Tight Junction Proteins as Therapeutic Targets to Treat Liver Fibrosis and Hepatocellular Carcinoma

Antonio Saviano, MD, PhD[1,2,3] Natascha Roehlen, MD, PhD[4,5] Thomas F. Baumert, MD[1,2,3,6]

[1] Inserm, U1110, Institute of Translational Medicine and Liver Disease, Strasbourg, France
[2] University of Strasbourg, Strasbourg, France
[3] Service d'hépato-gastroentérologie, Pôle Hépato-digestif, Institut-Hospitalo-Universitaire, Hôpitaux Universitaires de Strasbourg, Strasbourg, France
[4] Department of Medicine II, Gastroenterology, Hepatology, Endocrinology and Infectious Diseases, Freiburg University Medical Center, Faculty of Medicine, University of Freiburg, Freiburg, Germany
[5] Berta-Ottenstein-Programme, Faculty of Medicine, University of Freiburg, Freiburg, Germany
[6] Institut Universitaire de France, Paris, France

Address for correspondence Thomas F. Baumert, MD, Inserm U1110, ITM, 3 rue Koeberlé, Strasbourg 67000, France (e-mail: thomas.baumert@unistra.fr).

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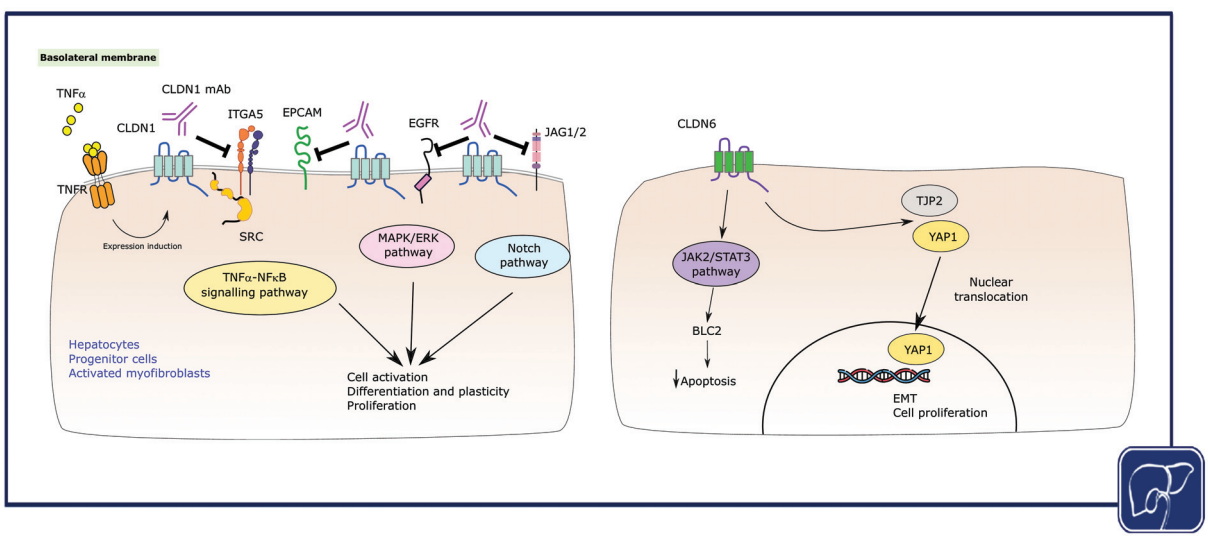

Abstract

In the last decade tight junction proteins exposed at the surface of liver or cancer cells have been uncovered as mediators of liver disease biology: Claudin-1 and Occludin are host factors for hepatitis C virus entry and Claudin-1 has been identified as a driver for liver fibrosis and hepatocellular carcinoma (HCC). Moreover, Claudins have emerged as therapeutic targets for liver disease and HCC. CLDN1 expression is upregulated in liver fibrosis and HCC. Monoclonal antibodies (mAbs) targeting Claudin-1 have completed preclinical proof-of-concept studies for treatment of liver fibrosis and HCC and are currently in clinical development for advanced liver fibrosis. Claudin-6 overexpression is associated with an HCC aggressive phenotype and treatment resistance. Claudin-6 mAbs or chimeric antigen receptor-T cells therapies are currently being clinically

Keywords
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investigated for Claudin-6 overexpressing tumors. In conclusion, targeting Claudin proteins offers a novel clinical opportunity for the treatment of patients with advanced liver fibrosis and HCC.

Lay Summary

Liver fibrosis and cancer are serious diseases with limited treatment options and poor outcome. Recent research has revealed that proteins relevant in cell–cell contact are also expressed on the surface of liver and cancer cells and can contribute to the disease. For example, Claudin-1 and Occludin mediate infection of hepatocytes by hepatitis C virus—a major cause of liver disease and cancer worldwide. Claudin-1 has been shown to play a function role in liver fibrosis and cancer development. Claudin-1 has been shown to be a target to treat liver fibrosis and cancer using monoclonal antibodies. In liver cancers, Claudin-6 overexpression is associated with aggressive behavior and treatment resistance. Clinical investigation is currently underway for CLDN-6 overexpressing tumors using Claudin-6 targeting therapies. In conclusion, targeting exposed Claudins offers a novel clinical opportunity for the treatment of liver fibrosis and cancer.

