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Design, Analysis, and Pitfalls of Clinical Trials Using Ex Situ Liver Machine Perfusion: The International Liver Transplantation Society Consensus Guidelines

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Design, Analysis, and Pitfalls of Clinical Trials Using Ex Situ Liver Machine Perfusion: The International Liver Transplantation Society Consensus Guidelines

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Background. Recent trials in liver machine perfusion (MP) have revealed unique challenges beyond those seen in most clinical studies. Correct trial design and interpretation of data are essential to avoid drawing conclusions that may compromise patient safety and increase costs. **Methods.** The International Liver Transplantation Society, through the Special Interest Group "DCD, Preservation and Machine Perfusion," established a working group to write consensus statements and guidelines on how future clinical trials in liver perfusion should be designed, with particular focus on relevant clinical endpoints and how different techniques of liver perfusion should be compared. Protocols, abstracts, and full published papers of clinical trials using liver MP were reviewed. The use of a simplified Grading of Recommendations Assessment, Development, and Evaluation working group (GRADE) system was attempted to assess the level of evidence. The working group presented its conclusions at the International Liver Transplantation Society consensus conference "DCD, Liver Preservation, and Machine Perfusion" held in Venice, Italy, on January 31, 2020. **Results.** Twelve recommendations were proposed with the main conclusions that clinical trials investigating the effect of MP in liver transplantation should (1) make the protocol publicly available before the start of the trial, (2) be adequately powered, and (3) carefully consider timing of randomization in function of the primary outcome. **Conclusions.** There are issues with using accepted primary outcomes of liver transplantation trials in the context of MP trials, and no ideal endpoint could be defined by the working group. The setup of an international registry was considered vital by the working group.

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INTRODUCTION

Machine perfusion (MP) preservation has been 1 of the most promising concepts in liver transplantation in the last 20 years.[1-19] Following extensive preclinical work,[20] liver MP entered the clinical arena a decade ago. To date, very few clinical trials have been published and the superiority of liver MP as a preservation method versus static cold storage is not yet established. Clinical trials investigating liver MP pose challenges beyond those of most clinical studies. Optimal trial design and interpretation of data may avoid incorrect conclusions that compromise patient safety, increase costs, and delay advancement of the science in the field.[21-31]

The International Liver Transplantation Society (ILTS) through the Special Interest Group (SIG) "DCD, Preservation and Machine Perfusion" established a working group to discuss the relevant literature and establish consensus statements and suggestions regarding how future clinical trials in liver perfusion should be designed, with particular focus on relevant clinical endpoints and

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how different techniques of liver perfusion should be compared. The Working Group presented the discussion at the ILTS consensus conference "DCD, Liver Preservation, and Machine Perfusion" consensus conference held in Venice, Italy, on January 31, 2020. This article describes the process followed by the Working Group and summarizes the discussion, recommendations, and guidelines it established.

METHODOLOGY

Early in 2019, the recently created ILTS SIG "DCD, Preservation and Machine Perfusion" received the task from the ILTS to establish a working group to discuss the relevant literature on "Clinical trials design in MP" and to write consensus statements and guidelines and assess the level of evidence. The ILTS and SIG "DCD, Preservation, and Machine Perfusion" leaderships selected a group of 7 ILTS members (all authors of this article). They were approached by the steering committee of the SIG and chosen based on their previous experience with MP experience and geographic distribution. All, except 1 (Rajiv Jalan-hepatologist), are transplant surgeons.

The working group was asked to consider the following questions regarding the design of clinical trials assessing liver MP:

1. Which preservation techniques should be compared in the next randomized trials?
2. What are clinically relevant trial endpoints?
3. Which grafts should be included?
4. Update on clinical trials

The expectation was to rate the level of evidence based on the Grading of Recommendations Assessment, Development and Evaluation working group (GRADE) system (Table 1), classifying it as strong, conditional, or not recommended (class 1–3), according to the level of evidence (level A to C), balance between patient benefit and harm, significance to patients, and cost-effectiveness http://www.gradeworkinggroup.org)[32] (Table 1).

The working group members identified published clinical trials investigating liver MP by using a PubMed search using keywords: liver MP, clinical trial, machine preservation, and searching open source platforms for trial registries (clinicaltrials.gov, EudraCT, ChiCTR). We also included metanalysis and cross-references from those articles.[33-35] These were shared via a cloud platform and discussed via email and 2 conference calls in the months preceding the final meeting in Venice, Italy. The results were presented to the delegates of the ILTS "DCD, Liver Preservation, and Machine Perfusion" consensus conference held in Venice, Italy, on January 31, 2020. The presentation is available for ILTS members online (at https://ilts.org/education/lectures/machine-perfusion-and-clinical-trials-session-special-considerations-and-pitfalls-in-clinical-trials-using-machine-perfusion/)

The ILTS invited 36 faculty that are experts in the field of DCD liver transplantation and MP transplantation (for a complete list and biography of invited faculty, please refer to https://s3.amazonaws.com/wp-ilts-media/wp-content/uploads/2020/01/29161208/02-Final-ILTS-Venice-2020-Meet-The-Faculty.pdf). The meeting was attended by 151 delegates from 25 countries.

After receiving feedback from the audience, a meeting was held with input from our working group (authors) and 15 delegates of different institutions, who voluntarily participated in this discussion group (list under acknowledgments). Data were discussed again in detail, and we established our consensus statements, level of evidence, and future recommendation guidelines.

After the consensus meeting, we discussed the article drafting through emails, edited using a cloud platform, and the final version was approved by all authors, the SIG, and ILTS leadership.

CHALLENGES IN LIVER MACHINE PERFUSION CLINICAL TRIAL DESIGN

Power and Primary Endpoints

It is very important when designing clinical trials to choose the appropriate primary endpoints.[21,23,30,36-38] The choice of endpoint can have a significant bearing on the study conclusions.[39-42] The primary endpoint needs to be clinically meaningful, and 1 should realize that a randomized controlled trial (RCT) can only be powered on 1 primary endpoint. Secondary endpoints are often defined as well, although the sample size is often too small for the analyses of the secondary endpoints to reach sufficient power. To reduce the potential for selective posttrial reporting and multiple testing, pretrial objective definition and reporting (eg, ClinicalTrials.gov) of the primary endpoint for which RCT is designed are strongly recommended.[25,28] The sample size calculation for an RCT is based on the

TABLE 1.

Simplified grading system of clinical evidence according to the GRADE system (http://www.gradeworkinggroup.org)

Level of evidence[a]		Confidence in the evidence
High	Data derived from meta-analyses or systematic reviews or from (multiple) RCTs with high quality	Further research is unlikely to change our confidence in the estimate of benefit and risk
Moderate	Data derived from a single RCT or multiple nonrandomized studies	Further research (if performed) is likely to have an impact on our confidence in the estimate of benefit and risk and may change the estimate
Low	Small studies, retrospective observational studies, registries	Any estimate of effect is uncertain
Grade of recommendation[b] (wording associated with the grade of recommendation)		
Strong	"Must," "should," or "ILTS recommends"	
Weak	Can," "may," or "ILTS suggests	

According to Guyatt GH et al.[32]
[a]Level was downgraded if there was poor quality, strong bias or inconsistency between studies; level was upgraded if there was a large effect size.
[b]Recommendations were reached by consensus of the panel and included the quality of evidence, presumed patient-important outcomes and costs.
ILTS, International Liver Transplantation Society; RCT, randomized controlled trial.

primary endpoint and includes a number of assumptions. The sample size calculation is essential to make sure that a statistically significant and clinically relevant difference can be detected with a high probability.

Trials in transplantation are particularly challenged by the difficulty to power studies for conventional "hard" endpoints such as graft loss and patient death in the first year because these events are uncommon, requiring very large numbers of patients.[21,38]

One way to overcome such a limitation is to focus the trial on a subgroup of subjects that are at higher risk to develop the event. Indeed, as the safety of liver MP is becoming established, it is now possible to design clinical trials that use extended-criteria grafts (DCD, older donors, steatotic grafts).[43,44] As these grafts have higher overall complication rates, with increased incidences of graft loss, ischemic type biliary injury (ITBL), primary nonfunction (PNF), or death in the first year, the sample size needed to show a clinically meaningful difference would be smaller than for trials including all donor types. There are important caveats to such an approach. There is no universal definition of extended-criteria donors. In addition, there are often concerns that trial participants are not a representative sample of the whole population because of stringent inclusion and exclusion criteria. External validation of findings also implies that the findings of a study will be applicable across the intended populations. The ability to make reliable statements about a broad population usually considers that the study groups represent a random sample from the population and comparisons of study arms assume that subjects are equally likely to be included in either arm.[44] Speich et al showed that in surgical RCTs, sample size calculation was only adequately reported in 53% of the cases.[31]

Trials in transplantation are often limited to the use of intermediate endpoints based on time and resource constraints unless intermediate endpoints have been validated and have independent clinical advantage (eg, improved graft function, fewer complications, lower cost); caution must be exercised in extrapolating results to an important long-term clinical finding (eg, graft and patient survival, biliary complications).[21,23,37,38]

Surrogate Endpoints (Laboratory Biomarkers)

A surrogate endpoint has been defined as "a biomarker that is intended to substitute for a clinical endpoint and generally is considered valid given a more rapid and frequent incidence and strong association with traditional endpoints."[37] The use of parameters more likely classified as intermediate endpoints, defined as a characteristic that is intermediate in the causal pathway between an intervention and the clinical endpoint, have become common substitutes for true surrogates. The primary limitation of intermediate endpoints is that they may not be predictive of the most important clinical endpoints (eg, graft loss).[21,37] To find statistical significance in a laboratory parameter without clear clinical significance may be meaningless.

