Eftilagimod alpha (soluble LAG-3 protein) combined with pembrolizumab as second-line therapy for patients with metastatic head and neck squamous cell carcinoma

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Running title: Eftilagimod alpha plus pembrolizumab in 2nd line HNSCC

Keywords: eftilagimod alpha, LAG-3 protein, anti-PD-L1, metastatic solid tumors

Financial support: This trial was funded by Immutep S.A.S.

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Authors' Disclosures

M. Forster reports grants from Boehringer Ingelheim, MSD and Merck; support for conferences and honoraria for advisory and consultancy work from Achilles, Amgen, AstraZeneca, Bayer, Boxer, Bristol-Myers Squibb, Celgene, EQRx, Immutep, Ixogen,

Janssen, Merck, MSD, Oxford VacMedix, Pharmamar, Roche, Takeda, Transgene and UltraHuman. **I. Brana** reports consulting work from Merck Sharp & Dohme, Sanofi, Achilles Therapeutics, eTheRNA Immunotherapies, Cancer Expert Now, Boeheringer Ingelheim and Merus; Speaker's bureau from Bristol-Myers Squibb; Merck Serono, Roche and MSD; research funding from AstraZeneca, Bristol-Myers Squibb, Celgene, Gliknik, GlaxoSmithKline, Janssen Oncology, Kura, Merck Sharp & Dohme, Novartis, Pfizer, Roche, Shattuck Labs, Nanobiotix, Seattle Genetics, Immutep, Debiopharm Group, Regeneron, Boehringer Ingelheim, ISA Pharmaceuticals, Merck Serono, Seattle Genetics, Northern Biologics, VCN Biosciences, Tolremo therapeutics and Merus and travel support from MSD Oncology. **M.G. Krebs** reports grants or contracts from Roche and Novartis; consulting fees from Bayer, Guardant Health, Janssen, Roche and Seattle Genetics; and honoraria and travel support from Janssen and Roche. **C. Mueller** holds employment with Immutep GmbH and owns stocks with Immutep Ltd. **C. Brignone** holds employment with Immutep SAS and owns stocks with Immutep Ltd and has one patent pending or issued and others planned. **F. Triebel** holds employment with Immutep SAS and owns stocks with Immutep Ltd, serves as a board member of Immutep Ltd, and has one patent pending or issued and others planned. **A. Lopez Pousa**, **B. Doger**, **P. Roxburgh**, **P. Bajaj**, **J. Peguero, E. Carcereny** and **G. Patel** declare no potential conflicts of interest.

Category: *Clinical Trials: Immunotherapy*

Word count: 3539/5000 (text), 246/250 (abstract)

Running Title: 54/60 characters

Translational Relevance: 147/150 words

Tables and Figures: 6/6

TRANSLATIONAL RELEVANCE

Head and neck squamous cell carcinoma (HNSCC) has proven responsiveness when treated with immunotherapy in a limited number of patients. However, there remain restricted treatment options and a high unmet need for patients with recurrent or metastatic (R/M) disease.

Increasing the number of patients responding to immune checkpoint inhibitors whilst maintaining the observed durability of responses may be a good first step to address this need. Non-responding patients have been shown to have limited or non-functional immune responses which may be overcome with other immune therapies.

In TACTI-002 R/M HNSCC patients, the strategy of adding the antigen-presenting cell (APC) activator eftilagimod alpha (efti) to the PD-1 antagonist pembrolizumab resulted in a promising durable objective response rate. Early and sustained increases of secondary targets such as T cells was linked to longer overall survival and progression-free survival. These findings may help to broaden the concept of combined immunotherapies.

Abstract (246/250 words)

Purpose: Eftilagimod alpha (efti), a soluble LAG-3 protein, activates antigen-presenting cells (APC) and downstream T-cells. TACTI-002 (Part C) evaluated whether combining efti with pembrolizumab led to strong anti-tumor responses in 2nd line recurrent or metastatic head and neck squamous cell carcinoma (R/M HNSCC) patients, while demonstrating good tolerability.

