



Application of *ex vivo* liver perfusion in hepatoma research

Ben Brown, Wen Yuan Chung, John Isherwood

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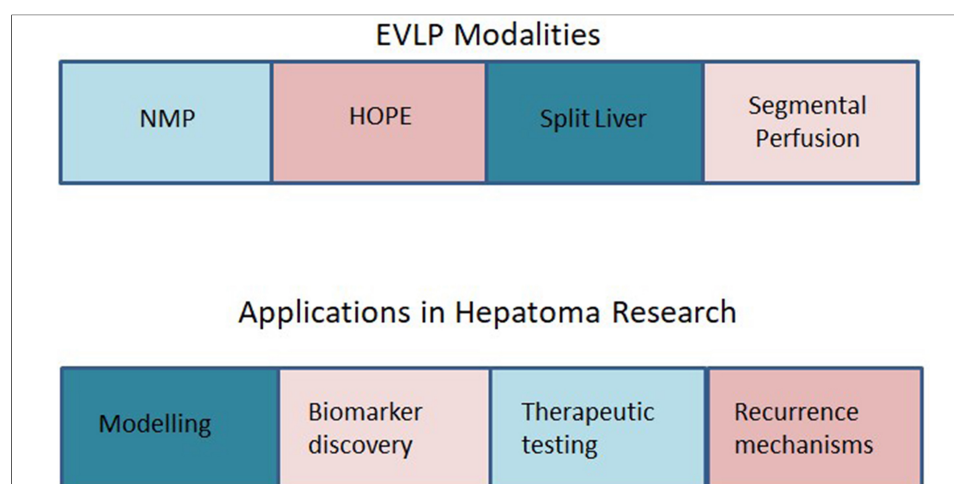
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Abstract

Hepatocellular carcinoma remains a leading cause of cancer-related mortality worldwide. Despite advances in surgical and systemic therapies, recurrence rates remain high, and translational models for therapeutic testing are limited. This review explores the evolving role of *ex vivo* liver perfusion (EVLP) as a translational platform in hepatoma research, highlighting its applications in tumour modelling, therapeutic testing, and biomarker discovery. A narrative synthesis of recent literature was performed, focusing on EVLP modalities such as normothermic machine perfusion, hypothermic oxygenated perfusion, split-liver perfusion, and segmental perfusion of resected tumour-bearing tissue. EVLP preserves hepatic architecture and metabolic function, enabling real-time study of tumour microenvironments, pharmacological responses, and recurrence mechanisms. Segmental perfusion provides an ethically viable translational model. Overall, EVLP represents a transformative tool in hepatobiliary oncology, bridging the gap between *in vitro* models and clinical practice, enhancing mechanistic understanding, and accelerating therapeutic innovation.



Department of Hepato-Pancreato-Biliary Surgery, Glenfield Hospital, University Hospitals of Leicester NHS Trust, Leicester GBR LE3 9QP, United Kingdom.

Correspondence to: Dr. Ben Brown, Department of Hepato-Pancreato-Biliary Surgery, Glenfield Hospital, University Hospitals of Leicester NHS Trust, Leicester GBR LE3 9QP, United Kingdom. E-mail: benjamin.brown23@nhs.net

INTRODUCTION

Management of primary liver cancer (PLC) and metastatic liver disease has attracted increasing attention in recent years due to the high mortality rate of PLC^[1] and the frequent metastasis of colorectal cancer to the liver^[2]. Globally, PLC has the 6th highest incidence and is the 3rd leading cause of cancer deaths among men and women, with approximately 80% of cases being hepatocellular carcinoma (HCC)^[1]. Colorectal cancer ranks 3rd in incidence and 2nd in global mortality^[1], with colorectal liver metastasis (CRLM) developing in the majority of patients with stage IV disease^[3]. Although advancements in neoadjuvant chemotherapy and surgical techniques have improved outcomes for CRLM^[4,5], HCC continues to present significant challenges due to high recurrence rates and treatment resistance. Liver transplantation and resection remain curative options; however, recurrence rates post-transplantation have been estimated at 15%-19% in selected cohorts^[6]. Traditional *in vitro* models fail to replicate the complexity of the tumour microenvironment (TME), while *in vivo* studies are limited by ethical and logistical constraints.

