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LDH correlation with survival in advanced melanoma from two large, randomised trials (Oblimersen GM301 and EORTC 18951) ☆

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ABSTRACT

Purpose: In a randomised study (GM301; dacarbazine with/without oblimersen), patients with advanced melanoma were stratified based on performance status, metastatic site and lactate dehydrogenase (LDH). Progression-free survival and response and durable response rates showed a highly significant difference favouring dacarbazine–oblimersen and a nearly significant survival difference. All efficacy parameters significantly favoured dacarbazine–oblimersen in patients with normal baseline LDH [$\leqslant 1.1 \times$ upper limit of normal (ULN)]. Each stratification factor was assessed for an interaction with treatment on survival and an interaction was detected only for LDH.

Experimental design: Baseline LDH values in Study GM301 treatment groups were combined and analysed using cutoffs above and below $1 \times$ ULN. Baseline LDH in EORTC study 18951 (dacarbazine, cisplatin, interferon-alfa-2b with/without interleukin-2 in advanced melanoma) was independently analysed using the same approach. In Study GM301, the relation between treatment effect and LDH, treatment effect and tumour size, LDH and tumour size and LDH and disease site were determined.

Results: In Study GM301 ($N = 760$) and Study 18951 ($N = 325$), LDH was within the upper range of normal for a large number of patients. This was not exhibited in the general population, suggesting such values may be elevated rather than normal in melanoma. A highly ordered and monotonic relationship was apparent between LDH and survival: survival

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worsened as LDH became more elevated, even when LDH remained within normal range. LDH and tumour size were poorly correlated; elevated LDH was not associated with any one disease site. LDH was highly predictive of oblimersen effect.
Conclusion: In designing studies, LDH should be considered, regardless of tumour size or disease site.

1. Introduction

An enhanced understanding of key prognostic variables in melanoma has emerged in the last 10 years, primarily due to new biologic techniques and large-scale, population-based analyses.[1] As a result, clinical trials now often include risk factors for stratification and identification of patient subpopulations that may achieve maximum therapeutic benefit.[2–4]

Serum lactate dehydrogenase (LDH) level is one of the most useful independent prognostic factors in metastatic melanoma.[1] Numerous studies investigating various prognostic factors confirm the correlation between increased LDH and decreased survival among patients with advanced melanoma.[5–13] This association is evident even after accounting for the number of metastases and location of distant metastatic involvement.[14,15]

Solid tumours require a blood supply to grow and metastasise and are highly adaptable in meeting this need, as well as thriving in a hypoxic state. Increased serum LDH is believed to be related to the hypoxic environment of tumour cells. Unlike normal cells that produce the majority of ATP from glucose through oxidative phosphorylation, many cancer cells produce ATP by converting glucose to lactate,[16] a process essential to the hypoxic environment in which many cancer cells exist. LDH is composed of 2 peptides – LDH-A and LDH-B. It is LDH-A (also known as LDH-5 in its tetrametric form) that is upregulated in the hypoxic environment. This enzyme catalyses the conversion of pyruvate to lactate, probably via the activity of the HIF1α transcription factor.[17,18] As LDH is not a secreted enzyme, the finding of elevated LDH in the serum of patients with advanced melanoma is probably due, at least in part, to melanoma-cell necrosis or apo-necrosis and the spillage of LDH, which likely occurs when part of a tumour outgrows its blood supply. The remaining poorly perfused, hypoxic tumour cells may be highly refractory to cytotoxic chemotherapy in light of abnormalities in their vasculature and microenvironment,[19] and they may eventually recover and proliferate.

Little progress in the treatment of advanced melanoma has been made over the last several decades. The standard therapy remains dacarbazine. Various agents, including cytotoxic drugs, interferon-α and IL-2 and tamoxifen, have been used in combination with dacarbazine in clinical studies.[20] Nevertheless, randomised trials of single-agent dacarbazine versus dacarbazine-containing combination regimens have failed to show a survival benefit with the multiple-drug regimens.[20]

The largest randomised study in advanced melanoma conducted to date is a multinational, Phase 3 trial (Study GM301) in 771 patients, including 760 for whom baseline serum LDH was recorded. This study was performed to evaluate the potential of the antisense oligonucleotide oblimersen to enhance the efficacy of dacarbazine in a chemotherapy-naïve population.[21] Prior to randomisation, patients were stratified according to 3 prognostic factors: Eastern Cooperative Oncology Group performance status (0 versus 1 or 2), liver metastases (present versus absent) and disease site in combination with baseline LDH (non-visceral disease and normal LDH, defined as a baseline serum LDH level $\leqslant 1.1$ times the upper limit of normal [×ULN], versus visceral disease [excluding liver metastases] or elevated LDH, defined as a baseline serum LDH level $>1.1 \times$ ULN). Every 3 weeks patients received either dacarbazine 1000 mg/m^2 intravenously (i.v.) or the same dacarbazine dose preceded by a 5-d continuous intravenous infusion of oblimersen 7 mg/kg/d. Patients were assessed by RECIST[22] at the end of every two cycles.

