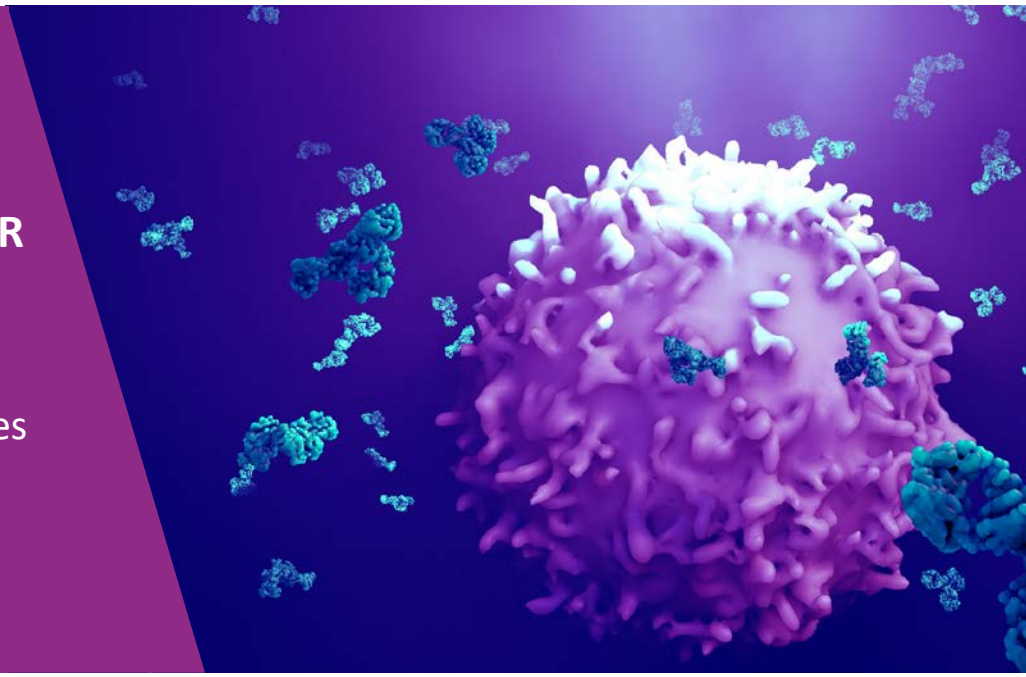
imSAVAR

IMMUNE SAFETY AVATAR

Nonclinical mimicking of the immune system effects of immunomodulatory therapies



Deliverable 2.4
CPI immune safety assessment research roadmap

DELIVERABLE REPORT

This project has received funding from the Innovative Medicines Initiative 2 Joint Undertaking (JU) under grant agreement No 853988.
The JU receives support from the European Union's Horizon 2020 research and innovation programme and EFPIA and JDRF INTERNATIONAL.

innovative medicines initiative

efpia

JDRF IMPROVING LIVES. CURING TYPE 1 DIABETES.

Abstract

Checkpoint inhibitors frequently trigger immune-mediated adverse events in patients. Similar to many other organs, liver toxicity is induced by aberrantly activated immune cells but likely less influenced by microbiota or other environmental factors. We discuss currently available in-vitro systems for their suitability to capture mechanisms of in-vivo drug effects. We conclude that an in-vitro workup of checkpoint inhibitor-induced hepatotoxicity is not advised in the consortium due to mechanistic complexity and insufficient translation of antigen specificity of T cells in experimental systems. We propose to refine hypotheses by patient-based workup, and potentially revisit in-vitro systems if this is successful.

Document Information

Deliverable Report	**D2.4: CPI immune safety assessment research roadmap**
Date	**20.05.2022**
Report prepared by	**Roche** Fraunhofer IGB, Fraunhofer ITMP, Fraunhofer ITEM, Fraunhofer IZI, Dynamic42 GmbH, Labcorb, Boehringer Ingelheim International GmbH, Merck, Novartis, MERCK SHARP & DOHME CORP
Project	**imSAVAR - Immune Safety Avatar: nonclinical mimicking of the immune system effects of immunomodulatory therapies** Grant Agreement No.: 853988 (IMI2-2018-15-04)
Project Coordinator	**Fraunhofer-Gesellschaft zur Foerderung der angewandten Forschung e.V.** Prof. Dr. Dr. Ulrike Köhl Dr. Kristin Reiche **Novartis Pharma AG** Dr. Jonathan Moggs Hannah Morgan, PhD
Type	**Deliverable Report | Public**

This project has received funding from the Innovative Medicines Initiative 2 Joint Undertaking (JU) under grant agreement No 853988. The JU receives support from the European Union's Horizon 2020 research and innovation programme and EFPIA and JDRF INTERNATIONAL.

