SYNOPSIS

Name of Company: Nerviano Medical Sciences, s.r.l. **Name of Finished Product:** Not Applicable **Name of Active Ingredient:** Nemorubicin Hydrochloride (PNU-152243A)	*(For National Authority Use only)*
Title of Study: Nemorubicin hydrochloride (PNU-152243A) administered via intrahepatic artery in combination with cisplatin in adult patients with unresectable hepatocellular carcinoma: Phase II study preceded by dose-escalation	
Protocol Number: NEMA-0027-017	
Investigators: 1) Francesco Izzo (Coordinating Investigator); *2) Carlo Barone*; 3) Giordano Beretta*; 4) Fabio Farinati*;* 5)Sergio Frustaci; 6) Cosmo Gadaleta; 7) Roberto Labianca; 8) Maurizio Grosso; *9) Franca Meloni*;* 10) Mark Middleton; *11) Eric Raymond**: 12) Juan W. Valle; 13) Thomas J Vogl; **= no recruitment in their sites*	
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Publication Reference: Not applicable	
Studied Period (Years): 19 December 2005 19 April 2012	**Phase of Development:** II
Objectives: ***Primary:*** Dose-escalation portion: determination of MTD and DLT; characterization of toxicities and their reversibility; identification of Recommended Dose for Phase II (RP2D). Phase II portion: evaluation of antitumor efficacy in terms of Response Rate (proportion of patients with confirmed CR or PR or downstaging). ***Secondary*** Both dose-escalation and phase II portion*:* evaluation of tumor response characteristics (i.e., duration of response, time to progression, changes in alpha-fetoprotein levels); overall survival; safety profile after repeated administrations; pharmacokinetic (PK) profile of the two compounds when administered in combination.	

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Methodology: This was a multicenter, single-arm, open-label, dose-escalation/Phase II study of nemorubicin administered by IHA in combination with cisplatin to unresectable HCC patients. Nemorubicin was to be administered first, as a 30 minutes infusion. Cisplatin was to be administered 15 minutes after the end of nemorubicin infusion, as a 30 minutes infusion. Both drugs were to be administered through a pump to standardize infusion time. IHA treatment was to be repeated every 4 weeks. Drugs administration was to be done through the hepatic artery, so to perfuse both lobes of the liver.

Number of Subjects (Planned and Analyzed):
- Dose escalation:
Intermediate risk population: 20-25 patients expected and 22 enrolled and treated.
Advanced risk populations: 20-25 patients expected and 15 enrolled and treated.
- Phase II:
Intermediate risk population: 40 patients expected, 44 enrolled and 42 treated.
Advanced risk population: 40 patients expected, 43 enrolled and 37 treated.

Diagnosis and Main Criteria for Inclusion:
Patients with unresectable, microscopically confirmed HCC, not amenable to ablation techniques, either newly diagnosed or relapsing after prior surgery/ablative techniques (including embolization, provided that the lesions at study entry are vascularized through hepatic artery) (if biopsy is contraindicated, CT scan/MRI evidence of hepatic tumor and AFP ≥400 ng/mL acceptable as evidence of HCC); CLIP 0-1 patients, Child A or B with bilirubin restriction to ≤ 1.5 x UNL, ECOG PS 0-1 and no portal vein thrombosis ("intermediate risk" population); CLIP 2 patients, Child A or B with bilirubin ≤ 2.5 mg/dL, ECOG PS 1 or 2, portal vein thrombosis admitted (in advanced risk population only as per Amendment N°2); at least one bidimensionally measurable disease ≥2 cm in at least 1 diameter with conventional CT scan/MRI or ≥1 cm in at least 1 diameter with spiral CT scan (for Phase II only); resolution of all acute toxic effects of any prior surgical/ablative procedure to NCI CTC Grade ≤1; 18-75 years of age (inclusive); adequate bone marrow function, defined as ANC ≥1.5 x 10^9/L; PLT ≥75 x 10^9/L, and Hb ≤ CTC Grade 1; adequate liver function defined as SGOT, SGPT, and ALP ≤ 2.5 x UNL for the Institution; adequate renal function defined as serum creatinine < 1.5 x UNL and creatinine clearance (Cockcroft formula) > 30 mL/min; coagulation parameters (PT and APTT) < 1.5 x UNL and INR ≤ CTC Grade 1; LVEF within normal limits for the Institution; no prior systemic or locoregional anticancer therapy for HCC (e.g., chemoembolization, chemotherapy, immunotherapy, etc.) or liver transplantation or radiotherapy to treat tumor lesions present at study entry; absence of extrahepatic metastatic disease, or current active second malignancy (with the exception of cone-biopsied in situ carcinoma of the cervix uteri, or non-melanoma skin cancer); absence of vascular invasion or complete obstruction of the portal vein (for intermediate risk patients only); absence of bleeding diathesis, or bleeding (within prior 6 months) from esophageal varices, or clinically detectable ascites or pleural effusion, or symptomatic ulceration of gastrointestinal ulcers (≤30 days prior study entry); absence (within the last year) of previous myocardial infarction, unstable angina, cerebrovascular accident or transient ischemic attack, pulmonary embolism, deep vein thrombosis, or other significant thromboembolic events, heart failure or uncontrolled arterial hypertension; absence of severe systemic diseases, or active infections (other than chronic active hepatitis).