Many surrogate markers of liver graft viability and injury have been utilized, however whether they are adequate predictors of long-term graft outcomes remains a topic of debate. None of them has been strongly validated in the clinical setting.[46,47] The ideal biomarker would be specific, easily processed and inexpensive with a quick "turn around" time that could be available before transplantation.[48] It would also have to predict long-term clinically relevant outcomes with a high degree of precision. Unfortunately, in MP trials, no single parameter (or combination of parameters) has been clearly established that meets strong criteria as a surrogate endpoint.[46-48] Additionally, MP introduces many variables that may affect intra- and postoperative parameters. For example, size of the liver, volume of perfusate, and temperature of perfusion may all impact on machine and even postreperfusion transaminases levels.

Composite Endpoints

To decrease the need of large sample size and to increase trial efficiencies in transplantation, a common strategy is the utilization of composite endpoints, which typically consist of selective adverse events, patient deaths, and graft losses. It has been suggested the use of the "comprehensive complication index" as primary endpoint, which is currently often used in surgery and transplantation with the availability of reference values provided in a recent multicenter benchmark study covering 1 year after transplantation.[36,43] Biochemical composite endpoints have been used in most MP trials as EAD scores. Clinical composite endpoints have already been used in a lung MP preservation trial.[49]

One limitation of these endpoints is the presumption of equivalent severity of individual outcomes. Trials utilizing composite endpoints should report distinct event rates for each component, but the interpretation of results should not extend to individual outcomes.

RESULTS

Summary of Clinical Trials

We analyzed the literature on clinical trials using liver MP (Tables 2 and 3). The majority of study protocols had been made public in advance in an open access registry of clinical studies (clinicaltrials.gov, EudraCT, ChiCTR). Most published studies were single center and had a small sample size and therefore likely underpowered. Several studies did not provide detailed description of the study, and nomenclature was not uniform. Only 2 papers were randomized and both used NMP.[50,51] A number of ongoing randomized studies had not been completed or published at the time this article was prepared. Follow-up was short (all these studies had an overall median follow-up <1 y).

In addition to the published NMP clinical trials, there are currently at least 10 ongoing clinical trials in clinicaltrials.gov and others in national registries. In addition to the published HMP clinical trials, there are at least 9 ongoing clinical trials (Tables 2 and 3).

Regarding the GRADE system classification of clinical evidence, our group agreed that the level of evidence for all questions is generally low.

1. Does machine preservation provide better outcomes compared with standard cold static preservation?

We were not able to reliably and systematically answer this question based on GRADE system because this would require much more complexes analysis of all complications and outcomes. There are only 2 published RCTs in NMP of the liver, both of which suggest positive evidence,

TABLE 2.
Clinical trials on ex situ liver hypothermic machine perfusion

Author	Y	Donor type (DCD/DBD)	No. total (HMP/SCS)	Perfusion characteristics	Perfusate	Total time of preservation (min) (range)	Endpoints	Outcome (HMP vs SCS)
Trial name: HOPE with cytokine filtration in liver transplantation (Cyto-HOPE) NCT04203004 PI: Stefania Camagni, Bergamo, Italy	Estimated completion 2022	Not reported	20 (20/0)	Device not reported. HA <30 mm Hg/PV <5 mm Hg. Time on machine: 4 h	UW-MPS	Results awaited	Primary: Incidence of PRS Secondary: Entity of IRI, incidence of EAD	Results awaited
Trial name: HOPE for extended criteria donors in liver transplantation (HOPEext) NCT03929523 PI: Mickael Lesurtel, Lyon, France	Estimated completion date 2022	DBD	266 (133/133)	Device: Liver assist. PV only. Time on machine: 1–4 h.	UW-MPS	Results awaited	Primary: Incidence of EAD Secondary: MEAF score, L-GrAFT, metabolic profiling, PRS. 90-d morbidity/mortality, length of hospital stay, MCRP within 1 y, 3-mo/1-y graft/patient survival, hospital costs	Results awaited
Trial name: Clinical trial of new HOPE system vs SCS NCT03837197 PI: Matteo Ravaioli, Bologna, Italy	Estimated completion date 2021	DBD	110	Device not reported. Oxygenated (500–600 mm Hg).	UW-MPS	Results awaited	Primary: incidence of EAD Secondary: surgical complications, liver function at 6/12 mo, patient survival at 6/12 mo	Results awaited
Trial name: Post-SCS HOPE in Bergamo Liver Transplant Program NCT03098043 PI: Stefania Camagni, Bergamo, Italy	Estimated completion date 2021	DCD/DBD	20	Device not reported. HA 25-30 mm Hg/PV <5 mm Hg. Time on machine: 1 h. Oxygenated (50–70 kPa)	UW-MPS	Results awaited	Primary: incidence of EAD Secondary: Dindo-Clavien complications, ischemic cholangiopathy, length of hospital stay, 30-d/1-y graft/patient survival,	Results awaited
Trial name: Study to evaluate performance of LifePort liver transporter system, a machine perfusion system, for liver transplant (PILOT) NCT03484455 Organ recovery systems	Estimated completion date 2021	Not reported	140	Device: LifePort liver transporter.	Vasosol	Results awaited	Primary: incidence of EAD	Results awaited
Trial name: DHOPE of DCD liver grafts in preventing biliary complications after transplantation (DHOPE-DCD) NCT02584283 PI: Robert Porte, Groningen, The Netherlands	Completion date: 2019	DCD	156 (78/78)	Device: Liver Assist HA 25 mm Hg/PV 5 mm Hg. 0.5 mL/min 100% O_2 Time on machine: 2 h	UW-MPS	Results awaited	Primary: incidence of NAS at 6 mo Secondary: graft/patient survival, PNF, IPF, recipient hemodynamics during LT, hospital length of stay, postoperative complications, liver function and injury markers, costs of treatment, quality of life.	Results awaited

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TABLE 2. (Continued)

Author	Y	Donor type (DCD/DBD)	No. total (HMP/SCS)	Perfusion characteristics	Perfusate	Total time of preservation (min) (range)	Endpoints	Outcome (HMP vs SCS)
Trial name: HOPE for Human ECD and DBD Liver Allografts (HOPE-ECD-DBD) NCT03124641 PI: Georg Lurje (Aachen, Germany)	Completion date: 2019	DBD	46 (23/23)	Device: Liver Assist PV <3 mmHg. Oxygenated (150-200 mmHg) Time on machine: 1 h	IGL-1	Results awaited	Primary: postoperative peak ALT in the first postoperative wk. Secondary: Dindo/Clavien classification, hospital- and ICU stay, IRI, 1-y patient/graft survival	Results awaited
Trial name: Interest of oxygenated hypothermix perfusion in preservation of hepatic grafts from ECD (PERPHO) NCT03376074 Renes University Hospital	Completion date: 2019	DBD	25 (25/0)	Device not reported. PV <3 mm Hg. Oxygenated (40 kPa) Time on machine: 2 h	UW-MPS	Results awaited	Primary: incidence of PNF/EAD. Secondary: nr of intraoperative transfusions, PRS, morbidity on d 7, graft survival at 3 mo, hospital length of stay, cost of initial stay, cost of the hospitalization stay	Results awaited
Trial name: HOPE vs SCS for Margina Graft (PIO) NCT03031067 PI: Matteo Ravaioli, Bologna, Italy	Completion date: 2018	DBD	10 (10/0)	Device: Exiper, Bologna machine perfusion oxygenated (80–100 kPa) Time on machine: 2 h	Not reported	Results awaited	Primary: graft function at 3 mo Secondary: graft/patient survival at 3 mo	Results awaited
Van Rijn et al[108]	2017	HMP 10/0 SCS 20/0	30 (10/20)	Device: LiverAssist HA 20–30 mm Hg/PV 5 mm Hg. 500 mL/min 100% O_2 Time on machine: 126 min (123–135)	UW-MPS	HMP: 521 (469–592) SCS: 503 (476–526)	Primary: graft survival at 6 mo. Secondary: 1-y graft/patient survival, technical safety, perfusate microbiology, postoperative complications	100% vs 80% 6-mo graft survival, 100% vs 67% 1-y graftt survival. 100% vs 85% 1-y patients survival. No technical problems. Peak ALT (IU/L): 966 vs 1858. NAS: 0/10 vs 5/20.
Dutkowski et al[109]	2015	HMP 25/0 SCS 50/0	75 (25/50)	Device: ECOPS device PV 120-180 mL/min Oxygenated time on machine: 118 min (101–149)	KPS-1	HMP: 317 (280–391) SCS: 395 (349–447)	Primary: incidence and severity of biliary complications within 1 y after LT. Secondary: liver IRI and function, graft survival	Ischemic cholangiopathy: 0% vs 22%. Biliary complications: 20% vs 46%. Peak ALT (IU/L): 1239 vs 2065. 90% vs 69% 1-y graft survival.
Guarrera et al[110]	2015	HMP 0/31 SCS 0/30	61 (31/30)	Device: Medtronic PBS 0.667 mL/g/liver/min No active oxygenation Time on machine: 258 ± 54 min	Vasosol	HMP: 564 ± 96 SCS: 534 ± 144	Primary: incidence of PNF, EAD and vascular complications, 1-y graft/patient survival. Secondary: incidence of biliary complications, AKI, hospital length of stay, liver/kidney function markers	PNF: 3% vs 7%. EAD: 19% vs 30%. Vascular complications: 9% vs 7%. 84% vs 80% 1-y patient survival. Biliary complications: 4/31 vs 13/30/ AKI: 10% vs 27%. Hospital stay: 13.6 vs 20.1 d.

