EBioMedicine xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.ebiomedicine.com

Research Paper

Neutrophil to Lymphocyte Ratio is Associated With Outcome During Ipilimumab Treatment☆

Michael R. Cassidy [a,*], Rachel E. Wolchok [a], Junting Zheng [b], Katherine S. Panageas [b], Jedd D. Wolchok [c,d], Daniel Coit [a,d], Michael A. Postow [c,d], Charlotte Ariyan [a,d]

[a] Department of Surgery, Memorial Sloan Kettering Cancer Center, New York, NY, United States
[b] Department of Epidemiology and Biostatistics, Memorial Sloan Kettering Cancer Center, New York, NY, United States
[c] Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, United States
[d] Weill Cornell Medical College, New York, NY, United States

ARTICLE INFO

Article history:
Received 3 March 2017
Received in revised form 20 March 2017
Accepted 20 March 2017
Available online xxxx

Keywords:
Neutrophil to lymphocyte ratio
NLR
Melanoma
Immunotherapy
BRAF inhibitors

ABSTRACT

Background: Ipilimumab (IPI) and BRAF inhibitors (BRAFi) improve survival in melanoma, but not all patients will benefit and toxicity can be significant. Pretreatment neutrophil to lymphocyte ratio (NLR) has been associated with outcome in IPI-treated patients, but has not been studied during treatment or in BRAFi-treated patients.
Methods: Using a prospectively maintained database, patients with unresectable stage III or IV melanoma treated with IPI or a BRAFi (vemurafenib or dabrafenib as monotherapy) from 2006 to 2011 were identified. NLR was calculated before treatment and at 3-week intervals after treatment initiation until 9 weeks. Baseline NLR was tested for association with overall survival (OS), progression free survival (PFS), and clinical response to treatment. On-treatment NLRs were tested for association with the same outcomes using landmark survival analyses and time-dependent Cox regression models. The association of relative change of NLR from baseline with outcomes was also examined. A multivariate model tested the association of NLR and OS/PFS with additional clinical factors.
Results: There were 197 IPI patients and 65 BRAFi patients. In multivariable analysis adjusting for M stage, and disease type (in OS)/gender (in PFS), an NLR value of 5 or above at every timepoint was associated with worse OS (HR 2.03–3.37, $p < 0.001$), PFS (HR 1.81–2.51, $p < 0.001$), and response to therapy (OR 3.92–9.18, $p < 0.007$), in the IPI cohort. In addition, a >30% increase in NLR above baseline at any timepoint was associated with a worse OS and PFS (HR 1.81 and 1.66, $p < 0.004$). In BRAFi patients, NLR was not consistently associated with outcomes.
Conclusions: A high NLR, whether measured prior to or during treatment with IPI, is associated with worse OS, PFS, and clinical response in patients with advanced melanoma. An increasing NLR from baseline during treatment was correlated with worse OS and PFS in IPI-treated patients. In comparison, as NLR was not associated with outcomes in BRAFi patients, NLR may have a uniquely predictive value in patients treated with immunotherapy.

1. Introduction

Although advanced melanoma still remains a challenging diagnosis, significant enthusiasm has been generated by new therapeutic agents that have demonstrated increases in survival. Ipilimumab (IPI) is a monoclonal antibody that inhibits cytotoxic T-lymphocyte associated antigen 4 (CTLA-4), thereby acting as a "checkpoint inhibitor" to enhance native immune function. In randomized trials, IPI produced objective responses in 11% of patients, but some degree of disease control in 29% (Hodi et al., 2010). Moreover, there is a pattern of delayed but often durable response, and long-term survival is possible in patients who respond to treatment (Prieto et al., 2012). In fact, some groups treated with IPI may have 4 year survival rates up to 49.5% (Wolchok et al., 2013a). Despite promising outcomes, immune-related adverse events have been described in a significant proportion of patients who receive the agent. These include diarrhea (30%), colitis (7%), hepatitis (3%), and hypophysitis (2%). In a randomized trial, 2.1% of enrolled patients died as a direct result of treatment (Hodi et al., 2010). The therapeutic effect of ipilimumab led to rapid investigation of other checkpoint blocking agents and antibodies blocking the PD-1 pathway have demonstrated 40–45% response rates with 35% > 3 year survival and reduced toxicity, compared with ipilimumab (Hamid et