The protocol was amended in November 2006 (Amendment No. 2) as regards eligibility criteria to allow the inclusion of patients previously treated for HCC: both systemic and locoregional therapy was allowed provided that ≥ 6 weeks had elapsed from prior therapy, prior chemotherapy regimen did not contain platinum derivatives, and liver lesion(s) were vascularized at study entry; in addition presence of vascular invasion was allowed, when documented, in advanced population only. Eligibility criteria were further amended in October 2007 (Amendment No. 3) to increase the threshold value for platelets at inclusion from ≥ 50,000/mm^3 (≤ Grade

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2) to $\geq 75{,}000/mm^3$ ($\leq$ Grade 1) and to exclude patients with allergy to the contrast agents.
The protocol was further amended in January 2011 (Amendement No. 4) to notify that the evaluation of CYP3A4 activity in plasma samples (biomarker reaction of quinine to 3-hydroxy quinine) was not performed due to the fact that some centers did not perform the quinine test.

Test Product, Dose and Mode of Administration, Batch Number:
Nemorubicin hydrochloride is formulated as powder for solution, to be supplied in freeze-dried vials containing 500 mcg of active drug substance to be dissolved in saline.
Cisplatin is formulated as 1 mg/ml sterile concentrate for solution for infusion. For the purpose of this study cisplatin (50 mg/vial) was purchased on the market and *ad hoc* relabeled by the Sponsor before being provided to the investigational sites.
The ***combination*** nemorubicin + cisplatin was to be given by an IHA administration on Day 1, in a 4-week cycle, at the following planned doses: 200+40, 200+60, 400+60, 600+60 and 600 mcg/m^2 nemorubicin + 70 mg/m^2 cisplatin, respectively. Nemorubicin was to be administered first, as a 30 minutes infusion. Cisplatin was to be administered 15 minutes after the end of nemorubicin infusion, as a 30 minutes infusion.

The batch numbers used for this study were:
Nemorubicin hydrochloride: N0500693, N0600620, N0600622, N0700316, N0700318, N0700484, N0700547, N0800077, N0800134, N0800146, N0800160, N0900001, N0900683, N0900922, N0900923, N0901189, N0901426, N0902052, N1000068, N1000275, N1000432, N1000933.

Cisplatin: N0500264, N0600348°, N0600609, N0600784, N0700324, N0700326, N0700477, N0700478, N0700479, N0700485, N0700631, N0700772, N0800029, N0800078, N0800079, N0800161, N0800287, N0800288, N0800289, N0800549, N0800550, N0800913, N0900002, N0900238, N0900239, N0900240, N0900575, N0900577, N0900676, N0900681, N0900684, N0900921, N0901302, N0901421, N0901422, N0901423, N0901795, N0901796, N0901797, N0901943, N0902054, N0902055, N0902285, N0902286, N1000403, N1000715.

Reference Therapy, Dose and Mode of Administration, Batch Number: Not applicable

Duration of Treatment: Patients were to continue treatment for a minimum of 2 cycles, until disease progression, patient refusal or withdrawal of patient consent, or the occurrence of unacceptable toxicity. A maximum of six cycles was recommended. For responding patients, additional cycles might have been given under strict monitoring of the cardiac function and after discussion with the Sponsor. The end of trial was defined as the time when all the protocol specified numbers of patients were fully evaluable for the primary endpoint and the time when the follow up monitoring up to two years after the patients' enrollment was completed.

