

# **IID Essay ICA**

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How could type 2 immunity-driven liver injury pathogenesis benefit treatment and diagnosis of hepatocellular carcinoma?

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## Introduction

Hepatocellular carcinoma (HCC) is the most usual form of liver cancer and the fourth most common cause of cancer-related death worldwide (Villanueva, 2019). Usually arising at the end stage of chronic liver diseases (CLDs), HCC is a clear example of inflammation-related cancer. Viral infections, alcoholism, and obesity are common causes of CLDs, where non-resolving inflammation is known to promote and exacerbate malignancy (Bishayee, 2014; Yu et al., 2018). Thus, it is crucial to understand the mechanisms underlying the abnormal immune responses that contribute to hepatocarcinogenesis.

Immune responses in the liver are orchestrated by both type 1 immunity (i1) and type 2 immunity (i2), which exhibit pro- and anti-inflammatory responses, respectively (Robinson et al., 2016). i1-i2 imbalance drives non-resolving inflammation in the liver, resulting in sequential development of fibrosis, cirrhosis, and eventually HCC (**Figure 1**), where there are common inflammatory cascades during the transition (Dondeti et al., 2016). It is recently found that i2 is crucial for sustaining the non-resolving inflammation in liver fibrosis, whereas i1 activation proves protective against chronic injury (Hart et al., 2017).

In light of the i2-driven pathology of liver fibrosis, this article aims to 1) appreciate anti-fibrotic treatment based on the pathology, 2) evaluate its applications in HCC, and 3) suggest further directions for therapeutic applications of i2 in HCC, with related diagnostic and therapeutic implications for HCC discussed in each section and summarised in the conclusion.

## Targeting cytokines in i1-i2 imbalance

The i1-i2 axis represents two opposing immune responses that shape tissue injury during the progression towards HCC (**Figure 1**). Distinct CD4<sup>+</sup> T helper subpopulations, termed Th1 and Th2, can cause different immune responses patterns, which are characterised by different patterns of cytokines (Yamaguchi et al., 2015). Th1 secrete Th1 cytokines, including interleukin (IL)-2, interferon (IF)- $\gamma$ , and lymphotoxin- $\alpha$  and activate i1, inducing intense phagocytosis, while Th2 produce Th2 cytokines including IL-4, IL-5, IL-9 and IL-13 and activate i2, resulting in high antibody titres (Spellberg & Edwards Jr., 2001).

(Hart et al., 2017) show that the serum levels of Th2 cytokines IL-4, IL-5 and IL-13 increase with the worsening of liver fibrosis in high-fat-fed mice (Gieseck et al., 2018). By comparing liver injury in i1 and i2 activated mice, they further find that i1 activation instead protects the

liver from chronic injury (Hart et al., 2017), which challenges the assumed key role of i1 in driving liver fibrosis (Dondeti et al., 2016) and indicates liver fibrosis as an "overhealing wound." (Townsend et al., 2019) further confirm i2 amplification in hepatic viral infection. Since fibrosis and sequent cirrhosis are found in more than 90% of HCC patients and drive hepatocarcinogenesis (Campbell et al., 2005; Ghouri et al., 2017). Therefore, different aetiologies of liver cancer share a common i2-driven pro-fibrotic pathway (**Figure 1**), which should be a promising diagnostic and therapeutic target.

Thus, potential clinical applications of Th2 cytokines have been actively investigated. For example, (Hou et al., 2010) show elevated expression of IL-13R $\alpha$ 2, a receptor of Th2 cytokine IL-13 (Dondeti et al., 2016), in cancer cell lines and tissues, which allows them to develop an effective targeted therapy using an IL-13 and diphtheria toxin fusion protein called DT389-hIL13-13E13K in liver cancer cell lines. However, further preclinical and clinical research is still needed. The association between HCC and Th2 cytokine IL-4 has been meta-analysed by (Wu et al., 2015). It is found that *IL-4 -590C>T* polymorphism, which shows increased IL-4 expression (Saxena & Kaur, 2015), is associated with a higher risk for HCC, especially in the Asian population, yet further data and analyses are needed to support IL-4 polymorphism as a predictive biomarker. Moreover, for IL-4 signalling is enhanced in HCC (Guo et al., 2017), whether IL-4 inhibition could be used in HCC treatment requires further exploration (Bankaitis & Fingleton, 2015).

Similarly, increased i1 activation may also produce an anti-tumour effect or predict disease outcome. Previous studies show IFN $\alpha/\beta$  promotes differentiation into Th1 cell in human T cells and i1 activation (McNab et al., 2015). Retrospective reviews also show a protective effect of IFN $\alpha/\beta$  against HCC among hepatitis C patients, independent of viral infection (Hsu et al., 2015). (Enomoto et al., 2017) further show IFN $\alpha$  can induce an anti-tumour effect *in vivo* but not *in vitro*. Thus, it is likely that IFN $\alpha$  could suppress tumour growth by initiating i1. However, despite reduced tumour recurrence, IFN treatment fails to reduce metastasis among melanoma patients in a phase III clinical trial (Parker et al., 2016). During metastasis, cancer cells must pass a series of structural barriers formed by collagen (Jiang et al., 2000), which is also a major component of liver fibrosis.