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TABLE 2. (Continued)

Author	Y	Donor type (DCD/DBD)	No. total (HMP/SCS)	Perfusion characteristics	Perfusate	Total time of preservation (min) (range)	Endpoints	Outcome (HMP vs SCS)
Guarrera et al[111]	2010	HMP 0/20 SCS 0/20	40 (20/20)	Device: Medtronic PBS 0.667 mL/g/liver/min No active oxygenation Time on machine: 228 ± 54 min	Vasosol	HMP: 558 ± 126 SCS: 516 ± 168	Primary: incidence of PNF, EAD and vascular complications, 1-y graft/patient survival. Secondary: incidence of biliary and vascular complications, AKI, hospital length of stay, liver/kidney function markers	No PNF in either group. EAD: 5% vs 25%. No vascular complications in either group. 90% vs 90% 1-y graft/patient survival. Biliary complications: 2/20 vs 5/20. Hospital stay: 10.9 vs 15.3 d. Peak ALT (IU/L): 560 vs 1358

AKI, acute kidney injury; C, control; D, duration; DBD, donation after brain death; DCD, donation after circulatory death; DHOPE, dual hypothermic oxygenated machine perfusion solution; EAD, early allograft dysfunction; ECD, extended criteria donor; HA, hepatic artery; HOPE, hypothermic oxygenated machine perfusion; IGL-1, Institute George Lopez solution; IRI, ischemia-reperfusion injury; KPS-1, kidney perfusion solution; L-GrAFT, liver graft assessment following transplantation risk factor; LT, liver transplantation; MEAF, model of early allograft function; MP, machine perfusion; NAS, non-anastomotic biliary strictures; PNF, primary nonfunction; PRS, postreperfusion syndrome; PV, portal vein; SCS, static cold storage. UW-MPS, University of Wisconsin machine perfusion solution.

TABLE 3.
Clinical studies on ex situ liver normothermic machine perfusion

Author	Y	Donor type DCD/DBD	No. total (NMP/SCS)	Perfusion characteristics	Perfusate	Total time of preservation (min) (range)	Endpoints	Outcome (NMP vs SCS)
Trial name: Safety and feasibility of NMP to preserve and evaluate orphan livers NCT03456284 PI: Cristiano Quintini, The Cleveland Clinic	Ongoing completion date: 2023	Results awaited	15	Device: institutional liver MP device	Not reported	Results awaited	Primary: 30 d posttransplantation rate of survival and PNF Secondary: EAD, 6 mo graft survival, liver function, and injury markers	Results awaited
Trial name: Efficacy of Ex situ NMP vs cold storage in the transplant with steatotic liver graft (ORGANOXLAFE) NCT03930459 Instituto de Investigacion Sanitaria La Fe	Ongoing completion date: 2023	Results awaited	50	Device: not reported.	Not reported	Results awaited	Primary: Peak of AST and ALT at 1, 3, 5, 7 d post-LT Secondary: PNF, graft/patient survival at 30 d, 6/12 mo, PRS, EAD, liver function and injury markers, hospital/ICU stay, RRT, intraop thromboelastogram result, biliary stenosis in MRS evidence	Results awaited

Continued next page

TABLE 3. (Continued)

Author	Y	Donor type DCD/DBD	No. total (NMP/SCS)	Perfusion characteristics	Perfusate	Total time of preservation (min) (range)	Endpoints	Outcome (NMP vs SCS)
Trial name: sequential hypo- and normo-thermic perfusion to preserve extended criteria donor livers for transplantation NCT04023773 PI: Cristiano Quintini, The Cleveland Clinic	Ongoing completion date: 2022	ECD	15	Device: Institutional Liver MP device	Not reported	Results awaited	Primary: Patient/graft survival at 1 mo Secondary: EAD, patient/graft survival at 6 mo, blood loss, liver function and injury markers, hospital/ICU length of stay	Results awaited
Trial name: using ex vivo NMP with the organox metra device to store human livers for transplantation NCT02478151 PI: David Grant, University Health Network, Toronto	Ongoing Completion date: 2021	Results awaited	40	Device: OrganOx metra	Not reported	Results awaited	Primary: incidence of PNF, re-LT, survival at 3 mo Secondary: Rate of device failures resulting in organ discard, recruitment rates to study, IRI, graft function, ability of perfusion parameters to predict clinical outcomes following LT	Results awaited
Trial name: Normothermic Liver Preservation Trial NCT03089840 PI: James Shapiro, University of Alberta	Ongoing Completion date: 2021	Results awaited	50	Device: OrganOx metra	Not reported	Results awaited	Primary: 30-d graft survival Secondary: 30-d patient survival, EAD	Results awaited
Trial name: Pilot Study to Assess Safety and Feasbility of NMP in Human Liver Transplantation NCT02515708 PI: Cristiano Quintini, The Cleveland Clinic	Ongoing Completion date: 2020	Results awaited	25	Device: Institutional Liver MP device	Not reported	Results awaited	Primary: incidence of EAD Secondary: PNF, 6-mo graft/ patient survival, 7-d peak liver function tests, intraop flow measurement, PRS, intraop surgical outcomes, kidney failure, biliary/vascular complications at 6mo, hospital/ICU stay, rejection rate at 6mo, opportunistic viral infection rate	Results awaited

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TABLE 3. (Continued)

Author	Y	Donor type DCD/DBD	No. total (NMP/SCS)	Perfusion characteristics	Perfusate	Total time of preservation (min) (range)	Endpoints	Outcome (NMP vs SCS)
Trial name: WP01-Normothermic Liver Preservation NCT02775162 PI: Stuart Knechtle, Duke University	Ongoing Completion date: 2020	Results awaited	266	Device: OrganOx metra	Not reported	Results awaited	Primary: Incidence of EAD Secondary: PNF, graft/patient survival, PRS, liver function and injury markers, biliary complications, incidence of livers randomized but not transplanted, organ utilization, healthcare costs, quality of life measures	Results awaited
Trial name: Viability Testing and Transplantation of Marginal Livers (VITTAL) NCT02740608 PI: Darius Mirza, University Hospital Birmingham	Ongoing Completion date: 2020	Results awaited	22	Device: OrganOx metra	Not reported	Results awaited	Primary: 90-d patient survival, use of NMP to identify the proportion of transplantable liver grafts from currently rejected donor organ pool Secondary: 12 mo liver graft function, 90-d morbidity associated with receipt of extended criteria graft, physiological response to reperfusion of perfused grafts	Results awaited
Trial name: TransMedics (OCS) Liver PROTECT NCT02522871 TransMedics	Ongoing Completion date: 2020	Results awaited	300	Device: TransMedics OCS	Not reported	Results awaited	Primary: Incidence of EAD, SAEs in first 30 d	Results awaited
De Vries et al[112]	2019	DHOPE-COR-NMP 7/0	7 (7/0)	Device: Liver Assist Pressure DHOPE: HA: 11 mm Hg PV: 5mmHG Pressure NMP: HA: 70 mm Hg PV: 11 mm Hg Flow NMP: HA: 0.55 L/min (0.24-0.73(PV: 1.7 L/min (01.46-1.74)	HBOC-201	Total MP time: 427 (283-517)	Primary: Graft survival at 3 mo	100% 3-mo graft survival

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TABLE 3. (Continued)

