International study on the outcome of locoregional therapy for liver transplant in hepatocellular carcinoma beyond Milan criteria
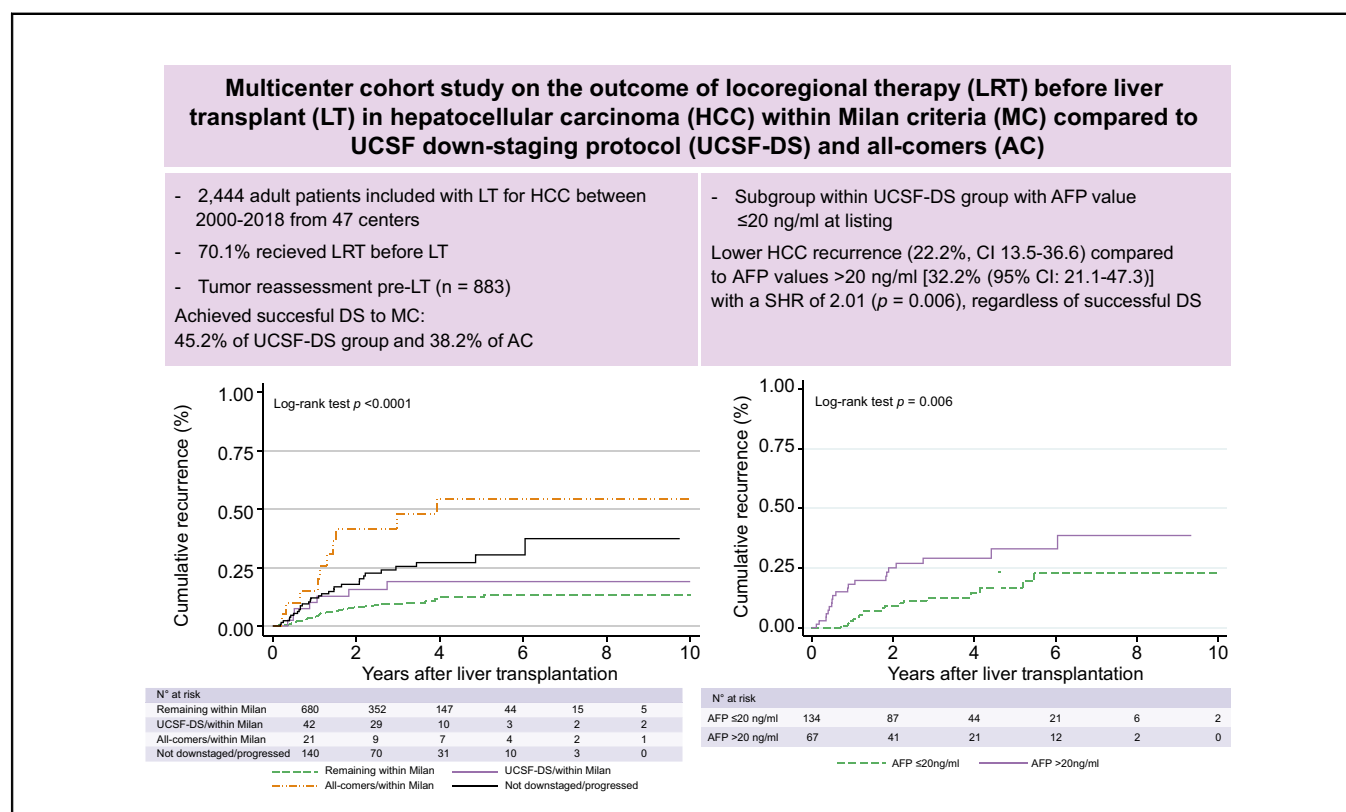
Authors

Helena Degroote, Federico Piñero, Charlotte Costentin, Andrea Notarpaolo, Ilka F. Boin, Karim Boudjema, Cinzia Baccaro, Aline Lopes Chagas, Philippe Bachellier, Giuseppe Maria Ettorre, Jaime Poniachik, Fabrice Muscari, Fabrio Di Benedetto, Sergio Hoyos Duque, Ephrem Salame, Umberto Cillo, Adrián Gadano, Claire Vanlemmens, Stefano Fagiuoli, Fernando Rubinstein, Patrizia Burra, Daniel Cherqui, Marcelo Silva, Hans Van Vlierberghe, Christophe Duvoux

Correspondence

Helena.Degroote@Uzgent.be (H. Degroote).

Graphical abstract

Multicenter cohort study on the outcome of locoregional therapy (LRT) before liver transplant (LT) in hepatocellular carcinoma (HCC) within Milan criteria (MC) compared to UCSF down-staging protocol (UCSF-DS) and all-comers (AC)

- 2,444 adult patients included with LT for HCC between 2000-2018 from 47 centers
- 70.1% recieved LRT before LT
- Tumor reassessment pre-LT (n = 883)
Achieved succesful DS to MC:
45.2% of UCSF-DS group and 38.2% of AC

- Subgroup within UCSF-DS group with AFP value ≤20 ng/ml at listing
Lower HCC recurrence (22.2%, CI 13.5-36.6) compared to AFP values >20 ng/ml [32.2% (95% CI: 21.1-47.3)] with a SHR of 2.01 (p = 0.006), regardless of successful DS

Highlights

- Successful downstaging from UCSF criteria resulted in a similar outcome compared with the group initially within Milan criteria.
- Patients within UCSF-DS criteria and with AFP values below or equal to 20 ng/ml at listing have excellent survival and recurrence rates.
- All-comers have a higher recurrence after downstaging to Milan criteria, with frequent microvascular invasion in the explant.

Lay summary

Patients with more extended HCC (within the UCSF-DS protocol) successfully downstaged to the conventional Milan criteria do not have a higher recurrence rate after LT compared with the group remaining in the Milan criteria from listing to transplantation. Moreover, in the UCSF-DS patient group, an ALP value equal to or below 20 ng/ml at listing might be a novel tool to further optimise selection of candidates for LT.

