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4 **Outcomes of Radiofrequency Ablation as First-Line Therapy for Hepatocellular**  
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6 **Carcinoma less than 3 cm in Potentially Transplantable Patients**  
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37 interpretation, critical appraisal of the and approved the final version.  
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45 **List of abbreviations:**  
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48 AFP: Alpha fetoprotein; CI: Confidence interval; CT: Computed tomography; HCC:  
49 Hepatocellular Carcinoma; HR: Hazard ratio; IQR: Interquartile range; LT: Liver  
50 transplantation; MC: Milan criteria; MR: Magnetic resonance; RFA: Radiofrequency ablation;  
51 TACE: Transarterial chemoembolization; UHN: University Health Network; US: Ultrasound  
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## Abstract

Background & Aim: Radiofrequency ablation (RFA) is an effective treatment for single hepatocellular carcinoma (HCC)  $\leq 3$ cm. Disease recurrence is common, and in some patients will occur outside transplant criteria. We aimed to assess the incidence and risk factors for recurrence beyond Milan criteria (MC) in potentially transplantable patients treated with RFA as first-line therapy.

Methods: We performed a retrospective cohort study of potentially transplantable patients with new diagnoses of unifocal HCC  $\leq 3$ cm that underwent RFA as first-line therapy between 2000-2015. We defined potentially transplantable patients as those aged  $< 70$  years without any comorbidities that would preclude transplant surgery. Incidence of recurrence beyond MC was compared across two groups according to HCC diameter at the time of ablation: (HCC  $\leq 2$ cm vs. HCC  $> 2$ cm). Competing risks Cox regression was used to identify predictors of recurrence beyond MC.

Results: We included 301 patients (167 HCC  $\leq 2$ cm and 134 HCC  $> 2$ cm). Recurrence beyond MC occurred in 36 (21.6%) and 47 (35.1%) patients in the HCC  $\leq 2$ cm and the HCC  $> 2$ cm groups, respectively ( $p=0.01$ ). The 1-, 3- and 5-years actuarial survival after RFA was 98.2%, 86.2% and 79.0% in the HCC  $\leq 2$ cm group vs. 93.3%, 77.6% and 70.9% in the HCC  $> 2$ cm group ( $p=0.01$ ). Tumor size  $> 2$ cm [HR 1.94 (95%CI 1.25-3.02)] and alpha fetoprotein levels at the time of ablation [100-1000ng/mL: HR 2.05 (95%CI 1.10-3.83)] were found to be predictors of post-RFA recurrence outside MC.

Conclusion: RFA for single HCC  $\leq 3$ cm provides excellent short- to medium-term survival. However, we identified patients at higher risk of recurrence beyond MC. For these patients, liver transplantation should be considered right after the first HCC recurrence after RFA.

## Lay Summary

Radiofrequency ablation (RFA) and liver transplantation are treatment options for early-stages of hepatocellular carcinoma (HCC). After RFA some patients will experience recurrence or metastatic spread of the initial tumor, or may develop new tumors within the liver. Despite close follow-up, these recurrences can in some cases progress rapidly and exceed transplant criteria, thereby preventing the patient from receiving a transplant and losing the potential for cure. In this study, we investigated the incidence and risk factors for recurrence beyond transplant criteria in patients treated with RFA that could have otherwise received a transplant. Among 301 patients, recurrence beyond transplant criteria occurred in 28%, despite undergoing close radiological follow-up. We identified that patients with HCC >2cm and higher serum alpha fetoprotein are at greater risk of recurrence beyond the transplant criteria. These data suggest that liver transplantation should be considered right after the first HCC recurrence for these patients.

## Highlights

- Most transplantable patients with single hepatocellular carcinoma  $\leq 3$ cm treated by RFA will eventually develop recurrent HCC distant to the ablation site.
- Many transplantable patients treated with HCC will recur beyond the Milan criteria despite close post-RFA surveillance, losing the opportunity for cure.
- Transplantable patients with tumors  $> 2$ cm and higher serum alpha fetoprotein have higher risk of recurrence beyond Milan criteria.

## Introduction

The optimal approach for transplantable patients with early unifocal HCC is unclear. Small HCC (BCLC-0 or A) can be treated with ablation, liver resection, or liver transplantation (LT) as first-line therapies.<sup>1</sup> Although the results of LT are excellent, most jurisdictions currently only assign MELD exception points to patients with larger HCC based on the belief that if resection or RFA do not work, transplantation can be undertaken as a second curative procedure.<sup>2</sup>

Radiofrequency ablation (RFA) of early HCC provides a 90% complete response<sup>3</sup> and a 5-year survival rate of 66-86% even in candidates unfit for resection.<sup>4,5</sup> Outcomes following RFA are similar to surgical resection, especially for lesions smaller than 3 cm.<sup>6</sup> HCC recurrence after ablation of small HCC occurs in up to 60-80% of patients by 5-years, mainly due to de novo tumors.<sup>7,8</sup> Unfortunately, there is little data on how often these recurrences exceed the criteria for a potentially curative liver transplant.<sup>9</sup> The present study was therefore undertaken to assess the incidence and risk factors for HCC recurrence outside of the Milan criteria (MC) after locoregional therapies in patients with unifocal HCC less than or equal to 3cm who were treated with RFA and had no contraindications for LT at the time of ablation. Our primary aim was to identify the characteristics of patients for whom transplantation might be the best first-line treatment.

## Methods

### *Study Design and Population*

This is a retrospective cohort study from a large academic institution, the University Health Network (UHN) in Toronto, Canada. We assessed all adults (age  $\geq 18$ -years old) with new

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4 diagnoses of solitary HCC up to 3cm who underwent RFA between February 1<sup>st</sup>, 2000 and  
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6 March 31<sup>st</sup>, 2015. All patients included in this study were reviewed by the UHN HCC  
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8 Multidisciplinary Board and were determined to be most appropriate for RFA as first-line  
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10 treatment. In our institution, all treatment decisions are individualized after a combined  
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12 consideration of tumor size, location, liver function, patient comorbidities, and functional status.  
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14 Patients with profound liver dysfunction (MELD >15) are typically referred for transplant  
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16 consideration as definitive therapy. In patients with well compensated liver function, either RFA  
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18 or surgery are offered as first line treatment. For lesions <2.0cm, RFA is offered as first line  
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20 therapy. For lesions between 2.0 and 2.5 cm, surgical resection or RFA are offered depending on  
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22 tumor location and patient characteristics. Patients with solitary tumor between 2.5 and 3 cm are  
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24 offered resection. Those that are not suitable for resection are evaluated on a case-by-case basis  
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26 for either RFA or transplantation. The choice between ablation or LT is made based on tumor  
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28 location and severity of underlying liver disease by consensus at the UHN HCC  
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30 Multidisciplinary Board. Portal hypertension was diagnosed in most cases with indirect measures  
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32 (platelet count <100.000 and or elevated bilirubin and/or elevated bilirubin and/or presence of  
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34 varices and/or splenomegaly). In cases where it was unclear, direct measurement was performed  
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36 with a hepatic venous pressure gradient, where significant portal hypertension was defined as a  
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38 gradient  $\geq 10$  mmHg. Supplementary Figure 1 shows the algorithm for the solitary HCC  $\leq 3$ cm  
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40 at our institution.

