Integrins as a drug target in liver fibrosis

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Abbreviation list:

CLD – Chronic liver disease

ECM – Extracellular matrix

NASH – Non-alcoholic steatohepatitis

IPF – Idiopathic pulmonary fibrosis

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C8 – Compound 8

HSC – Hepatic stellate cells

NAFLD – Non-alcoholic fatty liver disease

DAMP – Damage-associated molecular patterns

HMGB1 – High-mobility group box protein 1

ROS – Reactive oxygen species

CCL – Chemokine ligand

IL – Interleukin

TNFα – Tumour necrosis factor α

PDGF – Platelet derived growth factor

TGFβ – Transforming growth factor β

RGD – Arginine, glycine and aspartate

MAPK – Mitogen-activated protein kinase

PI3 – Phosphoinositide 3

IC_{50} – Inhibitory concentration

ALK – Activin receptor-like kinase

CCL_4 – Carbon tetrachloride

UUO – Unilateral ureteral obstruction

BDL – Bile duct ligation

TGFα – Transforming growth factor α

ERK – Extracellular-signal-regulated kinase

PET – Positron emission tomography

FXR – Farnesoid X receptor

THR-β – Thyroid hormone receptor β

CCR – Chemokine receptor type

FGF – Fibroblast growth factor

ACC – Acetyl-CoA carboxylase

DGAT2 – Diacylglycerol O-acyltransferase 2

FGFR – Fibroblast growth factor receptor

KLB – β-klotho

TLR4 – Toll-like receptor 4

K_D – Dissociation constant

ASK1 – Apoptosis signal-regulating kinase 1

Conflict of interest

JAR is a former employee of GlaxoSmithKline and current employee at Galecto Biotech and may own stocks or shares in both companies. KTP is a current employee at GlaxoSmithKline. GPA has served as an advisory board member for Medicines and Healthcare Products Regulatory Agency, Department of Health and Social Care, UK, Pfizer and GlaxoSmithKline; he has been a consultant to Amryt Pharmaceuticals and Astra Zeneca. SRR, JIG and AJB have no conflict of interest to declare.

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Abstract and keywords

As the worldwide prevalence of Chronic Liver Diseases is high and continuing to increase, there is an urgent need for treatment to prevent cirrhosis-related morbidity and mortality. Integrins are heterodimeric cell-surface proteins that are promising targets for therapeutic intervention. αv integrins are central in the development of fibrosis as they activate latent TGFβ, a known profibrogenic cytokine. The αv subunit can form heterodimers with β1, β3, β5, β6 or β8 subunits and one or more of these integrins are central to the development of liver fibrosis, however, their relative importance is not understood. This review summarises the current knowledge of αv integrins and their respective β subunits in different organs, with a focus on liver fibrosis and the emerging preclinical and clinical data with regards to αv integrin inhibitors.

Keywords:

Integrins, fibrosis, liver disease, non-alcoholic steatohepatitis, liver cirrhosis

- There are not any approved treatments for liver fibrosis with the current drug pipeline targeting anti-inflammatory pathways and/or lipid metabolism/steatosis rather than fibrosis.
- αv integrins which form heterodimers with β1, β3, β5, β6 or β8 subunits are central to the development of liver fibrosis and have the potential to be a promising antifibrotic target, however, their specific role is not completely understood.
- Preclinical models, as well as preclinical and clinical data using αv integrin inhibitors have shown certain αv integrins to be a potential target in various fibrotic diseases including liver fibrosis.

Key points:

Main text

Introduction

Liver fibrosis

Liver fibrosis occurs in most chronic liver diseases (CLD) (Bataller & Brenner, 2005), affecting an estimated 844 million people worldwide and having a mortality rate of two million deaths per year (Marcellin & Kutala, 2018). It is the excessive deposition of extracellular matrix (ECM) proteins and results from

persistent and chronic injury to the liver which may be caused by hepatitis C infections, alcohol excess and non-alcoholic steatohepatitis (NASH) (Bataller & Brenner, 2005).

The majority of CLDs follow a pattern where recurrent or persistent injury is associated with inflammation, followed by fibrosis and finally cirrhosis, leading to altered hepatic function (Brenner, 2009). The presence and severity of fibrosis predicts both cirrhosis development and long-term survival (Caballería et al., 2018).

Although fibrosis initially starts by being part of the tissue repair process, it becomes pathogenic when it is not controlled (Wynn, 2008). In tissue fibrogenesis, integrins, which are heterodimeric cell surface transmembrane receptors, mediate various cell-matrix and cell-cell interactions (Kimura et al., 2019; Schnittert et al., 2018). In idiopathic pulmonary fibrosis (IPF), αvβ6 integrin has been shown to be a potential biomarker of fibrosis (Kimura et al., 2019) as expression increases in the lung following injury (Henderson & Sheppard, 2013) and is correlated with poor prognostic outcome (Saini et al., 2015). Furthermore, in both rodent models of liver fibrosis and patients with CLD, αvβ6 integrin expression has been shown to be markedly upregulated (Popov et al., 2008). Alternative studies have suggested that the major integrin driving tissue fibrosis in the liver and lung is αvβ1 integrin. This is supported by data showing that administration of the αvβ1-specific small-molecule integrin inhibitor compound 8 (c8), resulting in the partial reversal of liver and lung fibrosis in rodent models (Reed et al., 2015).

Initially, fibrosis was thought to be irreversible, with tissue scarring being deemed permanent. However, there is now compelling evidence for the resolution of fibrosis in the liver (Jun & Lau, 2018). Patients with liver fibrosis due to viral hepatitis showed regression of fibrosis after treatment with antiviral therapies, suggesting that liver fibrosis can be reversed through addressing the underlying cause of the fibrosis (Jung & Yim, 2017). The liver also has an extraordinary capacity to regenerate in comparison to organs such as the lungs and kidneys. This may make the liver a more attractive target to reverse fibrosis compared to other organs.

Role of integrins in liver fibrosis

Cellular mechanisms of liver fibrosis

The progression of fibrosis is complex and involves parenchymal, non-parenchymal, as well as infiltrating immune cells. During liver injury, hepatocyte death via apoptosis, necrosis or necroptosis triggers activation of inflammatory and fibrogenic pathways. This occurs in cells such as hepatic stellate cells (HSCs), hepatocytes and Kupffer cells which all interact and promote the secretion of proinflammatory

and/or profibrogenic cytokines (Bataller & Brenner, 2005; Marra et al., 1999; Seki & Schwabe, 2015; Trautwein et al., 2015) (Figure 1). HSCs are the primary effector cells in liver fibrosis (Lee & Friedman, 2011) and are considered to be the main source of hepatic myofibroblasts, the major hepatic ECM-producing cells (Seki & Schwabe, 2015). In response to chronic liver injury, quiescent HSCs are activated and change their morphology transdifferentiating to myofibroblasts (Bataller & Brenner, 2005).

Transforming growth factor β (TGFβ) is a key regulator of liver physiology and pathology. It contributes to all stages of disease progression, from initial liver injury, to fibrosis and then cirrhosis. It is known as a profibrogenic cytokine due to its role in HSC activation and ECM production (Fabregat et al., 2016). HSCs become activated as a result of reactive oxygen intermediates, apoptotic bodies and paracrine stimuli from neighbouring cell types in response to liver injury (Friedman, 2008) and as a result HSCs secrete latent TGFβ. The activation of latent TGFβ by integrins is a major mechanism (Khan & Marshall, 2016) that leads to an autocrine positive feedback loop being formed which drives fibrogenesis (Higashi et al., 2017).