☆ This study was supported in part by NIH/NCI P30 CA008748 (Cancer Center Support Grant), with no role in the writing or publication of the manuscript.
* Corresponding author at: Department of Surgery, Memorial Sloan Kettering Cancer Center, 1275 York Avenue, C-1272, New York, NY 10065, United States.
E-mail address: cassidym@mskcc.org (M.R. Cassidy).

http://dx.doi.org/10.1016/j.ebiom.2017.03.029

al., 2013; Robert et al., 2015a; Hodi et al., 2016). Further, combined checkpoint blockade with ipilimumab + nivolumab results in response rates up to 60% in melanoma, albeit with higher rates of toxicity (Wolchok et al., 2013b; Postow et al., 2015).

Targeted agents are also important in the treatment of advanced melanoma. BRAF inhibitors (BRAFi), including the agents vemurafenib and dabrafenib, are beneficial in the population of melanoma patients whose tumors harbor a BRAF mutation. In a prospective randomized trial, the response rate to vemurafenib approached 50% and improved overall and progression free survival when compared to dacarbazine, although median progression free survival was only 6 months even with vemurafenib (Chapman et al., 2011). Combined inhibition of BRAF and MEK improves the objective response rate to 64% and improves 12 month overall survival from 65% to 72% when compared with single-agent BRAFI (Robert et al., 2015b; McArthur et al., 2014). Combined BRAF and MEK inhibition is also associated with less dermatologic toxicity (Robert et al., 2015b; Larkin et al., 2014).

Therefore, immunotherapy and targeted agents provide important therapeutic options with distinct mechanisms in advanced melanoma, each capable of improving survival. However, not all patients derive benefit, and the personal and financial cost can be significant. In this setting, establishing biomarkers capable of predicting response to these agents would provide an opportunity to identify patients most likely or unlikely to benefit, while allowing a more refined calculation of the risk/benefit ratio.

There is a complex interaction between tumors and the innate and adaptive immune responses. The immune system may work to eradicate tumors, as is the theoretical basis of immunotherapy. Yet inflammation may also be associated with disease progression and adverse outcomes, thought to be due to the inflammatory production of bioactive molecules in the tumor microenvironment (Hanahan and Weinberg, 2011). Systemic inflammation leads to neutrophilia and lymphocytopenia, and can be quantified by the neutrophil to lymphocyte ratio (NLR). Elevated NLR is associated with inferior disease-specific survival in a number of malignancies including gastric cancer (Wang et al., 2016), pancreatic cancer (Stotz et al., 2013), hepatocellular carcinoma (Mano et al., 2013), colorectal cancer (Malietzis et al., 2014), renal cell carcinoma (de Martino et al., 2013), non-small cell lung cancer (Pinato et al., 2014), gastrointestinal stromal tumor (Perez et al., 2013), ovarian cancer (Williams et al., 2014), multiple myeloma (Kelkitli et al., 2014; Templeton et al., 2014).

There is evidence that pretreatment NLR may be associated with outcome in patients treated with IPI (Ferrucci et al., 2015; Zaragoza et al., 2016). In particular, prior studies have found that a baseline NLR < 5 is associated with improved survival in patients treated with IPI (Ferrucci et al., 2015). However, the predictive value of change in NLR over time during treatment has not yet been investigated. We sought to determine if change in NLR could be associated with treatment outcome. Additionally, the role of NLR has not been established in patients treated with BRAFi. Given the differences in mechanisms between immunotherapy and BRAFi, we sought to determine the value of NLR in these different therapeutic subgroups.

2. Methods

We performed a retrospective review of a prospectively maintained database to identify all patients with unresectable stage III and IV melanoma treated with IPI or a BRAFi as monotherapy between 2006 and 2011 at Memorial Sloan Kettering Cancer Center (MSKCC). Patients without pretreatment complete blood count (CBC) values were excluded. Clinicopathologic features, including age, gender, performance status, melanoma type, AJCC stage, lines of prior therapy, and follow-up status were documented. We recorded absolute neutrophil and lymphocyte values obtained before the initiation of treatment with either IPI or a BRAFi, and at three week intervals after treatment initiation until 9 weeks. Timepoints were the same for both cohorts. The MSKCC institutional review board approved this study.

