Prognostic and predictive role of lactate dehydrogenase 5 (LDH5) expression in colorectal cancer patients treated with PTK787/ZK 222584 (Vatalanib) anti-angiogenic therapy

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Abstract

Purpose—The CONFIRM randomized trials, investigating the role of the VEGF-receptor inhibitor PTK787/ZK 222584 (vatalanib) in colorectal cancer (FOLFOX 4 ± vatalanib), showed some benefit in patients with high serum LDH levels. Here we investigated the expression of LDH5 (encoded entirely by the LDHA gene, regulated by the hypoxia inducible factors) in cancer tissues from patients recruited in the CONFIRM trials and relationship to response.

Experimental Design—Paraffin embedded materials from 179 patients recruited in the CONFIRM trials were analyzed by immunohistochemistry for the expression of the LDH5 protein. Correlations with serum LDH, response and survival were assessed.

Results—A significant association of tumor burden and of poor Performance Status (PS) with serum LDH was noted. Poor PS and high tumor LDH5 expression predicted for poor response rates. High tissue LDH5 was related to poor progression free survival (PFS) only in the placebo group of patients, while the addition of Vatalanib seemed to improved response and PFS in this subgroup. High serum LDH levels were linked with significantly poorer overall survival, which however was not sustained in multivariate analysis.

Conclusions—Serum LDH and tissue LDH5 levels are complementary features that help to characterize the activity of lactate dehydrogenase in colorectal cancer and have a potent value in

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predicting response to chemotherapy. The addition of vatalanib diminished the impact of LDH expression on the prognosis of patients.

Keywords

LDH; PTK/ZK; vatalanib; colorectal cancer; CONFIRM

INTRODUCTION

Lactate dehydrogenases (LDH) is involved in the reversible transformation of pyruvate, the end product of glycolysis, to lactate [1]. The LDH5 isoenzyme composed of 5 M-subunits encoded by the LDHA gene, is the most efficient in catalyzing the conversion of pyruvate to lactate [1]. The anaerobic glucose metabolism is intensified in cancer cells, even in presence of oxygen. This phenomenon, first described by Otto Warburg as aerobic glycolysis [2] has been recently elucidated, at least in part, by studies showing the direct control of the hypoxia inducible factors (HIF1α and 2α) on the transcription of a variety of genes involved in angiogenesis, glycolysis and apoptosis, including VEGF and LDHA [3].

VEGF, one of the most potent angiogenic factors in tumors, has been a major target for the development of anti-angiogenic therapies. The anti-VEGF monoclonal antibody bevacizumab, improves progression-free outcome of patients with a variety of human carcinomas [4,5]. The VEGF tyrosine kinase receptor inhibitor PTK787/ZK222584 (PTK/ZK, vatalanib), that blocks all known VEGF receptor tyrosine kinases, and also PDGFR, c-kit, and c-fms, after showing promising activity in combination with chemotherapy in phase I/II trials in patients with colorectal cancer [6,7], entered phase III evaluation in two large trials, CONFIRM 1 and 2, recruiting patients with advanced colorectal cancer and treating them with oxaliplatin/5-fluorouracil (FOLFOX) chemotherapy with or without vatalanib [8,9]. Although vatalanib seemed not to offer a survival advantage overall, an analysis according to the prospectively stratified serum LDH levels showed a 40% reduction in progression-free survival in patients with high LDH serum levels [10]. This finding was considered of particular clinical importance as colorectal cancer patients with high serum LDH levels have the worst prognosis [11].

The assumption that serum LDH may serve as a predictive marker of response to VEGFR-inhibitor combination with chemotherapy has a biological basis as both LDHA and VEGF are genes transcriptionally regulated by HIFs [3]. Indeed, in previous studies we confirmed that HIF1α overexpression is directly linked with the expression of LDH5, of VEGF and of the phosphorylated (activated) form of the VEGFR2/KDR receptor, in primary colorectal cancer, and correlated with metastasis and the post-operative prognosis [12,13,14].

To further investigate the role of LDH in predicting the efficacy of vatalanib in advanced colorectal cancer, we examined the expression status of LDH5 in cancer tissues obtained from patients recruited in the CONFIRM trials. The relation of tumor LDH5 to response and prognosis of patients is examined in parallel with the serum LDH levels and the performance status of patients.

