Prevention of Hepatocellular Carcinoma Recurrence With alpha-Interferon After Liver Resection in HCV Cirrhosis

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Tumor recurrence after resection of hepatocellular carcinoma (HCC) can occur early (<2 years) or late (>2 years) as metastases or de novo tumors. Interferon (IFN) has the potential for chemoprevention against hepatitis C virus (HCV)-related cirrhosis. A predetermined group of 150 HCV RNA-positive patients undergoing resection of early- to intermediate-stage HCC was stratified into 80 HCV-pure (hepatitis B anticore antibody [anti-HBc]–negative) and 70 mixed HCV+hepatitis B virus (HBV) (anti-HBc–positive) groups, then randomized to IFN-α (3 million units 3 times every week for 48 weeks [n = 76]) versus control (n = 74). The primary end point was recurrence-free survival (RFS); secondary end points were disease-specific and overall survival. Intention-to-treat and subgroup analysis on adherent patients were conducted. Treatment effects on early/late recurrences were assessed using multiple Cox regression analysis. No patient experienced life-threatening adverse events. There were 28 adherent patients (37%). After 45 months of median follow-up, overall survival was 58.5%, and no significant difference in RFS was detectable between the two study arms (24.3% vs. 5.8%; P = .49). HCC recurred in 100 patients (48 IFN-treated, 52 controls), with a 50% reduction in late recurrence rate in the treatment arm. HCC multiplicity and vascular invasion were significantly related to recurrence (P = .01 and .0003). After viral status stratification, while no treatment effect was apparent in the mixed HCV+HBV population and on early recurrences (72 events), there was a significant benefit on late recurrences (28 events) in HCV-pure patients adherent to treatment (HR: 0.3; 95% CI: 0.09-0.9; P = .04). In conclusion, IFN does not affect overall prevention of HCC recurrence after resection, but it may reduce late recurrence in HCV-pure patients receiving effective treatment. (HEPATOLOGY 2006;44:1543-1554.)
Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide, with an increasing incidence because of the spread of hepatitis C virus (HCV) infection. Only a minority of patients are eligible for potentially curative treatments, and the prognosis is poor in the remaining patients, with mortality rates almost equaling the yearly incidence.

Among the curative treatments, liver transplantation has limitations (graft availability, selection criteria, cost), and resection and physical local ablation are burdened by a 50% recurrence rate at 3 years. Recurrent tumors following resection are either subclinical metastases missed during primary treatment (early recurrences) or de novo HCC from persistent fibrosis and HCV-related carcinogenicity in the remaining liver (late recurrences). Potential benefits of interferon (IFN) in preventing HCC recurrence after resection or ablation have been suggested by three randomized controlled trials (RCTs) studying a total of 154 patients in Asia. In addition, a retrospective analysis of 913 Caucasian patients suggested that IFN-related HCC prevention may appear only in HCV-pure infections as opposed to hepatitis B virus (HBV)+HCV–infected patients, with previous HBV contact determined by serum hepatitis B anti-core antibody (anti-HBc).

Based on these findings, we designed a RCT for patients with HCV-related cirrhosis undergoing potentially curative HCC removal. The primary end point was to assess if IFN could prevent or delay cancer recurrence; therefore, after stratification based on concomitant anti-HBc status, patients were randomly assigned to IFN treatment versus control. The results reported here could contribute to a more systematic approach to the complex issue of HCC chemoprevention.

Patients and Methods

Study Design, Eligibility, and Randomization. The proposal for a multicenter clinical trial originated in 1997 at the National Cancer Institute of Milan, where hepatologists and surgeons from the four participating centers formed a committee together with independent experts. In June 1998, the protocol and informed consent form, which was prepared in accordance with the Declaration of Helsinki, were approved by the Institutional Scientific and Ethic Committee (#98/016, identification number NCT00273247; available at www.clinicaltrial.gov). The trial was intentionally designed as an unrestricted collection of patients with histologically proven HCC and HCV-related cirrhosis who had been selected for surgical resection as elective/rescue treatment. Inclusion and exclusion criteria, trial design, and study development are shown in Fig. 1.