IPI was administered intravenously at doses of either 3 mg/kg or 10 mg/kg every 3 weeks for up to 4 doses during the induction phase. Clinical assessments were conducted and patients with an objective response or stable disease were eligible to receive maintenance therapy at their assigned dose every 3 months. In the BRAFi group, patients received either vemurafenib (960 mg orally twice daily) or dabrafenib (150 mg orally twice daily).

Overall survival (OS) was calculated from date of treatment initiation to the date of death from any cause. Patients who were still alive were censored at the last follow-up. Progression-free survival (PFS) was calculated from the date of treatment initiation until progression, as documented by imaging, according to response evaluation criteria in solid tumors (RECIST) or clinical examination or death. Those alive and without progression were censored at last follow-up. Response to therapy was assessed at 12 weeks after treatment initiation by the treating physician and was classified as complete response (CR), partial response (PR), stable disease (SD), or no response (NR). For the purpose of this study, we considered clinical benefit to encompass CR, PR, and SD. NLR at each timepoint (pretreatment baseline, 3 weeks, 6 weeks, and 9 weeks after treatment initiation) was tested for association with OS, PFS, and clinical benefit. Landmark survival analyses with Cox regression models were fit for on-treatment timepoints. NLR values were stratified into ≥5 or <5 for consistency with previously published reports (Stotz et al., 2013; Pinato et al., 2014; Ferrucci et al., 2015; Cananzi et al., 2014). Additionally, we tested for associations with the same outcomes using the median NLR value at each timepoint as the threshold. The association of relative change of NLR over time from baseline with OS and PFS was also determined. Over the treatment course, we marked the first increase of NLR from baseline with magnitudes between 10% and 100%, and assessed its associations with OS/PFS using time-dependent Cox regression. Multivariate models tested the association of NLR and OS/PFS in the context of other known clinically relevant prognostic factors including M stage, and disease type (in OS) or gender (in PFS). Multivariable models assessing associations between NLR change from baseline and OS/PFS controlled for the same sets of clinical factors plus baseline NLR ≥ 5. All analyses were carried out using SAS (version 9.2, SAS Institute Inc., Cary, NC) and R version 3.1 (The R Foundation for Statistical Computing).

3. Results

The study flow diagram is shown in Fig. 1. Baseline characteristics of patients included in the final analysis are shown in Table 1. There were 197 patients treated with IPI and 65 treated with a BRAFi who had pretreatment CBC available. Median time from baseline CBC to first dose of treatment was 0 days, with a range of 0–28 days. Median follow-up was 54.3 months in the IPI group (range 5–109) and 53 months in the BRAFi group (range 4–56).

In the IPI group, median baseline NLR was 4.4 (range 0.9–87.5) and decreased to 3.2 (range 1–26.8) by week 9 (Table 1). In contrast, NLR remained similar over the course of treatment in the BRAFi group, with a median at baseline and at week 9 of 4.9 (range 1.3–31.9) and 4.6 (range 1.1–26.8), respectively.

Univariate associations of NLR with OS and PFS in the IPI group are shown in Table 2. NLR ≥ 5 at all time points, in addition to stage IV M1C disease, mucosal melanoma and Karnofsky performance status score < 90 were associated with significantly worse OS and PFS. In multivariate analysis, NLR ≥ 5 at every timepoint was associated with poor OS (HR 2.03–3.37) and PFS (HR 1.81–2.51) (Table 2). Stage IV M1C disease and mucosal melanoma were also associated with worse outcomes. The associations of NLR with outcome remained significant if the median NLR at each timepoint was used as the cutoff to stratify patients.

Please cite this article as: Cassidy, M.R., et al., Neutrophil to Lymphocyte Ratio is Associated With Outcome During Ipilimumab Treatment, EBioMedicine (2017), http://dx.doi.org/10.1016/j.ebiom.2017.03.029