MATERIALS AND METHODS

The phase III multinational CONFIRM trials 1 and 2 aimed to investigate the therapeutic role of vatalanib in combination with chemotherapy in patients with metastatic colorectal cancer [8,9,10]. In the CONFIRM 1 trial, 1,168 patients were randomly assigned to receive FOLFOX (oxaliplatin and 5-fluorouracil chemotherapy) with either vatalanib or placebo as first line therapy. Safety, progression free and overall survival were the end-points. The

CONFIRM 2 study randomized 855 colorectal cancer patients who had progressed after irinotecan-based first line chemotherapy. The primary end-points of the CONFIRM 1 and 2 trial were the progression free and the overall survival, respectively. The secondary end points in CONFIRM 2 were the overall and the progression free survival in patients with high serum LDH levels. In this study, where patients were stratified according to the PS and serum LDH levels, vatalanib improved the progression free survival of patients with high baseline serum LDH levels (Confirm 2 results for high LDH PFS: HR = 0.63; 95% CI, 0.48 to 0.83; P < .001 and Confirm 1 results for high LDH PFS: HR = 0.67; 95% CI, 0.49–0.91; P = .009).

In an attempt to investigate the association between LDH and vatalanib therapeutic activity, paraffin embedded material from 179 patents with metastatic colorectal adenocarcinoma recruited in the CONFIRM 1 and 2 trials were analyzed with immunohistochemistry for the expression of LDH5 protein. Tissue slides from the primary tumor (not from metastatic sites) were collected randomly by Bayer-Schering Pharma and Novartis from centers participating in the trials and sent to the department of Pathology, Democritus University of Thrace, Alexandroupolis, Greece [AG, ES]. The pathologists that assessed the LDH5 expression were blinded to the outcome of patients and to all other clinical and laboratory details. The LDH5 scoring system applied was the standard one established and used in previous clinicopathological studies performed by the Tumor and Angiogenesis Research Group [13]. The scoring obtained was sent to the central data management office of the CONFIRM trials before the clinical and outcome data could be made available. Although tissues from primary tumors may exhibit different biological features and behavior this was a risk we accepted before starting the whole project. In any case it was impossible to collect an adequate number of tissues from metastatic sites to allow a reliable analysis.

Although the CONFIRM 1 and 2 trials are not fully comparable, as they deal with two distinct populations (chemotherapy naïve and irinotecan resistant patients), the collected samples obtained from patients recruited in these two trials allow a reliable overall analysis, as the patients receiving or not vatalanib are well balanced in numbers within the CONFIRM1 and 2 tissue samples (40 vs. 44 and 51 vs. 44, respectively). Thus, analysis was performed in combined CONFIRM 1 and 2 data sets and no separate analysis was attempted due to the relatively low number of tissues collected. A direct comparison of the demographics and survival data, of the subset of patients analyzed herein, with the whole CONFIRM trial population is not available, as the clinical data of the studies have not been published, as yet. Nevertheless, the preliminary reports on median progression free survival (PFS) in the CONFIRM 1 trial was 7.7 and 7.6 months in patients receiving vatalanib and versus controls coincide at the 7.7 and 7.7 months of the subgroup of patients analyzed n the current study. Regarding the CONFIRM 2 study, the reported median PFS was 5.5 and 4.1 in patients receiving vatalanib and controls, while in the current subgroup analysis this was 3.9 and 4.0 months respectively, implying a slightly reduced median survival in the group of patients receiving vatalanib in the subgroup.

Patient characteristics and response rates according to treatment arm are shown in Table 1. Serum levels of LDH were available from the institutes where patients had been treated and since the normal range depends upon laboratory all values are transformed to the ratio of LDH levels divided by the upper normal limit given by the laboratory. Outcomes and response to treatment data were given by the independent data monitoring board. The follow-up of patients ranges from 1 to 1418 days (median 610 days). All patients were dead with progression of their disease at the time of analysis.