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Endpoints and Criteria for Evaluation:

Primary Endpoints - Dose escalation

- First cycle DLTs to be used for MTD definition;
- Laboratory and clinical safety parameters (including cardiac function monitoring);
- Adverse events emerging during the trial

Primary Endpoints - Phase II

Overall confirmed objective tumor response, defined as a best confirmed response of CR or PR, or a tumor downstaging allowing for tumor lesion surgery or ablation. Objective tumor responses were to be determined according to modified World Health Organization (WHO) criteria.

Secondary Enpoints (both Dose Escalation and Phase II portions):
- Down-Staging: regression of a previously unresectable lesion up to be considered resectable (ie, suitable for surgery or other ablative techniques, whether or not the patient actually undergoes resection);
- Duration of Response; The time from the date of the first documentation of confirmed objective tumor response to the date of first documentation of objective tumor progression, objective tumor recurrence (in patients with a CR or with a PR regarded as a down-staging and followed by surgery), or of death due to any cause (in the absence of previous documentation of objective tumor progression), whichever comes first.
- Time to Tumor Progression (TTP): The time from the date of enrollment to the date of first documentation of objective tumor progression, objective tumor recurrence (in patients with a CR or with PR / down-staging who underwent surgery), or of death due to any cause, whichever comes first.
- Overall Survival (OS), i.e. the time from the date of treatment start to the date of death from any cause; in the absence of documentation/confirmation of death, survival time was to be censored at the date of the last visit or contact documenting that the patient was still alive.
- Alpha-fetoprotein (AFP) decrease; Duration of AFP response; Time to AFP porogression.
- Safety of repeated administrations
- Pharmacokinetic profile of nemorubicin and cisplatin, when the two drugs are given in combination.

Statistical Methods:
Tumor staging at study entry was to be evaluated for each patient according to the CLIP Scoring system. Two populations were to be explored: 1) "intermediate risk" population, i.e. patients characterized by CLIP 0-1, Child A or B with bilirubin restriction to ≤ 1.5 x UNL, ECOG PS 0-1 and no portal vein thrombosis 2) "advanced risk" population, i.e. patients characterized by CLIP 2, Child A or B with bilirubin ≤ 2.5 mg/dL, ECOG PS 1 or 2, portal vein thrombosis admitted.
The "intermediate risk" population was to be evaluated first; the evaluation of the tolerability of the combo on the "advanced risk" population was planned to start as soon as the safety evaluation on the first two patient cohorts of the "intermediate risk" population was available.
Descriptive statistics and patients' data listings were to be used in the characterization of patients' disposition, demographic and baseline characteristics, treatment exposure and safety variables.
Dose-escalation portion. In each population, dose escalation was to be carried out in cohorts of 3 patients each. Patients treated at a given dose level were to be observed for at least three weeks of their first cycle of treatment, before the next cohort could start at an escalated dose. In each cohort, the first patient treated was to be followed for at least 3 weeks before entering the other two patients at the same dose level. In the event that 1 (and only 1) out of the first 3 patients at a given dose level developed DLT in the first cycle of treatment, the number of patients at this dose level was to be increased to 6 and toxicity evaluated for three weeks before continuing escalation. If ≥ 2/3 or ≥ 2/6 patients experienced a first cycle DLT, that dose level was to be

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considered the MTD. The previous dose level was to be considered the potential recommended Phase II dose. If at that dose level only 3 patients had been evaluated, 3 additional patients were to be enrolled and followed for at least 3 weeks of the first cycle. If no more than 1 out of 6 patients treated on that dose level experienced DLT, this dose level was to be established as the recommended Phase II dose. Otherwise the same procedure was to be followed with the next previous level until the recommended dose was identified.