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51 The study population was composed of those patients that fulfilled all of the following  
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53 inclusion criteria: 1) new diagnosis of HCC based on imaging criteria consistent with  
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55 international guidelines<sup>10</sup> or by histological assessment of a radiologically indeterminate tumor;  
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58 2) single HCC up to 3cm in maximum diameter; 3) not a resection candidate 4) the patient  
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4 underwent RFA as first-line therapy for their HCC; 5) patients had achieved tumoral complete  
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6 response after a single ablation procedure; 6) the patient was otherwise a transplantable  
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8 candidate at the time of ablation. Transplantability was defined as any patient younger than 70  
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10 years old, with no medical comorbidities that would preclude transplantation. Complete response  
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12 was defined with the mRECIST criteria.<sup>11</sup> Patients who did not achieve complete tumoral  
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14 response after the first RFA, who did not qualify as transplantable, or underwent any other HCC  
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16 treatment before the first HCC recurrence were excluded. Patients who did not have complete  
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18 response after the first ablation were excluded to homogenize the cohort, as we were most  
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20 interested in the natural history of post-ablation disease recurrence, rather than incompletely  
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22 ablated tumors. In patients with single HCC  $\leq 3$ cm RFA was considered a bridging treatment if  
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24 the patient was already listed for LT (due to decompensated cirrhosis) and thereafter developed  
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26 HCC while listed; or if the indication for transplant was decompensated cirrhosis in association  
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28 with HCC, and the patient was therefore listed for LT prior to any HCC recurrence. Patients that  
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30 received RFA as a bridge to transplant were excluded from this analysis. Patients were divided in  
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32 two groups according to the HCC size at the time of ablation: HCC  $\leq 2$ cm and HCC  $> 2$ cm. This  
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34 study was approved by the UHN Research Ethics Board (CAPCR ID 16-5285). This study  
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36 complies with the ethical guidelines of the 1975 Declaration of Helsinki and no organs from  
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38 prisoners were used in this research. This study complies with the STROBE Statement for  
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40 observational studies.<sup>12</sup>

### 41 42 43 44 45 46 47 48 49 50 51 52 53 *Ablation Techniques*

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55 Patients were considered eligible for RFA under the following conditions: single HCC  
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57 under 3cm, acceptable liver function (Child-Pugh A or B), no encephalopathy (unless previous  
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4 mild encephalopathy and currently well controlled medically), and tumors were amenable to an  
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6 image guided procedure [ultrasound (US), computed tomography (CT) or combined US and CT].  
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9 Patients with well controlled ascites were considered eligible if all other conditions were met.  
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11 RFA was performed as an outpatient procedure. All procedures were performed by the same  
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13 team of interventional radiologists. All procedures were performed percutaneously using local  
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15 anesthesia and moderate sedation. HCCs were treated with LeVeen™ electrodes (Boston  
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17 Scientific, Natick, MA) or Cool-tip™ electrodes (Medtronic, Minneapolis, MN), according to  
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19 the manufacturers' recommended protocols. The intraprocedural monitoring was made with  
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21 ultrasound for those lesions visible sonographically to ensure the hyperechoic ablation zone  
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23 produced encompasses with adequate tumoral margins. For lesions that were sonographically  
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25 occult, CT was applied to adequate needle positioning prior to ablation and post-ablation to  
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27 ensure ablation zone was appropriate location and size to encompass lesion. Following RFA  
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29 treatment, patients underwent serum alpha-fetoprotein (AFP) measurements and contrast  
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31 enhanced CT or magnetic resonance (MR) imaging at one-month after the primary procedure  
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33 and, thereafter at 3 monthly intervals for two years, before reverting to 6-month ultrasound if no  
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35 recurrent disease was identified. Chest CT was performed every 6 months for the first two years  
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37 after ablation.  
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#### 48 *Patterns of Recurrence after RFA*

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51 The primary outcome was HCC recurrence beyond MC. This was assessed as a time-to-  
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53 event variable. All patients underwent surveillance after ablation as previously described. HCC  
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55 recurrence following RFA was defined by a new lesion on imaging, either radiologically  
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57 compatible with HCC or confirmed by histology. Recurrence was classified as local intrahepatic  
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4 (when the enhancing tumor reappeared at the previous ablation zone), distant intrahepatic (a new  
5 intrahepatic HCC at a separate location to the ablation zone) or extrahepatic (HCC outside the  
6 liver). Patients with more than one type of recurrence were classified according to the size of the  
7 greater intrahepatic tumor or as extrahepatic recurrence if an extrahepatic HCC were present.  
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9 Recurrence beyond MC was defined as recurrence with tumor size greater than 5cm for a single  
10 tumor, greater than 3cm for up to 3 tumors, more than 3 tumor deposits, macrovascular invasion,  
11 or extrahepatic disease. Patients were classified as within or beyond MC at the time of first  
12 recurrence (i.e. at the first-time patient had recurrent HCC diagnosed after ablation). Patients  
13 who were deemed within MC at the time of first recurrence could have undergone further  
14 ablation and recurrence beyond MC could be diagnosed at any time during the follow-up.  
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### 31 *Treatments of recurrence after ablation*

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33 At the time of HCC recurrence all patients were re-presented at HCC multidisciplinary  
34 rounds. Our general policy has been to re-ablate the intrahepatic HCC recurrence applying the  
35 same criteria we use for the primary treatment. Otherwise, if unsuitable for repeat RFA, patients  
36 would be considered for surgery or ‘salvage’ LT based on a combined assessment of tumor  
37 stage, liver function, and comorbidities. Other locoregional therapies such as transarterial  
38 chemoembolization would be considered if patients were not candidates for either ablation,  
39 surgery or transplantation, or used as bridging therapy to transplantation. We recorded the first  
40 treatment that was provided after recurrence. For those listed for transplantation at the time of  
41 recurrence, we recorded their outcome in the waiting list (i.e. whether they dropped out or were  
42 actually transplanted). Causes of death were also recorded and classified as related to HCC  
43 progression or not.  
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### *Statistical Methods*

Statistical methods included a descriptive assessment of our cohort using mean/standard deviation for normally distributed continuous variables, median/interquartile range (IQR) for non-normally distributed continuous variables, and counts/percentages for categorical variables. Differences between groups in terms of baseline variables and outcomes post RFA were determined using Chi-squared test for categorical variables, student T-test for normally distributed continuous variables, and Mann Whitney U test for non-normally distributed continuous variables. The study's primary outcome was time to recurrence beyond MC. Other outcomes of interest were overall recurrence beyond MC, overall HCC recurrence, recurrence-free survival and overall survival. Time to recurrence beyond MC was defined between the date of RFA and the date diagnosis of recurrence beyond MC. We took into account the competing risks between death not related to HCC and recurrence beyond MC. Recurrence-free survival was defined between the date of ablation and the date of diagnosis of HCC recurrence or death. To avoid confounding disease-recurrence outcomes, patients who underwent LT as treatment for recurrent disease were censored at the date of transplant. The recurrence beyond MC probabilities and survival probabilities were estimated by the Kaplan-Meier method and compared between groups using the logrank test. Univariate and multivariate Cox regression were performed to assess predictors for post-ablation recurrence beyond MC. All variables at baseline were included in the model and the final model was selected by stepwise backward approach. AFP levels were categorized as previously validated by [Duvoux et al.<sup>13</sup>](#) Patients were divided in 3 categories according to their serum AFP: <100 ng/mL, 100-1000 ng/mL and >1000 ng/mL. Proportional-hazard assumptions were evaluated using Schoenfeld residuals when

