Inflammatory Stress Determines the Need for Chemotherapy in Patients with HER2-Positive Esophagogastric Adenocarcinoma Receiving Targeted Therapy and Immunotherapy

Joseph Tintelnot[1], Lisa Paschold[2], Eray Goekkurt[1,3], Christoph Schultheiss[4,5], Urte Matschl[6], Mariana Santos Cruz[1], Marcus Bauer[7], Claudia Wickenhauser[7], Peter Thuss-Patience[8], Sylvie Lorenzen[9], Thomas J. Ettrich[10], Jorge Riera-Knorrenschild[11], Lutz Jacobasch[12], Albrecht Kretzschmar[13], Stefan Kubicka[14], Salah-Eddin Al-Batran[15], Anke Reinacher-Schick[16], Daniel Pink[17,18], Carsten Bokemeyer[1], Marianne Sinn[1], Udo Lindig[19], Axel Hinke[20], Susanna Hegewisch-Becker[3], Alexander Stein[1], and Mascha Binder[4,5]

ABSTRACT

Anti–PD-1, trastuzumab, and chemotherapy are used in the treatment of patients with advanced HER2-positive esophagogastric adenocarcinoma, but long-term survival remains limited. In this study, we report extended follow-up data from the INTEGA trial (NCT03409848), which investigated the efficacy of the anti–PD-1 nivolumab, trastuzumab, and FOLFOX chemotherapy (FOLFOX arm) in comparison with a chemotherapy-free regimen involving nivolumab, trastuzumab, and the anti–CTLA-4 ipilimumab (Ipi arm) in the first-line setting for advanced disease. The 12-month overall survival (OS) showed no statistical difference between the arms, with 57% OS (95% confidence interval, 41%–71%) in the Ipi arm and 70% OS (95% confidence interval, 54%–82%) in the FOLFOX arm. Crossing of the survival curves indicated a potential long-term benefit for some patients within the Ipi arm, but early progressors in the Ipi arm underlined the need for biomarker-guided strategies to optimize treatment selection. To this end, metabolomic and cytokine analyses demonstrated elevated levels of normetanephrine, cortisol, and IL6 in immunotherapy-unresponsive patients in the Ipi arm, suggesting a role for systemic inflammatory stress in modulating antitumor immune responses. Patients with this signature also showed an increased neutrophil to lymphocyte ratio that persisted in the Ipi arm, but not in the FOLFOX arm, and strongly correlated with survival. Furthermore, a low neutrophil to lymphocyte ratio characterized patients benefiting from immunotherapy and targeted therapy without the need for additional chemotherapy. These data suggest that patient selection based on inflammatory stress–driven immune changes could help customize first-line treatment in patients with advanced HER2-positive esophagogastric adenocarcinoma to potentially improve long-term survival.

Introduction

On a global scale, there are approximately 1.7 million newly diagnosed cases of gastric and esophageal cancers annually. These cancers remain a substantial contributor to cancer-related deaths, accounting for more than 1.3 million fatalities each year (1). Approximately 75% of patients with esophagogastric adenocarcinoma experience recurrent or metastatic disease at some stage, underscoring the challenging nature of these malignancies. Palliative systemic therapy remains the primary approach for treating these patients.

For HER2-positive esophagogastric adenocarcinoma, identified through IHC with a score of 3+ or a score of 2+ and detection of amplification via ISH, the established standard of care entails combining the HER2-specific antibody trastuzumab with chemotherapy (2, 3). Moreover, for patients exhibiting a PD-L1 combined positive score (CPS) of ≥1, both the European Medicines Agency

[1]II. Medical Clinic and Polyclinic, University Cancer Center Hamburg (UCCH), University Medical Center Hamburg-Eppendorf, Hamburg, Germany. [2]Department of Internal Medicine IV - Oncology/Hematology, University Hospital, Martin-Luther University, Halle, Germany. [3]Hematology-Oncology Practice Eppendorf (HOPE), Hamburg, Germany. [4]Division of Medical Oncology, University Hospital Basel, Basel, Switzerland. [5]Laboratory of Translational Immuno-Oncology, Department of Biomedicine, University and University Hospital Basel, Basel, Switzerland. [6]Department of Virus Immunology, Leibniz Institute for Experimental Virology, Hamburg, Germany. [7]Institute of Pathology, University Hospital, Martin-Luther University, Halle, Germany. [8]Vivantes Hospital Friedrichshain, Berlin, Germany. [9]Rechts der Isar Hospital, Technical University of Munich, Munich, Germany. [10]University Hospital Ulm, Ulm, Germany. [11]University Hospital, Philipps-Universität Marburg, Marburg, Germany. [12]Practice for Hematology – Oncology, Dresden, Germany. [13]MVZ-Mitte, Leipzig, Germany. [14]Kreisklinik Reutlingen, Reutlingen, Germany. [15]Institute of Clinical Cancer Research, Krankenhaus Nordwest, Frankfurt, Germany. [16]St. Josef-Hospital Bochum, University of Bochum, Bochum, Germany. [17]Klinik und Poliklinik für Innere Medizin C, University Greifswald, Greifswald, Germany. [18]Klinik für Hämatologie, Onkologie und Palliativmedizin, Sarkomzentrum Berlin-Brandenburg, HELIOS Klinikum Bad-Saarow, Bad Saarow, Germany. [19]University Hospital, Jena, Germany. [20]CCRC, Düsseldorf, Germany.

A. Stein and M. Binder contributed equally to this article.

Corresponding Author: Joseph Tintelnot, University Medical Center Hamburg-Eppendorf, Martinistraße 52, Hamburg 20251, Germany. E-mail: j.tintelnot@uke.de

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(EMA) and FDA have authorized the incorporation of the PD-1 inhibitor pembrolizumab into this treatment regimen, based on data derived from the KEYNOTE-811 trial (4, 5). In instances of HER2-negative disease, the combination of anti–CTLA-4 and anti–PD-1 has shown prolonged survival in only a subset of patients compared with conventional chemotherapy (6). Whether also in the case of HER2-positive esophagogastric adenocarcinoma a specific subsets of patients might derive distinct benefits from the combination of anti–CTLA-4, anti–PD-1, and trastuzumab remains to be elucidated.