Overall survival (the primary end-point) showed an improvement in the oblimersen–dacarbazine group at 24-month minimum follow-up that approached statistical significance (median 9.0 months versus 7.8 months in the dacarbazine group; $P = 0.077$). Other efficacy end-points consistently demonstrated a highly significant between-treatment difference favouring the oblimersen–dacarbazine regimen, including progression-free survival (median 2.6 months versus 1.6 months; $P < 0.001$), overall response rate (13.5% versus 7.5%; $P = 0.007$) and durable response rate (7.3% versus 3.6%; $P = 0.03$).[21] Particularly noteworthy was a separation of the survival curves beginning at about 6 months, which suggested the existence of a heterogeneous population. Thus, we assessed whether there was a subset of patients who derived increased benefit from the addition of oblimersen to dacarbazine.

Each of the three stratification factors in this study (performance status, metastatic site and LDH) was assessed for an interaction with treatment on survival. An interaction was detected only between LDH and treatment.[21] Given the heterogeneity of the population, treatment effect was then analysed in both the normal and elevated LDH subpopulations. The normal LDH population included 508 of the 760 patients, representing a sample size greater than that used in any previous randomised study in advanced melanoma.[21] Statistically significantly superior results were observed for multiple efficacy end-points in patients with normal LDH who were treated with the oblimersen–dacarbazine regimen, including overall survival (median 11.4 months versus 9.7 months; $P = 0.02$), progression-free survival (median 3.1 months versus 1.6 months; $P < 0.001$), overall response rate (17.2% versus 9.3%; $P = 0.009$) and durable response rate (9.6% versus 4.0%; $P = 0.01$). In contrast, the elevated LDH population ($N = 252$) was neutral with respect to treatment effect;

no significant between-treatment differences in efficacy parameters were apparent.[21]

Per protocol, the cutoff for defining normal LDH and elevated LDH had been established as 1.1 × ULN. Given the interaction between baseline LDH and treatment, two important questions arose: (1) Would a different cutoff to define 'normal LDH' have led to different conclusions? and (2) What is the medical relevance of this observation? This article attempts to provide answers to these two questions, as well as the medical basis for our findings.

2. Patients and methods

LDH values from both treatment groups in Study GM301 were combined, and the distribution of these values was compared to the distribution in the general population (i.e. expected Gaussian distribution) from whom the normal reference range is established.

LDH cutoffs above and below 1 × ULN were used to assess the robustness of the definition of normal LDH.[23] Five LDH categories were considered: ⩽0.8 × ULN, >0.8 to ⩽ 1.1 × ULN (cutoff point used for stratification in Study GM301), >1.1 to ⩽2 × ULN (cutoff point used for stratification in another randomised study in advanced melanoma [EORTC Study 18951]),[24] >2 to ⩽5 × ULN, and >5 × ULN. Survival based on the data of both treatment groups in Study GM301 combined was presented in Kaplan–Meier curves by LDH category. A randomised study in advanced melanoma matched to Study GM301 for key eligibility criteria (EORTC Study 18951[24]; N = 363, including 325 patients for whom baseline serum LDH was recorded) was independently analysed using the same methodology.[23] In the previously reported EORTC study, therapy with dacarbazine, cisplatin and interferon-alfa-2b with or without interleukin-2 was compared.[24]

In Study GM301, survival, progression-free survival and response were analysed for the lowest LDH category. Survival and progression-free survival were analysed using the log-rank test, and hazard ratios were estimated by using an unadjusted Cox proportional hazard model. Response rates were analysed using the Chi-square test.

In Study GM301, the relation between treatment effect and baseline LDH and treatment effect and tumour size (based on total measurable disease, as defined by RECIST)[22] were examined by using a moving-average technique.[25] Curves of hazard ratios for survival (oblimersen–dacarbazine arm over dacarbazine arm) as a function of these baseline variables were drawn. The complete sample was divided into 11 equally sized, contiguous groups based on the lowest and highest LDH values observed. Every three adjacent groups were combined to obtain an adequate number of cases to estimate the hazard ratio for survival. Lastly, the relation between LDH and tumour size and LDH and disease site (based on the protocol-specified strata [see Table 1]) was analysed. The Spearman correlation coefficient was calculated to determine the correlation between LDH and tumour size.

