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ORIGINAL RESEARCH ARTICLE

Bintrafusp Alfa, a Bifunctional Fusion Protein Targeting TGF-β and PD-L1, in Patients with Esophageal Adenocarcinoma: Results from a Phase 1 Cohort

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Abstract

Background Esophageal adenocarcinoma patients have limited treatment options. TGF-β can be upregulated in esophageal adenocarcinoma, and blocking this pathway may enhance clinical response to PD-(L)1 inhibitors. Bintrafusp alfa is a first-in-class bifunctional fusion protein composed of the extracellular domain of the TGF-βRII receptor (a TGF-β "trap") fused to a human IgG1 mAb blocking PD-L1.

Objective The objective of this study was to investigate the efficacy and safety of bintrafusp alfa in patients with advanced, post-platinum esophageal adenocarcinoma, unselected for PD-L1 expression.

Patients and Methods In this phase 1 study, patients with post-platinum, PD-L1–unselected esophageal adenocarcinoma received bintrafusp alfa 1200 mg every 2 weeks until disease progression, unacceptable toxicity, or withdrawal. The primary endpoint was confirmed best overall response per RECIST 1.1 by independent review committee (IRC).

Results By the database cutoff of 24 August 2018, 30 patients (80.0% had two or more prior anticancer regimens) received bintrafusp alfa for a median of 6.1 weeks. The confirmed objective response rate (ORR) per IRC was 20.0% (95% CI 7.7–38.6); responses lasted 1.3–8.3 months. Most responses (83.3%) occurred in tumors with an immune-excluded phenotype. Investigator-assessed confirmed ORR was 13.3% (95% CI 3.8–30.7). Nineteen patients (63.3%) had treatment-related adverse events: seven patients (23.3%) had grade 3 events; no grade 4 events or treatment-related deaths occurred.

Conclusions Bintrafusp alfa showed signs of clinical efficacy with a manageable safety profile in patients with heavily pre-treated, advanced esophageal adenocarcinoma.

Clinical Trials Registration NCT02517398.

1 Background

In 2018, esophageal cancer caused approximately 500,000 deaths worldwide [1]. The prognosis of patients with esophageal cancer is poor, with age-standardized 5-year survival rates ranging from 10 to 30% after correction for background mortality [2]. The majority of patients have advanced disease at diagnosis, with 5-year survival rates below 10% [3].

Esophageal cancer comprises two subtypes: adenocarcinoma (AC) and squamous cell carcinoma (SCC). Although SCC remains the most prevalent histological type of esophageal cancer, incidence rates are decreasing worldwide [1]. In contrast, the incidence of AC is rapidly increasing in Western countries such as Australia, England, France, and the

USA [1]. This observed increase is most likely due to the escalating prevalence of risk factors for esophageal AC, such as obesity and chronic gastroesophageal reflux disease [1, 4].

Genetic profiling has identified tumor-specific alterations in esophageal AC affecting *ERBB2*, *VEGFA*, *GATA4*, and *GATA6*, as well as genes involved in DNA hypermethylation, but other factors in the tumor microenvironment (TME) are also known to impact cancer [5–7]. Transforming growth factor β (TGF-β) is one such factor in the TME. TGF-β is a cytokine with highly diverse functions that can act in either a tumor-suppressive or a tumor-promoting role, depending on the tumor type and stage, and genetic and/or epigenetic changes within the TGF-β pathway [8, 9]. Signaling through the TGF-β pathway can enable cancer progression and immune evasion in the TME through regulatory effects on immune cells, and may also influence angiogenesis and epithelial-to-mesenchymal transition (EMT) [9, 10]. Preclinical studies have shown that TGF-β can induce EMT in

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Key Points

Bintrafusp alfa is a first-in-class bifunctional fusion protein that was designed for co-localized, simultaneous inhibition of two nonredundant immunosuppressive pathways, TGF-β and PD-L1, within the tumor microenvironment.

Treatment with bintrafusp alfa had a manageable safety profile and demonstrated clinical activity in patients with advanced, post-platinum esophageal adenocarcinoma from a phase 1 expansion cohort.

These results, along with those reported in the accompanying article from an expansion cohort of Asian patients with pretreated, PD-L1-unselected esophageal squamous cell carcinoma in a separate phase 1 study, support further clinical investigation of bintrafusp alfa in esophageal cancer.

esophageal AC cell lines and that expression of TGF-β correlated with the expression of EMT-related genes [11, 12]. Furthermore, elevated expression of TGF-β in esophageal AC has been associated with advanced stages of disease, and correlates with poor overall survival (OS) [13].

Antibodies that target immune checkpoints have emerged in recent years as a novel therapeutic approach for the treatment of a wide range of cancers. The efficacy of anti-PD-1 agents has been investigated in advanced, pretreated esophageal cancer; however, these studies often had low enrollment of patients with esophageal AC [14, 15]. In the KEYNOTE-180 and KEYNOTE-181 clinical trials, pembrolizumab showed an objective response rate (ORR) of 5% in patients with PD-L1-unselected (all-comer), 3% in those with PD-L1-negative (combined positive score [CPS] < 10), and 18% in those with PD-L1-positive (CPS ≥ 10) esophageal AC [14, 15].

Preclinical studies have suggested that the efficacy of anti-PD-(L)1 agents may improve with the addition of TGF-β blockade [16–19]. Bintrafusp alfa is a first-in-class bifunctional fusion protein composed of the extracellular domain of the human TGF-β receptor II (TGF-βRII or TGF-β "trap") fused via a flexible linker to C-terminus of each heavy chain of IgG1 antibody blocking PD-L1 (anti-PD-L1) [20]. Bintrafusp alfa is designed for co-localized, simultaneous inhibition of two nonredundant immunosuppressive pathways (TGF-β and PD-L1) within the TME [20]. This may provide an enhanced treatment effect, potentially improving clinical benefit compared with anti-PD-(L)1 monotherapies [20–22].