Phase II portion. Once the recommended Phase II dose was identified in each patient population, additional patients were to be enrolled on that dose to evaluate the antitumor activity of the nemorubicin-cisplatin combination. This Phase II portion of the study was designed as a single arm, open label, two-stage study using Simon's minimax design with the following characteristics:

Population	H0 vs. Ha	α (1-sided), 1-β	Step 1 RR *	Final Analysis RR **
Intermediate Risk *(33 ptss evaluable for RR)*	p≤0.20 vs. p≥0.40	0.05, 0.80	≥ 5 / 18	≥ 11 / 33
Advanced Risk *(29 pts evaluable for RR)*	p≤0.10 vs. p≥0.30	0.05, 0.80	≥ 2 / 10	≥ 6 / 29

** RR = Response Rate; Number of patients with objective response (confirmed or unconfirmed) out of the set of 'step 1' evaluable patients, required to proceed from step 1 to the final analysis*
*** RR = Response Rate; Number of patients with confirmed objective response out of the set of all evaluable patients, required not to reject the experimental treatment in the final analysis*

Analyses: In each patient population, the dose-escalation and the Phase II portion of the study were to be analyzed separately. Patients enrolled at the recommended combo dose in the dose escalation phase and fulfilling the eligibility criteria for the Phase II evaluation were to be included also in the Phase II analyses.
Patients' disposition and reasons for ending the study were to be presented in frequency distribution tables and individual data listings. Deviations from eligibility criteria at study entry were to be identified and documented.
Baseline characteristics of treated patients were to be described. Summary statistics were to be calculated, as appropriate, for the quantitative variables. Descriptive analysis of safety data was to be performed considering adverse events, laboratory studies, vital signs and LVEF decline.
All *tumor assessment* data were to be documented in patients'data listings. In the Phase II portion of the trial, the primary analysis of efficacy was to be performed by evaluating the objective response rate in the evaluable patient population, according to the decision criteria above outlined. Both point and interval estimates were to be provided for the objective tumor response rate. Kaplan-Meier method was to be applied to estimate time-to-event variables.
Adverse events, hematological and biochemical toxicities were coded according to MedDRA dictionary and their severity was graded according to the NCI CTCAE (version 3.0).

Pharmacokinetics: Plasma sampling for the pharmacokinetic evaluation was to be performed in the dose escalation portion of the trial, during the first cycle (plasma samples to be collected up to 168 h from the end of cisplatin infusion). A simplified pharmacokinetic sampling was to be performed also during the second cycle of treatment to verify possible accumulation of the study drugs.

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SUMMARY OF RESULTS

Disposition of subjects and Baseline Characteristics

Between 19 December 2005 and 26 August 2010, 101 patients were treated (110 enrolled) in dose escalation and phase II part of the trial. Patients were treated in Italy (5 sites), UK (2 sites) and Germany (1 site).

Dose escalation: different dose levels were tested with the aim of reaching a recommended phase II dose (RP2D). A total of 37 patients were enrolled and treated, 22 in the "intermediate risk" and 15 in the "advanced risk" population. Once a population had attained the RP2D the enrollment was to be switched to the phase II portion.

In the phase II part of the study, 72 new patients were enrolled and 15 patients, enrolled in the dose escalation portion and fulfilling the eligibility criteria for phase II treatment, were also included (N=87).

In this report, except for efficacy results, these 15 patients are counted in both part of the study in all tables and appendices. Thus, in phase II portion, 87 patients are enrolled (79 treated), 44 (42 treated) belonging to the "intermediate risk" population and 43 (37 treated) to the "advanced risk" population.

Table 1. Disposition of Patients

	Dose Escalation				Phase II			
	Intermediate Risk Population		Advanced Risk Population		Intermediate Risk Population		Advanced Risk Population	
	N	%	N	%	N	%	N	%
Patients Enrolled	22	100	15	100	44	100	43	100
Patients Treated	22	100	15	100	42	95.5	37	86.0
Patients Not Treated	-	-	-	-	2	4.5	6	14.0

At the time of database lock (24 April 2013), all patients were off treatment. The primary reasons for ending treatment are provided in Table 2.

Table 2. Primary Reasons for Ending Treatment

	Dose Escalation				Phase II			
	Intermediate Risk Population (N=22)		Advanced Risk Population (N=15)		Intermediate Risk Population (N=42)		Advanced Risk Population (N=37)	
	N	%	N	%	N	%	N	%
Lack of Efficacy	14	63.6	10	66.7	16	38.1	17	45.9
Adverse Events	7	31.8	5	33.3	18	42.9	11	29.7
Treatment completed	1	4.5	-	-	4	9.5	5	13.5
Consent Withdrawn	-	-	-	-	3	7.1	4	10.8
Not reported	-	-	-	-	1	2.4	-	-

Demographic characteristics: The mean *age* at study entry was 66.6 and 64.9 years for the dose escalation part of the study and 69.2 and 65.4 years for the phase II, considering intermediate and advance risk populations, respectively. Male *gender* was predominant in both the treated populations at the two different steps of the trial.