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4 applicable. To assess the potential impact of tumoral differentiation in the outcome, we repeated  
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6 the analysis for the subgroup of patients who had tumoral differentiation information available.  
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8 Results of these analyses are presented with hazard ratios (HR) with a 95% confidence interval  
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10 (95% CI). All statistical testing was performed in Stata 15.0 (Stata Corp., College Station, TX).  
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12 The median follow-up was 3.6 (IQR 2.1-5.5) years and 23 (7.6%) patients had less than 2-years  
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14 of follow-up.  
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## 21 **Results**

### 22 *Study Population*

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26 Between February 2000 and March 2015, a total of 635 patients with unifocal HCC  
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28 smaller than 3cm underwent RFA. Of those, 195 patients were older than 70-years or had  
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30 medical contraindications for LT at the time of ablation, 35 patients did not achieve complete  
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32 response with the first RFA and 104 underwent ablation as a bridge to LT and were excluded  
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34 from the study. Of these 104 bridged patients, 93 had already been listed for LT for  
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36 decompensated cirrhosis and developed an HCC while listed, and 11 patients were listed for LT  
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38 for decompensated cirrhosis prior to any HCC recurrence. The study group was therefore  
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40 composed of 301 patients (Figure 1). The median age was 59-years old (IQR 53 – 64) and 221  
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42 (73.4%) patients were male. Regarding the etiology of liver disease, 135/301 (44.9%) patients  
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44 had chronic hepatitis C (HCV) and 106/301 (35.2%) had chronic hepatitis B (HBV). The median  
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46 MELD score at the time of RFA was 9 (IQR 7 – 11) and 261/301 (86.7%) patients were Child-  
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48 Pugh class A. No patients were Child-Pugh class C. The median tumor size at the time of RFA  
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50 was 2cm (IQR 1.6-2.5). HCC histology at the time of RFA was available in 180/301 (59.8%)  
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52 patients; 38/180 (21.1%), 134/180 (74.4%), and 8/180 (4.5%) of those patients had well,  
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4 moderate, and poorly differentiated HCC respectively. Prior to ablation, serum AFP was  
5 available in 276/301 (91.7%) patients and the median serum AFP was 9 ng/mL (IQR 5-46).  
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9 Characteristics of the study cohort are summarized in Table 1.

#### 10 11 12 13 14 *Comparison of patients with tumors $\leq 2$ and $> 2$ cm*

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16 According to the tumor size at the time of the first ablation, 167/301 (55.5%) patients had  
17 an HCC  $\leq 2$  cm whereas 134/301 (44.5%) patients had an HCC  $> 2$  cm. Patients in the  $\leq 2$  cm group  
18 were slightly younger (57.6 vs. 60.1,  $p=0.01$ ) and less likely to have had a biopsy of the tumor  
19 (50.9% vs. 70.9%,  $p=0.001$ ). There were no statistically significant differences between patients  
20 in terms of sex, MELD, Child-Pugh score and serum AFP. Among patients with an available  
21 biopsy, patients with HCC  $> 2$  cm had greater proportion of moderately differentiated HCC [HCC  
22  $\leq 2$  cm 55 (64.7%) patients vs. HCC  $> 2$  cm 79 (83.2%) patients,  $p=0.012$ ]. The baseline  
23 characteristics of the two groups are summarized in Table 1.  
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#### 38 *Tumor Recurrence*

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40 HCC recurrence was diagnosed in 199/301 (66.4%) patients. According to initial HCC  
41 diameter at the time of ablation, 105 (62.9%) patients in the HCC  $\leq 2$  cm and 94 (70.1%) patients  
42 in the in the HCC  $> 2$  cm group experienced eventual recurrence ( $p=0.18$ ). The median  
43 recurrence-free survival was 1.98 (95% CI 1.57-2.40) years for the whole cohort. The median  
44 recurrence-free survival was 2.5 (95% CI 1.85-3.15) years for those with HCC  $\leq 2$  cm and 1.52  
45 (95% CI 1.24-1.81) years for those with HCC  $> 2$  cm ( $p=0.01$ ) (Figure 2-A). The 1-, 3-, and 5-  
46 year cumulative recurrence rate was 26.9%, 53.9% and 62.9% in the HCC  $\leq 2$  cm group vs.  
47 34.3%, 67.9% and 70.9% in the HCC  $> 2$  cm group ( $p<0.001$ ).  
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### *Patterns of HCC Recurrence After Ablation*

The first episode of recurrence after ablation occurred as distant intrahepatic recurrence in 139 (46.2%) patients, whereas 50 (16.6%) patients had local recurrence and 10 (3.3%) patients had extrahepatic recurrence. The time to recurrence was 1.1 years (IQR 0.6-2.4) in those with distant intrahepatic vs. 0.9 year (IQR 0.5-1.8) in those with local recurrence, and 1.5 years (IQR 0.4-2.9) in those with extrahepatic disease ( $p=0.42$ ). Table 2 shows the patterns of recurrence among the study groups.

Among patients with chronic hepatitis C, 12 (8.9%) patients had achieved sustained virologic response at the time of ablation. The rate of recurrence was 8.9% for patients with HCV and SVR and 8.8% for patients with HCV without SVR ( $p=0.99$ ). Among the 106 patients with chronic hepatitis B, 91 (85.8%) were suppressed at the time of ablation. The rate of recurrence among patients with HBV and virologic suppression was 59.3% and among patients with HBV without virologic suppression was 66.7% ( $p=0.59$ ).

### *Tumor Recurrence Beyond Milan criteria*

During follow-up, 83 (27.6%) patients had eventual recurrence beyond MC within a median time of 0.9 (IQR 0.5-1.8) years. According to initial tumor size, 36 (21.6%) patients in the HCC  $\leq 2$ cm group and 47 (35.1%) patients in the HCC  $> 2$ cm group had recurrence beyond MC ( $p=0.01$ ). Between the two initial HCC diameter groups, there were no differences regarding the specific reason for exceeding MC (number of lesions, size of lesions, vascular invasion, extrahepatic disease). Notably, the first recurrence after ablation was already beyond MC in 38 (12.6%) patients: 15/167 (9.0%) in the HCC  $\leq 2$ cm group and 23/134 (17.2%) in the HCC  $> 2$ cm

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4 group ( $p=0.03$ ) (Figure 2-B) – this was despite post ablation radiological surveillance. The  
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6 median time to recurrence amongst patients with first recurrence beyond MC was 1.4 (IQR 0.7-  
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8 2.8) years. These patients had regular radiological assessment that showed no recurrence until  
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10 the first evidence of tumor recurrence was already beyond Milan criteria. Table 2 summarizes  
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12 patterns of recurrence after ablation. Amongst patients who were diagnosed with recurrence  
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14 beyond MC at their first recurrence, 13/38 (34.2%) patients had a size or number of tumors that  
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16 exceeded criteria, 14/38 (36.8%) had macrovascular invasion and 11/38 (28.9%) had metastatic  
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18 disease. Figure 3 provides two cases where recurrence occurred beyond MC.  
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### 26 *Treatment after recurrence*

