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Safety and efficacy of intra-arterial hepatic chemotherapy with doxorubicin-loaded nanoparticles in hepatocellular carcinoma

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ABSTRACT

Background Doxorubicin Transdrug (DT), a nanoformulation of doxorubicin, was demonstrated to overcome the chemoresistance of hepatocellular carcinoma (HCC) in preclinical models. Its efficacy and safety were thus investigated in phase I and randomised phase II trials in unresectable HCC.

Patients and methods Phase I was a single dose of DT through the hepatic intra-arterial (HIA) route, dose-escalating 3+3 trial, evaluating five-dose levels from 10 to 40 mg/m^2 with maximal tolerated dose (MTD) as primary endpoint. The multicentre phase II trial randomly assigned (2:1 ratio) patients to receive either 30 mg/m^2 of DT through HIA route every 4 weeks for up to three courses or best standard of care (BSC). Progression-free survival (PFS) rate at 3 months was the primary endpoint. Overall survival (OS) and disease control rate (DCR) were secondary endpoints.

Results In phase I, haematological and respiratory limited toxicities were reported at 35 and 40 mg/m^2, giving MTD at 30 mg/m^2. Partial response rate was 10%, and stable disease 70%. Phase II was discontinued due to three severe acute respiratory distress events in the DT group while 17 patients had received 30 mg/m^2 DT and 11 BSC. At 3 months, PFS was 64% (95% CI 31 to 89) vs 75% (95% CI 35 to 97), and DCR 35% vs 27% in DT and BSC, respectively (p=NS). Median OS was 32.6 months (95% CI 8.2 to 34.1) in DT group and 15 months (95% CI 8.0 to 18.8) in BSC group (p<0.05).

Conclusion DT increased OS in unresectable HCC but induced severe respiratory distress. Efficacy data deserve further investigation using a safer dosing and schedule regimen.

Trial registration number EUDRACT 2006-004088-77; Results.

Key questions

What is already known about this subject?

- Cytotoxic chemotherapy is not recommended in hepatocellular carcinoma (HCC) due to its toxicity and lack of efficacy.
- Doxorubicine showed benefit on overall survival but with high toxicity.
- Doxorubicine-loaded nanoparticles overwhelm chemoresistance of HCC cells and target the liver.
- Exciting preclinical data are available with this nanoformulation.

What does this study add?

- The maximal tolerated dose of doxorubicine-loaded nanoparticles has been determined in patients with HCC.
- Their hepatic intra-arterial administration in bolus led to severe acute respiratory adverse events.
- However, patients treated by doxorubicine-loaded nanoparticles tended to have better overall survival.
- Experimental data in rats demonstrated that prolonged infusion time over 2 hours of doxorubicine-loaded nanoparticles prevented these severe respiratory adverse events.
- Systemic therapies are needed for HCC. Doxorubicin efficacy and toxicity can be improved by nanoformulation. Doxorubicin -nanoparticles have a maximum tolerated dose of 30 mg/m^2, and seem to show trends to benefit on overall survival of patients with HCC. However, this drug displays acute lung toxicity, that compels to apply duration of infusion above 2 hours by intravenous route.

How might this impact on clinical practice?

- The schedule of administration has been changed in a 6-hour intravenous perfusion.
- A phase III trial is being conducted in HCC, testing doxorubicine-loaded nanoparticles after sorafenib failure.
- If positive, this trial could change the therapeutic algorithm of HCC.

INTRODUCTION

Hepatocellular carcinoma (HCC) is the second cause of cancer death worldwide. Majority of patients are not eligible for curative therapies, and only palliative options such as transarterial hepatic chemoembolisation (TACE) or systemic therapies can be