Author	Y	Donor type DCD/DBD	No. total (NMP/SCS)	Perfusion characteristics	Perfusate	Total time of preservation (min) (range)	Endpoints	Outcome (NMP vs SCS)
Ghinolfi et al[51]	2019	NMP 0/10 SCS 0/10	20 (10/10)	Device: LiverAssist Flow: HA: 0.205-0.420 L/min PV: 1.1-1.7 L/min Time on machine: 4.2h (3.25-4.7)	Gelofusine (B Braun) + ABO-compatible RBC concentrate	NMP: 246 (206-267) SCS: 394 (366-465)	Primary: Graft/patient survival at 6 mo Secondary: peak transaminases within 7 d, biliary complications at 6 mo	No PNF in either group. EAD: 2/10 vs 1/10. Peak ALT (IU/L): 332 (263-610) vs 428 (303-1162). Graft survival: 90% vs 100%. Patient survival: 100% vs 90%. Biliary complication: 1/10 vs 0/10.
Liu et al[11]	2019	NMP 8/13 SCS 17/68	105 (21/84)	Device: noncommercial, institutional apparatus Flow: HA: 0.5 L/min (0.2-0.7) PV: 1.6 L/min (1.1-2.1) Time on machine: 3.35-7.89h	4 units blood bank-obtained FFP + 4 units PRBC	NMP: 528 (462-594) SCS: 498 (408-588)	Primary: Safety, feasibility, and impact on intrahepatic hemodynamics of FFP Secondary: Prove safety and feasibility of a noncommercial, institutional perfusion apparatus	No PNF in either group. EAD: 19% vs 46.4%, $P = 0.02$. Peak ALT (IU/L): 363 ± 318 vs 1021 ± 999. No cases of ischemic. cholangiopathy. Patient survival: NMP: 95.2% (One patient died of intracranial hemorrhage on postoperative mo 8 with normal liver function). Mortality in the historical control group not reported.
Nasralla et al[50]	2018	NMP 34/87 SCS 21/80	221 (121/101)	Device: OrganOx metra Flow: HA ≈ 0.28 L/min PV ≈ 1.1 L/min Time on machine: 9.13h (1.42-24)	Gelofusine (B Braun) + 3-unit donor-matched PRBC	NMP: 714 (258-1527) SCS:465 (223-967)	Primary: Peak level of serum AST within 7 d after LT Secondary: Organ discard rate, PRS, PNF, EAD, length of hospital/ICU stay, RRT, cholangiopathy on MRCP at 6 mo, graft/patient survival at 1 yr	PNF: 0.8% vs 0%. EAD: 10% vs 30%. Peak AST (IU/L): 488 (408.9-582.8) vs 964 (794.5-1172.0). Patient survival at 1y: 95.8% vs 97%. Graft survival at 1y: 95% vs 96%.
Watson et al[92]	2018	NMP 35/12	47 (47/0)	Device: Liver Assist Pressure: HA: 60 mm Hg PV: 8-10 mm Hg Flow: not reported Time on machine: 4 h	Leukocyte depleted red cells + Gelofusine (B Braun) or Steen solution	NMP: 460-1388	Primary: observation of biochemistry and perfusion characteristics	22 livers were transplanted. 1 recipient died following PNF, 1 developed EAD, 4 developed ITBL (3 required reLT).
Watson et al[93]	2017	NMP 9/3	12 (12/0)	Device: Liver Assist Pressure: HA: 60 mm Hg PV: 8-10 mm Hg Flow: not reported Time on machine: 284 (122-530)	Leukocyte depleted red cells + Gelofusine (B Braun) or Steen solution	NMP: 778 (564-1561)	Primary: Assessment of viability in declined marginal livers and research livers	5/6 developed PRS, 4 sustained vasoplegia, 1 PNF, 3 DCD livers developed cholangiopathy

Continued next page

TABLE 3. (Continued)

Author	Y	Donor type DCD/DBD	No. total (NMP/SCS)	Perfusion characteristics	Perfusate	Total time of preservation (min) (range)	Endpoints	Outcome (NMP vs SCS)
Bral et al[113]	2017	NMP 4/6 SCS 8/22	39 (10/30)	Device: OrganOx metra Pressure: Not reported. Flow: Not reported. Time on machine: 11.5h (3.3-22.5)	Gelofusine (B Braun) + 3-unit type "O" PRBC	NMP: 786 (304-1631) SCS:235 (64-890)	Primary: 30-d graft survival Secondary: Patient survival at d 30, peak AST within 7 d, EAD, liver function and injury markers, Clavien-Dindo score, graft/patient survival at 6 mo, biliary complications at 6 mo.	No PNF in either group. EAD: 55.5% vs 29.6%. Peak AST (IU/L): 1252 (383-2600) vs 839 (153-2600). 6-mo graft survival: 80% vs 100% 6-mo patient survival: 89% vs 100% 6-mo biliary complications 0% (0/8) vs 14.8% (4/27) $P = 0.55$.
Mergental et al[114]	2016	NMP 4/2	6 (6/0)	Device: Liver Assist, OrganOx Pressure: not reported. Flow: HA: 0.53 L/min (0.36-0.62) PV: 1.1 L/min (0.7-1.5) Time on machine: 3 h	Blood-based	NMP: 798 (724-951)	Primary: Demonstrate feasibility of rejected allografts transplanted following assessment and resuscitation by NMP	Uneventful transplant procedure in all 5 transplanted patient and immediate function recovery in all grafts. Normalized liver tests at median follow-up of 7mo. One graft did not met viability criteria after 3 h of MP.
Selzner et al[115]	2016	NMP 2/8 SCS 8/24	40 (10/30)	Device: OrganOx metra Pressure: Not reported. Flow: HA: 0.3 L/min (0.2-0.4) PV: 1.25 L/min (1.2-1.3) Time on machine: 8h (5.7-9.7)	3-units PRBC + Steen solution	NMP: 586 (221-731) SCS: 634 (523-783)	Primary: assess safety and feasibility of NMP	Peak ALT (IU/L): 619 (55-2858) vs 949 (233-3073). 3-mo graft/patient survival 100% in both groups.
Ravikumar et al[116]	2016	NMP 4/16 SCS 8/32	60 (20/40)	Device: OrganOx metra Pressure: HA 60-75mmHg PV not reported. Flow: HA≈0.2 L/min PV≈0.8 L/min Time on machine: 9.3h (3.5-18.5)	3 units of cross-matched PRBC + 1 unit of Gelofusine (B Braun)	Not reported	Primary: 30-d graft survival Secondary: liver function and injury markers within 7 d after LT, patient/graft survival at 6 mo	No PNF in either grougs. EAD: 15% vs 22.5%. Peak AST (IU/L): 417 (84-4681) vs 902 (218-8786)/ 30-d graft survival: 100% vs 97.5%. 6-mo patient survival: 100% vs 97.5%.

Modified from Martins PN et al.[88] References in this table include studies by Liu et al,[11] Nasralla et al,[50] Ghinolfi et al,[51] Watson et al,[92] Watson et al,[93] De Vries et al,[112] Bral et al,[113] Mergental et al,[114] Selzner et al,[115] and Ravikumar et al.[116]
ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; C, control; C-NMP, continuous-normothermic machine perfusion; D, duration; DBD, donor after brain death; DCD, donor after circulatory death; EAD, early allograft dysfunction; ECD, extended criteria donor; FFP, fresh frozen plasma; GGT, gamma-glutamyl transferase; HA, hepatic artery; HAT, hepatic artery thrombosis; ICU, intensive care unit; INR, international normalized ratio; IRI, ischemia-reperfusion injury; LT, liver transplantation; MAP, mean arterial pressure; MP, machine perfusion; MRCP, magnetic resonance imaging scan of biliary tree; NCT, national clinical trial identifier; NEVLP, normothermic ex vivo liver perfusion; NMP, normothermic machine perfusion; PNF, primary nonfunction; POD, postoperative day; PRBC, packed red blood cells; PRS, postreperfusion syndrome; pSCS, poststatic cold storage; PV, portal vein; RRT, renal replacement therapy; SAE, serious adverse event; SCS, static cold storage.

although further corroboration from other trials would be desirable. None of the HMP trials in liver transplantation have reported yet. It is too early, therefore, to provide a definitive answer to this question.

2. What are clinically relevant trial endpoints?

The group agreed that, wherever possible, the use of direct clinically relevant endpoints as the primary endpoint is desirable (eg, 1-y graft survival, 1-y patient survival, ITBL/biliary complication rates, length of stay, ICU stay, acute kidney injury/ hemodialysis need, total complication rate, mortality on the waitlist, organ utilization, overall cost). We support the creation of an international registry of all cases of MP (including in situ normothermic regional perfusion as well as ex situ MP) in liver transplantation. The rigorous analysis of a large and comprehensive registry database enables questions to be addressed that are impractical as the objectives of randomized clinical trials. On the other hand, where practical, the establishment of multicenter consortia trials is strongly supported, with the intention to provide enough statistical power for relevant endpoints. We also support meta-analyses of existing trials to obtain datasets of great enough magnitude to investigate questions that cannot be reliably addressed individually. It is very important that clinical trials have standard nomenclature and reporting system (eg, using endpoints and metrics that are consistent) so that they can be meta-analyzed. Trials that establish new and reliable biomarkers of organ viability should be strongly encouraged and supported.