Table of Contents

1. Results

Background

Checkpoint inhibitors (CPI) have revolutionized the treatment options for cancer and are now indicated for various malignancies, e.g., metastatic melanoma or non-small cell lung cancer (NSCLC) (Lewis et al., 2020; Anderson et al., 2019). CPI induce an anti-tumor response by inhibiting immune checkpoints such as the cytotoxic T-lymphocyte antigen-4 (CTLA-4) or the programmed death-1/-ligand-1 (PD-1/PD-L1) pathway. These pathways are physiologically responsible for the downregulation of T-cell responses and hence protect the body from a possible damaging immune response. However, some tumors are able to hijack and exploit these checkpoints and consequently evade the immune response. By reactivating the immune system, checkpoint inhibitors restore a potentially efficacious anti-tumor immunity in a wide range of cancer types (Ramos-Casals et al., 2020).

In 2011, the Food and Drug administration (FDA) approved the first CIP, ipilimumab, for the treatment of metastatic or unresectable melanoma (Almutari et al., 2020). Ipilimumab is an inhibitor of a negative regulator of the anti-tumor T-cell response called **CTLA-4** (Tarhini et al., 2010). T-cell activation requires multiple stimulatory signals to turn into an effective response. The first step to achieve specificity of the T-cell activation is ensured by the binding of the T-cell receptor (TCR) with the antigen-presenting major histocompatibility complex (MHC) presenting an antigen recognized by the TCR. Additional co-stimulatory signals are obtained via the binding of CD28 molecules, located on the T-cell, with B7-1 or B7-2 molecules that are located on the antigen-presenting cells (APC). If sufficient, these two concomitant signals result in T-cell proliferation, increased survival and differentiation. Conversely, co-inhibitory signals such as CTLA-4 intervene at the priming stage of the naïve T-cell activation to delete potential autoreactive T-cells. CTLA-4 is a homolog of the CD28 with a higher affinity to the B7 molecules. Unlike the CD28/B7 binding, the CTLA-4/B7 binding does not result in a co-stimulatory response but rather in an inhibitory signal. In addition to this inhibitory signal, the competitive binding of CTLA-4 and CD28 may lead to a decreased CD28/B7 co-stimulatory signal. Thus, by adding an anti-CTLA-4 antibody, the inhibitory signal can be interrupted. However, the exact mode of action of anti-CTLA-4 antibodies in promotion of cancer immunity remains unclear. It has been proposed that blocking the CTLA-4 acts on the immune priming phase in the lymph node by aiding the activation and proliferation of effector T-cells and/or reducing the immune suppression mediated by Tregs. Thus, through the inhibition of CTLA-4 by ipilimumab an effective anti-tumor immune response occurs (Buchbinder et al., 2016).

Other approved CPI inhibit either the programmed death receptor 1 (**PD-1**) such as nivolumab and pembrolizumab or they inhibit the programmed-death ligand-1 (**PD-L1**) such as **atezolizumab**, durvalumab and avelumab (Akinleye et al., 2019, Alexander et al., 2016). These inhibitors act through a similar mechanism of action by modulating the immune cell-tumor cell interaction. PD-1 is expressed on activated T-cells located primarily in the peripheral tissue. The need to control the degree of inflammation wherever antigens are expressed and to secure normal tissue from damage has led to the emergence of the PD-1/PD-L1 pathway (Buchbinder et al., 2016, Alsaab et al., 2017). When an antigen presented by the MHC is recognized by a T-cell, inflammatory cytokines are produced and initiate the inflammatory process. As a consequence, these cytokines lead to the PD-L1 expression in the affected tissue and thus cause the activation of the PD-1 pathway in T-cells resulting in immune tolerance. However, tumor cells can exploit this protective PD-1 signaling pathway by overexpressing PD-L1 along the MHC on different types of cancer, e.g. melanoma or lung carcinoma, to evade the generation of an anti-tumor immune response. Through this inhibition, the T-cell suppression is lifted and the antitumor immunity restored. (Akinleye et al., 2019, Alsaab et al., 2017).

The main differences between the CTLA-4 and the PD-1/PD-L1 pathways are the timing of downregulation and the anatomic location of the immune inhibition. CTLA-4 operates during the priming phase of the T-cells (in the lymph node) while PD-1 functions during the effector phase in peripheral tissue. CTLA-4 expression is mostly restricted to T-cells, but PD-1 on the other hand is more broadly expressed, i.e. PD-1 can be found on activated T-cells, B-cells and myeloid cells (Alsaab et al., 2017, Zitvogel et al., 2012).

