1L1)16 and
E-cadherin,17 where attractive antiviral targets. Antibodies
targeting CD81,16 SR-BI19 and CLDN120–23 pan-genotypically
inhibit HCV infection in vitro and in vivo, and small molecules
targeting EGFR15 and NPC1L116 have similar anti-HCV activ-
ities. Several HTAs, including erlotinib,15 ezetimibe16 and the
antihistamine chlorcyclizine,24 are approved for other indications
and could be repurposed as HCV antivirals. HTAs act synergistic-
ly with DAAs25 and prevent emergence of DAA-resistant vari-
ants,26 highlighting their potential for combination therapy.
Furthermore, as the targets of HTAs are not encoded by highly
mutable viral genomes, a higher barrier to resistance is expected.

In the case of CLDN1-targeting HTAs, previous studies in cell
lines indicated the possibility of escape via other claudin sub-
types, namely CLDN6 and CLDN9.27 28 Indeed, exogenous
expression of CLDN9 in CLDN1-deficient Bel7402 hepatoma
cells27 and of CLDN6 and CLDN9 in CLDN-null 293T cells27 28
enabled entry of some HCV genotypes.29 CLDN1-specific anti-
odies only partially inhibited infection by genotypes with broad
CLDN tropism (ie, genotypes 1, 3, 4 and 6) in Huh7.5 cells.29
However, the clinical relevance of escape is still unknown and
the ability of CLDN1-specific antibodies to block entry into
human liver cells without escape remains unclear.30

We previously reported that rat anti-CLDN1 monoclonal anti-
bodies (mAbs) have robust anti-HCV activities.20 31 These anti-
bodies inhibit HCV entry by disrupting CD81–CLDN1 co-receptor
complex formation.31 The lead rat anti-CLDN1 mAb (OM-7D3-B3)
prevented de novo HCV infection and cleared chronic HCV infection in monotherapy without toxicity
in human-liver chimeric mice.21 These promising findings
warrant further clinical studies. However, humanisation of this
antibody to reduce immunogenicity in humans is critical for any
further clinical development.

In this context, we humanised the rat anti-CLDN1 antibody
into the human IgG4 subtype and functionally characterised the
humanised antibody using cell culture models, primary human
hepatocytes (PHH), and human-liver chimeric mice. We found
that the humanised anti-CLDN1 antibody inhibited entry of all
HCV genotypes into PHH without escape, suggesting that
escape is not relevant in the human liver, likely due to low
expression of alternative CLDN subtypes. The humanised anti-
body retained its functional activity and its ability to cure chron-
ically infected human-liver chimeric mice in monotherapy. It
was also active against DAA-resistant HCV and synergised with
current DAAs. Our findings highlight the clinical potential of
CLDN1-targeted therapies and pave the way for clinical devel-
opment of a humanised CLDN1 antibody as an alternative treat-
ment strategy for HCV.

EXPERIMENTAL PROCEDURES

**Sequencing of the variable region of rat anti-CLDN1 mAb
OM-7D3-B3**

Total RNA was isolated in pure RNase-free water from 10⁷
hybridoma cells using the Qiagen RNeasy mini kit. Vh and Vl
cDNAs were prepared using primers derived from the constant
domain (CH1) of rat IgG2b and rat kappa light chain by reverse
transcription. cDNAs were amplified using a pool of primers
corresponding to the signal sequence region and subsequently
cloned into pGem-T Easy vector (Promega). Clones were
screened and sequenced. Complementarity-determining regions
(CDRs) were identified with reference to other antibody
sequences as described.32

**Humanisation of rat anti-CLDN1 mAb OM-7D3-B3 and
antibody production**

Humanisation was performed by CDR grafting as described.33 34
Human germline VH and VL sequences most similar to the rat
anti-CLDN1 mAb OM-7D3-B3 in the IMGT database were
identified using the IGBLAST tool (http://www.ncbi.nlm.nih.
gov/igblast/) (figure 1A). The CDRs of mAb OM-7D3-B3 were
engrafted on frameworks of the identified VH and VL
human germline sequence. Framework regions of OM-7D3-B3
were compared with rat germline sequences to identify vari-
ations that could contribute to antibody specificity. Three variants
each of heavy chain (H1, H2, H3) and light chain (L1, L2, L3)
were generated (figure 1B). These VH and VL variants were
fused in-frame with constant domains of the human IgG4 heavy
and light chains, respectively, and cloned into a mammalian
expression vector. Nine full-length humanised antibodies were
produced by co-transfecting plasmids containing the appropriate
heavy and light chain variants into Chinese hamster ovary
(CHO) cells. Supernatants were harvested and antibodies puri-
plied using the MAbTrap Kit (GE Healthcare). Antibodies were
buffer-changed into phosphate-buffered saline (PBS) prior to
use.