3. Results

With a Gaussian distribution, one would expect 50% of LDH values to fall below the median. For the 760 patients in Study GM301, the median midpoint value between the upper and lower limits of the normal range was 0.75, but the median ratio of baseline LDH to the upper limit of normal range was 0.9, demonstrating a shift to the right in the median by approximately 20%. In fact, in Study GM301, one-third of the values fell between 0.8 and 1.1 × ULN, one-third fell below 0.8 × ULN and one-third fell above 1.1 × ULN. Thus, a relatively large number of patients in Study GM301 had increased LDH, but the value remained within normal limits – specifically in the upper range of normal. This differs from observations in the general population in which there are comparatively few values in the upper range of normal.

Kaplan–Meier survival curves based on Study GM301 data for the 2 treatment groups combined are shown in Fig. 1a by LDH category. Remarkably, a highly ordered and monotonic relationship was apparent. As expected, high baseline LDH values were associated with poor survival. Unexpectedly, survival was better in the lowest LDH category (⩽0.8 × ULN; N = 274) than in the central LDH category (>0.8 to ⩽1.1 × ULN; N = 234) ($P < 0.05$). A minor increase in LDH, even within the normal range as defined based on the general population, appeared to have an important prognostic impact on survival.[23] Certainly other factors, such as the oblimersen treatment benefit observed in patients with baseline LDH ⩽ 0.8 × ULN, could have contributed to this finding.

Data from EORTC Study 18951 for the two treatment groups combined showed the same highly ordered prognostic pattern (see Fig. 1b): survival was definitely improved in pa-

Table 1 – Protocol-specified strata in Study GM301.

Stratum	ECOG score	Liver metastases	Disease distribution and lactate dehydrogenase (LDH) status
1	0	Present	Not considered in light of the presence of liver metastasis
2	1 or 2	Present	Not considered in light of the presence of liver metastasis
3	0	Absent	Disease in visceral organ other than liver, or elevated LDH
4	1 or 2	Absent	Disease in visceral organ other than liver, or elevated LDH
5	0	Absent	Soft tissue only: disease in the skin, subcutaneous tissue, and/or lymph nodes without visceral metastases, and LDH not elevated
6	1 or 2	Absent	Soft tissue only: disease in the skin, subcutaneous tissue, and/or lymph nodes without visceral metastases, and LDH not elevated

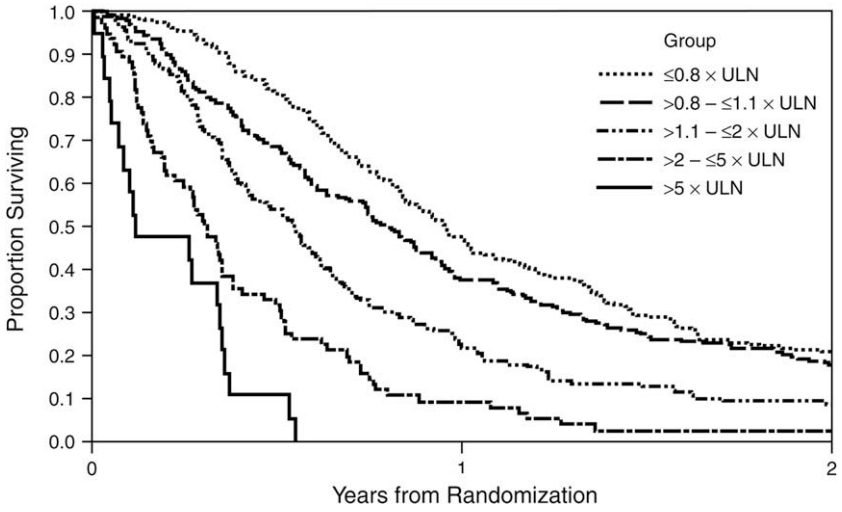

Fig. 1a – Kaplan–Meier survival curves at 24-month minimum follow-up by LDH category in Study GM301 (*N* = 760).