Clinical data from two phase 1 studies of bintrafusp alfa (NCT02517398 and NCT02699515) have demonstrated a manageable safety profile and early signs of clinical efficacy

in patients with heavily pretreated advanced solid tumors [23–25]. Here we report results from an expansion cohort of the phase 1 study, which investigated the efficacy and safety of bintrafusp alfa in patients with advanced, post-platinum esophageal AC, unselected for PD-L1 expression.

2 Patients and Methods

2.1 Study Design and Participants

This phase 1, open-label trial investigated the safety and efficacy of bintrafusp alfa in patients with heavily pretreated solid tumors and included multiple expansion cohorts in specific tumor types (NCT02517398). Patients were screened across the Asia-Pacific region, Europe, Canada, and the USA.

Eligible patients had histologically confirmed recurrent or metastatic, unresectable (stage III/IV) esophageal AC and measurable disease by Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST 1.1). Patients were at least 18 years old and must have received at least one prior platinum-based regimen. Patients with HER2-positive tumors must have received prior trastuzumab. Other inclusion criteria included Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, life expectancy of ≥ 12 weeks, and adequate renal, hepatic, and hematological function. Prior to enrollment, tumor samples (archival material or fresh biopsies obtained within 28 days) were collected from each patient; however, no selection was made on the basis of PD-L1 expression or other biomarkers. Prior therapy with immune checkpoint inhibitors was not permitted.

2.2 Treatment and Assessments

Patients received bintrafusp alfa at the recommended phase 2 dose of 1200 mg intravenously over 1 h once every 2 weeks until confirmed progressive disease, unacceptable toxicity, or trial withdrawal [26]. A flat dose of 1200 mg every 2 weeks was selected as the recommended phase 2 dose of bintrafusp alfa based on safety/tolerability, pharmacokinetic, and pharmacodynamic data, as well as preliminary population pharmacokinetics and exposure-response modeling of phase 1 data [26, 27]. The planned duration of treatment for patients who achieved a best overall response of complete response, partial response, or stable disease was 12 months. If the investigator believed that a patient may benefit, additional treatment beyond 12 months could be possible. Dose reductions were not permitted. Tumor responses were monitored by imaging every 6 weeks during the first year and every 12 weeks thereafter. Scans were reviewed by an independent review committee to assess disease response. Efficacy and safety were analyzed in all patients who received at

least one dose of bintrafusp alfa. Adverse events (AEs) were monitored throughout treatment and were assessed according to National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 4.03. An additional safety follow-up visit was planned 28 days after the last study dose or before the start of a new treatment (whichever occurred first). Treatment-related AEs (TRAEs) continued to be evaluated 10 weeks post-treatment. Any AE that was believed to be a potential immune-related or potential TGF-β-related event was considered an AE of special interest. Immune-related AEs (irAEs) were identified using a preselected list of terms from the Medical Dictionary for Regulatory Activities version 21.0.

2.3 Endpoints

The primary endpoint was confirmed best overall response according to RECIST 1.1 as adjudicated by the independent review committee and evaluated by the confirmed ORR; key secondary endpoints included the safety of bintrafusp alfa and best overall response per investigator assessment. Other efficacy measures included duration of response, disease control rate (DCR), progression-free survival (PFS), and OS. The evaluation of potential predictive biomarkers was an exploratory endpoint.

2.4 Exploratory Endpoints

PD-L1 expression was detected by immunohistochemistry staining of formalin-fixed, paraffin-embedded tumor tissue using an anti-PD-L1 antibody clone 73-10 (Dako PD-L1 IHC 73-10 pharmDx; Dako, Carpinteria, CA, USA). PD-L1 expression was measured on tumor cells and on cells of the TME. Data herein are reported based on the percentage of tumor cells expressing PD-L1. A threshold of 1% was used to characterize tumors as either PD-L1 positive ($\geq$ 1%) or PD-L1 negative (< 1%).

RNA sequencing (RNAseq) was also performed to assess the correlation between specific tumor characteristics and response to bintrafusp alfa. RNAseq used formalin-fixed, paraffin-embedded archival tumor samples and was performed by Asuragen (Austin, TX, USA) using standard protocols based on ribosomal depletion. Resulting sequencing reads were aligned against the Ensembl 75 human genome (GRCh37 February 2014) with Bowtie2 version 2.2.3 (Johns Hopkins University, Baltimore, MD, USA) [28]. Gene expression was determined using RSEM version 1.2.31 and Ensembl gene annotations, and hypothesis testing was performed by comparing RSEM-computed expected counts [29]. Transcript-per-million values were upper-quartile normalized and log transformed for further analysis. To test whether *TGFB1*, *TGFB2*, and *TGFB3* gene expression was higher in this cohort than in other expansion cohorts

across phase 1 trials of bintrafusp alfa (NCT02517398 and NCT02699515), we applied limma + voom, modeling expression as a function of indication [30]. The analysis included all patient samples that passed quality control (N = 537, including 27 esophageal AC samples) and tested all genes with $\geq$ 10 reads in $\geq$ 20 samples. We report p values for differential expression after adjustment for testing of all genes.

