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## **Personalised medicine: Development and external validation of a prognostic model for metastatic melanoma patients treated with ipilimumab**

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### **Abstract**

#### **Purpose**

The purpose of this study was to set up a prognostic model for the identification of survival predictors specific for melanoma patients treated with ipilimumab.

#### **Experimental design**

The following prospectively collected data were utilised: patient and primary tumour characteristics, relapse-free-interval, site and number of metastases, previous therapies and level of serum biomarkers (lactic dehydrogenase (LDH), C-reactive protein,  $\beta$ 2-microglobulin, vascular endothelial growth factor (VEGF), IL2, IL6, S-100, alkaline phosphatase (ALP), transaminases, leucocyte count, lymphocytes subpopulations). A multivariate prognostic model was developed using the Cox regression model fitted to the data of 113 consecutive metastatic patients treated with ipilimumab (3 mg/kg, q3w) at Veneto Institute of Oncology (IOV). External validation was obtained using the data of 69 and 34 patients treated at European Oncology Institute (IEO) and University of Torino (UT), respectively.

#### **Results**

Median survival was 8.3, 4.9 and 7.1 months from first ipilimumab administration at IOV, IEO and UT, respectively. Both higher baseline levels of LDH (Hazard Ratio [HR]  $v = 1.36$ , 95% Confidence Interval [CI] 1.16–1.58,  $P < .001$ ) and neutrophils (HR = 1.76, 95% CI 1.41–2.10,  $P < .001$ ) were associated with worse prognosis. Model performance was satisfactory both upon internal validation (Dxy = 0.42) and external validation (Dxy = 0.40). Serum LDH and neutrophil count discriminated patients who lived more (low neutrophils and low LDH) or less (high LDH or neutrophils) than 24 months.

#### **Conclusion**

Serum LDH and neutrophil count were significant independent prognostic factors. This externally validated prognostic nomogram, could help clinicians to identify the patients who would benefit most from ipilimumab and consequently to improve resource allocation. These easily available biomarkers deserve further validation.

### **1. Background**

Metastatic melanoma (MM) has been associated with a dismal prognosis, as underscored by the meta-analysis of Korn et al. that indicated a median survival of 6.2 months for patients with metastatic disease and a 1-year life expectancy of 25.5% [1]. Melanoma is an immunogenic tumour, its immunogenicity being possibly a consequence of the high rate of somatic mutations and expression of neoantigens [2] and [3]. Elucidation of the cellular and molecular mechanisms underlying the activating and suppressive immunological checkpoints has led to much more promising results [4] and [5]. Ipilimumab is a fully humanised monoclonal anti-Cytotoxic T-Lymphocyte Antigen 4 antibody that demonstrated a significant improvement of MM patient survival [6], with 5-year survival rates up to 16.5% and 17.0% in pre-treated and treatment-naïve patients, respectively, who were administered with ipilimumab 3 mg/kg q3w in phase II clinical trials [7] and [8]. However, treatment with ipilimumab may be associated with severe

immunological toxicity, usually according to a time pattern that presents consistent risk of delayed adverse events [9]. Moreover, even though cost-effectiveness analyses reported an acceptable profile for ipilimumab, nonetheless its cost represents a burden for the Healthcare System [10]. Given these issues, several research groups are active in analysing potential biomarkers to identify patients who are likely to benefit from treatment with ipilimumab, thus sparing toxicity and resources. A number of potential biomarkers have been investigated so far, including serum lactic dehydrogenase (LDH) [11], the absolute number of lymphocytes and C-reactive protein (CRP) [12]. The purpose of the present study was the identification of easily accessible independent prognostic factors for reliable stratification of MM patients receiving ipilimumab, in order to generate a new prognostic nomogram for the routine clinical practice.

## **2. Methods**

### **2.1. Patients and therapy**

The prospectively maintained melanoma databases of patients administered with ipilimumab 3 mg/kg w3w at Veneto Institute of Oncology (IOV) ( $N = 113$ , treated up to November 2014), at European Institute of Oncology (IEO) ( $N = 69$ , treated up to December 2013) and at University of Torino (UT) ( $N = 34$ , treated up to June 2013) were searched under Institutional Review Board approval.

The IOV database recorded the following variables: characteristics of primary melanoma, date and site of metastases at inoperable disease onset and at the time of first ipilimumab administration; type and outcome of previous therapies; baseline (within 4 weeks before first ipilimumab administration) serum levels of LDH (imputed as the ratio with the upper normal limit (UNL) LDH and transformed under square root in order to relax the linearity assumption of the Cox model), C-reactive protein (CRP), beta2-microglobulin, vascular endothelial growth factor (VEGF), interleukin 2 (IL2), interleukin 6 (IL6), S-100, alkaline phosphatase (ALP), transaminases, circulating blood leucocyte formula, lymphocytes subpopulations analysed with cytofluorometry; date of last follow up or death and cause of death. The IEO and UT databases were queried for the values of the significant prognostic factors identified in the prognostic model as well as for survival data of patients, to provide the validation cohort. Date and cause of death were collected from local registry offices and telephonic interviewing of the family or of the general practitioner for patients lost to follow up.