**Cells and viruses**

Huh7.5.1,21 HepG225 and human embryonic kidney 293T26 cells
were cultured as described. Production of HCVpp27 bearing
envelope glycoproteins of strains H77, HCV-J, JFH1, J8, NIH
S52, UKN3A1.28, UKN4.21.16, UKN5.14.4 and
UKN6.5.340 has been described previously. Strain gt3JSXB1
(genotype 3) was cloned from the serum of a patient chronically
infected with HCV and followed at the Strasbourg University
Hospitals. E1E2 sequences were amplified and inserted into the
pHCMV IRES vector using EcoRV restriction sites as described.36
Luciferase reporter HCVcc (Luc-Jc1) and DAA-resistant mutants (Luc-Jc1-A156S and
Luc-Jc1-R155K) were produced as described.26 38

**Primary human hepatocytes**

PHH were isolated from liver tissue obtained from patients
undergoing liver resection at the Strasbourg University
Hospitals. PHH from 12 donors were isolated and cultured as
described.31

**Flow cytometry**

To evaluate binding of the humanised antibodies to CLDN1
expressed on Huh7.5.1, HepG2 and 293T cells, 2×10⁴ cells
were incubated with 20 µg/mL mouse anti-CD81 mAb JS-81
(BD Biosciences), rat anti-CLDN1 mAb OM-7D3-B3, human-
ised anti-CLDN1 mAb or PBS for 1 hour at room temperature.
Cells were washed and incubated with phycoerythrin
(PE)-conjugated species-specific secondary antibodies at 4°C for
45 min to allow detection of binding. Cells were subsequently
washed and fixed with 2% paraformaldehyde (PFA) prior to
analysis by flow cytometry (BD LSRII, 293T cells; FACSscan,
Huh7.5.1 and HepG2 cells). Delta median fluorescence
intensities (ΔMFI) were obtained after subtracting the background fluorescence with isotype control antibody. To evaluate the binding specificity, 293T cells were transfected with either empty vector or pcDNA3.1 encoding human CLDN1. After 48 hours, binding was assessed by flow cytometry (BD LSRII).

We evaluated binding of one humanised antibody clone (H3L3) to PHH, as well as expression of CLDN subtypes on the surface of PHH. Cryopreserved PHHs (from the same donors used in inhibition experiments) were incubated with CLDN-specific mAbs (CLDN1, rat OM-7D3-B3 or humanised

Figure 1  Sequence alignment of the variable region of the heavy and the light chains of rat anti-tight junction protein claudin-1 (CLDN1) monoclonal antibody (mAb) OM-7D3-B3. (A) Protein sequences of rat OM-7D3-B3 heavy chain (top) and light chain (bottom) aligned with the nearest rat germline sequence. The most closely matched germline sequences had homology of 86.7% and 90.5% with heavy and light chain, respectively. (B) Sequence alignment of rat OM-7D3-B3 heavy (top) and light (bottom) chain with the nearest human germline sequences. Also shown are the humanised variants of heavy (H1, H2 and H3) and light (L1, L2 and L3) chains that were generated in the process of humanising rat OM-7D3-B3. The complementarity-determining regions (CDRs) are highlighted in yellow and the joining (J)-region is shown in pink. The framework region (FR), CDRs and the J-region are indicated.
H3L3; CLDN6, rat WU-9E1-G2; CLDN9, rat YD-4E9-A2) or isotype control at 20 μg/mL, as described. Cells were then stained with PE-conjugated secondary antibody. After washing, cells were fixed with 2% PFA and analysed by flow cytometry (FACScan). In all experiments, Huh7.5.1 cells and PHH were analysed in parallel.