From June 1998 to November 2002, of 190 consecutive HCV RNA–positive/hepatitis B surface antigen–negative Caucasian patients with HCC undergoing liver resection in four surgical centers, 161 met the predetermined selection criteria and 150 were eventually randomized within 6 weeks of surgery. Previous contact with HBV (determined by anti-HBc status) was considered to be a stratification criterion. Symptomatic treatment of underlying liver disease or complications was allowed at any time in both groups during follow-up. Modification of inclusion/exclusion criteria to improve patient compliance was not allowed.

Sequence generation, stratum assignment, and randomization were computer-driven and centralized at the National Cancer Institute of Milan (also accounting for two thirds of the operations) in a protected database that did not disclose individual or center-specific information. Patient allocation was performed via telephone from the coordinating office after confirmation of eligibility criteria based on faxed medical, surgical, and pathological reports. The RCT was not double-blind; participants knew the group assignment during follow-up. The protocol did not foresee any ad interim analysis, and serum/tissue banking of tumor/nontumor specimens was not required. The study followed the conditions for a good RCT, and the present report follows the CONSORT guidelines.

End Points. The primary study end point was recurrence-free survival (RFS); secondary endpoints were disease-specific survival (DSS) and overall survival. Recurrences were categorized a priori as early (≤2 yr after surgery) or late (>2 yr after surgery). Additional end points were the assessment of IFN tolerability and the observation of prognostic factors related to HCC reappearance.

Treatment (IFN) Group, Management of Toxicity, and Adherence to Therapy. Soon after trial approval, the protocol was amended to use IFN-α2b (Intron A; Schering-Plough, Kenilworth, NJ) rather than lymphoblastoid IFN-α. In the treatment arm, IFN-α was started within 6 weeks of surgery and was given subcutaneously (3 million units 3 times every week for 48 weeks). Adverse events during treatment were rated as mild, moderate, severe, or life-threatening as previously described. Therapy was discontinued if life-threatening events occurred. To increase patient compliance on treatment, for adverse events other than anemia, the IFN-α dosage was reduced to 1.5 million units 3 times every week. The full dosage was resumed if the event resolved but was discontinued if the event persisted. IFN-α was stopped in the
Fig. 1. Trial flowchart. Abbreviations: HCV, hepatitis C virus; HBsAg, hepatitis B surface antigen; HCC, hepatocellular carcinoma; US, ultrasonography; NMR, nuclear magnetic resonance; TACE, transarterial chemoembolization; RFA, radiofrequency ablation; PEI, percutaneous ethanol injection; IFN, interferon; Hb, hemoglobin; BMI, body mass index; antiHBc, hepatitis B anticore antibody.
case of cancer recurrence, because the primary end point of the trial was prevention of such an event.

The percentage of the total predicted dose of IFN actually used in each patient was recorded in addition to the time needed to resume regular therapy in case of dose reduction. Patients who used 80% of the total dose and were treated for at least 80% of the expected period were defined as adherent to treatment. Adherent patients were analyzed as a subgroup (per-protocol population) of patients who effectively received the treatment designed to prevent HCC recurrence.

Follow-up and Definition of Recurrence. All patients were followed in specific outpatient clinics. Clinical monitoring and management of adverse events, toxicity, or complications were referred and discussed with the referring physician and two trained assessors from the coordinating center. Case report forms were collected at each follow-up visit and were transmitted for independent compilation of the database. Biochemical and hematological testing were not centralized.

Patients received outpatient evaluations at 1, 2, 4, 6, 8, and 12 weeks, then every 3 to 4 weeks for up to 6 months, then every 2 months thereafter, unless symptoms of toxicity, adverse events, or other complications occurred. In such instances, closer intervals in the outpatient visits were tailored on an individual basis. IFN patients were asked to deliver used vials of IFN that were actually consumed between follow-up visits.