Following the first use of *ex vivo* organ perfusion (EVOP) in 1935^[7], EVOP has mainly been utilised in the field of organ transplantation and, in the context of *ex vivo* liver perfusion (EVLP) with the aim to expand the availability of donor organs^[8]. Alternative areas of research other than transplantation have also been investigated and this review will discuss how EVLP has been developed predominantly for transplantation, but how these modalities are also applicable to Hepatoma research. The use of animal experimentation in EVLP has helped improve our understanding in areas such as disease modelling^[9] and the evaluation of drug efficacy^[10] and metabolism^[11]. While smaller animal models provide more efficient forms of disease modelling and cost effectiveness, porcine models have a similar genome to that of humans as well as offering greater efficiency and practicality during EVLP^[12].

EVLP utilising diseased human livers unsuitable for transplantation has emerged as a translational research platform that maintains tissue viability under near-physiological conditions^[13], thus avoiding ethical concerns associated with animal models.

This review synthesises current applications of EVLP in hepatoma research and outlines future directions for its integration into experimental oncology.

PRINCIPLES AND MODALITIES

EVLP involves the extracorporeal circulation of oxygenated perfusate through the hepatic vasculature. The two main modalities employed are normothermic machine perfusion (NMP) and hypothermic oxygenated perfusion (HOPE). In both approaches, the equipment and tissue typically include the hepatic specimen, the perfusion system, and auxiliary components. Given the liver's dual blood supply, the portal vein (PV) is usually the afferent vessel, with the hepatic artery (HA) incorporated if required, and the hepatic veins serving as the efferent vessels. Once cannulated and connected, blood can be circulated in either pulsatile or continuous flow, depending on the pump type (centrifugal or roller). An oxygenator allows the blood to be re-oxygenated before returning to the liver. To maintain nutrition and metabolic support, the perfusate contains varying concentrations of constituents, such as glucose and vitamins, according to the specific protocol. In addition to controlling flow and pressure, the system temperature can be adjusted to achieve either physiological or hypothermic conditions.

An overview of the different EVLP modalities discussed herein is presented in [Table 1](#).

Normothermic perfusion

By maintaining the system at physiological temperature (37 °C), NMP aims to preserve resected livers in a

Table 1. Comparative overview of ex vivo liver perfusion modalities applied in hepatoma research

Perfusion modality	Temperature	Tissue format	Applications in hepatoma research	Advantages	Limitations	Representative studies
NMP	37 °C	Whole liver Split-liver Segmental	- Drug testing - TME modelling - Biomarker discovery - Gene therapy	- Preserves metabolic activity - Real-time functional assessment	- Complex setup - Higher cost	Nasralla <i>et al.</i> ^[14] Boteon <i>et al.</i> ^[18] Zimmerman and Carter ^[24] Kanani <i>et al.</i> ^[38] Trebo <i>et al.</i> ^[42] Brevini <i>et al.</i> ^[50]
HOPE	4-10 °C	Whole liver	- Recurrence mechanism studies - Transplant oncology outcomes	- Reduces ischemia-reperfusion injury - Enhances graft viability	- Limited metabolic activity - Less suitable for drug testing	Ravaoli <i>et al.</i> ^[26] Schlegel <i>et al.</i> ^[28] Jeddou <i>et al.</i> ^[29] Mueller <i>et al.</i> ^[52]
Split-liver perfusion	Variable	Split lobes from donor liver	- Internal control for comparative studies - Tumour vs. non-tumour analysis	- Enhances experimental rigour - Reduces organ demand	- Requires suitable donor anatomy - Limited availability	Attard <i>et al.</i> ^[34] Lau <i>et al.</i> ^[36] Krendl <i>et al.</i> ^[43] Cabanes-Creus <i>et al.</i> ^[49]
Segmental perfusion	37 °C	Resected tumour-bearing segments	- Ethical translational modelling - TME profiling	- Uses surgical waste tissue - High translational relevance	- Short perfusion duration - Limited tissue volume	Kanani <i>et al.</i> ^[38] Kanani <i>et al.</i> ^[39] An <i>et al.</i> ^[40]

Modalities are differentiated by temperature, tissue format, and translational utility. Segmental perfusion of resected tumour-bearing tissue offers a novel, ethically viable model for therapeutic testing and microenvironment analysis. NMP: Normothermic machine perfusion; TME: tumour microenvironment; HOPE: hypothermic oxygenated perfusion.

near-physiological state. This enables the assessment of metabolic activity and hepatic function, histological changes, pharmacological responses, and immune profiling.