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28 As treatment for first HCC recurrence: 81/105 (77.1%) patients with initial HCC  $\leq 2$ cm  
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30 and 60/94 (63.8%) patients with initial HCC  $>2$ cm underwent repeat RFA, whereas LT was the  
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32 treatment for first recurrence in 7/105 (6.7%) patients in the HCC  $\leq 2$ cm group and 13/94  
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34 (13.8%) patients in the HCC  $>2$ cm group ( $p=0.28$ ). During the entire duration of follow-up,  
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36 39/105 (37.1%) patients in the HCC  $\leq 2$ cm group and 33/94 (35.1%) patients in the HCC  $>2$ cm  
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38 group were listed for LT ( $p=0.76$ ), of whom 30/39 (76.9%) patients with HCC  $\leq 2$ cm and 18/33  
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40 (54.5%) patients with HCC  $>2$ cm were eventually transplanted ( $p=0.04$ ). The proportion of  
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42 patients that dropped-out due to tumor progression was significantly lower in the HCC  $\leq 2$ cm  
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44 group than the HCC  $>2$ cm group [9/39 (23.1%) vs. 15/33 (45.5%)] ( $p=0.04$ ). Table 3  
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46 summarizes treatments after recurrence.  
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### 56 *Predictors of Recurrence Beyond Milan Criteria after Ablation*

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4 Table 4 shows the results of univariate and multivariate analyses. Predictors of recurrence  
5 beyond MC were tumor size >2cm [HR 1.94 (95% CI 1.25-3.02)] and AFP at the time of  
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9 ablation [HR 2.05 (95% CI 1.10-3.83) for AFP 100-1000 ng/mL]. In a sensitivity analysis of  
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12 patients who had tumor biopsies, we identified that poorly differentiated HCC was associated  
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14 with increased risk of recurrence beyond MC [HR 4.45 (95% CI 1.20-16.61)] (Supplementary  
15  
16 Table 1).  
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### 21 *Overall Survival*

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23 Median survival after ablation for the entire cohort was 7.8-years. Figure 4 shows  
24 survival probabilities according to tumor size. The 1-, 3- and 5-years actuarial survival after  
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27 ablation was 98.2%, 86.2% and 79.0% in the HCC  $\leq$ 2cm group vs. 93.3%, 77.6% and 70.9% in  
28  
29 the HCC >2cm group (p=0.01). HCC progression was the cause of death in 75/301 patients  
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31 (24.9%) [35/167 (21.0%) patients in the HCC  $\leq$ 2cm group and 40/134 (29.9%) in the HCC >2cm  
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36 group (p=0.08)].  
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### 41 **Discussion**

42  
43 There are conflicting data on the best management for patients with early stage HCC who  
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46 are otherwise candidates for transplant. In this study, small HCC treated with RFA in patients  
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49 who would have also been transplant candidates had a 66% recurrence rate. 42% of these  
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52 recurrences exceeded the MC disqualifying them from a curative treatment option in  
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55 jurisdictions that limit transplantation to the MC. In contrast, if the early stage HCC patients in  
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65 this study had been treated with LT instead of RFA we would have expected a 5-year survival



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4 rate of ~75-80% and a 5-year recurrence rate of <15%.<sup>14</sup> Risk factors for recurrences beyond the  
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6 MC after RFA included tumors >2cm, and a serum AFP >100ng/mL.  
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9 The association between higher recurrence rates after RFA and larger tumor size and  
10 higher AFP levels has been previously documented. Tsuchiya et al. performed a retrospective  
11 study of 323 patients with HCC within MC (including patients with multifocal and larger  
12 tumors) that received first-line RFA<sup>9</sup> and identified an AFP level >100ng/mL and tumor size  
13 >2cm as risk factors for recurrence outside MC. Cho et al. included 438 patients with unifocal  
14 HCC <3cm who received first-line RFA.<sup>15</sup> Variables associated with initial recurrence beyond  
15 MC included tumor size >2cm and tumor location adjacent to the colon, presumably due to  
16 technical limitations of RFA. None of these studies specifically examined these risk factors in  
17 the select population of potentially transplantable patients who were the subjects of the present  
18 study. We identified a local recurrence rate of 13.2% in patients with HCC  $\leq$ 2 cm in accordance  
19 with other retrospective studies<sup>16,17</sup>, but slightly higher than reported in clinical trials<sup>18,19</sup>. The  
20 probable explanation for this higher incidence when compared to clinical trials is the ‘real-world’  
21 nature of our data.  
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40 Survival differences after RFA related to initial HCC size have also been previously  
41 reported. Kutlu et al. demonstrated that patients with tumors greater than 3 cm have impaired  
42 survival after RFA when compared to surgery and transplantation.<sup>20</sup> However, in contrast with  
43 the present study, survival differences between patients with HCC  $\leq$ 2cm and >2cm diameter  
44 were not significant. However, the Kutlu et al. study did not perform an intention-to-treat  
45 comparison, made no distinction about transplant candidacy of included patients at baseline, and  
46 had limited data regarding severity of liver disease, or of recurrence outcomes. In our study, the  
47 5-year overall survival of patients with tumors >2cm was 71%. However, recurrence was almost  
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4 universal. Patients with tumors >2cm had a higher recurrence rate beyond Milan when compared  
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6 to those with tumors <2 cm.  
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9 This study shows that up to 2/3 of transplantable patients with HCC  $\leq$ 3cm treated with  
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11 ablation as first line therapy, will recur. The most common location of recurrence was  
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13 intrahepatic and distant to the initial ablation site; this is not surprising, given that a past history  
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15 of HCC is a well-known risk factor for new lesions.<sup>21</sup> New ablation techniques continue to be  
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17 developed and utilized, including multi-bipolar<sup>22</sup> and microwave ablation<sup>23</sup>. Since the majority of  
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19 our recurrences beyond MC were not local, we speculate that these newer techniques may not  
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21 reduce recurrence rates although this question certainly merits further study.  
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26 It has been speculated that new direct acting antivirals against HCV may affect HCC  
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28 recurrence rate after RFA for HCCs.<sup>24</sup> In the present study, we did not find an impact of SVR on  
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30 the recurrence rate among patients with HCC treated with RFA but these agents only became  
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32 available in Ontario in 2014, towards the end of the interval reviewed in this study. The impact  
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34 of SVR on HCC recurrence after RFA remains unresolved and requires further study.  
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39 The treatment of recurrent HCC after first-line therapy with ablation is also not  
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41 standardized. Re-ablation, liver resection and LT are valid options.<sup>25</sup> Because of the organ  
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43 supply, many centers utilize further locoregional therapies for recurrence, with salvage transplant  
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45 considered on a case-by-case basis.<sup>26</sup> However, there is data suggesting that higher survival rates  
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47 can be achieved with salvage transplantation as compared with resection or re-ablation for HCC  
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49 recurrence.<sup>27,28</sup> Most of these studies do not take into account the LT waiting list dropout rate  
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51 which was 33% in our series, higher than previous reports.<sup>29-31</sup> Drop-out rates are likely higher at  
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53 our center because of our practice to list patients for LT with tumors beyond the MC, a group  
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4 with a higher risk of dropout.<sup>32</sup> Importantly, the dropout rate was higher for those patients with  
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6 initial tumors >2cm when compared to smaller tumors.  
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9 A limitation of this study is the retrospective, observational study design. However, in the  
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11 current era it would be very challenging to undertake a prospective, randomized trial of different  
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13 management strategies for very early/early stage HCC. We did not assess the impact of multi-  
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15 bipolar ablation, combined therapies for HCC recurrence (e.g. RFA and TACE) or emerging  
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17 therapies such as radioembolization. We do not believe this to be a significant limitation since  
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19 RFA remains the most commonly used treatment for early HCC in unresectable patients.<sup>5,33</sup>  
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21 Strengths of this study include the intention-to-treat and competing risks analysis (thereby more  
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23 accurately reflecting real-world outcomes) and an outcome measure (recurrence within MC) that  
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25 is relevant to most LT centers worldwide.  
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31 In summary, our study suggests that transplantation rather than ablation may offer  
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33 patients with >2cm tumors or serum AFP >100ng/mL the best chance of cure. In regions where  
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35 it is impossible to offer LT as the initial treatment to patients with these risk factors, they should  
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37 be considered for enhanced post-treatment surveillance after RFA with prompt referral for LT  
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39 whenever there are worrisome findings. The ideal surveillance protocol for patients with high-  
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41 risk of post ablation recurrence is still unknown and should be the aim of future investigation.  
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## 48 **Conclusion**