Despite the advantages of CPI, not every individual treated with CPI is responsive to them. In patients treated for solid tumors only between 15-30% of the patients respond to the treatment whereas patients treated for melanoma have a slightly higher response rate between 45-60%. However, this leaves a significant proportion of patients unresponsive to CIP treatment (Das et al., 2019). Furthermore, unique inflammatory unwanted effects termed **immune-related adverse events** (irAEs) may occur and pose a major challenge to CIP treatments (Ramos-Casals et al., 2020; Das et al., 2019). The mechanisms underlying the occurrence of these irAEs have not been completely elucidated yet (Das et al., 2019) and the biological triggers of irAEs may differ between the different types of CIP (Ramos-Casals et al., 2020) - exemplified by reports that CTLA-4 inhibitors cause irAEs in a dose-dependent manner while PD-1/PD-L1 inhibitors cause toxicities independently of dosages (Ramos-Casals et al., 2020). Data on patient subpopulations and animal models suggest a localized activation and expansion of tissue-resident adaptive cells, which are without therapy held in check by tissue-resident checkpoint expression (e.g. PD-L1 on macrophages; Damo et al, 2021). Other concepts propose a CPI-induced exacerbation of the immune response against latent infections with endemic viruses like CMV (Hutchinson et al., 2021). Irrespective of the trigger, the mechanism of irAEs might be tied to CPI's mode of action in immunological homeostasis, rendering an untangling of safety and efficacy challenging. Genetic factors of Th17 cell polarization, previously linked to psoriasis and vitiligo, can indeed be linked to overall survival after PD-L1 blockade - while concurrently, a cohort with mild skin irAE also shows increased overall survival (Khan et al., 2020). With phenotypic similarities between irAE and idiopathic skin diseases, it is intriguing to speculate that a genetically or environmentally driven adaptive immunity pattern is beneficial in anti-tumor immunity, but may induce irAE when dysbalanced or in unwanted tissues. This concept is also supported by multiple other mechanistic hypotheses of efficacy and safety in CPI therapy (Ramos-Casals et al., 2020).

Unlike for autoreactive T cells, the contributions of B cells and autoantibodies are less clarified. Still, anti-drug antibodies (ADA) are frequently observed in CPI-treated patients, and it is yet unclear whether the immune-stimulating MoA contributes to increased B cell activation, e.g. by Tfh activation. As some irAE are considered a complex interplay of therapeutics and immune-complex forming ADA, further mechanistic exploration promises to support patient prioritization or irAE mitigation. Furthermore, cytokine release might contribute to the occurrence of irAEs (13). Interestingly, ipilimumab is associated with different types of irAEs compared to PD-1/PD-L1 inhibitors, as it is linked to higher rates of gastrointestinal toxicities, pruritus and rash whereas PD-1/PD-L1 inhibitors are associated with higher rates of pneumonitis and hepatotoxicity (14). However irAEs are not considered to be organ-specific, although some organ systems are affected more frequently such as the liver, gastrointestinal tract and lung (13, 15) sometimes leading to severe consequences for the patients.

Given the potential severity of hepatotoxicity for patients, the consortium laid a first focus on modeling hepatic immune related adverse events with the help of in vitro tools. Other organ toxicities share underlying mechanisms, but may be significantly more complex due to influences of non-host, environmental factors (esp. Microbiome, nutritional cues, physical challenges).

<u>In-vitro Models of hepatic irAE and non-ir AE</u>

The liver is an important organ to consider during early drug development and often the subject of many off-target-related investigations. As the liver is responsible for the metabolism and detoxification of intrinsic and extrinsic molecules, including drugs, almost all compounds pass the liver. This can lead to undesired drug-induced liver injuries (DILI) and made in-vitro liver toxicity testing a mainstay of small molecule drug development.

For the assessment and investigation of DILI findings in vitro, different cell sources and cultivation methods have been described, all featuring benefits and drawbacks. **Hepatic cell lines** are generated from tumor tissue or by genetic engineering and are, due to their availability and high proliferation capacity, widely used cell sources in research. Although these cell lines are suitable tools in certain research fields, their phenotype and functionality differ substantially from the in vivo situation (Rodríguez-Antona et al., 2002; Sison-Young et al., 2015). Another promising approach includes the use of stem-cell derived hepatic cells, differentiated from either adult, embryonic, or **iPS**-derived stem cells (Snykers et al., 2011; Wills and Rajagopalan, 2019). Here, a lot of progress has been made over the last decades, although the differentiation into fully functional and mature hepatic cells is still not fully understood and the focus of many research groups (Hu et al., 2018; Koui et al., 2017; Takayama et al., 2012; Wang et al., 2016).