All the treated patients were of White *race* except for one Black patient and one Asian patient.

In the dose escalation portion of the study, ECOG *performace status* was reported at baseline in all the 37

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population, ECOG scored 0 (60.0%), 1 (33.3%) and 2 (6.7%). In the phase II portion, ECOG was collected in 77 (out of 79) treated patients, and scored 0 (88.1% of patients), and 1 (9.5%) in the intermediate risk population, and 0 (54.1% of patients), 1 (40.5%) and 2 (2.7%) in the advanced risk population.

Tumor history: All treated patients but one had a tumor diagnosis of unresectable HCC at study entry and at least one measurable lesion, as per protocol requirements. In addition to the measurable lesion(s), both evaluable and non-evaluable lesions were reported in all patient populations in both *dose escalation* and *phase II* portion of the trial.
In the *dose escalation* portion, 50% of the patients of the intermediate population had no prior therapy at study entry. Out of the remaining 50%, 10 patients underwent surgery only and one patient had surgery plus radiotherapy at study entry. In the advanced risk population 40% of treated patients (6 out of 15) were treatment *naïve* at study entry, and 9 patients had, respectively, previous surgery only (7 patients) and systemic therapy only (2 patients).
In the *phase II* portion, 28.6% of the patients of the intermediate risk population were treatment *naïve* at study entry in details. Out of the remaining patients, 27 underwent surgery only and 3 had systemic only, surgery plus radiotherapy and surgery plus systemic, respectively.
In the advanced risk population, 59.5% of treated patients (22 out of 37) had no prior therapies at study entry, and 40.5% of patients (15 out of 37) underwent surgery only (12 patients), systemic therapy only (1 patient) and surgery plus systemic therapy (2 patients).

Medical history: in both parts of the trial, the majority of the reported medical conditions at study entry were related to vascular hypertension (around 50% in both study populations) and chronic hepatic disorders (cirrhosis and/or hepatitis B and/or C), given that HCC is frequently associated with chronic liver disease. In 2 patients of the intermediate risk population (dose escalation) and in 8 patients of the advanced risk (phase II), also portal hypertension was reported at baseline. Overall, other baseline conditions such as cardiac disorders and blood and lymphatic disorders were reported across dosages and populations.
Vitals signs: in both part of the trial and study populations *systolic BP* exceeded the threshold value in almost 50% of evaluated patients (BP mean value: 129.8 mmHg [range 110.0-160.0] and 131.4 mmHg [range 95.0-174.0] in dose escalation portion in the two populations respectively, and 131.8 mmHg [range 110.0-150.0] and 135.0 mmHg [range 95.0-177.0] in the phase II portion), but this did not prevent them from entering the trial. *Diastolic BP* and pulse were within the normal ranges except for 7 patients, who, however, presented history of hypertension at study entry.
ECG tracings were reported almost in all treated patients and abnormalities were reported at baseline as follows: in the dose escalation study abormalities at baseline were reported in 6 patients of the intermediate risk population and in 3 of the advanced risk population; in the phase II study abnormalities at baseline were reported in 14 patients in intermediate risk population and in 12 patients of the advanced risk. Overall, abnormalities were classified as abnormalities in rhythm, in conduction and in ST-wave and QRS. One patient also presented abnormalities in P Wave Morphology (left atrial enlargement). None of the above reported tracing abnormalities were sufficient to prevent patients from entering the study. No QT prolongations were recorded at baseline in any of the patient populations of both dose escalation and phase II portion of the trial.
LVEF value was assessed at baseline, by MUGA scan or ultrasound. In the dose escalation part LVEF was assessed in 18 out of 22 intermediate risk patients and in 13 out of 15 advanced risk patients. In the phase II part LVEF was assessed in 32 out of 42 intermediate risk population patients and in 34 out of 47 advanced risk patients. No abnormalities were reported at baseline in any of the populations and doses explored.
Chest X-Ray: in the dose escalation was performed at baseline in 21 out of 22 treated patients of the intermediate risk population and in all the 15 treated patients in the advanced risk population. In the phase II portion, 41 out of 42 treated patients of the intermediate risk population had chest X-ray/CT scan baseline

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assessments and in all the 37 patients of the advanced risk population. Across both portions of the study, 5 patients presented clinically relevant abnormalities at baseline assessments, 3 patients in the intermediate risk population and 2 patients in the advanced risk population. All these patients were anyway eligible in the Investigator's opinion.
Alpha-fetoprotein -AFP: levels of AFP in blood were measured, at least at baseline, in all treated patients, in both phases of the trial.

