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Impact of Sequential Ramucirumab Plus Docetaxel After PD-1 Inhibitors on Anti-PD-1 Antibody-Bound T-Cell Dynamics and Clinical Outcomes

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ABSTRACT

Ramucirumab plus docetaxel (RAM+DOC) demonstrates clinical activity after programmed cell death-1 (PD-1) inhibitors in advanced non-small cell lung cancer (NSCLC); however, the underlying mechanisms remain unclear. We aimed to evaluate clinical efficacy and explore immunologic dynamics and benefit-associated biomarkers. Patients treated with RAM+DOC after PD-1 inhibitors were enrolled in a multicenter prospective cohort. Anti-PD-1 antibody bound ($IgG4^+$) T-cell subsets were measured at baseline (T0) and after 2–3 cycles (T1), reflecting residual anti-PD-1 antibody binding on circulating T cells. T1/T0 ratios of immune subsets were calculated to assess dynamics. Landmark analyses at T1 evaluated associations with progression-free survival (PFS) and overall survival (OS). Prognostic biomarkers were assessed at baseline. Among 27 evaluable patients, the objective response rate was 37.0%, median PFS 5.1 months, and OS 10.4 months. RAM + DOC

Abbreviations: DCR, disease control rate; DOC, docetaxel; ECOG-PS, Eastern Cooperative Oncology Group-performance status; ICIs, immune checkpoint inhibitors; NR, not reached; NSCLC, non-small cell lung cancer; ORR, objective response rate; OS, overall survival; PBMCs, peripheral blood mononuclear cells; PD-1, programmed cell death-1; PFS, progression-free survival; RAM, ramucirumab; RECIST, Response Evaluation Criteria in Solid Tumors; Tcm, central memory; Tem, effector memory; Temra, terminally differentiated effector memory RA^+; Tnaive, naïve; TPS, tumor proportion score; TRAE, treatment-related adverse events; Tregs, regulatory T-cells; VEGF, vascular endothelial growth factor; VIFs, variance inflation factors.

Kinnosuke Matsumoto and Yujiro Naito contributed equally to this work.

responders had higher IgG4$^+$CD8$^+$ T-cell and lower IgG4$^+$ Treg T1/T0 ratios (both $p < 0.001$). Higher IgG4$^+$CD8$^+$ ratios were associated with longer landmark PFS ($p = 0.002$) and OS ($p = 0.016$) and were inversely correlated with IgG4$^+$ Treg ratios ($p = 0.008$). Among the baseline factors, high IgG4$^+$CD8$^+$ Temra conferred survival benefits (OS, not reached vs. 8.2 months; $p = 0.006$), and low vascular endothelial growth factor (VEGF)-C levels were associated with longer OS (not reached vs. 8.5 months, $p = 0.044$). Both variables remained independent prognostic factors of PFS and OS in multivariable analysis. Our findings suggest that sequential strategy administering RAM+DOC during persistent binding of anti–PD-1 antibody to T cells may be beneficial. IgG4$^+$CD8$^+$ Temra and VEGF-C levels at RAM+DOC initiation may serve as biomarkers of survival benefit.

Trial Registration: UMIN-Clinical Trials Registry (UMIN000050478)

1 | Introduction

Immune checkpoint inhibitors (ICIs) have transformed the therapeutic landscape and markedly improved survival in patients with advanced non-small cell lung cancer (NSCLC). Nivolumab plus ipilimumab and pembrolizumab were approved on the basis of phase III randomized controlled trials, including CheckMate 227, CheckMate 9LA, and KEYNOTE-042, -189, and -407 [1–5]. These anti-programmed cell death-1 (PD-1) regimens are standard first-line options for patients with advanced NSCLC harboring *EGFR* and *ALK* wild-type. Ramucirumab (RAM), a monoclonal antibody targeting vascular endothelial growth factor receptor 2 (VEGFR2), in combination with docetaxel (DOC), is a promising subsequent-line option after PD-1 inhibitors. Several real-world analyses have shown favorable survival outcomes in this setting compared with those in ICI-naive patients [6, 7]. These observations have been consistently corroborated by some prospective studies in comparable post-ICI cohorts [8–10], collectively suggesting that anti-angiogenic modulation may restore responsiveness to PD-1 inhibitors in advanced NSCLC; however, the underlying mechanisms remain unclear.

Synergy between anti-angiogenic therapy and immunotherapy has been reported in previous studies [11, 12]. VEGFR2 is expressed on the surface of various immune cells, including dendritic cells, macrophages, and regulatory T cells (Tregs). Zhu et al. showed that Tregs expressed high levels of VEGFR2 and that VEGFR2 inhibition reduced their infiltration into tumor tissues [13]. VEGF blockade has also been linked to the suppression of the immunosuppressive phenotype of VEGFR-positive myeloid cells and enhanced T-cell activation by mitigating TOX-dependent exhaustion [14]. These findings support the premise that VEGFR2 inhibition can potentiate the efficacy of ICIs in solid tumors. We previously developed a simplified approach to monitor anti-PD-1 antibody–bound T cells (IgG4$^+$ T cells) by leveraging nivolumab's IgG4 isotype and demonstrated that anti-PD-1 antibody binding on T cells persists for more than 20 weeks after the last infusion [15]. Together, these data suggest that VEGFR2 blockade administered during the persistence of anti–PD-1 antibody binding may beneficially modulate IgG4$^+$ T cells.

Accordingly, we aimed to elucidate the clinical efficacy and safety of RAM plus DOC after prior immunotherapy in advanced NSCLC. We also sought to explore longitudinal peripheral blood mononuclear cells (PBMCs)- and plasma-based immunologic/molecular dynamics and candidate biomarkers measurable before RAM initiation.

2 | Materials and Methods

2.1 | Study Design

This multicenter prospective cohort study was conducted at 12 institutions in Japan between March 10, 2023, and August 30, 2024. The study was approved by the Central Institutional Review Board of the University of Osaka (IRB #22406(T14)-6), conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines, and registered with the UMIN-Clinical Trials Registry (UMIN000050478). Written informed consent was obtained from all participants before any study-related procedures.

2.2 | Patient Selection

Eligible patients were required to have histologically or cytologically confirmed NSCLC; stage III or IV disease or postoperative recurrence; receipt of RAM plus DOC within two subsequent lines of therapy after PD-1 inhibitors (nivolumab or pembrolizumab) with or without platinum-based chemotherapy; age ≥ 18 years; Eastern Cooperative Oncology Group-performance status (ECOG-PS) 0 or 1; and at least one measurable lesion. For patients with driver gene alterations, molecular targeted therapy was permitted before the initiation of PD-1 inhibitors. Detailed inclusion and exclusion criteria are provided in the clinical protocol (Appendix S1).

2.3 | Procedures

Patients received RAM (10 mg/kg) plus DOC (60 mg/m^2) as routine clinical care according to the approved labels; one cycle was defined as 3 weeks. Treatment was continued until disease progression or unacceptable toxicity occurred. Clinical data, including age, sex, ECOG-PS, smoking history, histology, PD-L1 tumor proportion score (TPS), clinical stage, malignant pleural effusion, liver and brain metastasis, prior treatments, treatment outcomes, and treatment-related adverse events (TRAEs), were extracted from electronic medical records. Tumor response was assessed after two to three treatment cycles according to the Response Evaluation Criteria in

Solid Tumors (RECIST) version 1.1 [16]. Patients were categorized as responders (complete response or partial response) or non-responders (stable disease or progressive disease). Overall survival (OS) was defined as the time from RAM plus DOC initiation to death from any cause, and progression-free survival (PFS) was defined as the time from RAM plus DOC initiation to disease progression or death from any cause. Safety was evaluated using Common Terminology Criteria for Adverse Events, version 5.0, based on TRAE incidence, treatment discontinuation, and treatment-related death [17]. The data cutoff date was July 30, 2025.