Liver fibrosis and cancer are major global health issues with increasing incidence due to prevalent risk factors including alcohol consumption, metabolic syndrome, and steatotic liver disease SLD.[1] The most prevalent chronic liver disease, metabolic dysfunction-associated steatotic liver disease MASLD, affects roughly 30 of the global population, and has shown an increase of 50.4 between 1990 to 2006 and 2016 to 2019.[2] MASLD and its inflammatory and aggressive form, metabolic dysfunction-associated steatohepatitis MASH, significantly contribute to liver fibrosis, which is the most significant risk factor for liver cirrhosis and decompensation. Research analyzing the natural progression of MASH patients indicates that approximately 20 of patients with F3 fibrosis develop cirrhosis or liver-related complications within 2 years.[3] Developing treatments for liver fibrosis has proven challenging. Clinical studies of compounds in late-stage development, such as glucagon-like peptide-1 GLP-1 and thyroid hormone receptor- agonists, have shown effects on metabolic and inflammatory endpoints, with limited reduction in liver fibrosis, particularly in patients with advanced fibrosis.[4–6]

Liver fibrosis is the key risk factor for liver cancer, which represent the third leading cause of cancer-related deaths in the United States. The available treatment options for hepatocellular carcinoma (HCC), which accounts for more than 80% of primary liver cancers, are inadequate. Curative treatment is available for only a small number of patients, and tumor recurrence often occurs after treatment. Moreover, no adjuvant treatment has been authorized yet. Palliative systemic therapy can only be recommended to advanced-stage patients with preserved liver function.[7] Therefore, innovative therapies targeting liver fibrosis and cancer remain an unmet medical need.

Tight junction (TJ) proteins have emerged as targets for chronic diseases and cancers, such as gastric cancer.[8] TJ proteins not only form intercellular junctions but are also exposed at the cell surface outside the TJs in a nonjunctional form (►**Fig. 1**). They have shown to play an important role in various liver diseases comprising hepatitis C, liver fibrosis, and HCC and a large body of data show that nonjunctional TJ proteins are therapeutic targets for these conditions.

In this article, we review recent advances and perspectives on TJ proteins focusing on Claudins as therapeutic targets for liver disease and HCC.

Functional Role of Tight Junction Proteins Expressed in and Outside the Junctions in the Biology of the Liver

TJs are intercellular junctions responsible for regulating paracellular transport and maintaining cell polarity. They are formed by transmembrane and cytosolic proteins.[9] Notably, transmembrane proteins forming TJs are also expressed outside of the junction where they participate in signal transduction, intercellular communication,[10–12] and can influence cellular processes such as cell proliferation, migration, and differentiation.[13,14] Nonjunctional TJ proteins comprise several proteins, including Claudins (CLDNs), TJ-associated marvel proteins like occludin (OCLN), tricellulin, Marvel D3, junctional adhesion molecules (JAMs), and angulins. Junctional and nonjunctional protein pools are interconnected. Studies on the dynamic behavior of TJ have shown that TJ proteins are highly mobile, constantly remodeling and exchanging between the junctional and nonjunctional membrane and intracellular pools.[15] Junctional and nonjunctional TJs proteins have been shown to transmit signals through various pathways to regulate cell differentiation and proliferation. These pathways include the Hippo-pathway transcriptional coactivators YES-associated protein 1 (YAP1) and transcriptional coactivator with PDZ-binding motif (TAZ),[16–18] AKT–mTOR[9,19] and JUN N-terminal kinase (JNK) mitogen-activated protein kinase (MAPK), and extracellular signal–regulated kinase (ERK) signaling pathways.[20–22] It has been suggested that these pathways play