So far, **primary hepatocytes** are considered the gold standard in DILI research, as they, compared to the cell sources described above, most closely resemble the in vivo situation upon isolation. The cultivation of these primary cells in suspension or traditional 2D culture settings, however, leads to rapid dedifferentiation, loss of viability, phenotype and liver-specific functions, which renders their use in more complex, and prolonged DILI investigations difficult (Elaut et al., 2006; Rowe et al., 2013)

Therefore, new and better ways to cultivate primary liver cells have been established, which help to avoid the dedifferentiation of hepatocytes, thereby stabilizing the viability and functionality over a prolonged period of time (Lauschke et al., 2016). Primary hepatocytes can be cultivated in a **sandwich** configuration between two layers of extracellular matrix (ECM), which consists of collagen or Matrigel. This configuration better mimics the microenvironment in the liver, which promotes the stability of the cellular phenotype over a prolonged period of time, improves hepatocyte polarization, and leads to the formation of functional bile canaliculi (Knobeloch et al., 2012; Schyschka et al., 2013). However, in a sandwich configuration, the dedifferentiation of the cells is substantially retarded, but not completely prevented (Rowe et al., 2013), and collagen and Matrigel, both rodent-derived materials, raise concerns due to batch-to-batch variability, immunogenicity and technical challenges during handling (Serban and Prestwich, 2008).

A step towards more complex liver systems has been introduced by **micro-patterning** of primary hepatocytes. Here, hepatocytes are cultured as “islands”, surrounded by fibroblasts, which reflects the physiological microenvironment in the liver (Khetani and Bhatia, 2008; Ukairo et al., 2013). This culture-system maintains its liver-specific functionality for up to 6 weeks and has been successfully used to predict the DILI potential of known drugs (Khetani et al., 2013), including slow-metabolizing compounds (Chan et al., 2013). However, the system’s throughput is rather low and liver specific (adaptive) immune cells are missing in this system.

The inclusion of the liver-adaptive immune system, comprising Kupffer cells (KCs), Stellate cells (SCs), and Liver Sinusoidal Endothelial cells (LSECs), has been identified as a critical component towards a more physiological relevant liver model as the inclusion of these cells (1) can additionally help to prolong the viability and functionality of primary hepatocytes (Dash et al., 2009) and (2) is necessary to address immune-related liver effects (Hasmall et al., 2000), thereby providing a liver in vitro tool which covers the broad range of DILI mechanisms.

Primary hepatocyte **spheroids** have emerged as a promising 3D tissue model, as they self-assemble by adhering to each other, so that no artificial scaffold is needed. Spheroid cultures require ~20x fewer cells compared to 2D cultures and can be produced at low costs and high throughput, even in an automated way. Several studies have demonstrated liver-specific functions and long-term viability of primary liver spheroids over multiple weeks, while they maintained transcriptomic and proteomic signatures similar to those observed in vivo (Bell et al., 2017, 2016; Messner et al., 2018; Ohkura et al., 2014; Vorrink et al., 2017). Spheroids have been used to assess the hepatotoxicity of drugs in experiments that require long-term exposure, metabolic activation, or the accumulation of bile acid or reactive oxygen species. The toxicity was measured at concentrations similar to relevant serum levels, which indicates that spheroids are a physiologically relevant system to assess and investigate DILI effects of compounds in early drug development (Hendriks et al., 2016; Messner et al., 2013; Tostões et al., 2012). Considering the composition of those spheroids and their architecture, liver spheroids do not reflect the in vivo morphology, as cells (particularly the NPCs) are randomly distributed throughout the spheroids.

Different advanced liver models including microfluidics – so called organ-on-chip - have been established in the last decades, which helped to gain important information about DILI and related mechanisms. The **CNBio** model (Domansky et al., 2010) features a mechanical (polystyrene) scaffold, were both, hepatocytes and NPCs can grow in a three-dimensional (3D) surrounding, while constantly applied flow of cell culture medium through the system generates physiological shear stress and an oxygen gradient. Both are important features which can hardly be provided in a 2D cell culture setting (Powers et al., 2002). The CNBio system can be maintained for up to 1 month, it has been used for metastatic cancer studies and to investigate the metabolism of hydrocortisone in an inflamed liver (Sarkar et al., 2015; Wheeler et al., 2014).

Microfluidic, multi-compartmental in-vitro systems can be used to mimic in-vivo tissue architecture (e.g. in commercial Emulate liver chips). Consortium members have previously used such systems in the past for tissue-immune interactions. They are complex to set up, and mononuclear immune cell recruitment from the “vascular” channel requires many modifications that introduce artefacts. We would advise against exploring these for hepatitis.

Despite the unquestionable success of these mentioned liver models in drug toxicity testing, irAE in cancer immunotherapy pose a challenge of biologic complexity. Unlike with most small molecules, CPI-induced liver toxicity (and almost all other organ damages) depend on a dysbalanced immune activation, especially of cytotoxic CD8 T-cells (see above). As these T cells are specific for antigen presentation in an MHC context, in-vitro models may face non-resolvable issues of antigen specificity and allogenicity, if tissues and cells from different donors are combined in vitro. T cell may be aberrantly activated by physiologic HLA-peptide presentation (Shlomchik, 2007). Using an autologous cell system, HLA-matching or benchmarking of donors will likely be necessary for any in-vitro workup, but questions on the existing pool of antigen-specific T cells will remain close to impossible to address.