2.4 | PBMCs Sampling and Flow Cytometry

PBMCs were collected at baseline (T0) and after two to three treatment cycles (T1). Samples were processed within 12 h, and PBMCs were separated using density-gradient centrifugation. Fractions containing 1.0×10^6 to 1.0×10^7 cells were cryopreserved using a cell-banking solution. Frozen PBMCs were thawed at room temperature (RT) and initially stained with the LIVE/DEAD Fixable Dead Cell Stain Kit (Invitrogen). Cells were subsequently incubated with a human Fc receptor–blocking reagent (Miltenyi Biotec) for 15 min at RT and stained with eight pre-titrated premixed antibody panels (Table S1) for 20 min at RT in the dark. Foxp3/Transcription Factor Staining Buffer (eBioscience) was used for intracellular staining. After washing, samples were analyzed using a FACSCanto II flow cytometer (BD Biosciences). Data were analyzed using FlowJo software, version 10.10.0 (BD Biosciences). Statistical analyses used cell proportions at T0 and T1, and the T1/T0 ratio.

2.5 | Plasma Sampling and Biomarker Assays

Plasma was collected at T0 and T1 in parallel with PBMC sampling. Biomarkers were measured using a Luminex multiplex assay kit (R&D Systems) on a Bio-Plex 200 system (Bio-Rad) according to the manufacturer's instructions. Each sample was assayed in triplicate, and the mean concentrations of seven angiogenesis-related analytes (VEGF-A, VEGF-C, angiopoietin-1, angiopoietin-2, FGF, PlGF, and Fas ligand) were reported in pg/mL. Statistical analyses were performed using plasma concentrations at T0 and T1, and paired changes (T1–T0).

2.6 | Endpoints and Sample Size Calculation

The primary endpoint was objective response rate (ORR) to RAM plus DOC after prior immunotherapy. Exploratory endpoints comprised $IgG4^+$ T cell-focused peripheral immune profiling and angiocrine factor analyses, including longitudinal pre/post-RAM comparisons between responders and non-responders. An additional exploratory objective was to identify candidate prognostic factors associated with the survival outcomes. Sample size was based on ORR. Assuming a conventional ORR of 19.0% and an expected ORR of 38% [18], a one-sample exact binomial test (one-sided $\alpha=0.10$; power $=0.80$) required 25 patients; allowing 10% dropout, 28 patients were planned (Appendix S1: Section 6.2). Immunologic and angiocrine analyses were hypothesis-generating endpoints, as post-ICI effect-size estimates were unavailable for a dedicated power calculation.

2.7 | Statistical Analysis

Survival was analyzed using the Kaplan–Meier method to estimate median survival with 95% confidence intervals (CIs) and compared by the log-rank test. Patients were dichotomized into high and low groups using the median cellular proportion or plasma level. A pre-specified 6-week landmark analysis after two treatment cycles was performed; PFS and OS were redefined from the landmark based on pre- and post-treatment cellular proportion ratios. T-cell subset ratios (T1/T0) and plasma changes (T1 − T0) were compared between responders and non-responders using a two-sided Wilcoxon rank-sum test, and associations between cellular and plasma dynamics were assessed with Spearman's rank correlation (ρ). Hazard ratios (HRs) and 95% CIs were estimated using Cox proportional hazards models. Multivariable models were exploratory and parsimonious, with covariates chosen for biological plausibility and univariable support and limited to reduce overfitting. Sensitivity analysis used leave-one-out refitting ($n=27$), sequentially excluding one patient and re-estimating the multivariable Cox model. Multicollinearity among T-cell subsets was evaluated using variance inflation factors (VIFs), with a VIF <5 considered acceptable. A two-sided $p<0.05$ was considered statistically significant. All analyses were conducted using R software (version 4.4.2; R Foundation for Statistical Computing, Vienna, Austria).

3 | Results

3.1 | Patient Characteristics

Figure 1A outlines the patient flow. Between March 2023 and August 2024, 28 eligible patients were enrolled. One patient experienced a severe adverse event after the first cycle and was excluded from subsequent analyses, leaving 27 evaluable patients. T1 samples were unavailable for 1 patient, yielding 26 patients for mechanistic analyses. At the data cutoff, 2 patients remained on protocol therapy without progression, and 25 patients had discontinued due to disease progression ($n=14$) or adverse events ($n=11$). The baseline characteristics of the 27 efficacy-evaluable patients are summarized in Table 1. The median age was 71 years (interquartile range [IQR], 67.5–74), and adenocarcinoma was present in 17 patients (63%). Twenty-one patients (78%) had ECOG-PS 1 and had previously received pembrolizumab plus chemotherapy as first-line therapy, while the remaining patients received platinum doublets followed by PD-1 inhibitor monotherapy. The median interval from the last PD-1 inhibitor dose to RAM initiation was 32 days (IQR, 25–50). During prior ICI therapy, the median PFS was 224 days (IQR, 150–278), and immune-related adverse events (irAEs) occurred in 14 of 27 patients (Table S2). Comprehensive genomic profiling was performed in 24 patients (88.9%), and oncogenic alterations were identified in 7 (*EGFR* exon20 S768I, $n=1$; *KRAS* G12A, $n=1$; *KRAS* G12V, $n=1$; *PIK3CA*, $n=1$; *TP53*, $n=3$). Although permitted per protocol, none of the patients had received prior tyrosine kinase inhibitor therapy (Table S3).