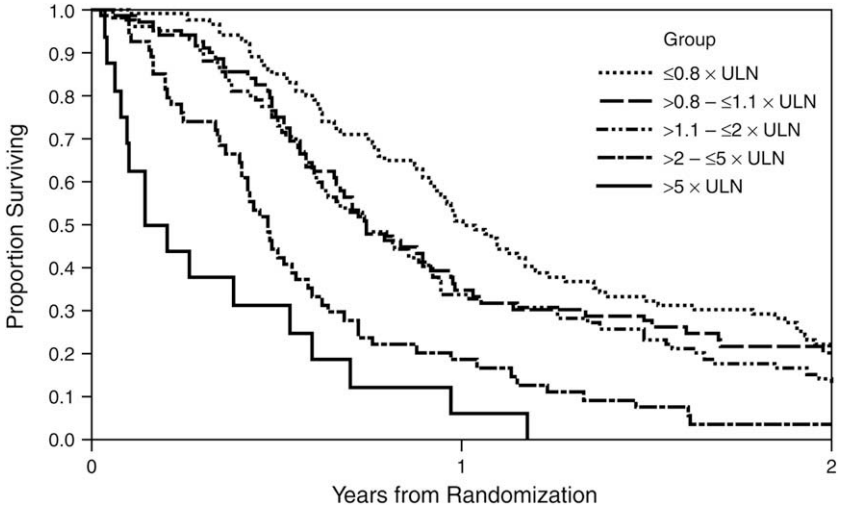

Fig. 1b – Kaplan–Meier survival curves at 24-month minimum follow-up by LDH category in EORTC Study 18951 (*N* = 325).

tients with baseline LDH $\leqslant 0.8 \times$ ULN.[23] The LDH categories of >0.8 to $\leqslant 1.1 \times$ ULN and >1.1 to $\leqslant 2 \times$ ULN were more separated in Study GM301 than those observed in EORTC Study 18951. This difference was perhaps due to the somewhat smaller sample size in the EORTC study.

In Study GM301, a nearly significant difference in overall survival ($P = 0.077$) favoured the oblimersen–dacarbazine regimen over dacarbazine alone. Maximum efficacy was observed in patients with a baseline LDH level of $\leqslant 0.8 \times$ ULN[23]: median overall survival improved by more than 2 months in the oblimersen–dacarbazine group (12.3 months versus 9.9 months, respectively; $P < 0.001$; hazard ratio = 0.64; see Fig. 2). The treatment effect in this LDH category was consistently demonstrated across multiple end-points, including progression-free survival (3.6 months versus 1.6 months; $P < 0.0001$), overall response rate (20.8% versus 7.2%; $P = 0.002$) and durable response rate (10.7% versus 2.4%; $P = 0.007$). This suggests that a lower baseline LDH is most likely to be the 'true normal' in patients with advanced melanoma, and that therapy in melanoma, such as the oblimersen–dacarbazine treatment, may be most efficacious in patients with a lower LDH value than is considered normal based on standard reference ranges.

Although the relationship of LDH to survival was identical in Study GM301 and EORTC Study 18951, no significant difference was detected between treatment arms in the EORTC study.[23] The treatments evaluated differed from those assessed in Study GM301, and the sample size was somewhat smaller than that in Study GM301.

Assessment of the relation between LDH and treatment effect (based on the hazard ratios for survival) in Study GM301 using a moving-average technique is shown in Fig. 3a. As is evident in this figure, the first two points are nearly on the same level, suggesting a lower plateau has been reached. Then there is a steep increase in hazard ratios up to a ratio of about 1. Thereafter, the hazard ratios plateau around 1. The probable explanation for this pattern follows: At an LDH value less than $0.8 \times$ ULN, the treatment effect reaches a plateau, as these values are all normal regardless of the specific value. Between $0.8 \times$ ULN and $1.1 \times$ ULN, the treatment ef-

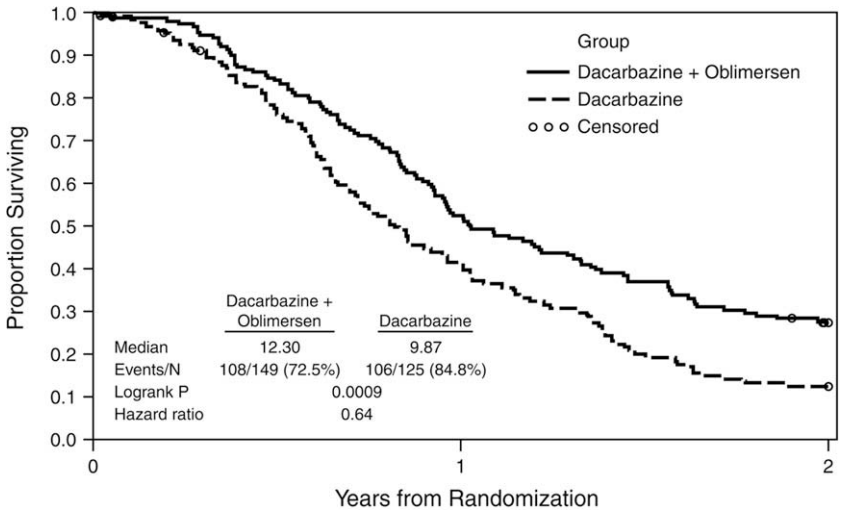

Fig. 2 – Kaplan–Meier survival curves at 24-month minimum follow-up among patients with baseline LDH value $\leqslant 0.8 \times$ ULN in Study GM301 (N = 274).