All patients gave informed consent to the treatments and to the use of their clinical records for scientific purposes.

### **2.2. Statistical analysis**

Disease-free interval (DFI) was defined as the time from initial MM diagnosis to first inoperable disease recurrence onset. Overall survival (OS) was defined as the time from first ipilimumab administration to the date of death or last follow-up, and was estimated with the Kaplan–Meier estimator, the log-rank test being used to compare survival estimates of different groups. We used Cox proportional hazards regression analysis on the IOV dataset to examine the association between potential prognostic variables and survival. Schoenfeld residual methodology was used to check the proportional hazard assumption of the Cox model. The Wald test with Bonferroni correction for multiple testing was used to assess the significance of each variable included in the full model, fast-backward method (with Akaike Information Criterion [AIC] as a stopping rule instead of  $P$ -values, in order to weight the probability of both significance and prediction strength) was used to select the covariates in the final model. Model performance was measured with the Receiver Operating Curve simulation of the hazard prediction estimates at 6, 12 and 24 months; shrinkage slope (after 100 bootstrap replications) was used to calibrate the overfitting of the model, and discrimination (a measure of the correlation with the hazard of death) was determined with Somer's Dxy (that is also equal to  $2 * (\text{Harrell's C-index} - 0.5)$ ). The prognostic model was then externally validated using the IEO and UT reunited datasets. A nomogram was tailored on the final regression model, the total

number of points derived by specifying values was used to calculate the expected survival probabilities at 6, 12 and 24 months. Missing values were estimated with multiple imputations using additive regression, bootstrapping and predictive matching; a correction on the estimation procedure, based on 20 multiple imputations, was performed. Patients lost to follow-up, or whose death was unrelated to metastatic melanoma progression were censored at last follow-up. Logistic regression was used to analyse the value of covariates to predict survival superior to 24 months. When analysing the impact on survival of therapies after ipilimumab, the model was adapted with landmark analysis (i.e. in which survival time was defined as the time from 12 weeks after first ipilimumab administration, at the time of first response assessment). All reported *P*-values are two-sided. Statistical analysis was performed with R 3.0.2 (survival and rms libraries, R Foundation for Statistical Computing, Vienna, Austria).

### 3. Results

#### 3.1. Patients' characteristics

Putative prognostic factors were collected from the IOV dataset. Table 1 summarises the characteristics of patients, of the disease at time of primary melanoma diagnosis and Table 2 shows the biomarker levels at the time of first ipilimumab administration.

Table 1. Characteristics of patients and melanoma.

| Variable                              | N (%)   | Median (range)   |
|---------------------------------------|---------|------------------|
| Weight (Kg)                           |         | 69 (45–135)      |
| Site of primary melanoma              |         |                  |
| Trunk                                 | 51 (45) |                  |
| Limb                                  | 38 (34) |                  |
| Extremities (Acral and Head and Neck) | 4 (4)   |                  |
| Mucosal                               | 5 (4)   |                  |
| Uveal                                 | 5 (4)   |                  |
| Unknown                               | 10 (9)  |                  |
| Ulceration                            |         |                  |
| Present                               | 21 (18) |                  |
| Absent                                | 12 (11) |                  |
| Not evaluated                         | 80 (71) |                  |
| Molecular alterations                 |         |                  |
| BRAF and NRAS wt                      | 29 (26) |                  |
| BRAF V600                             | 26 (23) |                  |
| NRAS                                  | 6 (5)   |                  |
| Not tested                            | 52 (46) |                  |
| Disease-free interval (months)        |         | 27.8 (1.1–192.0) |
| N of therapies before ipilimumab      |         | 1 (0–4)          |
| Performance status (PS)               |         |                  |
| 0                                     | 76 (67) |                  |
| 1                                     | 27 (24) |                  |
| ≥2                                    | 10 (9)  |                  |
| Number of metastatic organs           |         | 3 (1–6)          |
| Localisation of metastases            |         |                  |
| M1a or inoperable IIIC                | 16 (14) |                  |
| M1b                                   | 20 (18) |                  |
| M1c                                   | 77 (68) |                  |

Characteristics of patients and melanoma. Older cases did not report ulceration in the diagnosis of primary melanoma, and were not tested for BRAF or NRAS mutation. Most patients had M1c disease, and the median number of metastatic organs was 3, only a minority of patients had oligometastatic disease.