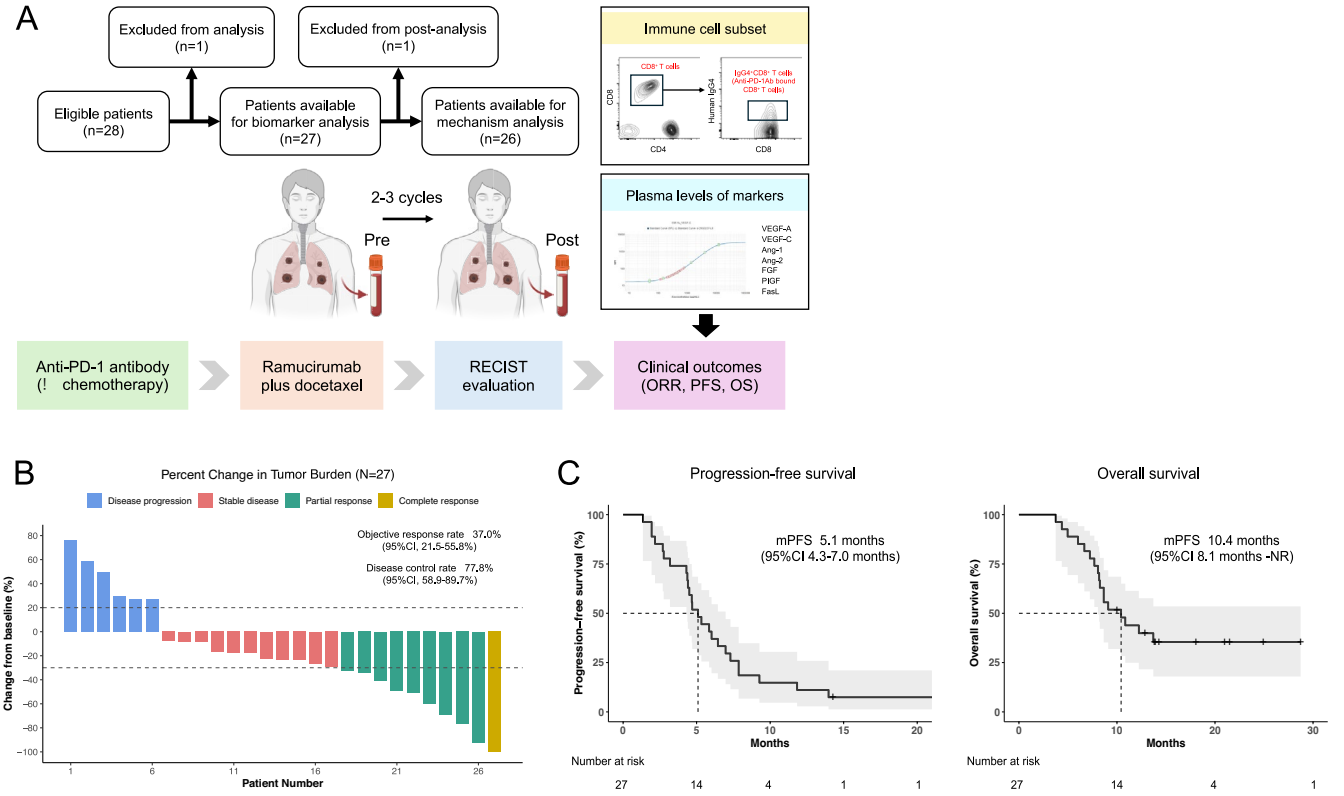

FIGURE 1 | Clinical efficacy and biomarker analysis of ramucirumab plus docetaxel after anti–PD-1 therapy. (A) Flowchart of patient enrollment and study design. (B) Waterfall plot of maximum percent change in tumor size from baseline. (C) Kaplan–Meier curves of progression-free survival and overall survival in all patients. ORR, objective response rate; OS, overall survival; PD-1, programmed cell death-1; PFS, progression-free survival; RECIST, Response Evaluation Criteria in Solid Tumors.

3.2 | Clinical Outcomes in the Overall Cohort

As shown in Figure 1B, 21 of the 27 patients (77.8%) achieved a decrease in target-lesion burden. The ORR and disease control rate (DCR) were 37.0% (95% CI, 21.5–55.8) and 77.8% (95% CI, 58.9–89.7), respectively. With a median follow-up of 16.2 months, disease progression was observed in 25 patients, and 17 deaths had occurred by the data cutoff. Median PFS and OS were 5.1 months (95% CI, 4.3–7.0) and 10.4 months (95% CI, 8.1–not reached [NR]), respectively (Figure 1C).

3.3 | Effect of RAM Plus DOC on Anti-PD-1 Antibody–Bound T Cells

The representative gating of IgG4$^+$ T cells is shown in Figure 2A [15]. Under RAM plus DOC, responders exhibited a higher T1/T0 ratio of IgG4$^+$CD8$^+$ T cells and a lower ratio of IgG4$^+$ Tregs compared with non-responders (both $p < 0.001$) (Figure 2B). Baseline (T0) and post-treatment (T1) frequencies and the paired changes are shown in Figure S1. Moreover, in the pre-specified 6-week landmark analysis after two cycles, patients with higher IgG4$^+$CD8$^+$ T-cell ratios had significantly longer PFS and OS than those with the lower ratios (HR, 0.24; 95% CI, 0.10–0.65; $p = 0.002$; and HR, 0.30; 95% CI, 0.11–0.84; $p = 0.016$, respectively). In contrast, patients with lower IgG4$^+$ Treg ratios experienced significantly longer PFS than those with higher ratios (HR, 7.24; 95% CI, 2.44–21.5; $p < 0.001$) (Figure 2C). The IgG4$^+$CD8$^+$ T-cell ratio was inversely correlated with the IgG4$^+$ Treg ratio (Figure 2D). The concentrations of each plasma marker at T0 and T1, along with the paired changes, are shown in Table S4. Among the angiocrine factors, responders exhibited a significantly smaller increase in VEGF-A levels than non-responders ($p = 0.025$) (Figure 2E). The IgG4$^+$ Treg ratio was positively correlated with ΔVEGF-A (T1–T0) (Figure 2F). Additionally, stratification by the median interval between the last PD-1 inhibitor and RAM initiation showed no significant differences in survival or in IgG4$^+$CD8$^+$ T-cell or IgG4$^+$ Treg ratios (both $p > 0.6$; Figure S2). Finally, neither prior ICI-PFS nor prior irAEs was associated with post/pre ratios of IgG4$^+$CD8$^+$ T cells and IgG4$^+$ Tregs (Figure S3).

3.4 | Prognostic Value of Baseline Cellular and Plasma Biomarkers in RAM Plus DOC

The gating strategy for lymphocytes is shown in Figure S4A. Across the overall T-cell panel, higher CD8$^+$ or CD56$^+$ T-cell levels were associated with significantly longer OS compared with low levels (Figure S4B). Within IgG4$^+$CD8$^+$ T-cell subsets, Temra showed a trend toward longer PFS in the high versus low groups (6.2 vs. 4.5 months, $p = 0.05$) and was significantly associated with longer OS (NR vs. 8.2 months, $p = 0.006$; Figure 3). In contrast, analyses of IgG4$^+$CD4$^+$ T-cell subsets revealed no significant association with either PFS or OS

TABLE 1 | Patient characteristics.

Characteristics	*N* (%)
All patients	27
Median age (IQR)	71 (67.5–74)
Age group, year	
<70	11 (40.7)
≥70	16 (59.3)
Sex	
Female	7 (25.9)
Male	20 (74.1)
ECOG-PS	
0	6 (22.2)
1	21 (77.8)
Smoking history	
Never	4 (14.8)
Current/past	23 (85.2)
Histology	
Adenocarcinoma	17 (63.0)
Other	10 (37.0)
PD-L1 TPS, %	
≥1	20 (74.1)
<1	7 (25.9)
Clinical stage	
IV	20 (74.1)
Others	7 (25.9)
Malignant pleural effusion	12 (44.4)
Liver metastasis	2 (7.4)
Brain metastasis	3 (11.1)
Interval from last ICI to RAM, day	
Median (IQR)	32 (25–50)
Number of prior treatment	
1	21 (77.7)
2	4 (14.9)
≥3	2 (7.4)
Prior ICI regimen	
Platinum + pemetrexed + pembrolizumab	13 (48.1)
Platinum + paclitaxel/ nab-paclitaxel + pembrolizumab	8 (29.6)
Pembrolizumab	1 (3.8)
Nivolumab	5 (18.5)
Prior ICI-PFS, day	
Median (IQR)	224 (150–278)
Prior irAE	14 (51.9)

Abbreviations: ECOG-PS, Eastern Cooperative Oncology Group-performance status; ICI, immune checkpoint inhibitor; IQR, interquartile range; irAE, immune-related adverse event; PD-L1 TPS, programmed death-ligand 1 tumor proportion score; PFS, progression-free survival; RAM, ramucirumab.