NMP has been shown to result in a lower proportion of graft injury compared to static cold storage (SCS) during transplantation^[14]. The functionality of hepatic tissue can be assessed by monitoring bile production and the synthesis of coagulation factors during NMP^[15]. In addition, Lascaris *et al.* evaluated metabolic activity by measuring concentrations of substrates in the exiting perfusate and collected histological samples after 72 h and 7 days^[16]. These observations revealed mild ischaemic changes but preserved tissue architecture. Longer durations of NMP have also been demonstrated; Cillo *et al.* analysed a liver undergoing NMP for 424 h^[17]. The specimen exhibited minimal cell death on days 13 and 15, and at the end of perfusion. Preserved metabolic and synthetic function was noted, highlighting the feasibility of long-term perfusion of resected livers.

Examples of pharmacological testing during NMP include analyses of lipid metabolism. Steatotic livers have been of particular interest in transplantation research due to the potential to expand the pool of extended criteria donors. This prompted studies investigating lipid metabolism in discarded human livers. Boteon *et al.* assessed the efficacy of a defatting regimen on hepatic triglyceride content^[18]. The defatting group received a combination of agents previously shown to be effective in animal models^[19], together with L-carnitine diluted in dimethylsulfoxide (DMSO), whereas the control group received an equivalent volume of DMSO alone. Over a 6-h period of NMP, the defatting group exhibited a significant reduction in tissue triglycerides compared to controls, reflecting enhanced lipid metabolism.

NMP has also enabled immune profiling of donor livers. Lee *et al.* and Hautz *et al.* demonstrated that cell lineages change during NMP, and the immunological response becomes less pro-inflammatory over time^[20,21]. Further work by Hautz *et al.* aimed to identify genetic patterns predictive of graft viability^[22]. They

found multiple genes that may be useful in determining graft outcomes, including genes such as *CD274* and liver enriched antimicrobial peptide 2 (*LEAP2*). Notably, programmed cell-death ligand 1 (PD-L1), encoded by *CD274*, is not only predictive of graft outcomes but has also been implicated in the prognosis of HCC^[23].

Despite the significant research opportunities offered by NMP, the cost-effectiveness of NMP and hypothermic machine perfusion in the UK remains a crucial issue^[24]. Zimmerman and Carter noted that cost-effectiveness should be re-evaluated once further long-term data on NMP and associated costs become available^[24].

HOPE

HOPE operates at a lower temperature than NMP (usually 4-10 °C). Maspero *et al.* elucidate the pathways involved in ischaemia-reperfusion injury (IRI) and show that HOPE reduces the extent of IRI during transplantation and promotes regenerative mechanisms^[25].

The single-centre randomised controlled trial (RCT) by Ravaioli *et al.* found that HOPE, following a period of SCS, reduced early graft dysfunction and resulted in a lower proportion of complications and re-admissions in the post-operative period compared to SCS alone^[26]. Positive 5-year graft and patient survival outcomes have been reported in the multicentre, observational HOPE-REAL study (NCT05520320)^[27], the largest study to date, with death-censored survival rates exceeding 80%. Schlegel *et al.* conducted a multicentre RCT comparing livers allocated to SCS alone or to SCS followed by HOPE^[28]. While HOPE did not reduce the number of patients developing at least one major complication (defined as Clavien-Dindo score > III), it did reduce the number of patients developing serious liver-related complications (CD > IIb).

A prominent issue highlighted in recent literature is the need for validated markers to assess graft viability during transplantation. Multiple markers and parameters have been investigated for their predictive value during NMP; however, no consensus exists, leading to discrepancies in parameters used to assess graft viability, as discussed by Jeddou *et al.*^[29]. Jeddou *et al.* question whether grafts failing proposed criteria could function adequately if transplanted and advocate for graft scoring systems instead of binary parameters^[29]. Although HOPE occurs at lower temperatures than NMP, reducing metabolism in perfused livers, a recent international observational study by Eden *et al.* demonstrated the potential for developing biomarkers of graft viability^[30]. Flavin mononucleotide (FMN), a mitochondrial complex I cofactor, is released upon mitochondrial damage. Eden *et al.* show that FMN can be detected during dual HOPE (DHOPE, perfusion of both PV and HA) and has predictive value for graft viability^[30]. As with NMP, no consensus exists on the optimal FMN levels to assess perfusate or judge graft viability.