fect is the result of the proportion of truly normal values and elevated values. At an LDH value clearly above $1.1 \times$ ULN, treatment effect is diminished.

Assessment of the relation between tumour size (based on total measurable disease [RECIST])[22] and treatment effect (based on the hazard ratios for survival) in Study GM301 using the same moving-average technique as described for LDH is shown in Fig. 3b. No particular pattern was seen and no relationship with treatment effect suggested.

There was a poor correlation between baseline LDH and tumour size ($R = 0.36$; see Fig. 4). No pattern with respect to baseline LDH and tumour burden (based on tumour size)

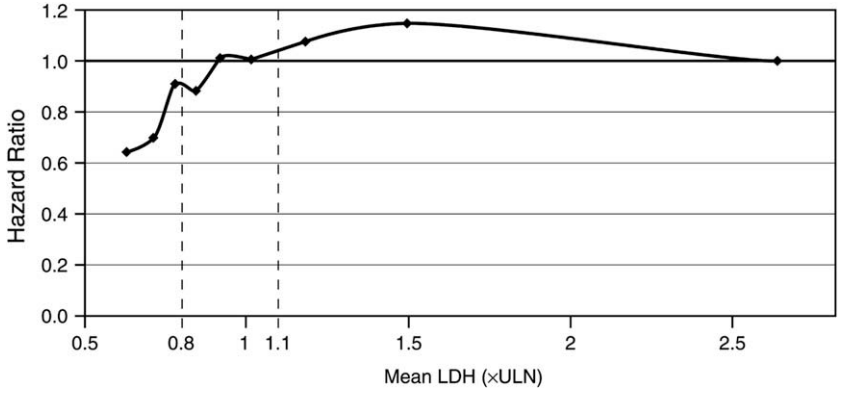

Fig. 3a – Treatment hazard ratio for survival as a function of baseline LDH in Study GM301.

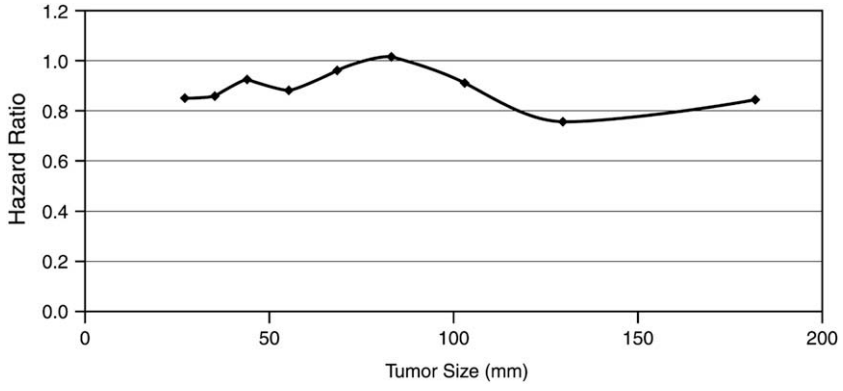

Fig. 3b – Treatment hazard ratio for survival as a function of tumour size (based on total measurable disease) in Study GM301.