(Figure S5). Because these markers were derived from related T-cell compartments, multicollinearity was assessed, and all VIFs were < 5 (Table S5). Among the angiocrine factors, only plasma VEGF-C was prognostic, with longer OS in the low group than in the high group (NR vs. 8.5 months, $p = 0.044$; Figure 4). In multivariable analyses, high IgG4$^+$CD8$^+$ Temra levels were independently associated with improved PFS and OS (HR, 0.38; 95% CI, 0.14–0.99; $p = 0.047$; and HR, 0.18; 95% CI, 0.03–0.47; $p = 0.002$, respectively), whereas high VEGF-C was independently associated with shorter PFS and OS (HR, 3.34; 95% CI, 1.16–9.65; $p = 0.026$; and HR, 2.70; 95% CI, 1.03–7.20; $p = 0.0044$, respectively; Table 2). In leave-one-out refitting ($n = 27$), HRs remained directionally consistent: IgG4$^+$CD8$^+$ Temra (PFS 0.44–0.68; OS 0.21–0.39) and VEGF-C (PFS 1.36–2.10; OS 1.12–2.67).

3.5 | Treatment-Related Adverse Events

Safety was assessed in 27 patients who received at least one cycle of RAM plus DOC. Any-grade TRAEs occurred in 26 patients (96.3%), and grade ≥3 TRAEs occurred in 15 patients (55.6%). The most frequent TRAE was fatigue, reported in 16 patients (59.3%). Grade ≥3 neutropenia and febrile neutropenia were observed in seven (25.9%) and two (7.4%) patients, respectively. Prophylactic pegfilgrastim was administered to 25 patients (92.6%). Any-grade and grade ≥3 pneumonitis occurred in four (14.8%) and three (11.1%) patients, respectively. One grade 5 TRAE (3.7%) due to sepsis was reported (Table 3).

4 | Discussion

This prospective study is the first to evaluate the antitumor activity of RAM plus DOC after PD-1 inhibitors using PBMC profiling. IgG4$^+$ T cells were present in all patients at baseline. In responders, IgG4$^+$CD8$^+$ T cells were largely maintained, whereas IgG4$^+$ Tregs markedly declined compared with non-responders, a pattern associated with improved response and survival. IgG4$^+$ Tregs frequency correlated with changes in VEGF-A levels. These findings suggest that administering RAM plus DOC during persistent anti–PD-1 antibody binding may be a promising sequential strategy. Moreover, IgG4$^+$CD8$^+$ Temra and VEGF-C may be independent prognostic factors of PFS and OS.

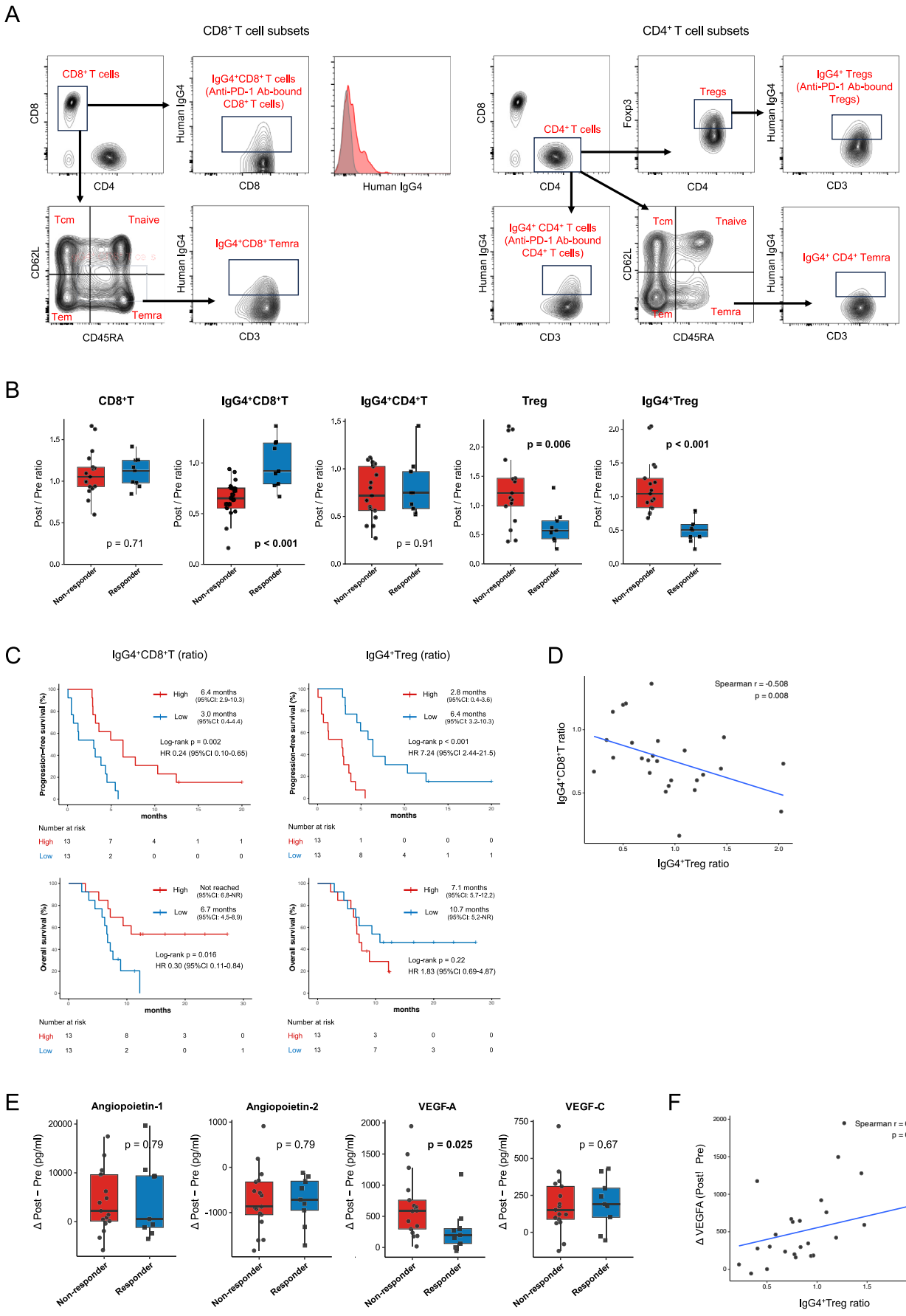

FIGURE 2 | Legend on next page.

FIGURE 2 | Ramucirumab plus docetaxel alters the dynamics of anti–PD-1 antibody–bound (IgG4$^+$) T cells associated with survival outcomes. (A) Representative gating strategy for IgG4$^+$ T cells (B) Post−/pre-treatment (T1/T0)* ratios of T-cell subsets (CD8$^+$ T cells, IgG4$^+$CD8$^+$ T cells, IgG4$^+$CD4$^+$ T cells, Tregs, IgG4$^+$ Tregs) in responders vs. non-responders; two-sided Wilcoxon rank-sum tests (p values shown) (C) Six-week landmark Kaplan–Meier curves for progression-free survival and overall survival stratified by high vs. low IgG4$^+$CD8$^+$ T-cell and IgG4$^+$ Treg ratios; log-rank p values and Cox hazard ratios (HRs) with 95% confidence intervals (CIs) are shown. (D) Spearman correlation between IgG4$^+$CD8$^+$ T-cell and IgG4$^+$ Treg ratios (E) Changes (Δ post–pre, pg/mL) in plasma angiocrine factors (angiopoietin-1, angiopoietin-2, VEGF-A, VEGF-C) in responders vs. non-responders; two-sided Wilcoxon rank-sum tests (F) Spearman correlation between ΔVEGF-A and the IgG4$^+$ Treg ratio. *T0, baseline; T1, after 2–3 cycles. PD-1, programmed cell death-1; VEGF, vascular endothelial growth factor.