International study on the outcome of locoregional therapy for liver transplant in hepatocellular carcinoma beyond Milan criteria

Helena Degroote,[1,*,†] Federico Piñero,[2,3,†] Charlotte Costentin,[4] Andrea Notarpaolo,[5] Ilka F. Boin,[6] Karim Boudjema,[7] Cinzia Baccaro,[8] Aline Lopes Chagas,[9] Philippe Bachellier,[10] Giuseppe Maria Ettorre,[11] Jaime Poniachik,[12] Fabrice Muscari,[13] Fabrio Di Benedetto,[14] Sergio Hoyos Duque,[15] Ephrem Salame,[16] Umberto Cillo,[17] Adrián Gadano,[18] Claire Vanlemmens,[19] Stefano Fagiuoli,[20] Fernando Rubinstein,[21] Patrizia Burra,[22] Daniel Cherqui,[23] Marcelo Silva,[2,3] Hans Van Vlierberghe,[1] Christophe Duvoux [24], for the French-Italian-Belgium and Latin American collaborative group for HCC and liver transplantation

[1]Department of Hepatology and Gastroenterology, Ghent University Hospital, Ghent, Belgium; [2]Hospital Universitario Austral, School of Medicine, Austral University, Argentina; [3]Argentina and Latin American Liver Research Educational and Awareness Network (LALREAN); [4]Grenoble Alpes University; Institute for Advanced Biosciences, Research Center UGA/Inserm U 1209/CNRS 5309; Gastroenterology, Hepatology and GI Oncology Department, Digidune, Grenoble Alpes University Hospital; 38700 La Tronche, France; [5]Arcispedale Santa Maria Nuova, Reggio Emilia, Italy; [6]Hospital das Clinicas UNICAMP Campiñas, Campiñas, Brazil; [7]Department of Hepatobiliary and Digestive Surgery, Pontchaillou Hospital Rennes 1 University, Rennes, France; [8]Lanciano's Hospital, Chieti, Rome, Italy; [9]Instituto do Câncer do Estado de São Paulo, Hospital das Clínicas, Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil; [10]Department of Hepatobiliary and Pancreatic Surgery and Liver Transplant, Hôpital de Hautepierre, CHU de Strasbourg, France; [12]Hospital Clínico de la Universidad de Chile, Santiago, Chile; [13]Digestive Surgery and Transplant Unit, Hôpital Rangueil, Toulouse, France; [14]Hepato-Pancreato-Biliary Surgery and Liver Transplantation Unit, Department of General Surgery, University of Modena and Reggio Emilia, Modena, Italy; [15]Hospital Pablo Tobón Uribe y Grupo de Gastrohepatología de la Universidad de Antioquía, Medellín, Colombia; [16]Digestive Surgery Unit, CHU de Tours, Tours, France; [17]Hepatobiliary Surgery and Liver Transplant Unit, Padova University Hospital, Padua, Italy; [18]Hospital Italiano de Buenos Aires, Buenos Aires, Argentina; [19]Hepatology Unit, Hôpital Jean Minjoz, Besançon, France; [20]Gastroenterology, Hepatology and Transplantation, Papa Giovanni XXIII Hospital, Bergamo, Italy; [21]Instituto de Efectividad Clínica y Sanitaria (IECS), Buenos Aires, Argentina; [22]Multivisceral Transplant Unit, Department of Surgery, Oncology and Gastroenterology, Padova University Hospital, Padua, Italy; [23]Paul Brousse Hospital, APHP – University Paris Saclay, Villejuif, France; [24]Liver and Medical Liver Transplant Unit, Hospital Henri Mondor, University of Paris-Est, Creteil, France

Background & Aims: Good outcomes after liver transplantation (LT) have been reported after successfully downstaging to Milan criteria in more advanced hepatocellular carcinoma (HCC). We aimed to compare post-LT outcomes in patients receiving locoregional therapies (LRT) before LT according to Milan criteria and University of California San Francisco downstaging (UCSF-DS) protocol and 'all-comers'.

Methods: This multicentre cohort study included patients who received any LRT before LT from Europe and Latin America (2000–2018). We excluded patients with alpha-foetoprotein (AFP) above 1,000 ng/ml. Competing risk regression analysis for HCC recurrence was conducted, estimating subdistribution hazard ratios (SHRs) and corresponding 95% CIs.

Results: From 2,441 LT patients, 70.1% received LRT before LT (n = 1,711). Of these, 80.6% were within Milan, 12.0% within UCSF-DS, and 7.4% all-comers. Successful downstaging was achieved in 45.2% (CI 34.8–55.8) and 38.2% (CI 25.4–52.3) of the UCSF-DS group and all-comers, respectively. The risk of recurrence was higher for all-comers (SHR 6.01 [*p* <0.0001]) and not significantly higher for the UCSF-DS group (SHR 1.60 [*p* = 0.32]), compared with patients remaining within Milan. The all-comers presented more frequent features of aggressive HCC and higher tumour burden at explant. Among the UCSF-DS group, an AFP value of ≤20 ng/ml at listing was associated with lower recurrence (SHR 2.01 [*p* = 0.006]) and better survival. However, recurrence was still significantly high irrespective of AFP ≤20 ng/ml in all-comers.

Conclusions: Patients within the UCSF-DS protocol at listing have similar post-transplant outcomes compared with those within Milan when successfully downstaged. Meanwhile, all-comers have a higher recurrence and inferior survival irrespective of response to LRT. Additionally, in the UCSF-DS group, an ALP of ≤20 ng/ml might be a novel tool to optimise selection of candidates for LT.

Clinical trial number: This study was registered as part of an open public registry (NCT03775863).

Lay summary: Patients with more extended HCC (within the UCSF-DS protocol) successfully downstaged to the conventional Milan criteria do not have a higher recurrence rate after LT compared with the group remaining in the Milan criteria from listing to transplantation. Moreover, in the UCSF-DS patient group, an ALP value equal to or below 20 ng/ml at listing might be a novel tool to further optimise selection of candidates for LT.