An overview of integrins and their role in health and disease

Integrins are present in all nucleated cells and multiple subtypes can be expressed simultaneously. There are 24 known integrin heterodimers which arise from noncovalent associations between 18 different α-subunits and 8 β-subunits (Hamidi & Ivaska, 2018; Springer & Dustin, 2012). Integrins can be broadly grouped into families according to these ligand binding or expression specificities. For example, Arg-Gly-Asp (RGD) integrins that recognise proteins containing the RGD peptide motifs (notably present on latent TGFβ), collagen receptors, laminin receptors and leukocyte-specific integrins (Goodman & Picard, 2012; Humphries et al., 2006).The regulatory effect of integrins on TGFβ activity appears to primarily involve αv-containing integrins that selectively bind to the RGD motif on latent TGFβ, activating TGFβ (Khan & Marshall, 2016; Margadant & Sonnenberg, 2010). Of the 8 members in this family of RGD integrins, 5 contain the αv subunit, with αvβ6 and αvβ8 integrins having the highest affinity for latent TGFβ (Humphries et al., 2006; Khan & Marshall, 2016; Margadant & Sonnenberg, 2010). In activated HSCs, αvβ1, αvβ3, αvβ5 and αvβ8 integrins have been shown to be expressed (Henderson et al., 2013) with αvβ3 and αvβ5 integrins expressed on endothelial cells. These cells promote activation of HSCs (Schuppan et al., 2018). Additionally, αvβ6 integrin has also been shown to be expressed on activated cholangiocytes (Hintermann & Christen, 2019) which are important drivers of fibrogenesis in biliary liver diseases and in more advanced stages of liver fibrosis of all etiologies (Schuppan et al., 2018).

Integrins are involved in a wide range of cellular processes and are the main cell adhesion receptors for the components of the ECM. They are activated by a variety of extracellular ligands which include ECM

ligands, causing them to undergo conformational changes (Goodman & Picard, 2012; Hamidi & Ivaska, 2018; Margadant & Sonnenberg, 2010) which are more complex than other cell surface receptors (J. Li & Springer, 2017; Springer & Dustin, 2012). Integrins sense both chemical and physical properties of the ECM, binding extracellularly to the ECM and intracellularly to the cytoskeleton, thereby linking the extracellular environment with the cell interior (Hamidi & Ivaska, 2018). Cooperation with growth factors, ECM components and co-receptors results in integrins being able to generate an integrated signal, fine-tuned to the precise external environment (Patsenker & Stickel, 2011; Thomas et al., 2019). Integrin activation and binding to the ECM triggers complex and highly dynamic machinery responsible for regulating aspects of cell fate such as survival, migration, polarity and differentiation (Hamidi & Ivaska, 2018; Margadant & Sonnenberg, 2010).

Interactions of integrins with ECM ligands specifically results in the remodelling of the ECM (Hamidi & Ivaska, 2018; Margadant & Sonnenberg, 2010). The pathways that are activated depend on the nature of the binding and include integrin-linked kinase and growth factor signalling pathways such as the mitogen activated protein kinase (MAPK) and phosphoinositol 3-kinase pathways (Patsenker & Stickel, 2011), pathways regulating cell proliferation, survival and growth (Engelman et al., 2006; Wei & Liu, 2002). Hence, the dysregulation of integrin-mediated adhesion, signalling and in particular, the activation of latent TGFβ, is a precursor in the pathogenesis of diseases such as fibrosis (Conroy et al., 2016; Goodman & Picard, 2012; Hamidi & Ivaska, 2018) (Figure 1).

Integrin antagonists have attracted attention as potential therapeutic targets for fibrosis as they are already well established therapeutics for cardiovascular diseases (Shimaoka & Springer, 2003). For example, αIIbβ3 integrin antagonists, abciximab, eptifibatide and tirofiban are used to inhibit thrombosis (Estevez et al., 2015). Small-molecule integrin antagonists fall into three different classes that each relate to a specific integrin conformation state (Figure 2). The antagonists can either interfere with ligand binding or stabilise a particular integrin conformation. Most antagonists stabilise the extended, high-affinity integrin conformation (Shimaoka & Springer, 2003). There have been efforts to develop antagonists that effectively inhibit high affinity conformations, however, none have been translated to successful therapies. Suggestions are that they have either insufficient specificity or systemic toxicity but details have not been disclosed (Ley et al., 2016). In addition to this, the functional consequences of the antagonist-induced active conformation has been debated with agonistic effects not clear. For example, αIIbβ3 inhibitors stabilise the high-affinity integrin conformation and failed phase 3 trials which could be due to the unintended partial agonist effects. Further structural work on integrins and their complexes in order to understand the conformation change during activation should accelerate the development of

current and novel classes of integrin antagonists, especially for allosteric inhibitors (Ley et al., 2016; Shimaoka & Springer, 2003).

TGFβ and αv integrins

A large body of evidence points to the importance of TGFβ and αv integrins in tissue fibrosis. TGFβ signalling has been shown to upregulate expression of specific integrins (Munger & Sheppard, 2011). For example, treatment of human WI-38 lung fibroblasts cell line with TGFβ led to increased αvβ3 and β1 integrin expression by ~3- and 2-fold respectively (Heino Jyrki et al., 1989; Ignotz et al., 1989). Similarly, in human keratinocytes, TGFβ increased the expression of αvβ5 integrin ~4-fold and it induced αvβ6 integrin expression, thereby further driving TGFβ activation effects (Zambruno et al., 1995).

In models of pulmonary and renal fibrosis, αvβ6 integrin has been shown to be the main class involved in latent TGFβ activation during fibrosis development. For example, the reduced fibrotic response from bleomycin and ureteral obstruction-induced fibrosis in β6 integrin knockout mice was suggested to be due to the lack of active TGFβ as shown through the little staining detected using immunohistochemistry (Ma et al., 2003; Munger et al., 1999). Both αvβ3 and αvβ5 integrins are suggested to activate TGFβ in scleroderma fibroblasts to promote the transformation of these cells into myofibroblasts. Assessment of TGFβ activation in a co-culture model comprising murine HSCs and a TGFβ reporter (plasminogen activator inhibitor 1-luciferase) cell line demonstrated TGFβ activation was inhibited by an αvβ1 integrin inhibitor, c8. Similar results were also observed using human IPF lung fibroblasts and in murine renal fibroblasts. Inhibitory concentrations (IC_{50}) of c8 in this co-culture system ranged from 0.35nM to 0.5nM for HSCs and lung fibroblasts, as well as 1nM for renal fibroblasts. These data suggest that αvβ1 integrin is an important integrin expressed by liver HSCs, lung and kidney fibroblasts, which is at least in part responsible for activation of latent TGFβ and drugs targeting αvβ1 integrin could prove useful for the treatment of fibrosis (Chang et al., 2017; Reed et al., 2015).

In addition to the above showing the important role of αv integrins in the activation of TGFβ, intrinsically linking it to fibrosis, further compelling evidence comes from studies in mice which demonstrated that the targeted conditional deletion of αv integrins from HSCs significantly inhibited fibrosis in the liver and similarly in models of lung and kidney fibrosis (Henderson et al., 2013). Hence, αv integrins are also central in the development of fibrosis (Conroy et al., 2016)

TGFβ as a target to treat fibrosis

As TGFβ plays a central role in the pathogenesis of liver fibrosis, targeting TGFβ signalling may present a therapeutic strategy for liver diseases (Xu et al, 2016). A variety of strategies to block TGFβ signalling by

targeting TGFβ isoforms or by interfering with TGFβ receptor activation have been evaluated over the years (Györfi et al., 2018). For example, the TGF-β type I receptor, also known as activin receptor-like kinase (ALK) 5 inhibitor inhibits TGFβ signalling which has been shown to prevent fibrosis in animal models of liver, lung and kidney (Bonniaud et al., 2005; De Gouville et al., 2005; Petersen et al., 2008).