HCC recurrence detection was accomplished through regular oncologic visits, assessing alpha-fetoprotein (AFP) levels and imaging techniques at 1, 2, 3, 6, 8, and 12 months, every 3 months in year 2, then every 4 months from year 3 onward. Recurrence was diagnosed on the basis of combined abnormal findings on ultrasonography and on one additional imaging technique confirming hypervascular lesions, as well as on AFP findings. All participating centers had high-quality and updated radiological facilities; because of this, the average size of HCC recurrence was 1.3 cm (range 0.8-3.1). Recurrences were treated whenever possible (36 IFN-treated, 31 controls; \( P \) value not significant)

Statistical Analysis. The study was designed to detect a 20% increase in 3-year RFS in the treatment arm from an anticipated 25% in the control arm (corresponding approximately to a 40% relative rate reduction). For the comparison, we calculated that an overall sample size of 150 patients, 75 per treatment arm, would provide an 80% power for a two-sided log-rank test at a 5% significance level.

RFS, DSS, and overall survival curves were calculated using the Kaplan-Meier method and were compared by means of the log-rank test or multiple Cox regression model in univariate and multivariate settings. Cumulative incidence curves considering deaths unrelated to tumor recurrence as competing events and the hazard rate function were calculated to estimate the pattern of HCC recurrence over time in each study arm. In particular, the hazard function describing the instantaneous risk of recurrence was estimated by means of a generalized linear model with Poisson error; smoothing was achieved using a four-knot restricted cubic spline.

All analyses were performed in the overall study population according to the intention-to-treat principle and, as reported in Results, additional exploratory analyses were performed in specific patient subgroups, defined a priori on the ground of biological rationales. SAS software (SAS Institute Inc., Cary, NC) and S Plus (StatSci; MathSoft, Seattle, WA) were used to perform modeling and statistical calculations. In all analyses, the conventional two-sided 5% significance level was adopted.

Results

General Characteristics of the Study and Baseline Parameters

Figure 1 summarizes the trial design and number of patients. With respect to HCV genotype, 108 patients (72%) were genotype 1b, while the remaining 42 cases were distributed among genotype 1a (n = 5), genotype 2 (n = 32), and genotype 3a (n = 5).

Baseline characteristics of the patients assigned to each group (76 IFN vs. 74 controls) are summarized in Table 1. At a mean ± SD of 9 ± 8.7 months preceding liver resection, 36 patients had experienced unsuccessful pre-surgical treatment of their HCC. Neither baseline parameter nor pre- or postsurgical features (e.g., kind of resection, blood transfusion, time to discharge, complication rate) were statistically different among randomized groups and viral strata. No postoperative death occurred. Tumor characteristics were distributed similarly among strata and groups. As summarized in Table 1, HCCs were recruited at different stages of disease (43% classified as B-C), and the overall median tumor size in case of a single nodule was 35 ± 24 mm. Vascular invasion (n = 32) was mainly microscopic, although there were 5 cases (16%) of macroscopic tumor invasion of the main branches of the portal and/or hepatic veins. Variations in AFP level were wide (range 0.4-6854; median 18 IU/mL) with no significant difference among groups. Grading was rated high in 31.6% of carcinomas, with more than 10 mitoses per high-power field.
Adhesion to Therapy and Adverse Events

Out of 76 IFN patients, 28 were adherent to protocol according to the definition19: 15 were HCV-pure and 13 were HCV+HBV. Twenty-five of these patients received 100% of the prescribed yearly dose of IFN, while the remaining 3 patients had at least 95%. As expected in the established cirrhosis population of the present study, sustained virological response was observed in only 2 patients (7%), one of whom relapsed within 12 months, while normalization of aminotransferases (biological response) was observed in 2 additional patients. Such a small group of responders (14% of the adherent group) did not show...
a statistical influence in the subsequent cancer recurrence analysis.