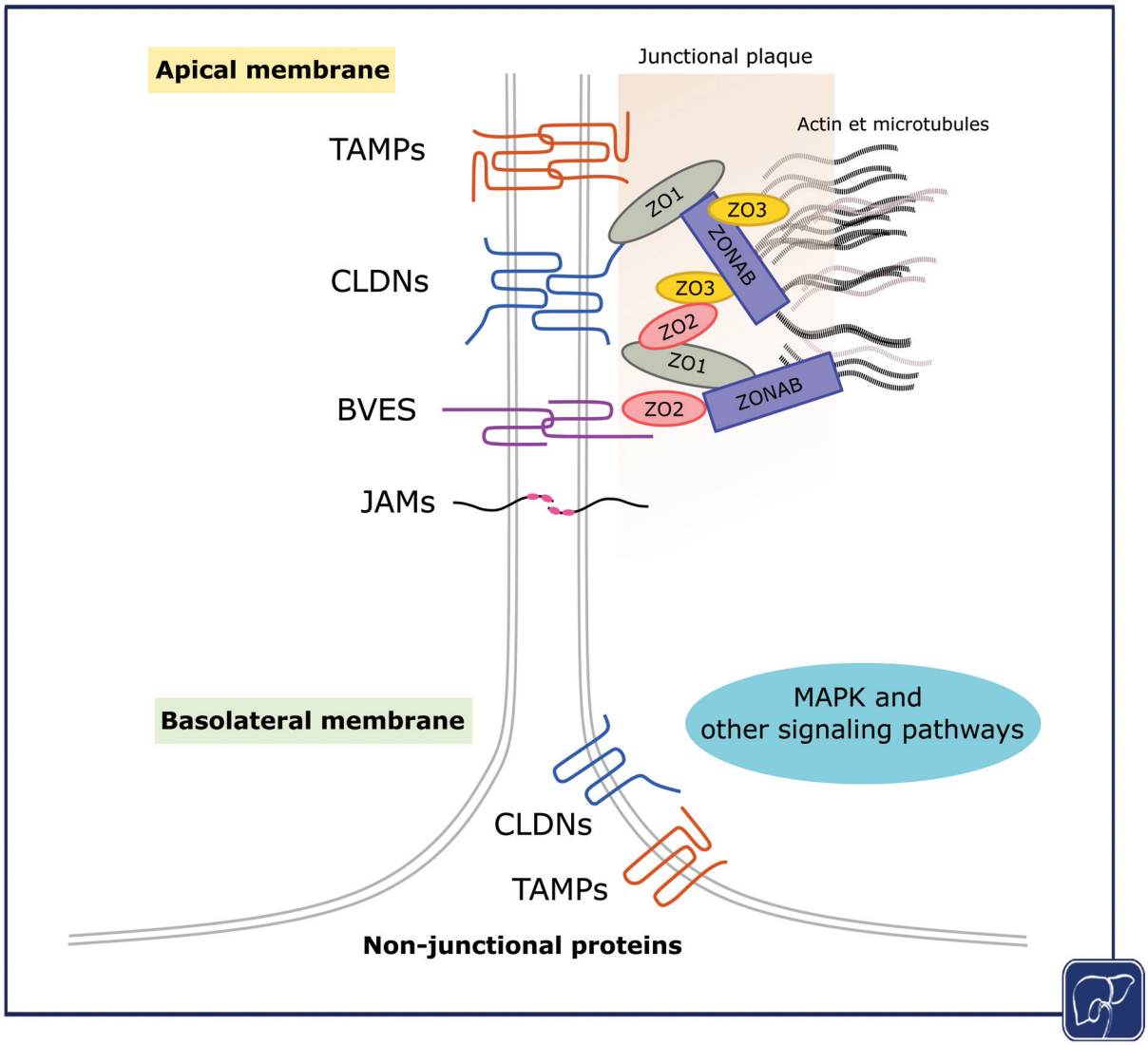

Fig. 1 **Fig. 1** Tight junction (TJ) structure and biology. TJs are formed by transmembrane proteins belonging to the claudin family (CLDNs), TJ-associated marvel proteins (TAMPs), blood vessel epicardial substance (BVES) and junctional adhesion molecules (JAMs). The transmembrane proteins are connected to the actin filaments and microtubules by the junctional plaque, which contains several adaptor proteins, protein phosphates, kinases, GTP-binding proteins, transcriptional, and posttranscriptional regulators such as ZO-1 associated nucleic acid binding proteins (ZONAB). TJ transmembrane proteins are also localized outside the junctions at the basolateral membrane. Nonjunctionally expressed proteins not only have a major intracellular signaling role but are also used by pathogens to enter in the cells. Nonjunctional proteins are accessible to drugs and are the main therapeutic targets of TJ-targeting agents.

important roles in controlling cellular functions. Moreover, both junctional and nonjunctional TJ proteins engage in crosstalk with focal adhesion, such as between integrins and claudins.[23–26] These interactions result in a complex interaction system with neighboring cells and extracellular matrix, which further modifies cell proliferation and migration.[9]

In the liver, TJ proteins are predominantly expressed in epithelial cells, specifically hepatocytes and cholangiocytes. CLDNs are the most prevalent and abundant TJ proteins. In hepatocytes, junctional TJ proteins establish the blood–biliary barrier, which separates the apical biliary pole from the basolateral membrane near the sinusoidal space. In cholangiocytes, junctional TJ proteins secure the bile ducts and canals and facilitate bile production and modification of composition.[27] In both types of cells, nonjunctional proteins are expressed at basolateral membrane and play a significant role in signal transduction and cell–matrix interactions. Recent studies have also shown that TJ proteins are also expressed in liver mesenchymal cells in their nonjunctional form and are upregulated during inflammatory processes.[28,29] Exposed, nonjunctional, TJ proteins are accessible to pathogens (such as hepatitis C virus) or drugs (such as antibodies) compared with their junctional counterparts. Indeed, TJ proteins exposed outside the TJs at the cell surface play a significant role in the pathogenesis of liver diseases, including viral infection, liver fibrosis, and cancer.

HCV infection biology exemplifies the role of nonjunctional TJ proteins in liver disease and their potential as therapeutic targets. Numerous studies have demonstrated that nonjunctionally expressed TJ proteins, such as CLDN1 and OCLN are crucial in HCV cell entry.[13,30] Together with CD81 and scavenger receptor BI, they are four of the primary host entry factors for HCV necessary for hepatocyte infection.[13,31] Several studies have elegantly demonstrated that both nonjunctional forms of CLDN1 and OCLN mediate HCV entry.[32–36] It is important to note that HCV does not directly bind to CLDN1.[37] Instead, CLDN1 serves as a coreceptor implicated in viral entry steps following viral binding of CD81.[32,38,39] Indeed, after HCV–CD81 binding, CLDN1 interacts with CD81 to facilitate viral internalization.[32,38,39] OCLN, as CLDN1, appears to not bind HCV and acts a cofactor necessary for late postbinding events involved in HCV entry.[40–42] Additionally, OCLN and CLDN1 contribute to cell-to-cell transmission of HCV.[13,43,44]

Research in HCV biology has opened new insights into the role of nonjunction TJ proteins in liver disease and their potential as therapeutic targets. CLDN1 was among the first TJ proteins uncovered as an essential host factor allowing the virus to enter and infect hepatocytes.[13] Monoclonal antibodies (mAbs) that recognize the conformation-dependent epitope within ECL1 prevent and disrupt CD81–CLDN1 association at the basolateral membrane of cells, thereby inhibiting viral internalization.[45,46] This mechanism confers to the CLDN1 mAbs a pan-genotypic effect enabling them to prevent HCV infection and cure chronic hepatitis C.[44,45] In chimeric mice engrafted with human hepatocytes, CLDN1-specific mAbs were able to prevent acute infection as well as cure of established chronic viral infection.[35] Moreover, these antibodies have an additional antiviral effect by modulating CLDN1 intracellular signaling, interfering with the MAPK pathway involved in maintaining HCV infection.[13,35] Other mAbs targeting the extracellular loop-2 (ECL2) of CLDN1 have demonstrated the ability to prevent HCV infection in chimeric mice with human liver.[13] Furthermore, CLDN1-derived peptides or recombinant proteins can compete with the endogenous CLDN1 and prevent viral entry.[30]

Similarly, mAbs against ECL1 and 2 of OCLN have also been developed.[47,48] Interestingly, a mAb targeting ECL2 of OCLN prevented HCV infection in human hepatoma Huh7.5.1 cells only when applied to the basolateral membrane instead of the apical one, confirming the nonjunctional protein's entry role at the basolateral membrane.[48] In addition, the ECL2-directed mAb demonstrated more effective antiviral activity, indicating that ECL2 is the primary site involved in HCV late entry steps.[13] Some of the OCLN antibodies have also been evaluated for safety and efficacy in human liver chimeric mice with no signs of major toxicity.[47]