In the INTEGA trial (NCT03409848), we performed a head-to-head comparison of trastuzumab and the anti–PD-1 nivolumab in combination with either FOLFOX or the anti–CTLA-4 ipilimumab in patients with HER2-positive esophagogastric adenocarcinoma. Our previous publication on the initial data from this trial confirmed the strong efficacy of the treatment with 5-fluorouracil, platinum, trastuzumab, and anti–PD-1, as proven by the KEYNOTE-811 trial, compared with historical controls (7). Additionally, although the median survival in the Ipi arm was comparable with historical controls, experimental biomarker analysis indicated an overall survival (OS) benefit for a subgroup of patients (8). Herein, we present an extended follow-up of 18.8 months, revealing that both treatment arms demonstrated strong performance. Although the Ipi arm showed a higher number of long-term survivors, some patients experienced a risk of early progression. Additionally, we report cytokine and chemokine screening in responder and non-responder patients from the Ipi arm and show that a lack of systemic inflammatory stress seems to be a strong predictor of benefit from chemotherapy-free treatment. These data inspire new concepts, such as patient selection based on systemic inflammation for chemotherapy–immunotherapy combinations or immunotherapy alone in the first-line setting.

Materials and Methods

Patient enrollment on the INTEGA trial

Eligible patients were 18 years of age or older with pathologically confirmed HER2-positive (local IHC 3+ or IHC 2+ and ISH positive), inoperable, locally advanced, or metastatic esophagogastric adenocarcinoma previously untreated for metastatic disease with either measurable or nonmeasurable disease according to RECIST v1.1. Prior neoadjuvant and/or adjuvant treatment was permitted if completed at least 3 months prior to randomization. Further inclusion criteria were Eastern Cooperative Oncology Group performance status 0 to 2 and adequate hematologic, hepatic, and kidney function. Exclusion criteria included reduced cardiac ejection fraction (<55%), other cancers within the past 5 years, substantial autoimmune disease or conditions requiring corticosteroids (>10 mg daily prednisone equivalent), and known peripheral neuropathy [defined as greater than grade 1 per NCI Common Terminology Criteria for Adverse Events (version 4.03)]. Patients were randomly assigned to receive trastuzumab and nivolumab in combination with either FOLFOX (FOLFOX arm) or ipilimumab (Ipi arm) as described before (7). The study was conducted following the principles of good clinical practice, all relevant regulatory requirements, and the Declaration of Helsinki. The protocol received approval from the Ethics Commission Hamburg (Ethikkommission der Ärztekammer Hamburg). All patients provided written informed consent before enrollment and before undergoing any study-specific procedures [ClinicalTrials.gov identifier: NCT03409848].

Material collection

Blood samples were collected between March 2018 and May 2020 using cell-free DNA BCT tubes (Streck) and processed at selected sites. This processing led to the isolation of a leukocyte pellet, which was subsequently preserved by freezing in 1 mL of heat-inactivated FBS (Life Technologies) with 10% DMSO (Sigma), and serum that was frozen in a naïve state. Serum samples from 16 patients in the Ipi arm and 14 patients in the FOLFOX arm were available for this exploratory analysis. Samples were stored at −80°C until processing. HER2 as well as PD-L1 CPS, tumor proportion score (TPS), and immune cells were centrally tested from available formalin-fixed, paraffin-embedded tissue as described elsewhere (8). Leukocyte counts and other laboratory parameters were assessed using routine laboratory procedures at each trial site. Quality-of-life assessment (European Organisation for Research and Treatment of Cancer Core Quality of Life Questionnaire QLQ-C30) was conducted every 8 weeks at each individual trial site. For emotional distress, baseline QLQ-C30 scores for questions Q21 to 24 were considered.

Tumor burden

Tumor burden was assessed by local radiologists using mostly CT scans. Target lesions were defined according to RECIST v1.1. The sum of target lesions was calculated by adding all defined target lesions at a given time point, such as before the start of therapy, as indicated in **Table 1**.

Classifying patients as responders or nonresponders based on OS

OS was defined as the primary endpoint of this trial, making it the key measure of response to the therapy. Patients were classified as responders (R) or nonresponders (NR) based on the median OS. Those with survival times longer than the median were considered R, whereas those with shorter survival times were considered NR. The median survival was 21.8 months in the FOLFOX arm and 23.2 months in the Ipi arm.

Metabolomic screening

An aliquot of 10 μL from each serum sample was diluted with either 90 μL of hydrophilic interaction chromatography dilution buffer (5% hydrophilic interaction chromatography mobile phase A/95% acetonitrile) or 90 μL of reverse-phase dilution buffer (water with 0.1% formic acid; ref. 9). LC/MS-MS was performed on an Agilent 1290 Infinity II UHPLC system coupled to an AB Sciex QTRAP 5500 triple quadrupole linear ion trap tandem mass spectrometer according to the method and analysis by McMillen and colleagues (9). In brief, the separation was performed with a flow rate ranging from 200 to 400 μL per minute. The ionspray voltages were set to 5,500 V for positive ionization and −4,500 V for negative ionization. The source temperature was maintained at 450°C. The curtain gas, ion source gas 1, ion source gas 2, and collision gas were set to 20, 30, 30, and 7 U, respectively. Both the entrance potential and the collision cell exit potential were consistently set at 10 V. Each sample was injected in duplicate for both positive and negative mode analyses. Data were captured using Analyst, version 1.6.2 software (Sciex), and peak integration was performed using Skyline (version 21.2.0.425, MacCoss Laboratory, University of Washington). An in-house R script was used for data normalization and QC analysis (version 4.2.0). Patient selection for the metabolomic screening was conducted in a blinded manner by an

Table 1. Baseline factors of total cohort or from patients of the Ipi or FOLFOX arm.

Baseline factor	All patients	Ipi arm	FOLFOX arm
Age, years, median (range)	60.5 (41–80)	63 (42–80)	59.5 (41–79)
Gender			
Female/male (%)	18/70 (20/80)	10/34 (23/77)	8/36 (18/82)
HER2-3+			
n (%)	63 (75)	33 (79)	30 (73)
HER2 2+/ISH			
n (%)	13 (15)	7 (17)	6 (15)
HER2 negative			
n (%)	8 (10)	3 (2)	5 (12)
Median tumor burden			
mm (range)	66 (10–334)	55.5 (21–219)	73 (10–334)
Histology			
Diffuse type, *n* (%)	14 (16)	8 (18)	6 (14)
Intestinal type, *n* (%)	44 (50)	23 (52)	21 (48)
Mixed, *n* (%)	1 (1)	—	1 (2)
NA, *n* (%)	29 (33)	13 (30)	16 (36)
Localization			
Stomach, *n* (%)	22 (25)	12 (27)	10 (23)
AEG I, *n* (%)	26 (30)	9 (20)	17 (39)
AEG II, *n* (%)	29 (33)	15 (34)	14 (32)
AEG III, *n* (%)	11 (13)	8 (18)	3 (7)
Grading			
Gx, *n* (%)	7 (8)	5 (11)	2 (5)
G1, *n* (%)	2 (2)	1 (2)	1 (2)
G2, *n* (%)	42 (48)	19 (43)	23 (52)
G3, *n* (%)	37 (42)	19 (43)	18 (41)
T stage			
Tx, *n* (%)	20 (23)	11 (25)	9 (20)
T1/2, *n* (%)	9 (10)	5 (11)	4 (9)
T3, *n* (%)	50 (57)	21 (48)	29 (66)
T4, *n* (%)	9 (10)	7 (16)	2 (5)
M stage			
M0, *n* (%)	21 (24)	9 (20)	12 (27)
M1, *n* (%)	67 (76)	35 (80)	32 (73)

Abbreviation: AEG, adenocarcinoma of the esophagus.
The number of patients meeting the indicated criterion is calculated. The absolute number of patients per arm is indicated, and the percentage of patients from the respective arm or total population is indicated in parentheses.

individual who was unaware of the outcome data. The final data were analyzed using MetaboAnalyst v. 6.0, the data were log-transformed, and the missing values were handled using default settings. Fold change analysis and *t* tests were then performed. The descriptive volcano plots display raw *P* values.