Table 2. Biomarkers.

| Biomarker (normal limits, unit)                        | Median (range)     |
|--|--------------------|
| Sex (M)  | 47 (42)            |
| White blood cells (4.40–11.00×10 <sup>6</sup> /L)      | 6.2 (2.3–17.5)     |
| Eosinophils (0–0.50×10 <sup>6</sup> /L)                | 0.08 (0.01–0.89)   |
| Neutrophils (1.80–7.8×10 <sup>6</sup> /L)              | 4.0 (1.1–16.2)     |
| Lymphocytes (1.10–4.80×10 <sup>6</sup> /L)             | 1.3 (0.7–2.5)      |
| CD3+ (7.0–27.0%)                                       | 71.0 (42.0–92.0)   |
| CD4+ (32–52%)  | 39.0 (17.0–73.0)   |
| CD8+ (16–33%)  | 23.0 (5.3–79.0)    |
| NK (7.0–27.0%)   | 18.0 (5.6–35.6)    |
| CD3/CD16/CD56+ (1–11%)                                 | 3.0 (1.0–13.0)     |
| Lactic dehydrogenase (LDH) (upper normal limit (UNL))  | 0.8 (0.3–6.8)      |
| C-reactive protein (CRP) (0–6mg/L)                     | 6.7 (2.9–214.0)    |
| β2-microglobulin (1.09–2.53ng/L)                       | 2.3 (1.2–7.2)      |
| IL6 (0–5.9ng/L)  | 3.5 (2.0–100.0)    |
| IL2 (0–2ng/L)  | 7 (2–28.3)         |
| S-100 (0.00–0.15μg/L)                                  | 0.6 (0.03–97.0)    |
| Vascular endothelial growth factor (VEGF) (62–707ng/L) | 431.5 (3.4–2100.0) |

Median values of biomarkers were within the normal ranges, with the exception of CD3 lymphocytes, IL2 and S-100.

Most patients ( $N = 78$ , 69%) completed the four cycles of therapy, 24 (21%) interrupted the treatment before completion because of toxicity and 11 (10%) developed symptomatic central nervous system (CNS) metastases or rapid performance status (PS) worsening that required interruption of therapy.

### 3.2. Survival and prognostic model

In the IOV cohort, 31 patients were alive after a median follow up of 11.5 months (95% Confidence Interval (CI) = 9.42–14.35). Median OS was 8.3 months, 1- and 2-year survival rates being 37.4% and 19.3%, respectively. After ipilimumab, 24 (22%) patients received at least one line of systemic treatment: among these patients, seven (6%) received BRAF inhibitors, seven (6%) were administered anti-PD1 drugs and one (1%) had surgical resection of residual disease.

In the validation cohorts, median OS was 4.9 months at IEO (95% CI 3.4-7.3; 15 patients alive after a median follow up of 14 months), the 1- and 2-year survival rates being 23.9% and 17.4%, respectively; at UT, median OS was 7.1 months (95% CI = 2.9–na; 10 patients alive after a median follow-up of 15 months), the 1- and 2-year survival rates being 41% and 26%, respectively. The survival analysis demonstrated no significant differences among the three cohorts ( $P = .486$ ). Covariates collected in the IOV cohort were tested for their relationship with survival, and Fig. 1 shows the discrimination (Somers' Dxy) performance of the variables tested in the full model (i.e. the model including all available covariates): the highest the Dxy, the strongest the relation with survival.

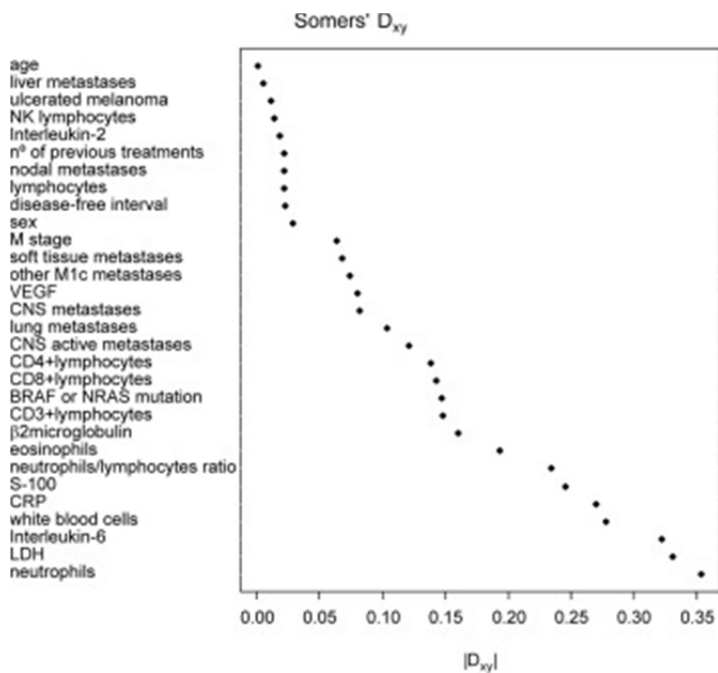


Fig. 1.