The mechanistic studies on nonjunctional CLDN1, OCLN, and HCV have provided crucial insights into the biology of TJ proteins, their role in liver disease, and as therapeutic targets. Studying the biology of HCV and targeting the intracellular signaling pathways of TJ proteins has facilitated the development of precise mAbs for the treatment of liver fibrosis and cancer, which are currently in clinical trials.[28,49]

Nonjunctional Tight Junction Proteins as Therapeutic Target for Liver Fibrosis

Liver fibrosis is a common endpoint of chronic liver diseases, marked by excessive extracellular matrix deposition, tissue remodeling, and liver regeneration. Advanced liver fibrosis stages can result in end-stage liver disease and is associated with increased risk of liver decompensation and HCC. The severity of liver fibrosis is the most important prognostic factor in patients with MASLD[50] and is linked to the occurrence of liver-related events. Moreover, impaired liver function in patients with HCC is a major barrier to cancer treatment. Treatment for liver fibrosis is a major unmet medical need, but the development of such treatments has been challenging, with no drug being approved so far. Inflammatory and metabolic drugs for SLD have shown no major efficacy on liver fibrosis, a condition where collagen remodeling and liver regeneration are impaired.

TJ proteins exposed at the basolateral membrane outside the TJs have been shown to have a role in liver fibrosis pathogenesis and to influence mesenchymal cell activation and liver cell regeneration. Among all the TJ proteins, CLDN1 has been the most extensively studied. Multiple pieces of evidence support the role of nonjunctional CLDN1 as a major contributing factor for the pathogenesis of liver fibrosis. Studies of *CLDN1* expression have shown overexpression in cirrhotic livers.[51,52] CLDN1 levels, and in particular the nonjunctional form, increase alongside the fibrosis stage.[28] Single-cell RNA-sequencing analysis revealed that *CLDN1* is expressed in liver epithelial cells (hepatocytes and cholangiocytes), bipotent progenitor cells, and stellate cells. Notably, individual cell expression appears to be the highest in bipotent progenitor cells. TNFα, a cytokine implicated in liver inflammation and regeneration, contributes to stellate cells activation into myofibroblast, major players in collagen production. Additionally, it enhances *CLDN1* expression levels in hepatocytes as well as myofibroblasts. Furthermore, in vivo knockdown of CLDN1 considerably reduced liver fibrosis and tumor burden in a chimeric NASH mouse model, providing a genetic validation for targeting CLDN1 for liver fibrosis and HCC prevention.[28]

An mAb that targets nonjunctional CLDN1 has demonstrated a potent antifibrotic and HCC prevention effect across several mouse and patient-derived models of liver fibrosis progressing to HCC.[28] Mechanistic studies have shown that targeting CLDN1 suppresses profibrotic signal transduction including a robust effect on the TNF-α–NFκB signaling pathway. CLDN1 interacts directly with other proteins, including epidermal growth factor receptor (EGFR), EPCAM, the ECM receptor integrin α 5 (ITGA5), and the ECM component laminin 5 (LAMA5). By modulating this interaction, CLDN1 mAb inhibits EGFR and ERK phosphorylation and suppresses SRC proto-oncogene and SRC signaling, a key downstream pathway of cell-ECM mechanoreceptors.[53] These changes result in modulation of the plasticity of hepatocytes, progenitor cells, and myofibroblasts, leading to a reversal of their pathological, immature, and profibrotic profile to more differentiated and functionals, thereby reversing fibrosis and reducing HCC development.[28,54–56] The function and