Introduction

In most countries, the gold standard protocol for liver transplantation (LT) in patients with hepatocellular cancer (HCC) is the Milan criteria (MC).[1] However, in recent years, the MC have been challenged for being too strict, unjustifiably excluding specific subgroups with very good outcomes following LT. To broaden the allocation criteria for LT in patients with HCC,[2] the expansion of transplant criteria beyond MC has been described based on extended morphometric variables such as the University of California San Francisco (UCSF) criteria[3] or by adding markers for biological behaviour such as alpha-foetoprotein (AFP) in the French model[4] or the Metro ticket 2.0 model.[2,5]

In patients exceeding MC, downstaging (DS) with locoregional therapies (LRT) to within conventional transplant criteria has been proposed. In a meta-analysis, the pooled (12 studies) recurrence rate after DS to MC was 0.16 (CI 0.11–0.23). Because of the heterogeneity in data and protocols, the post-LT survival could not be assessed.[6] Consequently, as a next step, *a priori* inclusion criteria were used to maximise the probability of success of DS protocols and reduce the risk of recurrence. Yao *et al.*[7] published the first small prospective study using the University of California San Francisco downstaging (UCSF-DS) protocol as eligibility criteria for enrolment for DS and a minimum of 3 months of observation after the last LRT before LT. The intention-to treat (ITT) outcome of this approach was encouraging, with excellent survival and low recurrence rates following LT.[6,7] On the contrary, in patients exceeding the UCSF-DS criteria, defined as 'all-comers' (AC), there was a higher waiting list dropout rate, lower DS success, and inferior survival.[8] However, from a very recently published retrospective analysis from the United Network for Organ Sharing (UNOS), lower 3-year post-LT survival for AC and higher 3-year HCC recurrence rates for both DS groups were observed when compared with MC (6.9% for MC, 12.8% for UNOS-DS, and 16.7% for AC-DS).[9] The authors proposed a threshold of AFP values below 100 ng/ml to better select candidates for DS, but even lower values should be addressed. The aim of the present work is therefore to further evaluate the effect of LRT during waiting time on the outcome after LT and for the first time to test the UCSF-DS protocol outside the USA in a large multinational cohort, by comparing the UCSF-DS group with patients within MC. Furthermore, we investigate the group of AC to address the controversy about the existence of an upper limit in tumour burden limiting successful LT after DS. As a third goal, we evaluate variables associated with better post-LT outcomes to define the best subgroup of patients eligible for DS.

Materials and methods

This is a retrospective, multicentre, multinational cohort study of patients with HCC who underwent LT in 47 different centres across Europe and Latin America. For that purpose, four databases dealing with patients who underwent transplantation for HCC from different centres in France,[4] Italy,[10] and Belgium[2]

between 2000 and 2018 and from Argentina, Uruguay, Chile, Brazil, Ecuador, Colombia, and Mexico[11] between 2005 and 2018 were considered. These 4 regional databases were merged, harmonised, quality controlled, and hosted on a central server, after agreement of all participating centres. We grouped patients in different 6-year periods (2000–2005, 2006–2011, and 2012–2018), and we chose a time point of comparison according to the first HCC European Association for the Study of the Liver (EASL) guideline.[12] All procedures were followed in accordance with Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.[13] The ethics committee of each centre approved this study, which complied with ethical standards and with the Helsinki Declaration of 1975, as revised in 2008. Each investigator was subject to a confidentiality agreement. This study was registered as part of an open public registry (NCT03775863; www.clinicaltrials.gov). We included adult patients (>17 years of age) who underwent a first LT, received any LRT before LT, and were followed up after transplantation. We excluded patients if (1) incidental HCC was found at explant pathology and (2) there were tumours other than HCC found in the explant.

DS groups and common exposure variables

The following variables were collected at each transplant site: recipient characteristics, tumour burden, and AFP serum levels at HCC diagnosis, at listing, and at the last evaluation before LT if available. MC or the AFP score[4] were the common standardised patient selection criteria in all centres, but according to local practices and allocation policies, patients exceeding MC or downstaged to MC were also considered and discussed at each transplant centre on a case-by-case basis.

Serum AFP values were categorised as reported by Duvoux *et al.*[4] and further grouped according to lower values including ≤20, 21–100, and 101–1,000 ng/ml (excluding patients with AFP values above 1,000 ng/ml, n = 43).[7] Patients were grouped according to tumour burden at listing into 3 groups: (a) meeting MC, (b) exceeding MC but within the UCSF-DS protocol, and (c) AC, exceeding UCSF-DS criteria. The UCSF-DS group was defined according to previously reported criteria, including the following: HCC exceeding MC but with at least 1 of the following tumour size criteria: (a) a single lesion ≤8 cm, (b) 2 or 3 lesions each ≤5 cm and the sum of total tumour diameter ≤8 cm, and (c) 4–5 lesions each ≤3 cm and the sum of total tumour diameter ≤8 cm, as well as in the absence of macrovascular invasion or extrahepatic spread or AFP values above 1,000 ng/ml.[7] Patients beyond the UCSF-DS protocol were defined as AC. Of the total study cohort, 1,711 patients received LRT before LT after listing. Tumour treatment and type of LRT were decided at each transplant centre on a case-by-case basis. We focused on patients receiving LRT during the waiting list period with available radiologic tumour reassessment and AFP values before LT (n = 883).[14] Successful DS was defined as tumour size and number meeting MC based on imaging measurements of each HCC nodule maximal diameter of viable enhancing lesions and

included a decrease in tumour size and number or complete tumour nonenhancement equivalent to complete tumour necrosis. Time on the waiting list after listing and time from LRT to LT were registered.

Pathological tumour features at explant analysis included the presence of microvascular invasion (MVI), tumour differentiation according to Edmondson and Steiner criteria,[15] and number and size of each nodule as assessed by experienced pathologists specialised in liver pathology. Necrotic nodules were measured, including necrotic and viable tumour diameter. MC were also assessed at explant pathology.