Therefore, it is likely that IFN treatment, like other i1-targeted treatment, enhances local immunosurveillance, but fails to restrict the cells with fibrosis, which questions the sole use of i1-targeted therapy and the role of fibrosis in HCC. Therefore, the role of i2 must be further discussed in the context of HCC.

## Immunomodulation of liver cell differentiation

A key challenge for anti-fibrotic therapies in HCC is the poorly understood links between liver injury and HCC. The liver is known to have a strong regeneration ability (Kung et al., 2010), while different liver injury levels cause hepatocytes into either proliferation or senescence (C. Wang et al., 2018). Upon acute liver injury, Th1 cytokine activation promotes liver regeneration, which is later resolved by Th2 cytokines (Markose et al., 2018).

However, it has long been observed that chronic liver injury impairs immune function, which results in immunodeficiency and systemic inflammation (Albillos et al., 2014; Q. Zhang et al., 2019). Specifically, liver-resident group 2 innate lymphoid cells (ILC2s) cooperates with Th2 cells to maintain the activation of M2 macrophages (**Figure 2**) (Bouchery et al., 2015), which can develop into or recruit tumour-associated macrophages (TAMs). TAM accumulation predicts worse prognosis due to its role in metastasis, angiogenesis, and immune regulation (Lin et al., 2019). Thus, a variety of treatment targeting TAMs has been proposed (**Figure 3**) to eliminate and convert M2 macrophages (Barry et al., 2020). Additionally, approved anti-tumour treatment, such as PD-1/PD-L1 inhibition (Shi & Line, 2020), has also been found to mediate the conversion from M2 to M1.

Alternate activation of innate immune cells is associated with liver regeneration (Garrido & Djouder, 2021; Markose et al., 2018). Liver-resident and recruited macrophages produce growth factors, especially TGF- $\beta$  and PDGF, which activate hepatic stellate cells (HSCs), a kind of T cell-tolerant liver-resident antigen-presenting cells (Crispe, 2014). The activated HSCs transdifferentiate into cancer-associated fibroblasts (CAFs) and myofibroblasts, producing ECM and accelerating fibrosis (Barry et al., 2020; Garrido & Djouder, 2021). In vitro co-culture and in vivo co-implantation of HSCs/myofibroblasts with tumour cells into mice indicate HSC activation's role in promoting cancer migration, growth and survival (Barry et al., 2020; Kang et al., 2011). Thus, i2 activation contributes to HCC onset and progression by promoting HSC activation through TAMs (**Figure 4A**). In light of this, HSCs can be a prognostic marker in HCC, while therapeutic strategies targeting HSC activation through TGF- $\beta$  and PDGF are also under research (Chan et al., 2020).

A remaining question is the origin of HCC. Using genetic lineage tracing in an HCC mouse model, (Tummala et al., 2017) show that HCC mostly originates from hepatocytes instead of hepatic progenitor cells, which primarily develop into benign lesions. With broad and deep-sequencing, (Zhu et al., 2019) suggest that mutations implicated in HCC happen long

before tumour initiation. Furthermore, single-cell transcriptomics reveals highly diverse molecular landscapes of hepatocytes (Henderson et al., 2020; Kho et al., 2004), which may provide cell heterogeneity to undergo selection pressure due to chronic liver injury, as suggested by increased mutation loads with the course of liver injury (Zhu et al., 2019).

Notably, distinct cirrhotic nodules are shown to have different disease involvements (**Figure 4B**), where most mutations promote survival, instead of oncogenesis, which suggests a potential protective role of i2-driven fibrosis during early carcinogenesis, though the role of noncoding mutations remains to be elucidated (Müller et al., 2019; Zhu et al., 2019). Therefore, fibrosis may limit both immunosurveillance and tumour expansion, which should be critically evaluated when designing a new therapy. Also, in light of cell heterogeneity and fibrosis restriction undermining drug intake, targeted drug delivery to specific cells, e.g., HSCs and tumour cells in specific cirrhotic nodules, should be developed (Chen et al., 2019).

## **Type 2 immunity: beyond liver immunity**

As an immunological organ highly exposed to circulating antigens and endotoxins, the liver exhibits restricted immune responses, allowing itself to recover through liver regeneration; yet, the restricted responses, termed liver tolerance, cause incomplete antigen clearance (Crispe, 2014). The tolerance may well contribute to the cell heterogeneity within the liver, allowing cancer growth (F. Li & Tian, 2013). In fact, the liver is a common target of metastasis, during which cytokines in the liver promotes cancer cell expansion (Clark et al., 2016), supporting the tumour-promoting role of liver immunity and suggesting inter-organ crosstalk.