Inhibition of HCV infection

Huh7.5.1 or PHH were preincubated with serially diluted antibodies (control mAb, rat OM-7D3-B3, humanised H3L3, anti-CLDN6 WU-9E1-G2 or combinations thereof) for 1 hour at 37°C and subsequently exposed to HCVcc or HCVpp, respectively. For HCVpp experiments, PHH from several different donors were evaluated. Following treatment, cells were incubated with HCVpp for 4 hours at 37°C. HCVpp entry/HCVcc infection was analysed by measuring intracellular luciferase activity after 72 hours (relative light units (RLU)). Inhibition was expressed as a percentage relative to cells treated with control antibody. The concentration of antibody required for 50% inhibition (IC50) and 90% inhibition (IC90) was calculated by non-linear regression analysis (constrained fit) using GraphPad Prism (V6.0, GraphPad Software, USA).

Cell–cell transmission assay

The HCV cell–cell transmission assay has been described. Huh7.5.1 cells were electroporated with HCV Jc1 RNA (virus-producer cells) and co-cultured with uninfected Huh7.5 target cells stably expressing green fluorescent protein (GFP) in the presence of H3L3, OM-7D3-B3 or isotype control mAb (11 μg/mL). Cell-free transmission was blocked by a neutralising HCV anti-E2 mAb, AP33 (25 μg/mL) as described. After 24 hours, cells were fixed with 2% PFA and stained with an NS5A-specific antibody (Viostar, 0.1 μg/mL). De novo infection (GFP and NS5A double-positive cells) was assessed by flow cytometry (BD LSRII). Cell–cell transmission was presented as a percentage of GFP+ NS5A+ infected cells relative to Huh7.5-GFP + target cells in the presence of HCV anti-E2 mAb AP33.

Assessment of antiviral synergy

Synergy was evaluated as described previously. Huh7.5.1 cells were pretreated with H3L3 in combination with sofosbuvir or target cells stably expressing green fluorescent protein (GFP) in the presence of H3L3, OM-7D3-B3 or isotype control mAb (11 μg/mL). Cell-free transmission was blocked by a neutralising HCV anti-E2 mAb, AP33 (25 μg/mL) as described. After 24 hours, cells were fixed with 2% PFA and stained with an NS5A-specific antibody (Viostar, 0.1 μg/mL). De novo infection (GFP and NS5A double-positive cells) was assessed by flow cytometry (BD LSRII). Cell–cell transmission was presented as a percentage of GFP+ NS5A+ infected cells relative to Huh7.5-GFP + target cells in the presence of HCV anti-E2 mAb AP33.

HCV infection and treatment of human-liver chimeric uPA-SCID mice

Experiments were performed according to local ethics committee approval (CREMEAS, project numbers 0201412041623 4981 (APAFIS#72.02) and 02014120511054408 (APAFIS#74.03)) at the Inserm U1110 animal facility. Severe combined immunodeficient mice homologous for urokinase-type plasminogen activator expression under control of the mouse albumin promoter (uPA-SCID) were engrafted with PHH as described. Human liver–chimeric mice were infected with HCVcc (Jc1; genotype 2a) by intraperitoneal injection as described. Three weeks after infection, mice were given intraperitoneal injections of 500 μg/mouse of H3L3 or isotype control mAb at weekly intervals for 4 weeks. Plasma HCV RNA, human albumin and IgG4 levels were monitored as described.
variability, we tested PHH from a panel of 11 additional donors. We focused on one strain of genotype 1b and three strains of HCV genotype 3a as these genotypes have been described to escape CLDN1-targeted therapies in cell lines.29 H3L3 fully inhibited entry of all genotypes in all PHH we tested, irrespective of donor (figure 3, see online supplementary table S1).

H3L3 inhibits HCVpp entry into PHH without escape via CLDN6 or CLDN9

Given that escape from CLDN1-directed therapies has been reported for certain HCV genotypes in CLDN6-expressing and/or CLDN9-expressing cell lines,27–29 we investigated the physiological relevance of escape in the context of the H3L3 antibody and PHH. We evaluated surface expression levels of CLDN6 and CLDN9 on PHH from four of the donors tested above. In contrast to robust CLDN6 expression in Huh7.5.1 cells, the PHH from these four donors (PHH 218, 235, 283 and S2310) and three additional donors (PHH 297, S1532 and S1533) expressed only very low surface levels of CLDN6, regardless of whether the cells were assessed immediately after isolation (fresh PHH) or following cryopreservation (frozen PHH) (figure 4A). Neither PHH nor Huh7.5.1 cells expressed detectable CLDN9 (figure 4A). Therefore, it is likely that low surface expression levels of CLDN6 in the human liver preclude escape from CLDN1 antibodies in vivo.