Cytokine and chemokine screen

To conduct the cytokine and chemokine screening, serum samples were thawed and analyzed for the following factors using the MILLIPLEX Human Cytokine/Chemokine Magnetic Bead Panel HCYTA-60K-PX38 (Merck): EGF, eotaxin, G-CSF, GM-CSF, IFNα2, IFNγ, IL1α, IL1β, IL1ra, IL2, IL3, IL4, IL5, IL6, IL7, IL8, IL10, IL12 (p40), IL12 (p70), IL13, IL15, IL17A, IL17E/IL25, IL17F, IL18, IL22, IP-10, MCP-1, M-CSF, MIG, MIP-1α, MIP-1β, PDGF-AA, PDGF-AB/BB, RANTES, TNF, and VEGF-A. Serum samples from 16 patients in the Ipi arm and 14 patients in the FOLFOX arm were available for this analysis. Analysis was conducted on a Bio-Plex 200 analyzer using Bio-Plex Analysis Software from Bio-Rad Laboratories.

Statistical analysis

Kaplan–Meier plots and linear regression were created and calculated using Prism V.9. Univariate and multivariate analyses as well as log-rank tests were conducted using SPSS V.28.0.0.1. *P* values are presented alongside the respective statistical tests for each figure.

Data availability

The data generated in this study are available in the article and its Supplementary Materials or upon request from the corresponding author.

Results

Clinical outcomes of the INTEGA trial with a median follow-up of 18.8 months

Between March 2018 and May 2020, 97 patients with advanced, untreated, HER2-positive esophagogastric adenocarcinoma were enrolled, and 88 of them were randomized across 21 German sites. Baseline characteristics were well balanced between treatment arms (**Table 1**). After a median follow-up of 18.8 months, the 12-month OS rate was 57% [95% confidence interval (CI), 41%–71%] in the Ipi arm and 70% (95% CI, 54%–82%) in the FOLFOX arm (**Fig. 1A**). There was no statistical difference between the groups. However, as time progressed, the two curves intersected, resulting in a nonsignificantly different ($P = 0.57$) median survival of 21.8 months in the

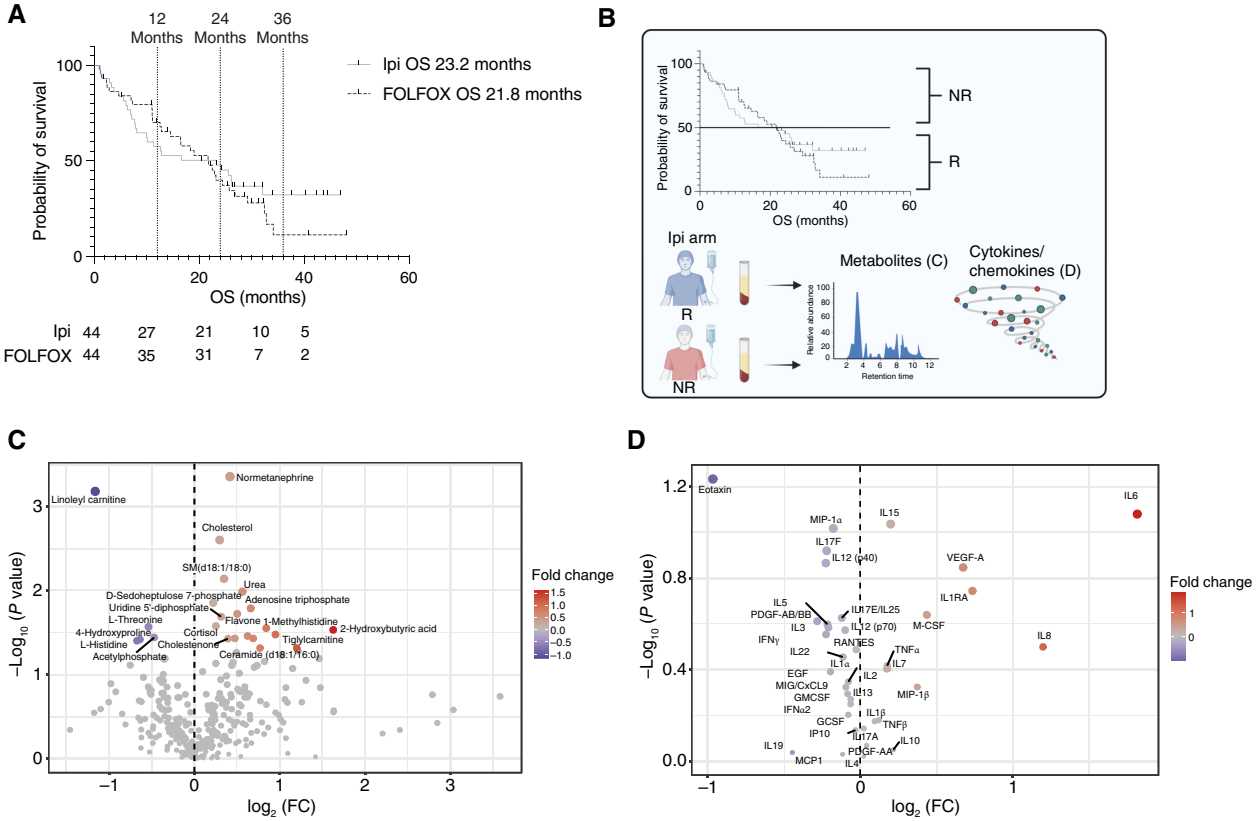

Figure 1.