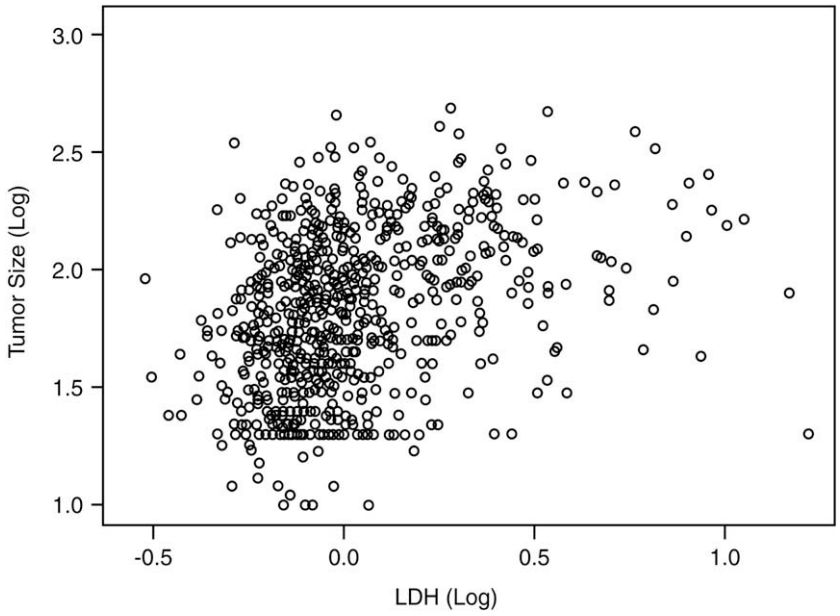

Fig. 4 – Tumour size based on total measurable disease versus baseline LDH in Study GM301.

Table 2 – Disease site by lactate dehydrogenase (LDH) category in Study GM301.

Disease site	LDH category (×ULN)					
	⩽0.8 *n* (%)	>0.8 to ⩽1.1 *n* (%)	>1.1 to ⩽2.0 *n* (%)	>2.0 to ⩽5.0 *n* (%)	>5.0 *n* (%)	Total *n* (%)
Liver	58 (21)	74 (32)	72 (46)	44 (58)	16 (84)	264 (35)
Other visceral	156 (57)	115 (49)	60 (38)	19 (25)	2 (11)	352 (46)
Soft tissue only	60 (22)	45 (19)	25 (16)	13 (17)	1 (5)	144 (19)

was evident. In addition, elevated LDH values were not solely associated with any one disease site (see Table 2), although the proportion of patients with the highest LDH levels was greatest among those with liver involvement.

4. Discussion

Elevated serum baseline LDH is widely recognised as an important prognostic factor in melanoma. As with all laboratory values, the normal limits for LDH are derived from the general healthy population. Based on our findings, the applicability of the upper limit of the normal range is questionable in the setting of advanced melanoma. The overrepresentation of values close to but not exceeding the upper limit of normal implies that these values may actually be elevated rather than normal in a melanoma population. Our data support that survival worsens as LDH becomes more elevated – even when baseline LDH remains within the normal range.

Elevated serum baseline LDH levels have historically been interpreted as a surrogate for large tumour burden. LDH is involved in the cellular production of energy, and, in the recent years, its overexpression has been reported as ensuring anaerobic/glycolytic metabolism within tumour cells and reduced cellular dependence on oxygen.[16–18] Of the 5 LDH isoenzymes identified to date, LDH-5 is widely expressed in cancer cells but absent from normal epithelia and is thought to be linked to tumour phenotype.[18] Moreover, in non-small cell lung cancer, LDH-5 expression was linked to high total serum LDH levels.[17,18] LDH isoenzyme data were not collected in Study GM301. Perhaps our patients with baseline LDH in the upper range of the normal limit have intracellular expression of LDH-5, suggesting a more aggressive phenotype, and patients with low baseline LDH represent those expressing less ominous LDH isoenzymes.

It has been hypothesised that the normalisation of vasculature abnormalities in solid tumours may increase the efficacy of chemotherapy through enhanced drug delivery (with attainment of effective drug concentrations) and improved oxygen perfusion.[19] In addition to its specific anti-Bcl-2 effect, oblimersen dramatically but non-specifically increases endothelial cell mitogenesis and tubular morphogenesis in vitro (Stein submitted), thus stimulating the development of new vessel sprouts. These effects may be mediated by oblimersen's very high (picomolar), non-specific affinity for collagen I, a major inducer of angiogenesis. Cells tend to undergo necrosis when they can no longer produce sufficient ATP to meet metabolic requirements.[26] Cellular necrosis and the resulting LDH spillage may critically depend on the balance between tumour growth and the ability to supply nutrients and oxygen, which, in turn, depend on the state of dynamic vascularisation. We speculate that oblimersen may affect this balance in light of its effects on endothelial cells and that

these effects may perhaps be observable only in tumours to which adequate amounts of oxygen can be delivered relative to the metabolic requirements of the tumour. If this is the case, oblimersen will not be effective in tumours with either insufficient vasculature or a very high growth rate, as both conditions will produce hypoxia and an increase in LDH.