Although TGFβ could be used as a target for antifibrotic therapies, there are many concerns. This is due to TGFβ having multiple essential roles in cell differentiation, regulating cell proliferation, immune regulation, cancer surveillance and wound healing. This explains that while there have been many ALK5 small molecule inhibitors developed, few have progressed to clinical investigation due to severe cardiac toxicities in animals (Herbertz et al., 2015; van Beuge et al., 2013). Moreover, studies in patients have not been promising, in the CAT-192 placebo-controlled clinical trial, people treated with anti-TGFβ1 monoclonal antibody reported more adverse effects than those on placebo. This re-emphasises the wide ranging critical functions of TGFβ (Morikawa et al., 2016) and systemic manipulation of levels is likely to incur unwanted toxic effects (Denton et al., 2007). This has spurred the idea for targeting specific steps in TGFβ activation in a more localised manner to reduce widespread toxicity (Mehal & Schuppan, 2015).

This can be achieved through targeting integrins such as αv integrins which have a key role in the activation of latent TGFβ. The targeting of tissue or cell specific over expression of integrins could potentially permit a more precise regulation of TGFβ, rather than targeting global TGFβ activity. This potential for αv integrins being an effective antifibrotic therapy is revealed by their upregulated expression in mouse models of fibrosis and by the promising integrin inhibitor and knockout studies (Henderson et al., 2013; Henderson & Sheppard, 2013).

Integrins as a target to treat liver fibrosis

αv integrins

The αv integrin subunit can form heterodimers with β1, β3, β5, β6 or β8 subunits (Henderson & Sheppard, 2013) and activated HSCs with a myofibroblastic phenotype have been shown to express all of the known αv-containing integrins except αvβ6 integrin (Henderson & Sheppard, 2013). Most αv integrin drug discovery programmes over the past three decades have primarily focused on cancer, with some attention to fibrotic diseases (Hatley et al., 2018). Pan-αv integrin antibodies, abituzumab (EMD 525797) and intetumumab (CNTO-95) both bind to the αv subunit and prevent their cognate ligands from binding, thereby inhibiting activity. Abituzumab has been in phase 2 clinical trials for colorectal cancer and has been investigated in prostate cancer and systemic-sclerosis-associated interstitial lung disease (O'Day et al., 2011; Raab-Westphal et al., 2017). Unfortunately, the clinical trial for scleroderma patients was

terminated early due to difficulties in identifying participants who met the eligibility criteria (Lodyga & Hinz, 2020). Intetumumab showed early promise in the treatment of melanoma patients and was in phase 2 of clinical trials (O'Day et al., 2011) but there has not been any progress since 2013 (NCT00246012).

It is important to note that αv integrins are widely expressed with wide ranging multimodal functions, hence, molecules targeting αv integrins can affect all 5 αv combinations and be prone to off-target effects (Hynes, 2002; Lowell & Mayadas, 2011; Schnittert et al., 2018). For example, cilengitide, (EMD121974), an αv integrin antagonist which exerts antiangiogenic and antitumour effects preclinically, inhibits αvβ3 and αvβ5 integrins (Alghisi et al., 2009) but failed to improve clinical outcome in phase 3 late-stage glioblastoma trials (Raab-Westphal et al., 2017).

Another small molecule, GLPG0187, a pan αv inhibitor entered the clinic for cancer treatment. However, like cilengitide, it did not show notable clinical efficacy. Although this inhibitor targets multiple integrins, it has been shown to have acceptable safety profiles. There is possibility that inhibition of multiple different integrins may protect against adverse effects in a similar way to that observed in pre-clinical experiments, which have shown an increased risk of vascular leak through αvβ3 integrin inhibition but protection from this may be provided through inhibition of αvβ5 integrin. From an efficacy perspective, it is not known whether inhibiting more than one αv integrin in any particular disease will deliver a better therapeutic agent clinically. Overall, application of integrin antagonists in oncology has been disappointing. One reason is the lack of pre-clinical evidence in the modulation of a particular biological mechanism being predictive of the clinical effect. This underpinning target validation is currently inadequate for αv integrins (Hatley et al., 2018).

As described previously, deletion of αv integrins in mouse models of liver, lung and kidney fibrosis protects mice from fibrosis. A novel inhibitor, CWHM12, a synthetic small molecule RGD peptidomimetic antagonist that targets all αv integrins, effectively treated fibrosis in both liver and lung mouse models (Henderson et al., 2013). Furthermore, CWHM12 significantly ($p<0.05$) reduced cardiac fibrosis in mouse models and a similar result was observed in skeletal muscle mouse models of fibrosis (Murray et al., 2017). In addition, MK-0429, another pan αv inhibitor, significantly ($P< 0.0001$) reduced kidney fibrosis in rat models (Xiaoyan Zhou et al., 2017). Murine HSCs have been shown to express αvβ1, αvβ3, αvβ5 and αvβ8 integrins which were significantly up-regulated during activation *ex vivo*.

In order to identify the principle αv heterodimer responsible for the antifibrotic effect, fibrosis was evaluated in mice individually depleted of the β3, β5, β6 or β8 subunits. In models of liver fibrosis in mice globally lacking αvβ3, αvβ5 and αvβ6 integrins, or conditionally deleted αvβ8 integrin, due to embryonic

lethality, protection was not observed. This suggests that either multiple αv integrins contribute to liver fibrosis with a high degree of redundancy or that the principal integrin is αvβ1 integrin. Unfortunately, it has not been possible to evaluate the role of αvβ1 integrin in liver fibrosis *in vivo* as global loss is lethal in mice (Henderson et al., 2013).

The potential role of other integrins in fibrosis

Increasing research has shown that other integrins have a similar role to αv integrins in tissue repair as matrix protein receptors. Hence, it is important to note that other integrin families beside αv integrins may also have a role in liver fibrosis and potential to be used as treatment targets (Margadant & Sonnenberg, 2010; Yokosaki & Nishimichi, 2021). Of the RGD-recognising integrins, αIIbβ3, α5β1 and α8β1 integrins do not contain αv integrin. Notably, α8β1 integrin shows the most promise. An anti-α8 integrin neutralising antibody was evaluated in three different mouse models of liver fibrosis and in all models, the pathology improved after administration with the α8 integrin neutralising antibody (Nishimichi et al., 2021; Yokosaki & Nishimichi, 2021). These findings were also shown in mouse models of pulmonary fibrosis (Nishimichi et al., 2013). Furthermore, α8 integrin expression was also upregulated in 90 liver fibrosis patients compared to the non-fibrotic patient controls (Nishimichi et al., 2021). α8 integrin was shown to be essential for the function and expression of lysyl oxidase 1, an enzyme which crosslinks and stabilises the ECM and was found to be upregulated in fibrotic liver tissue in humans as well as mice (Yang et al., 2021). In addition, α11 integrin has also been identified as a potential therapeutic target, knockdown studies of α11 integrin in HSCs in mice models of liver fibrosis inhibited differentiation and functionality of HSCs in response to TGFβ (Bansal et al., 2017).

Nevertheless, the αv family of integrins have to date been the main research focus as there has been more studies showing the successful treatment of fibrosis using pan αv inhibitors CWHM12 or MK-0429 in various preclinical disease models and specific αv integrins inhibitors are currently being tested in clinical trials. This indicates their potential as targets for antifibrotic therapies (Henderson et al., 2013; Henderson & Sheppard, 2013; Y. Li et al., 2019; Peng et al., 2016; Smith & Henderson, 2016).

Targeting specific αv heterodimers

One important question is whether the antifibrotic effects of pan αv inhibition are dependent upon the inhibition of multiple αv containing integrins which are found on myofibroblasts in the liver, or is there a single, dominant αv integrin mediating the antifibrotic effect observed in the preclinical models using the CWHM12 pan-αv inhibitor (Smith & Henderson, 2016). In general, the therapeutic targeting of specific αv integrins has shown potential as a possible treatment for patients with a broad range of fibrotic diseases

(Conroy et al., 2016). Evidence from rodent models of fibrosis combined with knockout studies or pharmacologic blockade of specific integrins (Chang et al., 2017; Henderson et al., 2013; Ma et al., 2003) suggest that the particular αv integrins (Table 1) or a potential combination of αv integrins involved in fibrosis is organ specific (Hatley et al., 2018). Consequently, the inhibition of specific αv integrin subunits should allow a more refined, targeted approach to TGFβ pathway inhibition to provide the desired antifibrotic effects, but with fewer undesirable side effects (Conroy et al., 2016).