Main outcomes and statistical analysis

The primary end point analysed was post-LT HCC recurrence. Recurrence was determined based on imaging criteria and serum AFP or by biopsy.[16] Secondary end points were successful DS, overall survival, discordance rate between imaging and explant pathology tumour burden, and proportion of MVI and tumour dedifferentiation between patients within MC, patients within UCSF-DS, and AC. Successful DS was defined when a patient exceeding MC at listing was finally within MC at the last tumour reassessment before LT. All patients were followed up until death or the last outpatient visit.

Categorical data were compared using Fisher's exact test or Chi-square (X^2) test (2-tailed), as appropriate. Continuous variables are shown in mean ± SD or median with IQR and were compared with Student's t test or the Wilcoxon rank-sum test according to their distribution, respectively. Dummies for ordinal or categorical variables were assessed. Multiple comparison for parametric and non-parametric variables were done with ANOVA and Kruskal–Wallis tests, respectively.

For the primary outcome, to avoid selection bias and an overestimation of the risk of recurrence in the presence of competing events, we conducted a multivariable competing risk regression analysis to identify independently associated variables with HCC recurrence, estimating cause-specific hazards (subdistribution hazard ratios [SHRs]) and its corresponding 95% CI using the Fine and Gray method.[17] Non-HCC deaths were considered as the competing event for HCC recurrence. To avoid overfitting, for each multivariate model, we followed the rule of 1 independent variable per 10 events included. Variables with a p value of <0.05 in the univariate analysis were included step by step in the multivariate model, evaluating its independent effect on the primary outcome and confounding effect (>20% of change in crude SHR).

For overall survival analysis since transplantation, Kaplan–Meier survival curves were compared using the log-rank test (Mantel–Cox), and Cox proportional regression model with hazard ratios (HRs) and 95% CI were estimated. Proportional hazard assumption was evaluated through the graphic and Schoenfeld residual test. Collected data were analysed with STATA 13.0 (StataCorp, College Station, TX, USA).

Results

A total of 2,441 LT patients were included, of which 55.5% were from Europe (n = 1,356) and 44.5% from Latin America (n = 1,085) (Fig. 1.) Participating centres included 20 centres from France (n = 352), 4 from Italy (n = 480), 6 from Belgium (n = 524), 5 from Argentina (n = 325), 3 from Brazil (n = 376), 3 from Chile (n = 90), 2 from Colombia (n = 157), 2 from Mexico (n = 63), and 1 from
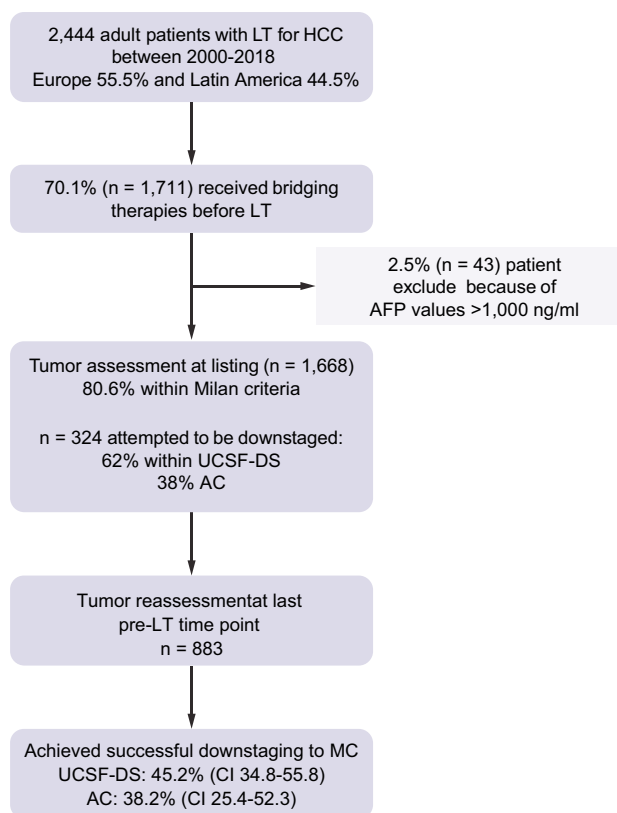

Fig. 1. Flow chart study design. AC, all-comers; AFP, alpha-foetoprotein; HCC, hepatocellular carcinoma; LT, liver transplantation; MC, Milan criteria, UCSF-DS, University of California San Francisco downstaging.

Ecuador (n = 13), Peru (n = 26), and Uruguay (n = 35). Overall, 70.1% of the patients received at least 1 LRT before LT (n = 1,711). Table S1 describes the main characteristics of patients who received LRT, after excluding 43 patients with AFP values higher than 1,000 ng/ml, according to the definition of the UCSF-DS protocol.[7] Overall, 61.6% of the patients (n = 1,027) underwent transplantation before the first HCC EASL guideline was published (2000–2012) and 38.4% after 2012 (n = 641). There was a lower proportion of patients meeting MC at the time of listing when comparing before and after HCC EASL guidelines publication (76.2% *vs.* 87.5%; *p* <0.0001). Most patients received transarterial chemoembolisation (TACE; 85.8%) and less frequently radiofrequency ablation (RFA; 20.5%), percutaneous ethanol ablation (PEI; 7.2%), or liver resection (LR; 6.2%). The use of TACE and LR was not significantly different between groups. In the UCSF-DS group, PEI was more frequently performed, and in the AC group, RFA was more frequently performed (Table S1).