Discrimination (Somers's  $D_{xy}$ ) performance of the variables tested in the full model. The Somers's  $D_{xy}$  was calculated for all the variables in the full model. The highest the  $D_{xy}$ , the strongest the relation with the hazard of death (in this representation, the result does not render if the correlation is direct or inverse, and if it is statistically significant). Baseline levels of IL6, lactic dehydrogenase (LDH) and neutrophils had the strongest relationship with the hazard, with  $D_{xy} > 0.30$ . Metastatic sites were analysed both according to AJCC stage (i.e. inoperable III plus M1a versus M1b versus M1c) and according to the specific location of metastases, focusing on the most common sites.

Baseline levels of IL6, LDH and neutrophils had the most significant relationship with the hazard of death. These three covariates were associated with prognosis in the full model after Wald test corrected for multiple testing (respectively,  $P = .046$ ,  $P = .010$ ,  $P = .001$ ), while the presence of CNS active metastases ( $P = .081$ ) and CD8 lymphocyte count ( $P = .052$ ) showed a trend. After fast backward variable selection, only LDH and baseline neutrophils satisfied the AIC rule and were retained in the final model. In particular, higher baseline levels of LDH (HR = 1.36, 95% CI 1.16–1.58,  $P < .001$ ) and neutrophils (HR = 1.76, 95% CI 1.41–2.10,  $P < .001$ ) were associated with a worse prognosis. Fig. 2 shows the predictive effect on the death relative hazard of LDH and neutrophil level.

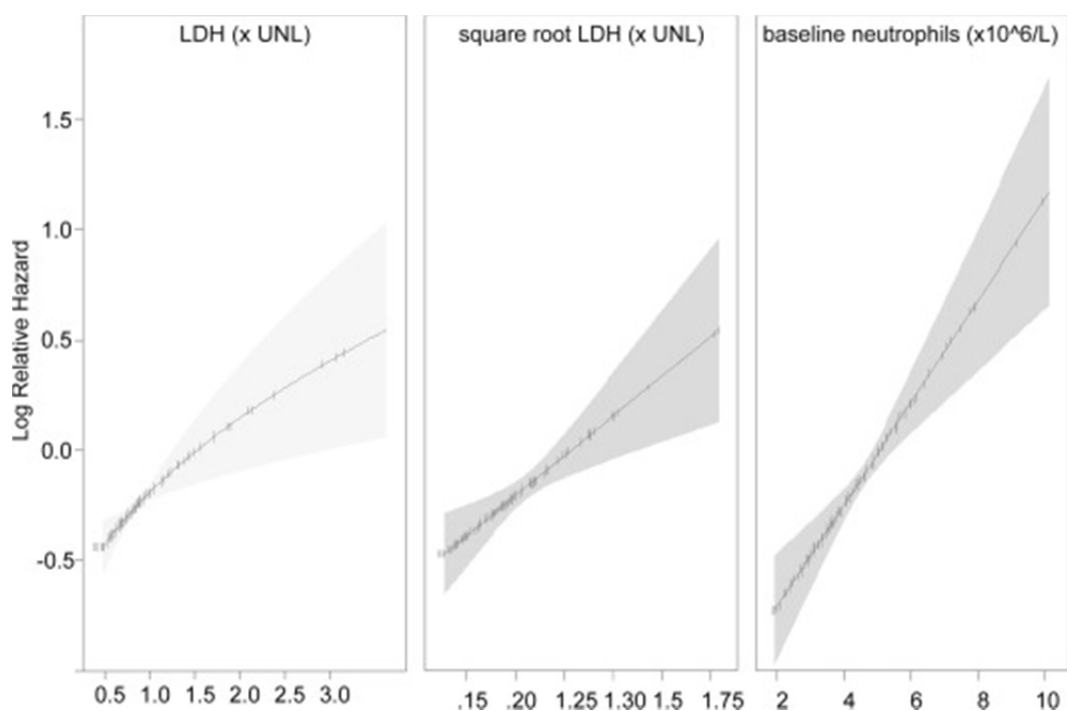


Fig. 2.