Tumor mutation count was measured by an RNAseq-based variant calling that used tumor RNAseq data combined with germline, normal whole-exome sequencing to produce a set of tumor-specific mutations. Tumor samples were sequenced at 2×50 to a target of 10^8 read pairs with an Illumina HiSeq System (Illumina, San Diego, CA, USA). Whole-exome sequencing was performed by Expression Analysis (Research Triangle Park, NC, USA) using matched peripheral blood samples and an Agilent SureSelect Human All Exon V5 kit (Agilent Technologies, Santa Clara, CA, USA). Sequencing was done on an Illumina HiSeq System with a target of 100 $\times$ coverage. Sequencing reads were mapped to hg19 and the Ensembl gene annotations (ensGene; University of California, Santa Cruz, CA, USA) using RNA-STAR version 2.5.0b; whole-exome reads were mapped to hg19 using BWA-MEM version 0.7.12 [31, 32]. Mutation calling was performed on paired BAM files using VarDictJava version 1.4.2 [33]. Results were annotated using Ensembl Variant Effect Predictor version 85 to determine the location and type of mutation [34]. Tumor mutation count was defined for each patient as the total count of all missense mutations discovered for that sample.

Immune phenotype was determined from tumor samples using available immunohistochemistry data (PD-L1 stain and negative control) and hematoxylin and eosin stain. A pathologist who was masked to the response data scored the scanned images and determined the corresponding immune phenotype using an exploratory classification system [35–38]. Inflamed samples had immune cells in direct physical contact with tumor cells. Immune-excluded samples had $\geq$ 1% of the tumor stroma area populated by lymphocytes where they may be near tumor cells without infiltration and infrequent physical contact between lymphocytes and tumor cells. Immune-desert samples had < 1% of the tumor stroma area populated by lymphocytes, with no dense immune cell infiltrates and no contact between immune and tumor cells.

2.5 Statistical Analysis

Thirty patients were planned for this expansion cohort to give the study approximately 87% power to rule out $\leq$ 15% ORR (null hypothesis) when the true ORR is 35% with 1-sided α = 0.1. The analysis presented here was performed based on a data cutoff approximately 1.5 years after the last patient enrolled in the study. The confirmed ORR was

defined as the proportion of patients achieving a confirmed complete or partial response. The uncertainty of the estimates was assessed by a two-sided 95% Clopper-Pearson CI. DCR was defined as the proportion of patients with a best overall response of complete response, partial response, stable disease, or non-complete response/non-progressive disease. PFS and OS time were analyzed using the Kaplan-Meier method.

3 Results

3.1 Patient Demographics and Characteristics

Between 12 September 2016, and 31 January 2017, 47 patients with advanced, post-platinum esophageal AC who had disease progression after at least one prior line of treatment were screened; 30 patients from 21 centers met the eligibility criteria and were treated with bintrafusp alfa. Of these patients, 14 (46.7%) were from the USA, ten (33.3%) were from Europe, five (16.7%) were from Australia, and one (3.3%) was from Taiwan. The median age was 61 (range 30–80) years, and most patients had an ECOG performance status of 1 ($n = 25$ [83.3%]). This was a heavily pretreated population; 80.0% of patients had received at least two prior anticancer therapy regimens, and 36.7% of patients had received at least two prior lines of therapy for locally advanced or metastatic disease. Most tumors ($n = 20$ [66.7%]) were negative for PD-L1 expression, with nine (30.0%) being positive and one (3.3%) that was not evaluable. Detailed baseline characteristics are described in Table 1.

As of 24 August 2018, 30 patients received bintrafusp alfa for a median duration of 6.1 (range 2.0–55.7) weeks. The Kaplan-Meier estimate of median duration of follow-up since first dose was 86.1 (range 1.3–91.4) weeks. All patients had discontinued treatment at the time of data cutoff. Reasons for discontinuation were disease progression ($n = 19$ [63.3%]), AEs ($n = 8$ [26.7%]), death ($n = 2$ [6.7%]), and other reasons ($n = 1$ [3.3%]; patient completed 12 months' treatment). Following treatment with bintrafusp alfa, eight patients (26.7%) received at least one type of subsequent anticancer treatment, of whom six patients received cytotoxic therapy.

3.2 Efficacy

Responses occurred in six patients per independent review committee assessment, all of which were partial responses (confirmed ORR, 20.0% [95% CI 7.7–38.6]) (Table 2, Fig. 1a). The median duration of response was 4.3 (range 1.3–8.3) months (Table 2, Fig. 1b). An additional four patients had stable disease (DCR, 33.3% [95% CI

Table 1 Patient baseline and disease characteristics

Characteristic	$N = 30$
Median age, years (range)	61 (30–80)
Sex, n (%)	
Male	28 (93.3)
Female	2 (6.7)
ECOG performance status, n (%)	
0	5 (16.7)
1	25 (83.3)
Number of prior anticancer therapy regimens, n (%)	
1	6 (20.0)
2	14 (46.7)
≥ 3	10 (33.3)
Number of prior lines of therapy for locally advanced or metastatic disease, n (%)	
0	5 (16.7)
1	14 (46.7)
≥ 2	11 (36.7)
Type of prior anticancer therapy for locally advanced or metastatic disease, n (%)[a]	
Cytotoxic therapy	25 (83.3)
Monoclonal antibodies therapy	6 (20.0)
Small molecules	2 (6.7)
Tumor cell PD-L1 expression, n (%)[b]	
Positive	9 (30.0)
Negative	20 (66.7)
Not evaluable	1 (3.3)

ECOG Eastern Cooperative Oncology Group

[a]Patients may be included in more than one category

[b]A threshold of 1% was used to characterize tumors as either PD-L1 positive (≥ 1%) or negative (< 1%) using an anti-PD-L1 antibody clone 73-10

17.3–52.8]). The investigator-assessed confirmed ORR was 13.3% (95% CI 3.8–30.7); agreement between independent review committee and investigator-assessed responses is presented in the Online Supplementary Material (OSM), Table S1.