Outcome after LRT in patients achieving effective DS to MC after LRT

Table 1 shows a comparative analysis between longitudinal tumour changes from listing to the last tumour reassessment before LT. In 52.9% of patients receiving LRT, tumour reassessment before LT was available (n = 883). Among these patients, 83.2% (n = 735) were within MC, 10.5% (n = 93) were within UCSF-DS, and 6.2% (n = 55) were AC at the time of listing. The success rate of DS to within the MC at imaging reassessment

Table 1. Last imaging reassessment before LT in patients receiving locoregional therapies.

| Variables at last reassessment	Categorisation at listing			
	Within MC, n = 735 (83.2%)	UCSF-DS, n = 93 (10.5%)	All-comers, n = 55 (6.2%)	*p* value
Months from reassessment to LT, median (IQR)	2.2 (1.0–4.3)	2.3 (0.8–4.2)	1.9 (0.9–2.9)	0.48
Within MC, n (%)	680 (92.5)	42 (45.2)	21 (38.2)	<0.0001
Successful DS (95% CI)		45.2 (34.8–55.8)	38.2 (25.4–52.3)	

Categorical data were compared using Fisher's exact test or Chi-square test (2-tailed), as appropriate. Continuous variables are shown in mean ± standard deviation (SD) or median with interquartile range (IQR) and were compared with Student's *t* test or Wilcoxon rank-sum test according to their distribution, respectively. DS, downstaging; LT, liver transplantation; MC, Milan criteria; UCSF-DS, University of California San Francisco downstaging.

before LT for the UCSF-DS group was 45.2% (CI 34.8–55.8), whereas for the AC, this was 38.2% (CI 25.4–52.3). In those patients meeting MC at listing, 92.5% (CI 90.4–94.3) remained within MC at the last tumour reassessment (Table 1).

For each group, the outcome after LT was compared according to effective DS to MC. Five-year HCC recurrence rate in patients successfully DS in the UCSF-DS group was 18.2% (CI 9.0–34.7), with a SHR of 1.60 (CI 0.69–4.05, *p* = 0.32) compared with those patients remaining with MC (13.0% [CI 9.4–17.9]). (Fig. 2). In contrast, the subgroup of AC that could be DS to within the MC had a higher 5-year recurrence rate of 54.7% (CI 33.7–78.2) with a SHR of 6.01 (CI 3.00–12.06; *p* <0.0001) compared with those remaining within MC. (Fig. 2). The subgroup of patients that progressed to beyond MC or were not DS (n = 140) had a 5-year recurrence rate of 24.3% (CI 11.6–46.8) and a SHR of 2.58 (CI 1.59–4.16, *p* <0.0001) compared with those remaining within MC (Fig. 2). Patients not DS in the UCSF-DS group presented a significantly higher recurrence rate (24.7% [14.3–40.8]) when compared with those within the UCSF-DS that could be DS with a SHR 3.7 (CI 1.26–11.01; *p* = 0.017). On the contrary, no significant differences were observed in the AC group either with or

without effective DS (SHR 1.86 [CI 0.69–5.63]; *p* = 0.22) (Table S2).

Corresponding 4-year survival rates were arithmetically lower in the AC [47.8% (CI 24.5–67.9)] and the group that progressed to beyond MC or were not DS (53.6% [CI 41.7–64.0]) when compared with those patients achieving effective DS in the UCSF-DS group (63.0% [CI 43.9–77.2]) and the MC (64.9% (CI 60.0–69.3)] with no significant difference in survival between groups (*p* = 0.58) (Fig. S1). Table 2 describes and compares explant pathology findings and tumour burden at the last evaluation in patients receiving LRT and reassessment before LT. In patients achieving MC at the last tumour reassessment, a significant higher discrepancy between radiological tumour burden and pathological tumour burden was observed in the AC group (71.4%) compared with those within the UCSF-DS group (45.2%) and those remaining within MC (27.1%) (Table 2). The proportion of MVI was similar between patients who were successfully DS in the UCSF-DS group (23.8%) and patients remaining within MC. There was a significant higher proportion of MVI observed in the AC group that was not successfully DS (44.1%) and interestingly even after successful DS (52.4%) (Table 2 and Table S2).
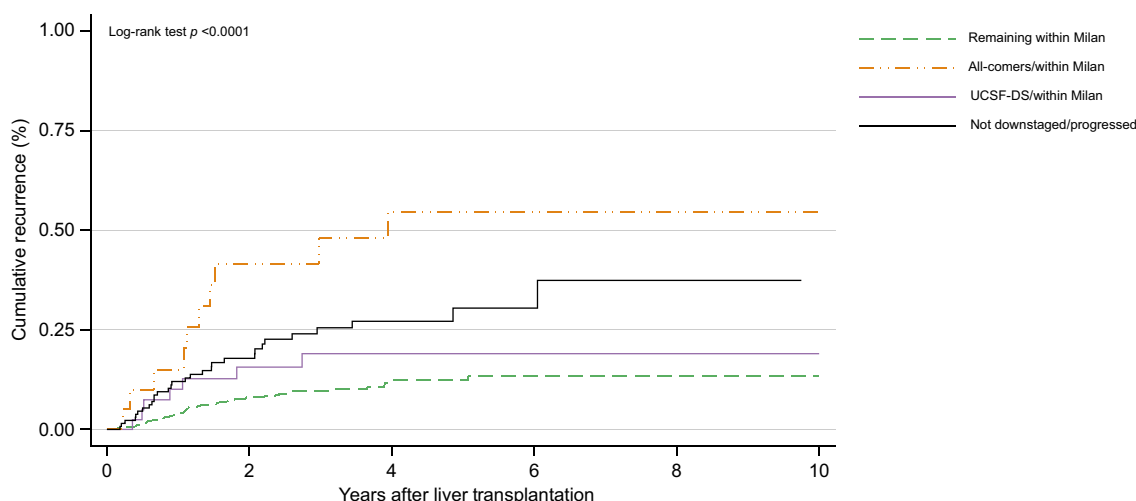
Fig. 2. Post-transplant cumulative recurrence among patients receiving locoregional therapies and with tumour reassessment before liver transplantation. unembolden. Kaplan Meier survival curves were compared using the log-rank test (Mantel-Cox). UCSF-DS, University of California San Francisco downstaging.

Table 2. Explant pathology findings according to groups at last evaluation in patients receiving locoregional therapies and reassessment before to liver transplantation.