Low levels of systemic stress hormones are associated with long-term survival in the Ipi arm. **A,** Final OS is shown for the Ipi vs. FOLFOX arm. The number of patients at risk is indicated, and a log-rank test was performed to determine differences in the curves. **B,** Graphical abstract of the translational analysis. R and NR patients were defined based on the median OS. Serum samples from patients who had higher or lower than median survival in the Ipi arm were screened for metabolites using metabolomic screening ($n = 8$; random subset of all available patients from the Ipi arm; **C**) or cytokine/chemokine screening ($n = 16$; all available serum samples from the Ipi arm; **D**) as described in the "Materials and Methods" section. Metabolites with a P value < 0.1 are depicted. Fold change (FC) and t test calculations were performed as described in the "Materials and Methods" section.

FOLFOX arm and 23.2 months in the Ipi arm (**Fig. 1A**). To better understand whether second- and third-line therapies received by treated patients in either arm may influence long-term benefit, we compared the use of different regimens. Overall, there were numerically more patients receiving second-line therapy ($n = 30$) or third-line therapy ($n = 18$) in the Ipi arm compared with patients from the FOLFOX arm ($n = 23$ and 15, respectively) (**Table 2**). Most patients receiving chemotherapy-free treatment in the Ipi arm received platinum-based second-line therapy (**Table 2**). Most patients (67%) within the Ipi arm continued trastuzumab beyond progression compared with only 23% in the FOLFOX arm. In addition, seven patients (16%) who were initially treated within the Ipi arm showed long-lasting disease control under trastuzumab monotherapy. Three of these seven patients had local tumor treatment by resection ($n = 1$) or radiotherapy ($n = 2$). One patient was treated for a short period with a platinum-based therapy before changing to trastuzumab monotherapy, and three patients never progressed under trastuzumab (Supplementary Fig. S1).

Safety profile and adverse events within the trial

The overall incidence of grade 3 or greater adverse events (AE) was similar in both arms (82% in the Ipi arm vs. 88% in the FOLFOX arm; **Table 3**). In the Ipi arm, the most frequently observed treatment-related AEs were anemia, infection, and diarrhea. Rates of grade 3 or greater autoimmune disorders, such as hepatitis,

Table 2. Second- and third-line therapies in the Ipi or FOLFOX arm.

Therapy		Ipi arm	FOLFOX arm
Second line	*n*	30	23
Ramucirumab ± paclitaxel	*n* (%)	3 (10)	12 (52)
Irinotecan based	*n* (%)	2 (7)	2 (9)
Platinum based	*n* (%)	22 (73)	1 (4)
Surgery	*n* (%)	1 (3)	2 (9)
Radiotherapy	*n* (%)	2 (7)	4 (17)
Trastuzumab	*n* (%)	20 (67)	5 (25)
Other	*n* (%)	—	2 (9)
Third line	*n*	18	15
Ramucirumab ± paclitaxel	*n* (%)	14 (78)	7 (47)
Irinotecan based	*n* (%)	4 (22)	5 (33)
Platinum based	*n* (%)		1 (7)
Surgery	*n* (%)		2 (13)

Treatment regimen is indicated. The absolute number of patients per arm is indicated, and the percentage of patients from the respective arm is indicated in parentheses.

Table 3. Adverse events ≥ grade 3.

Grade ≥3 AE		Ipi arm	FOLFOX arm
All grade ≥3 AEs	*n* (%)	36 (82)	38 (88)
Treatment-related AEs	*n* (%)	20 (46)	29 (67)
Leukopenia	*n* (%)	2 (5)	10 (23)
Anemia	*n* (%)	5 (11)	3 (7)
Infection	*n* (%)	5 (11)	7 (16)
Fatigue	*n* (%)	3 (7)	6 (14)
Diarrhea	*n* (%)	6 (14)	2 (5)
Pyrexia	*n* (%)	1 (2)	3 (7)
Neuropathy	*n* (%)	0 (0)	5 (11)
Pulmonary embolism	*n* (%)	3 (7)	1 (2)
Hypertension	*n* (%)	0 (0)	3 (7)
Autoimmune hepatitis	*n* (%)	4 (9)	0 (0)
Colitis	*n* (%)	3 (7)	0 (0)
Pneumonitis	*n* (%)	3 (7)	0 (0)
Endocrine disorders (hypophysitis/thyroiditis)	*n* (%)	3 (7)	1 (2)
SAEs	*n* (%)	34 (77)	28 (65)
Treatment-related SAEs	*n* (%)	17 (39)	15 (35)
Fatal SAEs	*n* (%)	1 (2)	4 (9)
Treatment-related fatal SAEs	*n* (%)	0 (0)	1 (2)

Treatment regimen is indicated. The absolute number of patients experiencing AEs of at least grade 3 per arm is indicated, and the percentage of patients from the total amount of patients treated per arm ($n = 44$ Ipi arm and $n = 43$ FOLFOX arm) is indicated in parentheses.

pneumonitis, colitis, and endocrine pathologies, were less than 10% and rarely seen in the FOLFOX arm (up to 2%). The FOLFOX arm most frequently experienced leukopenia, infection, fatigue, and neuropathy. Overall, both arms tolerated the treatment as expected. Treatment was discontinued because of an AE in nine patients (21%) in the Ipi arm and in seven patients (16%) in the FOLFOX arm. Serious AEs (SAE) occurred in 34 patients in the Ipi arm, with 17 being treatment related, compared with 28 patients in the FOLFOX arm, with 15 being treatment related. There were five fatal SAEs noted overall, with one treatment-related SAE (tumor lysis) in the FOLFOX arm.

An inflammatory stress signature is associated with resistance to therapy in the Ipi arm

The OS curves that intersected with longer follow-up pointed at biological heterogeneity that led to a broad variety of outcomes in the chemotherapy-free Ipi arm from early progression to long-term survival. We sought to identify specific tumor or host factors that could account for these differences. Our focus was to pinpoint a response/resistance signature to define patients with a need for additional chemotherapy.

The expression of PD-L1 on tumor cells (TPS), immune cells, or the combined score of PD-L1 on immune and tumor cells (CPS) has been shown to correlate with immunotherapy response in various solid cancers, including esophagogastric adenocarcinoma (10–12). However, in the INTEGA trial, despite both arms including the anti–PD-1 nivolumab, no association between PD-L1 expression and treatment outcomes was observed in either of the two arms (**Table 4**).

We conducted metabolomic and cytokine analyses on serum from immunotherapy R and NR patients from the Ipi arm (**Fig. 1B**; Supplementary Tables S1 and S2). NR patients exhibited increased levels of normetanephrine and cortisol, indicative of a systemic stress reaction before therapy initiation (**Fig. 1C**). This signature was not associated with discernible differences in emotional well-being scores as measured by the European Organisation for Research and Treatment of Cancer QLQ 30 questionnaire between R and NR patients in the Ipi arm (Supplementary Fig. S2A). This suggests that emotional distress might not be the primary contributor to the systemic stress signature in immunotherapy NR patients. In line with the established mutual influence of stress hormones and inflammatory

Table 4. Number of patients and median survival based on CPS, TPS, or IC.