Median PFS was 1.3 months (95% CI 1.0–5.3) by independent review committee, with a 6-month rate of 25.9% (95% CI 11.5–43.1) (Fig. 2a). Similar median PFS was observed by investigator assessment (1.3 months [95% CI 1.1–5.3]) (OSM Fig. S1). Median OS was 3.4 months (95% CI 2.5–10.3), with respective 6- and 12-month OS rates of 39.7% (95% CI 22.0–57.0) and 32.1% (95% CI 16.0–49.5) (Fig. 2b).

3.3 Biomarker Analyses

Response to bintrafusp alfa was observed in patients with PD-L1-positive and PD-L1-negative tumors (Table 2). PD-L1 expression in the TME was also evaluated, and

Table 2 Treatment response to bintrafusp alfa

Efficacy ($N = 30$)	Independent review committee	Investigator
Confirmed best overall response, n (%)		
Complete response	0	0
Partial response	6 (20.0)	4 (13.3)
Stable disease	4 (13.3)	7 (23.3)
Progressive disease	15 (50.0)	12 (40.0)
Not evaluable	5 (16.7)	7 (23.3)
Confirmed ORR, n (%)	6 (20.0)	4 (13.3)
95% CI	7.7–38.6	3.8–30.7
DCR, n (%)	10 (33.3)	11 (36.7)
95% CI	17.3–52.8	19.9–56.1
Median duration of response, months (range)	4.3 (1.3–8.3)	NR (3.6–12.6)
ORR by tumor cell PD-L1 expression, n/N (%)[a]		
Positive	1/9 (11.1)	0/9 (0)
Negative	4/20 (20.0)	4/20 (20.0)
Not evaluable	1/1 (100)	0/1 (0)

DCR disease control rate, *NR* not reached, *ORR* objective response rate

[a] A threshold of 1% was used to characterize tumors as either PD-L1 positive ($\geq$ 1%) or negative ($<$ 1%) using an anti-PD-L1 antibody clone 73-10

responses to bintrafusp alfa, as determined by either independent review committee or investigator assessment, were observed independently of PD-L1 expression (data not shown). The majority of responses ($n = 5$ [83.3%]) occurred in patients with an immune-excluded phenotype (Fig. 3a). The ORR by independent review committee was 12.5% in the inflamed, 27.8% in the immune-excluded, and 0% in the immune-desert phenotype. However, responses to bintrafusp alfa were observed independently of tumor mutation count, as well as expression of genes associated with an active immune pathway (such as *CD8A*, *CD8B*, and *IFNG*) or TGF-β activation (such as *TGFB1*, *TWIST1*, *VIM*) (Fig. 1a, Fig. 3b–g). Further investigation revealed different average levels of tumor *TGFB1* expression between patients with esophageal AC in this study and patients with esophageal SCC in a separate phase 1 study of bintrafusp alfa [39], with 41.8% lower expression in AC than in SCC (false discovery rate–adjusted $p < 0.002$). *TGFB3* expression was also lower in patients with esophageal AC than in those with esophageal SCC, but the difference was not significant (false discovery rate–adjusted $p = 0.426$). In contrast, *TGFB2* expression was 96.6% higher in esophageal AC than in esophageal SCC (false discovery rate–adjusted $p = 0.049$) (OSM Fig. S2). When compared across patients from phase 1 trials of bintrafusp alfa, *TGFB* expression levels in tumor samples from patients with esophageal AC were not significantly different from those from patients with other tumor types with various histologies (false discovery rate–adjusted $p = 0.465$ [*TGFB1*]; $p = 0.989$ [*TGFB2*]; $p = 0.082$ [*TGFB3*]).