	Categorisation at last evaluation				
Variable at explant analysis	Within MC, n = 680 (77.0%)	Within UCSF-DS and downstaged to MC, n = 42 (4.8%)	AC and downstaged to MC, n = 21 (2.4%)	Not downstaged or progressed, n = 140 (15.9%)	*p* value
Complete necrosis, n (%)	23 (3.4)	2 (4.8)	0	5 (3.6)	0.83
MVI, n (%)	142 (23.8)	10 (23.8)	11 (52.4)	48 (34.3)	<0.0001
Tumour grade >II, n (%)	171 (26.4)	17 (42.5)	4 (20.0)	40 (30.5)	0.12
Within MC, n (%)	496 (72.9)	23 (54.8)	6 (28.6)	50 (35.7)	<0.0001

AC indicates groups initially beyond the UCSF-DS and successfully downstaged to within MC. MC indicates group initially and remaining within MC between listing and last tumour reassessment. UCSF-DS indicates groups initially within the UCSF-DS and successfully downstaged to within MC. Categorical data were compared using Fisher's exact test or Chi-square test (2-tailed), as appropriate. AC, all-comers; MC, Milan criteria; MVI, microvascular invasion; UCSF-DS: University of California San Francisco downstaging.

Serum AFP values and cut-off as a useful tool for optimising DS selection criteria

We further evaluated independent prognostic factors at the time of listing associated with HCC recurrence after LT in all patients who received LRT as an ITT analysis for DS (Table 3). AC had a 2-fold increased risk for HCC recurrence, when compared with those meeting the UCSF-DS protocol (SHR 1.9 [CI 1.16–3.07]; *p* = 0.01). The presence of a major target HCC lesion >6 cm resulted in a 7-fold higher risk of recurrence compared with target lesions ≤3 cm (SHR 6.7 [CI 2.41–18.76]; *p* <0.0001). Patients presenting AFP values of 21–100 ng/ml (SHR 2.13 [CI 1.25–3.63]) and 101–1,000 ng/ml (SHR 2.59 [CI 1.36–4.94]) presented a higher risk of HCC recurrence independently of tumour burden

compared with those patients with AFP values of ≤20 ng/ml. Time on the waiting list, time since the last LRT to transplantation and LT period, and region (Europe *vs.* Latin America) were not associated with a higher risk of recurrence (Table 3).

Consequently, we evaluated if serum AFP values could identify better candidates for DS within the UCSF-DS group at listing. As shown in Fig. 3A, cumulative incidence of HCC recurrence proportionally increased in patients within UCSF-DS with AFP values from ≤20, 21–100, and 101–1,000 ng/ml, with the lowest cumulative incidence observed in those with AFP values below 20 ng/ml (Fig. 3A). From a competing risk regression analysis for HCC cumulative incidence of recurrence, AFP values below 20 ng/ml were associated with lower incidence of HCC recurrence

Table 3. Multivariable competing risk regression analysis of independently associated prognostic factors of HCC recurrence in patients receiving LRT attempted to be downstaged (n = 324).

	Unadjusted SHR (95% CI)	*p* value	Adjusted SHR (95% CI)	*p* value
Characteristics at listing				
Age	0.99 (0.96–1.02)			
Tumour burden				
UCSF-DS (n = 201)	--	-	-	-
All-comers (n = 123)	2.18 (1.37-3.45)	<0.0001	1.89 (1.16-3.07)	0.01
Major target lesion	1.18 (1.07-1.29)	<0.0001		
≤3 cm (n = 68)	-	-	-	-
3-6 cm (n = 223)	3.25 (1.29-8.22)	0.012	3.11 (1.22-7.89)	0.017
>6 cm (n = 50)	7.31 (2.70-19.77)	<0.0001	6.72 (2.41-18.76)	<0.0001
No. of HCC nodules	1.03 (0.91-1.16)	0.60		
1-3 nodules (n = 220)	–	-		
≥4 nodules (n = 121)	0.86 (0.53-1.42)	0.56		
AFP (ng/ml)	1.00 (1.00-1.01)	0.001		
≤20 ng/ml (n = 218)	-	-	-	-
21-100 ng/ml (n = 71)	1.79 (1.05-3.06)	0.032	2.13 (1.25-3.63)	0.005
101–1,000 ng/ml (n = 33)	2.46 (1.29-4.70)	<0.0001	2.59 (1.36-4.94)	0.004
WL characteristics				
WL time	0.99 (0.97-1.02)	0.81		
<3 months (n = 139)	–	-		
3-6 months (n = 55)	0.90 (0.48–1.68)	0.74		
>6 months (n = 130)	0.88 (0.53–1.47)	0.63		
Time from LRT to LT				
≤3 months (n = 59)	0.99 (0.85–1.15)	0.91		
>3 months (n = 27)	1.04 (0.43–2.52)	0.92		
Other confounding factors				
Region				
Europe (n = 214)	–			
Latin America (n = 110)	1.05 (0.64–1.72)	0.85		
Period of LT year				
2000-2012 (n = 244)	-	–		
2012-2018 (n = 80)	1.07 (0.59-1.93)	0.29		

