Doxorubicin-loaded nanoparticles for patients with advanced hepatocellular carcinoma after sorafenib treatment failure (RELIVE): a phase 3 randomised controlled trial

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Summary

Background Cytotoxic chemotherapy is generally ineffective in patients with hepatocellular carcinoma. We assessed the intravenous perfusion of doxorubicin-loaded nanoparticles in patients with hepatocellular carcinoma in whom previous sorafenib therapy had failed.

Methods We did a multicentre, open-label, randomised, controlled phase 3 trial at 70 sites in 11 countries. Patients with hepatocellular carcinoma with one or more previous systemic therapies, including sorafenib, were randomly assigned to receive 30 mg/m² doxorubicin-loaded nanoparticles (30 mg/m² group), 20 mg/m² doxorubicin-loaded nanoparticles (20 mg/m² group), or standard care using a computer-generated randomisation list prepared by the funder and stratified by geographic region. Patients in the experimental groups received perfusion of the drug every 4 weeks and those in the control group received any systemic anticancer therapy (except sorafenib) as per investigator decision. The primary endpoint was overall survival in the intention-to-treat population. Safety was assessed in the population of patients who received at least one dose of their assigned treatment. This trial is registered with ClinicalTrials.gov, number NCT01655693.

Findings Between June 15, 2012, and Jan 27, 2017, 541 patients were screened, of whom 144 were excluded and 397 were randomly assigned to one of the groups (133 to the 30 mg/m² group; 130 to the 20 mg/m² group; and 134 to the control group). Median follow-up was 22.7 months (IQR 11.2–34.9). After pooling the doxorubicin groups for the efficacy analysis, median overall survival was 9.1 months (95% CI 8.1–10.4) in the pooled doxorubicin-loaded nanoparticles group and 9.0 months (7.1–11.8) in the control group (HR 1.00 [95% CI 0.78–1.28], two-sided p=0.99). 227 (94%) of 242 patients who received doxorubicin-loaded nanoparticles and 100 (75%) of 134 patients in the control group had at least one treatment-emergent adverse event. The most common drug-related grade 3 or 4 treatment-emergent adverse events were neutropenia (25 [10%] of 242 treated with doxorubicin-loaded nanoparticles and eight [6%] of 134 in the control group), asthenia (six [2%] and four [3%]), and thrombocytopenia (three [1%] and ten [7%]). Six (2%) patients treated with doxorubicin-loaded nanoparticles and one (1%) of those in the control group were deemed by investigators to have had a drug-related death. Serious adverse events occurred in 74 (31%) patients who received doxorubicin-loaded nanoparticles and 48 (36%) in the control group.

Interpretation Doxorubicin-loaded nanoparticles did not improve overall survival for patients with hepatocellular carcinoma in whom previous sorafenib treatment had failed.

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Introduction

The treatment of hepatocellular carcinoma follows guidelines based on the Barcelona Clinic Liver Cancer (BCLC) staging system. Surgical resection, transplant, and thrombectomy are potential curative options for patients with early-stage hepatocellular carcinoma, whereas chemoembolisation is recommended as a palliative option for intermediate-stage hepatocellular carcinoma. For patients with advanced hepatocellular carcinoma, or with intermediate stage disease that is no longer a candidate for chemoembolisation, systemic strategies based on oral tyrosine kinase inhibitors such as sorafenib as first-line treatment and regorafenib as second-line treatment provide a clinically significant improvement in overall survival. Lenvatinib is non-inferior to sorafenib as first-line treatment and cabozantinib is efficacious as second-line or third-line treatment. Ramucirumab, a monoclonal antibody targeting vascular endothelial growth factor receptor 2, has shown efficacy in the subgroup of patients with hepatocellular carcinoma who have concentrations of alpha-fetoprotein of at least 400 ng/mL after sorafenib...
Research in context

Evidence before this study

We searched PubMed for phase 3 randomised controlled studies of doxorubicin-loaded hollow microspheres or nanoparticles, published between 2000 and 2018, and in English. We included studies published after 2000, with doxorubicin-loaded nanoparticles or microspheres which were included in phase 3 randomised controlled trials (RCTs) comparing doxorubicin-loaded hollow microspheres or nanoparticles with standard of care, including placebo or other systemic therapies. We excluded studies where the active component was free doxorubicin or studies where the active component was doxorubicin-loaded small interfering RNA (siRNA). We included studies comparing doxorubicinloaded nanoparticles or microspheres with other systemic therapies, including sorafenib, and with or without other systemic therapies, including sorafenib. We included patients with hepatocellular carcinoma confirmed by pathological or imaging assessment, and in whom the primary treatment had failed, in patients with advanced hepatocellular carcinoma. The absence of more effective therapies is an unmet clinical need, but so far, chemotherapy has been clearly demonstrated to be ineffective and toxic in these patients.