Methods: In this multinational phase 2 trial using Simon's 2-stage design, R/M HNSCC PD-L(1)-naïve patients who had failed first-line platinum-based therapy, unselected for PD-L1, received intravenous pembrolizumab (200 mg, Q3W) combined with subcutaneous efti (30 mg Q2W for 24 weeks and Q3W thereafter). The primary endpoint was objective response rate (ORR) per iRECIST by investigator assessment. Additional endpoints included duration of response (DoR), progression free survival (PFS), overall survival (OS) and tolerability. Pharmacodynamic effects (absolute lymphocyte count [ALC] and Th1 cytokine biomarkers [IFN-gamma/CXCL-10]) were evaluated in liquid biopsies.

Results: Between Mar 2019 – Jan 2021, 39 patients were enrolled; 37 were evaluated for response. All patients received prior chemotherapy and 40.5% were pretreated with cetuximab. 53.1% of patients had PD-L1 CPS <20. With a median follow up of 38.8 months, ORR was 29.7%, including 13.5% complete responders. Median DoR was not reached. Rapid and sustained ALC increase was observed in patients who had an objective response. Th1 biomarkers increased sustainably after first treatment. No unexpected safety signals were observed.

Conclusion: Efti plus pembrolizumab was safe and showed encouraging antitumor activity and pharmacodynamic effects in 2nd line HNSCC patients, thus supporting further evaluation of this combination in earlier treatment lines.

INTRODUCTION

Ranked as the seventh most prevalent cancer worldwide, head and neck squamous cell carcinoma (HNSCC) originates from the mucosal epithelium of various regions, including the oral cavity, nasopharynx, oropharynx, hypopharynx, and larynx and represents a large burden to public health (1). Recommendations for curative treatments at early or locally-advanced stages include surgery, radiation, or combined modalities. For patients with recurrent or metastatic (R/M) disease, however, there are limited treatment options that are often accompanied by poor prognoses (2,3).

At the time of trial initiation, first-line standard of care (SOC) for R/M HNSCC comprised platinum-based doublet chemotherapy with or without targeted therapies such as cetuximab, an anti-epidermal growth factor receptor (EGFR) monoclonal antibody, with median overall survival (OS) ranging between 10–13 months (4). Meanwhile, second-line treatment options were long-restricted to targeted therapy and chemotherapy, including cetuximab, methotrexate, and taxanes, with only marginal improvements observed in tumor response and progression-free survival (PFS) (4).

In recent years, the emergence of immunotherapy represented a transformative shift in the HNSCC treatment landscape. Initially, in second-line patients, the immune checkpoint inhibitors (ICI) pembrolizumab and nivolumab, which specifically target the programmed cell death protein 1 (PD-1) receptor on immune cells, were approved for patients with R/M HNSCC after progression on chemotherapy (5,6). These studies showed good tolerability, and clinically-meaningful antitumor activity with objective response rates (ORR) of up to 18% (7) that included long-lasting responses, a phenomena not typically observed with chemotherapy (5). Compared to SOC single agent chemotherapy, pembrolizumab (KEYNOTE-040) or nivolumab (CheckMate 141) showed longer OS (HR 0.8; 95% confidence internal [CI], 0.65–0.98; P = 0.0161) and HR 0.70; 95% CI, 0.51–0.96; P = 0.01], respectively (8,9)). Importantly, however, objective responses were only achieved only in <20% of these patients (9), underscoring the need for advancements in treatment options for this particular patient population.

The KEYNOTE-048 trial subsequently established pembrolizumab alone or in addition to platinum-based chemotherapy as a current approved SOC in first-line R/M HNSCC, after demonstrating a longer OS than the EXTREME-regimen (cetuximab, cisplatin and 5-fluoracil) (13.0 months versus 10.7 months; HR, 0.77; P = 0.0034) (10).

Understanding the reasons why most patients do not initially respond to ICIs, specifically to the PD-1 and programmed cell death ligand 1 (PD-L1) blockade, is key to developing new therapy combinations. Analysis of tumor biopsies showed that non-responding patients had either little to no tumor-infiltrating lymphocytes (TILs) or

a non-functional immune response with PD-1/PD-L1 negative TILs (11). One approach to overcome this deficiency is to stimulate antigen-presenting cells (APCs) such as immature dendritic cells (DC) to prime and activate effector-memory T-cells. Competent APCs can be activated by lymphocyte activation gene-3 (LAG-3), which interacts with the MHC class II on the surface of the APCs and is a key mediator of the immune response (12-15).