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50 RFA as first-line therapy for HCC  $\leq 3$ cm provides excellent short- to medium-term  
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52 survival rates. However, recurrent or new tumors develop in more than 2/3 of these patients. We  
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54 identified risks factors for developing recurrence beyond MC including AFP level and tumor size  
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56 greater than 2cm. This analysis suggests that patients at higher risk of recurrence beyond MC  
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4 should be considered for LT earlier in their treatment pathway, ideally after their first HCC  
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6 recurrence.  
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4 **Figures legends:**  
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9 **Fig. 1. Study flowchart.**  
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11 **Fig. 2. A – Recurrence-free survival probabilities among patients with HCC >2cm and**  
12 **≤2cm treated with first-line ablation. B – Cumulative hazards of recurrence beyond Milan**  
13 **criteria among patients with HCC >2cm and ≤2cm treated with first-line ablation.**  
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19 **Fig. 3. Computed tomography scans showing the control image at 3-months after ablation**  
20 **(left) and at the time of first recurrence (right). Patient A with multifocal HCC recurrence**  
21 **12 months after ablation. Patient B with local recurrence and tumoral portal vein**  
22 **thrombus 18 months after ablation.**  
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28 **Fig. 4. Survival probabilities between transplantable patients with HCC >2cm and ≤2cm**  
29 **after first-line ablation.**  
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Variable	Overall n = 301	Tumor Size		
		HCC ≤2cm n = 167	HCC >2cm n = 134	p*
Male (%)	221 (73.4)	128 (77.2)	92 (68.7)	0.11
Age, median (IQR)	58.8 (53.3-64.1)	57.6 (51.5-63.5)	60.1 (55.5-64.6)	0.01
Etiology (%)				0.8
HCV	135 (44.9)	71 (42.5)	64 (47.8)	
HBV	106 (35.2)	61 (36.5)	45 (33.6)	
ETOH	24 (8)	15 (9)	9 (6.7)	
NASH	28 (6)	11 (6.6)	7 (5.2)	
Other	18 (6)	9 (5.4)	9 (6.7)	
Biopsy, yes (%)	180 (59.8)	85 (50.9)	95 (70.9)	0.01
Differentiation (%)				0.01
Well	38 (21.1)	26 (30.6)	12 (12.6)	
Moderate	134 (74.4)	55 (64.7)	79 (83.2)	
Poor	8 (4.5)	4 (4.7)	4 (4.2)	
AFP, ng/mL, median (IQR)	9 (5-45.8)	8 (5-28)	12 (5-67)	0.06
AFP categories, ng/mL (%)				0.01
0 – 100	263 (87.4)	150 (89.8)	113 (84.3)	
101 – 1000	28 (9.3)	16 (9.6)	12 (9.0)	
>1000	10 (3.3)	1 (0.6)	9 (6.7)	
Child-Pugh Score (%)				0.09
A	261 (86.7)	150 (89.8)	111 (82.8)	

B	40 (13.3)	17 (10.2)	23 (17.2)	
C	0	0	0	
MELD Score, median (IQR)	9 (7-11)	9 (7-11)	9 (7-11)	0.65
Total bilirubin ( $\mu\text{mol/L}$ ), median (IQR)	16 (10-23)	15 (10-23)	16 (9-24)	0.94
INR, median (IQR)	1.1 (1.0-1.2)	1.1 (1.03-1.2)	1.09 (1.0-1.2)	0.80
Creatinine ( $\mu\text{mol/L}$ ), median (IQR)	72 (65-83)	72 (65-83)	72 (64-84)	0.57
Albumin (g/L), median (IQR)	39 (35-42)	39 (36-42)	39 (35-42)	0.36
Platelet count ( $\times 10^9/\text{L}$ ), median (IQR)	101 (75-151)	106 (75-155)	98 (75-150)	0.86
Hepatitis C with SVR, yes (%) <sup>†</sup>	12 (15.8)	6 (15.0)	6 (16.7)	0.85
Hepatitis B with suppression, yes (%) <sup>††</sup>	91 (85.8)	51 (83.6)	40 (88.9)	0.44

\* Chi-squared test for categorical variables and the Mann-Whitney U test for continuous variables. <sup>†</sup>

Among 135 patients with viral hepatitis C. <sup>††</sup> Among 106 patients with hepatitis B

HCC: Hepatocellular carcinoma; HCV: Chronic hepatitis C; HBV: Chronic hepatitis B; ETOH: alcoholic liver disease; NASH: Nonalcoholic steatohepatitis; AFP: Alpha fetoprotein.

**Table 2: Patterns of recurrence after RFA as first-line therapy for patients with HCC  $\leq$ 3cm.**

Recurrence patterns	Overall n = 301	According to HCC size		
		HCC $\leq$ 2cm n = 167	HCC >2cm n = 134	p*
Recurrence, yes (%)	199 (66.1)	105 (62.9)	94 (70.1)	0.18
First recurrence type (%)				
Local	47 (15.6)	20 (12)	27 (20.1)	0.07
Distant intrahepatic	139 (46.2)	81 (48.5)	58 (43.3)	0.38
Distant extrahepatic	10 (3.3)	2 (1.2)	8 (5.9)	0.02
Beyond Milan criteria (%)				
At first recurrence	38 (12.6)	15 (9.0)	23 (17.2)	0.03
At any time during the follow-up	83 (27.6)	36 (21.6)	47 (35.1)	0.01
Reason to being classified as beyond Milan criteria <sup>¶</sup> (%)				
Tumor size and/or number	29 (34.9)	11 (30.6)	18 (38.3)	0.78
Macrovascular invasion	30 (36.1)	15 (41.7)	15 (31.9)	0.36
Metastatic disease	24 (28.9)	10 (27.8)	14 (29.8)	0.84

\* Chi-squared test for categorical variables and the Mann-Whitney U test for continuous variables.

† Among the 83 patients who had recurrence beyond Milan Criteria. HCC: Hepatocellular carcinoma.

Recurrence treatment	Overall n = 199	According to HCC size		
		HCC ≤2cm n = 105	HCC >2cm n = 94	p*
First treatment after recurrence (%)				0.45
Repeated ablation	141 (70.9)	81 (77.1)	60 (63.8)	
Liver Transplant	20 (10.1)	7 (6.7)	13 (13.8)	
TACE	7 (6.4)	3 (2.9)	6 (6.4)	
Liver resection	6 (3.0)	3 (2.9)	3 (3.2)	
SBRT	11 (5.5)	6 (5.7)	5 (5.3)	
Sorafenib	2 (1.0)	0	2 (2.1)	
Best supportive care	10 (5.0)	5 (4.8)	5 (5.3)	
Listed for liver transplant anytime during follow-up (%)	72 (36.2)	39 (37.1)	33 (35.1)	0.76
Wait list outcome (%)				
Dropout	24 (33.3)	9 (23.1)	15 (45.5)	0.04
Underwent LT	48 (66.7)	30 (76.9)	18 (54.5)	

\* Chi-squared test for categorical variables and the Mann-Whitney U test for continuous variables.