Table 1 lists the inhibitors that have been developed against the specific αv heterodimers and have been assessed in active clinical trials (i.e. have not been withdrawn or terminated), with the exception of the inhibitor IDL-2965 where the phase 1 study was recently terminated due to the SARS-CoV-2 pandemic (NCT03949530). It also summarises the animal research related to specific αv integrins as a therapeutic target for the treatment of fibrosis.

To date, the most widely studied integrin for fibrosis is αvβ6, with αvβ6 integrin inhibitors progressing to phase 2 of clinical trials (Hatley et al., 2018). However, BG00011, an αvβ6 integrin blocking antibody, also known as STX-100, in phase 2 of clinical trials for IPF (NCT03573505), was terminated due to safety concerns. There are end-of-study and follow-up visits currently underway. Function-blocking αvβ6 integrin monoclonal antibodies have showed promise in preclinical mouse model of renal fibrosis (Hahm et al., 2007) and BG00011 has also previously been used in phase 2 trials for interstitial fibrosis in kidney transplant patients (NCT00878761), but similarly was withdrawn with unspecified safety concerns. GSK3008348 is another αvβ6 integrin inhibitor which was the first inhaled small molecule αvβ6 integrin inhibitor for the treatment of IPF. Preclinical studies of this inhibitor have shown reduced TGFβ signalling and fibrotic endpoints in a mouse model of lung fibrosis, in addition to binding to the αvβ6 integrin with high affinity and selectivity in human fibrotic lung tissue, isolated lung epithelial cells and reduced TGFβ signalling in human IPF tissue slices *ex vivo* (John et al., 2020). It was well tolerated in phase 1 clinical trials (NCT02612051) using healthy participants. Although this study was completed with the data reported (Maden et al., 2018), there have not been any updates in further trials.

In contrast, inhibitors for liver fibrosis have not progressed as far in clinical trials. Although, there have been numerous integrin inhibitors used to attenuate liver fibrosis in preclinical models (see Table 1), this has not, as yet, translated into human studies. This is likely due to the suboptimal preclinical models (Buzzetti et al., 2016) and less attention on the liver compared to the lung and kidney. However, recently Pliant Therapeutics, Inc in collaboration with Novartis have developed a small molecule inhibitor of αvβ1 integrin, PLN-1474 for the treatment of end-stage liver fibrosis in NASH and there is currently a phase 1 trial underway (Slack et al., 2021). Currently, there are no approved clinical therapies for fibrosis or other

diseases using αv integrin inhibitors (Yokosaki & Nishimichi, 2021). The current advantage for αv integrin inhibitors in clinical trials is their good tolerability, shown through phase 1 and 2 trials with GSK3008348 (Maden et al., 2018) and PLN-74809 (Turner et al., 2019). However, many αv integrin inhibitors are not specific enough due to the pharmacophore of the inhibitors for the 5 integrins being based on the same RGD structure (Slack et al., 2021; Yokosaki & Nishimichi, 2021) and a common problem is the termination of trials due to safety concerns as demonstrated through BG00011.

The role of αvβ1 integrin in fibrosis has been difficult to study due to the promiscuity of the β1 integrin in forming heterodimers with 11 other α subunits (Smith & Henderson, 2016). Investigations of the role of this integrin are therefore challenging, requiring non-standard transgenic mouse approaches and the development of new experimental tools. For example, specific function blocking antibodies against αvβ1 integrin with which to effectively and selectively study αvβ1 integrin are not yet available despite great efforts to generate this heterodimer-specific antibody (Hatley et al., 2018; Song et al., 2016).

Despite this, a highly potent and specific inhibitor of the αvβ1 integrin, known as c8 has been developed. A concentration-dependent cell adhesion assay of c8 against αvβ3, αvβ5, αvβ6 and αvβ8 integrins showed IC_{50}'s of >100,000 nM with αvβ1 integrin having an IC_{50} of 0.089 ± 0.02 nM which demonstrates the selectivity of c8 for inhibiting αvβ1 integrin mediated cell adhesion. Reports of the antifibrotic effects of c8 provide evidence that αvβ1 is the predominant integrin on pathologic fibroblasts responsible for activating latent TGFβ and driving tissue fibrosis in the liver and lungs. For example, administration of c8 to mice with liver or lung fibrosis, resulted in a significant down regulation of phosphorylated Smad3 protein, a downstream mediator of TGFβ signalling as observed through fluorescence intensity mapping (Reed et al., 2015). Additional evidence for the role of β1 integrin is supported through recent studies identifying αvβ1 integrin as the major αv integrin expressed in activated primary human HSCs. C8 completely abolished TGFβ induced procollagen-1 production. Interestingly, the observed inhibition of αvβ1 integrin through these inhibitors suggest that αvβ1 integrin-mediated regulation of TGFβ-activated pro-collagen-1 production in HSCs is Smad3 independent. The study further suggests that αvβ1 integrin regulated procollagen-1 production is through the activation of phosphorylated extracellular-signal-regulated kinase 1/2 (ERK) also known as MAPK, a non-canonical TGF-β signalling pathway as using those inhibitors significantly inhibited TGFβ induced ERK1/2 phosphorylation levels after TGFβ treatment (Han et al., 2021).

Furthermore, using fetal lung fibroblasts which showed adhesion to the latency associated peptide of TGFβ was blocked by antibodies to either β1 or αv integrins, but not by antibodies to either αvβ3, αvβ5, αvβ6, or αvβ8 integrins (Reed et al., 2015). However, additional studies using c8 have suggested that the

selectivity of this compound within the β1 integrin family is poor, with high affinity also observed for α4β1 integrin. A saturation experiment with c8 and α4β1 integrin produced a dissociation constant (K_D) of 0.78nM, comparable to αvβ1 integrin which was 0.21nM. These studies also report that c8 had a moderate affinity for α2β1, α3β1 and α8β1 integrins which have a K_D of 11.2nM, 13.5nM and 8.13nM respectively (Wilkinson et al., 2019), an especially pertinent finding given the emerging role of α8β1 in liver fibrosis (Nishimichi et al., 2021). This raises the question of whether the observed antifibrotic effects of c8 can be attributed to the inhibition of a single integrin target.

Cilengitide is a specific inhibitor of αvβ3 and αvβ5 integrins. This small molecule was suggested to be beneficial in hepatic fibrosis due to its antiproliferative and antifibrotic activity in HSCs *in vitro*. Compounds that target αvβ3 integrin also usually show activity at αvβ5 integrin (Z. Liu et al., 2008), with most existing small molecule αvβ5 integrin inhibitors displaying near-equipotency with the highly homologous αvβ3 integrin (Lippa et al., 2020). This makes it difficult to produce a selective αvβ3 and αvβ5 integrin inhibitor. Surprisingly, administering cilengitide in rat models with secondary biliary fibrosis and panlobular fibrosis due to thioacetamide worsened liver fibrosis (Patsenker et al., 2009). This is in contrast to data showing β3 integrin being expressed at very low levels in quiescent rat HSCs (Xiaoying Zhou et al., 2004), increased αvβ3 integrin expression in both rat and human HSCs after activation *in vitro*, as well as αvβ3 integrin being required to sustain HSC proliferation and survival in culture. Moreover, hepatic expression of αvβ3 integrin is upregulated in bile duct ligation (BDL) and thioacetamide fibrotic livers in mice (Y. Li et al., 2019). One explanation is that either cilengitide could have caused a mild increase in macrophage activation which resulted in the release of inflammatory mediators or hepatic hypoxia which could have contributed to the progression to fibrosis (Patsenker et al., 2009). This highlights the need for greater understanding of the role and pattern of integrin expression both in healthy tissue and longitudinally as diseases progress, rather than only targeting integrins that are predominant in the disease state, as well as emphasising the use of human tissue samples instead of artificially induced rodent models of fibrosis.