Immunohistochemistry

A modified streptavidin technique was used for immunohistochemistry, as previously reported [12,13]. The primary antibody (ab9002 recognizing the LDH5, Abcam Cambridge, UK) was used. The percentage of cancer cells expressing the proteins under investigation was assessed in all optical fields containing cancer at magnification x200. The percentage of positive cells per optical field was recorded and the mean value of all fields was used to obtain the final score for each case. LDH5 reactivity is both nuclear and cytoplasmic. The cytoplasmic and the nuclear expression of these proteins was first assessed separately and subsequently combined in a grading system, as previously proposed and tested [16,17,19]. Briefly, cases with nuclear LDH5 expression in >10% of cancer cells and/or strong cytoplasmic expression in >50% of cancer cells were considered as being of high LDH5 reactivity. Nonetheless, nuclear and cytoplasmic scoring, which was assessed separately, was also used for continuous variable analysis, where appropriate. All staining scoring was performed separately by two independent observers and the pathologists were blinded to the clinical data. Discordance was resolved on the conference microscope.

Statistical analysis

Statistical analysis was performed using the GraphPad Prism 5.0 and the Instat 3.1 package (GraphPad Software Inc., USA). A Fisher's exact test was used for testing relationships between categorical variables (contingency tables). Linear regression analysis was used to assess correlation with continuous variables. Multinomial regression analysis was used to assess the impact of dichotomous variables on the response rates. Progression free (PFS) and overall (OS) survival were estimated using Kaplan-Meier curves. Curves were compared using the log-rank test (Mantel-Haenszel). The Gehan-Breslow-Wilcoxon method that gives more weight to events at early time points, a feature that may be important when analyzing progression events following therapy or death events expected to occur at a high rate early in the course of follow-up, was also used'. A Cox proportional hazard model including all pre-outcome variables was used to further test the independent significance of variables proved of significance at univariate analysis (in at least one of the two statistical methods applied). A stepwise selection with backward elimination of subsets was used to determine the final model. A two tailed p-value of <0.05 was used for significance.

RESULTS

Serum LDH vs. cancer LDH5 expression and tumor burden

Out of 179 patients analyzed in the current study, 98 (54.7%) had serum LDH levels lower than the upper normal limit, 38 (21.3%) had levels 1.01-1.49 fold and 43 (24%) had 1,5 fold higher value than the upper normal limit. Thus, three groups were formed for subsequent analysis (normal, medium and high serum LDH levels).

Cytoplasmic expression in cancer cells ranged from 0 to 100% reactivity (median 20%), while nuclear expression was lower ranging from 0 to 50% (median 0%). Nuclear expression in more than 10% of cancer cells was noted in 25/179 cases and for 11 of these this pattern was decisive for their grouping in the high LDH5 expression group (Figure 1). Using the established scoring system, 77/179 (43%) of cases had high cancer cell LDH5 reactivity.

Linear regression analysis of serum LDH levels and of the % of cancer cells with cytoplasmic or nuclear expression (separately or combined) of LDH5 did not show any significant correlation. Group analysis of the % of cancer cells in patients with normal vs. abnormal (medium and high) serum LDH levels showed a trend for higher tissue LDH5

expression in patients with high serum LDH levels (mean±standard error: 26±2.8 vs. 33±3.4 p=0.10) (Figure 2a).

On the contrary, the tumor burden as expressed by the sum of maximum assessable tumor diameters showed a significant direct association with the serum LDH levels but none with the tissue LDH5 expression (Figure 2b). Linear regression analysis showed a significant association of tumor burden with serum LDH (p<0.0001, r=0.52). Analysis in patients with abnormal serum LDH levels showed a significant association of cytoplasmic LDH5 expression with tumor burden (Figure 2c). No correlation was found in the group of patients with LDH serum levels lower than the upper normal LDH value.

Serum and tumor LDH association with patient variables

High serum LDH values were significantly more frequent in patients with PS 1,2 (low, median and high serum LDH levels were noted in 38/81, 15/81 and 28/81 patients with PS 1,2 vs. 60/98, 23/98 and 15/98 in those with PS 0; p=0.01). Tissue LDH5 was not linked with PS which is explained by the fact that tissue biopsy was performed at surgery and could not be linked to the PS of patients at diagnosis of metastasis. Female patients also had significantly higher serum LDH levels (low, median and high serum LDH levels were noted in 76/124, 19/124 and 29/124 male patients vs. 22/55, 19/55 and 14/55 female patients; p=0.007). Age was not related to LDH levels. Tumor burden was strongly associated with the PS of patients. The median tumor dimensions (±standard deviation) were 9.4±6 cm in patients with PS=0 vs. 13.5±9cm in patients with PS=1 or 2 (p=0.0008).