The efficacy of NMP and HOPE has been established in comparison to SCS; however, few studies have directly compared NMP and HOPE. Lurje *et al.* demonstrated that immune cell populations differ between the two modalities, with smaller populations of leucocytes and granulocytes observed in the effluent of HOPE-treated livers^[31]. They also found that PD-L1 expression on the cell surface differed depending on whether NMP or HOPE was utilised. The clinical relevance of this finding remains unclear, and further studies are required to assess its significance.

Split-liver perfusion and segmental perfusion

Following advancements with NMP and HOPE in transplantation, innovations such as split-liver and segmental perfusion using resected tumour-bearing livers have expanded the translational utility of EVLP, enabling ethical and reproducible experimentation on human livers.

Split-liver perfusion involves separating the two physiological lobes of the liver, which can then undergo machine perfusion. The advantages and limitations of NMP and HOPE in split-liver perfusion have been discussed, with themes similar to those observed in whole-liver perfusion^[32,33]. Beyond transplantation, improvements in split-liver perfusion protocols may facilitate more in-depth preclinical testing of therapeutics, as the lobes exhibit near-equal metabolism and functionality^[34] and can maintain long-term perfusion over multiple days^[35]. Lau *et al.* further developed long-term split-liver perfusion, reporting two livers declined for transplantation that were split and maintained on NMP for over 12 days^[36]. Throughout this period, metabolic and functional parameters remained stable, and tissue architecture was preserved. However, bacterial cultures of the perfusate were positive at 306 h, despite being negative at 96 h, suggesting that protocol optimisation is required to extend the safe duration of these studies^[37].

Segmental liver perfusion is an exciting new area for research opportunities. Kanani *et al.* have demonstrated viability of hepatic cells in segmented diseased livers undergoing NMP^[38]. Nine livers were resected due to HCC or colorectal liver metastases, with healthy segments being taken from each of the segments to be perfused. There was a comparison made between the perfusion of expired O negative red blood cells (RBCs) and Oxyglobin/Volplex. Following 6 h of NMP, 4 of 5 segments perfused with the oxyglobin solution demonstrated intact histology compared to 1 of 4 segments perfused with expired RBCs. Though the sample size used in this study was small, it highlights an exciting opportunity for further preclinical research and the development of longer-term models similar to those of whole or split-liver perfusion.

The potential use of segmental perfusion in the context of disease modelling is also evidenced by Kanani *et al.*^[39], who used concepts from previous animal studies (An *et al.*^[40]) and evaluated comparative data in human tissue. Experimentation in animal models has shown that Kupffer cells play a key role during *Escherichia coli* (*E. coli*) and *Streptococcus pneumoniae* (*S. pneumoniae*) infection^[40]. An *et al.* provide evidence that different virulence strains of these bacteria can avoid these mechanisms in murine models^[40]. However, when applied in human segmental-liver models, Kanani *et al.* did not find that *S. pneumoniae* was eliminated based on virulence strain^[39]. This is a clear example of the translational issues from animal to human models, in keeping with ongoing debates over the efficacy of animal testing in the context of clinical research^[41].

APPLICATIONS IN HEPATOMA RESEARCH

TME modelling

Trebo *et al.* have conducted an impressive study utilising EVLP to model CRLM^[42]. Six livers obtained from patients undergoing either resection or transplantation due to CRLM underwent NMP. Throughout the study (median duration of NMP was 41 h, with maximum duration of 144 h), tissue architecture of the livers was sustained as were the metabolic/functional activities of both healthy and tumoral tissue. Though not directly examined, implications of the constant perfusion pressure/flow during NMP imply that the vascular integrity of the specimens, on a macro/microscopic level, is maintained. An interesting area of further research would be to directly assess microvascular integrity during NMP. The population of immune cell-types present varied greatly between livers, with most of this variability arising not from healthy tissue, but from the cancerous environment. Trebo *et al.* also showed how immunosuppressive cell types play a role in the maintenance of the TME by creating a hypoxic and fibrotic environment in which the tumour cells can reside^[42].