All αv integrins apart from αvβ6 integrin are present on HSCs (Hintermann & Christen, 2019). Studies have shown that mouse HSCs express αv, β1, β3, β5 and β8 integrins which implies expression of these specific dimers (Henderson et al., 2013). Reports from recent studies using human HSCs show similar results and highlighted that β1 and β3 integrins represent the two most abundant integrins with β5, β6 and β8 integrins showing lower abundancy in activated human HSCs via TGFβ (Han et al., 2021). It has been shown that β6 integrin knockout mice are protected from renal and pulmonary fibrosis (Hahm et al., 2007; Pittet et al., 2001). In particular, αvβ6 integrin has been shown to be a promising target in lung

fibrosis as the administration of low doses of blocking αvβ6 integrin antibodies attenuated bleomycin-induced lung fibrosis in mouse models (Horan et al., 2008) and increased levels of αvβ6 integrin were observed in patients with IPF, compared with control lung tissue (Saini et al., 2015). This is a potential advantage as targeting αvβ6 integrin which is upregulated in diseased tissue and low in healthy could have reduced side effects compared to a target that is essential in normal tissue. There is also the additional benefit through treating the affected tissue directly and in relative isolation using inhaled drugs, such as the αvβ6 integrin inhibitor GSK3008348, which limits the potential of off-target systemic effects (John et al., 2020; Maden et al., 2018). In contrast to this, in liver fibrosis, the role of αvβ6 integrin is not clear. Rodent models show αvβ6 integrin expression increases over time with liver fibrosis progression (Peng et al., 2016) but this data is not available for human liver fibrosis. This needs to be collected to inform integrin-centred strategies for pharmacological therapy of human liver disease.

Considerations for future integrin inhibitors

Fibrosis progression requires numerous signalling pathways that interact with each other (Lee & Friedman, 2011) and the effective abrogation of fibrosis might also require targeting these multiple pathways in concert (Conroy et al., 2016). For example, even following genetic ablation of the β6 integrin in mice there was a significant degree of lung fibrosis which developed (Madala et al., 2014). This could be due to compensation from increased expression of other integrins, as is the case for αvβ3 and αvβ5 integrins which have been shown to compensate for each other's functions in activating TGFβ in cardiac fibroblasts (Sarrazy et al., 2014). This suggests that drugs targeting multiple integrins should be considered or a single compound with varying efficacy for different integrins should be used as they could be more successful in treating fibrosis. Furthermore, there are precedents that promiscuous drugs may offer some advantages as multiple factors contribute to the pathogenesis of a particular disease and that does not necessarily make it more toxic or cause pathways to be completely shut down or excessively activated (Mencher & Wang, 2005). For example, PLN-74809, a dual αvβ6/αvβ1 integrin inhibitor may have some benefit and has demonstrated good tolerability, currently being in phase 2 clinical trials as shown in table 1 (Turner et al., 2019).

In general, there is little data on specific αv integrin expression in human liver disease. As the primary source of ECM in the liver, activated HSCs have been widely considered the most promising cellular target in liver fibrosis and their activation is a process commonly induced by various hepatic injuries (He et al., 2020). HSCs and integrin expression in fibrosis are not fully elucidated and the molecular mechanisms underlying fibrosis is important for developing therapies for treatment of liver fibrosis. Additionally, as integrins are not exclusively expressed on HSCs, expression profiles for all liver cells should also be

established and their contribution to liver fibrosis for each integrin in each cell type should be explored (Schuppan et al., 2018). Progress has been made through recent studies showing α8β1 and α11β1 integrins exhibiting pathology-specific expression through their selective mRNA expression on activated rat HSCs in culture. Unfortunately, this was not demonstrated for αv integrins (Yokosaki & Nishimichi, 2021). Hence, more studies exploiting the expression of specific integrin subunits and its cellular localisation within pathological tissues and other healthy organs are crucial to determine novel integrin-based therapeutic targets. The design of dimer specific drugs for specific diseases will decrease the risk of off-target effects (Hynes, 2002; Schnittert et al., 2018).

It is important to develop drugs that have selectivity between the different αv integrins as there are potential liabilities associated with the engagement of certain integrins. For example, αvβ3 and β5 integrins are involved in angiogenesis/vascular permeability and αvβ8 integrin in autoimmunity (Rowedder et al., 2017). In terms of the adverse side effects of targeting specific integrin heterodimers for the treatment of liver fibrosis, the information is limited to the side effects reported from the clinical trials using BG00011 and the BDL and thioacetamide rat models of liver fibrosis using cilengitide (Patsenker et al., 2009). Unfortunately, obtaining acceptable selectivity for any particular integrin can be difficult due to similarities in some of the binding sites (Anderson et al., 2019). Furthermore, sufficient potency is required to drive an antifibrotic response at a realistic clinical dose, but a compromise may be required between the ideal potency and ideal selectivity (Adams et al., 2014). Complete blockage of a specific integrin may not be necessary as studies have suggested that low-dose partial inhibition of αvβ6 integrin-mediated TGFβ activation is sufficient to attenuate fibrosis in bleomycin-induced fibrosis in mice (Horan et al., 2008). In addition to this, studies have been performed to assess the structure–activity relationships of a series of novel analogues of αv integrin antagonists. The activity of aryl substitutes in these analogues were compared against different αv integrins. They found that by simple variation of the position of the aryl ring, the cell adhesion potency against αvβ6 integrin can be increased and potency against αvb3 and αvβ5 integrins reduced (Adams et al., 2014). Further studies using a variety of aryl substituents have been performed to improve this αvβ6 integrin potency and selectivity with the aim of treating IPF in which key structure-activity relationships were identified (Procopiou et al., 2018). Similar studies may be required for developing specific integrin compounds to treat liver fibrosis.

Another consideration is RGD-mimetic inhibitors or non-RGD-mimetic inhibitors. Earlier studies have shown a synthetic RGD analogue, SF-6,5, was effective in alleviating hepatic fibrosis induced by thioacetamide in rats (Bruck et al., 1997). RGD-mimetics are designed to make the same interactions as the native ligand and they include cilengitide and CWHM12 (Hintermann & Christen, 2019; Miller et al.,

2017). They have shown good potency but have the potential to activate integrins (Miller et al., 2017; Wilkinson et al., 2019). Many RGD-binding integrin inhibitors are RGD mimetics with physicochemical properties that are not compatible with oral bioavailability and compromises may have to be made between the pharmacological profile and sufficient bioavailability for clinical use (Slack et al., 2021). There is little research showing the intracellular signalling effects of RGD-mimetics when it engages the RGD site. An approach to avoid the unwanted activation is through non-RGD-mimetic inhibitors. For instance, the non-RGD-mimetic inhibitor of αIIbβ3 integrin did not activate the integrin upon binding and will consequently prevent any unwanted signalling. However, the development of efficacious and safe drugs for this new generation of inhibitors is yet to be achieved (Miller et al., 2017; Slack et al., 2021).

Current treatments

Current therapeutic options for liver fibrosis are limited (Henderson et al., 2013), especially in comparison to other organs. In IPF, treatments such as pirfenidone in which the mechanism of action is not fully understood and nintedanib, an inhibitor of multiple receptor-associated tyrosine kinases have received Food and Drug Administration approval (Trawinska et al., 2016). Unfortunately, most patients continue to experience disease progression and/or exacerbation despite treatment. This emphasises, that although pirfenidone and nintedanib have been approved as antifibrotic drugs, current treatments are insufficient and there is a need to develop alternative compounds (Somogyi et al., 2019). Both these drugs have been used in rodent and/or patient studies to reduce fibrosis in the liver and kidney (F. Liu et al., 2017; Lopez-de la Mora et al., 2015; Wollin et al., 2020) and pirfenidone is currently undergoing a phase 2 clinical trial for advanced liver fibrosis (ClinicalTrials.gov identifier NCT04099407). However, both these drugs have not progressed beyond clinical trials which could be due to the limited understanding of their mechanisms of action and differences in fibrotic processes between organs such as the lung and liver.