Treatment exposure: four dose levels of the combination nemorubicin (mcg/m^2) + cisplatin (mg/m^2) were explored: 200/40, 200/60, 400/60 and 600/60, administered IHA in a 4-week cycle.

Dose escalation: in the intermediate risk population, 97 cycles were administered at any dose level; the median was 4 cycles (range 1 – 18 cycles), with a median treatment duration of 18.7 weeks (range 3.9-72.1 weeks). Three DLTs occurred out of the 22 treated patients, namely Grade 3 thrombocytopenia (200/40), Grade 2 prolonged fatigue and Grade 3 thrombocytopenia (600/60). Treatment administration was modified due to logistical reasons in the most of the occurred cases (64%), to hematological reasons (23%), to non-hematological toxicity (9%) and due to combination of both hematological and non –hematological toxicities in one patient (5%).
The RP2D was indicated as 600/60 for intermediate risk population.
In the advanced risk population, the treatment cycles administered were 46; the median was 3 cycles (range 1-6 cycles), with a median treatment duration of 12.1 weeks (range 2.6-36.7 weeks). Three DLTs occurred out of the 15 treated patients, namely Grade 4 thrombocytopenia (400/60), Grade 3 hyperbilirubnemia and Grade 3 blood bilirubin increased/fatigue (600/60). Treatment administration was modified due to logistical reasons in the most of the occurred cases (40%), hematological reasons (13%), non-hematological toxicity (7%) and due to combination of both hematological and non –hematological toxicities in one patient (13%). The RP2D was indicated as 400/60 for advanced risk population.

Phase II: in the intermediate risk population, 150 cycles were administered at the RP2D (600/60); the median was 3 cycles (range 1 – 6 cycles), with a median treatment duration of 17.4 weeks (range 4.0 -51.0 weeks). Treatment modifications occurred in 75 out of 150 (50%) cycles, due to logistical reasons (57%), hematological toxicity (33%), non-hematological toxicity (12%) and combination of hematological and non-hematological toxicities (6%).
In the advanced risk population, the treatment cycles administered were 153; the median was 3 cycles (range 1-13 cycles), with a median treatment duration of 17.9 weeks (range 4.0 – 93.0 weeks). Treatment modifications occurred in 84 out of 153 (55%) cycles, due to logistical reasons (73%), hematological toxicity (19%), non-hematological toxicity (11%) and combination of hematological and non-hematological toxicities (5%).

EFFICACY RESULTS:

In the present study, 79 patients out of the 101 treated, were considered for the the phase II part of the trial. According to the CLIP score evaluated, 42 patients were allocated to the intermediate risk population and 37 patients to the advanced risk population.
The investigated combination nemorubicin + cisplatin administered by IHA infusion met the protocol criteria to conclude for activity in the advanced risk population only, with 8 responses (7 PRs and 1 downstaging) out of the 27 evaluable patients (RR 29.6%).

Objective tumor response: in the intermediate risk population, 33 out of 42 patients treated at the RP2D (600/60) were evaluable for the primary efficacy analysis. According to WHO criteria, 7 out of 33 confirmed objective tumor responses (i.e., 2 CRs and 5 PRs) were reported (success rate: 21.2%, 95% CI: 9.0-38.9) as compared to ≥11 objective responses required for evidence of success. Among the 7 patients showing objective

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tumor response, the median duration of the responses was 15.7 months (95% CI: 3.7-26.9).

Best tumor response was NC in 19 patients (57.6%) and the observed stabilization lasted ≥3 months (range 3.0-25.6+ months) in 18 of them, including 3 patients presenting unconfirmed PR and 5 patients showing meaningful tumor shrinkage not qualyfing for a PR.
In the advanced risk population, 27 out of 37 patients treated at the RP2D (400/60) were evaluable for the primary efficacy analysis. According to WHO criteria, 8 confirmed objective tumor responses were reported (i.e., 7 PRs and 1 **downstaging**, success rate of 29.6%, 95% CI: 13.8-50.2), meeting the number of responses required to conclude for activity of the combination investigated in this trial (≥ 6 responses out of 29 evaluable patients). Among the 8 responding patients, the median duration of the response was 14.5 months (95% CI: 5.0-16.3). Best tumor response was NC in 13 patients (48.1%) and in 10 patients the observed stabilization lasted ≥3 months (range 3.2 - 13.7 months), including one patient presenting unconfirmed PR.
In addition, it was observed that in the dose escalation part of the study the combination induced two objective responses (confirmed PRs) also at the MTD.