Predictive effect on the death Relative Hazard of lactic dehydrogenase (LDH) and neutrophil count. The figure shows the predictive effect on the death relative hazard of serum LDH and neutrophil count. On the y-axis the Log of relative hazard is represented; this means that a Hazard Ratio (HR) of 1 corresponds to 0, upper values correspond to HR >1 and lower values correspond to HR <1. Pointwise .95 confidence bands (shadowed area) are also shown. 'Rug plots' on curves show the density of the predictor. LDH is represented both before (left) and after (centre) square root transformation, to show the relaxation of the relation: the curve is, in fact, straight in the centre graph, as for neutrophil count (right), indicating a linear relation with the hazard.

The analysis was adapted to include the therapy regimens after ipilimumab for patients who prosecuted active treatments at disease progression, and showed a non-significant trend for survival benefit for PD1 inhibitors and, in the subgroup of patients BRAF mutated, for BRAF inhibitors (not shown).

The shrinkage factor (slope) of the prognostic model was 0.95 (range of the parameter 0–1, where 1 would be the ideally fitted model). Supplemental Fig. 1 shows the performance of survival prediction at 6, 12 and 24.

The Proportional Hazard Hypothesis was confirmed both in the full and the final models. The prognostic model was validated internally with bootstrap (200 replications) and the Dxy (the possible range of the parameter being 0–0.5, where 0.5 is the ideal model) was 0.42 (standard error [SE] .006). The Dxy of the external validation, performed using the conjoined IEO and UT datasets, was 0.40 (SE .007). A prognostic nomogram was tailored on the final prognostic model (Fig. 3); an example of its use is shown in Supplemental Fig. 2.



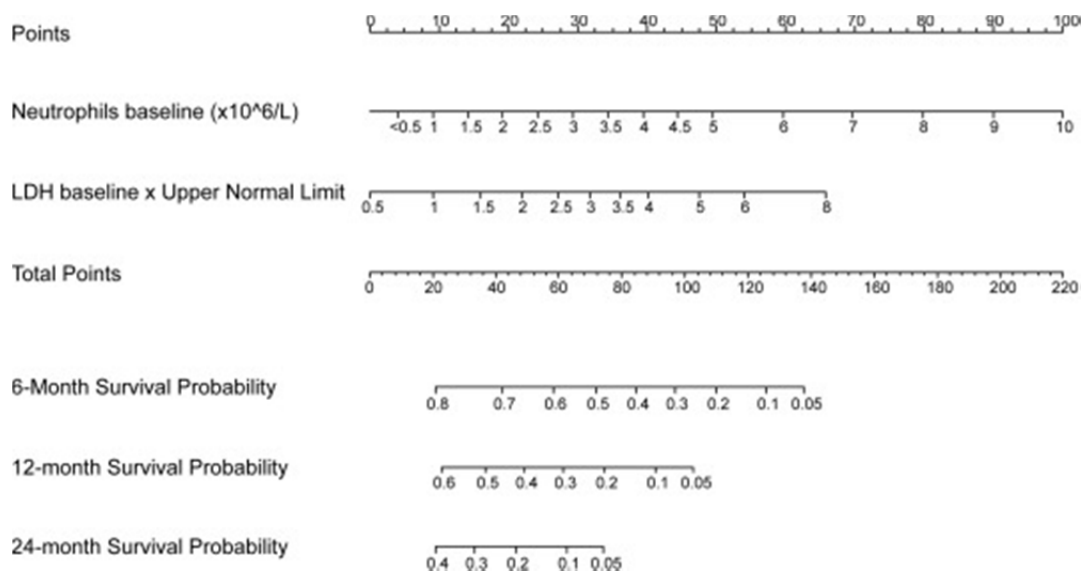


Fig. 3.

Nomogram tailored on the final model. The sum of the prognostic factor points corresponds to the survival probability at 6, 12 and 24 months.