Importantly, this mechanistic background separates checkpoint inhibitors from targeted therapies like BiTEs or CAR-T cells. For the latter, an activation in-vitro may be comparable to in-vivo, which makes them valuable tools for the preclinical safety assessment. They are already established to some degree, and used in regulatory processes by consortium members and other pharmaceutical companies.

Aside from the mere activation of the immune system, the recruitment of immune cells to the actual organ is tightly regulated in vivo by inflammatory and homeostatic lymphocyte homing mechanisms (Butcher & Picker 1996). The recapitulation of immune cell migration into tissues is a challenge faced by the entire field of in-vitro models, and likely requires diligent separation into individual mechanistic steps

if this biology is relevant for CPI-induced toxicity (e.g. via the chemokine-dependent recruitment of T cells into the intestine after activation of resident memory T cells; Luoma et al., 2020).

2. Conclusion

This complexity in setup and raw tissue sourcing, in conjunction with the only superficial mechanistic description of CPI-induced toxicity (Adam et al., 2021; Affolter et al., 2019, Malnick et al., 2021) lead us to conclude that an experimental workup of hepatotoxicity is not feasible in the consortium. We rather propose to focus on a different angle of workup, e.g. starting with the deep phenotypical or transcriptional characterization of patient tissue with observed side effects. Upcoming technologies (spatial transcriptomics, multiplex IHC) are available in some of the consortium members' labs, and should be assessed for their suitability to generate mechanistic hypotheses. If this is successful, a second step can be to emulate these specifically in in-vitro systems.

3. References

1. Adam K, Iuga A, Tocheva AS, Mor A. A novel mouse model for checkpoint inhibitor-induced adverse events. PLoS One. 2021 Feb 11;16(2):e0246168.
2. Affolter T, Llewellyn HP, Bartlett DW, Zong Q, Xia S, Torti V, Ji C. Inhibition of immune checkpoints PD-1, CTLA-4, and IDO1 coordinately induces immune-mediated liver injury in mice. PLoS One. 2019 May 21;14(5):e0217276.
3. Akinleye, A., Rasool, Z. Immune checkpoint inhibitors of PD-L1 as cancer therapeutics. J Hematol Oncol 12, 92 (2019).
4. Alexander W. The Checkpoint Immunotherapy Revolution: What Started as a Trickle Has Become a Flood, Despite Some Daunting Adverse Effects; New Drugs, Indications, and Combinations Continue to Emerge. P T. 2016 Mar;41(3):185-91.
5. Almutairi AR, McBride A, Slack M, Erstad BL, Abraham I. Potential Immune-Related Adverse Events Associated With Monotherapy and Combination Therapy of Ipilimumab, Nivolumab, and Pembrolizumab for Advanced Melanoma: A Systematic Review and Meta-Analysis. Front Oncol. 2020 Feb 11;10:91.
6. Alsaab HO, Sau S, Alzhrani R, Tatiparti K, Bhise K, Kashaw SK, Iyer AK. PD-1 and PD-L1 Checkpoint Signaling Inhibition for Cancer Immunotherapy: Mechanism, Combinations, and Clinical Outcome. Front Pharmacol. 2017 Aug 23;8:561.
7. Anderson R, Theron AJ, Rapoport BL. Immunopathogenesis of Immune Checkpoint Inhibitor-Related Adverse Events: Roles of the Intestinal Microbiome and Th17 Cells. Front Immunol. 2019 Sep 26;10:2254.
8. Bell CC, Lauschke VM, Vorrink SU, Palmgren H, Duffin R, Andersson TB, Ingelman-Sundberg M. Transcriptional, Functional, and Mechanistic Comparisons of Stem Cell-Derived Hepatocytes, HepaRG Cells, and Three-Dimensional Human Hepatocyte Spheroids as Predictive In Vitro Systems for Drug-Induced Liver Injury. Drug Metab Dispos. 2017 Apr;45(4):419-429.
9. Bell CC, Hendriks DF, Moro SM, Ellis E, Walsh J, Renblom A, Fredriksson Puigvert L, Dankers AC, Jacobs F, Snoeys J, Sison-Young RL, Jenkins RE, Nordling Å, Mkrtchian S, Park BK, Kitteringham NR, Goldring CE, Lauschke VM, Ingelman-Sundberg M. Characterization of

primary human hepatocyte spheroids as a model system for drug-induced liver injury, liver function and disease. Sci Rep. 2016 May 4;6:25187.