applied.[1] Sorafenib has been the first systemic therapy to show benefit on overall survival (OS).[2 3] Other targeted therapies have failed in phase III trials (erlotinib, everolimus, brivanib and sunitinib) except regorafenib in second-line therapy.[4–8] Recently, lenvatinib demonstrated equivalent efficacy to sorafenib.[9] In contrast to many other cancers, HCC cells demonstrate chemoresistance. However, doxorubicin was among the most convincing, although associated with high morbidity in cirrhotics.[10] Other trials comparing different chemotherapies to doxorubicin showed an advantage for doxorubicin versus nolatrexed,[11] or equivalence versus oxaliplatin/fluorouracil/leucovorin combination.[12] By the way, doxorubicin did not demonstrate any additive or synergic effect to sorafenib.[13] The complex mechanisms of HCC chemoresistance to doxorubicin involve the efflux pumps encoded by the *MDR* (multiple drug resistance) genes. Some evidence indicates that high expression of P-glycoproteins, also known as MDR protein 1 encoded by the ABCB1 (ATP-binding cassette subfamily B member 1) gene, is a major mechanism of HCC resistance to a broad range of chemotherapeutic drugs.[14] Furthermore, other proteins such as MDR-related proteins have been known to induce MDR to hydrophobic drugs by decreasing their intracellular accumulation via an ATP-dependent efflux mechanism.[15] Doxorubicin Transdrug (DT) is a nanoformulation consisting of a molecular complex of doxorubicin adsorbed on polyethylbutylcyanoacrylate (PEBCA) polymer nanoparticles (NP), defined as submicronic (100–200 nm) and ultradispersed colloidal systems. PEBCA is taken up and concentrated inside the liver due to opsonisation processes with cell internalisation, efficient drug protection, controlled release and reversion of MDR.[16 17] Experimentally, DT is able to bypass MDR in vitro, and is effective in vivo at much lower doses than doxorubicin, which is assumed to improve the benefit/risk ratio.[18–20] Objectives of the present study were to assess in human HCC the maximal tolerated dose (MTD) of DT in phase I, and to confirm safety and efficacy of DT in a randomised comparative phase II trial.

PATIENTS AND METHODS

Study designs and endpoints

Phase I was a multicentre, non-randomised '3+3' dose-escalating study. DT was a single hepatic intra-arterial (HIA) injection. Primary endpoint was to determine MTD following dose-limiting toxicities (DLT) observed within the 4 weeks after injection according to National Cancer Institute (NCI)/Common Terminology Criteria for Adverse Events (CTCAE). Testing increasing doses (10, 20, 30, 35 and 40 mg/m^2) of DT, patients were included in a stepwise manner, each patient being included after assessment of DLTs in the previous one and agreement of the Coordination Committee. Secondary endpoints were OS and objective response rate (ORR) by CT scan using Response Evaluation Criteria in Solid Tumors (RECIST) by an independent radiology committee.

Phase II (EUDRACT No 2006-004088-77) was a multicentre, open, randomised (2:1) with a web-based dynamic minimisation, controlled study comparing the efficacy and safety of three HIA injections of 30 mg/m^2 DT at 4-week intervals in arm A versus the best standard of care (BSC, treatment at free choice of each investigator) in arm B. Primary endpoint was progression-free survival (PFS) at month 3 in arm A based on RECIST as assessed by an independent radiology committee on 50 planned patients. Secondary endpoints were tolerance, OS and ORR. Patients were monitored every 3 months by hepatic CT scan until progressive disease (PD). Adverse events were recorded since the first injection of DT up to 30 days after the last injection according to the NCI/CTCAE, and safety was monitored by an independent safety board.

Patient selection

In phase I and II studies, inclusion criteria were: (1) HCC ineligible for curative options (surgical resection, liver transplantation, radiofrequency ablation) according to international guidelines,[21] and absence of objective response to TACE when applicable; (2) absence of decompensated cirrhosis; (3) Eastern Cooperative Oncology Group ≤2; and (4) 18–80 years of age. Non-inclusion criteria were: (1) HCC invasion >50% liver parenchyma; (2) HCC occurring on a transplanted liver; (3) impaired clotting tests (platelet count <100 × 10^9/L or prothrombin activity <60%); (4) contraindication to doxorubicin (neutrophils <1.500/mm^3, cardiac left ventricular ejection fraction <50%, previous cumulative dose of doxorubicin ≥550 mg/m²); (5) respiratory insufficiency (forced vital capacity (FVC) <80%, total lung capacity <80%, carbon monoxide diffusing capacity (or transfer factor) <80%, forced expiratory volume/FVC <65%, PaO_2<75 mm Hg); (6) presence of high risk bleeding of oesophageal varices; and (7) anticancer therapy within 6 weeks prior to inclusion.