### 3.3. predictive value of the prognostic factors

We grouped patients from the two cohorts (IOV and the validation cohort consisting of IEO plus UT), separating the patients according to LDH and neutrophil baseline levels, to assess the ability of the prognostic factors to discriminate the patients who lived longer than 24 months. In the Cox regression final model, the value of neutrophils and LDH for which the HR was 1 was, approximately,  $4.7 \times 10^6/L$  and  $1.5 \times UNL$ , respectively; therefore, we used these cut-offs to group patients. Both cut-offs rendered three groups of patients: (a) high LDH; (b) low LDH and high neutrophils; (c) low LDH and low neutrophils; survival of patients with high LDH did not diverge according to neutrophil value (data not shown). The three groups were significantly different at the survival analysis, the median OS of patients with low LDH and low neutrophils (c) being notably superior to that of the other two groups (Fig. 4 A and B, and Table 3). Additionally, we also used the previously proposed prognostic cut-off for neutrophils [13] and LDH [11] of  $7.5 \times 10^6/L$  and  $2 \times UNL$ , respectively, to gather the patients of IOV and validation cohort according to (a) high LDH; (b) low LDH and high neutrophils; (c) low LDH and low neutrophils. Again, at the survival analysis the three groups were significantly different both for IOV and the validation cohort patients (Fig. 4C and D, and Table 3); moreover, these cut offs were more specific in discriminating long survivors. In particular, median OS of patients with baseline LDH superior to  $2 \times UNL$  (a) or more than  $7.5 \times 10^6/L$  neutrophils (b) was far below 6 months, with zero patients alive at 24 months. Neutrophil baseline levels were associated to an Odds Ratio (OR) for death before 24 months of 3.9 (95% CI 1.7–9.4,  $P = .001$ ) and LDH had an OR of 6.4 (95% CI = 1.7–28.2,  $P = .014$ ).

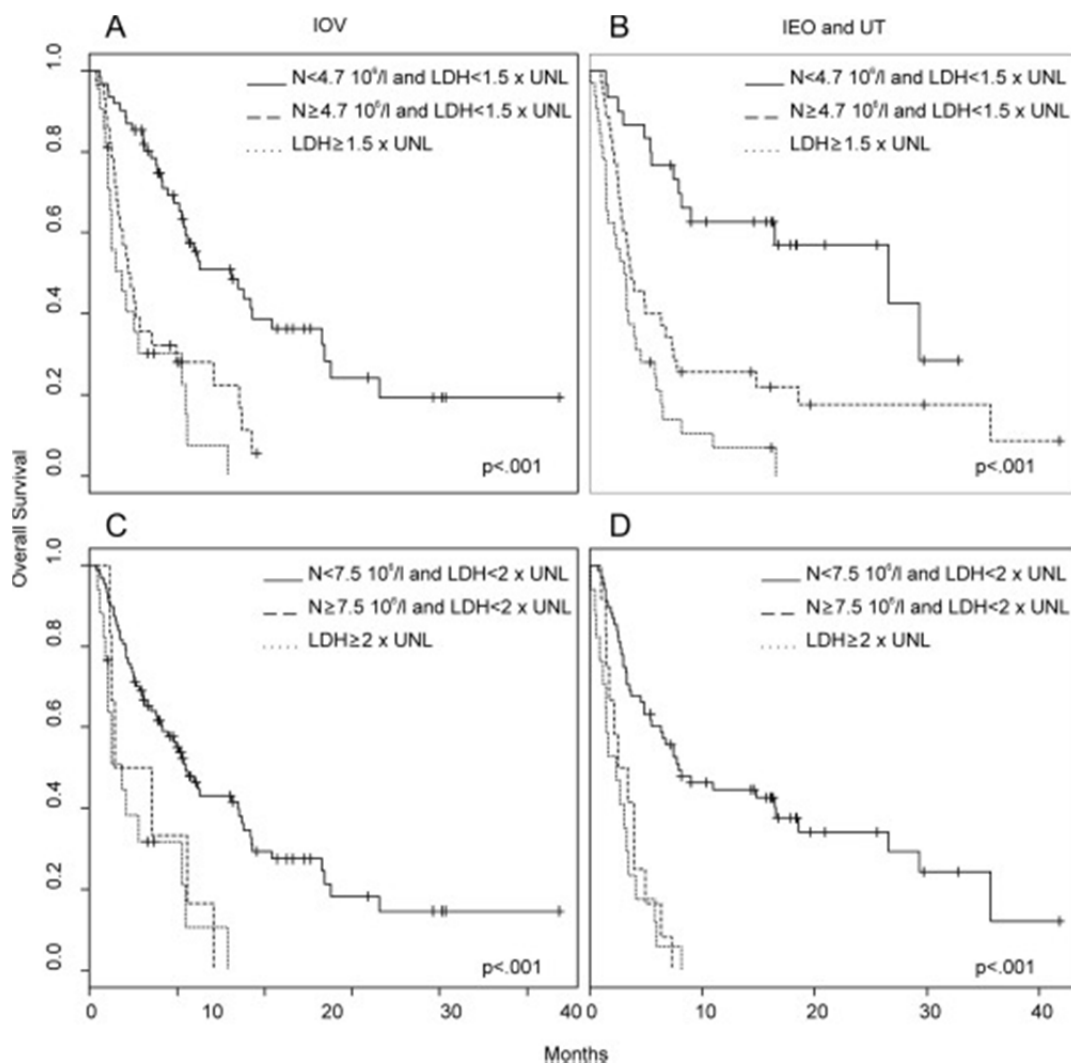


Fig. 4.