Methods

Study design

This multicentre, open-label, randomised, controlled phase 3 trial was done at 70 sites in 11 countries in Europe, the USA, the Middle East, and North Africa. The trial was approved by each centre’s ethics committee or institutional review board and complied with Good Clinical Practice guidelines, the Declaration of Helsinki, and applicable local laws. The protocol is available online.

Patients

Eligibility criteria were age of at least 18 years; hepatocellular carcinoma confirmed by pathological or imaging assessment, and in whom the primary treatment had failed in patients with advanced hepatocellular carcinoma. The absence of more effective therapies is an unmet clinical need, but so far, chemotherapy has been clearly demonstrated to be ineffective and toxic in these patients.

treatment failure, whereas ramucirumab did not show any significant benefit in non-selected patients with hepatocellular carcinoma. All other systemic drugs in phase 3 trials showed no efficacy in this population. Further, radioembolisation with yttrium-90 has not shown superiority to sorafenib in a randomised phase 3 study in patients with advanced hepatocellular carcinoma or in those with intermediate hepatocellular carcinoma in whom chemoembolisation has failed. The best observed overall survival in a systemic setting was for sorafenib followed by regorafenib (median 26–0 months [95% CI 22.6–28.1]). More effective systemic therapies are needed to increase overall survival of patients with advanced hepatocellular carcinoma.

To date, no phase 3 trials of doxorubicin-loaded nanoparticles or microspheres have shown evidence of efficacy. Doxorubicin was a potential candidate, but the administration of free doxorubicin is associated with high morbidity in cirrhosis; it also did not show any additive or synergistic effects when added to sorafenib. Doxorubicin-loaded nanoparticles in the liver overwhelm the efflux pumps encoded by multiple drug resistance genes. A phase 1–2 trial suggested a potential benefit of doxorubicin-loaded nanoparticles on overall survival of patients with hepatocellular carcinoma, although the trial was prematurely stopped because of lung toxicity associated with doxorubicin-loaded nanoparticles injected by the hepatic arterial route. Preclinical data from Wistar rats showed that this lung toxicity was reduced when doxorubicin-loaded nanoparticles were infused over 2 h. Thus, here, we assessed the efficiency of doxorubicin-loaded nanoparticles administered by a 6 h intravenous infusion in patients with hepatocellular carcinoma after failure of sorafenib therapy.
past 5 years; HIV infection; hepatocellular carcinoma on transplanted liver; risk of variceal bleeding; previous cumulative dose of more than 300 mg/m² doxorubicin; ongoing immunosuppressive treatment; unstable medical or surgical conditions, particularly uncontrolled diabetes, that might disrupt study participation; uncontrolled systemic infection; life expectancy less than 2 months; receipt of an experimental drug in another clinical trial in the past 30 days; and unwillingness or inability to use two forms of contraception for 6 months after final study drug administration. All patients provided written informed consent.

Randomisation and masking
Patients were randomly assigned to receive either 30 mg/m² doxorubicin-loaded nanoparticles (30 mg/m² group) or 20 mg/m² doxorubicin-loaded nanoparticles (20 mg/m² group) or standard care (1:1:1) using a computer-generated randomisation list prepared by the funder. This list was stratified by geographic region (Europe, USA, or Middle East and North Africa) using blocks (size 6). Investigators, patients, and the funder were unmasked to treatment assignment in this open-label trial. However, independent central review as per RECIST, version 1.1, and data review by the data review committee before database lock were performed blindly. The assignment of number and code for patient identification ensured patient anonymity.

Procedures
In both experimental groups, doxorubicin-loaded nanoparticles were delivered by intravenous perfusion every 4 weeks with a maximum allowed cumulative dose of doxorubicin of 550 mg/m². Patients assigned to the
standard care control group received any systemic anticaner therapy (except sorafenib) according to the centre’s practice and the decision of the principal investigator at that centre, being aware that any type of these systemic therapies had not shown efficacy in phase 3 trials at the time of randomisation. In all groups, patients received best supportive care. Treatment continued until disease progression as defined by RECIST, version 1.1, or clinical progression, death, unacceptable toxicity, withdrawal of consent by the patient, or decision by the principal investigator. Patients were followed up for tumour assessments every 8 weeks. Treatment could be continued beyond progression at the decision of the principal investigator. To prevent the occurrence of acute respiratory adverse events that we observed in our phase 1–2 trial,24 perfusion of doxorubicin-loaded nanoparticles was done over 6 h intravenously with safety measures (premedication with methylprednisolone 32 mg orally and one antihistamine drug given 24 h and 1 h before perfusion and 24 h after perfusion). Respiratory symptoms and oxygen saturation were continuously monitored during the 6 h of perfusion: in case of dyspnoea or oxygen saturation decrease from 95% or more to 93% or less, the infusion rate was reduced by half (to a 12 h infusion) without changing the total dose; in case of persistence of dyspnoea beyond 1 h or oxygen saturation decrease to 90% or less, perfusion was immediately and definitively stopped. Safety was monitored continuously throughout the study and patients had safety assessments every 4-week treatment cycle. Blood tests were assessed every 2 weeks. Adverse events were graded using National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.03.