Low compliance or toxicity led to nonadhesion to therapy in 35 patients, while 13 others were excluded because they never started treatment for personal reasons. Sixteen patients (21%) withdrew from the study because of tumor recurrence during the first year of follow-up. In 9 cases of mild to moderate toxicity (12%), IFN dose was reduced by 50%, with 6 patients eventually discontinuing the drug because of adverse events: thrombocytopenia and neutropenia (n = 3), depression and severe malaise (n = 1), hyperthyroidism (n = 1), and deterioration in Child’s score (n = 1). In 3 of 9 cases with moderate toxicity, IFN was resumed and the patients eventually were considered adherent. Life-threatening adverse events were not observed, and none of the 61 deaths was related to treatment or to surgical procedures.

### Intention-to-Treat Analysis

The entire series of 150 patients was analyzed after 45 months of median follow-up. Only 1 patient randomized to IFN-α treatment was lost to follow-up and censored at 1 month after randomization. A total of 100 patients had recurrence, and 61 patients died (41 related to cancer, 20 related to cirrhosis or other reasons). Global patient survival was 73% and 58.5% at 3 and 5 years, respectively.
whereas at the same time intervals RFS was 40% and 16%. After 5 years, the RFS of patients who received IFN was 24.3%, while 5.8% of controls were free from recurrence.

Considering the overall series of patients (Fig. 2A), RFS and DSS curves overlapped substantially in the two study arms ($P = .499$ and $P = .471$, respectively; log-rank test). Similarly, there was no significant difference for overall survival (data not shown). When stratifying the analysis by viral status, favorable trends of both RFS and DSS were observed for patients receiving IFN in the HCV-pure group (Fig. 2B) but not in the HCV+HBV group (Fig. 2C). In particular, among HCV-pure patients, RFS curves tended to diverge starting from 2 years of follow-up. However, the difference between IFN and control arms (42% vs. 29.9% at 3 yr; 24.7% vs. 3% at 5 yr) failed to reach statistical significance ($P = .240$). Coherent findings were observed for DSS ($P = .349$).

On intention-to-treat analysis, the primary trial hypothesis (IFN prevention of HCC recurrence in an unselected population of patients with HCV-related cirrhosis) was not satisfied.

**Timing of Recurrence and Per-Protocol Analysis**

The smoothed hazard function curves (Fig. 3) showed that the time trends in HCC recurrence were similar in the two study arms over the first 2 years of follow-up (72 out of the 100 recurrences observed), while they diverged thereafter in relation to a lower rate of tumor recurrence in the experimental arm, reduced by 50% with respect to controls (0.25/yr vs. 0.5/yr). The curve in the control arm, starting 2 years after surgery was confirmed in favor of the pure-HCV subgroup ($P = .001$), as previously shown in the overall series.

Cumulative incidence curves of early (within 2 years) and late recurrences were plotted separately for HCV-pure and HCV+HBV populations (Fig. 4). The only significant result was observed for late recurrences among HCV-pure patients ($P = .032$). Within this subgroup, the risk of HCC recurrence stabilized at 36.4% in the treated arm, while it approached 100% in controls. In all the remaining subgroups, no benefit was apparent in relation to IFN administration.

Analysis of HCC recurrence using the multiple Cox regression model is summarized in Table 2. Preliminary exploratory analyses showed that tumor multiplicity ($>1$ nodule) and vascular invasion consistently had a significant prognostic effect on RFS ($P = .016$ and $P = .0003$, respectively) and on secondary end points, multinodular HCC being the strongest predictor of recurrence (hazard ratio 1.89, 95% CI: 1.215-2.938; $P = .005$).

These factors combined with viral status when appropriate were entered into the Cox model for the purpose of obtaining adjusted estimates of IFN treatment effect. Whereas almost no effect on early recurrences was apparent, a favorable trend on late recurrence was observed in treated patients. Such a trend was statistically significant in HCV-pure patients adherent to treatment (Table 2). The hazard ratio estimate of 0.30 (95% CI: 0.094-0.989; $P = .048$) identified a 70% reduction of HCC recurrence rate in the subgroup of HCV-pure patients effectively receiving IFN, possibly because of a lower incidence of de novo tumors.