Based on our findings and consistent with these hypotheses, there was a poor correlation between baseline LDH and tumour size. Regarding oblimersen treatment effect, the prognostic impact of serum LDH and tumour burden on survival clearly differed. Overall, LDH was strongly prognostic for survival and not a simple marker of tumour burden. Although our analysis focused on a single prognostic variable among several currently identified, we cannot ignore the finding of a survival benefit in favour of patients with low baseline serum LDH ($\leqslant 0.8 \times$ ULN) in these two independently analysed, large, randomised studies.

We have also shown that a baseline LDH value of $\leqslant 0.8 \times$ ULN is predictive of the benefit attainable with oblimersen in combination with dacarbazine. This treatment effect was observed in patients with LDH $\leqslant 0.8 \times$ ULN, a number of whom had extensive disease, as well as disease at unfavourable sites. Particularly noteworthy was the consistent treatment effect across all efficacy end-points in patients with a baseline LDH value of $\leqslant 0.8 \times$ ULN. The Kaplan–Meier survival curves for these patients separated early and then remained separated in a fairly consistent manner, suggesting a more homogenous population with respect to treatment effect.

Based on the normal and elevated LDH categories described in Study GM301, the *P* value for the overall treatment comparison for survival was influenced by the relative proportions of patients in these categories. Clearly, inclusion of patients with high baseline LDH levels can obscure a treatment effect in clinical trials. This observation is extremely important in the evaluation of new therapeutics for melanoma. It may be difficult to demonstrate a survival benefit in patients with baseline LDH above $2 \times$ ULN, as these patients may fail quite rapidly. A large, randomised study (the AGENDA Trial) is underway to prospectively confirm the safety and efficacy of oblimersen plus dacarbazine in chemotherapy-naïve patients with advanced melanoma and baseline LDH $\leqslant 0.8 \times$ ULN.

5. Conclusions

The consistency in efficacy demonstrated in Study GM301 has not previously been observed in any other completed, randomised study in advanced melanoma. In this study, low baseline LDH ($\leqslant 0.8 \times$ ULN) was found to be predictive of the treatment effect of oblimersen in combination with dacarbazine. A confirmatory study is ongoing. Whether the low LDH cutoff point of $0.8 \times$ ULN is disease- or drug-specific warrants exploration.

In the setting of advanced melanoma, baseline serum LDH is not a simple marker of tumour burden. Rather, it is a discriminating prognostic variable. As observed in the dacarbazine–oblimersen trial, baseline serum LDH may also be predictive of the effect of some treatments. Thus, the cutoff value for baseline LDH warrants careful consideration in clinical trial design.

Trial registration numbers

Study GM301 – NCT00016263; EORTC Study 18951 – NCT00002669

Conflict of interest statement

Sanjiv S. Agarwala – none declared.
Ulrich Keilholz – none declared.
Erard Gilles – employment (Genta; compensated).
Agop Y. Bedikian – none declared.
Jane Wu – employment (Genta; compensated).
Richard Kay – consultant (Genta Incorporated; compensated).
Cy A. Stein – consultant (Genta; uncompensated); honoraria (Genta); expert testimony (Genta, compensated).
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REFERENCES

1. Homsi J, Kashani-Sabet M, Messina JL, Daud A. Cutaneous melanoma: prognostic factors. *Cancer Contr* 2005;**12**:223–9.
2. Buzaid AC, Anderson CM. The changing prognosis of melanoma. *Curr Oncol Rep* 2000;**2**:322–8.
3. Linette GP, Carlson JA, Slominski A, Mihm MC, Ross JS. Biomarkers in melanoma: stage III and IV disease. *Expert Rev Mol Diagn* 2005;**5**:65–74.
4. Petro A, Schwartz J, Johnson T. Current melanoma staging. *Clin Dermatol* 2004;**22**:223–7.
5. Sirott MN, Bajorin DF, Wong GYC, et al. Prognostic factors in patients with metastatic malignant melanoma. A multivariate analysis. *Cancer* 1993;**72**:3091–8.
6. Eton O, Legha SS, Moon TE, et al. Prognostic factors for survival of patients treated systemically for disseminated melanoma. *J Clin Oncol* 1998;**16**:1103–11.
7. Franzke A, Probst-Kepper M, Buer J, et al. Elevated pretreatment serum levels of soluble vascular cell adhesion molecule 1 and lactate dehydrogenase as predictors of survival in cutaneous metastatic malignant melanoma. *Br J Cancer* 1998;**78**:40–5.
8. Proebstle TM, Fuchs T, Scheibenbogen C, Sterry W, Keilholz U. Long-term outcome of treatment with dacarbazine, cisplatin,

