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A randomized, placebo-controlled phase III trial of masitinib plus gemcitabine in the treatment of advanced pancreatic cancer

G. Deplanque[1*], M. Demarchi[2], M. Hebbar[3], P. Flynn[4], B. Melichar[5], J. Atkins[6], E. Nowara[7], L. Moyé[8], D. Piquemal[9], D. Ritter[9], P. Dubreuil[10,11,12,13,14], C. D. Mansfield[14], Y. Acin[14], A. Moussy[14], O. Hermine[14,15,16,17,18,19,20] & P. Hammel[21]

[1]Department of Medical Oncology, Saint Joseph Hospital, Paris; [2]Department of Medical Oncology, University Hospital of Besançon, Besançon; [3]Department of Medical Oncology, University Hospital, Lille, France; [4]Metro-Minnesota Community Clinical Oncology Program, Park Nicollet Institute, Minneapolis, USA; [5]Department of Oncology, Palacký University Medical School & Teaching Hospital, Olomouc, Czech Republic; [6]Southeastern Medical Oncology Center, Goldsboro, USA; [7]Department of Clinical and Experimental Oncology, Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice, Poland; [8]Department of Biostatistics, University of Texas School of Public Health, Houston, USA; [9]Clinical Development, Acobiom, Montpellier; [10]Signaling, Hematopoiesis and Mechanism of Oncogenesis, Inserm U1068, CRCM, Marseille; [11]Institut Paoli-Calmettes, Marseille; [12]Aix-Marseille University, UM 105, Marseille; [13]CNRS, UMR7258, CRCM, Marseille; [14]Clinical Development, AB Science, Paris; [15]Department of Clinical Hematology, Necker Hospital, Paris; [16]INSERM UMR 1163, Laboratory of Cellular and Molecular Mechanisms of Hematological Disorders and Therapeutic Implications, Paris; [17]Paris Descartes – Sorbonne Paris Cité University, Imagine Institute, Paris; [18]CNRS ERL 8254, Paris; [19]Laboratory of Excellence GR-Ex, Paris; [20]National Reference Center on Mastocytosis (CEREMAST), Paris; [21]Department of Gastroenterology, Hôpital Beaujon, Clichy, France

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Background: Masitinib is a selective oral tyrosine–kinase inhibitor. The efficacy and safety of masitinib combined with gemcitabine was compared against single-agent gemcitabine in patients with advanced pancreatic ductal adenocarcinoma (PDAC).

Patients and methods: Patients with inoperable, chemotherapy-naïve, PDAC were randomized (1 : 1) to receive gemcitabine (1000 mg/m^2) in combination with either masitinib (9 mg/kg/day) or a placebo. The primary endpoint was overall survival (OS) in the modified intent-to-treat population. Secondary OS analyses aimed to characterize subgroups with poor survival while receiving single-agent gemcitabine with subsequent evaluation of masitinib therapeutic benefit. These prospectively declared subgroups were based on pharmacogenomic data or a baseline characteristic.

Results: Three hundred and fifty-three patients were randomly assigned to receive either masitinib plus gemcitabine ($N = 175$) or placebo plus gemcitabine ($N = 178$). Median OS was similar between treatment-arms for the overall population, at respectively, 7.7 and 7.1 months, with a hazard ratio (HR) of 0.89 (95% CI [0.70; 1.13]. Secondary analyses identified two subgroups having a significantly poor survival rate when receiving single-agent gemcitabine; one defined by an overexpression of acyl–CoA oxidase-1 (*ACOX1*) in blood, and another via a baseline pain intensity threshold (VAS > 20 mm). These subgroups represent a critical unmet medical need as evidenced from median OS of 5.5 months in patients receiving single-agent gemcitabine, and comprise an estimated 63% of patients. A significant treatment effect was observed in these subgroups for masitinib with median OS of 11.7 months in the '*ACOX1*' subgroup [HR = 0.23 (0.10; 0.51), $P = 0.001$], and 8.0 months in the 'pain' subgroup [HR = 0.62 (0.43; 0.89), $P = 0.012$]. Despite an increased toxicity of the combination as compared with single-agent gemcitabine, side-effects remained manageable.

Conclusions: The present data warrant initiation of a confirmatory study that may support the use of masitinib plus gemcitabine for treatment of PDAC patients with overexpression of *ACOX1* or baseline pain (VAS > 20mm). Masitinib's effect in these subgroups is also supported by biological plausibility and evidence of internal clinical validation.