Additional research shows the impact of i2 through neural regulation and metabolism, which also contributes to immunosuppression, which provides further targets in i2-driven pathogenesis of HCC. For example, it has recently been appreciated that gut microbiota plays a role in sustaining tissue homeostasis in the gut-liver axis, whereas translocation of metabolites derived from gut microbiota to the liver also interplays with liver immune cells. Especially, certain species of gut microbiota sensitise immune checkpoint therapy, signifying additional therapeutic insights beyond the liver (**Figure 5**) (Ohtani & Kawada, 2019).

Another example is the role of neural regulation via neuropeptide neuromedin U (NMU). Cardoso et al. (2017) and (Klose et al., 2017) reveal that neuronal signals can fast and potently induce i2 against helminth infection in the liver via ILC2 activation mediated by the NMU. (Wallrapp et al., 2017) further report the binding of NMU to its receptor NMUR2

activates ILC2 activation, making NMU/NMUR2 signalling a potential therapeutic. Besides, (Q. Li et al., 2020) support NMU as a prognostic marker in HCC, where they also prove that worse prognostic is associated with increased M2 macrophages and Th2 cytokines.

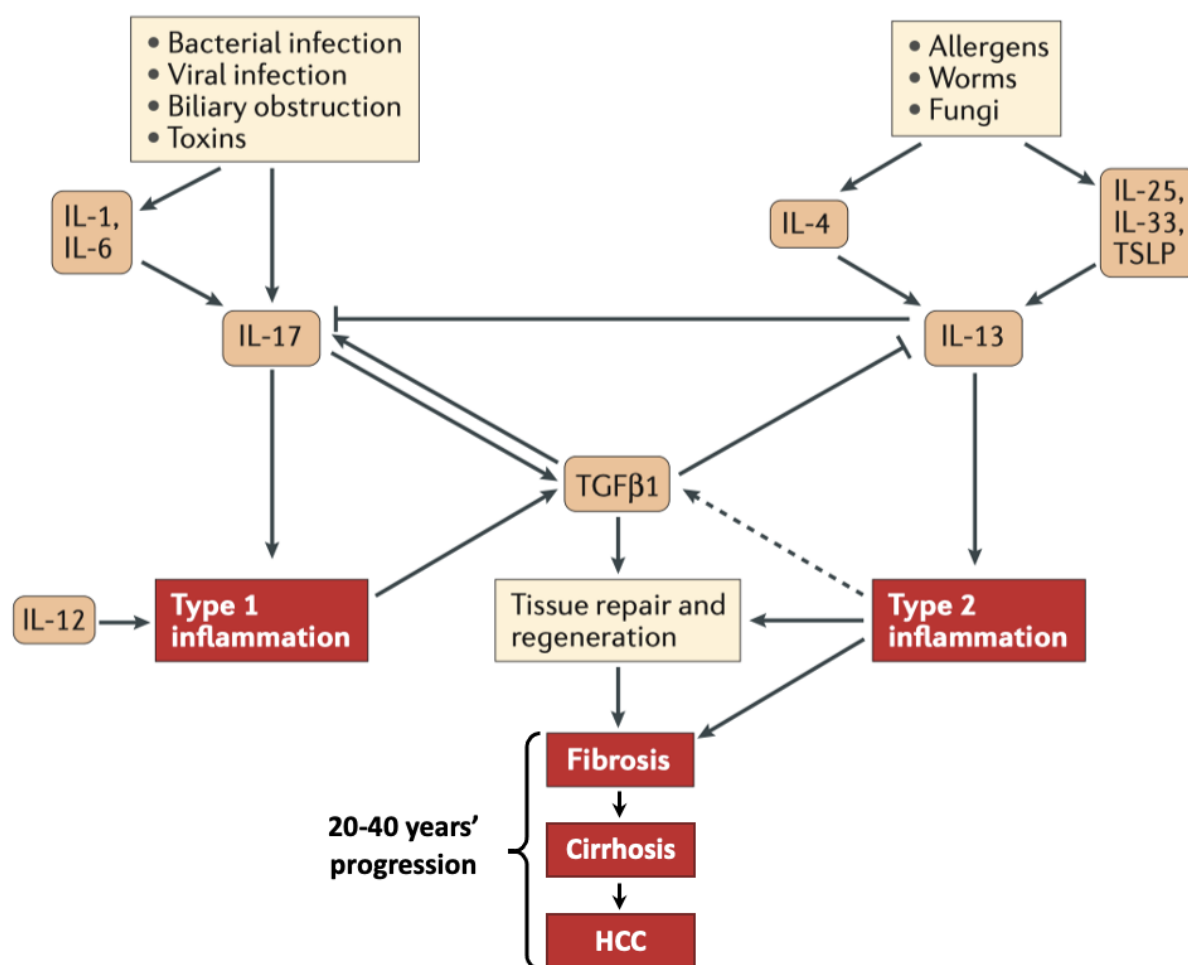
Considering dose-dependents effect of NMU in metabolism and thermoregulation (**Figure 6**) (Abdullahi et al., 2019; Klose et al., 2017) and its extensive roles in cancers, as reviewed by (Przygodzka et al., 2019), it is worthwhile to explore neuro-immune communication, which may provide new therapeutic and diagnostic targets (Kefeli et al., 2017). For example, (Teranishi et al., 2018) show that hepatic NMU overproduction accelerates liver inflammation. Considering i2's role in liver tumorigenesis as described previously, whether NMU overproduction-induced inflammation mediates tumorigenesis and whether liver-specific inhibition of NMU can mitigate HCC are worth further exploration.