Baseline factor	All patients	Ipi arm	FOLFOX arm	*P* value
CPS ≥ 1				
n (%)	59 (72)	31 (74)	28 (70)	
Median OS	22.1	17	22.8	0.83
CPS < 5				
n (%)	36 (44)	18 (43)	18 (45)	
Median OS	26.2	32.3	22.1	0.36
CPS ≥ 5				
n (%)	46 (56)	24 (57)	22 (55)	
Median OS	21.9	12.6	22.7	0.91
IC < 5				
n (%)	56 (68)	29 (69)	27 (68)	
Median OS	19	16.6	22.1	0.87
IC < 5				
n (%)	26 (32)	13 (31)	13 (33)	
Median OS	24.8	not reached	23.2	0.63
TPS < 5				
n (%)	72 (87)	37 (86)	35 (88)	
Median OS	23	23	23	0.80
TPS ≥ 5				
n (%)	11 (13)	6 (14)	5 (12)	
Median OS	11.2	11.35	11.2	0.46

Abbreviation: IC, immune cells.

All patients within the Ipi or FOLFOX arm were stratified based on CPS, IC score, or TPS. The percentage represents the fraction of patients selected by the respective marker within each treatment arm. The *P* value was determined by comparing survival in the Ipi and FOLFOX arms using a log-rank test.

cytokines and chemokines, IL6 was increased in NR patients compared with R patients in the Ipi arm (**Fig. 1D**). A similar increase of the metabolites normetanephrine and cortisol and the cytokine IL6 was observed if the cohort was divided into R and NR patients based on progression-free survival (PFS; Supplementary Fig. S2B). This IL6 increase was not observed when comparing R and NR patients from the FOLFOX arm, suggesting that systemic inflammatory stress might play a more important role in the chemotherapy-free setting (Supplementary Fig. S2C). To better understand whether tumor size triggers systemic inflammation, we correlated tumor burden, measured as the sum of target lesions, with levels of IL6, cortisol, and normetanephrine. We did not observe any association with any of them (Supplementary Fig. S2D), indicating that tumor size alone may not contribute to promoting systemic inflammation.

Given the impact of systemic inflammatory stress on the immune system and on the efficacy of immunotherapies (13, 14), we analyzed the peripheral immune composition in R and NR patients from both arms. In the Ipi arm, we observed decreased lymphocyte counts in NR patients as well as an increased neutrophil to lymphocyte ratio (NLR; **Fig. 2A**). No NLR shifts were identified between R and NR patients in the FOLFOX arm (**Fig. 2B**). Similarly, we did not observe any difference in other laboratory parameters linked to immunotherapy response pertinent to immune function, including urea (15), magnesium (16), or lactate dehydrogenase (17), when comparing R and NR from either treatment arm. Further analysis showed that the NLR correlated especially with plasma normetanephrine and, to a lesser extent, with cortisol (**Fig. 2C**). Additionally, the serum IL6 concentration was increased in patients with higher NLR, which is compatible with the notion that there is

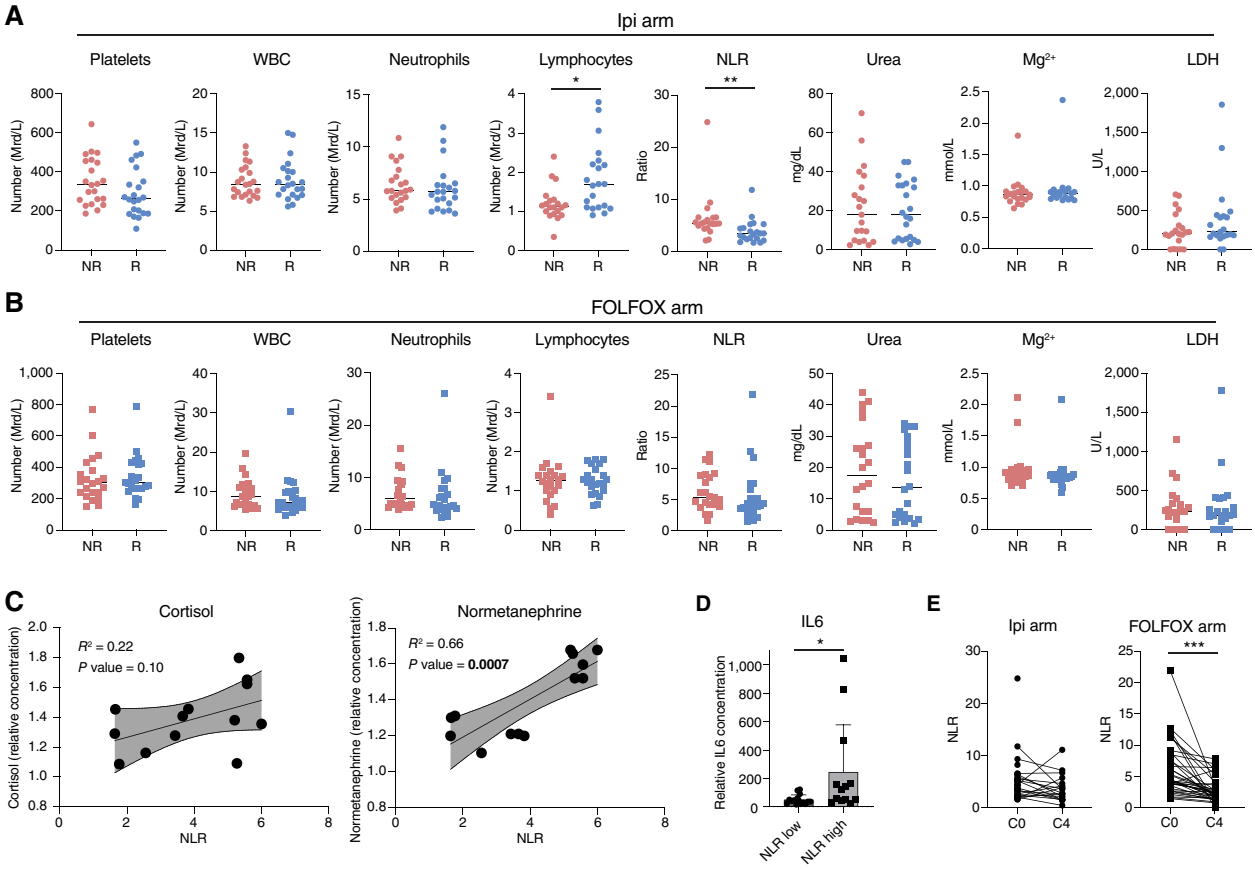

Figure 2.