**Subgroup Analysis on Risk of Recurrence**

**Single HCC <3 cm Without Vascular Invasion.** To evaluate IFN suppression on de novo carcinogenesis (after 2 years), a subgroup analysis was conducted in patients with a single HCC measuring <3 cm in diameter without vascular infiltration, criteria that in previous reports have strongly suggested the absence of remaining tumor in the liver. Such HCCs at early stages were present in 60 out of 150 patients (40%): 32 in the IFN group (90% adherent to therapy), 28 in the control group. The risk of HCC recurrence was significantly lower in the IFN group with respect to the control group at both intention-to-treat and per-protocol analysis (hazard ratio 0.4, 95% CI: 0.19-0.89; $P = .02$). When subgroup analysis was conducted in HCV-pure versus HCV+HBV subgroups, the considered populations were too small to achieve reliable statistical results.

**AFP Normalization After Surgery.** Out of 150 patients, 72 (48% of the series) already had a normal level of AFP (<15 IU/mL) at the time of diagnosis, while 45 patients normalized AFP after surgery (30%). Patients who normalized AFP had a lower risk of tumor recurrence (both early and late) in comparison with the remaining patients (hazard ratio 0.3, 95% CI: 0.15-0.48; $P < .0001$). On average, AFP returned to normal values within 2 months (range 1-5). In the per-protocol analysis, the advantage for patients with normalization of AFP after surgery was confirmed in favor of the pure-HCV subgroup ($P < .0001$), as previously shown in the overall analysis of the series.

**Discussion**

Clinical research in the context of prevention of HCC recurrence after tumor removal or ablation has been difficult with only 3 RCTs, all from the Asian experience and with no firm conclusions. The present RCT, which was based on a sample size of 150 patients, matching the cumulative population of all previous trials, allows insights into the heterogeneous population of HCV patients with HCC, introducing a subset of patients (pure...
Fig. 2. Intention-to-treat analysis of RFS (primary end point) and DSS (secondary end point). RFS was the primary end point of the study because all patients already had a resected HCC. Curves were estimated using the Kaplan-Meier method. (A) At the end of follow-up, there were no significant differences between the IFN and control arms. (B,C) Favorable trends of both RFS and DSS were observed for patients receiving treatment with IFN in the HCV-pure group but not in the HBV/HCV group. However, the difference in tumor recurrence between IFN and controls at 5 years (24.7% vs. 3%) failed to reach statistical significance. Abbreviations: IFN, interferon; Ctrl, control; DSS, disease-specific survival; RFS, recurrence-free survival; HCV, hepatitis C virus; HBV, hepatitis B virus.
HCV-related HCC, effectively receiving treatment) and tumor recurrences (de novo, at late intervals from resection) possibly more sensitive to the chemopreventive effect of IFN-α.

Prevention of HCC by IFN has been reported to be significant but relatively small in most previous trials9-14 in which patient selection bias, spurious associations, and method constraints have been reported.28-34 The present study, which was conducted in Caucasian patients, confirmed the original observations from Japan, describing a double-peaked incidence of recurrence after HCC resection,7 and demonstrated the efficacy of IFN only on the late peaks occurring more than 2 years after tumor resection. It has been shown that in patients with cirrhosis, despite the extremely low chances of HCV clearance as those reported herein (only 7% of sustained virological response), the reduction in inflammation and progression of fibrosis produced by IFN could lead to carcinogenesis inhibition and reduction in the incidence of new tumor foci at a late interval after HCC removal or ablation.21,35,36

The overall results of the present series (58.5% survival after 5 years, 0% perioperative death rate) are notable in light of the liberal selection criteria used, with many patients beyond the conventional criteria for liver resection (Table 1). Despite an expected 5-year survival not exceeding 40%,5,6 the benefit of surgical resection in cirrhotic patients with HCC was confirmed in this study (Fig. 2), which tested the chemopreventive potentials of IFN through the choice of RFS rather than patient survival as primary end point, because all patients already had HCC.