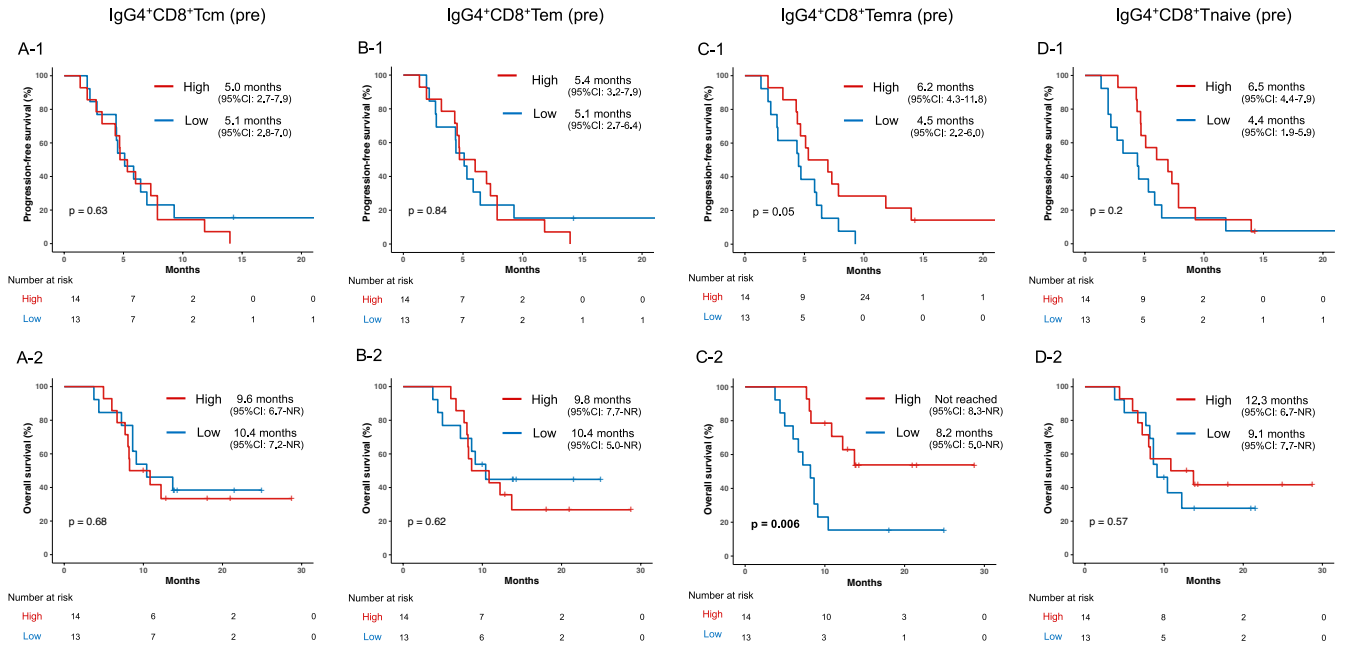

FIGURE 3 | Kaplan–Meier curves for progression-free survival (A1–D1) and overall survival (A2–D2) stratified by high vs. low baseline (T0) proportions of IgG4$^+$CD8$^+$ T-cell subsets (A: Tcm; B: Tem; C: Temra; D: Tnaive). Log-rank p values and 95% confidence intervals (CIs) are shown. NR, not reached; Tcm, central memory; Tem, effector memory; Temra, terminally differentiated effector memory RA$^+$; Tnaive, naïve.

Real-world data support the efficacy of RAM plus DOC after ICI exposure [7–10]. In the SCORPION trial ($n = 33$) after chemoimmunotherapy, ORR was 34.4%, DCR 81.3%, and median PFS 6.5 months [8]. Similarly, in the REACTIVE study ($n = 288$), ORR was 28.8%, DCR 69.8%, and median PFS 4.1 months [9]. Consistently, our study demonstrated ORR 37.0%, DCR 77.8%, and median PFS 5.1 months. OS appeared slightly shorter than in previous reports, possibly because approximately 20% of our cohort received RAM plus DOC as third-line or later therapy.

In a phase I trial of RAM monotherapy in solid tumors, plasma VEGF-A levels increased after RAM, consistent with compensatory upregulation following VEGFR2 inhibition [19]; similar patterns were observed with RAM monotherapy in hepatocellular carcinoma and with RAM combined with chemotherapy in colorectal cancer [20, 21]. Conversely, the phase III RAINBOW trial showed that RAM decreased plasma angiopoietin-2 levels [22], a trend also observed in our study. Thus, these concordant pharmacodynamics results support the reliability of our plasma biomarker measurements. VEGF-C and VEGF-D are closely related VEGF family members and preferentially signal through VEGFR3, reflecting lymphangiogenic programs linked to tumor progression [23, 24]. Prior NSCLC studies associate high VEGF-C or VEGF-D with poor survival, whereas VEGF-A was not prognostic in a prior prospective post-ICI RAM plus DOC cohort, consistent with our findings [25–27]. These differences may reflect distinct VEGF biology (VEGFR2-driven angiogenesis vs. VEGFR3-linked lymphangiogenesis) and greater variability in VEGF-A measurement; thus, our findings remain hypothesis-generating and require validation in larger comparative cohorts.

This study provides evidence that RAM plus DOC maintains IgG4$^+$CD8$^+$ T cells while diminishing IgG4$^+$ Tregs. We previously reported that, beyond canonical regulators of PD-1 expression (FoxO1, NF-κB, and NFAT), the VEGF signaling pathway was enriched in IgG4$^+$CD8$^+$ T cells relative to IgG4$^-$CD8$^+$ T cells [15, 28], suggesting that upregulation of these pathways reflects tumor-specific CD8$^+$ T cells strongly recognizing tumor antigens. Converging evidence indicates that VEGF signaling modulates PD-1 expression in T cells [13, 14, 29, 30], and bispecific antibodies targeting both the VEGF and PD-1 pathways, such as ivonescimab, have demonstrated enhanced antitumor activity via dual-axis inhibition [31–33]. Our data suggest that VEGFR2 inhibition by RAM may transiently reactivate