10. Buchbinder EI, Desai A. CTLA-4 and PD-1 Pathways: Similarities, Differences, and Implications of Their Inhibition. Am J Clin Oncol. 2016 Feb;39(1):98-106.
11. Butcher EC, Picker LJ. Lymphocyte homing and homeostasis. Science. 1996 Apr 5;272(5258):60-6.
12. Chan TS, Yu H, Moore A, Khetani SR, Tweedie D. Meeting the Challenge of Predicting Hepatic Clearance of Compounds Slowly Metabolized by Cytochrome P450 Using a Novel Hepatocyte Model, HepatoPac. Drug Metab Dispos. 2019 Jan;47(1):58-66.
13. Damo M, Cui C, William I, Hornick NI, Kwok D, Clulo K, Damsky WE, Leventhal JS, Joshi NS. The PD-1 checkpoint receptor maintains tolerance of self-reactive CD8 T cell in skin. Available at SSRN: https://ssrn.com/abstract=3883633 or http://dx.doi.org/10.2139/ssrn.3883633. This version of the paper has not been formally peer reviewed.
14. Das S, Johnson DB. Immune-related adverse events and anti-tumor efficacy of immune checkpoint inhibitors. J Immunother Cancer. 2019 Nov 15;7(1):306.
15. Dash A, Inman W, Hoffmaster K, Sevidal S, Kelly J, Obach RS, Griffith LG, Tannenbaum SR. Liver tissue engineering in the evaluation of drug safety. Expert Opin Drug Metab Toxicol. 2009 Oct;5(10):1159-74.
16. Domansky K, Inman W, Serdy J, Dash A, Lim MH, Griffith LG. Perfused multiwell plate for 3D liver tissue engineering. Lab Chip. 2010 Jan 7;10(1):51-8.
17. Elaut G, Henkens T, Papeleu P, Snykers S, Vinken M, Vanhaecke T, Rogiers V. Molecular mechanisms underlying the dedifferentiation process of isolated hepatocytes and their cultures. Curr Drug Metab. 2006 Aug;7(6):629-60.
18. Hasmall SC, DA, Olsen K, Roberts RA. Role of hepatic non-parenchymal cells in the response of rat hepatocytes to the peroxisome proliferator nafenopin in vitro. Carcinogenesis, Volume 21, Issue 12, 1 December 2000, Pages 2159–2165.
19. Hendriks DF, Fredriksson Puigvert L, Messner S, Mortiz W, Ingelman-Sundberg M. Hepatic 3D spheroid models for the detection and study of compounds with cholestatic liability. Sci Rep. 2016 Oct 19;6:35434.
20. Hu H, Gehart H, Artegiani B, LÖpez-Iglesias C, Dekkers F, Basak O, van Es J, Chuva de Sousa Lopes SM, Begthel H, Korving J, van den Born M, Zou C, Quirk C, Chiriboga L, Rice CM, Ma S, Rios A, Peters PJ, de Jong YP, Clevers H. Long-Term Expansion of Functional Mouse and Human Hepatocytes as 3D Organoids. Cell. 2018 Nov 29;175(6):1591-1606.e19.
21. Hutchinson, J.A., Kronenberg, K., Riquelme, P. et al. Virus-specific memory T cell responses unmasked by immune checkpoint blockade cause hepatitis. Nat Commun 12, 1439 (2021).
22. Khan Z, Di Nucci F, Kwan A, Hammer C, Mariathasan S, Rouilly V, Carroll J, Fontes M, Ley Acosta S, Guardino E, Chen-Harris H, Bhangale T, Mellman I, Rosenberg J, Powles T, Hunkapiller J, Chandler GS, Albert ML. Polygenic risk for skin autoimmunity impacts immune checkpoint blockade in bladder cancer. Proc Natl Acad Sci U S A. 2020 Jun 2;117(22):12288-12294.
23. Khetani SR, Kanchagar C, Ukairo O, Krzyzewski S, Moore A, Shi J, Aoyama S, Aleo M, Will Y. Use of micropatterned cocultures to detect compounds that cause drug-induced liver injury in humans. Toxicol Sci. 2013 Mar;132(1):107-17.

24. Khetani SR, Bhatia SN. Microscale culture of human liver cells for drug development. Nat Biotechnol. 2008 Jan;26(1):120-6.

25. Knobeloch D, Ehnert S, Schyschka L, Büchler P, Schoenberg M, Kleeff J, Thasler WE, Nussler NC, Godoy P, Hengstler J, Nussler AK. Human hepatocytes: isolation, culture, and quality procedures. Methods Mol Biol. 2012;806:99-120.