**Correspondence to:* Dr Gaël Deplanque, Service d'Oncologie, Groupe Hospitalier Paris Saint Joseph, 185 rue Raymond Losserand, Paris 75674, France. Tel: +33-(0)1-44-12-76-43. Fax: +33-(0)144-12-32-59. E-mail: gdeplanque@hpsj.fr

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introduction

Pancreatic cancer continues to be a disease with high unmet medical need, requiring new active agents. For over a decade, single-agent gemcitabine has been the standard first-line treatment for unresectable, locally advanced or metastatic pancreatic ductal adenocarcinoma (PDAC). Median overall survival (OS) is between 6 and 7 months and 1-year survival rates range between 17% and 25% [1, 2]. Numerous gemcitabine-based combination regimens evaluated in randomized trials have either failed to demonstrate significant improvement in OS or have shown statistically significant but rather modest survival benefits compared with gemcitabine alone; e.g. nab-paclitaxel plus gemcitabine recently reported a significant median OS gain of +1.8 months when compared with single-agent gemcitabine [1–3].

The potential therapeutic benefit of masitinib in combination with gemcitabine for the treatment of advanced PDAC has been previously reported in preclinical studies, wherein masitinib was shown to enhance the antiproliferative activity of gemcitabine in gemcitabine–refractory pancreatic cancer cell lines, and also in a clinical phase II trial [4, 5]. Exploratory analysis from the clinical study revealed two distinct patient subgroups with respect to masitinib treatment susceptibility, as evidenced by a plateau in the OS Kaplan–Meier curve between 9 and 17 months (see section A of the Supplementary Material, available at *Annals of Oncology* online). This observation could not be explained by patient–disease status leading to a hypothesis that there may be at least one subgroup of PDAC patients with particularly poor survival and susceptibility to masitinib plus gemcitabine treatment, the said subgroup being identifiable via a gene expression profile and/or another biological or clinical marker. Hence, future trials of masitinib in this indication would need to perform prospectively declared secondary subgroup analyses.

This observation is consistent with evidence that heterogeneity in tumor biology and microenvironment may be an important determinant of survival difference amongst groups of PDAC patients (i.e. aggressive versus relatively slow disease progression, as seen in routine clinical practice), which in turn leads to variability in terms of treatment susceptibility and potential failure of targeted drugs in the overall population [1, 6, 7]. It has been reported that such heterogeneity in PDAC patients may be associated with increased mast cell infiltration into the tumor or tumor microenvironment, both of which are prognostic factors for poor survival in PDAC [8, 9]. Masitinib is a potent oral tyrosine–kinase inhibitor (TKI) that targets a limited number of receptor tyrosine kinases including c-Kit, Lyn and Fyn, making it a highly selective inhibitor of mast cell function and activity [10].

methods

study design

The present study was a prospective, multicenter, randomized, double-blind, two-parallel group, placebo-controlled phase III trial evaluating the safety and efficacy of masitinib plus gemcitabine against placebo plus gemcitabine in chemotherapy-naïve PDAC patients. Masitinib (9 mg/kg/day) was administered orally in two daily doses, while gemcitabine (1000 mg/m^2) was administered according to standard clinical practice. The composition and dispensing of masitinib and placebo capsules were identical except for the amount of the active ingredient contained. Treatments were administered until progression, intolerance, or patient withdrawal, with disease progression assessed via CT scan according to RECIST criteria every 8 weeks. In the event of a treatment-related grade 3 or 4 adverse event (AE), treatment interruption or blinded dose reduction was permitted according to predefined criteria. The investigation was carried out in accordance with the Declaration of Helsinki and approved by the national health authorities and local ethics committees.

patients and randomization

Eligible patients were chemotherapy-naïve with histologically or cytologically confirmed inoperable advanced or metastatic PDAC. Other eligibility criteria included: age 18 years or older; Eastern Cooperative Oncology Group (ECOG) performance status ≤1; a life-expectancy of >12 weeks; bilirubin <3×ULN, adequate renal, cardiac, and hepatic functions. At baseline, patients were centrally randomized to treatments groups (1:1) using an Interactive Voice Response System (IVRS), with treatment allocated according to a modified minimization method. Stratification was done according to geographic region and disease status (locally advanced versus metastatic). The investigators, patients, data analysts, and the trial sponsor were blinded to the randomization sequence and treatment assignment.

statistical analysis

Safety was assessed throughout the study in all patients who received at least one dose of masitinib or placebo using the Common Terminology Criteria for Adverse Events version 3 (CTCAE v3) for classification of AE. Quality of life (QoL) was assessed using the EORTC QLQ-C30 questionnaire.