3. Which preservation techniques should be compared in the next randomized trials?

In the current era, with SCS the standard of care in liver preservation, the group believe that novel perfusion techniques should be compared with this before comparison between different perfusion methods. The majority of published trials to date have been safety (phase 1) or nonrandomized (phase 2) trials. These studies have effectively established the claims that can be made for the use of these novel technologies; this is an essential prerequisite in advance of RCTs designed to test efficacy. The results of a number of properly powered randomized trials are awaited: the results of these should provide the stimulus to design trials to establish the relative merits of different perfusion methodologies. Logically, there will be trials which compare NMP with HMP and NRP. However, there will be numerous permutations to be considered, including the variations of timing of perfusion (eg, continuous perfusion, post-SCS perfusion) and combinations of HMP and NMP. The primary and secondary endpoints will be key to the value of these trials. Health economic and logistic endpoints may prove as important as graft injury endpoints.

4. Which grafts should be included in clinical trials?

Preliminary studies, such as the majority of the single-arm studies carried out to date, have been designed for proof of feasibility and safety, and therefore, have most commonly enrolled livers that would be acceptable in current practice. Now, that the feasibility of perfusion is more widely accepted, trials are addressing issues of efficacy. In these trials, the enrollment criteria may be selective (eg, DCD only) or general (eg, all organs). Although all grafts may benefit from MP preservation, our recommendation is to focus on extended-criteria grafts (DCD, older, steatotic grafts) in the next trials because these are the organs that logically should have the greatest benefit. Indeed, it is likely that financial and logistical constraints will likely limit the use of perfusion to high-risk organs.[44] Studies that show cost-effectiveness of MP in high-risk organs are important because this is the context in which higher up-front costs may be associated with downstream cost savings and broader acceptance of the technology, as the potential to save money and increase organ utilization is appreciated.[52] The problem is that there is no standard definition of extended-criteria donors, and such definition would be important to compare clinical trials.

DISCUSSION

Limitations and Pitfalls of MP Trials

In general, transplant clinical trials are considered to be of limited quality when compared with pharmacological intervention trials.[21,25,28,29] However, many of the flaws of these studies can be prevented by well-designed trials. There are several reasons for the compromised quality of many of the trials that have been conducted in liver MP.

Different Nomenclature of Perfusion Settings/Lack of Standardization

With the number of publications on liver MP to date exceeding 450, the last 15 years have seen a significant increase in the volume of both experimental and clinical liver MP preservation research.[20] Several groups have described different methods of MP with respect to temperature, the addition of oxygenation, and whether the perfusion is flow or pressure controlled. It is very important to clearly describe perfusion settings (flow, pressure, resistance), to correct for graft weight (eg, mL/min/100g), temperature of perfusion, dual (PV+HA) versus single perfusion, oxygen saturation, and partial pressure, composition of the perfusate, supplementation of therapeutic agents, Varying definitions for reporting DCD data (eg, functional warm ischemia) is also a source of inconsistencies among studies.

Because liver MP preservation is a relatively new technology with a wide variety of technical aspects continuing to be explored by several groups worldwide, the publications on MP have shown significant inconsistencies. These include the nomenclature used to describe the different MP techniques (abbreviations included), the temperatures considered to be hypo-, subnormo-, or normothermic, and the details of the methodology are reported. The lack of standardized nomenclature and guidelines for reporting technical details makes it difficult to reproduce experiments, compare different studies, and perform meta-analyses. With the number of clinical studies on MP of donor livers rapidly increasing, a team of international experts proposed a nomenclature consensus and standardized set of guidelines for reporting the methodology of future studies on liver MP.[53] It is the suggestion of our group that this nomenclature is adopted.

Whenever possible, investigators should agree on the development of a "master design" of clinical trial for a more comprehensive analysis and to allow comparisons among studies (eg, a standard set of specimens like perfusate, blood, bile, and tissue to be collected at predetermined timepoints). This would significantly increase the power of subsequent laboratory analysis in helping find biomarkers of viability.

Our group also recommended that study protocols should be made public in advance in an open access registry of clinical studies (clinicaltrials.gov, EudraCT, ChiCTR) or peer-reviewed publications.

Sample Size and Costs

Transplant clinical trials in general require a large number of individuals to be enrolled.[23,38] For example, a proposed reduction in event incidence from 30% to 20%, with 2-sided type-I error probability of 0.05 and 80% power, the estimated sample size necessary in each study arm is 294 without accounting for patients lost to follow-up.[21]

Small sample sizes are a common limitation in liver MP clinical trials. Although single center, single-arm studies are helpful to provide preliminary data, it is important to progress to multicenter and adequately powered randomized trials as soon as the focus moves to efficacy. Very few, if any, transplant units in the world have the case volume needed to carry out randomized trials in organ preservation as a single center, and the need to collaborate in multicenter trials is therefore paramount.

As noted above, some of the drawbacks of underpowered single center retrospective trials might be overcome by the creation of international or national data registries for all machine perfused livers.[54] This would be an ideal resource to allow us to compare different techniques when the right variables are collected, and the methodology is standardized. With artificial intelligence or computerized analysis of all biomarkers obtained during perfusion and posttransplant, we may be able to create and validate viability criteria.

Decisions to adopt interventions at the policy level depend not only on the evidence around their effects on clinical outcomes but also on costs of care.[52] Clinical trials involving MP are very expensive. Costs of acquisition of the pump itself and the expensive disposable cassettes required for each case are limiting for many institutions. The necessary ties of such trials to industry potentially create conflicts of interests,[56] but these can be managed by complete transparency and by ensuring that the trials are run and data analyzed with independent oversight. MP requires equipment that may cost hundreds of thousands of dollars for the device itself, in addition to which there are costs of disposables (as high as US $50 000 per graft), and perfusate components.[44] Trials that require initiation of MP at the donor hospital can potentially add additional logistical challenges and costs. An extra member of the perfusion team is needed to set up and run the liver perfusion. Transporting the machine and additional personnel can add to the complexity of the transportation logistics to and from the donor hospital.[44]

Trial designs for liver MP must be intelligently restructured to ensure that the trial cost is reduced and the maximum amount of questions are reliably answered. There is also opportunity to incorporate novel trial designs in MP that would allow researchers to potentially test multiple hypotheses without the need for large and expensive trials using master protocols for new study designs—namely platform, basket, and umbrella- or adaptive trial designs (ATDs).[38] Master protocols are novel designs that investigate multiple hypotheses through concurrent sub-studies (eg, multiple treatments or populations or that allow adding/removing arms during the trial), offering enhanced efficiency and a more ethical approach to trial evaluation. It allows to evaluate multiple hypotheses, and the general goals are improving efficiency and establishing uniformity through standardization of procedures in the development and evaluation of different interventions. Master protocols may be tailored and adapted to suit the research objectives of multiple clinical indications, but master protocols have not been well established in fields outside of oncology.[56] It may be possible through a coordinated effort by researchers, the pharmaceutical industry, and regulatory bodies, that master protocols can be implemented in transplantation.[38] They may feasible and especially important when clinical trials involving target molecular therapy during machine preservation are implemented.[57] For a literature review as a landscape analysis of master protocols, please see Park et al.[58] Other alternative is to use ATDs. This is a methodology in which a clinical trial adapts as the trial proceeds depending on the outcomes of patients enrolled. The criteria for these decisions are set before the beginning of the trial. An adaptive design is best used in trials with short-term endpoints. Endpoints of ATDs can be traditional clinical endpoints or surrogate endpoints (biomarkers).

Appropriateness of Control Arms

The specific selection of a control arm is of critical importance to the utility of an RCT and extrapolation based on the assumed therapeutic benefits of other treatments not tested in the trial are invalid. In most cases, the control arm of an RCT should represent the standard of care. A standard of care may be defined as a national authority approved regimen (as the Food and Drug Administration in United States), a consensus based "most common treatment" or the standard protocol utilized at a particular center.[21,45] In MP trials, controls have generally been standard static cold preservation (SCS) using UW or HTK solution. However, there is increasing interest by the transplant community to compare different MP techniques. In contrast to trials in paired organs (kidneys, lungs), liver MP clinical trials have distinct challenges to prove superiority, as there is no natural ideal control arm (the paired organ). In liver preservation studies, therefore, there are both donor and recipient confounding variables, some of which might require stratification (eg, DBD/DCD status, age, degree of steatosis), and all of which contribute to the need for a larger sample size.

Nonblinding Nature of MP Trials

As a general principle of clinical trials, the blinding of both patients and investigators to the treatment investigated is important to eliminate unconscious bias of data reporting by both.[59-61] In trials assessing nonpharmacological interventions (eg, surgical randomized clinical trials), blinding is usually more difficult or impossible. A systematic review of surgical trials showed that blinding was explicitly stated for practitioners, patients, and outcome observers in 3%, 37%, and 52%, respectively.[62]

Unfortunately, in clinical trials with liver MP, it is extremely difficult for investigators (ie, the transplant team) to be blinded; this constitutes an important limitation. This is intrinsic to the nature of the surgical procedure, as MP cannulation, and backtable preparation of the allograft are usually performed by members of the same team and MP often occurs in the same operating room as the liver transplant procedure itself.[63] MP can be complex and requires surgeons (usually investigators) to perform the backtable dissection, cannulation, and perfusion initiation. Due to the staffing limitations and availability at most transplant centers, it is difficult to replace surgeons involved with the investigation with other surgeons or technicians not involved with the trial. Even if this were not the case and a separate trial team carries out the cannulation and perfusion, it is almost impossible for the transplanting team to remain unaware of the arm to which a particular liver belongs. It is vital therefore that as far as possible, the endpoints of the trial should be based on objective data-points and not vulnerable to subjective observer bias. For example, a surgeon's impression of the quality of organ reperfusion is subjective (and therefore a poor endpoint), whereas an anesthetist's assessment of the magnitude of the reperfusion syndrome, based on the measured effect on blood pressure, can be objective (and therefore a better endpoint).