**Outcomes**

The primary endpoint was overall survival, defined as the time from randomisation to death from any cause.
Secondary endpoints were safety, and overall survival for patients with Child–Pugh score A, progression-free survival (defined as time from randomisation to radiological or clinical disease progression or death), the proportion of patients achieving an objective response (defined as a complete or partial response), and the proportion of patients achieving disease control (defined as complete response, partial response, or stable disease maintained for ≥8 weeks). Responses to treatment were assessed using RECIST (version 1.1), with independent central review. Safety was assessed by adverse events, laboratory abnormalities, vital signs, chest x-ray, and left ventricular ejection fraction by cardiac echography and electrocardiography.

Exploratory endpoints were progression-free survival and objective response as assessed by investigators and time to progression (time from randomisation to radiological or clinical disease progression) assessed by independent central review per RECIST version 1.1.

Statistical analysis
At study initiation in 2011, the initial sample size calculation was done on the basis of an estimated median survival of 6–6 months in the control group and 10–9 months in the experimental groups (hazard ratio [HR] 0·60); an accrual period of 36 months; and a one-sided α of 2·5%. The required sample size to achieve a 90% power was 130 patients per group (for the two tests of the two doses at a 5% level).

In 2016, Bruix and colleagues' published the results of a phase 3 trial of regorafenib, with a median overall survival of 7·8 months (95% CI 6·3–8·8) in the placebo group. The revised power of the estimated required sample size for our study in view of these new results and according to our accrual period of 54 months would be decreased from 90% to 58%.

Considering these results, study feasibility, and the need for results in this serious, life-threatening disease, the statistical analysis plan was amended after validation by the US Food and Drug Administration (July 24, 2017) and signed off by the scientific committee (Aug 25, 2017) before database lock (Aug 28, 2017). The revised statistical analysis plan pooled the two doxorubicin-loaded nanoparticle groups; assuming a median overall survival in the control group of 8 months, and aiming for a hazard ratio of 0·69, the required total sample size to achieve 85% power to compare the experimental groups with control (two-sided α of 5%) was 348 patients (116 patients in the control group and 232 patients in the pooled experimental group). We expected recruitment to take 55 months, with 6 months of follow-up after the last inclusion (total follow-up 61 months), and around 10% of patients to be lost to follow-up. Thus the recalculated total sample size was 390 patients. The analysis was planned for when 285 events (deaths) occurred. For the primary efficacy endpoint of overall survival and the secondary endpoint of progression-free survival, the groups were compared using a non-stratified log-rank test. The HR for overall survival and its 95% CI were calculated using the stratified Cox model.

The primary analysis was done in the intention-to-treat (ITT) population, defined as all patients who had been randomly assigned to a group; safety analyses included all patients who received at least one dose of the study drug. The study was overseen by an independent data safety monitoring committee. To
assess the primary endpoint of overall survival in the ITT population and the secondary endpoints of overall survival in the subgroup of patients with Child–Pugh score A and progression-free survival and objective response in the whole population and Child–Pugh score A subgroup. We used a hierarchical sequential closed-test procedure to control the overall type I error rate of 5%, with the following sequence: overall survival in the ITT population, overall survival in the Child–Pugh A subgroup, progression-free survival in the Child–Pugh A subgroup, and objective response in the Child–Pugh A subgroup. If the closed-test procedure fails, all other analyses will be presented as exploratory.

We did a sensitivity analysis using a Cox model adjusting for predefined selected prognostic factors. We first analysed these prognostic factors in separate univariate analyses and then in multivariate analysis. More specifically, we tested each potential predictor in a univariate analysis and then in multivariate analysis.