Treatment

DT (Livatag, Onxeo, Paris, France) was injected at day 0 in a 15 min perfusion by non-selective HIA route after premedication by 32 mg methylprednisolone at 24 hours and 2 hours before, and 24 hours after DT injection. Neither lipiodol nor embolisation procedure was associated with DT. Patients were discharged of hospitalisation 72 hours later. Physical examination and blood tests were done at days 1, 2, 3, 7, 14, 21 and 28. BSC was applied in each patient with PD in phase I or II, or in arm B in phase II.

Statistical analysis

Data were analysed using SAS (V.9.1.3). Tolerance and efficacy analyses were conducted in intention to treat. In phase I, all analyses were descriptive without any test of significance. Phase II was designed as a classical one-stage trial with inactivity cut-off 50%, activity cut-off 75%, the hypothesis of interest being H_0: r≤50% against H_A: r≥75%, 'r' being the proportion of patients free of PD at

month 3. The type I error rate (α) was 5%, and the type II error rate (β) 10%. Under these assumptions, 50 patients were required: 33 in DT arm and 17 in BSC arm. Times to events were estimated using the Kaplan-Meier method and plotted as curves by arm. The comparisons between two arms used log rank, χ^2 or t-tests.

Ethical and legal considerations

Phase I/II trials were conducted in accordance with the recommendations of the Declaration of Helsinki, the Edinburgh amendment of October 2000, the French regulatory requirements (Loi Huriet), the rules of the International Conference on Harmonisation and the European Good Clinical Practice standards. All patients gave a written informed consent.

RESULTS

Patient recruitment and safety

Phase I included 21 patients, 20 treated from February 2004 to July 2006, and 1 withdrawn before therapy due to renal failure. Four DT dose levels (10, 20, 30 and 40 mg/m²) were evaluated in 12 patients. MTD was presumed at 30 mg/m^2 since 2 of 3 patients showed grade 4 neutropenia at 40 mg/m^2. An amendment allowed us to test 35 mg/m^2 dose level, 2 of 5 patients developing acute respiratory distress syndrome (ARDS), transient and rapidly reversible. Thus, three additional patients were treated at 30 mg/m^2 dose level to ensure its safety. All the patients experienced at least one treatment-emergent adverse event (TEAE) of any grade at any dose level, the most frequent being lymphopaenia (65%), neutropenia (60%), hypertransaminasaemia (60%), hyper-GGT (gamma-glutamyltransferase; 35%), nausea (50%) and respiratory events (10%). Grade 3–5 TEAEs were (table 1): blood disorders, hypertransaminasaemia and hyper-GGT, headache, peripheral arterial hypotension, dyspnoea, cough and ARDS. A dose effect was identified with a marked increase of TEAE incidence and severity at 35 mg/m^2 and 40 mg/m^2, giving 30 mg/m^2 as MTD. For phase II, recruitment started on December 2006, but was prematurely discontinued upon recommendation of the independent safety board on July 2008 because of four cases of severe unexpected ARDS with three deaths occurring in arm A. At this time, 28 patients had been randomised and treated: 17 in arm A (9 patients with 3 DT injections, 4 patients with 2 injections, 4 patients with 1 injection) and 11 in arm B (11 patients received TACE and 1 patient transarterial chemolipiodol). Both arms shared similar demographics and other baseline characteristics, except arm A which had more advanced versus intermediate HCCs than arm B (table 2). Every patient in both arms experienced at least one TEAE. The most frequent grade 3–5 TEAEs in arm A were neutropenia (41%), lymphopaenia (29%), ARDS (24%) and leucopenia (18%) (table 3). Among the three ARDS-related deaths in arm A, the first patient was a 74-year-old man with dysmetabolic comorbidities, ARDS occurring 24 hours after the first DT injection, and death 20 days later; the second patient was a 68-year-old woman without any comorbidity, ARDS occurring 48 hours after the second DT injection, and death 3 days later; the third patient was a 64-year-old woman without any comorbidity, ARDS occurring 48 hours after the second DT injection, and death 13 days later.