We further evaluated escape using a CLDN6-specific mAb (WU-9E1-G2). PHH were treated with H3L3 alone or in combination with 20 μg/mL of WU-9E1-G2 prior to incubation with HCVpp genotype 1b (HCV-J) or 3a (NIH S52). If escape...
in PHH is indeed possible via CLDN6 (even at low expression levels), we expected that addition of a CLDN6-specific antibody would enhance the antiviral potency of H3L3. To address this question, we used a previously described antibody WU-9E1-G2 that specifically binds cell surface CLDN6 with high affinity on Huh7.5.1 cells. Combination of H3L3 with WU-9E1-G2 did not affect the inhibition profile for either genotype 1b or 3a in PHH from two different donors (figure 4B). In CLDN6-expressing Huh7.5.1 cells, however, addition of WU-9E1-G2 under the same conditions blocked escape and restored the antiviral effect of H3L3 (figure 4C). Collectively, these data confirm previous findings that HCV can use CLDN6 as an entry factor in some cell lines. However, low levels of CLDN6 surface expression in PHH largely exclude a functional role during HCV entry, suggesting that escape from CLDN1-targeting therapies is highly unlikely in the human liver.

H3L3 efficiently inhibits HCV infection and spread

We evaluated the dose–response profile of H3L3, using HCVcc (Luc-Jc1; genotype 2a) and Huh7.5.1 cells. H3L3 dose-dependently inhibited HCVcc infection of Huh7.5.1 cells with an IC$_{50}$ of 0.24 μg/mL, similarly to OM-7D3-B3 (IC$_{50}$ 0.13 μg/mL) (figure 5A). Furthermore, H3L3 inhibited infection by DAA-resistant HCVcc NS3 mutants Jc1-A156S and Jc1-R155K (IC$_{50}$ 0.47 and 0.51 μg/mL, respectively), as did OM-7D3-B3 (IC$_{50}$ 0.23 and 0.36 μg/mL, respectively) (figure 5A). Therefore, both antibodies retained their potency against DAA-resistant HCVcc (figure 5A). Considering the important role of cell–cell transmission in HCV persistence and the demonstrated ability of the rat anti-CLDN1 mAb OM-7D3-B3 to block this route of HCV entry, we tested H3L3 in a cell–cell transmission assay in the presence of anti-E2 envelope antibody to inhibit cell-free transmission. As expected, H3L3 efficiently blocked cell–cell transmission of HCV (figure 5B), with similar potency as observed for OM-7D3-B3 (figure 5C).

Antiviral synergy is an attractive feature for combination therapies. We used the Prichard method to determine whether H3L3 synergises with DAAs, as observed for the parental rat antibody. Indeed, combining H3L3 with sofosbuvir or daclatasvir resulted in significant synergistic activity (figure 5D). The increased potency was >20% above that predicted from additive effects, indicating synergy.

H3L3 cures chronic HCV infection in human-liver chimeric uPA-SCID mice

We next tested the ability of H3L3 to cure human-liver chimeric uPA-SCID mice chronically infected with HCV. Chronically HCV-infected mice were given weekly intraperitoneal injections of 25 mg/kg of H3L3 (n=3) or isotype control human IgG4
Figure 4  Tight junction protein claudin-6 (CLDN6) is not a functional HCV entry factor in primary human hepatocytes (PHH). (A) PHH express only very low surface levels of CLDN6 and CLDN9. PHH from four donors tested in inhibition experiments and three additional donors were treated with isotype control or CLDN1-specific, CLDN6-specific or CLDN9-specific monoclonal antibodies (20 μg/mL). Labelling was done either immediately following the isolation of PHH (fresh PHH) or using cryopreserved cells (frozen PHH). Expression of CLDN1, CLDN6 and CLDN9 is shown as delta median fluorescence intensity (ΔMFI).