Additionally, a growing body of evidence shows that neuronal guidance cues, such as netrin-1, are involved in promoting liver regeneration (Schlegel et al., 2016), avoiding hepatocyte death (Lahlali et al., 2016), inducing epithelial-mesenchymal transition (Schlegel et al., 2016), etc. In renal ischemia-reperfusion injury, netrin-1 suppresses Th1/Th2/Th17 cytokines production, thus protecting against renal injury (Tadagavadi et al., 2010). However, additional verification in the liver will be beneficial for evaluating the therapeutic potentials.

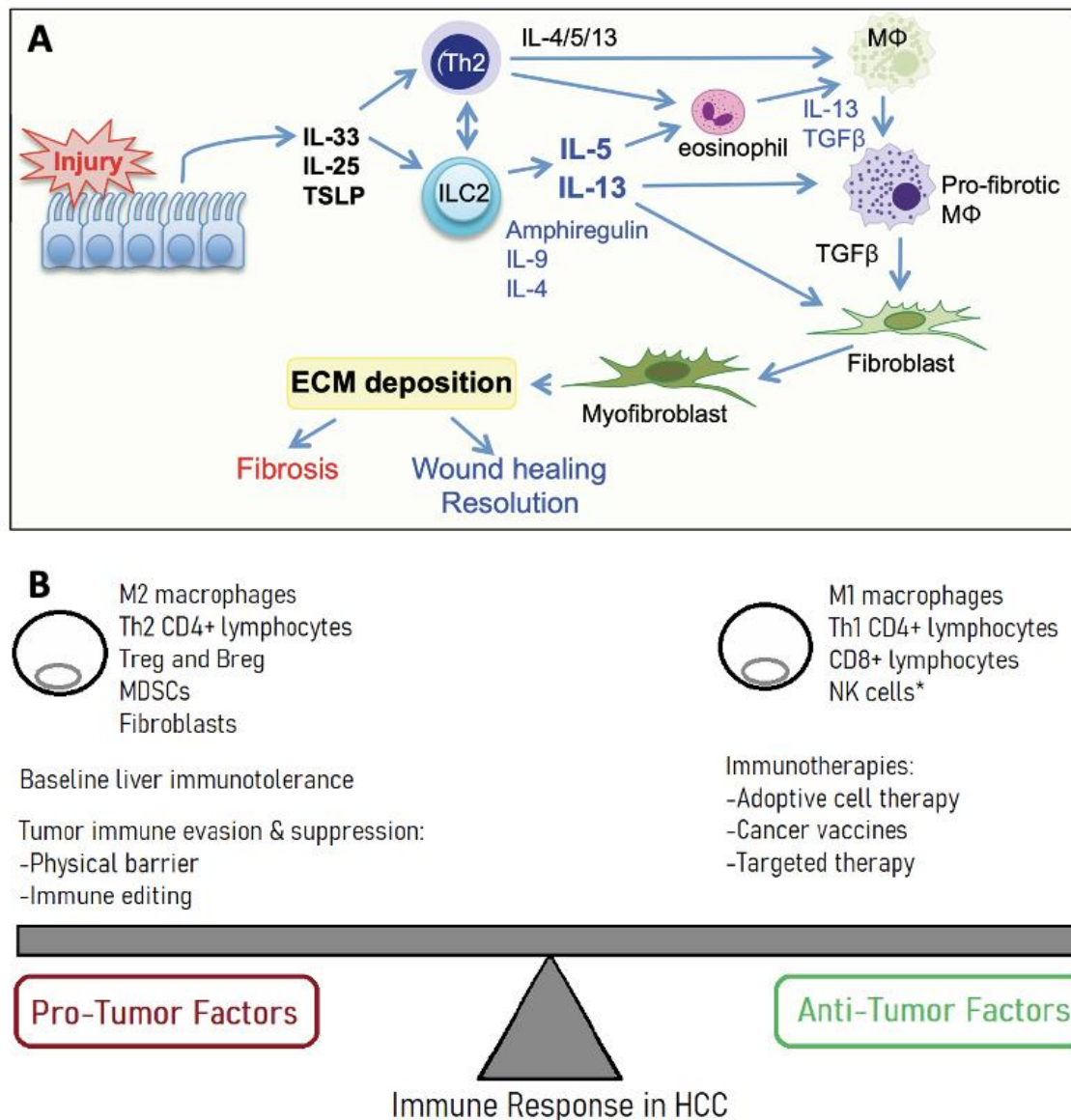
## **Conclusion**

The expanded understanding of i2 in liver repair and fibrosis has enabled new insights into HCC treatment. A balanced i1-i2 axis is vital to liver homeostasis; in contrast, dysregulated liver homeostasis skewing towards i2 profile causes liver fibrosis. During the process, molecular events such as cytokine release and activation, cellular activities such as macrophage polarisation, and tissue-level rearrangements such as fibrosis and cirrhosis happen in the liver, which is regulated by liver immunity, especially i2. This provides significant therapeutic and diagnostic insights, including molecular and cellular targets such as Th2 cytokines and HSCs, along with an emphasis on drug delivery methods. The extensive role of Th2 cytokines in metabolism and neural regulation links HCC to more broad fields such as inter-organ crosstalk, which remains to be explored. Undoubtedly, research into i2's role in liver fibrosis and HCC will provide further insights into i2-related clinical applications and innovations in HCC, with more solid theoretical and practical foundations.