Lack of Reliable Biomarker and The "Wash-Out" Phenomenon

There is no reliable biomarker to predict clinical outcomes in liver transplantation. In most clinical and experimental liver ex situ studies, posttransplant serum transaminases or early allograft dysfunction (EAD)[64] are used as an injury marker to compare the quality of liver preservation.[20,46,47] The majority of clinical trials in liver MP have also used EAD or transaminase peak as their primary end-point[7] (Tables 2 and 3). It should be noted that these endpoints have been used in the context of livers preserved by SCS but not confirmed in the context of MP.[65,66]

Perfusate transaminases (as opposed to postoperative systemic levels of transaminase) have been used (typically in combination with graft lactate clearance and bile production) during NMP to determine the viability of a particular graft for implantation.[1,7,48,67] Transaminase levels may be influenced by the age of the donor, steatosis, ischemia time, among other factors. Perfusate transaminases should be normalized for liver weight and perfusate volume to allow comparability with other perfusion systems and different livers.

There are several reasons why peak transaminases and consequently EAD are not primary endpoints of choice in a MP clinical trial. Evidence comes from a number of sources:

1. Transaminase levels in acute hepatitis: In ischemic and toxic hepatic injury, transaminase levels fall rapidly with both recovery and necrosis; these are therefore a poor indicator of recovery.[68] Serum transaminase levels do not correlate with survival in the context of acute autoimmune hepatitis: indeed, in the study of Al-Chalabi et al patients in the highest tertile of AST level had superior survival (avoidance of liver transplantation or death) to those in the lower tertiles, although it is notable that the latter patients had higher incidences of cirrhosis. There was some correlation between histological necroinflammatory activity and AST level.[69]
2. Transaminase levels following nontransplant liver resection surgery: In an analysis of 651 hepatic resections, of which 58% underwent inflow occlusion, Boleslawski et al showed that peak postoperative transaminase levels did not correlate with duration of inflow occlusion or with postoperative complications.[70]
3. Transaminase levels in the deceased liver donor: Donor transaminase is a poor predictor of posttransplant graft survival. Cuende et al analyzed data from 5150 liver transplants, showing no significant association between donor peak transaminase and graft survival in a Cox regression analysis.[71] In a retrospective study of UNOS data (2007–2016), Feng et al analyzed SRTR data from 20 023 liver transplants, showing that donor AST levels were not an independent predictor of graft outcome: donor AST level is therefore not a component of the donor risk index calculation.[72] Similarly, the Eurotransplant Donor Risk Index, based on analysis of 5939 transplants, does not include donor transaminase because this was not shown to be a significant independent variable with respect to graft survival.[73] In a retrospective study of UNOS data on all deceased donors liver transplants between 2007 and 2016 ($n = 59\,050$), Kaltenbach et al categorized donors into 6 study groups according to peak ALT (<499, 500–749, 750–999, 1000–1999, 2000–2999, and >3000 IU/L). They found evidence that preretrieval transaminase level does not predict posttransplant outcome.[74] Single center cases series have reported successful transplants even when the donor peak transaminases are extremely high.[75-77]
4. Posttransplant transaminase levels: There is evidence of an association between peak levels and transplant outcome, and this has been traditionally used as a surrogate endpoint for liver preservation studies in clinical and experimental transplantation. However, there is no linear correlation between the levels of transaminases and poor outcomes. Rosen et al showed the primary nonfunction rates were significantly correlated with peak postoperative AST levels and 12-month graft survival when the AST was >2000 IU/L. The effect on 12-month patient survival was limited to patients with the most extreme AST levels (>5000 IU/L)—the difference in the effects on graft and patient survival being a function of Retransplantation.[78] Eisenbach et al analyzed 328 patients and demonstrated that high peak levels of AST were significantly correlated to graft loss or death.[65] Robertson et al analyzed 1272 patients from a single institution, showing that AST levels correlate strongly with early graft failure on day 3 and on day 7 postoperatively.[66] Conversely, Gaffey et al correlating the peak of AST and ALT with postop biopsy finding concluded that transaminase levels are not useful in the diagnosis of preservation injury.[79] Anecdotally, good graft function has been reported even when the early posttransplant AST level was as high as 17 600.[80]
5. Dilution and wash-out of transaminase: Postoperative transaminase levels are likely to be influenced by the size of the liver, the process of MP and volume of perfusate ("wash-out" phenomenon).[43,44,54,63] Most studies have not normalized the transaminases by the liver weight. Organs that are machine perfused either are flushed with a larger amount of preservation solution (extra liters) or reperfused and oxygenated leading to release of transaminases accumulated in the graft to the perfusion circuit (perfusate) and not in the recipient immediately posttransplant. This leads to different concentrations of metabolites and biomarkers

such as cytokines, AST, and ALT in the graft at the time of implantation, leading to different levels postoperative (wash-out phenomenon). Because transaminases have a long half-life (17 ± 5 h for AST, 47 ± 10 h for ALT),[68,81] the posttransplant transaminase levels in recipients of grafts that were not machine perfused often have higher levels, while recipients that received MP grafts have artificially or "falsely" lower levels.[43,44,54,63]

Little is known about the early postoperative parameters that can be used as valid predictive indices for liver transplant outcomes and several early posttransplant tests and scores (composite endpoints) have been proposed.[82] The most commonly used definition of EAD was by Olthoff et al[64] uses transaminase peak (AST or ALT > 2000 IU/L) within the first 7 days, Bilirubin ≥10 mg/dL on day 7, INR ≥ 1.6 on day 7, and is therefore prone to bias. MEAF uses the same parameters as EAD by Olthoff but the max value at the first 3 days. This score has been shown to be more granular, with scores that varies from 0 to 10, and more reliable that EAD by Olthoff.[83-85] There is likely underestimation of EAD in MP livers due to lower transaminase peak after passive release into the perfusate after large volume of flush solution of liver grafts or active release of transaminases into the perfusate after reoxygenation under normothermic temperature. The transaminase peak usually happens in the first 24 hours posttransplant, affecting the EAD rate as well.[86,87] To support this finding of the influence of transaminases on EAD, in a large randomized study, Nasralla et al found that the difference in EAD rate between MP and SCS preservation was largely due to the transaminase values.[50] Therefore, transaminases peak and commonly used definition of EAD that takes into account transaminases peak should preferably not be used as a primary endpoint in MP trials.[7,43,44,54,88] EAD likely needs to be redefined, modeled, and validated in the setting of machine preservation. Attempts to add other parameters like platelet count or factor V as a biomarker of EAD have been recently proposed.[89,90] A new EAD formula involving both liver synthetic function and injury markers as a continuum instead of a binary use as previously described by Olthoff et al should address this limitation. In fact, the newly proposed parameter, the L-GrAFT risk score, is claimed to be highly accurate, predict 3-month graft failure posttransplant that is more accurate than existing EAD and MEAF scores.[89,91]

Viability Markers Used During Machine Perfusion

Ex situ liver MP is believed to offer a platform to assess viability of grafts before transplantation. They can be assessed for appearance and consistency, hydro/hemodynamics, metabolic, and excretory function (Figure 1). NMP is most commonly used to assess liver viability because the