(B) Treatment of PHH with a CLDN6-specific mAb (WU-9E1-G2) had no effect on HCVpp infection. PHH were treated with serially diluted WU-9E1-G2 or H3L3, or with serially diluted H3L3 in combination with 20 μg/mL WU-9E1-G2, for 1 hour prior to incubation with HCVpp genotype 1b (HCV-J) or genotype 3a (S52). HCVpp entry was assessed by measuring luciferase activity after 72 hours. (C) Treatment of HuH7.5.1 cells with H3L3 in combination with WU-9E1-G2 inhibited HCVpp entry, in contrast to treatment with H3L3 alone or WU-9E1-G2 alone. HuH7.5.1 cells were treated with serially diluted WU-9E1-G2 or H3L3, or with serially diluted H3L3 in combination with 20 μg/mL WU-9E1-G2, for 1 hour prior to incubation with HCVpp genotype 1b (HCV-J) or genotype 3a (S52). HCVpp entry was assessed by measuring luciferase activity after 72 hours. Graphs show one experiment performed in duplicate (A and B) or two experiments performed in triplicate (C).

Hepatology

mAb (n=1) for 4 weeks. For the control mouse and one H3L3-treated mouse, HCV RNA levels as well as serum human albumin and IgG4 levels were measured for 12 or 13 weeks post-treatment. For the second H3L3-treated mouse, the viral load was monitored for 5 weeks post-treatment until clearance of HCV RNA. Both H3L3-treated mice showed undetectable HCV RNA levels at the end of the study period (figure 6A). Stable human albumin levels in mice confirmed engraftment of human PHH (figure 6B). Increasing serum levels of the antibody (figure 6C) did not affect the levels of human albumin (figure 6B), indicating that H3L3 did not adversely affect liver function. Like the parental rat antibody, therefore, H3L3 clears chronic HCV infection in human liver chimeric mice without overt toxicity.

DISCUSSION
To enable the clinical development of a CLDN1-targeted HCV therapy, we generated humanised anti-CLDN1 antibodies by grafting CDRs of rat anti-CLDN1 mAb OM-7D3-B3 on to the human IgG4 backbone. All of the humanised antibodies bound to CLDN1 and inhibited HCVcc infection with similar potency. We selected one mAb, H3L3, for detailed characterisation. Given that genotype-dependent escape from CLDN1-targeted therapies...
through CLDN6 and/or CLDN9 has been described in cell lines,27–29 we evaluated the functional relevance of escape from the H3L3 antibody. H3L3 pan-genotypically inhibited HCVpp entry into PHH from up to 12 different donors, without any apparent escape (figure 3), reflecting low surface expression levels of CLDN6 and CLDN9 (figure 4A). This is consistent with the lack of CLDN6 protein expression in liver sections30 and lack of CLDN6 and CLDN9 protein expression in PHH.28 Furthermore, CLDN6 did not appear to play a role in HCV entry into PHH (figure 4B). Therefore, escape from CLDN1-directed therapies such as the H3L3 antibody is likely not relevant in vivo, at least for the majority of patients. Ultimately, clinical trials will be needed to address the clinical relevance of escape.

The humanised anti-CLDN1 antibody demonstrates promising activities that address some limitations of current DAA-based therapies. H3L3 potently inhibited HCVcc infection in cell culture (figure 5A). H3L3 dose-dependently inhibited HCV infection. Huh7.5.1 cells were incubated with rat OM-7D3-B3, humanised H3L3 or isotype control antibodies prior to infection with HCVcc (Jc1 wild type, Jc1-A156S or Jc1-R155K). Infection was assessed by measuring luciferase activity after 72 hours. Results are expressed as log relative luciferase units from three independent experiments performed in triplicate. (B and C) H3L3, like OM-7D3-B3, inhibits HCV cell–cell transmission. Huh7.5.1 cells electroporated with HCV Jc1 RNA (producer cells) were co-cultured with naive Huh7.5.1-GFP cells (target cells) in the presence of control or anti-CLDN1 antibody (11 μg/mL). Co-cultured cells were fixed with paraformaldehyde after 24 hours and stained with an NS5A antibody (B). The extent of cell–cell transmission was determined by calculating percentage of GFP+NS5A+ cells (C). Results from a single experiment performed in duplicate are shown. (D) H3L3 synergises with direct-acting antivirals. Huh7.5.1 cells were pretreated with H3L3 in combination with sofosbuvir (SOF) or daclatasvir (DCV) prior to infection with HCVcc. Infection was assessed after 72 hours by luciferase activity. Synergy (20% above that expected for additive effects, shown in black) was assessed according to the Prichard and Shipman method.40 Results from a representative experiment are shown.
Figure 6  H3L3 cures chronically HCV-infected human-liver chimeric uPA-SCID mice. (A) Two mice chronically infected with HCV Jc1 were treated with 500 µg of H3L3 anti-tight junction protein claudin-1 monoclonal antibody weekly for 4 weeks. As a control, one mouse was similarly treated with 500 µg of isotype control human antibody. Human albumin (B) and IgG4 antibody (C) levels were monitored. Red + symbols indicate the times of antibody treatment. The horizontal dashed line indicates limit of quantification (LOQ).