In the study by Trebo *et al.*, assessment of tumour and healthy liver tissue was performed^[42]. A slight, but important alternate view is shown by Krendl *et al.*, in which 2 different pathologies were examined within the same liver^[43]. By analysing a resected cirrhotic liver with HCC *in situ*, they were able to maintain NMP for 96 h, finding similar tissue viabilities and histological preservation in both tumour and cirrhotic biopsies.

Though not directly assessing the TME, Krendl *et al.* explain how their study allows assessment of two different pathologies within the same specimen^[43]. This hopefully will aid in the development of more in-depth studies of the TME of HCC, the *ex vivo* study of mechanisms involved in cirrhosis and the mechanisms leading to development of HCC in cirrhotic livers.

As an aside, given the difficulties in translational application of animal studies, microphysiological systems (MPSs) have been developed to attempt to bridge the gap of studying *ex vivo* models with near *in vivo* conditions at a cellular level. These MPSs use human cells to develop a micro-environment similar to that of *in vivo* situations, as discussed by Clark *et al.*^[44]. This paper discusses the different MPSs available and how they provide an opportunity to study the beginnings of tumour progression/metastasis in the controlled micro-environment. Though MPSs hold promise in the establishment of early metastatic models, only one validated MPS exists for investigating metastases, and similar problems to those of EVLP remain, such as the translation of findings to *in vivo* conditions over longer periods.

Therapeutic testing, pharmacological profiling and genetic testing

There were no studies examining the role of oncological treatments during EVLP in this narrative review search. This is clearly an area of great potential going forward with future studies. Smaller-scale models have been able to culture cancerous hepatic tissue and interrogate them with anti-cancer therapies. Precision-cut tissue slicing (PCTS) allows the use of resected HCC livers for testing of immunotherapy and chemotherapy agents. After developing the cultured specimens, Jagatia *et al.* administered doxorubicin (a chemotherapeutic drug) and multiple immunotherapeutic drugs, including atezolizumab with bevacizumab (a combination often used for HCC treatment) to the PCTS^[45]. Results were monitored over 8 days showing changes in the degree of proliferation and viability of tumour cells. Given the evidence for NMP and HOPE in the maintained viability of resected tissue over several days, there are numerous exciting avenues for further investigations. One avenue would obviously be the testing of novel chemotherapy/immunotherapy drugs in HCC/CRLM livers. Once administered, specimens could then be taken at different time points for analysis. With the use of split-liver perfusion, one lobe could be exposed to this drug as the experimental wing and be compared to the control lobe, aiming at minimising heterogeneity between livers. This would allow assessment of drug efficacy, the pharmacodynamics/kinetics of the compound, and potential resistance mechanisms. Given the advancements in prolonged EVLP (as previously discussed in this review), monitoring could be carried out over a longer term than that seen in smaller models, such as that reported by Jagatia *et al.*^[45]. A second interesting area of further study would be to subject tumour preclinical locoregional therapy such as embolisation and ablative techniques.

EVLP has been used in the development of therapeutics aimed at reducing IRI. Nanoceria (NC) is a nanoparticle (NP) that acts as an antioxidant. Del Turco *et al.* administered NC to human livers with an endpoint objective to assess telomere attrition and mitochondrial DNA deletion (markers of DNA damage) compared to controls^[46]. Liver used in this study underwent NMP, with five livers receiving NC and 4 controls receiving NMP alone. Del Turco *et al.* found that mitochondrial DNA deletion was significantly reduced in the experimental arm^[46]. On the other hand, telomere attrition was not shown to be significantly reduced. Preclinical animal studies have produced encouraging findings in potential uses of NC discussed by Tang *et al.*, but there is still limited evidence of NC use in humans^[47]. Again, there is an exciting opportunity for further research into the role of NC in the context of HCC, given the link between IRI and HCC recurrence post-transplant^[25].