There have been numerous other promising agents for the treatment of IPF, but these were terminated once they reached phase 3 clinical trials due to lack of efficacy (Aryal & Nathan, 2018) or adverse events (Saito et al., 2019). Similar problems are observed in kidney fibrosis in which antifibrotic treatment targets such as epidermal growth factor receptor identified in preclinical studies were not successfully translated into clinical use (Klinkhammer et al., 2017). In the case of liver fibrosis, antifibrotic agents have had even less success than for IPF (Schuppan et al., 2018). Instead transplantation is often the only effective treatment for end-stage of cirrhosis. However, with the limited donor organ availability, high cost and morbidity of transplantation (Henderson et al., 2013), there is an urgent need for effective antifibrotic therapies (Böttcher & Pinzani, 2017).

Apart from therapies that address the underlying cause of liver fibrosis such as antivirals, the most promising approaches are those that focus on cellular and molecular targets that are involved in the progression or reversal of fibrosis. A prime example is developing molecules that target integrins with a disease-specific expression in myofibroblasts, one of the main drivers of disease progression (Schnittert et al., 2018).

Drug pipeline

Many antifibrotic agents are currently under investigation for liver fibrosis (Trivella et al., 2020), with 377 interventional studies listed on the clinical trials website compared with 236 for IPF. Although this demonstrates the concerted biotech/pharmaceutical effort for developing antifibrotic therapies, none have so far been licensed for the treatment of liver fibrosis. The development of novel drugs that are well tolerated and have minimal systemic side effects has been challenging. This is not a problem exclusive to liver fibrosis (Trivella et al., 2020). Furthermore, this challenge is exacerbated by the fact that existing biomarkers for liver fibrosis in clinical practice lack specificity and sensitivity (Nallagangula et al., 2018) which makes it difficult to determine drug efficacy. Currently, liver biopsies are the gold standard for fibrosis assessment, however, due to their invasive nature and relative risk, biopsies deter patients from enrolling in trials and are an added cost for study sponsors (Trivella et al., 2020).

In an effort to overcome these difficulties, innovative methods are being developed to detect and measure fibrotic disease. This includes using high-affinity ligands and positron emission tomography (PET) to distinguish between diseased and healthy tissues. Accumulation of PET tracers in tissues provides a way to image the molecular signature of disease (Kimura et al., 2019). Furthermore, information from target engagement *in vivo* using PET tracers can help increase the success of drugs translated into clinical trials (Campbell et al., 2016). Studies have demonstrated that cysteine knot PET tracers have the ability to detect fibrotic lung disease in IPF patients and were associated with elevated levels of αvβ6 integrin. This showed the potential of αvβ6 integrin as a therapeutic target for IPF (Kimura et al., 2019), but has yet to be demonstrated for liver fibrosis.

Research on the mechanisms underlying fibrosis has revealed several major signalling pathways, as well as potential targets for different treatment strategies highlighted in table 2. Strategies that target the activation of the HSCs, the major producers of excessive ECM in liver fibrosis, are also being explored (Santoro & Mangia, 2019). For instance, obeticholic acid is a ligand for Farnesoid X receptor (FXR) receptors which is known to inhibit HSC activation and as such is being tested in clinical trials for NASH and fibrosis (Santoro & Mangia, 2019; Trivella et al., 2020).

There are currently several agents in the drug pipeline for NASH with a few in phase 3 (Table 2). However, none of these drugs target the fibrosis pathway directly and integrin targeting compounds are clearly absent from the list despite their pivotal role as important mediators of progressive fibrosis and integrin antagonism being proposed as an attractive concept to counteract liver fibrosis (Patsenker & Stickel, 2011). Many of the drugs in development act on the inflammatory processes or metabolic pathways rather than fibrosis directly (Romero et al., 2020; Santoro & Mangia, 2019). Additionally, although there have been a few antifibrotic drugs reaching phase 3 studies, compounds have failed to progress beyond this stage. Selonsertib, an inhibitor of apoptosis signal-regulating kinase 1 (ASK1), demonstrated potent activity against its target, but in phase 3 studies it was not associated with regression of fibrosis and failed to perform better than the placebo. One possible reason for the failure of ASK1 inhibition when used alone could be due to the contribution of other pathways that mediate fibrosis in NASH (Harrison et al., 2020). Combinations of treatments which target multiple interconnected pathways present in NASH may effectively slow or reverse disease progression. Presently, there are several ongoing phase 2 combination studies and the results from these trials will play a significant role in the development of treatments for NASH (Albhaisi & Sanyal, 2021; Romero et al., 2020). Overall, this reveals the paucity of drugs in development that directly target fibrosis (Cho & Kopp, 2010; Romero et al., 2020; Santoro & Mangia, 2019) and is an area that integrin targeted therapies have the potential to address (Patsenker & Stickel, 2011).

Concluding remarks

As the worldwide prevalence of CLDs is already high and increasing, there is an urgent need for effective treatment to prevent cirrhosis-related mortality (Marcellin & Kutala, 2018) and targeting integrins could provide this solution (Patsenker & Stickel, 2011). For αv integrins, selectivity has been demonstrated pre-clinically for peptides at αvβ1 and αvβ6 integrins (Hatley et al., 2018; Reed et al., 2015). Furthermore, rodent models of liver fibrosis have shown αvβ1 and αvβ6 integrin inhibitors successfully attenuating fibrosis (Patsenker et al., 2008; Reed et al., 2015).

Preclinical models have shown integrins to be a promising target in various fibrotic diseases, unfortunately, in clinical trials the same results have not been seen which emphasises the need for more data from human disease samples. There is progress being made through investment via pharmaceutical companies, such as Indalo, Pliant and Morphic (Rauchman & Griggs, 2019; Slack et al., 2021). Indalo currently has a small molecule that inhibits αvβ1, β3 and β6 integrins in clinical trials and Pliant has PLN-74809, a dual selective inhibitor of αvβ1 and αvβ6 integrins in clinical trials for IPF and liver fibrosis (Table

1). Additionally, the use of a dual integrin inhibitor is noteworthy as a potential strategic shift and this inhibitor was compared with pirfenidone and nintedanib in lung tissue and was found to have a higher potency (Decaris et al., 2019). This further signifies the potential of integrins as an antifibrotic treatment. Moreover, Morphic is performing investigational new drug-enabling studies with a selective αvβ6 integrin small molecule inhibitor (Rauchman & Griggs, 2019).

To date there are not any approved treatments for liver fibrosis (Schuppan et al., 2018). Expectations are set high as even in a large multicentre phase III trial demonstrating the significant, yet modest effect of obeticholic acid in reducing liver fibrosis in NASH (Younossi et al., 2019), the Food and Drug Administration declined accelerated approval for the drug due to the predicted benefits being uncertain and the drug not sufficiently outweighing the potential risks (Intercept Pharmaceuticals, Inc., 2020; Vuppalanchi et al., 2021). Hence, integrins could provide a solution as an effective antifibrotic agent. However, integrin expression in fibrosis is not fully understood, although αv integrins are described as the front runner, emerging evidence suggests α8β1 and α11β1 integrins may also be promising targets (Nishimichi et al., 2021; Yokosaki & Nishimichi, 2021). Studies exploiting the expression of specific integrin subunits and its cellular localisation within pathological tissues and other healthy organs are crucial to determine more successful therapeutic targets for liver fibrosis (Schnittert et al., 2018).