## Figures



**Figure 1. Th1 and Th2 cytokine in hepatocarcinogenesis**, adapted from (Gieseck et al., 2018). Regardless of the aetiologies, the CLD progression into HCC consists of three stages, including fibrosis, cirrhosis, and HCC, which takes about 20-40 years (Dhar et al., 2020). Nonresolving inflammation, due to the imbalanced activation of i1 and i2, as illustrated above, disrupts normal tissue repair and regeneration, contributes to the progression, Regardless of the causes of CLDs. However, it should be noted that HCC could rarely happen without prior cirrhosis or fibrosis; in contrast, cirrhosis is found in more than 90% of the HCC patients (Ghouri et al., 2017), which suggests the universality of the three-stage progression discussed in this article. However, the causal links between liver cirrhosis and HCC is questioned by (Garrido & Djouder, 2021)

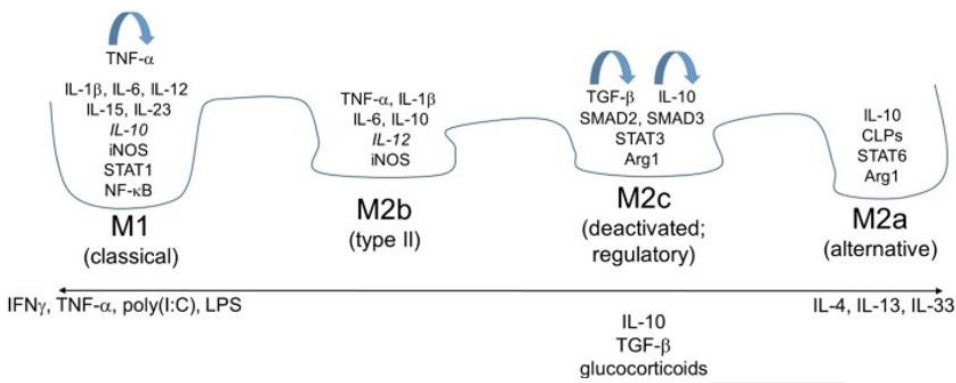


**Figure 2. Immune cells in liver fibrosis and HCC.**

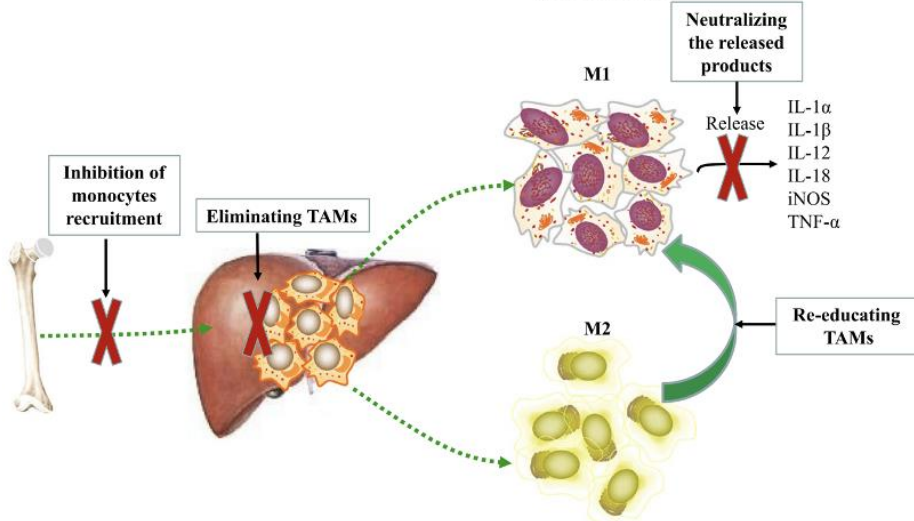
- **2A)** Upon liver injury, alarmins, e.g., IL-33, IL-25, thymic stromal lymphopoietin (TSLP) activate ILC2 and Th2, which produces Th2 cytokines, causing eosinophil recruitment and pro-fibrotic M2 macrophage differentiation. M2 macrophages further differentiate into fibroblasts in the presence of TGF- $\beta$ , myofibroblasts, causing ECM deposition and sequent fibrosis, if there is not wound-healing resolution (Hams et al., 2015).
- **2B)** A number of cells are believed to be pro-tumour and anti-tumour factors, as shown in the figure. In addition to these cells, high liver immunotolerance in the liver, along with physical barriers of ECMs and immune editing, favours tumour onset and growth. Not all therapeutic implications listed are mentioned in the main text, with details of therapies, such as cancer vaccines, reviewed by (S. Z. Wang et al., 2021).