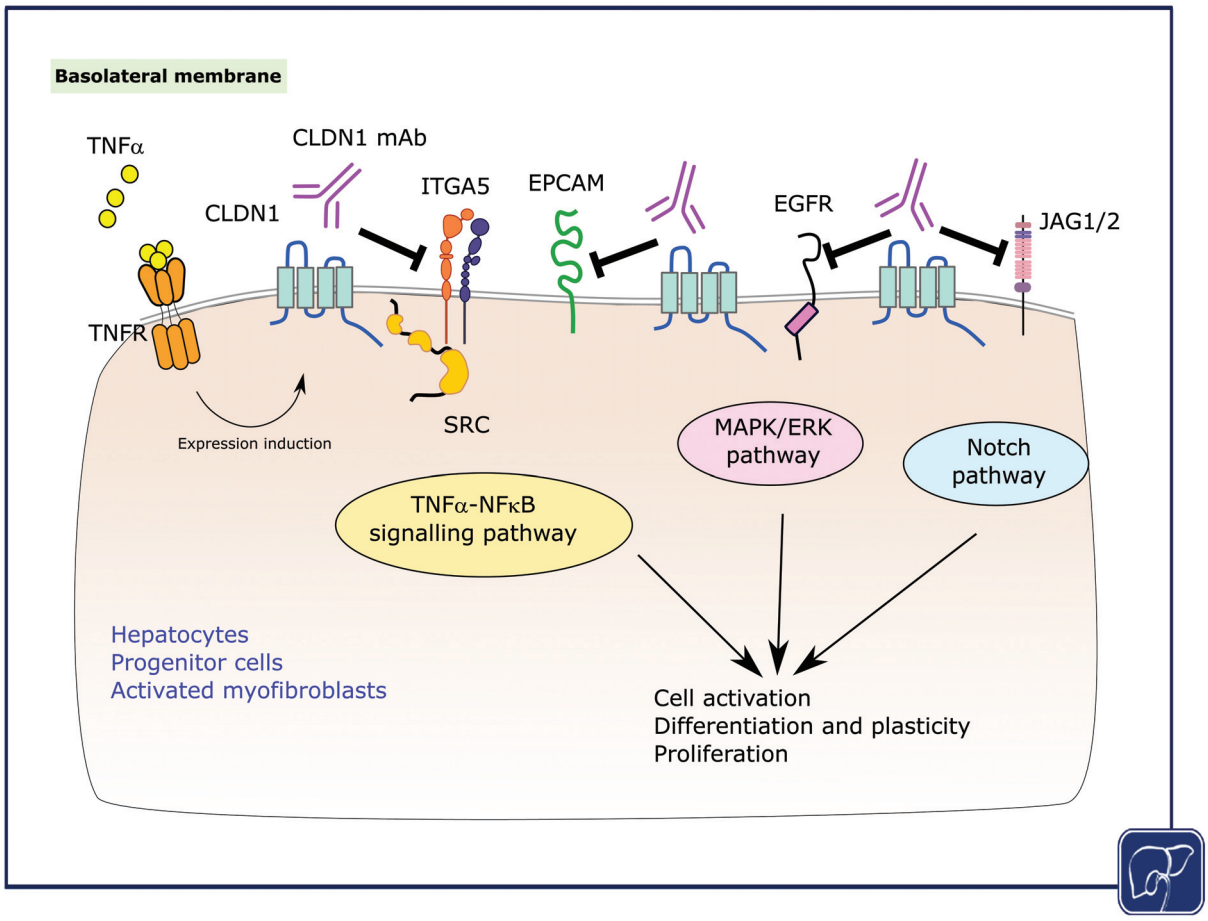

Fig. 2 Nonjunctional CLDN1 as therapeutic target for liver fibrosis and cancer. Nonjunctional CLDN1 (njCLDN1) interacts with other transmembrane proteins such as ITGA5 (Integrin Subunit Alpha 5), EPCAM (epithelial cell adhesion molecule), EGFR (epidermal growth factor receptor), and JAG1/2 (Jagged canonical Notch ligand) as described previously.[28] This interaction activates TNFα–NFκB signaling, MAPK/ERK, SRC, and Notch signaling pathways, which control cell activation, differentiation, and plasticity as well as proliferation. A CLDN1-specific mAb targeting exposed CLDN1 on hepatocytes or liver cancer cells has demonstrated to robustly inhibit liver fibrosis and prevent and treat HCC.[28,84] HCC, hepatocellular carcinoma; mAb, monoclonal antibody.

intracellular signaling of CLDN1, along with the effects of the nonjunctional CLDN1-specific mAb are presented in ►**Fig. 2**. In addition, the role of CLDN1 in fibrosis seems not to be limited to the liver. CLDN1 has been found to be upregulated in both lung and kidney patients and models.[57–59] Moreover, treatment with CLDN1 mAbs efficiently reduced lung and kidney fibrosis in several state-of-the-art mouse and patient models.[28,60]

The clinical development of an mAb targeting nonjunctional CLDN1 in liver and other organ fibrosis is currently ongoing. A fully humanized anti-human CLDN1 mAb has undergone safely testing in cynomolgus monkeys[28] and healthy volunteers. Moreover, a phase Ib clinical trial is currently ongoing for patients with advanced liver fibrosis or compensated cirrhosis (FEGATO-01, NCT05939947). A phase II clinical trial is under way for patients with anti-neutrophil cytoplasmic antibody-associated vasculitis with rapidly progressive glomerulonephritis, which induces kidney fibrosis (RENAL-F02, NCT06047171).

Other TJ proteins have also been described in liver fibrosis and regeneration, although their role as therapeutic target has been poorly investigated with no differentiation between junctional and nonjunctional proteins. JAMs affect leukocyte recruitment and migration, vascular permeability, and remodeling. In healthy livers, epithelial cells, endothelial cells, and hepatic stellate cells express JAM-A, whereas JAM-B and -C are restricted to endothelial cells. Complete and selective knockout of *Jam-a* in bone marrow-derived cells and endothelial cells exacerbates liver fibrosis in CCl_4-induced model regulating nonsinusoidal vascular immune cell recruitment, liver sinusoid capillarization, and hematopoietic stem cell quiescence.[61] In a mouse model of CCL4-induced fibrosis, JAM-B and -C expression increases in endothelial cells and JAM-C expression is induced in myofibroblasts, thereby enhancing their contractility capacity. These results suggest a potential role of these TJ proteins in regulating liver immune cell recruitment and fibrosis-induced portal hypertension.[62] Tight-junction protein 2 (TJP2) is a TJ protein required for normal cortical distribution of radixin, bile canalicular volume regulation, and biliary microvilli density. *Tjp2* knockout deregulates CLDN1, and key bile acid transporters and detoxification enzymes and it is associated with liver injury and fibrosis.[63] Experiments with mice fed a choline-deficient, ethionine-supplemented, or

diethoxycarbonyl-1,4-dihydrocollidine diet, models for chronic liver disease, biliary–blood barrier injury, and ductular reaction, show reduced expression of several TJ proteins such as CLDN3, 5, and 7.[64] In the healthy liver, CLDN4 and CLDN7 are expressed only in hepatic progenitor cells and cholangiocytes. However, in cirrhotic livers, CLDN4 and CLDN7 are detected in hepatocytes at the edges of regeneration nodules, suggesting a role of these TJ proteins in liver regeneration during extensive fibrosis.[65] Livers from patients with alcohol-related hepatitis, a condition usually associated with extensive fibrosis, demonstrated upregulations of several CLDNs and gap junction molecules linked to hepatocyte regeneration and hepatic stellate cell activation. These include CLDN5, CLDN10, CLDN1 and connexin 26, 32, 43, 46.6 as well as gap junction protein 12.[66] In the CCl_4 fibrosis model, the expression of hepatic and small intestinal zonula occludens-1 (ZO-1) and OCLN significantly decreased, and these changes can be reverted by a Ginkgolide-A treatment.[67] Finally, in rats treated with thioacetamide, a model of liver fibrosis with pronounced ductular reaction, a deregulation of OCLN et CLDN2 driven by MAP-kinase, p38 MAP-kinase, and PI3-kinase in response to IL-1β has been observed.[68] During liver regeneration after partial hepatectomy in rats, p38 MAP-kinase induces downregulation of connexin 32 and upregulation of CLDN1. In vivo administration of p38 MAP-kinase inhibitor SB203580 can effectively hinder connexin 32 downregulation and promote CLDN-1 upregulation, suggesting a possible contribution of this compound toward the restoration of hepatocytes following liver damage.[69,70]