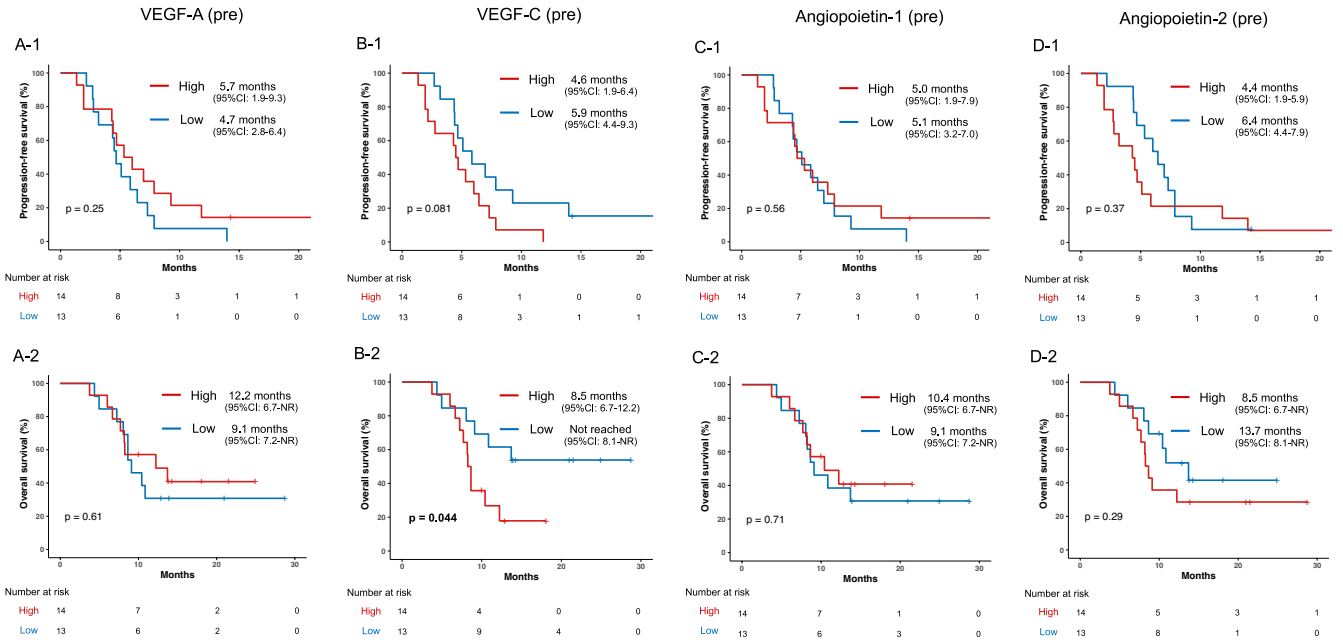

FIGURE 4 | Kaplan–Meier curves for progression-free survival (A1–D1) and overall survival (A2–D2) stratified by high vs. low baseline (T0) serum levels of angiocrine factors (A: VEGF-A; B: VEGF-C; C: Angiopoietin-1; D: Angiopoietin-2) Log-rank *p* values and 95% confidence intervals (CIs) are shown. NR, not reached.

exhausted tumor-specific IgG4$^+$CD8$^+$ T cells under disease progression. Additionally, anti-PD-1 antibodies restore dysfunctional PD-1$^+$CD8$^+$ T cells but also bind PD-1$^+$ Tregs, potentially enhancing immunosuppression [34, 35]. PD-1–inhibited Tregs can suppress CD8$^+$ T cells and dendritic cells in the tumor microenvironment and may contribute to hyperprogressive disease [36, 37]. VEGFR2 expression is enriched in FoxP3-high effector Tregs, and prior studies suggest VEGFA/VEGFR2 inhibition reduces effector Tregs infiltration and proliferation [38–40]. Consistently, activated IgG4$^+$ Tregs were significantly reduced in responders in our study. Moreover, increases in VEGFA positively correlated with IgG4$^+$ Treg proportions and tended to inversely correlate with IgG4$^+$CD8$^+$ T-cell proportions, supporting immunomodulatory effects of VEGF-pathway inhibition. Together, these findings provide a rationale for sequential PD-1/PD-L1 and VEGF pathways blockade and warrant prospective validation with other VEGF pathway inhibitors and in other tumor types.

With respect to timing, no differences were observed in survival or IgG4$^+$CD8$^+$ T-cell and IgG4$^+$ Treg dynamics by the interval from the last ICI to RAM initiation. Because anti–PD-1 antibodies can remain bound to T cells for more than 20 weeks, intervals in this cohort may have been generally too short to detect a temporal effect [15]. Thus, anti–PD-1 antibody binding itself may be more relevant than the absolute inter-treatment interval. Nevertheless, because the persistence of anti–PD-1 antibody binding shows considerable inter-individual variability, the timing of RAM initiation requires further validation. We examined whether prior ICI-PFS or prior irAEs were associated with RAM plus DOC efficacy [41, 42]; however, neither was associated with PFS/OS after RAM plus DOC or with IgG4-bound T-cell dynamics. This may reflect the small sample size and the fact that all patients had progressed on/after ICI. The immune milieu at treatment transition may differ from that during ICI exposure. Thus, outcomes after RAM plus DOC may not be consistently related to prior ICI-PFS or prior irAE.

In this study, most patients started RAM plus DOC within 60 days of their last ICI and overall safety was manageable; however, severe pneumonitis was relatively frequent (grade ≥ 3: 11%), comparable to or higher than (REACTIVE: 4.9%; SCORPION: 9%) [8, 9]. Although we could not determine whether prior ICI exposure increases the risk of severe pneumonitis, these data underscore the need for careful monitoring during RAM plus DOC.

This study had certain limitations. First, the sample size was modest, limiting statistical power. Restricting enrollment to NSCLC patients previously treated with PD-1 antibody–based therapy may introduce selection bias and limit generalizability. In addition, the prognostic value of IgG4$^+$CD8$^+$ Temra and VEGF-C should be interpreted as hypothesis-generating and requires external validation in independent cohorts. Second, residual confounding factors may have influenced the observed associations; therefore, we performed multivariate analyses to adjust for potential confounders. Moreover, because functional assays (e.g., ex vivo T-cell proliferation, cytokine production, or cytotoxicity assays) were not performed, the observed immune-cell dynamics should be interpreted as correlative rather than mechanistic evidence of enhanced antitumor immunity. Third, the follow-up duration was relatively short, necessitating caution when interpreting OS. Finally, as this was a single-arm prospective cohort study without a DOC-alone comparator, randomized controlled trials with designs similar to Kato et al.'s are warranted to rigorously assess RAM's antitumor activity after immunotherapy [43].

TABLE 2 | Cox proportional hazards analysis of factors associated with progression-free survival and overall survival in patients receiving RAM plus DOC.

	Progression-free survival				Overall survival			
	Univariate		Multivariate		Univariate		Multivariate	
Parameter	**HR (95% CI)**	***p***	**HR (95% CI)**	***p***	**HR (95% CI)**	***p***	**HR (95% CI)**	***p***
Age (≥70 vs. <70)	1.01 (0.45–2.25)	0.98			0.62 (0.24–1.60)	0.32		
Sex (Male vs. Female)	1.61 (0.63–4.01)	0.32			2.98 (0.68–13.1)	0.15		
ECOG-PS (1 vs. 0)	1.26 (0.47–3.36)	0.65			0.79 (0.26–2.42)	0.68		
Smoking (Current/Past vs. Never)	2.61 (0.77–8.81)	0.12			3.54 (0.47–26.8)	0.22		
Histology (Non-Ad vs. Ad)	2.81 (1.22–6.48)	0.015	6.04 (1.88–19.4)	<0.001	1.05 (0.39–2.86)	0.92		
PD-L1 TPS (<1% vs. ≥1%)	0.89 (0.36–2.16)	0.78			0.69 (0.23–2.13)	0.52		
Clinical stage (IV vs. Others)	0.78 (0.32–1.87)	0.57			0.94 (0.33–2.68)	0.91		
Malignant pleural effusion (Yes vs. No)	1.47 (0.65–3.31)	0.36			1.30 (0.50–3.42)	0.59		
Liver metastasis (Yes vs. No)	2.23 (0.49–10.1)	0.30			3.61 (0.76–17.2)	0.11		
Brain metastasis (Yes vs. No)	0.75 (0.22–2.53)	0.64			1.20 (0.27–5.26)	0.81		
Prior ICI-PFS (≥180 vs. <180)	1.07 (0.47–2.45)	0.87			0.84 (0.32–2.20)	0.72		
Prior irAE (Yes vs. No)	1.08 (0.49–2.38)	0.85			0.73 (0.27–1.91)	0.52		
$CD8^+$ T (High vs. Low)	0.49 (0.22–1.08)	0.08	0.67 (0.28–1.58)	0.36	0.33 (0.12–0.88)	0.026	0.19 (0.05–0.70)	0.013
$CD56^+$ T (High vs. Low)	0.62 (0.27–1.40)	0.25			0.23 (0.08–0.67)	0.007	0.27 (0.08–0.87)	0.028
$IgG4^+CD8^+$ T (High vs. Low)	0.70 (0.31–1.56)	0.38			1.33 (0.51–3.51)	0.56		
$IgG4^+CD8^+$ Temra (High vs. Low)	0.44 (0.19–1.02)	0.056	0.38 (0.14–0.99)	0.047	0.26 (0.09–0.72)	0.009	0.18 (0.03–0.47)	0.002
$IgG4^+$ Treg (High vs. Low)	0.81 (0.36–1.82)	0.61			1.35 (0.51–3.62)	0.55		
VEGF-C (High vs. Low)	2.07 (0.91–4.72)	0.084	3.34 (1.16–9.65)	0.026	2.80 (1.00–7.87)	0.05	2.70 (1.03–7.20)	0.044