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Tables

Table 1: Specific αv integrins and their potential involvement in fibrotic diseases through both animal and human studies with their respective inhibitors used in various animal models/clinical trials. These inhibitors developed for clinical trials have the potential to be used for other types of tissue fibrosis written in *italics* which were stated via press releases from the respective pharmaceutical company and further information on the inhibitors were provided

from ClinicalTrials.gov. Unfortunately, the NCT03949530 study using IDL-2965 was terminated early due to development challenges associated with the SARS-CoV-2 pandemic and emerging non-clinical data. It is important to note that the compounds that target αvβ3 integrin also target αvβ5 (Patsenker et al., 2009). The cell-based potency of IDL-2965 was 1.5, 1.4 and 0.4 nM against αvβ1, αvβ3 and αvβ6 integrins, respectively, with no data available for αvβ5 integrin (Kossen et al., 2019). The inclusion criteria for the inhibitors in the preclinical studies was whether they could successfully attenuate fibrosis. N/A means that there is not an inhibitor exclusively for that integrin undergoing clinical trial. In the case of αvβ8 integrin, it has not passed the preclinical stage (Raab-Westphal et al., 2017) with few studies using αvβ8 integrin and it has undergone less target validation for fibrosis which is partly due to the lack of suitably selective inhibitors (Hatley et al., 2018).

Integrin	**Target organ where role in fibrosis is suggested**	**Inhibitors undergoing clinical trials [*fibrotic disease, clinical phase, trial number*] and potential to work on other types tissue fibrosis**	**Inhibitors used in preclinical studies that lead to attenuated fibrosis [*animal model*]**	**Associated efficacy data**
αvβ1	Liver, renal & pulmonary fibrosis (Chang et al., 2017; Reed et al., 2015)	N/A	Small-molecule **compound 8** (c8) [*bleomycin/CCL_4/UUO-induced pulmonary/liver/renal fibrosis in mice models*] (Chang et al., 2017; Reed et al., 2015)	Ligand binding assay IC_{50} 0.089 nM IC_{50} against TGFβ activation using fibroblasts from the IPF patient lungs and murine HSCs ranged from 0.35 to 0.50 nM with IC_{50} of 1nM using renal fibroblasts (Chang et al., 2017; Reed et al., 2015)

αvβ3	Cardiac & lung fibrosis (Fiore et al., 2018; Sarrazy et al., 2014)	N/A	Small molecule **cilengitide** (EMD121974) [*cutaneous and pulmonary fibrosis induced by hypochlorous acid in mice models*] (Bagnato et al., 2018; Patsenker et al., 2009)	Ligand binding assay IC_{50} 3nM (Patsenker et al., 2009)
αvβ5	Cardiac fibrosis & localized scleroderma (Asano et al., 2006; Sarrazy et al., 2014)	None	Small molecule **cilengitide** (EMD121974) [*cutaneous and pulmonary fibrosis induced by hypochlorous acid in mice models*] (Bagnato et al., 2018; Patsenker et al., 2009)	Ligand binding assay IC_{50} 37nM (Patsenker et al., 2009)

αvβ6	Renal, pulmonary & biliary fibrosis (Coward et al., 2010; Ma et al., 2003; Peng et al., 2016)	N/A	Non-peptide antagonist **EMD527040** [*secondary biliary fibrosis using BDL in rat models*] (Patsenker *et al*, 2008)	Ligand binding assay IC_{50} 6nM (Patsenker *et al*, 2008)
			Antibody 3G9 [*biliary fibrosis using mouse models of biliary injury/BDL, lung fibrosis using bleomycin/TGFα-induced mouse models, renal fibrosis using mouse model of Alport syndrome*] (Hahm et al., 2007; Horan et al., 2008; Madala et al., 2014; Peng et al., 2016; Wang et al., 2007)	IC_{50} against TGF-β activation of 3.5 pM using human β6-transfected SW480 cells (Weinreb et al., 2004)
αvβ8	Small airway fibrosis, biliary atresia & renal fibrosis (Iordanskaia et al., 2014;	None	None	

	McCarty, 2020; Minagawa et al., 2014)			
αvβ1, αvβ3 & αvβ6	N/A	**IDL-2965** [*IPF, phase 1, NCT03949530*] (Kossen et al., 2019) *Liver + kidney*	None	
αvβ1, & αvβ6	N/A	**PLN-74809** [*IPF, phase 2, NCT04072315 & primary sclerosing cholangitis and suspected liver fibrosis, phase 2, NCT04480840*] (Decaris et al., 2019; Turner et al., 2019)	None	Ligand binding assay IC_{50} <10 nM for both murine and human αvβ6 and αvβ1 IC_{50} against TGF-β activation of <200 nM (Turner et al., 2019)

IPF, idiopathic pulmonary fibrosis; CCL_4, carbon tetrachloride; UUO, unilateral ureteral obstruction; BDL, bile duct ligation; TGFα, transforming growth factor α; IC_{50}, inhibitory concentration

Table 2: Overview of phase 2 and 3 clinical trials for the treatment of liver fibrosis. These trials do not directly treat fibrosis but are mostly anti-inflammatory and/or target lipid metabolism/steatosis. Drugs listed on clinicaltrials.gov are described along with their mechanism of action and their targets (Esler & Bence, 2019; Finan et al., 2021; Harrison et al., 2021; Hsu et al., 2017; Fishman et al., 2019; Romero et al., 2020; Sonoda et al., 2017; Trivella et al.,

2020). The drugs included have a primary or secondary outcome measure as improvement in liver fibrosis and are treatments for either NAFLD, NASH, which is a subgroup of NAFLD (Buzzetti et al., 2016), liver fibrosis, cirrhosis or a combination of these diseases. It includes studies that are active with the exception of the Tropifexor & Cenicriviroc study (NCT03517540) in which results are still being reviewed, studies that are at phase 2 and 3 and omit drugs at phase 4, as well as ones that have been previously used for or to treat other diseases such as Type 2 Diabetes Mellitis.

Drug trade name (manufacturer)	Mechanism of action	Target	Modality of the drug	Phase	Condition/ Disease	Trial identifier
Resmetirom (Madrigal Pharmaceuticals)	Agonist	THR-β	Small molecule	3	NASH	NCT03900429
Obeticholic Acid (Intercept Pharmaceuticals)	Agonist	FXR	Small molecule	3	NASH with fibrosis, NASH & compensated cirrhosis	NCT03439254 NCT02548351
Tropifexor & Cenicriviroc (Novartis Pharmaceuticals)	Agonist & Antagonist	FXR & CCR2/5	Small molecule	2b	NASH	NCT03517540
EDP-305 (Enanta Pharmaceuticals)	Agonist	FXR	Small molecule	2b	NASH	NCT04378010
Aldafermin (NGM Biopharmaceuticals, Inc)	Analogue	FGF19	Peptide	2b	NASH & compensated cirrhosis	NCT04210245
Namodenoson (Can-Fite BioPharma)	Agonist	A3 adenosine receptor	Small molecule	2b	NASH	NCT04697810
Efruxifermin	Analogue	FGF21	Peptide	2b	NASH	NCT04767529

(Akero Therapeutics, Inc)						
Pegbelfermin (Bristol-Myers Squibb)	Analogue	FGF21	Peptide	2 & 2b	NAFLD, NASH & liver fibrosis, NAFLD, NASH, liver fibrosis & cirrhosis, NASH	NCT04267393 NCT03486899 NCT03486912
BIO89-100 (89bio, Inc)	Analogue	FGF21	Peptide	2	NASH	NCT04048135
PF-06865571 & PF-05221304 (Pfizer)	Inhibitor	ACC & DGAT2	Small molecule	2	NAFLD & NASH with liver fibrosis	NCT04321031
BFKB8488A (Genentech, Inc)	Agonist	FGFR1/KLB receptor	Antibody	2	NASH	NCT04171765
LPCN 1144 (Lipocine)	N/A	N/A	N/A	2	NASH	NCT04134091 NCT04685993
JKB-122 (TaiwanJ Pharmaceuticals Co., Ltd)	Antagonist	TLR4	N/A	2	NASH with fibrosis	NCT04255069