In summary, CLDN1 is the first nonjunctional TJ protein that has undergone extensive preclinical studies with completed proof of concept to treat liver fibrosis and prevent liver cancer. A highly specific mAb targeting nonjunctional CLDN1 modulates the plasticity of hepatocytes, progenitor cells, and myofibroblasts, restoring their differentiated and functional cell phenotype that is disrupted by fibrosis and inflammation in chronic liver disease and during the development of HCC.[28] The mAbs act mainly on CLDN1 expressed on hepatocytes and progenitor cells as well as CLDN1 additionally expressed on stellate cells and myofibroblasts. The CLDN1-specific mAbs exhibit a dual effect on liver fibrosis and HCC prevention and thus have potential as a treatment for patients with active chronic liver disease and advanced fibrosis or at a high risk of HCC development. Moreover, these CLDN1-specific mAbs may be employed as HCC adjuvant treatment to reduce recurrence risk after surgery or locoregional therapies or, once safety study have been completed, in patients with advanced cirrhosis who are not eligible for liver transplantation. Compared with other compounds in clinical development for MASH and fibrosis, the CLDN1 mAb displays a very robust antifibrotic profile and has a shown a unique potential for preventing HCC in preclinical models. Combining antimetabolic drugs such as GLP-1 and thyroid hormone receptor-β agonists with the CLDN1 mAb will provide opportunities for treatment strategies that simultaneously target MASH's metabolic and fibrotic hallmarks.

Targeting Tight Junction Proteins to Treat Hepatocellular Carcinoma

HCC represents the most frequent type of primary liver cancer and the fourth-leading cause of cancer-related death worldwide.[71,72] A key characteristic of HCC carcinogenesis is its development in a chronically inflamed and most frequently fibrotic liver microenvironment.[73] In this regard, Claudins as key regulators of cell–cell and cell–matrix interactions have gained increasing interest as drivers of hepatocarcinogenesis. In fact, numerous studies report dysregulated expression of different members of the TJ protein family in HCC: while CLDN3 and 14 have been shown to be downregulated in HCC,[74,75] CLDN1, 5, and 10 are overexpressed in tumorous HCC liver tissue.[52,76–78] Indicating a functional impact of its differential abundance, CLDN1 overexpression has been shown to be correlated with poor patients' survival.[79] Similarly, high levels of CLDN10 were found to be associated with worse patients' outcome and tumor recurrence.[76–78] Only limited data exist on the expression of other TJ proteins, such as OCLN and ZO in HCC. Ram et al reported decreased expression of ZO-1 in HCC tissue.[80] In line, Nagai et al described decreased ZO-1 levels in HCC as a predictive marker of poor prognosis.[81] The data on the expression level of OCLN in HCC are controversial and both up- and downregulation have been described, potentially reflecting a correlation of OCLN expression with tumor cell differentiation[76,82] (for a review see[83]). Strikingly, the expression of Claudins has been associated with a specific differentiation of the corresponding liver tumor. Hereby, not only the overall expression level, but also particularly the subcellular localization seems to impact on tumor cell plasticity. Thus, CLDN1 overexpression in HCC tumor cells was found to be accompanied by aberrant nonjunctional delocalization in the cytoplasm and even nucleus.[84] These CLDN1 overexpressing liver tumors were further found to show a stem cell or progenitor like phenotype.[84] CLDN4 and CLDN7 overexpressing liver tumors on the other hand show a ductular differentiation.[85] Similar observations were made in other solid cancer types: cytonuclear delocalization of ZO-1 has been shown to be associated with epithelial–mesenchymal transition (EMT) and highly invasive human lung cancer cells.[86] In breast cancer, ZO-1 is associated with a glandular differentiation.[87] Cytosolic OCLN expression was further associated with a well differentiation of gastric tumor cells.[88]

Genetic and epigenetic alterations, as well as signaling pathways, can regulate the overexpression and delocalization of TJ proteins, potentially through posttranslational modifications. Recent research from our group has shown that Claudin-1 is upregulated in AXIN1-mutated HCC, whereas downregulated in tumors with CTNNB1 mutations.[84] Additionally, claudin gene amplification has been described to affect expression patterns.[89] Potential mechanisms of claudin upregulation also include epigenetic alterations. In fact, specific histone modifications have been shown to contribute to CLDN14 overexpression in HCC.[74] Signaling pathway-mediated posttranslational

modifications are believed to have the greatest impact on dynamic changes in TJ protein expression. A recent study found that TNFα-NFκB signaling strongly upregulates Claudin-1 expression in liver parenchymal and nonparenchymal cells in the context of inflammation.[28] Furthermore, our group identified hypoxia as a significant factor in the upregulation of CLDN1 expression in HCC cancer cells.[84] Other studies suggest that oncogenic signaling pathways, including PI3K/AKT/mTOR or MAPK/ERK signaling, can upregulate hepatic expression of different claudin family members.[90,91] However, the molecular mechanisms underlying protein overexpression have not yet been fully defined. The mechanism of TJ protein delocalization is also not yet understood. Studies on colon cancer cell lines suggest that the cell adhesion protein EPCAM, which is often overexpressed in cancer, stabilizes Claudin 1 and 7 at nonjunctional locations and prevents their lysosomal degradation.[83,92]