**A. i1-i2 axis of macrophage differentiation**



**B. Therapeutic strategies targeting tumour associated macrophages (TAMs)**

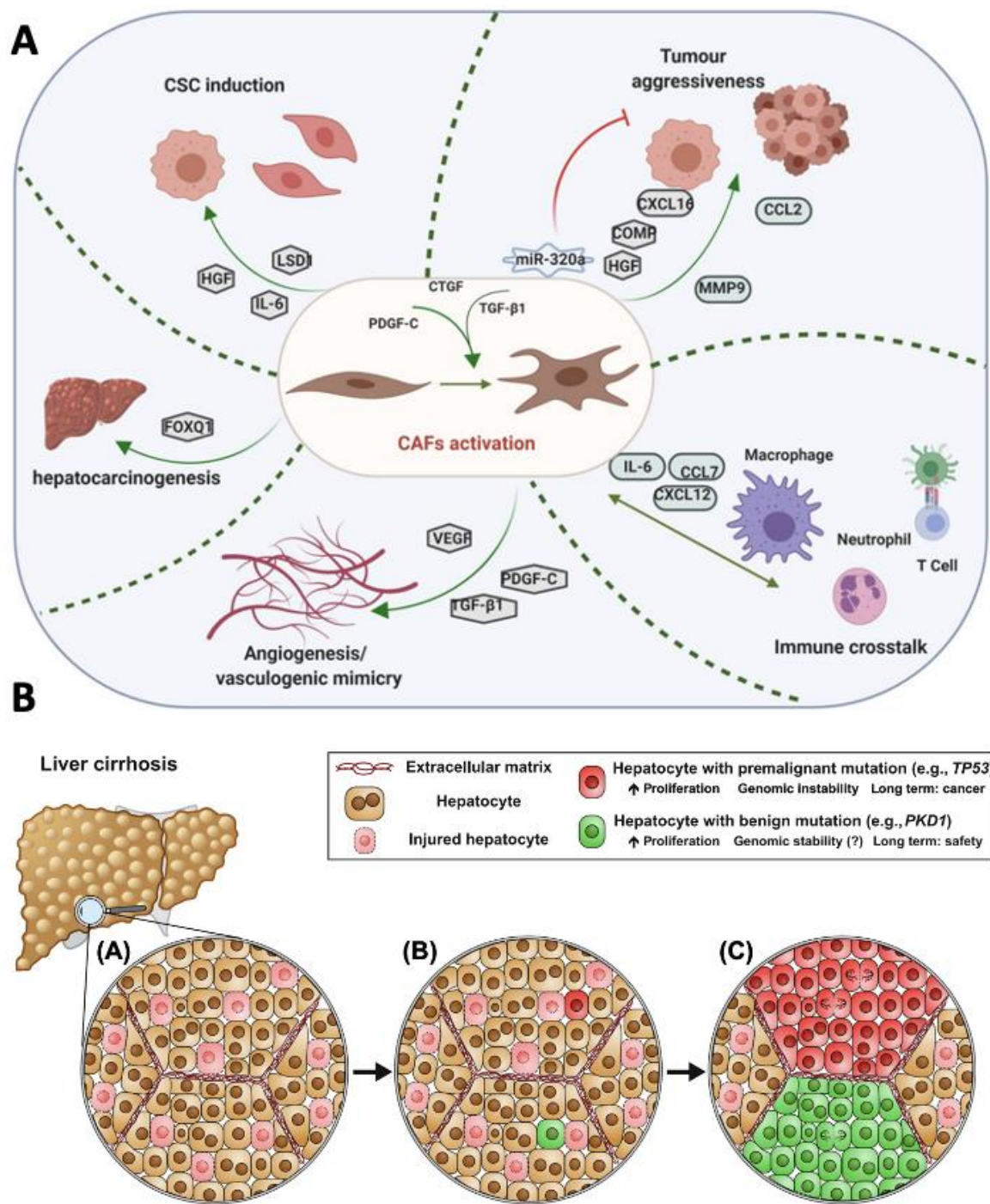


**B. Examples of current preclinical drugs targeting TAMs**

Mechanism of action	Compound	Target	Results
Inhibition of monocytes recruitment	RDC018 or knockout of CCR2	CCR2 antagonist	Inhibit malignant growth and metastasis, reduces postsurgical recurrence
	747	CCR2 antagonist	Anticancer properties and potentiated the efficacy of sorafenib in mouse models of HCC
	CCL2-neutralizing antibody	CCL2	Reduce liver damage, HCC incidence, and tumor burden in mouse models
Eliminating TAMs	GC33	Glypican-3	Phase I study for advanced HCC
	Clodrolip or Zoledronic acid		Enhance the inhibitory effect of sorafenib in nude mouse models
Re-educating TAMs	Zoledronic acid		Enhance the effects of TACE in rat HCC models
	PLX3397	CSF-1 receptor	Delayed tumor growth and increase in CD8 <sup>+</sup> T cells