Antitumour activity

In phase I, 16/20 patients were evaluable at month 1 (CT scans not available for 4 patients), showing 6% complete response (CR) (1 patient at 30 mg/m^2), 6% partial response (PR) (1 patient at 35 mg/m^2), 69% SD and 19% PD, thus 81% disease control rate (DCR). In phase II, only 11/17 patients in arm A were evaluable at month 3 versus 8/11 patients in arm B due to premature withdrawals of the study. ORRs between arms A and

Table 1 Grade 3–5 TEAE in the phase I trial by dose level (incidence >20%)

DT dose level (number of patients)	10 mg/m² (n=3)	20 mg/m² (n=3)	30 mg/m² (n=6)	35 mg/m² (n=5)	40 mg/m² (n=3)
Lymphopaenia				3	2
Neutropenia				2	2
Thrombocytopenia			1	1	1
ALAT increased	1			2	
ASAT increased			2	1	1
GGT increased			2	1	
Dyspnoea			1		
Cough		1			
Acute respiratory distress syndrome				2	
Headache				1	
Hypotension		1			

ALAT, alanine amino transferase; ASAT, aspartate amino transferase; DT, Doxorubicin Transdrug; GGT, gamma-glutamyltransferase; TEAE, treatment-emergent adverse event.

Table 2 Baseline characteristics of patients with HCC in the phase II trial

Treatment group	Arm A: DT 30 mg/m^2 (n=17)	Arm B: BSC (n=11)
Age (years): mean (range)	71 (51–78)	64 (52–80)
Gender (M/F)	13/4	10/1
Aetiological factors: virus/alcohol/others	3/13/3	4/6/1
Cirrhosis	16	11
ECOG (0/1/2)	10/6/1	6/4/0
AFP>400 µg/L	1 (6%)	3 (27%)
Multinodular, n (%) (nodule size: range in mm)	15 (88%) (29–251)	11 (100%) (26–175)
Portal invasion	11 (69%)	5 (45%)
Extrahepatic metastasis	0	0
BCLC stage B-C	6/11 (35%/65%)	6/5 (55%/45%)*

*p<0.05 vs DT 30 mg/m^2.
AFP, alpha-fetoprotein; BCLC, Barcelona Clinic Liver Cancer; BSC, best standard of care; DT, Doxorubicin Transdrug; ECOG, Eastern Cooperative Oncology Group; HCC, hepatocellular carcinoma.

B were respectively 0% vs 0% CR, 9% vs 38% PR, 55% vs 38% SD and 36% vs 24% PD, thus 64% vs 76% DCR (p=NS).

Survival

In phase I, OS (including all levels of DT) was 19.5 months (95% CI 5.6 to 29.4) with seven patients alive at 2 years. In phase II, OS was 32.6 months (95% CI 8.2 to 34.1) in arm A vs 15.0 months (95% CI 8.0 to 18.8) in arm B (p=0.0494) (figure 1A). Cox proportional hazards regression showed a proportional increase in survival probability depending on the number of DT injections (3 or <3) (figure 1B). This latter should be carefully interpreted since ARDS-related premature death occurred at the first or second injection. PFS at month 3 was similar between arm A and arm B: 64% (95% CI 31 to 89) vs 75% (95% CI 35 to 97) (p=NS). After the prematurely study treatment discontinuation, 6/17 patients (35%) and 5/11 patients (45.5%) of arm A and arm B, respectively, were treated according to each investigator decision. In arm A were given sorafenib (four patients), everolimus (one patient), TACE (four patients) and conformal radiotherapy (two patients). In arm B were given sorafenib (three patients), additional TACEs (three patients), erlotinib (one patient) and liver transplantation (one patient).

Table 3 Grade 3–5 TEAEs in the phase II trial by treatment group (DT 30 mg/m^2 and BSC: TACE in 10/11 patients) with incidence >10%

Group (number of patients)	Arm A: DT 30 mg/m^2 (n=17)	Arm B: BSC (n=11)
Lymphopaenia	5 (29%)	1 (9%)
Leucopenia	3 (18%)	–
Neutropenia	7 (41%)	–
Thrombocytopenia	2 (12%)	–
ASAT increased	1 (6%)	5 (45%)
GGT increased	2 (12%)	1 (9%)
Dyspnoea	2 (12%)	–
Acute respiratory distress syndrome	4 (24%)	–
Renal failure	2 (12%)	1 (9%)
Headache	2 (12%)	
Abdominal pain	1 (6%)	2 (18%)

ASAT, aspartate amino transferase; BSC, best standard of care; DT, Doxorubicin Transdrug; GGT, gamma-glutamyltransferase; TACE, transarterial chemoembolisation; TEAE, treatment-emergent adverse event.