Beyond its role as downstream targets of signaling cascades, Claudins and other members of the TJ protein family also actively impact on key oncogenic cell processes by forming bidirectional signaling hubs that connect the extracellular to the intracellular compartment (for a review see[93]). As a key event during hepatocarcinogenesis, EMT has consistently been associated with TJ protein alterations. However, given the role of TJ in maintaining cell polarity as a key feature of epithelial cells, the questions arise whether alterations in the expression reflect an active role of these proteins in the mechanism of EMT or rather depict its consequence. While numerous reports in other solid cancers, including breast, pancreatic, or colon cancer suggest a direct interaction of diverse TJ proteins with EMT mediators (for a review see[93,94]), conclusive perturbation studies in HCC are mostly limited to Claudins. Suh et al reported CLDN1 to induce EMT in HCC via c-Abl-ERK signaling induction and upregulation of the transcription factors Slug and Zeb 1.[95] Moreover, Yoon et al revealed CLDN1 to promote EMT via c-Abl-OKC-δ-mediated upregulation of MMP2.[96] Conversely, CLDN3 suppresses EMT in liver cancer by downregulation of Wnt-β-catenin signaling.[75]

Beyond its impact on EMT, Claudins, and other TJ proteins have been associated with cell–cell or cell–matrix interactions and key oncogenic cascades regulating cell growth. Corroborating the role of CLDN1 in cell–cell communication, a recent study identified nonjunctional CLDN1 to control Notch signaling upon cell–cell contact.[84] Zhang et al reported ZO-1 overexpression in liver cancer cells to inhibit cell proliferation and migration in vitro.[97]

Given its active role in key oncogenic events, including tumor cell differentiation and stemness, as well as EMT and invasion, TJ proteins have been studied extensively as potential targets of cancer therapy, including HCC. Yet, treatment strategies for patients with advanced HCC that are not eligible for surgical or locoregional therapies are highly limited.[7] Currently, the combination therapy with atezolizumab and bevacizumab or durvalumab and tremelimumab represent the preferred first line therapy for advanced HCC. However, objective treatment response is below 30%,[98] and alternative treatment strategies such as receptor tyrosine kinases show only limited efficacy in immunotherapy-resistant HCC's (for a review see[99,100]). Moreover, HCC recurrence after curative treatments such as resection or local ablation is high, with rates exceeding 70% after 4 years. To date, there is no approved adjuvant treatment to mitigate the risk of HCC recurrence. Thus, urgent development of novel HCC therapies is imperative.

CLDN1 and CLDN6 are currently the targets with the most promising and advanced clinical development. Targeting nonjunctional CLDN1 by a mAb has been shown to suppress tumor initiation and progression in patient-derived ex vivo and in vivo models of HCC.[28,84] Mechanistically, tumor cell suppression was associated with broad inhibitory effects on oncogenic signaling cascades, EMT, as well as cancer stemness.[84] These oncogenic pathways are also modified in other cancers such as head and neck cancer.[101] Currently, a phase I/II clinical trial is investigating a CLDN1 mAb with or without combination with pembrolizumab in patients with head and neck cancer (NCT06054477).

CLDN6 is highly expressed in embryonic stem cells and in HCC compared with normal tissue. CLDN6 expression in HCC is associated with a biliary transdifferentation phenotype and sorafenib resistance. CLDN6 overexpression activates the JAK2/STAT3 signaling pathway, leading to increased BLC2 expression and inhibition of apoptosis in cancer cells.[102] Additionally, CLDN6 competes with YAP1 for TJP2 binding, preventing YAP1 cytoplasmic retention. This results in elevated levels of free YAP1, facilitating its nuclear translocation and transcription of genes that promote cell proliferation and trigger EMT[49,103] (►**Fig. 3**). Anti-CLDN6 mAbs conjugated with cytotoxic agents further showed potent antitumor efficiency as well as sorafenib synergy in mouse models of HCC.[49] Safety and efficacy of CLDN6 targeting chimeric antigen receptor (CAR)-T cells, with or without a CAR-T Cell amplifying RNA vaccine, for any type of $CLDN6^{+}$ cancers, including HCC, are currently being evaluated in a phase I/IIa trial (NCT04503278). A nonprespecified interim analysis of 22 patients treated in this trial has been recently published. Safety data show manageable toxicity with 46% of patients experiencing cytokine release syndrome. The unconfirmed objective response was 33% with one complete response. However, no enrolled patient had liver cancer.[104]

While data on small molecule inhibitor mediated targeting of ZO protein is yet restricted to gastrointestinal cancers other than HCC (for a review see[105]), reduction of proliferation and migration of liver cancer cells upon transgenic ZO-1 overexpression may catalyze further research on ZO-targeting therapies in HCC.

In summary, targeting Claudins is a promising strategy for HCC treatment. CLDN1- and CLDN6-based treatment approaches have shown strong efficacy in preclinical models and clinical development has started for solid tumors outside the liver. Future studies should prioritize the development of companion biomarkers and response predictors as well as the investigation of combined therapies using immune checkpoint inhibitors and/or antiangiogenic drugs to enhance treatment efficacy.