Survival curves. Survival curves in patients treated with ipilimumab at Veneto Institute of Oncology (IOV) (left) and in the validation cohort (right, European Oncology Institute (IEO) and University of Torino (UT) patients), according to cut off of lactic dehydrogenase (LDH) and neutrophils of  $1.5 \times$  upper normal limit (UNL) and  $4.7 \times 10^6/L$  (upper) and of  $2 \times$  UNL and  $7.5 \times 10^6/L$  (down).

Table 3. Survival according to lactic dehydrogenase (LDH) and neutrophils.

| Cohort/prognostic group  |                             | N<br>(deaths) | Median<br>survival<br>(months) | 95%<br>confidence<br>interval (CI) | 6 m<br>survival<br>(%) | 12 m<br>survival<br>(%) | 24 m<br>survival<br>(%) | P     |
|--|-----------------------------|---------------|--------------------------------|------------------------------------|------------------------|-------------------------|-------------------------|-------|
| Cut-off: LDH $1.5 \times$ upper normal limit (UNL) and neutrophils $4.7 \times 10^6/L$ |                             |               |                                |                                    |                        |                         |                         |       |
| Veneto Institute of<br>Oncology (IOV)  | Low LDH low<br>neutrophils  | 61 (34)       | 16.4                           | 10.7–26.8                          | 85.2                   | 57.4                    | 36.4                    | <.001 |
|  | Low LDH high<br>neutrophils | 28 (24)       | 4.5                            | 3.3–14.2                           | 35.7                   | 28.1                    | 0                       |       |
|  | High LDH                    | 21 (16)       | 3.7                            | 2.4–11.1                           | 22.7                   | 7.6                     | 0                       |       |
| European Oncology<br>Institute (IEO) and<br>University of Torino<br>(UT)               | Low LDH low<br>neutrophils  | 30 (14)       | 26.6                           | 8.9–na                             | 73.2                   | 57.0                    | 8                       | <.001 |
|  | Low LDH high<br>neutrophils | 35 (29)       | 3.7                            | 2.8–7.5                            | 37.1                   | 22.0                    | 8                       |       |
|  | High LDH                    | 32 (30)       | 3.3                            | 2.3–5.8                            | 25.8                   | 8.6                     | 0                       |       |
| Cut-off: LDH $2 \times$ UNL and neutrophils $7.5 \times 10^6/L$                        |                             |               |                                |                                    |                        |                         |                         |       |
| IOV  | Low LDH low<br>neutrophils  | 87 (56)       | 10.9                           | 8.3–17.4                           | 68.9                   | 48.0                    | 27.6                    | <.001 |

| Cohort/prognostic group | N (deaths)               | Median survival (months) | 95% confidence interval (CI) | 6 m survival (%) | 12 m survival (%) | 24 m survival (%) | P    |       |
|-------------------------|--------------------------|--------------------------|------------------------------|------------------|-------------------|-------------------|------|-------|
| IEO and UT              | Low LDH high neutrophils | 6 (6)                    | 5.1                          | 2.5–na           | 50.0              | 16.7              | 0    | <.001 |
|                         | High LDH                 | 17 (12)                  | 3.7                          | 2.1–na           | 31.9              | 10.6              | 0    |       |
|                         | Low LDH low neutrophils  | 68 (44)                  | 7.9                          | 5.5–26.6         | 58.7              | 42.7              | 29.4 |       |
|                         | Low LDH high neutrophils | 12 (12)                  | 2.9                          | 1.7–na           | 8.3               | 0                 | 0    |       |
|                         | High LDH                 | 17 (17)                  | 2.9                          | 1.6–5.8          | 0                 | 0                 | 0    |       |
|                         |                          |                          |                              |                  |                   |                   |      |       |

#### 4. Discussion

With the advent of targeted therapies, oncologists eagerly need a reliable tool to personalise treatments in order to reduce the economic burden for the National Health systems as well as exposition to toxicity for patients who are unlikely to benefit from therapies. The aim of our study was to identify, in a real-world setting, prognostic factors that could be easily introduced in routine clinical practice, to screen the patients who are candidates to ipilimumab, an anti-CTLA4 monoclonal antibody that revolutionised the management of metastatic melanoma.