Assessment of DT-related lung toxicity in rats

We aimed at reproducing in Wistar rats the DT-related ARDS events observed in humans, and at evaluating the factors that might influence their occurrence and severity. DT was compared with free doxorubicin (DOX), PEBCA NPs devoid of loaded doxorubicin and excipient (EXC) alone (table 4). The intravenous bolus did not induce any mortality with 7.5 mg/kg DOX by comparison to 62% mortality with the same dose of doxorubicin in DT (DT-Dox/7.5 mg/kg). DT-induced mortality correlated with the dose of doxorubicin loaded in DT: 0% with DT-Dox/5 mg/kg and 83% with DT-Dox/10 mg/kg. Further, the prolonged duration of infusion decreased mortality: 7% with 2-hour infusion of DT-Dox/7.5 mg/kg, and 0% with 2 hours 30 min. All deaths were observed within 48 hours postinjection, closely related to ARDS events, mimicking lung-related mortality observed in humans in the phase I/II clinical trial. Macroscopic examination of rats showed acute lung injury with exudates in thoracic cavity, increased lung weight (average, +144%) due to oedema and presence of lung parenchyma haemorrhages with dark dots (figure 2). Microscopy highlighted the enlargement of perivascular areas around large veins, consisting of oedema with erythrocytes and fibrinous material, devoid of inflammatory cells. The alveolar compartment was normal in most cases except focal areas of macrophagic or haemorrhagic alveolitis. Focal abrasion of the bronchial epithelium was also noted. The perivascular oedema was 22-fold increased in the DT-Dox/7.5 mg/kg bolus-treated group as compared with controls. In contrast, DT-Dox/5 mg/kg bolus group and DT-Dox/7.5 mg/kg slow infusion group showed only mild perivascular oedema (ratio: 4 vs EXC or DOX). Further, heart examination showed absence of any abnormality in all groups except slight pericardial fibrosis or focal myocardial oedema with polymorphous inflammatory infiltrates in the DT-Dox/7.5 mg/kg and 10 mg/kg bolus-treated