Analysis of Response Rates

Response rates (complete and partial) were 42% (37/88) in the placebo group and 82% (39/89) in the group treated with vatalanib (p=0.87; for two patients response was not available).

Analysis of all patients showed a better response rate in those with good PS (50/98, 51% vs. 26/79, 33%; p=0.02) and low tissue LDH5 score (50/101, 50% vs 26/76, 34%; p=0.04). Multivariate analysis of all parameters showed that good PS and low tissue LDH5 expression were significantly linked with response to therapy (p=0.02, odds ratio 1.5 and p=0.05, odds ratio 1.4, respectively).

The predictive value of these two parameters was maintained in the placebo group, whilst it was lost in patients receiving vatalanib. In the placebo group, the response rate was 25.8% (8/31) when LDH5 score was high vs. 50.8% (29/57) when this was low (p=0.02). In the vatalanb group the response rate was 40% (18/45) when LDH5 score was high vs. 47.7% (21/44) when this was low (p=0.52). In a bi-variate model of response to therapy, LDH5 but not PTK/ZK showed an independent predictive value (p=0.03, and p=0.57 respectively).

Survival Analysis

All patients had progressive disease and were dead at the time of analysis. Univariate analysis of all patients showed that response to therapy and PS were the most important parameters linked with PFS and with OS (p<0.0001 and p 0.03, respectively; Table 2). The addition of Vatalanib did not show a significant impact on PFS or OS. High serum LDH levels, age and tumor burden were also linked with poor OS (p=0.003, 0.02 and 0.02, respectively, using the Gehan-Breslow-Wilcoxon method). There was not a significant prognostic relevance of vatalanib administration (PFS: p=0.86 and OS: p=0.65). In multivariate analysis of progression events, the response to therapy was the most significant independent variable linked progression events (p<0.0001, HR 4.3). Excluding response rate

(a post-outcome variable), PS and sex were independent predictors of progression events, while PS and age were independent predictors of death events (Table 2).

In the placebo group, univariate analysis showed that response rate was the most significant prognostic factor of PFS and OS (p<0.0001; Table 2). Tissue LDH5 score was significant linked with PFS (p=0.009; Gehan-Breslow-Wilcoxon method), while PS and serum LDH were linked with OS (p=0.005 and 0.007, respectively; Table 2). Excluding response rates, a multivariate model including parameters significant at univariate (Table 2) showed that none of the parameters were independent predictor of death events.

In the group of patients treated with vatalanib, the response rate was once again the most important factor defining the PFS and OS in univariate analysis (p<0.0001; Table 2). PS and Gender were significantly linked wit PFS (p=0.002 and 0.0003, respectively), while PS and Age were linked with OS (0.0003 and 0.03, respectively). Tissue LDH5 did not show a prognostic relevance. Excluding the response rate from a multivariate model and including parameters significant at univariate, PS and gender had an independent prognostic relevance regarding relapse events, while PS was the only independent predictor of death events (Table 2).

Figure 3 shows the Kaplan-Meier progression free survival curves according to tissue LDH5 expression, a parameter suggested to have an impact in defining time to progression following therapy. Figure 4 shows the Kaplan-Meier overall survival curves according to serum LDH, a parameter suggested to be important in defining time to death.

DISCUSSION

High serum lactate dehydrogenase levels is a common finding in human malignancies, including colorectal cancer. In two studies from the Memorial Sloan-Kettering Cancer Center, high serum LDH levels were significantly associated with resistance to fluoxuridine [15,16]. In a meta-analysis by Watine et al, serum LDH was one of the most important prognostic variables in colorectal cancer [17]. Recent studies confirm the adverse predictive role of serum LDH in response of colorectal cancer to chemotherapy [18,19]. The CONFIRM trials ended in the same conclusion, that serum LDH was one of the most important prognostic factors [10]. Why the addition of the VEGF tyrosine-kinase inhibitor vatalanib offered a progression free survival advantage in the high serum LDH group of patients remained to be elucidated.