3.4 Safety

Among the 30 patients, 19 (63.3%) experienced a TRAE of any grade, of which diarrhea, pruritus ($n = 4$ [13.3%] each), and asthenia ($n = 3$ [10.0%]) were most common (Table 3). Seven patients (23.3%) experienced at least one grade 3 TRAE, including anemia ($n = 2$ [6.7%]), Bowen's disease (also known as SCC in situ), cancer pain, gastritis hemorrhagic, hypophysitis, hypopituitarism, rash generalized, and SCC of skin ($n = 1$ [3.3%] each). No grade 4 TRAEs or treatment-related deaths occurred. TRAEs led to permanent discontinuation in two patients (6.7%). One patient developed a cough 2 days after the first dose of bintrafusp alfa. The cough persisted, and 4 weeks later the patient developed dyspnea (grade 1), determined as treatment related by the investigator. Imaging showed lymphangitis carcinomatosis and findings that were suggestive of multiple small lung metastases. Bintrafusp alfa was permanently discontinued, and the patient withdrew from the study due to confirmed disease progression. The second patient permanently discontinued treatment due to grade 3 gastritis hemorrhagic, also determined as treatment related by the investigator. This patient had a history of pseudoaneurysm of the celiac trunk and developed treatment-emergent gastritis hemorrhagic 3 weeks after the first study dose of bintrafusp alfa (9 days after the preceding dose). Gastrointestinal bleeding of unknown origin was found upon hospitalization. This event improved after 3 weeks following blood transfusions and treatment with parenteral tranexamic acid, somatostatin, and proton pump inhibitors. Following resolution of the AE and
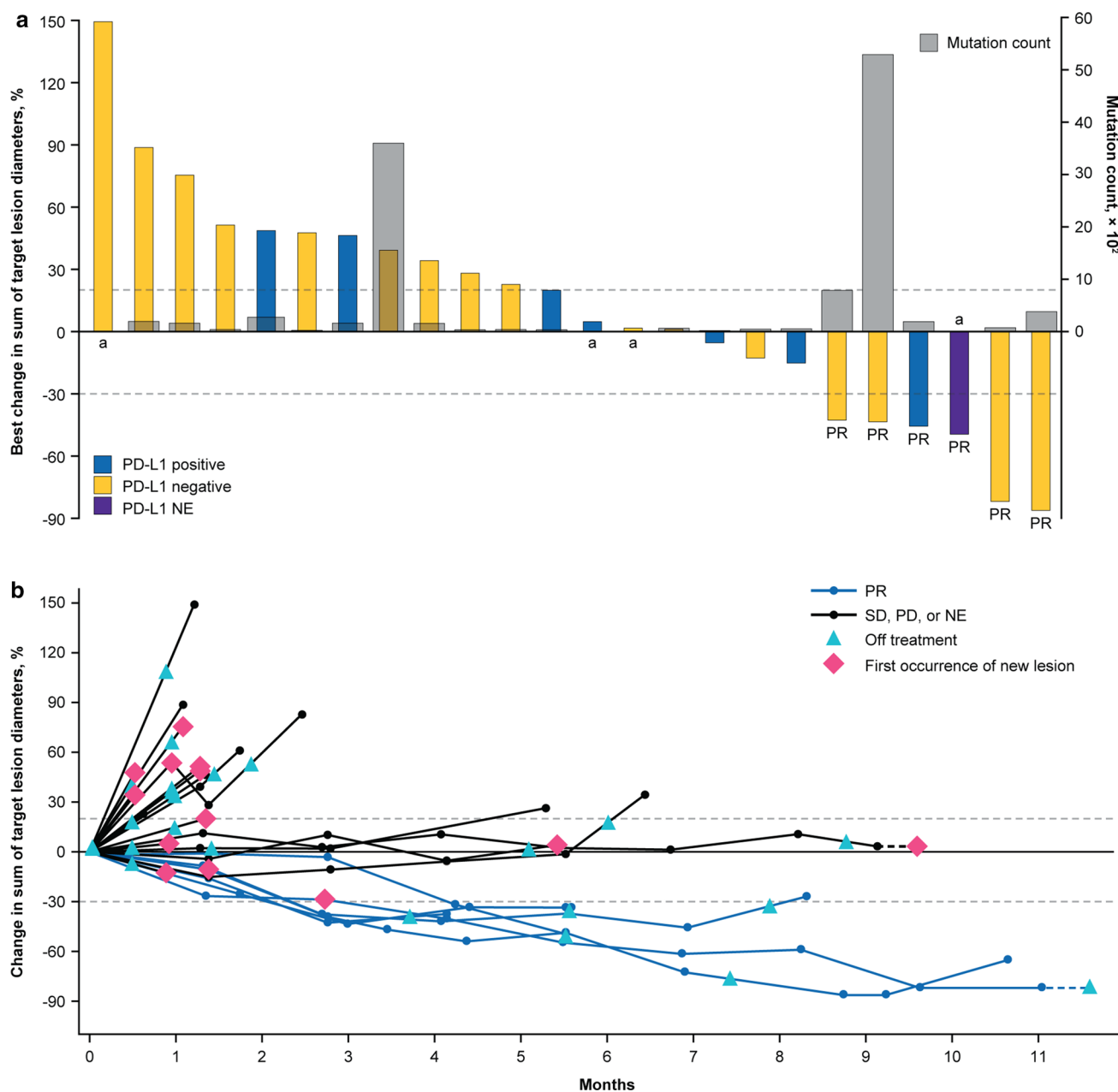
Fig. 1 Tumor response to bintrafusp alfa assessed by independent review. **a** Best change in sum of diameters and tumor mutation count. A threshold of 1% was used to characterize tumors as either PD-L1 positive (≥ 1%) or negative (< 1%) using an anti-PD-L1 antibody clone 73-10. One patient had non-evaluable PD-L1 expression. **b** Time to and duration of response. The upper dashed line represents progression at 20% increase in size of target lesions, and the lower dashed line represents the RECIST boundary for partial response at 30% decrease in size of target lesions. Six patients are not shown because they had no post-baseline assessments due to death within 6 weeks after starting treatment (n = 2), had no baseline and post-baseline target lesion measurement (n = 1), or due to other reasons (n = 3). [a]Tumor mutation count data unavailable. *NE* not evaluable, *PD* progressive disease, *PR* partial response, *SD* stable disease

reinitiation of bintrafusp alfa, grade 3 gastritis hemorrhagic recurred and was determined to be related to bintrafusp alfa. The patient permanently discontinued study treatment and received the same medical management that was previously used to treat their gastritis hemorrhagic for 3 weeks until the event resolved. Seven patients (23.3%) had irAEs, none