PFS: in the intermediate risk population the median PFS, evaluated in the 42 treated patients, was 6.3 months (95% CI: 5.4 – 9.0). In the advanced risk population the median PFS, evaluated in the 37 treated patients, was 6.3 months (95% CI: 3.4-7.9).

Overall survival: in the intermediate risk population, evaluated in the treated patients (N=42), ranged from 0.9 to 38.5 months with a median value of 19.5 months. In the advanced risk population OS, also evaluated in the treated patients (N=37), ranged from 2.4 to 33.2 months with a median value of 15.9 months.

AFP response: in phase II part of the trial, 6 patients (3 in each population) showed response to AFP, but no specific relationship between AFP response/duration and activity of the investigated combination can be detailed.

SAFETY RESULTS:

Adverse Events:
Dose escalation: all 37 treated patients were evaluable for safety and experienced at least 1 AE in the first or subsequent cycles. In the intermediate risk population (22 patients) the most frequent AEs (frequency of ≥10%), were fatigue (40.9%, drug related 36.4%), nausea (22.7%, all drug related), vomiting (22.7%, drug related 18.2%), constipation and anorexia (13.6%, 9.1% drug related for both events). CTC Grade 3-4 events were reported by 12 patients (54.5%, 50% reported as drug related).
In the advanced risk population (15 patients) the most frequent AEs, were fatigue (40.0%, drug related 33.3%), nausea and vomiting (33.3% and 20.0%, respectively, all drug related), constipation (20.0%, drug related 13.3%) and abdominal pain NOS, abdominal pain upper, asthenia, urinary tract infection, flank pain and cough (13.3% each) all unrelated except for 1 case each (6.7%) of asthenia, urinary tract infection and cough. Grade 3-4 events were reported by 8 patients (53.3%) and in 4 (26.7%) were reported as drug related. One Grade 5 event (hepatorenal syndrome) was reported as possibly related to the study combination

Phase II: all 79 treated patients were evaluable for safety and experienced at least 1 AE in the first or subsequent cycles. In the intermediate risk population (42 patients) the most frequent AEs (frequency of ≥ 10%), were fatigue (42.9%, drug related 35.7%), nausea (35.7%, drug related 28.6%), vomiting (31.07%, drug related 26.2%), constipation (16.7%, drug related 9.5%), diarrhoea (16.7%, drug related 11.9%), anorexia and epixtaxis (11.9% each, 7.1% and 2.4% drug related, respectively). Grade 3-4 events were reported by 28 patients (66.7%), in 21 (50.0%) were reported as drug related. No Grade 5 events were reported.
In the advanced risk population (37 patients) the most frequent AEs, were nausea (40.5%, drug related 35.1%),

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fatigue (37.8%, drug related 16.2%), vomiting (32.4%, drug related 27.0%) asthenia and anorexia (29.7% each, drug related 10.8% and 18.9, respectively), constipation (27.0%, drug related 10.8%), diarrhoea (24.3%, drug related 2.7%), malaise (21.6%, drug related 8.1%), abdominal pain upper and pain in limb (18.9% each, drug related 10.8% and 5.4%, respectively), ascites, dizziness and headache (16.2% each, drug related 5.4%, 2.7% and 5.4%, respectively), lethargy (13.5%, drug related 10.8%), back pain and peripheral swelling (10.8% each, 2.7% and 5.4% drug related, respectively) and nasopharyngitis (10.8%, none drug related). CTC Grade 3-4 events were reported by 25 patients (67.6%) and in 14 patients (37.8%) were reported as drug related. Two CTC Grade 5 events (bronchopneumonia NOS and sudden cardiac attack) were reported as unrelated and unlikely related, respectively. The events led patients to death.