Indeed, this investigation on secondary prevention of HCC revealed the negative/positive nature of the RCT: negative for the overall prevention of tumor recurrence after resection but positive for a significant effect of IFN-α in reducing late recurrences in HCV-pure patients effectively receiving treatment.

Whether or not HCV patients had previous contact with HBV (anti-HBc status), adjuvant IFN-α did not affect recurrence rate or survival, although a trend was observed in favor of HCV-pure infections that became significant in the subgroup of patients adherent to therapy (Figs. 2-4). In this particular subgroup, allocation to treatment emerged as the only significant factor reducing the late recurrence rate (Table 2); such a result could provide the basis for specific trials focused on HCV-related late-recurring HCC.

In agreement with previous studies, tumor size, multiplicity, and vascular invasion were confirmed as independent factors affecting recurrence. Preoperative detection of such factors did not limit case eligibility, provided that surgery was potentially curative. In fact, the preventive effect of IFN on recurrence was clear in the subgroup of patients with single, small HCC (<3 cm and with no vascular invasion) who had a nearly complete rate of compliance to treatment.

The preventive effect of IFN was also significant on the subgroup of early HCCs when surgery had a presumed eradication of cancer foci by means of postoperative AFP normalization, even though the Lens Culinaris agglutinin–reactive fraction of AFP surely would have been a better marker of biological aggressiveness and tumor recurrence compared with standard AFP.37 Such a determination was not available in our laboratories at the time of the study design. In the near future, new predictors of prognosis will contribute to better stratification criteria according to different risks of metastatic spread.38

Strata assignment of the present study was based on anti-HBc reactivity. The method may now be considered obsolete, even though anti-HBc serum determination is still quite common and inexpensive. Studies on liver specimens of HCV patients with HCC have shown occult HBV infection in 80%-90% of the anti-HBc-positive cases and in up to 40%-50% of the anti-HBc–negative individuals.39 Unfortunately, analysis on occult HBV could not be completed in the present series because serum/tissue banking was not foreseen. However, even if cryptic HBV infection is an identified risk factor for HCC, no information is available concerning its role in the specific setting of tumor recurrence.
Several IFN molecules are reported to have a preventive effect on HCC,\textsuperscript{9} with IFN-\textbeta inducing tumor apoptosis in liver and other cancer cell lines through alternative signaling pathways with respect to IFN-\textalpha.\textsuperscript{40,41} In the present clinical trial, as in others,\textsuperscript{10-14} IFN-\textalpha was chosen because of its additional effect on hepatitis C and widespread clinical use.

Pegylated IFNs have produced significantly higher sustained virological responses and better tolerability than treatment with IFN-\textalpha alone.\textsuperscript{42,43} In our study, the use of

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\caption{Influence of IFN treatment on timing of recurrence of HCC (early vs. late) in 150 patients with HCV-related cirrhosis stratified on viral status (anticore B antibody-positive vs. anticoar B antibody-negative). Risk of late recurrence (occurring during follow-up at more than 2 years from tumor resection) was significantly reduced among HCV-pure patients receiving IFN. In all the remaining subgroups no benefit was apparent in relation to IFN administration. Abbreviations: HCV, hepatitis C virus; IFN, interferon; HBV, hepatitis B virus.}
\end{figure}
the conventional schedule of IFN in patients with established cirrhosis and the unrestricted selection with respect to viremia and genotype allowed only a 7% rate (2 cases) of sustained virological response. The enhanced efficacy of new IFN formulations could achieve a higher reduction of HCC incidence, also thanks to a higher rate of patient compliance obtained with pegylated IFN. The low compliance observed in the present study (37%) was probably a limiting factor for a more definitive demonstration of IFN efficacy in preventing de novo HCC developments.

In conclusion, the results of this RCT suggest that interferon is not recommended as a single chemopreventive agent after resection in patients with HCV-related HCC. However, IFN could be indicated in the subgroup of patients with HCV-pure infection in whom late recurrences can be significantly reduced. Further exploration on chemoprevention with more potent antiviral combinations, such as pegylated IFN and ribavirin, should be pursued in larger cohorts of this subgroup of individuals.

References