The LDHA gene is directly controlled by the HIF1 transcriptional factor, which at the same time regulates the transcription of the angiogenic factor VEGF [3]. Hypoxia-triggered or oncogene-activated HIF pathways are common events in human malignancy, linked to aggressive clinical behavior and resistance to chemotherapy [20]. This pathway leads to the activation of the LDHA gene, encoding the M-subunit of LDH. We also confirmed the selective up-regulation of the LDH5 in colorectal cancer cells compared to the adjacent stroma [21] and its association with invasion, metastasis and the expression of HIFs and VEGF [12]. The strong prognostic relevance of tissue LDH5 with the post-operative outcome of patients with colorectal cancer and its close association with the expression of VEGF and VEGF-receptor expression in cancer cells has also been shown [13,22].

In the present study we examined the prognostic role of the LDH serum levels and tissue expression in a series of colorectal carcinomas recruited in two randomized trials investigating the therapeutic role of the anti-angiogenic drug vatalanib. Although this study suffers from the limited number of cases available for analysis, we had 179 cases assessed in a randomized population analysed by a blinded technique. This is a retrospective subset analysis, but represents a series of steps based on current biological understanding, to

produce hypotheses for future testing in such trials. This is essential to justify the costs of prospective tissue collection, storage and analysis.

The first step we examined was the relation between serum and tissue LDH. We concluded that the serum levels are defined mainly by the tumor burden. The fact that the LDH5 cancer cell expression was assessed on primary tumor tissues, while the serum LDH levels were measured at documentation of metastasis does not allow a reliable comparison of these two parameters, which in any case did not show a significant association.

It is also stressed that, as the serum levels of LDH before the development of the carcinoma are unknown, patients with serum LDH levels falling into the normal LDH range of the general population may in fact be elevated above the normal level for the patient and this cannot be discriminated on the basis of post-diagnosis serum LDH assessment. Thus, 'normal' serum LDH may not have low cancer cell LDH activity. On the other hand, low tumor burden may result in low serum LDH levels even in tumors with intense LDHA activity. Thus, serum LDH and tissue LDH5 expression are complementary and their combined use better delineates the LDH tumor activity profile. The factors causing release of LDHA are also unknown.

Analysis of response rates to chemotherapy showed that poor PS and cancer cell LDH5 content were linked with a 2-fold reduced response probability to chemotherapy. The addition, however, of vatalanib to chemotherapy diminished the adverse effect of tissue LDH5 on tumor responsiveness. In fact, tumors with high LDH5 content seemed to have better response to chemotherapy / vatalanib combination compared to tumors with low LDH5 expression. It is notable that although the tissue LDH5 was measured in the primary tumor, there was a relationship to response of metastases years later, indicating that this hypoxia regulated pathway may persist in secondary deposits.

Analysis of the progression free survival (PFS) showed that the response to chemotherapy was the most significant predictor of the progression free interval. Taking into pre-outcome parameters only, LDH5 was a significant predictor only in cases who did not receive vatalanib, as addition of this angiogenesis inhibitor reversed the adverse effect of high LDH5 on the efficacy of chemotherapy. In overall survival analysis serum LDH and PS were significantly related to overall survival but no effect of vatalanib on overall survival was noted.

It is stressed that the current study has several limitations as this is a retrospective attempt to evaluate a role of a tumor molecular variable, namely LDH, following a previous confirmation of its prognostic role when assessed in the serum of patients (a secondary end point of CONFIRM trial 2). Retrospective collection of tissues is not always feasible and the random collection of tissue blocks from less than 10% of the total number of patients recruited in the CONFIRM trials may have bias. This is further aggravated by the fact that the two CONFIRM trials were not addressed to the same patient population and also because the LDH5 expression in metastatic tumors may not be reliably reflect the results we obtained from primary tumors. The relatively low number of samples and the multiple variable analysis also have a risk of bias from multiple comparisons.

Despite the above limitations, the present study provides evidence that serum LDH and tissue LDH5 levels are complementary markers of lactate dehydrogenase activity in colorectal cancer. Clinical studies have already established serum and tissue LDH levels as strong prognostic indicators in colorectal cancer [12,13,15-19]. The new information from the current study is that tissue LDH5 is also associated with resistance to standard chemotherapy and worse progression free survival (PFS) in patients with advanced colorectal cancer. Addition of the anti-angiogenic agent vatalanib seemed to reduce the

adverse predictive and prognostic relevance of LDH5, at least in the current series of patients. This retrospective analysis is the first to provide evidence that a specific marker may be of value in future randomized trials investigating low molecular weight VEGF kinase inhibitors. Our pilot data suggest combined selection of patients on clinical grounds and by appropriate tumor biomarkers would increase cost-effectiveness of these therapies.