LDH levels and neutrophil count were independent prognostic factors, regardless of melanoma origin and other clinical characteristics and biomarkers: the higher their values, the worse patient prognosis. The nomogram we developed based on the findings of the prognostic model was externally validated, with satisfactory calibration and discrimination comparable to previously published models for rare tumours [14], and we believe this nomogram could be very useful if implemented in the routine clinical practice. Interestingly, LDH and neutrophils also resulted predictive factors of significant benefit from treatment with ipilimumab, i.e. a survival longer than two years. Objective response was not considered an end-point, because of the peculiar pattern of responses of ipilimumab: in fact, tumour response rates (TRR) are relatively low and it may take months for tumour shrinkage to occur, even after an initial disease progression [15]. This is why the most important results obtained with ipilimumab are usually measured in terms of OS and not in terms of TRR [6]. Our findings are consistent with previous works, concluding that neutrophils and LDH are independent prognostic factors for melanoma, in particular when candidate to immunotherapy [11], [13] and [16]. Moreover, neutrophils were found to be an independent poor prognostic factor in metastatic renal cell carcinoma as well [17] and [18].

The relationship between neutrophils and prognosis of melanoma is not fully known. One possible explanation is that the tumour microenvironment, which is believed to play an essential role in determining the response to immunotherapy [19] and [20], could be influenced by neutrophils themselves, for example by producing tumour-stimulating or immunosuppressive cytokines [21], [22] and [23], or coadjuvating tumour invasion with the release of metalloproteinases [24] and [25]. An alternative hypothesis is that the circulating neutrophil count is the consequence of a cytokine stimulus conditioned by melanoma [26]: this way the neutrophils could be the expression of an immunosuppressive environment induced by the tumour itself. The present findings should prompt researchers to focus on the potential explanations of the importance of this class of white blood cells, because they may be linked to the mechanisms of refractoriness to immunotherapy.

The confirmation of the independent prognostic value of LDH is consistent with the well established importance of this marker for melanoma [11] and [27], at the point that it is included in the AJCC TNM classification for stage IV melanoma [28]. Although the importance of both neutrophils and LDH in melanoma patient prognosis was previously known, this is the first time that these parameters have been evaluated together with a large series of clinical and biochemical markers; moreover, they were compared

both for their prognostic and predictive implication, and resulted equally significant to be considered and helpful to stratify patient prognosis.

In our study, only LDH and neutrophils were retained in the final model, but interleukin-6 showed a trend of association with survival and maybe implementing the number of observations this factor reaches statistical significance; anyway, a planned extended analysis should give more insight into the prognostic value of an inflammatory marker as interleukin-6.

The present study includes data from patients mainly treated before the approval of BRAF inhibitors and clinical implementation of PD1 inhibitors, in fact, patients treated with these drugs at disease progression after ipilimumab are a minority. Patients who progressed after ipilimumab had limited options, or their conditions allowed no active therapy. This reflects the oncologic treatment repertoire before the era of BRAF and PD1 inhibitors and enabled us to assess the value of prognostic factors without the confounding effect represented by post-ipilimumab therapies which are expected to impact on survival [29], [30], [31], [32] and [33]. BRAF status was unknown in a number of cases, because at the time no targeted therapies were available and as consequence the costs of the test were not justified; anyway, BRAF mutations have no influence on response to ipilimumab [8]. In the near future, due to the ever growing diffusion of other target therapies, the conduction of a study on prognostic factors in patients treated with ipilimumab could be unfeasible because of the interference of subsequent treatments. In our opinion, the relevance of neutrophils and LDH as prognostic factors is worthy to be studied with the future therapeutic options. Interested in the possible predictive value of neutrophils and LDH, we looked for a cut-off that could discriminate the patients who did not survive more than 24 months. The values previously published were more specific than the cut-offs obtained from our model, even if less sensible (not shown), and for this reason we would consider them more useful to discriminate the poor-prognosis group. Additional studies are needed to clarify the best therapeutic approach for patients with high LDH or high neutrophils because the benefit of ipilimumab treatment for patients with LDH superior to  $2 \times \text{UNL}$  and neutrophils superior to  $7.5 \times 10^6/\text{L}$  appears, notwithstanding the difficulty to effectively quantify it in absence of a control group, unimpressive, so raising the question whether the patients with high LDH and neutrophils are refractory to ipilimumab, or are affected by a more aggressive variant of the disease. In these subgroups of patients with worse prognosis, one possibility would be to test if newer therapies such as anti-PD1, combined anti-PD1 and anti-CTLA4, or anti-BRAF/anti-MEK inhibitors, could be more effective than ipilimumab alone. Moreover, although our results may give some indications regarding a subset of patients with adverse prognosis, confounding factors are numerous and we encourage the research of efficient, reliable and possibly usable clinical practice biomarkers applicable to metastatic melanoma patients candidate to immunotherapies.

In conclusion, clinicians could evaluate neutrophils and LDH to select patients before treatment initiation. Ipilimumab may not be the best treatment in patients with higher neutrophil count and LDH, in particular when superior to  $7.5 \times 10^6/\text{L}$  and  $\times 2 \text{ UNL}$ , respectively.

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