The primary endpoint was OS in the modified intent-to-treat (mITT) population, i.e. all randomized patients, excluding those withdrawn prematurely from the study for a well-documented non-treatment related cause, with OS measured from the date of randomization to the date of death. It was estimated that at least 320 patients were required to detect a difference in median OS between treatment-arms with a power of 80% using a two-sided log-rank test and significance level of 0.05 (assuming 264 events after 12 months follow-up). Comparative analyses were based on an alpha of 5% (two-sided), with results presented according to a two-sided 95% confidence interval (CI), unless otherwise stated.

Consistent with study rationale, secondary analyses on OS were pre-specified in the protocol with the objectives of: (a) characterizing a subgroup based upon pharmacogenomic data with poorer survival while under gemcitabine standard-of-care, (b) evaluating the therapeutic benefit of added masitinib in this genetic subgroup, (c) characterizing a subgroup based upon a baseline variable that negatively impacts survival while under gemcitabine standard-of-care, and (d) evaluating the therapeutic benefit of added masitinib in this baseline variable subgroup. Sample size for the prospectively declared subgroup analyses was predefined prior to unblinding. For the subgroup based on a baseline variable predictive of poor survival, it was estimated that 220 patients would be needed for 80% power to detect a hazard ratio (HR) of 0.66 (masitinib plus gemcitabine versus placebo plus gemcitabine) using a two-sided log-rank test with a significance level of 0.05. Overall survival was investigated in patients from the placebo plus gemcitabine treatment-arm according to each baseline variable (a total of 16 baseline characteristics were tested) through a univariate analysis,

thereby, identifying characteristics that impact OS independently of treatment (see section B of the Supplementary Material, available at *Annals of Oncology* online). Multivariate analysis of OS was performed using a Cox proportional-hazard model to evaluate the treatment effect with adjustment for the stratification factors. For the subgroup based on pharmacogenomic data, it was estimated that 100 patients per treatment-arm would be required for 80% power to detect a HR of 0.50 (masitinib plus gemcitabine versus placebo plus gemcitabine) using a two-sided log-rank test with a significance level of 0.05.

pharmacogenomic analysis

Prospectively declared secondary subgroup analyses included pharmacogenomic examination of the RNA expression in peripheral blood samples collected using the PAXgene Blood RNA System prior to treatment. Genome-wide analysis of RNA expression using a high-throughput method of next-generation sequencing was performed by Acobiom, Montpellier, France. The methodology used for identification of the genetic biomarker subgroup is described in section C of the Supplementary Material, available at *Annals of Oncology* online.

results

A total of 353 patients from 73 active centers (predominantly located in France, United States and the Czech Republic) were randomly assigned to receive masitinib plus gemcitabine or placebo plus gemcitabine. The safety population comprised all randomized patients who received at least one dose of either masitinib or placebo ($N = 349$). A CONSORT flow diagram for the study population as well as subgroups of interest and description of patient baseline characteristics are provided in sections D and E of the Supplementary Material, available at *Annals of Oncology* online. Baseline characteristics were generally well-balanced. The average number of post-study treatments was similar between treatment-arms at 1.1 ± 1.3 for the masitinib plus gemcitabine treatment-arm, and 1.0 ± 1.0 for the placebo plus gemcitabine arm, with the majority of patients receiving either single-agent gemcitabine (25% and 11%, respectively) or no additional treatment-line (27% and 31%) upon study discontinuation. Median exposure to masitinib or placebo in the safety population was 1.6 and 3.7 months, respectively, while median exposure to gemcitabine in the masitinib or placebo treatment-arms was 1.4 and 3.3 months, respectively; $P = 0.001$. At the data cut-off date, corresponding to a median follow-up of 26 months, one patient was ongoing treatment in the masitinib plus gemcitabine treatment-arm.