In their review, Liu *et al.* discuss the current status of gene therapies to treat disease, including several ongoing clinical trials assessing the efficacy of gene-editing therapies^[48]. In the context of EVLP and gene therapy, Cabanes-Creus *et al.* provide a proof-of-concept study utilising two discarded human livers

commenced on NMP^[49]. Following this, the livers were split and maintained on split-liver NMP, allowing comparison between control and experimental lobes. Multiple adeno-associated viral (AAV) vector variants were used during the study. To allow further comparisons, one of the livers used was perfused with anti-AAV antibodies, whilst the other was perfused with plasma containing low anti-AAV antibody levels. Cabanes-Creus *et al.* demonstrated that in the presence of circulating anti-AAV antibodies, the variant AAV8 was shown to have the highest functionality of those investigated based on vector clearance from perfusate, vector uptake and gene expression^[49]. Further work utilising AAV8 has been carried out by Brevini *et al.*^[50]. Here they use split-liver NMP on a liver resected from a patient with mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) for 8 days. MNGIE is characterised by mutations to the gene encoding thymidine phosphorylase (TP, *TYMP*). This leads to the development of nucleoside accumulation. By splitting the liver, the left lobe was the experimental lobe receiving AAV8-*TYMP*, whilst the right lobe was maintained as the control. AAV8-*TYMP* vector expression was noted from day 2 to day 5, after which expression plateaued. The production of TP in the experimental lobe using immunofluorescence was noted after 3 days, with enzyme functionality confirmed with enzymatic activity assays. Clear reductions in nucleosides were noted from day 6 in the experimental lobe, with lack of clearance from the control lobe.

As yet, EVLP has not been routinely used for pharmacological profiling, but its potential for drug profiling has been demonstrated in a pilot study by Tingle *et al.*^[51]. In this study, the drug 2,4-dinitrophenol (DNP) was administered to steatotic livers deemed unsuitable for transplantation and maintained using NMP. DNP is an oxidative phosphorylation uncoupler that was previously used as a weight-loss agent. A key manifestation of DNP toxicity is hyperthermia. Tingle *et al.* show that liver hyperthermia was transient and that the increase in oxygen consumption was dependent on DNP concentration^[51]. Although this was a small study, it highlights the potential of EVLP for pharmacokinetic assessment.

Investigating recurrence mechanisms post-transplant

As previously discussed, recurrence rates of HCC can lie between 15%-19% in selected cohorts^[6]. The association between IRI and tumour progression has led to multiple studies investigating whether the use of HOPE during liver transplantation has a protective effect in HCC recurrence. Mueller *et al.* compared the outcomes of livers transplanted having received HOPE perfusion, or not perfused at centre A^[52]. Centre A included 70 livers that received HOPE following donation after circulatory death (DCD) and 70 unperfused livers following donation after brainstem death (DBD). In Centre B, outcomes of 70 unperfused DCD livers and 70 unperfused DBD livers were compared. In Centre A, unperfused livers were four times more likely to develop recurrence than HOPE-perfused livers. Patients receiving HOPE-perfused livers had a higher 5-year recurrence-free survival rate. Comparisons were also made between HOPE-perfused livers in Centre A and the unperfused DCD cohort in Centre B. Mueller *et al.* further demonstrated that unperfused DCD livers had a twofold higher recurrence rate than HOPE-perfused DCD livers^[52].

Similarly favourable outcomes with HOPE have been reported by Dajti *et al.*^[53]. In this retrospective cohort study, 60 livers underwent HOPE perfusion, whereas 177 received SCS. The risk of recurrence was lower and recurrence-free survival higher in HOPE-treated livers. In contrast, Rigo *et al.* observed no significant difference in recurrence rates between 80 DHOPE-treated livers (66 DBD, 14 DCD) and 246 SCS-treated livers^[54]. These conflicting findings underscore the need for further studies to clarify the potential benefits of HOPE in reducing HCC recurrence.

The results of the HOPE4CANCER trial (NCT06717919) are eagerly anticipated. This ongoing, multicentre international RCT compares HOPE with SCS^[55]. Livers in the study are allocated 1:1 to either cold storage alone or HOPE following cold storage, with recurrence-free survival as the primary endpoint.

Maspero *et al.* provide a review of HCC recurrence and IRI^[25]. They discuss how recurrence following transplantation can be attributed to three main processes: (1) systemic release of cancer cells during surgery due to mechanical pressures experienced by the diseased liver; (2) mitochondrial damage during surgery leading to dysfunction and IRI; and (3) activation of innate hepatic repair mechanisms that may promote tumour recurrence.

Biomarker discovery and omics integration

EVLP enables serial sampling of perfusate and tissue, supporting identification of circulating tumour markers, transcriptomic and proteomic profiling, and metabolomic analysis. Integration with multi-omics platforms enhances mechanistic insights and supports precision oncology.