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**Figure 3**: Forest plot of overall survival in predefined subgroups

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| Age, years | Pooled doxorubicin group (n/N) | Control group (n/N) | Median (months) | Hazard ratio (95% CI) | p value |
|------------|-------------------------------|---------------------|----------------|----------------------|---------|
| <65        | 77/172                        | 34/57               | 8.1           | 1.09 (0.73–1.63)     | 0.682   |
| ≥65        | 174/351                       | 58/77               | 10.0          | 0.96 (0.70–1.31)     | 0.796   |
| Sex        |                               |                     |                |                      |         |
| Women      | 29/39                         | 9/18                | 9.4           | 1.18 (0.55–2.51)     | 0.670   |
| Man        | 147/312                       | 49/71               | 9.0           | 0.96 (0.74–1.25)     | 0.778   |
| Geographical region |                   |                     |                |                      |         |
| Europe or USA | 182/242                     | 88/222              | 9.7           | 0.95 (0.74–1.23)     | 0.706   |
| North Africa and Middle East |   | 14/21                      | 5.8           | NE                   | 0.182   |
| Race       |                               |                     |                |                      |         |
| White      | 183/239                       | 86/125              | 9.1           | 1.03 (0.79–1.33)     | 0.845   |
| Asian      | 1/4                           | 2/2                 | NE            | 0.24 (0.02–2.77)     | 0.254   |
| Other      | 11/18                         | 3/6                 | 10.2          | 0.66 (0.18–4.20)     | 0.524   |
| ECOG performance status |                   |                     |                |                      |         |
| 0          | 100/150                       | 45/69               | 11.0          | 1.12 (0.78–1.59)     | 0.540   |
| >0         | 96/113                        | 41/65               | 5.9           | 0.90 (0.64–1.28)     | 0.563   |
| Child–Pugh |                               |                     |                |                      |         |
| A          | 159/233                       | 73/113              | 10.1          | 0.95 (0.72–1.26)     | 0.724   |
| B          | 36/19                         | 19/21               | 3.8           | 1.35 (0.77–2.38)     | 0.299   |
| Macrovascular invasion |                   |                     |                |                      |         |
| No         | 124/170                       | 58/89               | 10.6          | 0.97 (0.71–1.33)     | 0.872   |
| Yes        | 73/93                         | 34/45               | 7.0           | 0.97 (0.64–1.46)     | 0.876   |
| Extrahepatic spread |                   |                     |                |                      |         |
| No         | 114/152                       | 57/83               | 8.6           | 0.90 (0.65–1.32)     | 0.507   |
| Yes        | 77/103                        | 31/45               | 10.0          | 1.27 (0.84–1.94)     | 0.258   |
| Intrahepatic spread |                   |                     |                |                      |         |
| No         | 100/150                       | 45/69               | 11.7          | 1.18 (0.83–1.69)     | 0.352   |
| Yes        | 86/109                        | 49/63               | 6.0           | 0.90 (0.63–1.38)     | 0.566   |
| Alpha-fetoprotein concentration, ng/mL |                   |                     |                |                      |         |
| >400       | 110/154                       | 42/70               | 12.4          | 0.70 (0.44–1.11)     | 0.332   |
| <400       | 94/124                        | 48/68               | 8.8           | 0.94 (0.67–1.37)     | 0.820   |
| Number of previous treatments |                   |                     |                |                      |         |
| 1          | 150/204                       | 70/99               | 9.2           | 1.00 (0.75–1.33)     | 0.984   |
| >1         | 46/59                         | 22/35               | 8.8           | 0.94 (0.67–1.37)     | 0.820   |
| Anticancer therapy |                   |                     |                |                      |         |
| Yes        | 177/242                       | 58/79               | 9.5           | 0.96 (0.71–1.29)     | 0.775   |
| No         | 94/124                        | 48/68               | 8.8           | 0.80 (0.59–1.13)     | 0.351   |
| Last reason for sorafenib discontinuation |                   |                     |                |                      |         |
| Progression | 130/174                       | 65/97               | 8.4           | 1.13 (0.84–1.52)     | 0.422   |
| Intolerance | 58/78                         | 26/35               | 12.4          | 0.71 (0.44–1.11)     | 0.332   |
| Previous sorafenib within 10 weeks* |                   |                     |                |                      |         |
| Yes        | 95/126                        | 45/63               | 8.8           | 0.97 (0.68–1.38)     | 0.851   |
| Alcohol use |                               |                     |                |                      |         |
| Yes        | 94/124                        | 48/68               | 8.8           | 1.12 (0.79–1.59)     | 0.531   |
| No         | 58/80                         | 22/38               | 8.6           | 0.91 (0.55–1.49)     | 0.703   |
| Non-alcoholic steatohepatitis |                   |                     |                |                      |         |
| Yes        | 29/35                         | 18/23               | 10.1          | 1.11 (0.61–2.00)     | 0.738   |
| No         | 15/23                         | 10/14               | 11.3          | 0.69 (0.30–1.56)     | 0.372   |
| Hepatitis B virus |                   |                     |                |                      |         |
| Yes        | 15/23                         | 10/14               | 11.3          | 0.69 (0.30–1.56)     | 0.372   |
| No         | 97/130                        | 92/134              | 10.1          | 0.99 (0.74–1.32)     | 0.947   |
| Treatment group |                   |                     |                |                      |         |
| Doxorubicin 20 mg/m² | 97/130                     | 92/134              | 10.1          | 0.99 (0.74–1.32)     | 0.947   |
| Doxorubicin 30 mg/m² | 99/131                     | 92/134              | 8.9           | 1.00 (0.75–1.33)     | 0.984   |