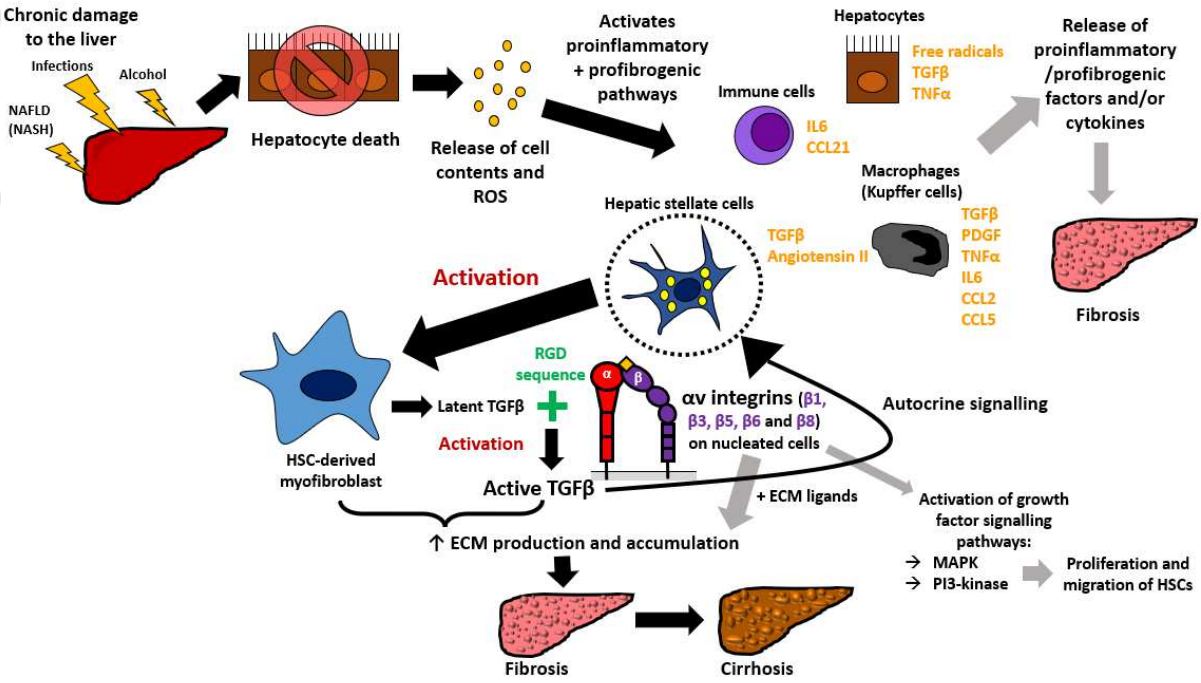
THR-β, thyroid hormone receptor β; FXR, farnesoid X receptor; CCR, chemokine receptor type; FGF, fibroblast growth factor; ACC, acetyl-CoA carboxylase; DGAT2, diacylglycerol O-acyltransferase 2; FGFR, fibroblast growth factor receptor; KLB, β-klotho; TLR4, Toll-like receptor 4; NASH, non-alcoholic steatohepatitis; NAFLD, non-alcoholic fatty liver disease.

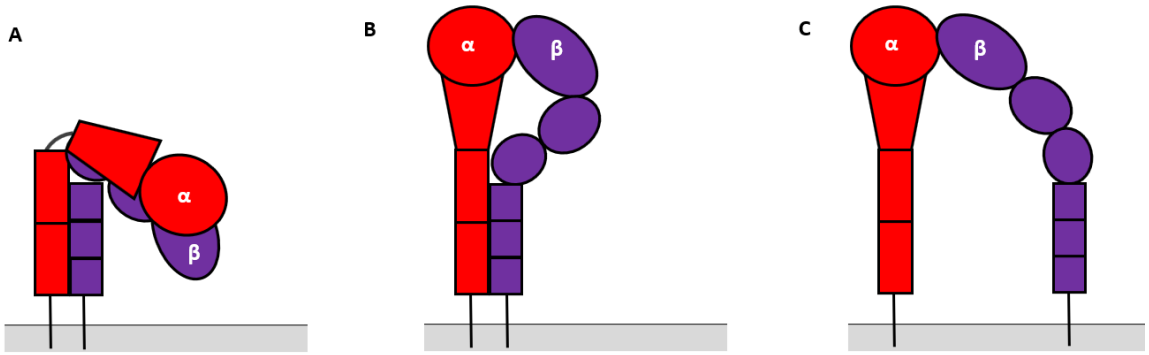
Figure legends

Figure 1: Overview of the cellular mechanism of fibrosis in the Liver. Chronic damage as a result of infection, alcohol abuse and/or NAFLD leads to hepatocyte death which releases cellular contents (e.g. DNA and potentially DAMPs such as ATP, formyl peptides and HMGB1) and ROS. This induces the activation of proinflammatory and

profibrogenic pathways in various cells present in the liver. These cells are involved in driving fibrosis by releasing proinflammatory/profibrogenic factors and/or cytokines/chemokines (highlighted in orange) and these can drive the activation of HSCs into myofibroblasts. IL6 and TNFα are both pro-inflammatory cytokines. TNFα induces hepatocyte apoptosis, neutrophil activation and promotes HSC survival and proliferation. The chemokine CCL21 results in the induction of proinflammatory genes in HSCs and CCL2 and CCL5 stimulate the influx of immune cells. Furthermore, CCL5 recruits and activates HSCs. Resident macrophages known as Kupffer cells are notably known to promote the survival of activated HSCs through releasing TGFβ and PDGF. They are the main producers of PDGF which is a predominant mitogen for activated HSCs. HSCs are considered to be the major source of myofibroblasts which contribute to 90% of the ECM. HSCs release angiotensin II which stimulates cell proliferation, migration, collagen synthesis and secretion of pro-inflammatory cytokines. More importantly, activated HSCs secrete latent TGFβ and form an autocrine positive feedback loop. The latent TGFβ contains an RGD sequence which has a high affinity for integrin molecules and once this interaction occurs, active TGFβ is released. TGFβ has a role in HSC activation and ECM production. Integrins αvβ1, αvβ3, αvβ5, αvβ6 and αvβ8 in the extended open conformation state have the highest affinity for TGFβ and once they are activated it leads to stimulation of growth factor signalling pathways such as MAPK and PI3-kinase pathways that regulate cell proliferation, survival and/or growth. These pathways mediate proliferation and migration of HSCs and are necessary for PDGF-induced proliferation. The release of pro-inflammatory factors, growth factors, and cytokines from various cells, in addition to the activities of myofibroblasts, binding of integrins both to the ECM and TGFβ, results in ECM accumulation and fibrosis which then ultimately leads to cirrhosis. NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; DAMP, damage-associated molecular patterns; HMGB1, high-mobility group box protein 1; ROS, reactive oxygen species; CCL, chemokine ligand; IL, interleukin; TNFα, tumour necrosis factor α; PDGF, platelet derived growth factor; HSC, hepatic stellate cell; RGD, arginine, glycine and aspartate; TGFβ, transforming growth factor β; MAPK, mitogen-activated protein kinase; PI3-kinase, phosphoinositide 3-kinase; ECM, extracellular matrix.

Figure 2: The conformational states of integrins. Overall, there are three integrin conformational states. These are known as the (A) bent (closed), (B) extended closed and (C) extended open conformations. In a resting, bent-closed conformation, the ligand-binding site lies close to the plasma membrane and has a low ligand affinity. In the two extended states, the ligand-binding site faces away and extends above the cell surface. Activation of the integrin requires the extended open state, which binds the ligand with higher affinity than the bent closed and extended closed states. The three different classes of integrin antagonists that have been discovered so far stabilise a specific integrin conformation with most antagonists stabilising the extended high affinity integrin conformation (J. Li & Springer, 2017; Shimaoka & Springer, 2003; Springer & Dustin, 2012).





Bent (closed)

Stabilised by:

- α I allosteric antagonists

Extended closed

Extended open

Stabilised by:

- α/β I-like competitive antagonists (e.g αIIbβ3, αvβ3 and α4β1 antagonists)
- α/β allosteric antagonists