	Baicalin		Inhibition of tumor growth
Neutralizing products of TAMs	8-Bromo-7-methoxychrysin	CD163	Disrupt the interaction of liver cancer stem-like cells and TAMs
	Tocilizumab	IL-6 receptor	Inhibit TAM-stimulated activity of human HCC stem cells in vitro and in vivo

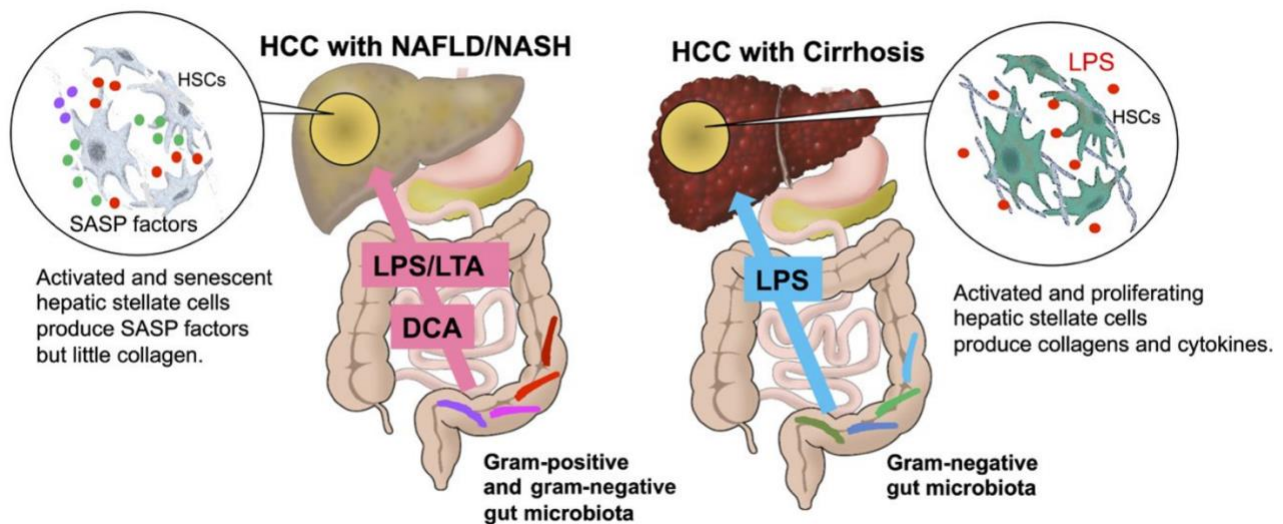
**Figure 3. TAMs in HCC**

- **3A)** The continuum of macrophage polarisation states (Yamaguchi et al., 2015) indicates that macrophage transdifferentiation is mediated by the centration of cytokines along the i1-i2 axis, where i1 is predominantly characterised by IFN- $\gamma$ , TNF- $\alpha$ , poly(I:C), LPS, and i2 characterised by IL-4, IL-13, IL-33.
- **3B & 3C)** Therapeutic strategies targeting TAMs (Tian et al., 2019). Proposed strategies include inhibition of monocyte recruitment, eliminating TAMs, M2-M1 conversion, neutralising M1 release products are proposed (3B), with examples listed in 3C.