A summary of safety data is presented in Table 1. Overall toxicity increased for masitinib combined with gemcitabine when compared with single-agent gemcitabine. A higher frequency of serious and severe (grade 3 and 4) AEs, discontinuations, temporary interruptions and dose reductions was reported in the masitinib plus gemcitabine treatment-arm, although the occurrence of AE related deaths was lower in this treatment-arm than in the placebo plus gemcitabine arm. Hematological AEs contributed strongly to the discrepancy between treatment-arms, with the higher frequency reported for masitinib-treated patients due predominantly to an increase in neutropenia. No deaths were reported due to neutropenia in the masitinib plus gemcitabine treatment-arm, moreover, the occurrence of febrile neutropenia was similar between treatment-arms (1.7% for masitinib plus gemcitabine versus 0.6% for placebo plus gemcitabine), as were infections (30.6% versus 37.5%, respectively). Non-hematological AEs were typical of previously reported toxicity for masitinib, including vomiting, nausea and rash, but these generally resolved without sequelae and were not associated with any deaths.

Patient QoL at baseline was similar between the treatment-arms (mean global health score of 53.5 ± 22.4 versus 53.9 ± 21.1 for the masitinib plus gemcitabine and placebo plus gemcitabine treatment-arms, respectively), as well as at the last patient visit

Table 1. Safety according to the number of patients with at least one reported adverse reaction (safety population)

Number of patients (%)	M + G ($n = 173$)	P + G ($n = 176$)	P-value[a]
Summary of AE			
All grades	173 (100%)	173 (98%)	0.248
Severe non-hematological[b]	132 (76%)	124 (71%)	0.010
Severe hematological[b]	109 (63%)	73 (42%)	<0.001
Non-fatal serious	107 (68%)	94 (53%)	0.111
Deaths[c]	14 (8%)	19 (11%)	0.388
AE leading to:			
Study discontinuation[d]	73 (42%)	48 (27%)	0.003
Temporary interruption[d]	129 (75%)	90 (51%)	<0.001
Dose reduction[d]	28 (16%)	16 (9%)	0.046
AEs of interest[e]			
Back pain	10 (6%)	27 (15%)	0.004[f]
Constipation	38 (22%)	62 (35%)	0.006[f]
Pulmonary embolism	4 (2%)	12 (7%)	0.044[f]
Vomiting	87 (50%)	57 (37%)	<0.001
Nausea	100 (58%)	82 (47%)	0.036
Rash	60 (35%)	22 (13%)	<0.001
Thrombocytopenia	83 (48%)	48 (27%)	<0.001
Thrombosis	8 (5%)	0 (0%)	0.003
Hypokalemia	34 (20%)	16 (9%)	0.005
Pyrexia	70 (41%)	48 (27%)	0.009
Neutropenia	87 (50%)	65 (37%)	0.012
Anemia	105 (61%)	84 (48%)	0.015

Adverse Events (AE) classified according to the Common Terminology Criteria for Adverse Events version 3.

[a]The Fisher exact test or Chi-squared test was used for comparison of qualitative variables; analysis of variance was used for comparison of quantitative variables.

[b]Severe adverse events correspond to CTCAE v3 grade 3 and 4 adverse events.

[c]Toxicity related deaths under study treatment.

[d]Adverse events leading to discontinuation (except death), interruption or dose reduction of study drug (masitinib or placebo).

[e]Adverse events reported with a significantly higher frequency in one treatment-arm.

[f]Adverse event reported at a statistically significant higher frequency in placebo plus gemcitabine-treated patients than in the masitinib plus gemcitabine-treated patients.

AE, adverse event; GEM, gemcitabine; P + G, placebo plus gemcitabine; M + G, masitinib plus gemcitabine.

Table 2. Summary of treatment effect according to overall survival for masitinib plus gemcitabine versus placebo plus gemcitabine in the mITT population (primary analysis) and also in two subgroups with a demonstrated poor survival while under standard-of-care, comprised patients with a genetic biomarker ('ACOX1 subgroup') and patients with baseline pain intensity of VAS > 20 ('pain subgroup')

	N	Median OS [95% CI] (months)	[a]Median OS Gain (months)	HR [95% CI]	*P*-value
Overall (mITT)	348				
P + G	175	7.0 [6.1;10.6]	+0.7	0.89 [0.70;1.13]	0.695
M + G	173	7.7 [6.1;10.6]			
'ACOX1' subgroup	40				
P + G	20	5.6 [3.7;12.9]	+6.1	0.23 [0.1;0.51]	0.001
M + G	20	11.7 [8.3;19.9]			
'Pain' subgroup	137				
P + G	73	5.4 [4.5;8.0]	+2.6	0.62 [0.43;0.89]	0.012
M + G	64	8.0 [5.8;11.5]			

Median follow-up of 26 months; multivariate model.
[a]Difference in median OS between treatment-arms (M + G minus P + G).
OS, overall survival; HR, hazard ratio of death; P + G, placebo plus gemcitabine; M + G, masitinib plus gemcitabine; mITT, modified intent-to-treat population.