TRANSLATIONAL RELEVANCE

There is major need for biomarkers to assess patients likely to benefit from anti-angiogenic therapy. We analysed a subset of 179 patients from two randomised trials (CONFIRM trials) that investigated the combinations of FOLFOX with or without the VEGF kinase receptor inhibitor, PTK787/ZK222584. High tumour LDH5 predicted poor response to chemotherapy, and vatalanib improved the response rate and progression-free survival in the patients. Tumour LDH5 and tissue LDH give complementary information as neither alone fully characterises the expression of the protein in colorectal cancer, and they have a potential value in predicting a benefit from chemotherapy and anti-angiogenic therapy. Justification is provided for further investigation of genes regulated by the HIF pathway as potential important markers for the effectiveness in subgroups of patients to VEGF kinase inhibitors. Selection of patients on clinical grounds and by appropriate tumor biomarkers would increase cost-effectiveness of these therapies.

Acknowledgments

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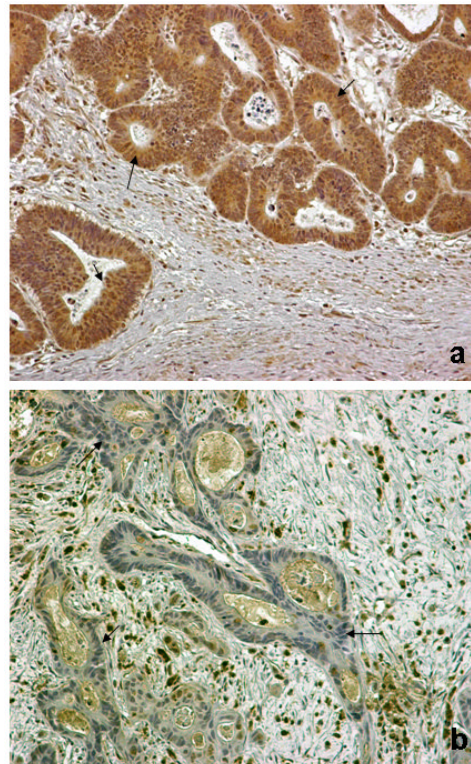
FIGURE 1.
Immunohistochemical LDH5 expression patterns in colorectal cancer tissues: (a). strong cytoplasmic and nuclear LDH5 expression (arrows). (b). Low LDH5 expression in cancer cells (arrows).
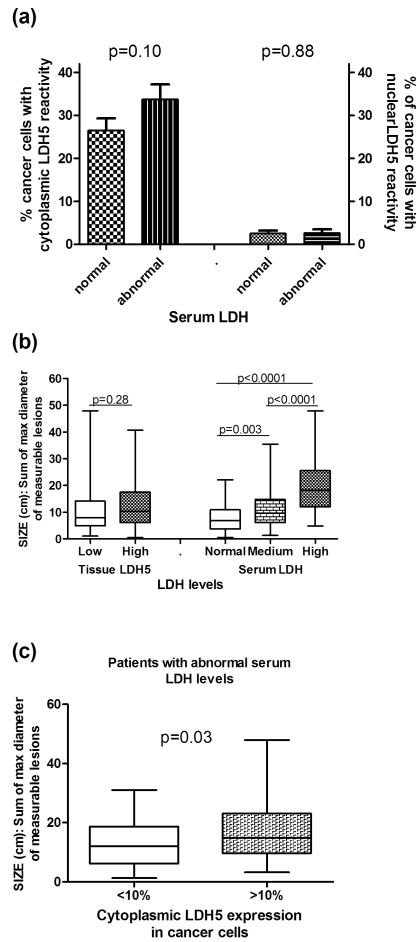
FIGURE 2.
Analysis of serum and tissue LDH levels: (a). serum LDH levels vs. tissue cytoplasmic and nuclear expression (bars show the standard error). (b). serum LDH levels vs. size of the measurable lesions (c). cytoplasmic LDH expression vs. size of the measurable lesions in patients with serum LDH levels higher than normal laboratory values (in 2b and 2c, boxes show the 1st and 3rd quartile of values, the orizontal line in the box shows the median value and the whiskers show the range of values).
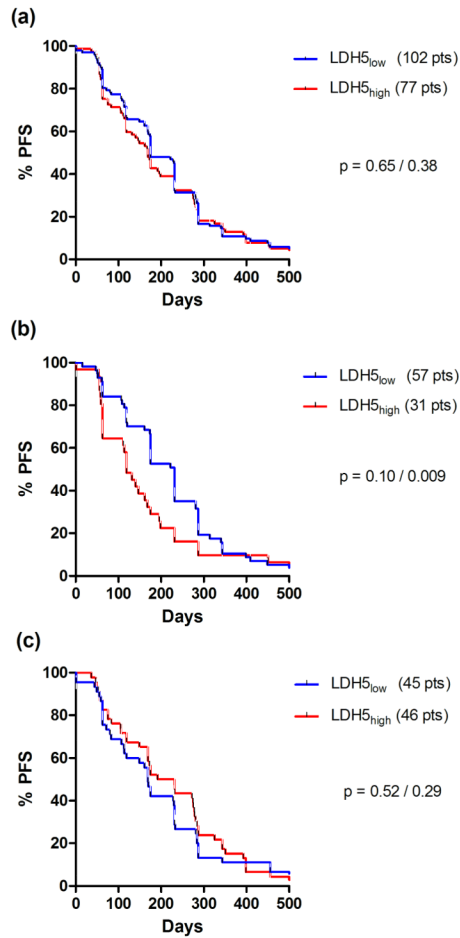
FIGURE 3.
Kaplan-Meier progression free survival (PFS) according to the LDH5 tissue expression in all patients (a), in patients receiving placebo (b) and in patients receiving vatalanib (c). The two p-values correspond to the Mantel-Haenszel and the Gehan-Breslow-Wilcoxon methods, the latter giving more weight to events at early time points.