HCC: Hepatocellular carcinoma; TACE: Transarterial chemoembolization; SBRT: Stereotactic body

radiation therapy.

**Table 4: Multivariable regression model to predict recurrence beyond Milan criteria in transplantable patients with single HCC  $\leq 3$ cm treated with first-line RFA**

Variable	Univariable		Multivariable	
	HR (95% CI)	p	HR (95% CI)	p*
Sex [ref.: male]	1.37 (0.80-2.32)	0.25	-	-
Age	1.00 (0.97-1.03)	0.84	-	-
Etiology of liver disease [ref.: HCV]				
HBV	0.75 (0.46-1.23)	0.26	-	-
ETOH	0.58 (0.24-1.37)	0.21	-	-
NASH	0.16 (0.02-1.21)	0.08	-	-
Other	1.04 (0.48-2.25)	0.92	-	-
HCC size, cm	1.46 (0.88-2.44)	0.14	-	-
HCC size [ref.: $\leq 2$ cm]				
>2cm	1.94 (1.27-2.99)	0.01	1.94 (1.25-3.02)	0.01
AFP, ng/mL	1.00 (0.99-1.01)	0.90		
AFP [ref.: $\leq 100$ ng/mL]				
101 – 1000ng/mL	1.98 (1.06-3.70)	0.03	2.05 (1.10-3.83)	0.02
>1000ng/mL	2.64 (1.06-6.55)	0.04	2.06 (0.82-5.17)	0.12

Child-Pugh score [ref.: A]				
B	0.83 (0.40-1.75)	0.63	-	-
MELD	1.05 (0.98-1.13)	0.16	-	-
Platelets (x10 <sup>9</sup> /L)	1.00 (0.99-1.01)	0.21	-	-
Hepatitis C with SVR [ref.: no]	0.82 (0.29-2.31)	0.71	-	-
Hepatitis B with suppression [ref.: no]	0.97 (0.38-2.81)	0.96	-	-
<p>* Stepwise backwards Cox regression.</p> <p>HCV: Chronic hepatitis C; HBV: Chronic hepatitis B; ETOH: alcoholic liver disease;</p> <p>NASH: Nonalcoholic steatohepatitis; HCC: Hepatocellular carcinoma; AFP: Alpha fetoprotein.</p>				

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Figure 1  
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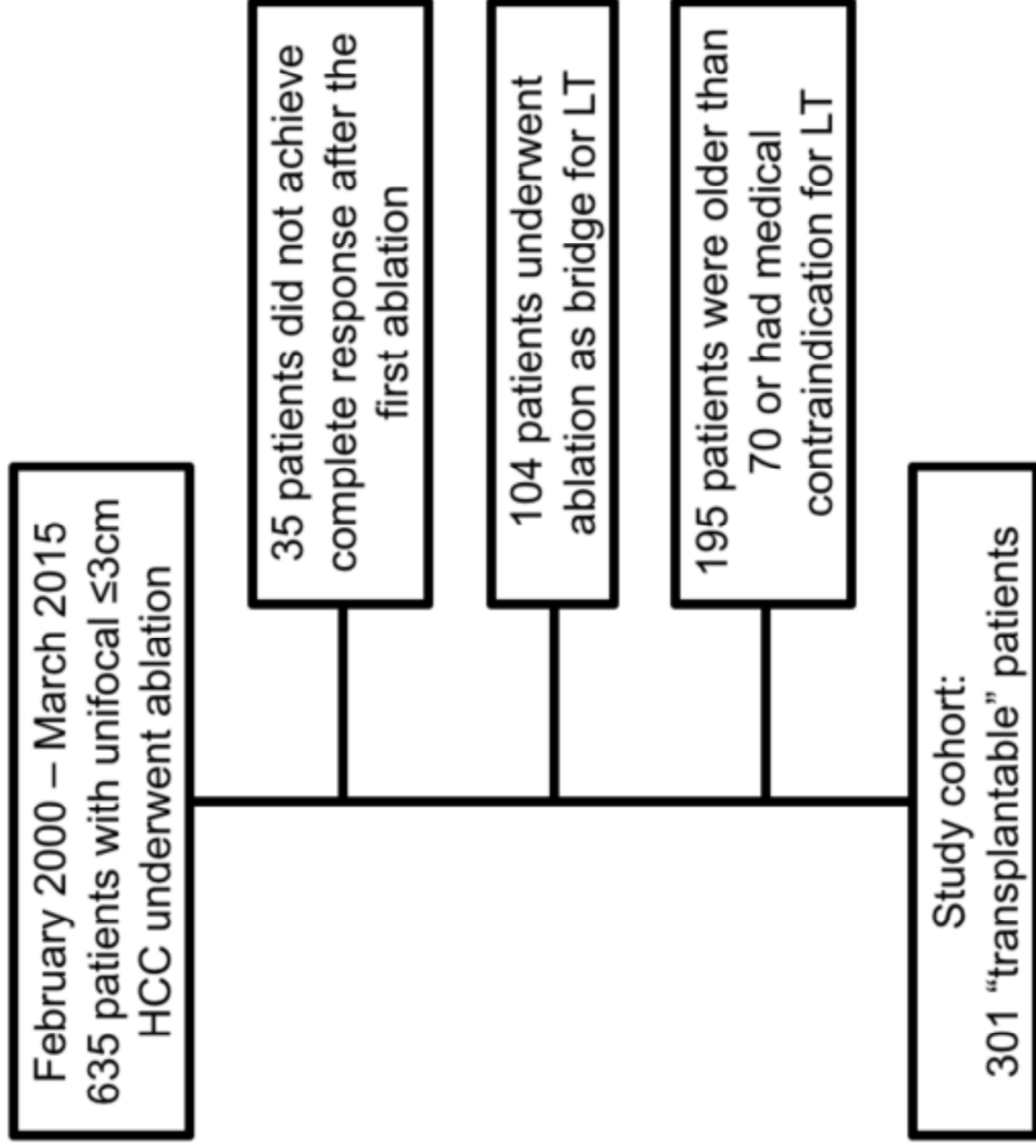