(46.3 ± 23.7 versus 49.7 ± 21.7, respectively). The combination of masitinib plus gemcitabine did not, therefore, accelerate the decline in QoL with respect to single-agent gemcitabine.

The median OS for the overall population, the primary efficacy analysis, was similar for both treatment-arms; 7.7 months [95% CI (6.1; 10.6)] for masitinib plus gemcitabine and 7.0 months [95% CI (6.1; 10.6)] for placebo plus gemcitabine (all results reported hereafter relate to the multivariate analysis unless otherwise stated) (Table 2). The corresponding HR was 0.89 [95% CI (0.70; 1.13)]. Secondary analyses on surrogate survival endpoints of the overall population, e.g. progression-free survival or time-to-progression, were also similar between treatment-arms (data not shown).

Secondary analyses on OS did, however, show two subgroups of patients having particularly poor survival with single-agent gemcitabine, which was consistent with the study's hypothesis and prospectively declared subgroup analysis. These subgroups comprised patients with a genetic biomarker (overexpression of *ACOX1* in blood), and patients with baseline pain intensity above a threshold of 20 mm as measured on a 100 mm visual analog scale (VAS). In both cases, the placebo plus gemcitabine patient cohorts divided into two distinct subgroups with survival reflecting aggressive or relatively slow disease progression (Figure 1), thus characterizing the defining variables of the prospectively declared secondary subgroup analysis (for further subgroup description see sections C and F of the Supplementary Material, available at *Annals of Oncology* online). Subsequent evaluation of the interaction between these variables and the combination of masitinib plus gemcitabine revealed a significant treatment benefit in both subgroups with respect to the placebo plus gemcitabine treatment-arm (Table 2).

Considering the patient cohort with pharmacogenomic data, 119 patients enrolled for the study had peripheral blood samples collected at baseline and were randomly assigned to the masitinib plus gemcitabine or placebo plus gemcitabine treatment-arms ($n = 60$ and $n = 59$ patients, respectively). The *ACOX1* subgroup was determined following a pre-specified methodology as patients with overexpression of *ACOX1* in blood defined as a delta cycle threshold (DCt) value of ≤3.05 (see section C of the Supplementary Material, available at *Annals of Oncology* online). In the overall pharmacogenomic population, a total of 40/119 patients (34%) were identified as being in the *ACOX1* subgroup while 79/119 patients (66%) were assigned to its complement subgroup (i.e. absence of *ACOX1* overexpression or non-*ACOX1*). In the *ACOX1* subgroup, median exposure to masitinib or placebo was 1.8 and 2.4 months, respectively, while median exposure to gemcitabine in the masitinib or placebo treatment-arms was 2.1 and 1.9 months, respectively; $P = 0.78$. In the placebo plus gemcitabine treatment-arm, patients without *ACOX1* overexpression ($n = 39$) had a significantly longer median OS compared with patients having *ACOX1* overexpression ($n = 20$); 8.8 months [95% CI (5.6; 15.0)] versus 5.5 months [95% CI (3.4; 8.3)] (univariate model). The corresponding HR was 0.46 [95%CI (0.26; 0.82)], $P = 0.007$ (Figure 1A).

In the aforementioned *ACOX1* subgroup, those patients treated with masitinib plus gemcitabine ($n = 20$) had a median OS of 11.7 months [95% CI (8.3; 19.9)] compared with a median OS of 5.6 months [95% CI (3.7; 12.9)] for the placebo plus gemcitabine treatment-arm ($n = 20$) (multivariate model), a statistically significant OS gain of + 6.1 months. The corresponding HR was 0.23 [95% CI (0.10; 0.51), $P < 0.001$] (Table 2). Overall survival rates at 6, 12, 18, and 24 months were respectively, 82%, 48%, 15%, and 11%, in masitinib plus gemcitabine treatment-arm versus 45%, 8%, 0.6%, and 0.3%, in the placebo plus gemcitabine treatment-arm. Safety in the *ACOX1* subgroup was similar to the overall safety population (data not shown).