*Exposure for at least 20 days at a concentration of at least 400 mg and discontinuation less than 10 weeks before randomisation. ECOG=Eastern Cooperative Oncology Group. n=events. N=group size. NE=not estimable.
less than 0.10 for the multivariate analysis. We then included the selected predictors in a multivariate Cox model (treatment not included in the model) and further selected them with a backward selection procedure eliminating covariates with a p value above 0.10 in presence of the other covariates. We then introduced treatment and well known predictors (macroscopic vascular invasion, extrahepatic spread, Child–Pugh, hepatitis B virus infection, and alpha-fetoprotein) as an additional covariate in the reduced model obtained at the end of the backward selection procedure. We tested the covariate by treatment interactions by adding in a separate model all interactions corresponding to the finally retained covariates. We compared overall survival using a naive test based on the comparison of Kaplan–Meier estimates of survival. We compared proportions of patients achieving responses and disease control in the two groups using Fisher’s exact test.

We did statistical analyses with the SAS software, version 9.4. This trial is registered with ClinicalTrials.gov, number NCT01655693.

Role of the funding source
The funder was involved in study design, data collection, analysis, and interpretation, and writing of the report. Data management was done by Lincoln Pharmaceuticals and Axial and statistical analyses were done by Chiltern International, both supervised by eXYZSTAT. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results
541 patients were screened between June 15, 2012, and Jan 27, 2017, and 144 were excluded because they did not meet eligibility criteria. 397 patients were randomly assigned to either the 30 mg/m² group (n=133), the 20 mg/m² group (n=130), or the control group (n=134) and included in ITT analysis (figure 1). 376 (95%) patients started treatment (120 in the 30 mg/m² group, 122 in the 20 mg/m² group, and 134 in the control group) and comprised the safety population. Of the patients who started treatment, 113 (94%) of 120 receiving 30 mg/m² doxorubicin-loaded nanoparticles, 118 (97%) of 122 receiving 20 mg/m² doxorubicin-loaded nanoparticles, and 130 (97%) of 134 patients in the control group discontinued study treatment. The most common reason for discontinuation was radiological progression (77% [68%] of 113 in the 30 mg/m² group, 91% [77%] of 118 in the 20 mg/m² group, and 58% [45%] of 130 in the control). The mean treatment duration was 3.7 months (SD 3.9) in the 30 mg/m² group, 3.4 months (3.9) in the 20 mg/m² group, and 5.8 months (2.6) in the control group. In both experimental groups, the mean number of cycles was 4.6 (SD 4.0); 64 (26%) of 244 patients who received their allocated intervention delayed at least one treatment cycle, and 17 (7%) patients had at least one dose reduction; the mean dose-intensity of doxorubicin-loaded nanoparticles was 99% (SD 7). In the control group, 55 (41%) of 134 patients received only best supportive care whereas 79 (59%) were administered a systemic anticancer therapy considered as the best standard of care by the investigator, the most common of which was oxaliplatin plus gemcitabine in 37 (28%) patients (appendix p 12).

At the cutoff date for the final analysis (May 28, 2017), median follow-up was 22.7 months (IQR 11.2–34.9) and 288 (73%) of the 397 randomised patients had died (99 [74%] of 133 in the 30 mg/m² group; 97 [75%] of 130 in the 20 mg/m² group; and 92 [69%] of 134 in the control group). Baseline demographics were similar between the pooled experimental groups and the control group (table 1). Median previous time on sorafenib was 4.1 months (IQR 2.4–9.3) in the pooled doxorubicin group and 4.9 months (2.6–8.7) in the control group. About a quarter of patients received additional systemic treatment lines after sorafenib and before RELIVE inclusion. Treatments received after withdrawal from RELIVE are shown in the appendix (p 11).

There was no significant difference in overall survival between the groups in the ITT population; median overall survival was 9.1 months (95% CI 8.1–10.4) in the pooled doxorubicin-loaded nanoparticle group and 9.0 months (7.1–11.8) in the control group (HR 1.00 [95% CI 0.78–1.26], two-sided p=0.74; figure 4). In exploratory analyses, overall survival since start of sorafenib was also not significantly different between the groups, (HR 1.04 [95% CI 0.81–1.32], p=0.77). Overall survival in the Child–Pugh A subpopulation was also similar between groups: median overall survival was 10.1 months (95% CI 8.8–11.6) in the pooled experimental group and 10.7 months (7.2–12.4) in the control group (HR 0.95 [95% CI 0.72–1.26], p=0.74;
No differences in overall survival were noted in any of the predefined subgroups (figure 3).