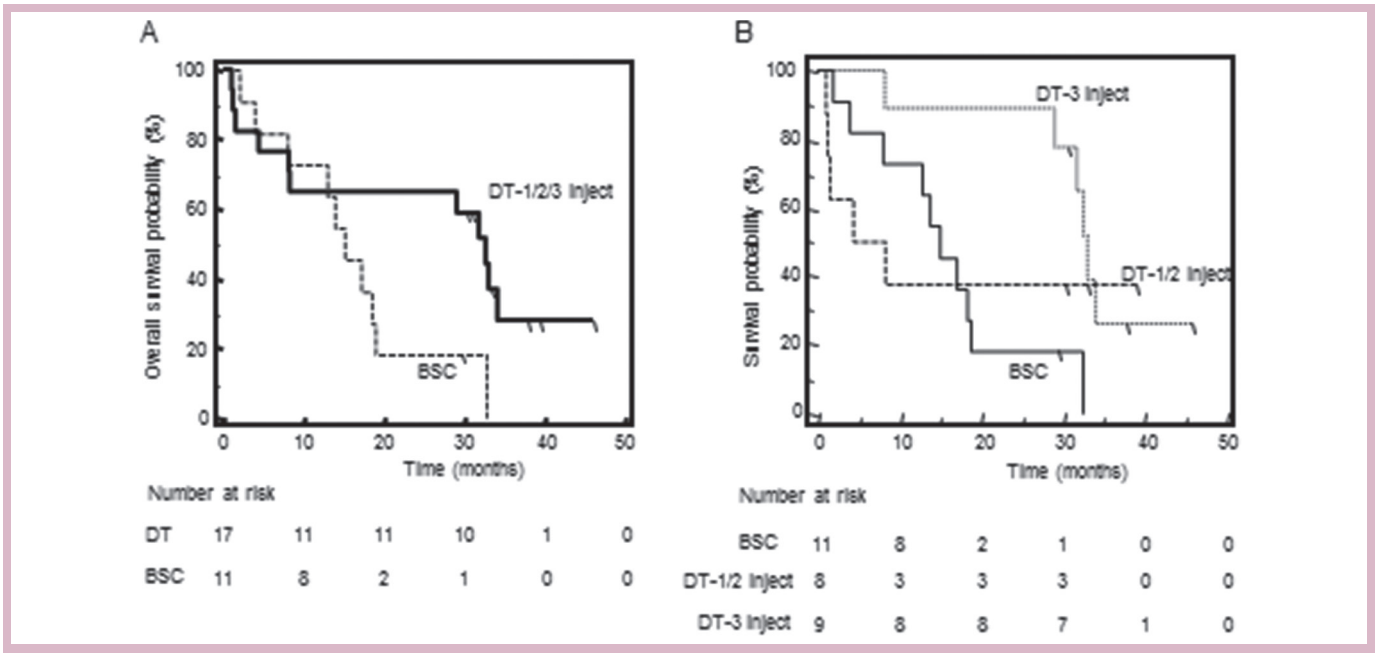

Figure 1 Kaplan-Meier curves for overall survival (OS) probability. (A) Doxorubicin Transdrug (DT; one, two or three injections) and best standard of care (BSC) groups. Median OS of respectively 32.6 months (95% CI 17.9 to 34.5) for DT vs 15.0 months (95% CI 10.3 to 21.5) for BSC. Comparison of survival curves by log-rank test, p=0.0494. (B) Survival curves depending on the number of DT injections: three injections (DT-3 inject) or one to two injections (DT-1/2 inject).

groups. No abnormalities were observed in livers and kidneys (weights and histology).

DISCUSSION

Efficient systemic therapies are dramatically lacking for advanced HCCs although sorafenib, regorafenib and tivantinib have shown significant benefit.[2 8 9] Doxorubicin could be of interest but showed substantial morbidity and modest benefit in controlled trials.[10] Indeed, HCC cells efflux anthracyclines via the MDR-encoded pumps.[14] It has clearly been shown that NP formulations could carry cytotoxic agents such as doxorubicin into the cancerous cells, overwhelming these pumps, thus rendering the drug much more efficient.[16] Herein, we tested DT in phase I and II trials for patients with HCC. Whereas phase I determined the MTD, phase II showed ARDS toxicity leading to premature withdrawal of the trial. However, exciting preliminary data on survival encouraged us to carry on some experimental investigations on DT.

We used the Wistar rat model to figure out parameters governing lung toxicity of DT. ARDS started within 48 hours following DT injection. This unexpected and severe TEAE had never been reported so far with DOX, and thus could be attributed to the nanoformulation of DT. Indeed, hypersensitivity reactions are frequent for infused nanomedicines or biological agents.[22–25] Immediate postinfusion reactions non-explained as IgE-mediated allergy have been described in 7%–45% with liposomal drugs including liposomal doxorubicin (Doxil, Myocet), micellar drugs with Cremophor EL (Taxol) or with poloxamer-188 (Fasturec), monoclonal antibodies and radio/ultrasound contrast agents. They are usually

Table 4 Cumulative death in Wistar rats 48 hours after intravenous injection of excipient (EXC), unloaded PIHCA nanoparticle (NP), free doxorubicin (DOX) or Doxorubicin Transdrug (DT)

Treatment/Wistar rats (n=244)	Dose level of doxorubicin	Duration of infusion	Cumulative death
EXC (n=54)	–	Bolus	0%
NP (n=6)	Equivalent to PIHCA in DT 7.5 mg/kg	Bolus	0%
DOX (n=24)	7.5 mg/kg	Bolus	0%
DT (n=44)	7.5 mg/kg	Bolus	62%
DT (n=50)	10 mg/kg	Bolus	83%
DT (n=31)	5 mg/kg	Bolus	0%
DT (n=12)	7.5 mg/kg	2 hours	7%
DT (n=23)	7.5 mg/kg	2.5 hours	0%

PIHCA, polyisohexylcyanoacrylate.

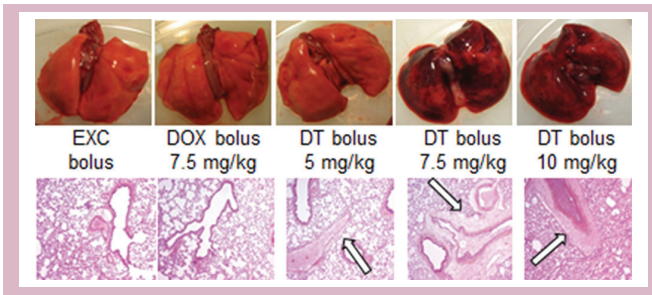

Figure 2 Macroscopical and microscopical examination of Wistar rat lungs after different treatment schedules: EXC (excipient) as control, DOX (doxorubicin) bolus, DT (Doxorubicin Transdrug) bolus at three different dosages (5, 7.5 and 10 mg/kg).