Hautz *et al.* use transcriptomic analysis to develop a predictive signature of graft quality^[22]. During this analysis, they demonstrated alterations in gene expression, signalling pathways, and the dynamics of immune-parenchymal cell interactions. Although not directly related to transcriptomic analysis of HCC or CRLM, these findings demonstrate how NMP can be applied to transcriptomic profiling. In their comprehensive modelling of CRLM, Trebo *et al.* provide strong evidence of the interplay between multiple signalling pathways, diverse cellular phenotypes, and altered gene expression within the TME, and demonstrate that these features were preserved during NMP^[42]. For example, C-X-C chemokine receptor type 4 (CXCR4) signalling, which is known to be involved in the migration of immunosuppressive cells and in various malignancies^[56], showed increased activity within the TME.

Currently, radiological investigations [ultrasound scan (USS) and CT] and non-specific laboratory investigations [e.g., the serum protein alpha fetoprotein (AFP)] are used for the diagnosis and surveillance of HCC^[57]. Although these investigations are useful, their low specificity and operational dependence limit their effectiveness, driving interest in the development of biomarkers and validated risk scores for HCC^[58-60]. Cillo *et al.* demonstrated that carcinoembryonic antigen (CEA), primarily used in colorectal cancer diagnosis, can be detected and quantified during NMP, with perfusate levels similar to those measured pre-transplantation^[17]. A group of novel biomarkers identified with respect to CRLM are microRNAs (miRNAs). The narrative review by Barjasteh *et al.* describes multiple functions of miRNAs, including the promotion of angiogenesis/metastasis (miRNA-25-3p), inhibition of cell migration (miRNA-432-5p), and diagnostic potential (miRNA-21), and also reports how altered expression of these miRNAs occurs in various neoplastic processes^[61]. HCC also exhibits altered miRNA expression, with miRNA-122 (inhibiting angiogenesis, among other functions) down-regulated and miRNA-224 (promoting cell proliferation and migration) up-regulated, as shown by Amr *et al.*^[62]. Using resected or transplanted livers from CRLM and HCC patients under EVLP, miRNAs could be profiled and compared, with promising candidates identified for further investigation.

Though the studies discussed in this review show promising results, they involve varying, but generally small, sample sizes. As EVLP is increasingly used in clinical practice, results from future studies will provide stronger evidence and enable more targeted therapeutic studies using discarded human livers.

The long-term perfusion capabilities of EVLP, its ability to support multi-omic profiling, and the ongoing interest in sensitive and specific biomarkers provide promising avenues for future research. For example, a steatotic liver unsuitable for transplantation could be split and perfused. The experimental lobe could be exposed to an oncogenic stimulus, and the multi-omic profiles of the experimental and control lobes could be compared to identify novel biomarkers, uncover potential therapeutic targets, and further elucidate HCC pathophysiology in a human model.

ETHICAL BENEFITS OF EVLP

Historically, animal studies have been used to support the progression from preclinical studies to clinical trials and to improve understanding of disease concepts^[63]. The 3Rs principle (replace, reduce, and refine) has been developed to optimise animal welfare and should be followed throughout animal experimentation. This principle is supported by international bodies, such as the European Medicines Agency (EMA)^[64]. Although animal models have provided valuable insights into disease fundamentals^[63], challenges remain in translating findings to humans and reproducing results in clinical trials^[65]. These limitations have driven the development of alternatives, including in silico modelling and tissue engineering^[66].

Although still relatively new, EVLP enables real-time study of human tissue obtained from patients undergoing resection or transplantation. This approach mitigates the ethical concerns associated with animal testing and overcomes the translational limitations inherent in animal models.

CONCLUSION

EVLP represents a transformative platform in hepatoma research. Its ability to preserve human liver tissue both functionally and structurally enables mechanistic studies, therapeutic development, and biomarker discovery. Segmental perfusion of resected tumour-bearing tissue provides an ethically viable model with strong translational relevance. As protocols mature and multi-omics integration progresses, EVLP is poised to redefine experimental standards in hepatobiliary oncology.

DECLARATIONS

Authors' contribution

Data curation, writing - original draft: Brown B

Conceptualisation, methodology, supervision, review: Chung WY, Isherwood J

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Consent for publication

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