Median progression-free survival was 2·3 months (95% CI 2·1–2·6) in the pooled experimental group and 2·3 months (2·1–2·8) in the control group (HR 0·95 [95% CI 0·74–1·22]; two-sided p=0·70; figure 4). Progression-free survival in the Child–Pugh A subpopulation was also similar between groups (median 2·4 months [95% CI 2·2–2·8] in the pooled doxorubicin group and 2·4 months [2·1–2·8] in the control group). No differences in progression-free survival were noted in any of the predefined subgroups (figure 5). Results were similar for progression-free survival per investigator assessment (appendix p 9).

In other exploratory analyses, time to progression by independent central review showed similar results, with an HR of 0·96 (95% CI 0·74–1·23; two-sided p=0·74; appendix p 10) and a median time to progression of 2·3 months (95% CI 2·1–2·6) in the pooled group and 2·3 months (2·1–2·8) in the control group.

Independent imaging central review was possible in 276 (70%) of the 397 randomised patients (204 [78%] of...
263 patients in the pooled doxorubicin-loaded nanoparticles group and 72 [54%] of 134 in the control group; table 2). Review was not possible for 121 patients because of absence of imaging data (n=29), poor quality imaging or identification issues (n=11), and presence of baseline imaging but no follow-up imaging (n=81). Among those with available data, the proportion of patients achieving an objective response or disease control was similar in both groups, by both independent and investigator review (table 2). In the Child–Pugh A subpopulation, response were also similar between the groups (no patients achieved a complete response in either group, partial response in two [1%] of 179 in the pooled doxorubicin group vs one [2%] of 64 in the control group, stable disease in 72 [40%] vs 28 [44%], and progressive disease in 105 [59%] vs 35 [55%]).

227 (94%) of 242 patients in the pooled doxorubicin-loaded nanoparticles group and 100 (75%) of 134 patients in the control group had at least one treatment-emergent adverse event (table 3). These were deemed related to the study drug in 177 (73%) patients in the pooled doxorubicin group and 58 (43%) of the patients in the control group. Serious adverse events occurred in 74 (31%) patients receiving doxorubicin-loaded nanoparticles and 48 (36%) in the control group, and were attributed to the study drug in 31 (13%) cases in the doxorubicin group and 13 (10%) in the control group. The most common grade 3 or 4 drug-related treatment-emergent adverse events were anaemia (six [3%] of 242 patients) and neutropenia (25 [10%]) in the pooled doxorubicin group and ashenia (four [3%] of 134), neutropenia (eight [6%]), and thrombocytopenia (ten [7%]) in the control group. Neutropenia was more frequent in those treated with doxorubicin-loaded nanoparticles than in those in the control group; by contrast, thrombocytopenia was less common in those treated with doxorubicin-loaded nanoparticles than in those in the control group (table 3).

Cardiorespiratory toxicity was rare and not severe for most patients treated with doxorubicin-loaded nanoparticles. Asymptomatic decreases of left ventricular ejection fraction below 50% occurred in five (2%) of 242 patients; respiratory symptoms in 11 (5%); and oxygen desaturation in 24 [10%] of 134 in the control group (n=204) and neutropenia (25 [10%]) in the control group. Neutropenia was more frequent in those treated with doxorubicin-loaded nanoparticles than in those in the control group; by contrast, thrombocytopenia was less common in those treated with doxorubicin-loaded nanoparticles than in those in the control group (table 3).

Table 2: Objective responses in evaluable patients by independent central review and per investigator review

| Best overall response | Independent central review | Investigator review |
|-----------------------|---------------------------|---------------------|
|                       | Pooled experimental group (n=204) | Control group (n=72) | Pooled experimental group (n=219) | Control group (n=78) |
| Complete response     | 0                          | 0                   | 0                                  | 0                   |
| Partial response      | 2 (1%)                     | 1 (1%)              | 6 (3%)                             | 4 (5%)              |
| Stable disease        | 80 (39%)                   | 31 (43%)            | 112 (51%)                          | 41 (53%)            |
| Progressive disease   | 121 (59%)                  | 40 (56%)            | 101 (46%)                          | 33 (42%)            |
| Objective response    | 2 (1%)                     | 1 (1%)              | 6 (3%)                             | 4 (5%)              |
| Disease control       | 82 (40%)                   | 32 (44%)            | 118 (54%)                          | 45 (58%)            |

Table 2: Objective responses in evaluable patients by independent central review and per investigator review


discussion

The absence of more effective therapies for hepatocellular carcinoma is an unmet clinical need; however, chemotherapy has been clearly shown to be ineffective and toxic in patients with advanced disease. In this phase 3 trial assessing doxorubicin-loaded nanoparticles as subsequent-line treatment for patients in whom sorafenib has failed, no difference was detected between doxorubicin-loaded nanoparticles and control in terms of overall survival.