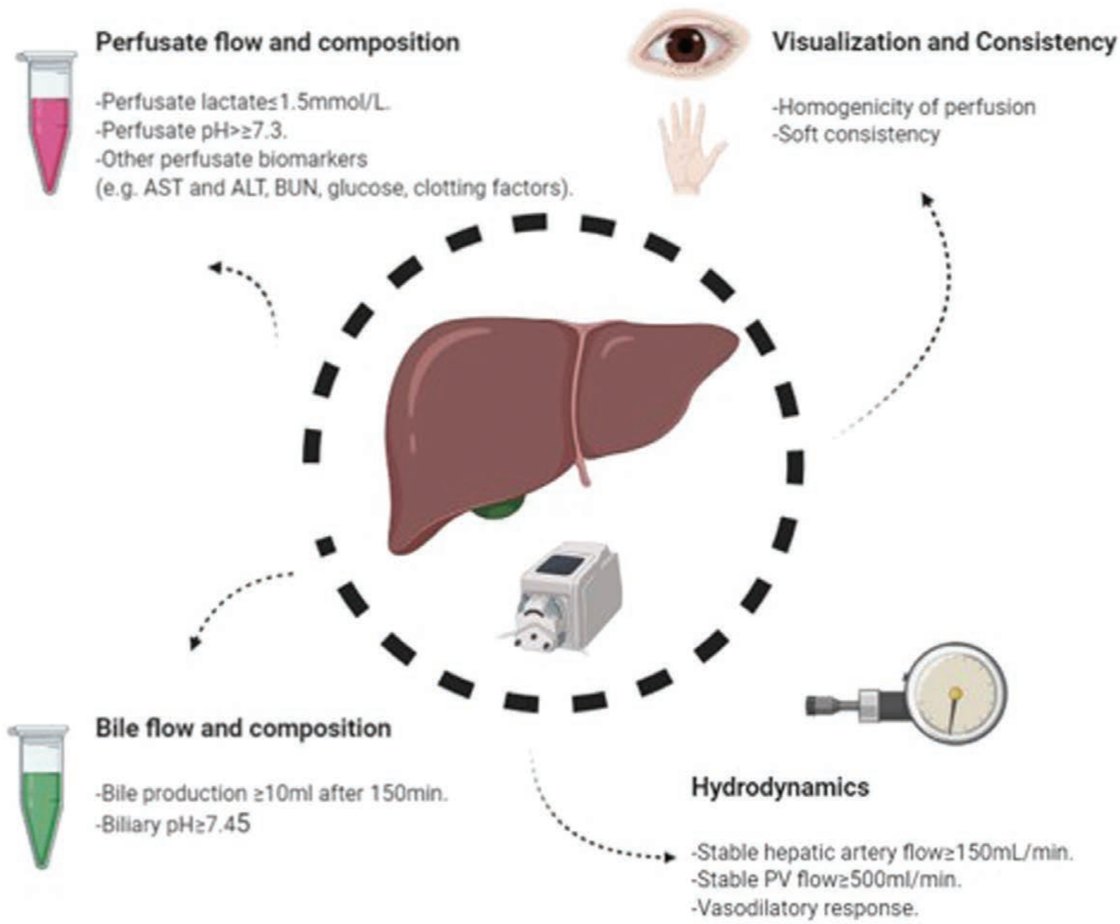

FIGURE 1. Viability criteria proposed during liver machine perfusion. Hepatocyte function can be tested by evaluating hydro-/hemodynamics (flow, resistance, and pressure), perfusate and bile composition, and other biomarkers. Cholangiocyte function (bile duct) can be assessed by evaluating bile flow and composition. ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen.

organ is maintained in a near-physiological state. Viability testing during hypothermic machine perfusion (HMP) is possible but more challenging since hepatic metabolism is markedly reduced and bile production is minimal. There is no consensus on the viability criteria, but the main candidates are perfusate lactate clearance, maintenance of a physiological pH in the perfusate, maintenance of glucose metabolism, bile production (if NMP), Bile pH, among others.[10,18,92-96] For viability assessment during HMP, the only injury biomarker that has been proposed is real time measurement of flavin mononucleotide (FMN), which is released upon injury to mitochondrial complex I.[96] There are few clinical studies investigating viability assessment during MP with promising results. However, there is to date no randomized clinical study that validated these criteria with posttransplant outcomes. This is of critical importance because it is the only way to prove MP can reliably make nontransplantable organs transplantable.[16,17,19]

With artificial intelligence/machine learning analysis of all biomarkers obtained during perfusion and posttransplant, we hope to create and validate more reliable viability criteria to predict EAD.

Selection Bias, Randomization, and Intention to Treat Analysis

As with all clinical trials, it is essential to identify and mitigate sources of selection bias in trials of perfusion technology. There is a general presumption that clinical trials are not susceptible to selection biases that are common to observational studies. However, selection biases can have marked impact on the findings of clinical trials.[21,25,28,60-62] There are several measures that we can take when designing clinical trials (Table 4). The International Committee of Medical Journal Editors recommends that all journal editors require the registration of clinical trials in a public trials registry at or before the time of first patient enrollment as a condition of consideration for publication[97] (Clinical trial registration. A statement from the International Committee of Medical Journal Editors. Available from: http://www.icmje.org/recommendations/browse/publishing-and-editorial-issues/clinical-trial-registration.html). A detailed description of the trial in open source platforms for trial registries preferably in English language (clinicaltrials.gov, EudraCT, ISCRNT, and other national registries) or, when possible, manuscript publication of study protocols[98-100] would allow us to enhance transparency of research, reduce publication bias, and prevent selective reporting of research outcomes.[28,63]

Common sources of selection bias in RCTs that can artificially increase treatment effects include poor application or design of the allocation process and incomplete or lack of blinding (discussed above). The proper time of randomization for MP depends on the objective of the study. For example, if the primary intention is to assess superiority of the preservation and compare posttransplant outcomes, the randomization time should be after final organ acceptance (after graft assessment by the procuring surgeon and liver biopsy). Randomization before final acceptance of the graft might enable selection bias, although we recognize that this may create logistical challenges depending on whether the trial design involves perfusion initiation at the donor hospital or at the transplant center. Achieving good outcomes with perfused grafts that were declined by all other local centers does not necessarily mean that MP was responsible for graft rescue or transplantability of the organ. At this time, there are no definitive viability criteria and the decision whether to transplant or discard a liver is subjective and often dependent on the particular practices of the transplant center itself.[102] There are several reports showing good outcomes with livers that were declined by all other centers without machine preservation.[101-106] The primary disadvantage of randomization at the time of final acceptance is that the perfusion device would need to be transported to the donor center regardless of which study arm the organ is randomized to in studies designed to initiate perfusion at the donor hospital. Alternatively, if the objective of the study is to assess organ utilization, then randomization should be done as early in the process as possible, ideally at the time of the organ offer or even at the time of listing the patient for transplant.

It is very important that the statistical analysis is based on an intention to treat analysis. Intention to treat analysis is a comparison of the treatment groups that includes all patients as originally allocated after randomization. This is the recommended method in superiority trials to avoid any bias. An additional "as treated" analysis will give some impression of the possible effect of "cross-over" allocation—grafts that were allocated to 1 group but treated with the other protocol (eg, allocated to MP but cold-stored because the MP machine was not available or not functioning). We also recommend a detailed description of all grafts that were discarded in each study arm (before or after perfusion) or any equipment failure so that the trial report can provide a narrative of every organ that has been randomized: this is an important way to detect selection bias (eg, the decision to exclude an organ from a trial may be subject to investigator/clinician bias).

Reallocation of Grafts When the Accepting Center Declines a Graft or the Intended Recipient Is No Longer a Candidate for Transplant

Transplant centers and Organ Procurement Organizations should develop a contingency plan to reallocate perfused liver grafts to avoid allocation delays or graft discard if a perfused liver cannot be used. This situation arises when the intended recipient, who had consented to the trial, becomes ineligible

TABLE 4.

Review criteria for the analysis of quality of clinical trials (modified from J Schold JD200821)

- Is there documentation on nonparticipants and characteristics of excluded subjects?
- Is the method of randomization and allocation appropriate and well described?
- Is the analysis conducted on an intention-to-treat or on-treatment basis?
- Is the interpretation of the trial results concordant with the data, particularly for the primary end
- Are all relationships of investigators, handlers, and analyzers of the study data third parties disclosed?

TABLE 5.

ILTS SIG "DCD, Preservation and Machine Perfusion" 12 recommendations for conducting clinical trials in liver MP preservation

ILTS SIG recommendations of the working group
1 Nomenclature standardization/Consensus (allow comparisons and meta-analysis) according to Karangwa et al.[53]
2 Pretrial registration of study protocol in public trial registries like (clinicaltrials.gov, EudraCT, others) and publication in peer-reviewed journals.
3 Preference of randomized trials and meta-analyses of existing trials. Preference to include ECD grafts (DCD, older, steatotic grafts). Support of trials looking into organ viability criteria as well.
4 Randomization time should depend on the primary outcome: - At the time of patient listing (to assess/compare organ utilization rate) - At the time of organ offer (to assess/compare organ utilization rate) - At final organ acceptance (after visualization/biopsy at the donor hospital): To assess/compare posttransplant outcomes
5 Support for multicenter consortia trials.
6 Creation of an international registry of all cases of machine perfusion/NRP in Liver transplant.
7 Preference to use of clinical data (1-y graft survival, 1-y patient survival, ITBL/biliary complication rates, LOS, ICU stay, AKI/HD need, overall complication rate, costs, etc) as primary outcomes instead of surrogate laboratory endpoints (until there is a validated endpoint). Consideration of mortality on the waitlist as endpoint.
8 Support for trials that compare specific MP techniques with standard preservation technique (static cold preservation) first before comparing different MP techniques. Then, compare HMP with NMP/NRP.
9 Redefinition of Early allograft dysfunction (Validation of composite endpoints of EAD in MP trials).
10 Intention-to-treat analysis. Detailed description/report of every graft that was damaged/lost during MP.
11 Collection of biospecimen (perfusate, bile, liver, and bile duct). Postreperfusion protocol biopsies and assessment of IRI by standard damage scores (eg, Suzuki for liver parenchyma, and Op den Dries/Hansen for Bile duct).[117,118]
12 Contingency plan. Back-up allocation system in case the primary team declines the graft after reperfusion because of graft performance or the intended recipient of a perfused liver can not undergo transplant (avoid surprises and allocation delays).