of which were above grade 3 or led to discontinuation of treatment (OSM Table S2). No patient experienced a grade ≥ 3 infusion-related reaction that was assessed by the investigator as being related to bintrafusp alfa. Potentially TGF-β-related skin lesions were reported in two patients (6.7%), one of whom experienced multiple events (OSM Table S2).
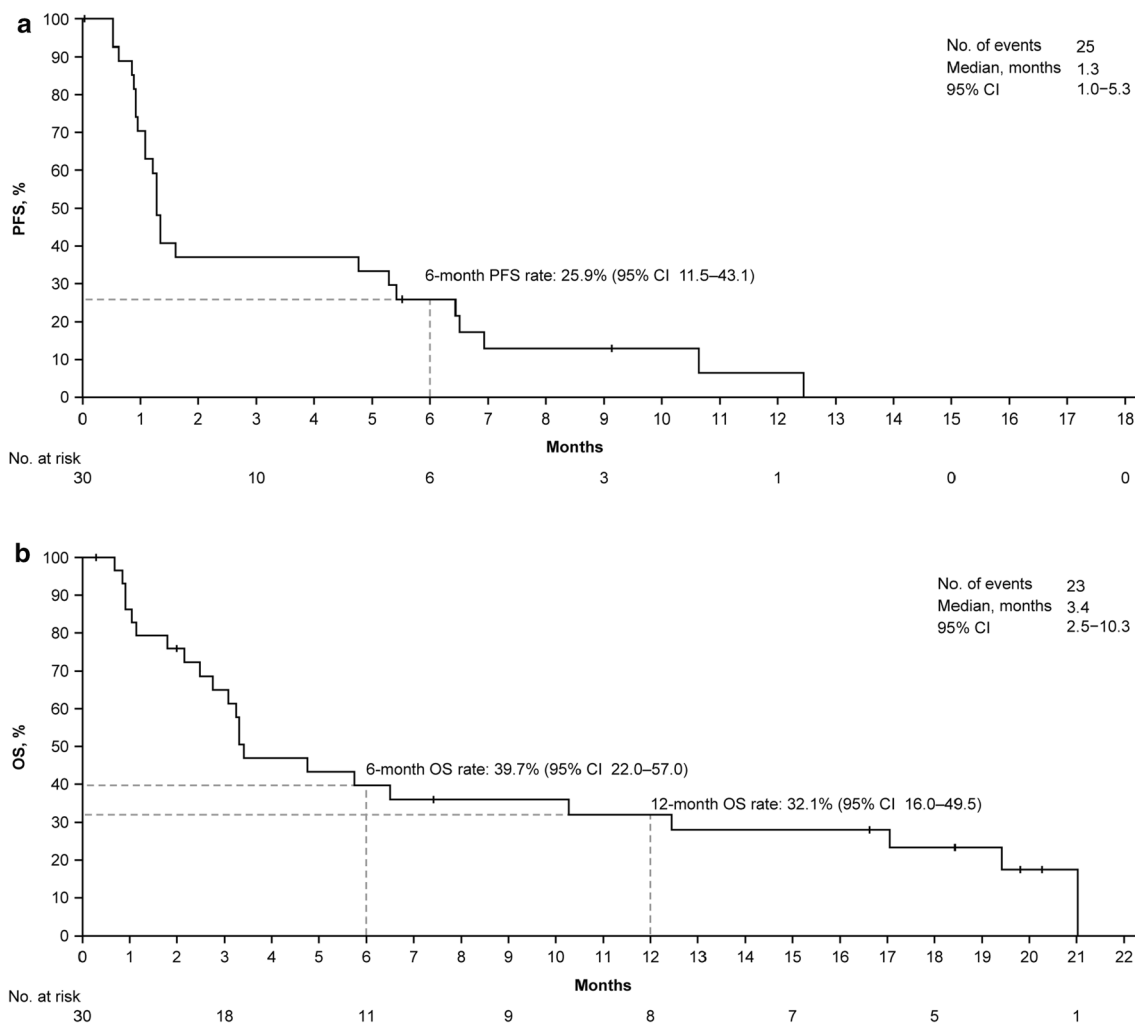
Fig. 2 Survival outcomes. Kaplan–Meier survival curves for **a** independent review committee-assessed progression-free survival (PFS) and **b** overall survival (OS)

These skin lesions were generally managed well with complete excision when needed and did not lead to treatment discontinuation.

4 Discussion

In this phase 1, dose-expansion cohort of 30 patients with advanced, post-platinum esophageal AC, bintrafusp alfa showed evidence of clinical activity with an ORR of 20.0% per independent review committee assessment and a 12-month OS rate of 32.1% in the overall, PD-L1-unselected cohort. Additionally, responses were independent of PD-L1 expression on tumor cells or on cells of the TME. Phase 2 and 3 studies of pembrolizumab showed ORRs that ranged from 3 to 18% and 12-month OS rates that were between 22 and 24% in patients with pretreated esophageal AC, depending on PD-L1 expression [14, 15]. However, the results observed here for bintrafusp alfa cannot be directly compared with those reported for pembrolizumab because of key differences in study design. Larger studies are needed to confirm the impact of PD-L1 expression on clinical activity with bintrafusp alfa in esophageal AC.