26. Koui Y, Kido T, Ito T, Oyama H, Chen SW, Katou Y, Shirahige K, Miyajima A. An In Vitro Human Liver Model by iPSC-Derived Parenchymal and Non-parenchymal Cells. Stem Cell Reports. 2017 Aug 8;9(2):490-498

27. Kumar V, Chaudhary N, Garg M, Floudas CS, Soni P, Chandra AB. Current Diagnosis and Management of Immune Related Adverse Events (irAEs) Induced by Immune Checkpoint Inhibitor Therapy. Front Pharmacol. 2017 Feb 8;8:49.

28. Lauschke VM, Hendriks DF, Bell CC, Andersson TB, Ingelman-Sundberg M. Novel 3D Culture Systems for Studies of Human Liver Function and Assessments of the Hepatotoxicity of Drugs and Drug Candidates. Chem Res Toxicol. 2016 Dec 19;29(12):1936-1955.

29. Lewis AL, Chaft J, Girotra M, Fischer GW. Immune checkpoint inhibitors: a narrative review of considerations for the anaesthesiologist. Br J Anaesth. 2020 Mar;124(3):251-260.

30. Luoma AM, Suo S, Williams HL, Sharova T, Sullivan K, Manos M, Bowling P, Hodi FS, Rahma O, Sullivan RJ, Boland GM, Nowak JA, Dougan SK, Dougan M, Yuan GC, Wucherpfennig KW. Molecular Pathways of Colon Inflammation Induced by Cancer Immunotherapy. Cell. 2020 Aug 6;182(3):655-671.e22.

31. Malnick SDH, Abdullah A, Neuman MG. Checkpoint Inhibitors and Hepatotoxicity. Biomedicines. 2021 Jan 21;9(2):101.

32. Messner S, Fredriksson L, Lauschke VM, Roessger K, Escher C, Bober M, Kelm JM, Ingelman-Sundberg M, Moritz W. Transcriptomic, Proteomic, and Functional Long-Term Characterization of Multicellular Three-Dimensional Human Liver Microtissues. Appl In Vitro Toxicol. 2018 Mar 1;4(1):1-12.

33. Messner S, Agarkova I, Moritz W, Kelm JM. Multi-cell type human liver microtissues for hepatotoxicity testing. Arch Toxicol. 2013 Jan;87(1):209-13.

34. Postow MA, Sidlow R, Hellmann MD. Immune-Related Adverse Events Associated with Immune Checkpoint Blockade. N Engl J Med. 2018 Jan 11;378(2):158-168.

35. Powers MJ, Domansky K, Kaazempur-Mofrad MR, Kalezi A, Capitano A, Upadhyaya A, Kurzawski P, Wack KE, Stolz DB, Kamm R, Griffith LG. A microfabricated array bioreactor for perfused 3D liver culture. Biotechnol Bioeng. 2002 May 5;78(3):257-69.

36. Puzanov I, Diab A, Abdallah K, Bingham CO 3rd, Brogdon C, Dadu R, Hamad L, Kim S, Lacouture ME, LeBoeuf NR, Lenihan D, Onofrei C, Shannon V, Sharma R, Silk AW, Skondra D, Suarez-Almazor ME, Wang Y, Wiley K, Kaufman HL, Ernstoff MS; Society for Immunotherapy of Cancer Toxicity Management Working Group. Managing toxicities associated with immune checkpoint inhibitors: consensus recommendations from the Society for Immunotherapy of Cancer (SITC) Toxicity Management Working Group. J Immunother Cancer. 2017 Nov 21;5(1):95.

37. Ramos-Casals M, Brahmer JR, Callahan MK, Flores-Chávez A, Keegan N, Khamashta MA, Lambotte O, Mariette X, Prat A, Suárez-Almazor ME. Immune-related adverse events of checkpoint inhibitors. Nat Rev Dis Primers. 2020 May 7;6(1):38.

38. Rodríguez-Antona C, Donato MT, Boobis A, Edwards RJ, Watts PS, Castell JV, Gómez-Lechón MJ. Cytochrome P450 expression in human hepatocytes and hepatoma cell lines: molecular mechanisms that determine lower expression in cultured cells. Xenobiotica. 2002 Jun;32(6):505-20.

39. Rowe C, Gerrard DT, Jenkins R, Berry A, Durkin K, Sundstrom L, Goldring CE, Park BK, Kitteringham NR, Piper Hanley K, Hanley NA. Proteome-wide analyses of human hepatocytes during differentiation and dedifferentiation. Hepatology Volume 58 Issue 2 2013, pages 799-809.

40. Rowe C, Goldring CEP, Kitteringham NR, Jenkins RE, Lane BS, Sanderson C, Elliott V, Platt V, Metcalfe P, Park BK. Network Analysis of Primary Hepatocyte Dedifferentiation Using a Shotgun Proteomics Approach. J. Proteome Res. 2010, 9, 5, 2658–2668.