(a)

(b)

(c)
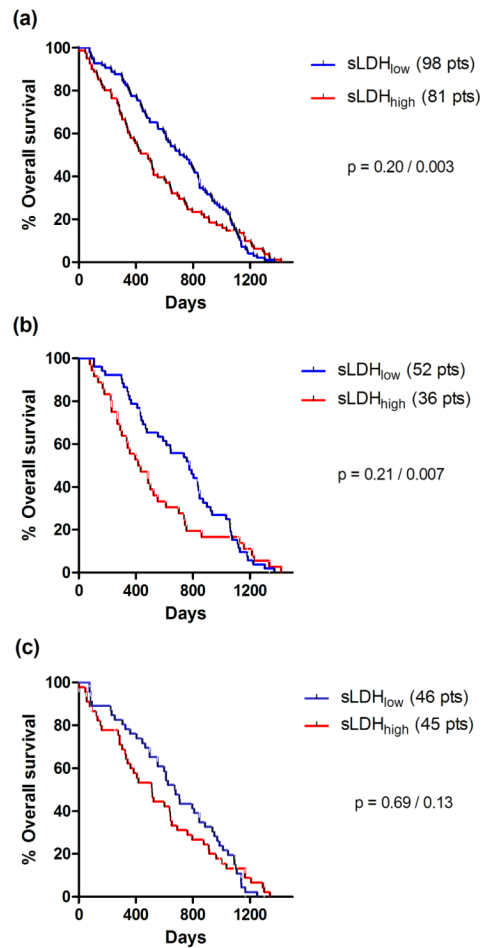
FIGURE 4.
Kaplan-Meier overall (disease specific) survival according to the serum LDH (sLDH) levels in all patients (a), in patients receiving placebo (b) and in patients receiving vatalanib (c). The two p-values correspond to the Mantel-Haenszel and the Gehan-Breslow-Wilcoxon methods, the latter giving more weight to events at early time points.

Table 1

Patient characteristics

	Placebo	Vatalanib
No	88	91
CONFIRM 1/2 [*]	44/44	40/51
PS 0/1,2	51 / 37 (57.9%)	47 / 44 (51.6%)
Sex M/F	60 / 28 (68%)	64 / 27 (70%)
Age (median,range)	62 (38-82)	63 (28-83)
Size	8.9 (1-35.4)	9.3 (0.5-47.9)
LDH serum	0.9 (0.3-8.5)	1.0 (0.2-24)
CR/PR	37/88 (42%)	39/89 (43.8%)
Median PFS (days)	175	175
Median OS (days)	600	610
Number of PFS events	88	91
Number of deaths	88	91

[*] tissue samples collected from patients recruited in the CONFIRM 1 or 2 studies; CR/PR complete and partial response; PFS progression free survival; OS overall survival

Table 2

Univariate and Multivariate analysis of all patients (including pre-outcome variables significant at univariate), of those treated with placebo and those treated with vatalanib. All patients had progressed and were dead at the time of analysis. The two p-values in the univariate analysis correspond to the Mantel-Haenszel and the Gehan-Breslow-Wilcoxon methods, the latter giving more weight to events at early time points.