Figure 2

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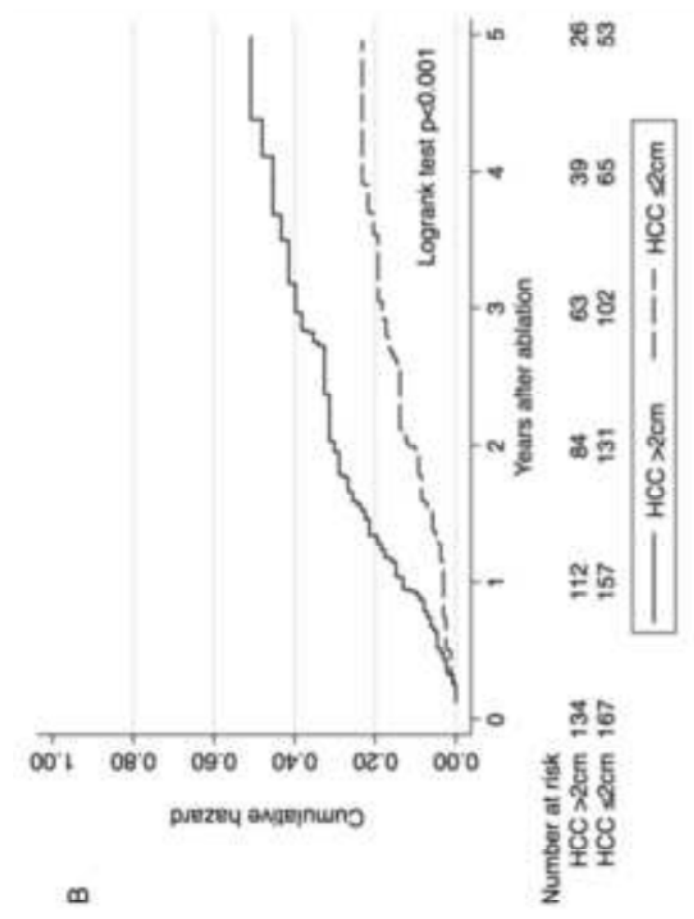
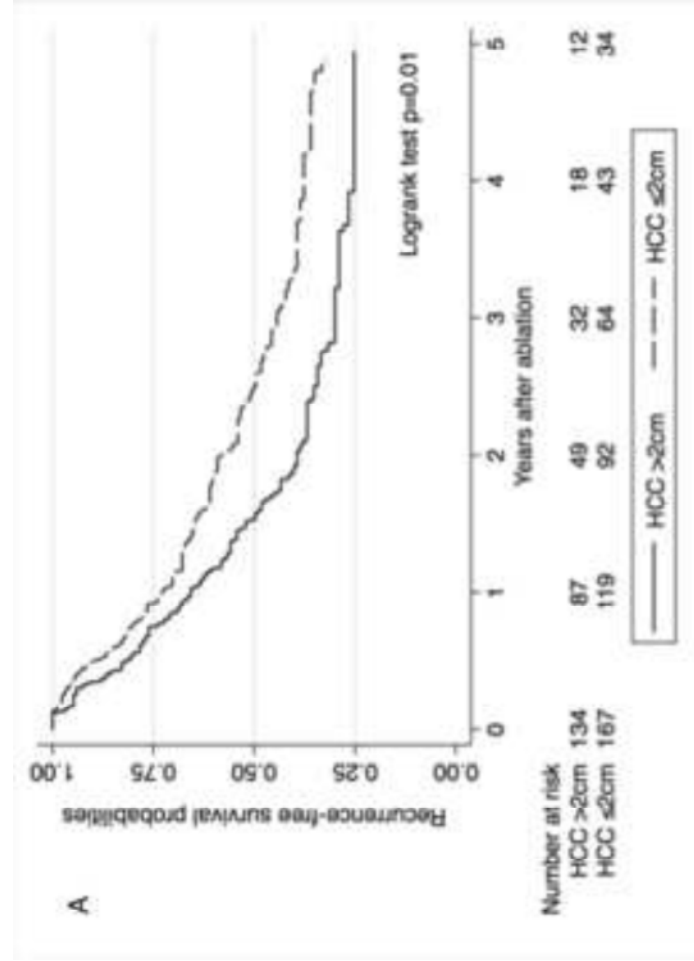