41. Sarkar U, Rivera-Burgos D, Large EM, Hughes DJ, Ravindra KC, Dyer RL, Ebrahimkhani MR, Wishnok JS, Griffith LG, Tannenbaum SR. Metabolite profiling and pharmacokinetic evaluation of hydrocortisone in a perfused 3D human liver bioreactor. Drug Metabolism and Disposition 2015 April 29.

42. Schyschka L, Sánchez JJ, Wang Z, Burkhardt B, Müller-Vieira U, Zeilinger K, Bachmann A, Nadalin S, Damm G, Nussler AK. Hepatic 3D cultures but not 2D cultures preserve specific transporter activity for acetaminophen-induced hepatotoxicity. Arch Toxicol. 2013 Aug;87(8):1581-93.

43. Serban MA, Prestwich GD. Modular extracellular matrices: solutions for the puzzle. Methods. 2008 May;45(1):93-8.

44. Shlomchik WD. Graft-versus-host disease. Nat Rev Immunol. 2007 May;7(5):340-52.

45. Sison-Young RL, Mitsa D, Jenkins RE, Mottram D, Alexandre E, Richert L, Aerts H, Weaver RJ, Jones RP, Johann E, Hewitt PG, Ingelman-Sundberg M, Goldring CE, Kitteringham NR, Park BK. Comparative Proteomic Characterization of 4 Human Liver-Derived Single Cell Culture Models Reveals Significant Variation in the Capacity for Drug Disposition, Bioactivation, and Detoxication. Toxicol Sci. 2015 Oct;147(2):412-24.

46. Snykers S, De Kock J, Tamara V, Rogiers V. Hepatic differentiation of mesenchymal stem cells: in vitro strategies. Methods Mol Biol. 2011;698:305-14.

47. Takayama K, Inamura M, Kawabata K, Katayama K, Higuchi M, Tashiro K, Nonaka A, Sakurai F, Hayakawa T, Furue MK, Mizuguchi H. Efficient generation of functional hepatocytes from human embryonic stem cells and induced pluripotent stem cells by HNF4α transduction. Mol Ther. 2012 Jan;20(1):127-37.

48. Tarhini AA, Iqbal F. CTLA-4 blockade: therapeutic potential in cancer treatments. Onco Targets Ther. 2010 Jun 24;3:15-25.

49. Tostões RM, Leite SB, Serra M, Jensen J, Björquist P, Carrondo MJ, Brito C, Alves PM. Human liver cell spheroids in extended perfusion bioreactor culture for repeated-dose drug testing. Hepatology. 2012 Apr;55(4):1227-36.

50. Ukairo O, McVay M, Krzyzewski S, Aoyama S, Rose K, Andersen ME, Khetani SR, Lecluyse EL. Bioactivation and toxicity of acetaminophen in a rat hepatocyte micropatterned coculture system. J Biochem Mol Toxicol. 2013 Oct;27(10):471-8.

51. Vorrink SU, Ullah S, Schmidt S, Nandania J, Velagapudi V, Beck O, Ingelman-Sundberg M, Lauschke VM. Endogenous and xenobiotic metabolic stability of primary human hepatocytes

in long-term 3D spheroid cultures revealed by a combination of targeted and untargeted metabolomics. FASEB J. 2017 Jun;31(6):2696-2708.

52. Wang B, Jakus AE, Baptista PM, Soker S, Soto-Gutierrez A, Abecassis MM, Shah RN, Wertheim JA. Functional Maturation of Induced Pluripotent Stem Cell Hepatocytes in Extracellular Matrix-A Comparative Analysis of Bioartificial Liver Microenvironments. Stem Cells Transl Med. 2016 Sep;5(9):1257-67.

53. Wheeler SE, Clark AM, Taylor DP, Young CL, Pillai VC, Stolz DB, Venkataramanan R, Lauffenburger D, Griffith L, Wells A. Spontaneous dormancy of metastatic breast cancer cells in an all human liver microphysiologic system. Br J Cancer. 2014 Dec 9;111(12):2342-50.

54. Wills LR, Rajagopalan P. Advances in Human Induced Pluripotent Stem Cell-Derived Hepatocytes for Use in Toxicity Testing. Ann Biomed Eng. 2020 Mar;48(3):1045-1057.

55. Zitvogel L, Kroemer G. Targeting PD-1/PD-L1 interactions for cancer immunotherapy. Oncoimmunology. 2012 Nov 1;1(8):1223-1225.

Acknowledgement

The authors would like to express their gratitude to the Innovative Medicines Initiative 2 Joint Undertaking (JU) for the financial support of this research under grant agreement No 853988. The JU receives support from the European Union's Horizon 2020 research and innovation programme and EFPIA and JDRF INTERNATIONAL.