Survival in patients with hepatocellular carcinoma is influenced by many factors related not only to tumour burden but also to underlying liver conditions, and minor imbalances in prognostic factors can have a meaningful effect on overall survival. Nonetheless, baseline characteristics were similar between the groups and thus are not a possible explanation for these results. One explanation could be insufficient power, because patients with a better prognosis than in previous trials were enrolled.8-11 Indeed, median overall survival in the control group was unexpectedly high in the whole population (9–0 months [95% CI 7–1–11–8]) as well as in the subpopulation of patients with Child–Pugh score A (10–7 months [7–2–12–4]). By contrast, median overall survival results in the Child–Pugh A populations of other phase 3 trials such as SHARP8 (sorafenib) and RESORCE9 (regorafenib) were lower: median overall survival was 7–9 months in the control group of both studies, and was 8–0 months in the cabozantinib trial.9 Equivalent data were observed for overall survival in the control groups of other phase 3 trials of drugs being tested after failure of sorafenib: in the brivanib trial it was 8–2 months,10 7–3 months in the trial of everolimus,10 and 7–6 months in the REACH trial of ramucirumab,10 overall survival in the control group in a phase 3 study of tivantinib was 9–1 months.2 In the RELIVE trial, only three-quarters of patients had previously received only sorafenib as systemic treatment whereas a quarter had received at least two lines of treatment (sorafenib plus one or more additional lines). Thus, the RELIVE trial might have selected patients with...
| Treatment-emergent adverse events | Control group (n=134) | Pooled experimental group (n=242) | Any grade | Grade 3 | Grade 4 | Leading to death |
|---------------------------------|-----------------------|----------------------------------|-----------|--------|--------|-----------------|
| Asthenia                         | 99 (4%)               | 59 (24%)                         | 1 (5%)    | 1 (<1%)| 0      | 41 (31%)        |
| Nausea                           | 59 (24%)              | 44 (18%)                         | 2 (1%)    | 0      | 0      | 24 (18%)        |
| Diarrhoea                        | 44 (18%)              | 27 (11%)                         | 2 (1%)    | 0      | 1 (1%)| 20 (15%)        |
| Peripheral oedema                | 35 (14%)              | 27 (11%)                         | 12 (5%)   | 2 (1%) | 0      | 20 (15%)        |
| Ascites                          | 33 (14%)              | 22 (9%)                          | 6 (3%)    | 1 (1%) | 0      | 16 (7%)         |
| Vomiting                         | 33 (14%)              | 22 (9%)                          | 6 (3%)    | 1 (1%) | 0      | 16 (7%)         |
| Constipation                     | 33 (14%)              | 22 (9%)                          | 6 (3%)    | 1 (1%) | 0      | 16 (7%)         |
| Fever                            | 23 (10%)              | 16 (7%)                          | 4 (3%)    | 1 (1%) | 0      | 16 (7%)         |
| Headache                         | 23 (10%)              | 16 (7%)                          | 4 (3%)    | 1 (1%) | 0      | 16 (7%)         |
| Abdominal pain                   | 13 (6%)               | 9 (4%)                           | 2 (1%)    | 0      | 0      | 7 (7%)          |
| Back pain                        | 13 (6%)               | 9 (4%)                           | 2 (1%)    | 0      | 0      | 7 (7%)          |
| Dyspnœa                          | 12 (5%)               | 8 (3%)                           | 2 (1%)    | 0      | 0      | 7 (7%)          |
| Cough                            | 12 (5%)               | 8 (3%)                           | 2 (1%)    | 0      | 0      | 7 (7%)          |
| Anaemia                          | 12 (5%)               | 8 (3%)                           | 2 (1%)    | 0      | 0      | 7 (7%)          |
| Neutropenia                      | 12 (5%)               | 8 (3%)                           | 2 (1%)    | 0      | 0      | 7 (7%)          |
| Thrombocytopenia                 | 12 (5%)               | 8 (3%)                           | 2 (1%)    | 0      | 0      | 7 (7%)          |
| Data are n (%)                   |                      |                                  |           |        |        |                 |

Table 3: Treatment-emergent adverse events and drug-related treatment-emergent adverse events of any grade occurring in at least 10% of patients in either treatment group (safety population).

The most indolent hepatocellular carcinomas, with good ECOG performance statuses (0–1) and acceptable liver functions (Child–Pugh A5–B7), and a tumour burden small enough to keep the patients alive after several lines of systemic treatment before randomisation in RELIVE. Indeed, the patients with the most aggressive forms of hepatocellular carcinoma might have either died during the previous systemic lines or did not meet the eligibility criteria to enter RELIVE because of end-stage liver disease.