Variable	Progression Free Survival				Overall Survival			
	Univariate		Multivariate		Univariate		Multivariate	
	HR (95%CI)	p-value	HR (95%CI)	p-value	HR (95%CI)	p-value	HR (95%CI)	p-value
ALL CASES								
Response	2.7 (1.9-3.8)	<0.0001/<0.0001	-------		3.0 (2.2-4.2)	<0.0001	-------	
Vatalanib	1.0 (0.7-1.3)	0.99/0.88			0.9 (0.6-1.2)	0.52/0.65		
PS	1.4 (1.0-1.9)	0.03/0.001	1.3 (1.0-1.8)	0.03	1.5 (1.1-2.1)	0.009/<0.0001	1.4 (1.0-1.9)	0.01
LDH5 score	1.1 (0.7-1.4)	0.65/0.38			1.0 (0.7-1.3)	0.84/0.86		
LDH serum	1.3 (0.9-1.7)	0.13/0.10			1.2 (0.9-1.7)	0.20/0.003	1.2 (0.9-1.7)	0.11
Age	1.1 (0.8-1.5)	0.43/0.13			1.4 (1.0-1.9)	0.03/0.02	1.5 (1.1-2.0)	0.01
Sex	1.5 (1.1-2.0)	0.05/0.002	1.3 (1.0-1.9)	0.05	1.0 (0.7-1.4)	0.88/0.80		
Tumor burden	1.2 (0.9-1.6)	0.19/0.25			1.2 (0.9-1.6)	0.26/0.02	1.1 (0.7-1.4)	0.67
PLACEBO GROUP								
Response	3.2 (2.0-5.2)	<0.0001/<0.0001	-------		3.5 (2.2-5.8)	<0.0001/<0.0001	-------	
PS	1.1 (0.7-1.8)	0.54/0.12			1.3 (0.8-2.0)	0.26/0.005	1.2 (0.8-1.9)	0.31
LDH5 score	1.5 (0.9-2.5)	0.10/0.009			1.0 (0.6-1.6)	0.86/0.50		
LDH serum	1.3 (0.8-2.1)	0.27/0.20			1.3 (0.8-2.1)	0.21/0.007	1.3 (0.8-2.0)	0.26
Age	1.0 (0.6-1.6)	0.89/0.57			1.4 (0.9-2.1)	0.18/0.31		
Sex	1.1 (0.7-1.8)	0.58/0.46			0.8 (0.5-1.3)	0.35/0.35		
Tumor burden	1.5 (0.9-2.4)	0.08/0.22			1.1 (0.7-1.7)	0.62/0.11		
VATALANIB GROUP								
Response	2.4 (1.5-3.8)	0.0002/<0.0001	-------		2.5 (1.6-3.9)	<0.0001/<0.0001	-------	
PS	1.7 (1.1-2.8)	0.01/0.002	1.6 (1.1-2.5)	0.01	1.9 (1.2-2.9)	0.004/0.0003	1.7 (1.1-2.7)	0.01
LDH5 score	0.8 (0.5-1.3)	0.52/0.29			0.8 (0.5-1.3)	0.57/0.20		
LDH serum	1.2 (0.7-1.8)	0.41/0.31			1.1 (0.7-1.7)	0.69/0.13		
Age	1.3 (0.8-1.9)	0.26/0.18			1.5 (0.9-2.2)	0.08/0.03	1.3 (0.8-2.0)	0.17
Sex	1.9 (1.1-3.3)	0.01/0.0003	1.6 (1.1-3.3)	0.02	1.2 (0.7-2.0)	0.36/0.25		
Tumor burden	1.0 (0.6-1.5)	0.94/0.71			1.3 (0.8-2.0)	0.23/0.08		