of mild intensity, but they can occasionally be severe and lethal (<5%). They can arise after the first injection, commonly become milder and resolve spontaneously after repeated injections, and finally can disappear. Their pathogenesis involves activation of complement, resulting in the release of several vasoactive mediators including histamine, tryptase, platelet activated factors (PAF) and leukotrienes by mast cells, basophils and macrophages, and thus have been called CARPA (complement-activated related pseudoallergy) syndrome. Several risk factors have been identified such as elderly, female gender, history of drug allergy and concomitant administration of β-blockers. One could expect that incidence of severe DT-related hypersensitivity reactions observed in our study was quite similar to cases reported with other drugs (3%–45% with liposomal drugs, 2% of severe forms). However, preliminary investigations showed that DT did not induce complement activation (C3b, Bb, C4d) using human plasma from healthy donors. Several risk factors were present in our patients: 2 of 3 older than 65 years, 2 of 3 female (reported incidence of 50% in women vs 7.5% in men) and 2 of 3 having β-blockers. Cirrhosis might have played a key role due to the common reduction of reticuloendothelial activity leading to absence of phagocytosis, and the intrahepatic vascular shunts allowing rapid distribution of DT towards lungs. Comparatively, similar moderate to severe reactions were reported in 45% of patients after the first injection of Doxil, whereas any prophylaxy with corticosteroid and antihistamine drugs reduced this prevalence to 8% (0%–25%). The arising of lung symptoms has been described to be correlated with the dose and speed of Doxil infusion. Thus, we set up a paradigm of DT-induced ARDS in Wistar rats (online supplementary material), clearly confirming that the specific ARDS toxicity of DT depends of both the dose and duration of DT infusion with absence of toxicity when exceeding 2 hours.

Because phase II was prematurely stopped, any statistical analysis did not seem very robust. However, although no difference could be observed in terms of tumour response (RECIST 1.1 criteria) and PFS at month 3 between DT and BSC arms, OS appeared as significantly improved in the DT arm, reaching 32.6 months. The study population included about half/half patients with Barcelona Clinic Liver Cancer (BCLC)-B/C HCC, well known as having respectively 20 months/11 months OS when treated by TACE (BCLC-B) or sorafenib (BCLC-C), and respectively 15 months/7 months in absence of above cited treatments.[3 26] The best OS was observed in patients receiving three DT injections, suggesting a beneficial cumulative effect of DT. OS in the BSC arm reached very rationally 15 months. After the 3-month study period, there was no significant difference in treatment regimen subsequently delivered between DT and BSC arms: abstention (65% vs 55%), TACE (24% vs 27%) and sorafenib (24% vs 27%). Thus, the difference in OS between the two groups could not be attributed to an uneven distribution of treatments after the trial withdrawal, but rather to DT injections. These exciting data on OS with DT, and at the view of a better understanding and prevention of lung toxicity, led us to redesign a new clinical study in patients with advanced HCC with a longer duration of DT infusion (6 hours by intravenous route) at the MTD of 30 mg/m^2, and a second arm with lower dose of 20 mg/m^2 (ClinicalTrials.gov Identifier: NCT01655693).

In summary, herein we have shown that DT might improve OS in patients with BCLC-B/C HCC. Global tolerance was manageable with the exception of the occurrence of some severe DT-related ARDS. Experiments in Wistar rats helped us set up a new schedule of prolonged DT infusion to minimise lung toxicity for subsequent trials.

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Competing interests PM: participant to boards and consultancy for Onxeo and Bayer; participant to boards for BMS. JPZ: honorary from Abbvie and consultancy for Gilead, BMS and Janssen. MB: consultancy for Bayer Pharma and Bristol-Myers Squibb; advisory board for Bayer Pharma and Bristol-Myers Squibb; received honoraria from Bayer Pharma and Sirtex. JT: honorary from Roche, Merck, Amgen, Celgene, Baxalta, Sanofi, Lilly and Sirtex. BV is an employee of Onxeo. PA is a shareholder of Onxeo and a patent owner.

Patient consent Obtained.

Ethics approval Ethics Committee (CCPPRB) of Lyon A, France, and the French Drug Agency (Agence Française de Sécurité Sanitaire pour les Produits de Santé).

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