and HCVpp entry of different genotypes, blocked cell–cell transmission and cured chronically HCV-infected human-liver chimeric uPA-SCID mice in monotherapy. H3L3 has a complementary mechanism of action compared with currently approved DAAs, which results in marked synergy as shown for sofosbuvir and daclatasvir (figure 5D). Such synergy could allow shortening of treatment duration, thus reducing costs and the possibility of side effects. Furthermore, H3L3 is active against DAA-resistant HCV NS3 mutants (figure 5A), another attractive feature for a potential combination therapy. Given that CLDN1 is a host-encoded target, a higher genetic barrier to resistance is expected. In contrast to current DAAs, H3L3 (figure 6A) cured chronically infected human-liver chimeric mice in monotherapy, without resistance or escape. Finally, host-targeting entry inhibitors such as H3L3 are expected to efficiently block HCV de novo infection of the liver graft. Therefore, H3L3 is ideally positioned to address DAA failure in chronic HCV infection and prevent acute liver graft infection.

Organ shortage is a major challenge facing transplants in all contexts (eg, kidney). In the USA, there are currently >120 000 patients on waiting lists for transplants, yet only approximately 30 000 transplants were performed in 2015. This medical burden can be eased by transplantation of organs from HCV-positive patients to HCV-negative recipients, which is no longer overtly contraindicated. Nonetheless, transplantation of organs from HCV-positive patients to HCV-negative recipients is challenged by de novo HCV infection of the recipient and is often associated with less positive outcomes. H3L3 could protect patients receiving HCV-positive organs from HCV infection, which would increase the pool of donor organs. In contrast to DAAs (which are administered postinfection), the humanised anti-CLDN1 antibody could prevent infection of the recipient.

Humanisation of anti-CLDN1 mAb OM-7D3-B3 is the first step towards its clinical development. Any potential anti-receptor antibody will have to be safe and non-immunogenic. In order to avoid destruction of healthy hepatocytes from antibody-mediated effector functions, we humanised anti-CLDN1 mAb OM-7D3-B3 into the IgG4 isotype, which can neither sensitise natural killer cells nor activate the complement system. Thus, IgG4 does not induce antibody-dependent cell-mediated cytotoxicity or complement-mediated lysis of target cells. Although humanisation of the rat antibody is likely to reduce its immunogenicity in humans, further evaluation in an appropriate animal model with functional immune responses will be required. However, rat anti-CLDN1 does not cause any toxicity in immunocompetent mice, this is likely also to be the case for the humanised antibody. The humanised antibodies did not exert any cellular toxicity in vitro (see online supplementary figure S3) and we did not observe any overt toxic effects in human-liver chimeric mice (figure 6B).

In conclusion, we successfully humanised a rat anti-CLDN1 mAb without loss of function. This is a critical step for further development of anti-CLDN1 antibodies as an alternative treatment strategy for HCV infection. The humanised antibody has pan-genotypic activity in PHH, without observable escape in PHH from multiple donors, highlighting the clinical potential of CLDN1-targeted therapies such as H3L3. Further evaluation of this antibody in preclinical efficacy and toxicity studies in suitable animal models will pave the way for clinical trials in humans. Overall, H3L3 represents a promising alternative approach for HCV therapy for patients who fail current therapies or to prevent post-transplantation HCV infection.

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Contributors CCC and RGT contributed equally. TFB initiated and supervised the study. CCC, RGT, LM, CT, LH, SCD, FX and ER performed the experiments. PP provided essential reagents. CCC, RGT, MBZ and TFB analysed data. CCC, RGT, LM, CT, LH, SCD, FX and ER performed the experiments. PP provided essential reagents. CCC, RGT, MBZ and TFB analysed data. CCC, RGT, MZB and TFB wrote the manuscript.
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