Considering the prospectively declared subgroup based on a baseline clinical characteristic, i.e. pain intensity tested once at baseline, 312 patients from the mITT population had VAS data available. The 'pain' subgroup, 137/312 patients (44%), included all patients reporting a VAS score of >20 mm, this threshold being consistent with established precedent and defined prior to unblinding (see section F of the Supplementary Material, available at *Annals of Oncology* online). Comparison was made

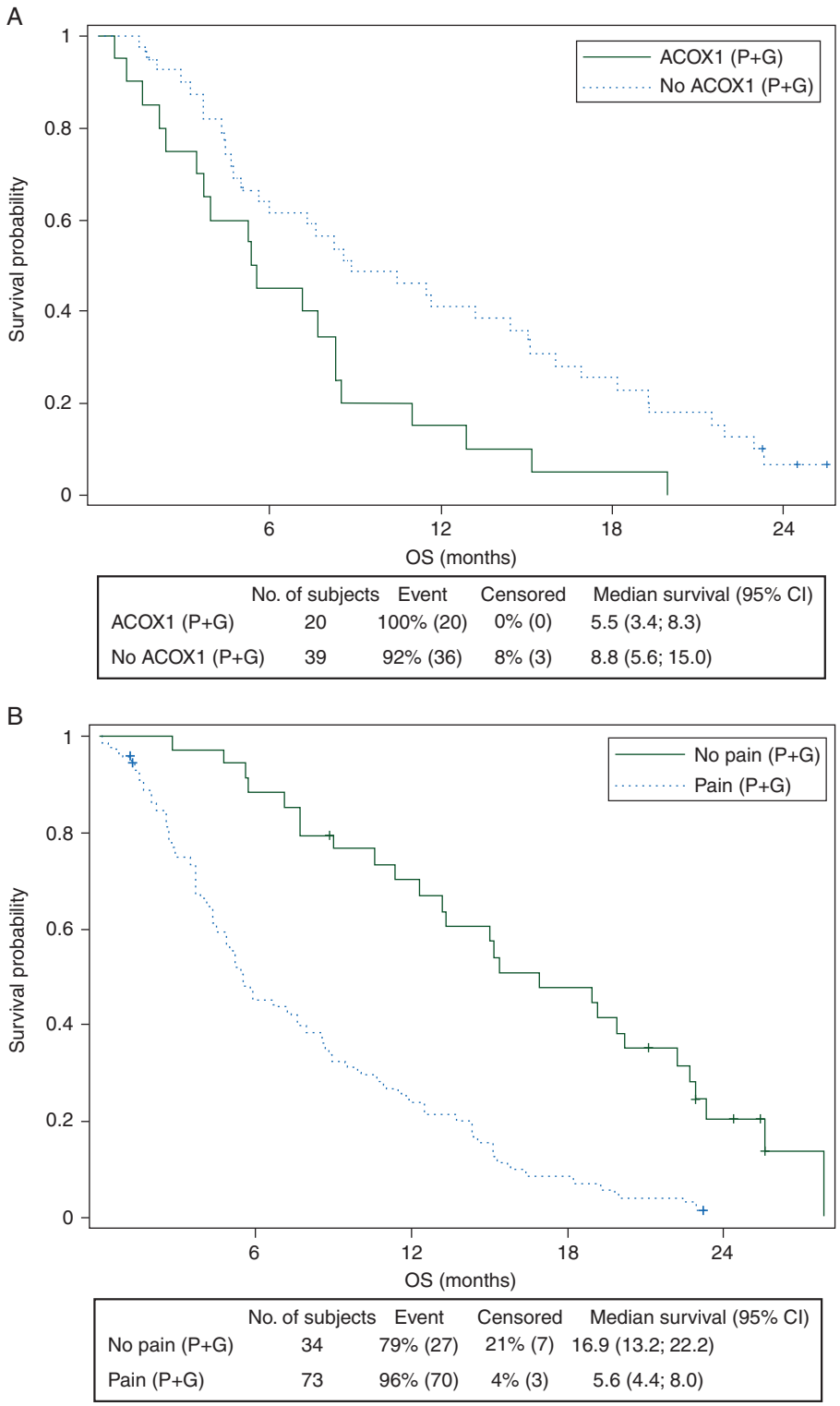

Figure 1. (**A**) Overall survival analysis in patients with advanced PDAC and treated with placebo plus gemcitabine (standard-of-care) according to subgroups defined via pharmacogenomic data (i.e. the 'ACOX1' subgroup versus its complement 'non *ACOX1*' subgroup); corresponding HR was 0.46 [95% CI (0.26; 0.82), $P = 0.007$]. (**B**) Overall survival analysis in patients with advanced PDAC and treated with placebo plus gemcitabine according to subgroups defined via a baseline variable (i.e. the 'pain' subgroup versus the 'no pain' subgroup); corresponding HR was 0.30 [95% CI (0.18; 0.48), $P < 0.001$]. These data demonstrate the prognostic value of *ACOX1* overexpression in blood and baseline pain intensity, thereby revealing two patient subgroups with remarkably poor survival and a critical unmet medical need. Median follow-up of 26 months; univariate model.

against patients reporting negligible baseline pain intensity, defined by a VAS < 5 and not requiring opioid analgesics to manage disease-related pain, referred to hereafter as the 'no pain' subgroup ($n = 68/312$ patients, 22%). All remaining patients, i.e. those with a baseline VAS ≥ 5 but <20 or VAS < 5 but taking analgesics opioids ($n = 107/312$, 34%) formed a third subgroup. In the 'pain' subgroup, median exposure to masitinib or placebo was 1.5 and 2.5 months, respectively, while median exposure to gemcitabine in the masitinib or placebo treatment-arms was 1.4 and 2.3 months, respectively; $P = 0.17$. In the placebo plus gemcitabine treatment-arm, patients from the 'no pain' subgroup ($n = 34$) had a significantly longer median OS than patients in the 'pain' subgroup ($n = 73$), 16.9 months [95% CI (13.2; 22.2)] versus 5.6 months [95% CI (4.4; 8.0)] (univariate model). The corresponding HR was 0.30 [95% CI (0.18; 0.48), $P < 0.001$] (Figure 1B).