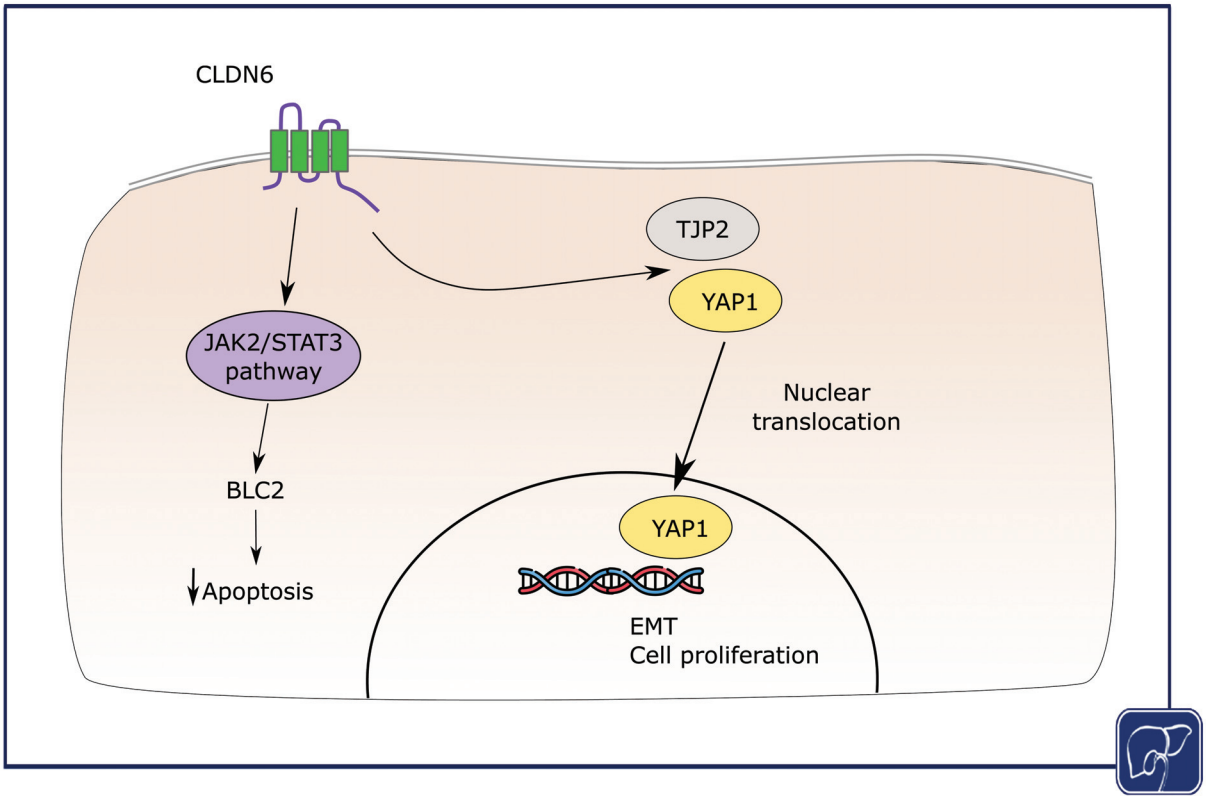

Fig. 3 Biology of CLDN6-overexpressing liver cancer. CLDN6 overexpression activates the JAK2/STAT3 signaling pathways that upregulates BLC2 (B cell lymphoma 2) and reduces cancer cell apoptosis. CLDN6 also competes with YAP1 (Yes-associated protein) for binding to TJP2 (tight junction protein 2), preventing YAP1 translocation to the nucleus. CLDN6 overexpression increases the levels of unbound YAP1 and favors YAP1 nuclear translocation and transcription of genes that promote cell proliferation and induce EMT. Anti-CLDN6 mAbs have been shown to have preclinical efficacy against HCC.[49] EMT, epithelial–mesenchymal transition; HCC, hepatocellular carcinoma.

Conclusions

Studies on HCV biology have unveiled a key role of TJs proteins in liver pathophysiology and development of fibrosis and cancer. Nonjunctionally exposed TJ proteins have been reported to play a key role in oncogenic intracellular signaling and mediate cell proliferation, differentiation, and function. As a treatment for liver fibrosis, CLDN1 is currently the most advanced target with ongoing clinical development. CLDN1 exists in a junctional and nonjunctional form, and it is expressed not only in liver epithelial cells but also in liver progenitors and activated myofibroblasts. Nonjunctional CLDN1 is has been uncovered as a driver for liver fibrosis and HCC via the activation of multiple signaling pathways such as TNF-α–NFκB, EGFR, ERK, Notch-1, and SRC resulting in modulation of plasticity and fate of fibrosis driver cells including hepatocytes and myofibroblasts. Preclinical data demonstrate robust and significant antifibrotic and HCC prevention efficacy, highlighting an opportunity for this mAb in patients to treat advanced liver fibrosis (F3/4), those at high risk of HCC, and patients with cirrhosis who are ineligible to liver transplantation.

Several studies investigated the role of TJ proteins such as CLDN1, CLDN4–7, OCLN, ZO-1 in the biology of HCC. Preclinical studies have identified CLDN1 and 6 as therapeutic targets. CLDN1- and CLDN6-based treatment approaches have shown robust efficacy in preclinical models and clinical development has started for solid tumors outside the liver. The antifibrotic activity of CLDN1 targeting therapies is a particular advantage for HCC treatment, since the dual anticancer and antifibrotic effect may also improve the underlying liver disease, which is often a key denominator of survival in HCC patients. Further studies are needed for clinical proof of concept in HCC.

Authors' Contribution

A.S. conceptualized, wrote the manuscript, and prepared the figures. N.R. wrote the manuscript. T.F.B. conceptualized, wrote, and edited the manuscript.

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Conflict of Interest

Inserm, the University of Strasbourg, Strasbourg University Hospitals, and the Institut Hospitalo-Universitaire have filed patents and patent applications: US62/153,727 (Clinical gene signature–based human cell culture model and uses thereof; inventors: T.F.B.), US13/119,233 (anti-Claudin-1 antibodies for the inhibition of hepatitis C virus infection; T.F.B.), US15/979,609 PCT/EP2016/055942 (anti-Claudin-1 monoclonal antibodies for the prevention and treatment of HCC; T.F.B.), US16/086,934 PCT/EP2017/056703 (humanized anti-Claudin 1 antibodies and uses thereof; T.F.B.), and PCT/EP2020/081941 (Anti–Claudin 1 monoclonal antibodies for the prevention and treatment of fibrotic disease; T.F.B., N.R., A.S.) which have all been licensed to Alentis Therapeutics, Basel. T.F.B. is the founder, owns shares and serves as a consultant for Alentis. Any potential conflict of interest is managed independently by the SATT Conectus and Inserm Transfert for the authors of the University of Strasbourg and Inserm.

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