Results of exploratory analyses showed that response to bintrafusp alfa was independent of tumor mutation count. Patients in this esophageal AC cohort had lower mutation counts than patients with esophageal SCC enrolled in a different phase 1 study of bintrafusp alfa (NCT02699515) [39]. Furthermore, the average expression levels of *TGFB1*, *TGFB2*, and *TGFB3* in tumor samples from patients in this study were similar to those from patients with other tumors across phase 1 studies of bintrafusp alfa; however, the levels of *TGFB1* and *TGFB2* were significantly different (lower and higher, respectively) than those observed in an esophageal SCC cohort of the separate phase 1 study of bintrafusp alfa. Previous reports have shown a link between TGF-β
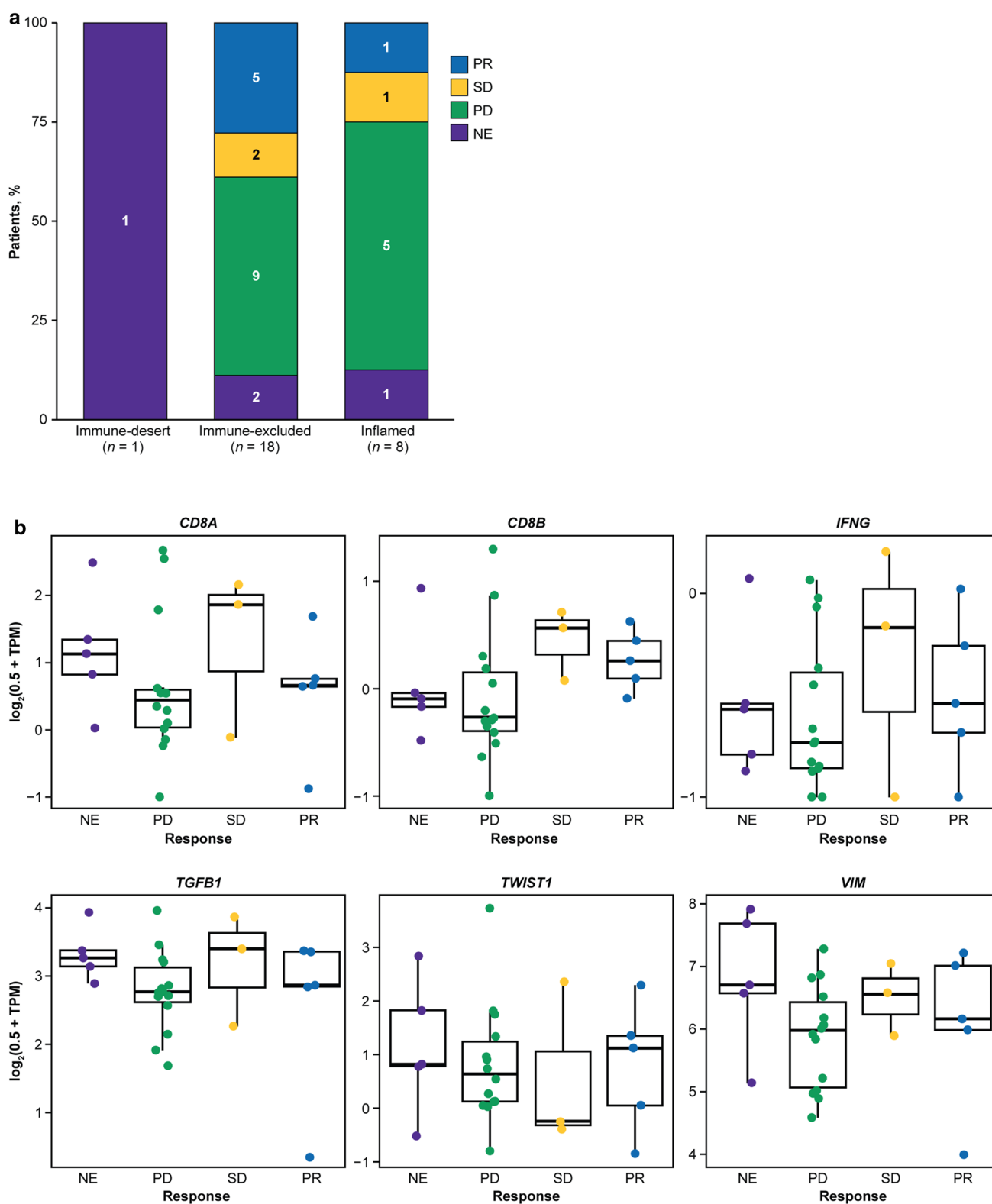
Fig. 3 Exploratory biomarker analysis by confirmed best overall response to bintrafusp alfa per independent review committee assessment. **a** Immune phenotype analysis; three patients with immune phenotypes of indeterminate ($n = 1$) or not annotated ($n = 2$) are not shown. **b** Gene expression analysis; three patients with confirmed best overall responses of partial response, stable disease, and progressive disease ($n = 1$, each) were not included in the RNAseq analysis due to failing QC or lack of RNAseq data. *IFNG* interferon gamma, *NE* not evaluable, *PD* progressive disease, *PR* partial response, *SD* stable disease, *TGFB1* transforming growth factor beta 1, *TPM* transcript per million, *TWIST1* twist family bHLH transcription factor 1, *VIM* vimentin

Table 3 TRAEs occurring at any grade in ≥ 5% of patients or at grade 3 severity, and any AEs of special interest

Preferred term, n (%)	N = 30	
	Any grade	Grade 3[a]
TRAEs		
TRAE	19 (63.3)	7 (23.3)
Diarrhea	4 (13.3)	0
Pruritus	4 (13.3)	0
Asthenia	3 (10.0)	0
Anemia	2 (6.7)	2 (6.7)
Rash generalized	2 (6.7)	1 (3.3)
Alanine aminotransferase increased	2 (6.7)	0
Aspartate aminotransferase increased	2 (6.7)	0
Dyspnea	2 (6.7)	0
Fatigue	2 (6.7)	0
Hyperthyroidism	2 (6.7)	0
KA	2 (6.7)	0
Nausea	2 (6.7)	0
Rash	2 (6.7)	0
Rash, macular	2 (6.7)	0
Bowen's disease[b]	1 (3.3)	1 (3.3)
Cancer pain	1 (3.3)	1 (3.3)
Gastritis hemorrhagic	1 (3.3)	1 (3.3)
Hypophysitis	1 (3.3)	1 (3.3)
Hypopituitarism	1 (3.3)	1 (3.3)
SCC of skin	1 (3.3)	1 (3.3)
Any AE of special interest		
Skin lesions[c]	2 (6.7)	1 (3.3)
Any irAE	7 (23.3)[d]	2 (6.7)
Immune-related rash	5 (16.7)	1 (3.3)
Immune-related pituitary dysfunction	1 (3.3)	1 (3.3)
Immune-related pneumonitis	1 (3.3)	0
Immune-related thyroid disorders	1 (3.3)	0