References in this table include studies by Karangwa et al,[53] Suzuki et al,[117] and Op den Dries et al.[118]
AKI, acute kidney injury; DCD, donor after circulatory death; EAD, early allograft dysfunction; ECD, extended criteria donor; HMP, hypothermic machine perfusion; ICU, intensive care unit; ILTS, International Liver Transplantation Society; IRI, ischemia-reperfusion injury; ITBL, ischemic type biliary injury; LOS, length of stay; MP, machine perfusion; NMP, normothermic MP; SIG, special interest group.

at or shortly before the planned start time of the transplant because of pre or intraoperative hemodynamic instability of discovery of findings that were not known in advance (eg, intraoperative finding of advanced cancer). There may be other instances in which the accepting program places the organ on the perfusion device as part of the trial and then declines it because of poor graft performance during the perfusion. If possible, the organ should be allocated according to the standard organ allocation rules, to the next recipient on the match run list even if not enrolled in the trial, or in a nonparticipating center (ie, not simply the next patient consented in the trial). If the graft is being preserved using a still-experimental technology (not yet approved by regulatory authorities), the recipient would have to provide consent to receive this graft and it may require ethical approval by the institutional review board. Centers enrolled in trials should address the issue of reallocation with other centers in their allocation area in advance to ensure that sharing protocols are already in place to prevent delays in the organ reallocation process.[54] As part of this, centers should agree whether the graft should remain on perfusion until arrival in the other center or if it should be repacked in standard cold static preservation.

Conflict of Interests and Relation With Industry

It is well known that any trial can be affected by conflicts of interest.[55] Machine perfusion clinical trials are very expensive, and some have been supported or partially supported by industry. We acknowledge that the relationship of academic institutions with industry is important. Conflicts of interest should be clearly stated, and the way to do this is well established. The role of external (particularly commercial) parties on trial design and analysis should be clearly stated, including holders of data and the responsible parties for analysis, as these relationships have the potential to impact study validity and interpretation.[110]

RECOMMENDATIONS

Our working group attempted to provide recommendations based on the GRADE methodology and acknowledge the current knowledge gap in this recent field. The first guidelines proposal for MP trials was initiated by the American Society of Transplant Surgeons' (ASTS) Standards Committee in 2018.[54] Some of our recommendations overlap this report. After thorough analysis and discussion, we concluded that we do not have all the elements to make recommendations based on the GRADE methodology. However, based on expert opinion, our working group proposed 12 recommendations (Table 5).

CONCLUSIONS

Machine perfusion preservation is a promising approach in liver transplantation.[12-14] In the last 10 years, many clinical trials in ex situ liver MP have been of limited quality and with specific limitations and pitfalls.[7,15,17,43] Many of these flaws can be avoided in future studies by well-designed protocols. The majority of MP clinical trials have been underpowered and some do not have clinically significant primary endpoints. Although some of the evidence is very promising, there is clear need for more information from high quality and appropriately powered trials. Scores to predict EAD need to be validated in the setting of liver MP trials. As we are moving from an early phase to maturation phase, certain key elements of the design and reporting of clinical trials in liver MP should be standardized. Standardization of data collection and reporting will allow comparisons of trials and meta-analysis. Optimum trial design and interpretation of data will increase the quality of the output, contributing to patient safety and advancing the field.

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APPENDIX

ILTS Special Interest Group "DCD, Preservation and Machine Perfusion":

Chair: Paulo N. Martins MD, PhD, FAST, FEBS, FACS (Univ Massachusetts, United States) Vice-chair: Michael Rizzari MD (Henry Ford Hospital, United States); Members: Magdy Attia MD (Leeds, United Kingdom) David Ghinolfi MD, PhD (Pisa, Italy); Ina Jochmans MD, PhD (Leuven, Belgium) Rajiv Jalan MBBS, MD, PhD, FRCP, FRCPE, FAASLD. University College London (United Kingdom) Peter Friend MD (Oxford, United Kingdom); Attendees of the smaller-subgroup workshop: Dieter Broering, Al Faisal University, Riyadh, Saudi Arabia; Michael Grat, Medical University of Warsaw, Warsaw, Poland; Jean Gugenheim and Zhiyong Guo, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou, China; Andrew Jacques, Kysela Marek, and Valeria Mas, School of Medicine the University of Tennessee Health Science Center Memphis, TN; Damiano Patrono, University of Turin Medical School Hospital, Turin, Italy; Daniele Dondossola, Fondazione IRCCS Ca'Granda, University of Milan Medical School Hospital, Milan, Italy; Elizabeth Pomfret, Colorado University, Denver-Co, United States; Patricia Ruiz, Biocruces Bizkaia Health Research Institute. Liver Transplantation Unit, Hospital Universitario Cruces, Bilbao, Spain; Sandra Spiritelli and Waldemar Patkowski, Medical University of Warsaw, Warsaw, Poland.; Peter DeMuylder, Organ Recovery Systems, Zaventem, Belgium.; Rutger Ploeg, University of Oxford, Oxford, England; Hynek Mergental, Queen Elizabeth Hospital, University Hospitals Birmingham NHS Foundation Trust, Birmingham, United Kingdom; Invited Faculty (panel of experts): For a list of their biographie please go to: https://wp-ilts-media.s3.amazonaws.com/wp-content/uploads/2020/01/29161208/02-Final-ILTS-Venice-2020-Meet-The-Faculty.pdf; Faculty listed in alphabetic order of last name: Peter L. Abt, MD, Hospital of the University of Pennsylvania, Philadelphia, PA; Magdy Attia, MD, MS, FRCSGen, MBBCh, Leeds Teaching Hospitals, Leeds, United Kingdom; PIERRE-A. CLAVIEN, MD, PhD, FACS, ASA, FRCS, FRCS, University Hospital Zurich, Zurich, Switzerland; Miriam Cortes Cerisuelo, MD, PhD, King's College Hospital, London, United Kingdom; Kristopher P. Croome, MD, Mayo Clinic, Jacksonville, Florida, FL; Olivier Detry, MD, PhD, University of Liege, Liege, Belgium; Federica Dondero Pozzo, MD, Beaujon Hospital, Paris, France; Philipp Dutkowski, MD, FEBS, University Hospital Zurich, Zurich, Switzerland; David Foley, MD, University of Wisconsin School of Medicine and Public Health, Madison, WI; Constantino Fondevilla, MD, PhD, Hospital Clínic, Barcelona, Spain; Juan Carlos García-Valdecasas Salgado, MD, PhD, Hospital Clinic University of Barcelona, Barcelona, Spain; Mikel Gastaca, MD, Cruces University Hospital, Bilbao, Spain; Davide Ghinolfi, MD, PhD, Universita di Pisa, Pisa, Italy; James Guarrera, MD, FACS, New Jersey Medical School, Newark, NJ; Zhiyong Guo, MD, PhD, Hospital of Sun Yat-sen University, Guangzhou, China; Nigel Heaton, MD, FRCS, King's College Hospital, London, United Kingdom; Roberto Hernandez-Alejandro, MD, University of Rochester Medical Center, Rochester, NY; Amelia Hessheimer, MD, Hospital Clínic, Barcelona, Spain; Rajiv Jalan MD, PhD, MBBS, FRCPE, FRCP, FAASZD, University College London, London, United Kingdom; Ina Jochmans, MD, PhD, University Hospitals Leuven, Leuven, Belgium; Marit Kalisvaart, MD, PhD, University Hospital Zurich, Zurich, Switzerland; Daniel Maluf, MD, UT/Methodist Transplant Institute Memphis, Memphis, TX; Paulo Martins, MD, PhD, The University of Massachusetts Medical School, Worcester, MA; Eduardo Miñambres, MD, PhD, Hospital Universitario Marques de Valdecilla, Santander, Spain; Paolo Muiesan, MD, The Queen Elizabeth Hospital, Birmingham, United Kingdom; David Nasralla, BMBCh, MA, MRCS, University of Oxford, Oxford, United Kingdom; Gabriel Oniscu, MD, Royal Infirmary of Edinburgh, Edinburgh, United Kingdom; Jacques Pirenne, MD, MSc, PhD, UZ Gasthuisberg, Leuven, Belgium; Wojciech Polak, MD, PhD, Erasmus MC, Rotterdam, The Netherlands; Robert J. Porte, MD, PhD, FEBS, University Medical Center Groningen, Groningen, The Netherlands; Cristiano Quintini, MD, Cleveland Clinic, Cleveland, OH; Michael Rizzari, MD, Henry Ford Transplant Institute, Detroit, MI; Eric Savier, MD, University Hospital Pitié-Salpetrière, Paris, France; Andrea Schlegel, MD, The Queen Elizabeth Hospital, Birmingham, United Kingdom.; C. Burcin Taner, MD, FACS, Mayo Clinic Florida.; Christopher J.E. Watson, MD, Cambridge University Hospitals, Cambridge, United Kingdom.