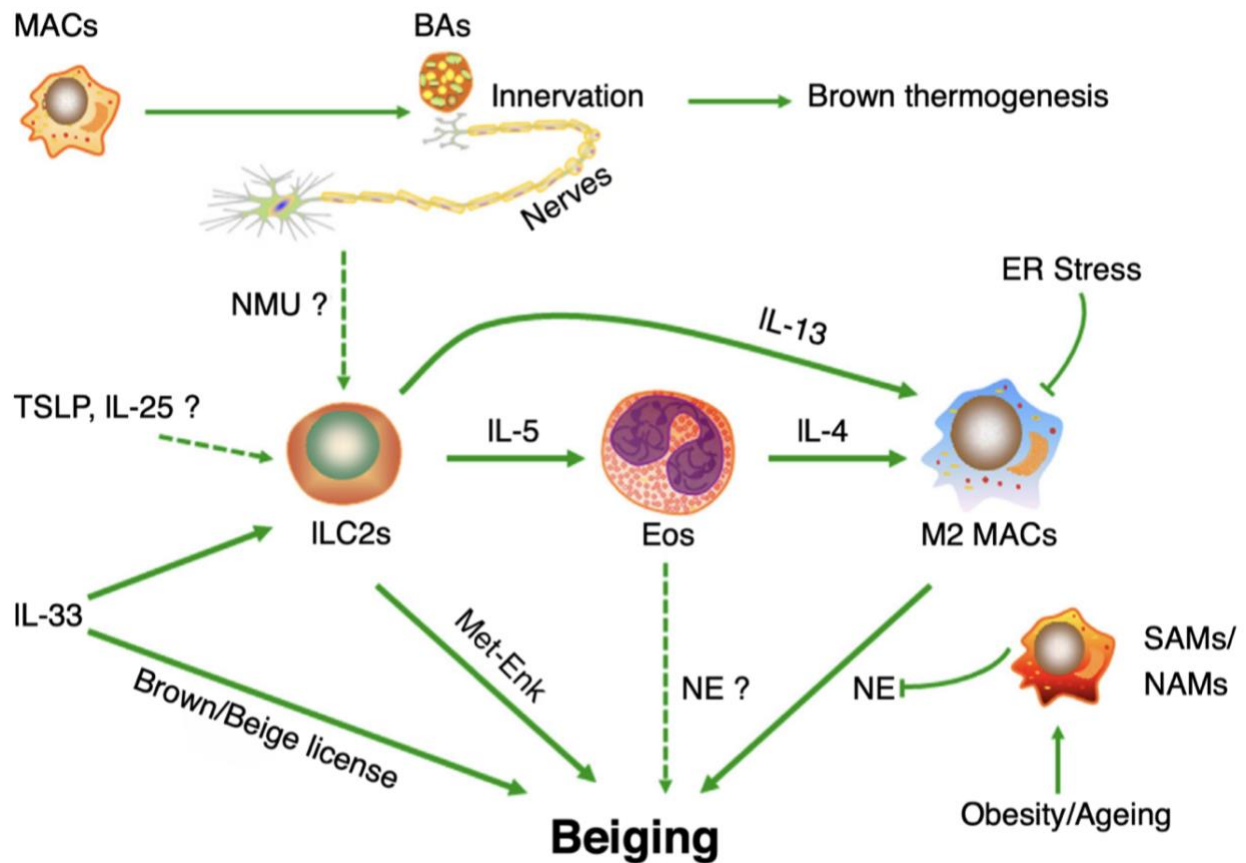


**Figure 4. Hepatocarcinogenesis mechanisms.**

- **4A)** With immune crosstalk to initiate HSC differentiation into CAFs upon PDGF and TGF- $\beta$  activation, activated CAFs promote tumour aggressiveness, hepatocarcinogenesis, angiogenesis, etc, impacting HCC onset (J. Zhang et al., 2020).
- **4B)** Mutations arise during chronic liver injury. (Zhu et al., 2019) suggest most mutations are adaptively beneficial, with cirrhotic nodules residing different colons (Müller et al., 2019).



**Figure 5. Gut microbiota in HCC show different impacts on HCC** (Ohtani & Kawada, 2019). As gut inflammation or other diseases causes a leaky gut, microbiobial components and metabolites flow into the liver. Both HCCs in the cirrhotic liver and NASH/NAFLD are closely associated with LPS exposure from gram-negative bacteria. However, additional gram-positive bacteria-derived LTA may well be involved in NASH-associated HCC, which undergoes cellular senescence. Thus, both activated and senescent HSCs need to produce SASP factors. In contrast, in cirrhostic liver with HCC, LPS directly activates HSC and allows its production of collagens and cytokines. (DCA: deoxycholic acid; LTA: lipoteichoic acid; LPS: lipopolysaccharide; SASP: senescence-associated secretory phenotype; NASH nonalcoholic steatohepatitis; NAFLD: nonalcoholic fatty liver disease.)



**Figure 6. Immune-neuro-thermogenic interplay** (Kane & Lynch, 2019), where Th2 cytokines, such as IL-4, IL-33, IL-13, etc., are extensively involved to produced a variety impact on beiging through ILC2-M2 macrophage axis, which is modulated by tissue injury charaterised by IL-33, ER stress and metabolism, characterised by sympathetic neuro-nassociated macrophage (SAM) and nerve-associated macrophage (NAM) inhibition of NE. Especially, the role of NMU and NE is not detailedly examined, which requires further research.

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