AE adverse event, *irAE* immune-related adverse event, *KA* keratoacanthoma, *SCC* squamous cell carcinoma, *TRAE* treatment-related adverse event

[a]No patients experienced a grade > 3 TRAE

[b]Also known as SCC in situ

[c]Includes actinic keratosis, basal cell carcinoma, Bowen's disease, hyperkeratosis, KA, lip SCC, and SCC of skin NCI-CTCAE v4.03 preferred terms

[d]Three patients experienced multiple different irAEs

and immune phenotype, suggesting TGF-β can restrict T-cell infiltration in the TME and limit antitumor immunity [16, 40]. In preclinical studies, the combined use of a TGF-β inhibitor with a PD-L1 inhibitor significantly increased tumor-infiltrating T cells and significantly reduced tumor burden in mouse models of immune-excluded tumors [16]. Interestingly, all but one response occurred in immune-excluded tumors in this esophageal AC cohort. Similar results were also observed in patients with esophageal SCC from the other study, in which responses were seen exclusively in immune-excluded tumors [39]. Taken together, the results of the exploratory analyses did not identify predictive biomarkers of response to bintrafusp alfa in this small cohort.

Treatment with bintrafusp alfa was well tolerated in this patient population, with no grade 4 TRAEs or treatment-related deaths. The safety profile, including incidence of irAEs, was similar to that seen with checkpoint inhibitors, except for potentially TGF-β-related skin lesions, which were reported in two patients [14, 15, 41–48]. These AEs were generally managed well and did not lead to discontinuation from the trial. Importantly, skin lesions, such as keratoacanthomas, are anticipated AEs for TGF-β inhibition [49]. The overall safety profile reported here for bintrafusp alfa in esophageal AC was consistent with those previously observed with bintrafusp alfa treatment in other solid tumors, including esophageal SCC [23, 24, 39].

Limitations of this study include the small enrollment size and the lack of a comparator arm, which preclude any definitive conclusions regarding comparisons of bintrafusp alfa with other available treatment options.

The clinical activity and manageable safety profile observed in this study of heavily pretreated patients with esophageal AC suggest that bintrafusp alfa could provide a novel treatment approach for these patients. Further clinical investigation of bintrafusp alfa in esophageal cancer is warranted based upon these results.

or when the product has been out-licensed, the responsibility for disclosure might be dependent on the agreement between parties. Under these circumstances, Merck KGaA will endeavor to gain agreement to share data in response to requests.

Declarations

Conflicts of interest/Competing interest B. Tan received research grants from EMD Serono Research & Development Institute, Inc., Billerica, MA, USA; an affiliate of Merck KGaA, Darmstadt, Germany. A. Khattak has consulted for Merck Healthcare Pty. Ltd., Australia; an affiliate of Merck KGaA, Darmstadt, Germany; received research grants from Merck Sharp & Dohme; and reports honoraria from Bayer, Bristol Myers Squibb, Merck Sharp & Dohme, Pfizer, Merck Healthcare Pty. Ltd., and Novartis. E. Felip has consulted for AbbVie, AstraZeneca, Blueprint Medicines, Boehringer Ingelheim, Bristol Myers Squibb, Eli Lilly, Guardant Health, Janssen, Merck KGaA, Darmstadt, Germany, Merck Sharp & Dohme, Novartis, Pfizer, Roche, Samsung, Takeda, and GlaxoSmithKline; has received research grants from Fundación Merck Salud; reports honoraria from AstraZeneca, Boehringer Ingelheim, Bristol Myers Squibb, Eli Lilly, prIME Oncology, Medscape, and touchIME; and is an independent member of the Grifols board. K. Kelly has consulted for AbbVie, AstraZeneca, Bristol Myers Squibb, EMD Serono Research & Development Institute, Inc., Billerica, MA, USA; an affiliate of Merck KGaA, Darmstadt, Germany, Eli Lilly, Inviata, Novartis, Regeneron, and Takeda; received research grants and other research support from AbbVie, Bristol Myers Squibb, Celgene, EMD Serono Research & Development Institute, Inc., Five Prime, Genentech, Eli Lilly, Lycera, Merck, Novartis, Regeneron, Tizona, and Transgene; reports honoraria from Merck; and reports royalties from UpToDate. P. Rich is an employee of Cancer Treatment Centers of America. D. Wang declares no competing interests. C. Helwig is an employee of and has ownership interest in Merck KGaA, Darmstadt, Germany. I. Dussault is an employee of EMD Serono Research & Development Institute, Inc., Billerica, MA, USA; an affiliate of Merck KGaA, Darmstadt, Germany; and has ownership interest in EMD Serono Research & Development Institute, Inc., and Amgen. L.S. Ojalvo is an employee of and reports intellectual property interests with EMD Serono Research & Development Institute, Inc., Billerica, MA, USA; an affiliate of Merck KGaA, Darmstadt, Germany. N. Isambert has consulted for Ipsen and Transgene.

Ethics approval and consent to participate The protocol for this study was approved by all relevant regulatory authorities and ethics committees at participating institutions, and the study was conducted in accordance with the International Conference on Harmonisation Topic E6 Good Clinical Practice and the Declaration of Helsinki. Each patient provided written informed consent before study enrollment.

Consent for publication All authors gave final approval of the version to be published.

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Author contributions CH, ID, and LSO contributed to study conception and design; BT, AK, EF, KK, PR, DW, and NI contributed to data acquisition. All authors contributed to data interpretation and drafting

of the manuscript. All authors reviewed and approved the final manuscript and approved the final manuscript for publication.

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