In the aforementioned 'pain' subgroup those patients treated with masitinib plus gemcitabine ($n = 64$) had a median OS of 8.0 months [95% CI (5.8; 11.5)] compared with a median OS of 5.4 months [95% CI (3.7; 8.3)] for the placebo plus gemcitabine treatment-arm ($n = 73$) (multivariate model), a statistically significant OS gain of + 2.6 months. The corresponding HR was 0.62 [95% CI (0.43; 0.89), $P = 0.012$] (Table 2). Overall survival rates at 6, 12, and 18 months, were respectively, 58%, 32%, and 18%, in the masitinib plus gemcitabine treatment-arm versus 44%, 18%, and 8%, in the placebo plus gemcitabine treatment-arm. Safety in the pain subgroup was similar to the overall safety population (data not shown). One also notes that the frequency of patients reporting back pain as an AE during treatment was significantly lower ($P = 0.004$) in the masitinib plus gemcitabine treatment-arm than in the placebo plus gemcitabine treatment-arm of the safety population (Table 1).

Internal validation of masitinib's effect in patients from the 'pain' subgroup is provided through analysis of survival data in patients consuming high doses of opioid analgesics at baseline (>1 mg/kg/day), referred to hereafter as the 'high opioid' subgroup ($n = 34$). Briefly, it is a fair assumption that such patients were experiencing moderate to severe cancer-related pain to justify initiation of such pain management measures and are, therefore, comparable to patients from the 'pain' subgroup. Patients in the exploratory 'high opioid' subgroup and treated with masitinib plus gemcitabine ($n = 20$) had a median OS of 8.5 months [95% CI (6.0; NA)], whereas patients treated with placebo plus gemcitabine ($n = 14$) had a median OS of 6.0 months [95% CI (3.5; NA)]. This corresponds to a survival benefit of 2.5 months and HR of 0.43 [0.17; 1.06]; $P = 0.23$.