Fine and Gray method for multivariable competing risk regression analysis to identify independently associated variables with HCC recurrence estimating cause-specific hazards and its corresponding 95% confidence intervals. AFP, alpha-foetoprotein; HCC: hepatocellular carcinoma; LT, liver transplantation; LRT, locoregional therapies; SHR, subdistribution hazard ratio; UCFS-DS, University of California San Francisco downstaging; WL, waiting list.
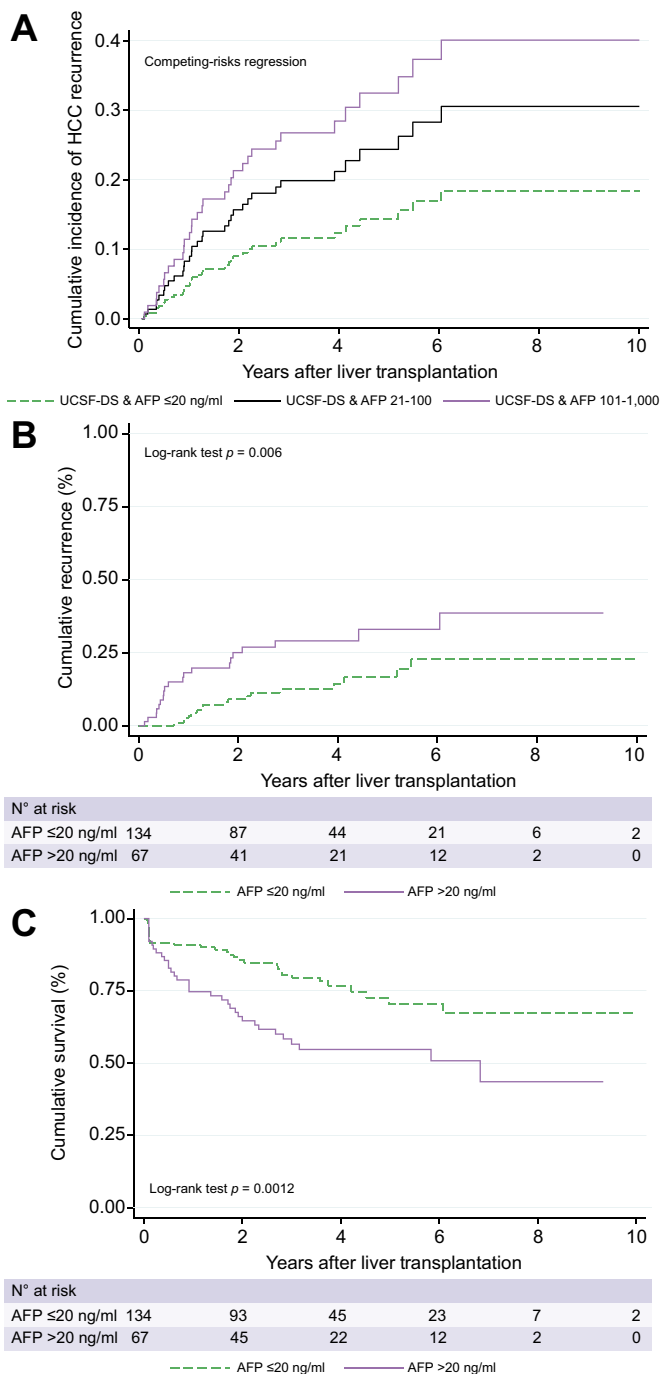
Fig. 3. Post-LT recurrence (A, B) and survival curves (C) among patients receiving locoregional therapies within UCSF-DS protocol according to AFP values at listing. (A) Grouped according to AFP values ≤20 ng/ml, 21–100 ng/ml, and 101–1,000 ng/ml. Cumulative incidence curves according to the Fine and Gray method for competing risk regression analysis. (B, C) Grouped according to AFP values ≤20 ng/ml and >20 ng/ml. Kaplan Meier survival curves were compared using the log-rank test (Mantel-Cox). AFP, alpha-foetoprotein; HCC, hepatocellular carcinoma; LT, liver transplantation; UCSF-DS, University of California San Francisco downstaging.

(22.2% [CI 13.5–36.6]) compared with AFP values higher than 20 ng/ml (32.2% [CI 21.1–47.3]) with a SHR of 2.01 (CI 1.02–4.00, p = 0.006). (Fig. 3B). This was also reflected in a higher 5-year

survival rate with AFP values of ≤20 ng/ml (70.9% [CI 59.8–79.5]) compared with AFP>20 ng/ml (50.0% [CI 36.1–62.5]; p = 0.001) (Fig. 3C). In the AC group, AFP values below 20 ng/ml were associated with lower risk of recurrence (SHR 2.48 [CI 1.39–4.48]; p = 0.002); however, 5-year recurrence rates were significantly high for both groups of AC presenting AFP ≤20 or >20 ng/ml (32.5% [CI 21.5–47.4] *vs.* 64.5% [CI 46.1–82.3]). Finally, AFP values below 20 ng/ml at the time of listing and achieving effective DS (n = 63) was associated with a lower risk of recurrence in this subgroup compared with those that were not DS (13.7% [CI 5.3–32.7] *vs.* 55.0% [CI 31.0–82.1]; SHR 4.43 [CI 1.41–13.92], p = 0.011).

Discussion

The outcome of patients with HCC lesions beyond the conventional MC receiving LT after DS is controversial. The reported higher recurrence rate might be as a result of heterogeneity in data and protocols. In prospective studies, using a stricter inclusion protocol and mandatory waiting period before LT results in similar outcomes compared with MC. This multinational cooperation is the first large, multicentre, cohort study outside the USA to validate the UCSF-DS protocol. We show, for the first time in a non-US cohort, that patients meeting UCSF-DS criteria at listing and successfully downstaged within MC have a similar risk of recurrence when compared with those patients initially and remaining within MC and also have similar 4-year survival rates. A second major finding of this study was that, among the group meeting UCSF-DS, AFP values at listing below or equal to 20 ng/ml identified a subgroup of patients with excellent post-LT survival and lower recurrence rates, thus suggesting that this biomarker should be added as a selection tool in these patients. This trend was even more impressive in patients with AFP <20 ng/ml at listing and achieving effective DS. These two important findings point out the need to maximise DS effort and deliver high consistency in the quality of DS, in particular in patients with favourable tumour biology, to reduce the risk of recurrence in patients with reasonably expanded criteria at listing. Interestingly, although Mehta *et al.*[9] found an AFP value below 100 ng/ml associated with better post-LT outcomes, our results show that a cut-off of 20 ng/ml is even more accurate in selecting the best candidates for DS protocols. This upper limit of AFP was also very recently identified in a study by Lai *et al.*[18] On the contrary, the AC group presented lower survival and higher recurrence rates even when successful DS was achieved. These results confirm that an upper limit in tumour burden exists beyond which successful LT after DS becomes an unrealistic goal.