Abbreviations: CI, confidence interval; DOC, docetaxel; ECOG-PS, Eastern Cooperative Oncology Group-performance status; HR, hazard ratio; ICI, immune checkpoint inhibitor; irAE, immune-related adverse event; PD-L1, programmed cell death protein 1; PFS, progression-free survival; RAM, ramucirumab; TPS, tumor proportion score; VEGF, vascular endothelial growth factor.

TABLE 3 | Treatment-related adverse events (TRAEs).

	Total	Grade 1–2	Grade 3–4	Grade5
	N (%)	*N* (%)	*N* (%)	*N* (%)
TRAEs	26 (96.3)	23 (85.2)	15 (55.6)	1 (3.7)
General malaise	16 (59.3)	13 (48.1)	3 (11.1)	0 (0)
Neutropenia	8 (29.6)	1 (3.7)	7 (25.9)	0 (0)
Anemia	8 (29.6)	7 (25.9)	1 (3.7)	0 (0)
Interstitial lung disease	7 (25.9)	4 (14.8)	3 (11.1)	0 (0)
Rash	7 (25.9)	6 (22.2)	1 (3.7)	0 (0)
Fever	6 (22.2)	6 (22.2)	0 (0)	0 (0)
Diarrhea	5 (18.5)	4 (14.8)	1 (3.7)	0 (0)
Peripheral edema	5 (18.5)	4 (14.8)	1 (3.7)	0 (0)
Peripheral neuropathy	4 (14.8)	3 (11.1)	1 (3.7)	0 (0)
Stomatitis	4 (14.8)	4 (14.8)	0 (0)	0 (0)
Nausea/Vomiting	4 (14.8)	4 (14.8)	0 (0)	0 (0)
Epistaxis	4 (14.8)	4 (14.8)	0 (0)	0 (0)
Hypertension	3 (11.1)	2 (7.4)	1 (3.7)	0 (0)
Thrombocytopenia	3 (11.1)	2 (7.4)	1 (3.7)	0 (0)
Myalgia/Arthralgia	3 (11.1)	3 (11.1)	0 (0)	0 (0)
Proteinuria	3 (11.1)	3 (11.1)	0 (0)	0 (0)
Dysgeusia	3 (11.1)	3 (11.1)	0 (0)	0 (0)
Febrile neutropenia	2 (7.4)	0 (0)	2 (7.4)	0 (0)
Hepatic dysfunction	2 (7.4)	0 (0)	2 (7.4)	0 (0)
Pneumonia	2 (7.4)	0 (0)	2 (7.4)	0 (0)
Alopecia	2 (7.4)	2 (7.4)	0 (0)	0 (0)
Sepsis	1 (3.7)	0 (0)	0 (0)	1 (3.7)
Hypokalemia	1 (3.7)	0 (0)	1 (3.7)	0 (0)
Pneumothorax	1 (3.7)	0 (0)	1 (3.7)	0 (0)
Anorexia	1 (3.7)	1 (3.7)	0 (0)	0 (0)
Gastrointestinal bleeding	1 (3.7)	1 (3.7)	0 (0)	0 (0)
Gastrointestinal disorder	1 (3.7)	1 (3.7)	0 (0)	0 (0)
Pleural effusion	1 (3.7)	1 (3.7)	0 (0)	0 (0)
Alveolar hemorrhage bleeding	1 (3.7)	1 (3.7)	0 (0)	0 (0)
Gingival bleeding	1 (3.7)	1 (3.7)	0 (0)	0 (0)

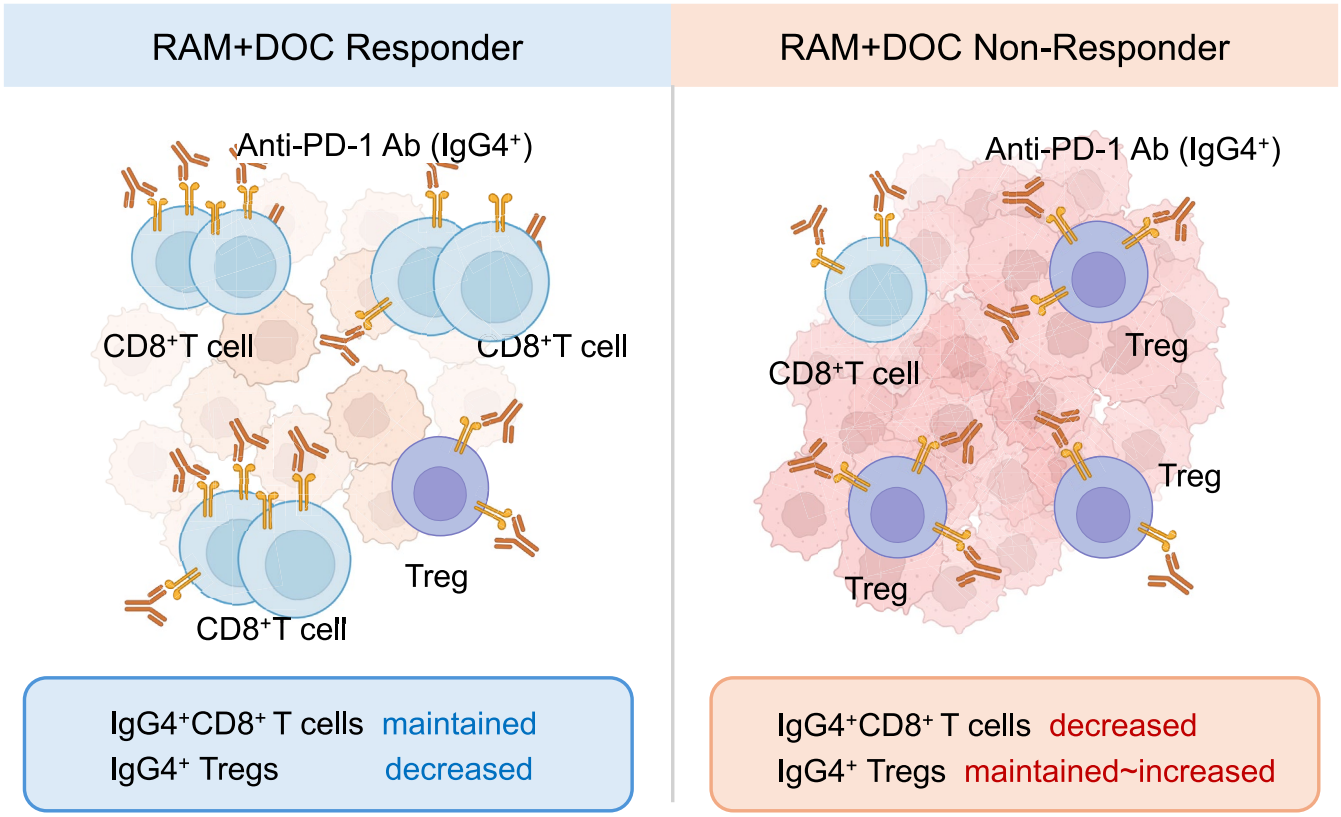

FIGURE 5 | Diagram showing that differential dynamics of anti–PD-1 antibody–bound $CD8^+$ T cells and Tregs are associated with tumor response to ramucirumab plus docetaxel (RAM+DOC).