discussion

Although no discernible difference between treatment-arms was observed for the primary endpoint in the overall population, this study did identify subgroups with remarkably poor survival while under single-agent gemcitabine. Patients with overexpression of *ACOX1* or baseline pain (VAS > 20 mm) had a worse prognosis (median OS of 5.6 and 5.4 months, respectively) with respect to the overall population (median OS of 7.0 months) and historical median OS data for gemcitabine-treated patients (typically 6.5 months) [1]. Such data illustrate that the markers of *ACOX1* expression in blood and baseline pain intensity may have prognostic value, with patients from these subgroups experiencing aggressive disease progression while receiving single-agent gemcitabine. It is estimated that together, these subgroups encompass 63% of the entire PDAC population (i.e. 34% of patients who were identified as belonging to the '*ACOX1*' subgroup and 29% of patients who were identified as belonging to the 'pain' subgroup with no overexpression of *ACOX1*, amounting to 63% of patients in one or the other subgroup).

Both parameters of *ACOX1* and baseline pain (VAS > 20 mm) also suggested predictive value with the masitinib plus gemcitabine treatment-arm showing a statistically significant median OS gain of + 6.1 months [HR = 0.23 (0.10; 0.51)] in the *ACOX1* subgroup and + 2.6 months [HR = 0.62 (0.43; 0.89)] in the pain subgroup when compared with single-agent gemcitabine. Although there was increased toxicity with the addition of masitinib to gemcitabine, safety remained within acceptable limits with application of appropriate risk management measures and there was no overall detrimental effect on QoL. Therefore, the combination of masitinib and gemcitabine for the treatment of advanced PDAC appears to exhibit a positive benefit–risk ratio for these subpopulations. Of note, the pharmacogenomic examination of RNA expression in peripheral blood samples also identified a set of ten genes with high discriminatory power, albeit ambiguous biological plausibility, with *ACOX1,* representing the single most important gene to explain OS (see section C of the Supplementary Material, available at *Annals of Oncology* online).

There is an emerging consensus that under certain circumstances it is possible for a subgroup to be considered of clinical significance (see section G of the Supplementary Material, available at *Annals of Oncology* online). The present study has met these criteria. For example, internal consistency supporting the clinical plausibility of each subgroup is provided from independent patient samples (see sections C and F of the Supplementary Material, available at *Annals of Oncology* online). Considering biological plausibility, it is thought that the presence of baseline pain (VAS > 20 mm) or an overexpression of *ACOX1* effectively identifies those patients with a pro-tumoral T-helper cell type-2 (Th2) immune response, a condition caused in part by increased mast cell activity in the tumor microenvironment or by transcriptional or physiological alterations favoring M2-polarization of tumor-associated macrophages (TAM) (see section H of the Supplementary Material, available at *Annals of Oncology* online). For instance, mast cells have been implicated with the development of neuropathic pain in PDAC patients and skewing macrophage polarization towards a pro-tumoral M2-type [11, 12]. Furthermore, recent preclinical data from KrasG12D driven mouse models of PDAC with pain or spontaneous chronic pancreatitis show that pancreatic tumor lesions of masitinib-treated mice have decreased mast cell count and reduced intra-tumoral vascularization and innervation when compared with control mice (Dubreuil P, 2014; personal communication). Other nascent research suggests masitinib may induce the recruitment of macrophages with a potential for antitumoral activity within the tumor (Hermine O, 2014; personal communication). Thus, mechanisms of action associated with masitinib apparently converge towards favoring a preferential accumulation of antitumoral M1-macrophages in the tumor microenvironment with concomitant reduction of oxidative stress effects. Presentation of these

supportive data fall beyond the scope of the current clinical paper with additional translational research needed to fully elucidate such mechanisms; as such, these preclinical data will be reported in full elsewhere.

In conclusion, the survival benefit observed for PDAC patients with overexpression of *ACOX1* in blood or reporting baseline pain of VAS > 20 mm when treated with masitinib plus gemcitabine, coupled with manageable toxicity suggests a positive benefit–risk ratio. This has led to the initiation of a confirmatory study that may support the use of masitinib plus gemcitabine as a new treatment option for these two subgroups of PDAC patients.

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disclosure

Masitinib is under clinical development by the study sponsor, AB Science (Paris, France). AM, CM, and YA are an employees and shareholders of the study sponsor AB Science. OH and PD are consultants and shareholders of AB Science. LM served as a paid consultant to AB Science. DP and DR are employees and shareholders of the study sponsor Acobiom. All other authors declare no conflicts of interest.

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