The lower overall outcome in our cohort compared with that in other studies[9,19] can be a result of several factors. All the patients within MC from listing to the last assessment before LT also received LRT to remain within this subgroup. Most cohorts include patients only marginally outside MC before DS. Our study population in the UCSF-DS group was more advanced (22.7% of patients had at least 4 lesions; in 67.8%, the diameter of the largest lesion was 3–6 cm, and in 9.5%, it was more than 6 cm). The success rate of DS in our cohort was also lower than that reported by Yao *et al.*,[19] which was higher than 60%. Two meta-analyses including patients who underwent LT following DS after initial presentation outside the MC showed success rates of DS between 24 and 69%[20] or a pooled success rate exceeding 40%.[21]

We found that only half of the patients who were successfully DS in the UCSF-DS group were finally within MC on explant analysis. When looking further into the results, on explant pathology, 33.3% of the UCSF-DS group had MVI. This indicates that number and size of lesions cannot be the only predictive factor of outcome. Although in the sucessfully UCSF-DS group the rate of MVI was reduced to 23.8%, similarly to the group remaining within MC from listing, the group of AC, although successfully DS, showed a significant higher rate of MVI (52.4%). In other studies, there were no statistically significant differences between the DS and MC groups in all the histologic characteristics,[19] or the MVI rate was lower (14.4% of the MC group, 16.9% of UNOS-DS, and 23.7% of AC).[9] The importance of MVI as a determinant of tumour behaviour has be extensively studied. The drawback is that this marker can only be assessed on explant, which stresses the need for other surrogates such as AFP values.[9,22–25] In this regard, we assessed the role of the AFP level in predicting HCC recurrence in the DS group of our cohort and found that the subgroup of patients within the UCSF-DS protocol at listing with an AFP-value of >20 ng/ml had a 2-fold increasing rate of cumulative recurrence compared with the subgroup with an AFP value of ≤20 ng/ml. Consequently, to better select the best candidates for DS within the UCSF-DS protocol, we propose stratification according to AFP values as additional refinements to improve post-LT outcome. The exclusion of patients with an AFP value of >20 ng/ml should be the subject of further research and certainly deserves to be tested in external populations. Future studies should also address if DS to MC is really necessary or if extended criteria taking into account biological factors of tumour behaviour can serve as valuable new targets.[26–29] Very recently, the first open-label, multicentre randomised controlled trial was published including patients with HCC beyond the MC, defining successful DS as partial or complete response to LRT (both surgical and systemic treatments), taking also into account

the evolution in AFP and using a 5-year estimated post-LT survival of at least 50% as an inclusion criterion. The study showed that LT improved tumour event-free and overall survival after effective and sustained DS of eligible HCC beyond the MC compared with non-transplant options.[30]

The limitations of our study include the retrospective acquisition of the data, not taking into account dropouts or ITT outcome given that all included patients underwent transplantation. However, our aim was to evaluate only the post-LT outcomes. Because of the retrospective design of the study, there can be a heterogeneity in LRT regimens and assessment of radiological response between the different centres and within a centre in time. However, to avoid any information bias, data collection and outcome definitions were homogenous across cohorts, and central quality control of all registered data was performed. Furthermore, neither region (Europe *vs.* Latin America) nor LT period (2001–2011 and 2012–2018) could be withheld as a prognostic factor in multivariable competing risk regression analysis. We also acknowledge that the assessment of DS efficacy was performed on only 50% of the population entering a DS procedure.

In conclusion, a successful DS from UCSF criteria to MC resulted in similar recurrence and survival rates, compared with MC. Moreover, patients within UCSF-DS criteria and with AFP values below or equal to 20 ng/ml at listing have excellent survival and recurrence rates, not only after successful DS, but also when taking into account at the time point of listing. This biomarker might be therefore an additional clinical tool to better select candidates in the UCSF-DS group. On the contrary, AC have a higher recurrence and lower survival rates even after apparently successful DS, with biologically more aggressive tumours observed in the explant pathology analysis, notably frequent MVI. Their exclusion from LT should therefore be considered.

Abbreviations

AC, all-comers; AFP, alpha-foetoprotein; DS, downstaging; EASL, European Association for the Study of the Liver; HCC, hepatocellular carcinoma; HR, hazard ratio; ITT, intention to treat; LR, liver resection; LRT, locoregional therapies; LT, liver transplantation; MC, Milan criteria; MVI, microvascular invasion; PEI, percutaneous ethanol ablation; RFA, radiofrequency ablation; SHR, subdistribution hazard ratio; TACE, transarterial chemoembolisation; UCSF-DS, University of California San Francisco downstaging; UNOS, United Network for Organ Sharing; WL, waiting list.

Conflicts of interest

There are no conflicts of interest to declare.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

Conceptualisation and methodology: HD, FP, CD. Data collection: FP, HD, CC, AN, IFB, KB, CB, AC, PB, GME, JP, FM, FD, SHD, ES, UC, AG, CV, SF, FR, PB, HVV, DV, MS, CD. Data analysis: FP, FR, HD, CD

Data interpretation: FP, FR, HD, CD. Writing – original draft, review, and editing: HD, FP, CD. Read and agreed to the published version of the manuscript: All authors.

Data availability statement

The dataset during and/or analysed during the current study are available from the corresponding author on reasonable request.

Michele Colledan, Domenico Pinelli, Paolo Magistri, Giovanni Vennarecci, Marco Colasanti, Valerio Giannelli, Adriano Pellicelli, Cizia Baccaro.

Belgium: Helena Degroote, Hans Van Vlierberghe, Callebout Eduard, Iesari Samuele, Dekervel Jeroen, Schreiber Jonas, Pirenne Jacques, Verslype Chris, Ysebaert Dirk, Michielsen Peter, Lucidi Valerio, Moreno Christophe, Detry Olivier, Delwaide Jean, Troisi Roberto, Lerut Jan Paul.

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Supplementary data

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Author names in bold designate shared co-first authorship

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