Another possible explanation for the failure to detect a treatment difference in the RELIVE study is based on the fact that all the phase 3 trials used a placebo as the control group, whereas the control group in our trial was standard treatment, at the decision of each principal investigator. In our control group, although 55 (41%) of 134 patients received only best supportive care, 79 (59%) received a systemic anticancer therapy, of whom 37 (47%) were given gemcitabine plus oxaliplatin (GEMOX). It is possible that GEMOX might be of benefit for patients with hepatocellular carcinoma, thus concealing the potential benefit of doxorubicin-loaded nanoparticles. Data from phase 3 randomised controlled trials using GEMOX in advanced hepatocellular carcinoma are still needed. In a prospective cohort study by Taieb and colleagues,25 overall survival with GEMOX was 12 months and in a phase 2 single-arm study of 32 patients by Louafi and colleagues,26 it was 11–5 months. In addition, in a large multicentre retrospective study of 204 patients, overall survival with GEMOX was 11 months.27 By contrast, overall survival with free doxorubicin was only 4–9 months in a phase 2 randomised controlled trial in Asia.28

Furthermore, the antitumour activity of doxorubicin-loaded nanoparticles might not be strong enough to extend survival. This idea is supported by the negative findings of the secondary and exploratory endpoints such as progression-free survival, objective response, and time to progression, as well as subgroup analyses, which all clearly show no difference between the 20 mg/m² and the 30 mg/m² groups. Furthermore, the 30 mg/m² group had more patients with drug-related treatment-emergent adverse events than the 20 mg/m² group, thus demonstrating a dose-dependent toxicity of doxorubicin.

Consistent with previously published data, clinically significant (observed in at least 10% of patients) drug-related treatment-emergent adverse events of any grade attributable to doxorubicin were mostly asthenia, nausea, vomiting, and neutropenia, whereas thrombocytopenia (probably due to gemcitabine) and paraesthesia (probably due to oxaliplatin) were observed in the control group. Although acute respiratory distress syndrome occurred in some patients due to intrahepatic arterial injection of doxorubicin-loaded nanoparticles in the phase 1–2 trial,24 no clinically significant pulmonary treatment-emergent adverse events were observed after 6 h of intravenous perfusion in RELIVE. Headache was more prevalent in the patients who received the nanoparticle-loaded
doxorubicin, which might be specific to the nanof ormulation, since this is not commonly reported with free doxorubicin, but is reported with other forms of nanof ormulation of doxorubincin such as liposomal doxorubicin.29 Causes of three deaths (in the pooled experimental group) considered by investigators and the data safety monitoring board to be treatment related were not unusual for this patient population (interstitial lung disease, lung infection, and peritoneal haemorrhage). Of note, only about a tenth of patients in the pooled doxorubicin-loaded nanoparticle group withdrew from the trial prematurely because of drug toxicity.

In conclusion, this first phase 3 study of doxorubicin-loaded nanoparticles in patients with advanced hepatocellular carcinoma who have already been treated with sorafenib did increase overall survival. The results of our trial could inform the design of future studies in this patient population.

Contributors
PM, PA, and BV conceived and designed the study. Principal investigators at each site enrolled patients. PM and JLeB collected the data. PM, BV, and JLeB analysed and interpreted the data. All authors participated in the drafting, review, and approval of the manuscript and the decision to submit for publication.

Declaration of interests
PM has received consultancy and advisory fees from Onxeo, Bayer, Lilly, Ipsen, Bristol-Myers Squibb (BMS), and Merck Sharp & Dohme (MSD). J-FB has received consultancy and advisory fees from Onxeo, Bayer, Lilly, Ipsen, and BMS. J-PB has received consultancy and advisory fees from Onxeo and Bayer. GP has received consultancy and advisory fees from Bayer, AbbVie, Novartis, and Gilead. AA has received consultancy and advisory fees from AbbVie, MSD, and Gilead. IW has received consultancy and advisory fees from Janssen, AbbVie, MSD, Marycy, Pharco, and Gilead. NSY reports grants from Penn State Cancer Institute, Halozyme, Boston Biomedical, Caris Life Sciences, Foundation Medicine, Pharmacyclics, EMD Serono, Onxeo, Regeneron, Momenta, Merck, Daichip Sankyo, Eli Lilly, Novartis, Takeda, Bayer, Celgene, Lexicon, Incyte, Pfizer, and BMS. PA is an Onxeo shareholder and an author of a patent for nanoparticles loaded with chemotherapeutic antitumoural drug (US Patent and Trademark Office 9763874). All other authors declare no competing interests.

Data sharing
Data collected for this study (including individual participant data and a data dictionary defining each field in the set) will be made available to others after approval by the corresponding author. Additional, related documents will be also available (case report forms, statistical analysis plan, and informed consent forms) on request by the corresponding author and sponsor’s approval. These data will be available after publication.

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