Peripheral immune composition in R and NR patients. Pretreatment immune composition and routine laboratory parameters that are potentially associated with response to immunotherapy are shown for R and NR patients in the Ipi (**A**) or FOLFOX (**B**) arm. R patients were defined by more than median OS in each arm. Each dot represents one patient. For the Ipi arm ($n = 44$), data were available for parameters, except for urea (43 values), neutrophils, and LDH (42 values each), and lymphocytes, NLR, and Mg^{2+} (41 values each). In the FOLFOX arm ($n = 44$), data were complete for most parameters, except for LDH (43 values) and Mg^{2+} (42 values). The median is indicated. **C,** The concentration of cortisol or normetanephrine was measured using LC-MS and was correlated with the NLR of the individual patient. Serum from 16 patients was analyzed. One sample was excluded because of an outlying NLR value, and two samples were missing an NLR value ($n = 13$ in the presented data). Each dot represents one patient. Values from two separate measurements were pooled. 95% CI is shown. **D,** Patients from the Ipi and FOLFOX arms were separated into NLR high (>5) and NLR low (NLR <5). Relative IL6 concentration as measured in **Fig. 1D** and Supplementary Fig. S2B was compared between NLR-high and NLR-low patients; $N = 30$ patients. Each dot represents one patient. Mean and upper SD are indicated. **E,** The NLR was defined per patient at time point pretreatment or cycle 4 of therapy. Each dot represents one patient. Values from similar patients are connected. *P* values (*, < 0.05; **, < 0.01; ***, < 0.001) were determined by the Mann–Whitney *U* test (**A**, **B,** and **D**), simple linear regression and Pearson R (**C**), or Wilcoxon paired-rank test (**E**). LDH, lactate dehydrogenase; WBC, white blood cells.

modulation of the peripheral immune compartment by this systemic stress reaction (**Fig. 2D**).

As a high NLR was only associated with adverse outcomes in the Ipi arm, but not in the FOLFOX arm, we asked if chemotherapy was able to reduce the NLR. To study this, we compared the baseline NLR with the on-treatment NLR measured after cycle 4 in both treatment arms. A reduction was only observed in the FOLFOX arm; the NLR remained unchanged in patients treated in the chemotherapy-free Ipi arm (**Fig. 2E**). We also observed that a decrease in the NLR upon chemotherapy (more than median decrease) and/or a low NLR after four cycles of chemotherapy could identify patients with improved survival in the FOLFOX arm (Supplementary Fig. S3A).

NLR as a biomarker for long-term survival in the chemotherapy-free Ipi arm

Given that the NLR is readily available compared with the serum stress signature (cortisol, normetanephrine, and IL6) and a high NLR has been previously found to be associated with diminished response to immunotherapy in the melanoma setting (18), we investigated whether the NLR could be used to identify patients with HER2-positive esophagogastric adenocarcinoma that benefit from chemotherapy-free treatment. To address this, our initial goal was to establish a baseline NLR cutoff that predicted optimal response to therapy within the Ipi arm. We found that patients with an NLR lower than 5, 4, or 3 experienced the most favorable OS (Supplementary Fig. S3B and S3C). The criteria of NLR lower than 5 was met by 22 of 41 (53.7%) patients, lower than 4 was observed in 17 of 41 (41.4%) patients, and lower than 3 was only met by 9 of 41 (22%) patients, which is why we focused on NLR lower than 5 in the subsequent analysis. In contrast to patient-specific (age and gender), tumor-specific (location, histologic subtype, grading, median tumor burden, lab values, or tumor markers), or other predictive markers like PD-L1 CPS of ≥1, 5, or 10, which are commonly used in immunotherapy studies for esophagogastric adenocarcinoma (4, 6), an NLR lower than 5 indicated increased OS as reflected by a decreased HR (**Fig. 3A**). It is worth noting that we also identified HER2-3+ to indicate patients with increased OS as measured by a decreased HR in the Ipi arm (**Fig. 3A**). NLR lower than 5 and HER2-3+ remained significant in the multivariate analysis (**Fig. 3A**).

Lastly, we set out to evaluate the robustness of patient preselection by NLR in conjunction with HER2-3+ status. Patient preselection based on both HER2-3+ and a NLR <5 showed a 24-month survival rate of 87.5% in the Ipi arm compared with 44% in the FOLFOX arm ($P = 0.0008$). The median OS was not reached in the Ipi arm compared with 23 months in the FOLFOX arm (**Fig. 3B**). This pattern was similarly observed in a preselected patient population characterized by a CPS ≥1, which aligns with the patient subset currently receiving FDA- and EMA-approved treatment with chemotherapy, trastuzumab, and pembrolizumab (**Fig. 3C**). Patients in the Ipi arm with CPS ≥1, HER2-3+, and NLR <5 demonstrated strong PFS with this therapy (Supplementary Fig. S4A). Additionally, 50% of these patients (6/12) achieved partial remission at the first follow-up assessment, 42% had stable disease (5/12), and only one patient (8%) experienced progressive disease (Supplementary Table S3). These results suggest that the treatment regimen in the Ipi arm has a direct therapeutic effect rather than acting through subsequent therapy lines. In contrast, patients with a strong inflammatory stress signature, including a high NLR, had a trend for improved survival in the FOLFOX arm compared with the Ipi arm (Supplementary Fig. S4B).

Discussion

The INTEGA trial was conducted in more than 20 German sites including universities, community hospitals, and private practices. It therefore reflects well the spectrum of daily clinical practice. The longer follow-up data presented here showed an objective response rate of 57% and a median OS of 22.1 months in the FOLFOX, nivolumab, and trastuzumab treatment arms, which is comparable with the results of the KEYNOTE-811 study that led to both FDA and EMA approval of chemotherapy in combination with anti-HER2 and anti–PD-1 treatment for patients with PD-L1 CPS ≥1 and HER2-positive esophagogastric adenocarcinoma (4, 5). Despite the shorter duration of response to first-line therapy and numerically decreased 12-month OS, the chemotherapy-free Ipi arm of the INTEGA trial indicated a potential long-term benefit for some patients treated with this regimen (8). In terms of tolerability, the main treatment-related AEs in the Ipi arm were autoimmune in nature, whereas in the FOLFOX arm, they were predominantly hematologic and oxaliplatin-induced neuropathy. The overall incidence of grade 3 or worse AEs was more than 80%. Moreover, the treatment discontinuation rate was slightly higher in the Ipi arm (21%) compared with the FOLFOX arm (16%). Overall, these side effects seem slightly higher than historical controls without immunotherapy or recent findings of pembrolizumab, trastuzumab, and chemotherapy (2, 4). We observed that patients who benefited from chemotherapy-free treatment exhibited reduced systemic inflammatory stress, in contrast to those who did not benefit from this treatment. The latter group showed high levels of normetanephrine, cortisol, and IL6 associated with systemic immune changes such as an increased NLR. This is in line with other recent studies pointing to a role of systemic stress in reducing the response to immunotherapy in patients with melanoma (19).