Hematological toxicity: in both part of the present study an increase in frequency and severity has been observed, compared with previous studies when nemorubicin was administered IHA as single agent. In particular, during phase II, hematological toxicity was more evident in the intermediate risk population rather than in the advanced patients (i.e: Grade 3-4 neutropenia: 65% vs 25%; Grade 3-4 thrombocytopenia: 58% vs 28%). No cases of febrile neutropenia were recorded. The observed increased in toxicicy is mainly due to the presence of cisplatin in the combination. In both patient populations, during repeated cycles, a trend toward increased incidence and severity of hematological toxicity was observed in the dose escalation portion. This was not confirmed in the phase II, except for platelets in the intermediate risk population.

Blood chemistry alterations, in both part of the study, were qualitatively similar in the two populations and were characterized mainly by an asymptomatic, reversible increase of transaminases and bilirubin.

Cardiac toxicity was monitored by ECG (on 55 patients) and LVEF (on 48 patients). Twelve treatment emergent abnormalities of ECG occurred in 10 intermediate risk patients and 17 abnormalities in 14 advanced risk patients. In both populations abnormalities were mostly observed in rhythm (7 and 10 cases, respectively), conduction (2 and 5 cases, respectively), QRS (2 cases in intermediate patients only) and ST-T wave (1 and 2 cases, respectively). Occasional LVEF declines (in the range of 1-17 absolute change from baseline) were observed remaining generally within normal limits. In one intermediate risk patient one occasional decrease from 53 to 48 (normal limit 50) was observed, however the patient continued on treatment and the LVEF level returned within normal limit values. One asymptomatic LVEF decline ≥15 absolute change has been reported in one advanced risk patient.
Overall, no toxic effect on cardiac function is present in the combination nemorubicin + cisplatin administered by IHA at the dose levels tested in both the dose escalation and the phase II part of the trial.

Discontinuations: 45 patients (5 during dose escalation step of the trial and 40 patients of the phase II) discontinued treatment due to the occurrence of one or more adverse events all concurring to patient's withdrawal.

SAEs: 59 SAEs occurred in 36 patients (15 intermediate risk patients and 21 advanced risk), 26 (44.1%) were considered unrelated to the study treatment, 23 (39.0 %) clearly related (possible, probable or definite relationship) and 10 (16.9%) events were considered as unlikely related.

Deaths: no patients died on study (i.e., within 30 days from the last dose administered).

Pharmacokinetic Results

Overall, plasma samples from 28 patients were available for the pharmacokinetic assessment. Twenty-six patients were treated during the dose-escalation phase and 2 were treated during the phase II part of the study. Nineteen subjects were enrolled as intermediate risk and 9 as advanced risk patients.
Plasma concentrations of nemorubicin (and its two main metabolites) were measured using a validated

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LC-MS/MS method. The calibration range of the assay in plasma was 0.1-5 ng/mL for all analytes. Plasma concentrations of total and free cisplatin were measured using a validated ICP-MS method. The calibration range of the assay in plasma was 2-1000 ng/mL. The pharmacokinetic evaluation on nemorubicin and cisplatin were performed using standard non-compartmental analysis.
Plasma concentrations of nemorubicin, when detectable, were close to the lower limit of quantification of the compound (0.10 ng/mL). Overall, no differences related to risk population or CLIP score were observed. Taking into account the inter-patient variability, patients classified as "advanced risk" or characterized by a CLIP score >1 did not resulted more exposed to nemorubicin compared to patients with intermediate risk or CLIP 1.

Plasma levels of the two nemorubicin metabolites were always below the limit of quantification of the analytical method in all patients, preventing any evaluation of the metabolite pharmacokinetic profile.

No differences in the pharmacokinetics of total and free cisplatin were observed by administering the drug in combination with different doses of nemorubicin in both intermediate and advance risk population.

CONCLUSIONS

In conclusion, the trial was successful in advanced risk population, with 8 responses (7 PRs and 1 downstaging) out of the 27 evaluable patients (RR 29.6%), and not formally successful in intermediate risk population. Anyway, although the IHA infusion of the combination nemorubicin with cDDP did not meet protocol criteria to conclude for activity in intermediate risk population, it showed some interesting signs of benefit also in this setting of patients, even at doses lower than the RP2D. The combination of nemorubicin and cisplatin administered by IHA infusion in adult patients with unresectable HCC presents a manageable safety profile at the dosages and treatment schedules investigated in both intermediate and advanced risk population.

These encouraging results warrant further investigation of nemorubicin in HCC, especially in multinodular type, indicating a wide therapeutic window, good safety profile and ease of combination with other antineoplastic agents.

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