In conclusion, this study demonstrated that RAM plus DOC after PD-1 inhibitors provides antitumor activity and survival benefit in advanced NSCLC. RAM plus DOC efficacy was associated with maintenance of anti-PD-1 antibody–bound $CD8^+$ T cells and a marked decline in anti-PD-1 antibody–bound Tregs (Figure 5). A sequential strategy of administering RAM plus DOC during persistent anti–PD-1 antibody binding may be beneficial. Furthermore, anti-PD-1 antibody–bound $CD8^+$ Temra cells and VEGF-C may serve as novel biomarkers of survival benefit. Further studies are warranted to validate these findings.

Author Contributions

Kinnosuke Matsumoto: conceptualization, data curation, formal analysis, investigation, methodology, resources, visualization, writing – original draft, writing – review and editing, funding acquisition, software. **Yujiro Naito:** conceptualization, data curation, formal analysis, visualization, writing – original draft, methodology, investigation, writing – review and editing, resources, software. **Takayuki Shiroyama:** conceptualization, data curation, formal analysis, investigation, methodology, project administration, resources, supervision, visualization, writing – original draft, writing – review and editing, validation. **Motohiro Tamiya:** investigation, resources, writing – review and editing. **Kazumi Nishino:** investigation, writing – review and editing, resources. **Akihiro Tamiya:** investigation, writing – review and editing, resources. **Kyoichi Okishio:** investigation, writing – review and editing, resources. **Yuhei Kinehara:** writing – review and editing, investigation, resources. **Tomoki Kuge:** investigation, writing – review and editing, resources. **Masahide Mori:** investigation, writing – review and editing, resources. **Shingo Satoh:** investigation, writing – review and editing, resources. **Hidekazu Suzuki:** investigation, writing – review and editing, resources. **Satoshi Tetsumoto:** investigation, writing – review and editing, resources. **Toshie Niki:** investigation, writing – review and editing, resources. **Yasuhiko Suga:** investigation, writing – review and editing, resources. **Akio Osa:** investigation, writing – review and editing, resources. **Toshiyuki Minami:** investigation, writing – review and editing, resources. **Shohei Koyama:** investigation, writing – review and editing, resources. **Yoshito Takeda:** investigation, writing – review and editing, resources. **Nobuyuki Takakura:** project administration, supervision, writing – review and editing, funding acquisition. **Atsushi Kumanogoh:** supervision, project administration, writing – review and editing.

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Ethics Statement

The study was approved by the Central Institutional Review Board of the University of Osaka (IRB #22406(T14)-6) and was conducted in

accordance with the Declaration of Helsinki and Good Clinical Practice guidelines.

Consent

Written informed consent was obtained from all participants before any study-related procedures.

Conflicts of Interest

Akihiro Tamiya reports receiving grants from AstraZeneca, BeiGene, Taiho Pharmaceutical and Daiichi-Sankyo; and honoraria for lectures from Amgen, AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb, Chugai Pharmacaceutical, DNA Chip Research, Eli Lilly, Kyowa-Kirin, Merck BioFarma, Merck Sharp & Dohme, Nihon-Kayaku, Novartis, Ono Pharmacaceutical, Pfizer, Pulmonx, Taiho Pharmaceutical, Takeda Pharmaceutical, Thermo Fischer Scientific, and Tsumura outside of the submitted work. Hidekazu Suzuki reports receiving honoraria for lectures from AstraZeneca, Chugai Pharmaceutical, and Merck Sharp & Dohme outside of the submitted work. Kazumi Nishino reports receiving grants from Ono Pharmaceutical, Taiho Pharmaceutical, Merck Sharp & Dohme, AbbVie, Daiichi-Sankyo, Amgen, Eisai, Sanofi, Janssen Pharmaceutical, Novartis, Pfizer, Eli Lilly Japan, Merck Biopharma, Takeda Pharmaceutical, Chugai Pharmaceutical, Merus; and honoraria for lectures from AstraZeneca, Chugai pharmaceutical, Nippon Boehringer Ingelheim, Eli Lilly Japan, Roche Diagnostics, Novartis, Pfizer, Merk, Janssen Pharmaceutical, Bristol-Myers Squibb, Nippon Kayaku outside of the submitted work. Kinnosuke Matsumoto reports receiving grants from Eli Lilly Japan, and honoraria for lectures from Bristol-Myers Squibb, Chugai Pharmaceutical, Eli Lilly Japan, Merck Sharp & Dohme, and Ono Pharmaceutical outside of the submitted work. Masahide Mori reports receiving honoraria for lectures from AbbVie, AstraZeneca, Boehringer Ingelheim, Chugai Pharmaceutical, Daiichi-Sankyo, Eli Lilly, Kyowa-kirin, MSD, Nihon-kayaku, Novartis, Ono Pharmaceutical, Phizer, Taiho, Takeda outside of the submitted work. Motohiro Tamiya reports receiving grants from Boehringer Ingelheim, Bristol-Myers Squibb, and Ono Pharmaceutical; and honoraria for lectures from Asahi Kasei Pharmaceutical, AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb, Chugai Pharmaceutical, Eli Lilly, Merck Sharp & Dohme, Ono Pharmaceutical, and Taiho Pharmaceutical outside of the submitted work. Shohei Koyama reports receiving grants from Otsuka Pharmaceutical and Chugai Pharmaceutical; and honoraria for lectures from MSD and Chugai Pharmaceutical. Nobuyuki Takakura recently established a start-up company, RevascularBio Co. Ltd., focusing on the clinical application of endothelial stem cells. Yuhei Kinehara reports receiving honoraria for lectures from AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb, Chugai Pharmaceutical, Merck Sharp & Dohme, and Ono Pharmaceutical outside of the submitted work. The other authors declare no conflicts of interest. Nobuyuki Takakura is an editorial board member of Cancer Science.

Data Availability Statement

The datasets used in this study are available from the corresponding author upon reasonable request for additional research.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Appendix S1:** Study protocol. **Table S1:** Flow cytometry antibodies and panel list. **Table S2:** Prior irAE before initiation of RAM plus DOC. **Table S3:** Driver gene mutation. **Table S4:** Trend of plasma markers at baseline and first radiological evaluation. **Table S5:** Assessment of multicollinearity among T-cell subsets. **Figure S1:** Baseline and post-treatment dynamics of anti-PD-1 antibody bound T-cell subsets stratified by tumor response. **Figure S2:** Exploratory analyses stratified by the interval between the last PD-1 inhibitor administration and RAM plus DOC initiation. **Figure S3:** Exploratory analyses stratified by the prior ICI-PFS and prior irAEs. **Figure S4:** Prognostic value of lymphocytes in ramucirumab plus docetaxel. **Figure S5:** Kaplan–Meier curves stratified by high vs. low baseline (T0) proportions of IgG4+CD4+ T-cell subsets.