The association among IL6, cortisol, and normetanephrine levels is not unexpected. Existing *in vivo* studies illustrate the capacity of IL6 to trigger cortisol production in chromaffin cells in the adrenal gland (20). Conversely, increased levels of stress hormones, such as cortisol and normetanephrine, might stimulate the expression of IL6 in cancer cells. This notion is substantiated by *in vitro* cancer models and supported by correlations observed in human patients with cancer (21, 22). Moreover, these soluble factors may affect the NLR because of their regulation of tissue migration and release of neutrophils and lymphocytes. Although IL-6 is involved in the release of neutrophils from the bone marrow (21), cortisol results in lymphopenia by preventing lymphocyte egress from lymphoid tissues (22, 23). Of note, tumor size alone, measured as the sum of target lesions, was not sufficient to explain increases in either IL-6, cortisol, or normetanephrine. The negative impact of inflammatory stress in the chemotherapy-free Ipi arm in contrast to the FOLFOX arm may point at a causal role of chemotherapy in normalizing systemic stress or its consequences. This hypothesis is underscored by the normalization of the NLR selectively in the chemotherapy-containing arm as evidenced by our analysis after cycle 4 of treatment. Whether the normalization of the NLR also increases the chance of the immunotherapy to benefit the patients or whether in this subgroup the chemotherapeutic effect is mostly responsible for the survival benefit needs further investigation. Such study could also clarify whether the addition of anti–CTLA-4 to chemotherapy, anti–PD-1, and trastuzumab could be beneficial in patients with

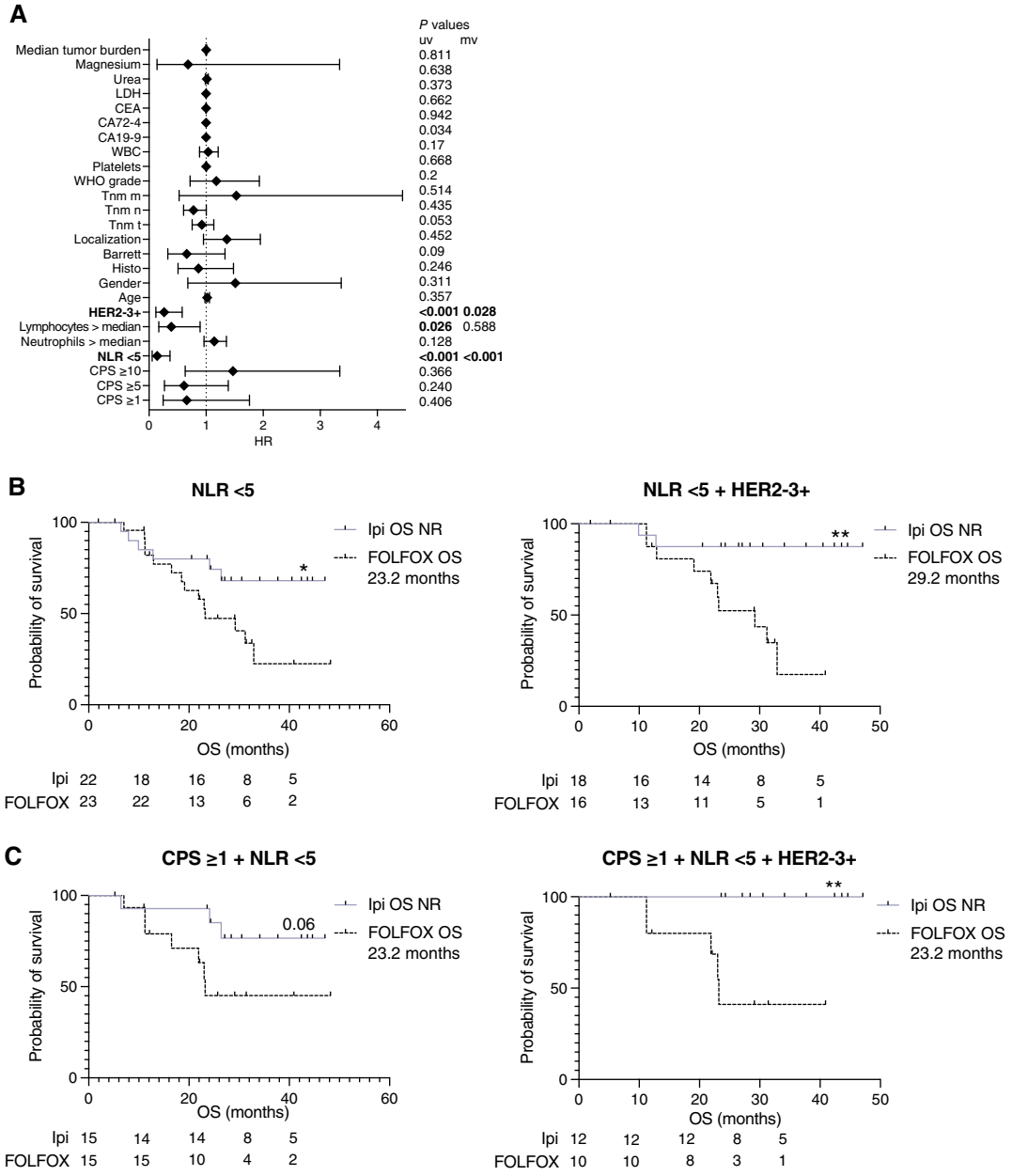

Figure 3.

Low NLR and HER2-3+ define patients with superior survival in the Ipi arm. **A,** HRs of indicated patient- or disease-specific parameters. Univariant (uv) Cox regression analysis was performed in the Ipi arm ($n = 44$ patients overall). Significant parameters with an HR different than 1 were further tested in a multivariant analysis (mv). OS is shown for patients selected by an NLR <5 (**B**) or CPS ≥1 + NLR <5 (**C**) alone or together with HER2-3+ within the Ipi and FOLFOX arms, respectively. The number of patients at risk is indicated. OS not reached is indicated as NR. *P* values (*, < 0.05; **, < 0.01) were determined by Cox regression analysis (**A**) or log-rank test (**B** and **C**). CEA, carcinoembryonic antigen; LDH, lactate dehydrogenase; WBC, white blood cells.

high systemic inflammation. In this case, toxicities would need to be closely monitored as such a regimen could result in toxicities outweighing the potential benefit. Our data, thereby, contribute to an emerging body of evidence on neutrophils and the NLR as prognostic and predictive biomarkers in immunotherapy of cancer (24, 25).