Figure 3

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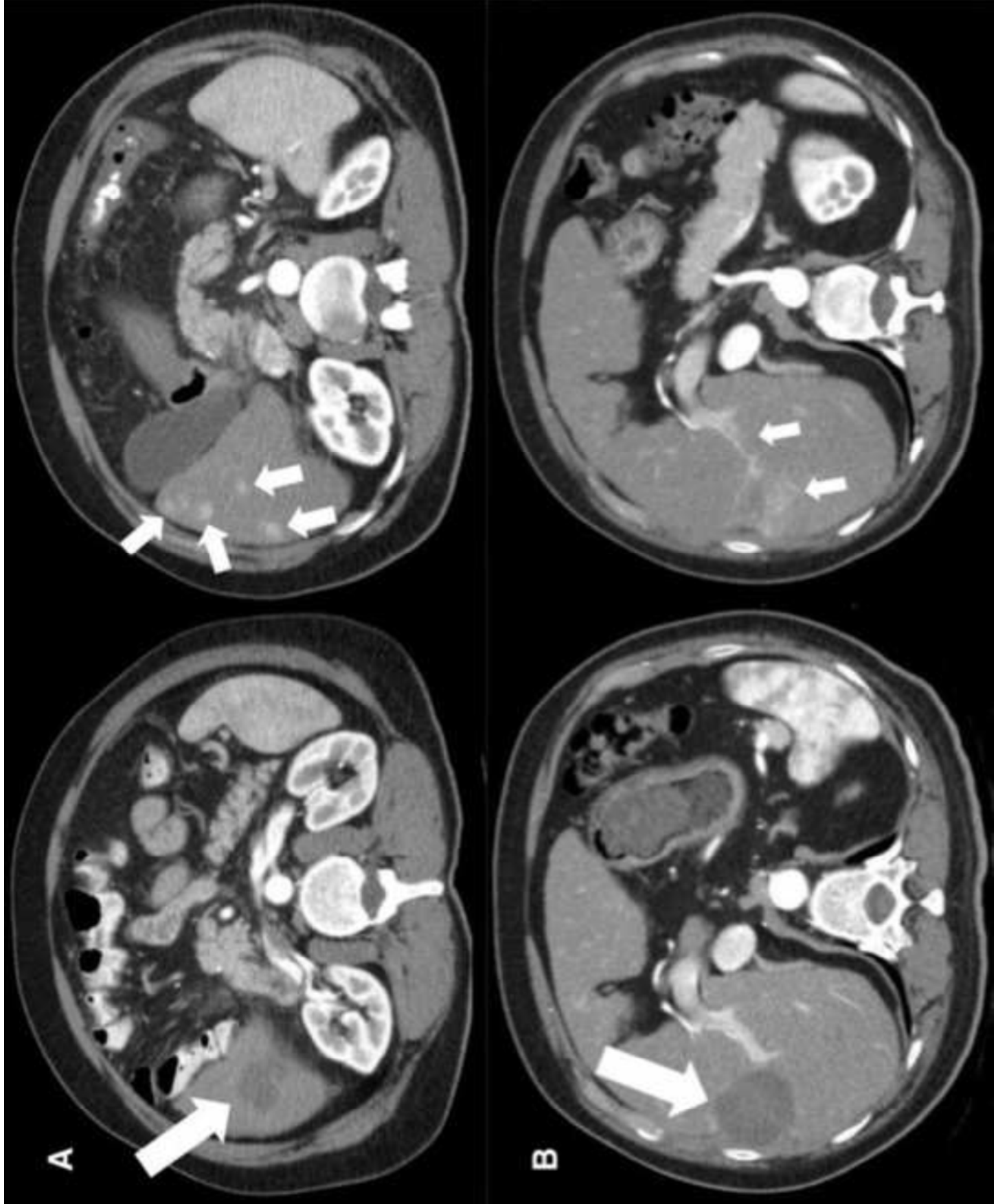
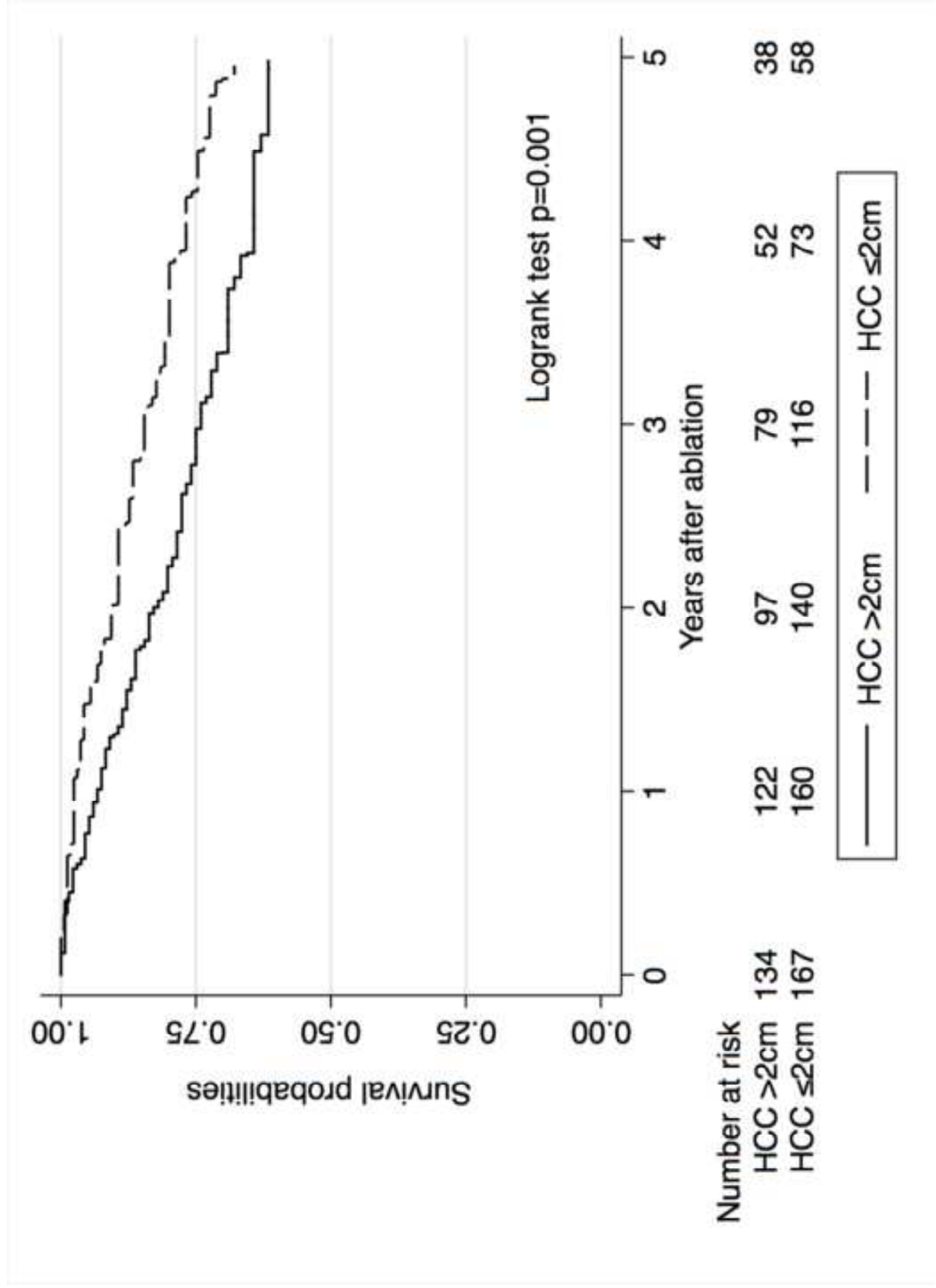
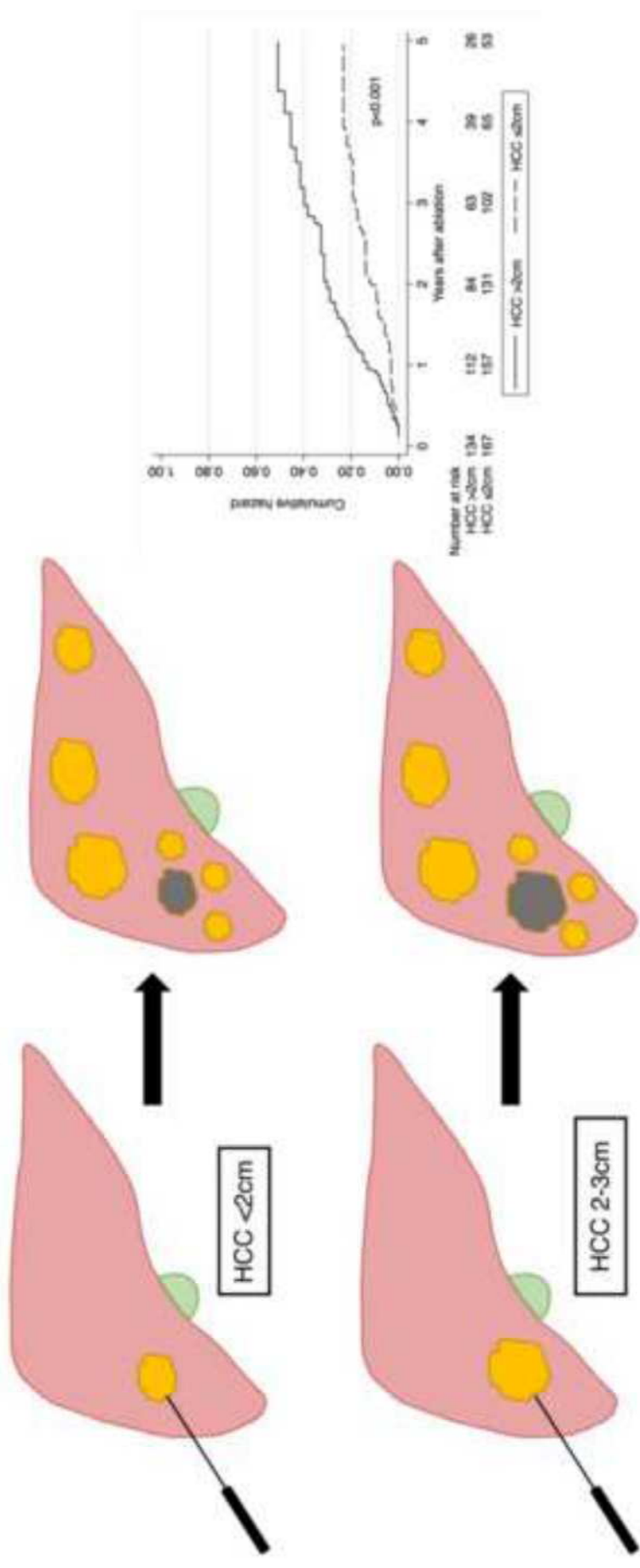


Figure 4  
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## Highlights

- Most transplantable patients with single hepatocellular carcinoma  $\leq 3$ cm treated by RFA will eventually develop recurrent HCC distant to the ablation site.
- Many transplantable patients treated with HCC will recur beyond the Milan criteria despite close post-RFA surveillance, losing the opportunity for cure.
- Transplantable patients with tumors  $> 2$ cm and higher serum alpha fetoprotein have higher risk of recurrence beyond Milan criteria.