interferon-α, and intravenous high dose interleukin-2 in poor risk melanoma patients. *Melanoma Res* 1998;**8**:557–63.
9. Manola J, Atkins M, Ibrahim J, Kirkwood J. Prognostic factors in metastatic melanoma: a pooled analysis of Eastern Cooperative Oncology Group Trials. *J Clin Oncol* 2000;**18**:3782–93.
10. Banfalvi T, Boldizsar M, Gergye M, et al. Comparison of prognostic significance of serum 5-S-cysteinyldopa, LDH and S-100B protein in stage III–IV malignant melanoma. *Pathol Oncol Res* 2002;**8**:183–7.
11. Keilholz U, Martus P, Punt CJA, et al. Prognostic factors for survival and factors associated with long-term remission in patients with advanced melanoma receiving cytokine-based treatments: second analysis of a randomized EORTC Melanoma Group trial comparing interferon-α2a (IFNα) and interleukin-2 (IL-2) with or without cisplatin. *Eur J Cancer* 2002;**38**:1501–11.
12. Smit LHM, Korse CM, Hart AAM, et al. Normal values of serum S-100B predict prolonged survival for stage IV melanoma patients. *Eur J Cancer* 2005;**41**:386–92.
13. Tas F, Oguz H, Argon A, et al. The value of serum levels of IL-6, TNF-alpha, and erythropoietin in metastatic malignant melanoma: serum IL-6 level is a valuable prognostic factor at least as serum LDH in advanced melanoma. *Med Oncol* 2005;**22**:241–6.
14. Balch CM, Soong S-J, Atkins MB, et al. An evidence-based staging system for cutaneous melanoma. *CA Cancer J Clin* 2004;**54**:131–49.
15. Kounalakis N, Goydos JS. Tumor cell and circulating markers in melanoma: diagnosis, prognosis, and management. *Curr Oncol Rep* 2005;**7**:377–82.
16. Fantin VR, St-Pierre J, Leder P. Attenuation of LDH-A expression uncovers a link between glycolysis, mitochondrial physiology, and tumor maintenance. *Cancer Cell* 2006;**9**:425–34.
17. Koukourakis MI, Giatromanolaki A, Sivridis E, et al. Lactate dehydrogenase-5 (LDH-5) overexpression in non-small-cell lung cancer tissues is linked to tumour hypoxia, angiogenic factor production and poor prognosis. *Br J Cancer* 2003;**89**:877–85.
18. Koukourakis MI, Giatromanolaki A, Sivridis E, Gatter KC, Harris AL. Lactate dehydrogenase 5 expression in operable colorectal cancer: strong association with survival and activated vascular endothelial growth factor pathway – a report of the Tumour Angiogenesis Research Group. *J Clin Oncol* 2006;**24**:4301–8.
19. Jain RK. Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. *Science* 2005;**307**:58–62.
20. Eggermont AMM, Kirkwood JM. Re-evaluating the role of dacarbazine in metastatic melanoma: what have we learned in 30 years? *Eur J Cancer* 2004;**40**:1825–36.
21. Bedikian AY, Millward M, Pehamberger H, et al. Bcl-2 antisense (oblimersen sodium) plus dacarbazine in patients with advanced melanoma: the Oblimersen Melanoma Study Group. *J Clin Oncol* 2006;**24**:4738–45.
22. Therasse P, Arbuck SG, Eisenhauer EA, et al. New guidelines to evaluate the response to treatment in solid tumors. *J Natl Cancer Inst* 2000;**92**:205–16.
23. Keilholz U, Suciu S, Bedikian AY, et al. LDH is a prognostic factor in stage IV melanoma patients (pts) but is a predictive factor only for bcl2 antisense treatment efficacy: re-analysis of GM301 and EORTC 18951 randomized trials. *J Clin Oncol* 2007;**25**:485s [Suppl.; abstr 8552].
24. Keilholz U, Punt CJA, Gore M, et al. Dacarbazine, cisplatin, and interferon-alfa-2b with or without interleukin-2 in metastatic melanoma: a randomized Phase III trial (18951) of the European Organisation for Research and Treatment of Cancer Melanoma Group. *J Clin Oncol* 2005;**23**:6747–55.
25. Greenland S. Analysis of polytomous exposures and outcomes. In: Rothman KJ, Greenland S, editors. *Modern Epidemiology*. 2nd ed. Philadelphia: Lippincott-Raven Publishers; 1998. p. 317.
26. Pouyssegur J, Dayan F, Mazure NM. Hypoxia signalling in cancer and approaches to enforce tumour regression. *Nature* 2006;**441**:437–43.