We report that most patients in the Ipi arm received platinum-based chemotherapy as second-line therapy. Additionally, numerically more patients in the Ipi arm received second- or third-line therapy, which may have contributed to the observed survival benefit in some cases. However, most patients preselected with CPS ≥1, HER2-3+, and NLR <5 had a prolonged PFS and overall response to the first-line treatment. This suggests a strong antitumor immune reaction by the combination of ipilimumab, nivolumab, and trastuzumab, also enabling some patients to undergo additional local therapies such as radiotherapy or surgery, which in this

combination induced long-term disease control. Altogether, these data suggest that this combination induces the observed effect rather than the second- or third-line therapy. However, further studies with larger cohorts are needed to confirm this assumption. One possible explanation could be that trastuzumab induces innate and adaptive immune responses through its Fc receptor–dependent mechanisms as a result of binding to surface HER2 (26, 27). Moreover, an NLR lower than 5 and HER2-3+ identified patients with an exceptional OS in the chemotherapy-free Ipi arm that by far exceeded the survival of this same subset in the FOLFOX arm. This improved survival may also be attributed to the anti–CTLA-4 ipilimumab, as evidenced in other contexts in which this combination has shown efficacy, such as in melanoma (28). However, the favorable survival outcome could also imply that chemotherapy is impeding the antitumor immune response in a specific subset of patients with low systemic inflammatory stress. This aligns with previous studies indicating that chemotherapy may hinder the expansion of mature T cells (29). Future investigations are warranted to elucidate the respective roles of CTLA-4 inhibition versus immune response modulation by chemotherapy in this setting. What is particularly noteworthy is that nearly half of the patients in this study met the criteria (NLR <5 and HER2-3+) that were associated with benefit from chemotherapy-free treatment. Additionally, these criteria can be easily assessed in routine laboratories and pathology departments, rendering the implementation of these biomarkers even more feasible.

The limitations of the study include the retrospective nature of the subgroup analysis that was performed and the fact that the systemic inflammatory signature was only analyzed in a subset of patients with available serum samples. However, immune modulations such as NLR were analyzed in nearly the entire cohort with available values. Another potential limitation of the study is that the histologically defined intestinal subtype was overrepresented in the cytokine/chemokine screening cohort from the FOLFOX arm, which could have potentially influenced the results. Nevertheless, most analyses were conducted in the ipilimumab biomarker cohort, which was like the overall Ipi arm cohort. Furthermore, survival analysis and NLR frequencies were also examined in the entire FOLFOX arm and compared with the Ipi arm, with nearly identical numbers of patients with the intestinal subtype in both cohorts. Although we cannot entirely exclude this bias in the biomarker analysis cohort of the FOLFOX arm, we believe that it does not significantly affect our conclusions.

Taken together, our data inspire new concepts of precision oncology and treatment selection in patients with advanced HER2-positive esophagogastric adenocarcinoma. A prospective clinical trial is warranted to compare the combination of anti–PD-1 and anti–CTLA-4 treatment with trastuzumab versus the chemotherapy-containing standard of care in low-NLR and HER2-3+ preselected patients.

Authors' Disclosures

E. Goekkurt reports grants from Bristol Myers Squibb during the conduct of the study, as well as personal fees from Bristol Myers Squibb outside the submitted work. P. Thuss-Patience reports personal fees from Astellas, MSD, Bristol Myers Squibb, Eli Lilly and Company, Servier, and Roche, personal fees and nonfinancial support from AstraZeneca, BeiGene, and Daiichi Sankyo, and nonfinancial support from Merck Serono and Celltrion outside the submitted work. T.J. Ettrich reports personal fees from MSD, Roche, Sanofi, Bristol Myers Squibb, AstraZeneca, Merck Serono, Pierre Fabre, Servier, Eli Lilly and Company, Ipsen, Daiichi Sankyo, AbbVie, and Takeda, and grants from Eli Lilly and Company and Servier outside the submitted work. A. Reinacher-Schick reports grants from Bristol Myers Squibb and personal fees from Bristol Myers Squibb during the conduct of the study, as well as grants from Roche, BioNTech, and Novartis and personal fees from Roche, Boehringer Ingelheim, Servier, Amgen, MSD, and Pierre Fabre outside the submitted work. D. Pink reports grants and personal fees from PharmaMar and Boehringer Ingelheim and grants from Roche, Deciphera, Recordati, and Bristol Myers Squibb outside the submitted work. C. Bokemeyer reports personal fees from AOK Germany, BioNTech, Lindis Biotech, Sanofi Aventis, med update GmbH, Merck Serono, Roche Pharma, and AstraZeneca and grants and personal fees from Bayer Healthcare outside the submitted work. M. Sinn reports personal fees from Falk and MSD, personal fees and other support from Roche and Pierre Fabre, and other support from Amgen, AstraZeneca, BeiGene, Bristol Myers Squibb, GSK, and Servier outside the submitted work. A. Stein reports grants from Bristol Myers Squibb during the conduct of the study, as well as grants from MSD and BeiGene and other support from Astellas outside the submitted work. M. Binder reports grants from MSD during the conduct of the study. No disclosures were reported by the other authors.

Authors' Contributions

J. Tintelnot: Conceptualization, resources, data curation, formal analysis, funding acquisition, investigation, methodology, writing–original draft, writing–review and editing. **L. Paschold:** Resources, data curation, writing–original draft. **E. Goekkurt:** Conceptualization, resources, data curation, funding acquisition, project administration. **C. Schultheiss:** Resources, data curation, formal analysis, writing–review and editing. **U. Matschl:** Resources, formal analysis, methodology. **M. Santos Cruz:** Resources, data curation, writing–review and editing. **M. Bauer:** Resources, formal analysis, methodology. **C. Wickenhauser:** Resources, formal analysis, methodology. **P. Thuss-Patience:** Resources. **S. Lorenzen:** Conceptualization, resources, writing–review and editing. **T.J. Ettrich:** Resources. **J. Riera-Knorrenschild:** Resources. **L. Jacobasch:** Resources, writing–review and editing. **A. Kretzschmar:** Resources. **S. Kubicka:** Resources. **S.-E. Al-Batran:** Conceptualization, resources, data curation. **A. Reinacher-Schick:** Resources. **D. Pink:** Resources. **C. Bokemeyer:** Conceptualization, resources, writing–review and editing. **M. Sinn:** Resources, writing–review and editing. **U. Lindig:** Conceptualization, resources. **A. Hinke:** Formal analysis, methodology. **S. Hegewisch-Becker:** Conceptualization, resources, project administration, writing–review and editing. **A. Stein:** Conceptualization, resources, data curation, funding acquisition, investigation, writing–original draft, project administration, writing–review and editing. **M. Binder:** Conceptualization, resources, data curation, funding acquisition, investigation, writing–original draft, project administration, writing–review and editing.

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Note

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