Eftilagimod alpha (efti; eftilagimod alfa; IMP321), a recombinant soluble LAG-3 protein, is a highly potent first-in-class activator of APCs. *Ex vivo* evaluations and proof-of-concept phase 1/2 studies in cancer patients showed that efti, in repeated subcutaneous doses of up to 30 mg, was generally well-tolerated and sustainably activated primary (monocytes, DC) and secondary target cells ($CD4^+$ and $CD8^+$ T cells) and led to elevated levels of Th1 cytokines when administered as a monotherapy or together with chemotherapy (16-21). Thus, the "Two ACTive Immunotherapies" (TACTI) concept of combining the well-tolerated APC activator efti with a PD-1 inhibitor such as pembrolizumab holds the potential to unlock more frequent, durable responses compared to PD-1 monotherapy. The TACTI-mel phase 1 trial confirmed that efti in combination with pembrolizumab was well-tolerated with encouraging antitumor activity in metastatic melanoma reacting suboptimally to anti-PD-1 therapy (22). Based on these results, TACTI-002 was designed to further evaluate the efficacy of efti in combination with pembrolizumab in three different indications, including in second-line patients with HNSCC, anti-PD-(L)-1-naïve, after failure of platinum-based chemotherapy, unselected by PD-L1 expression.

MATERIALS AND METHODS

Trial Design

TACTI-002 was a multicenter, open-label, phase 2 trial. There were three independent parts (A – C), each a different indication. Part A and B included patients with non-small cell lung cancer (NSCLC) in first and second line therapy, respectively and will not be reported in this publication.

All patients received intravenous pembrolizumab (200 mg) on day 1 of each 3-week cycle for up to 2 years (up to 35 cycles). In addition, patients received efti (30 mg, subcutaneously) in the first year, bi-weekly for the first six months (including cycle 8 of pembrolizumab), thereafter shifted to 3-week cycles (cycles 9 – 18 of pembrolizumab) (**Figure S1**).

Therapy was terminated prematurely for unacceptable toxicity, disease progression, death, or at the patient's request. After the end of treatment without progression, patients were followed-up until withdrawal of consent, disease progression. end of trial, or death, whichever occurred first.

Patients

Eligible patients for Part C of the TACTI-002 trial had recurrent HNSCC (not manageable with curative intent) and/or metastatic disease (disseminated), who

failed prior platinum-based therapy. At time of recruitment, anti-PD-1 therapy had not yet become first-line SOC treatment for HNSCC patients. Further key eligibility criteria included *i)* no more than 1 prior line of therapy, *ii)* no prior treatment with ICIs and/or anti-LAG-3 therapy, *iii)* ECOG 0–1, i*v)* measurable disease according to Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 modified for immune-based therapy (iRECIST), *v)* adequate laboratory values, and *vi) no* primary tumor originating from nasopharynx.

Outcomes

The primary objective of TACTI-002 was to determine the ORR according to iRECIST. Radiological assessments were performed at baseline and every 9 weeks thereafter for the first 9 months and then every 12 weeks afterwards until withdrawal of consent, loss to follow-up, disease progression, the end of the trial or death. Local assessments of responses are reported.

Secondary objectives included ORR according to RECIST 1.1 and disease control rate (DCR), duration of response (DoR) and PFS according to both iRECIST and RECIST 1.1, OS and assessment of safety including grading of adverse events (AE) according CTCAE version 5.0. Immune response was evaluated via liquid biopsies to identify and correlate biomarkers with clinical outcomes.

Central assessment of PD-L1 immunohistochemistry was retrospectively performed in a Clinical Laboratory Improvement Amendments (CLIA)-certified, accredited laboratory (Labcorp Central Laboratory Services, Meyrin, Switzerland) and was tested using the Dako PD-L1 IHC 22C3 pharmDx assay (Agilent, Carpenteria, California; RRID:AB_2889976). The assay was performed according to the package insert with appropriate controls. Combined Positive Score (CPS) testing was performed by certified pathologists specifically trained in PD-L1 22C3 CDx scoring for HNSCC.

Absolute lymphocyte count (ALC) was obtained from patients' full blood and was locally collected pre-dose on day 1 of each 3-week cycle. The cut-off was set at 0.2 x 10^9/L of blood to discriminate a subgroup of patients with relevant ALC increase from baseline. EDTA plasma samples for Th1 biomarkers were collected pre-dose on day 1 of cycles 1, 5 and 9 in all patients and 1, 2, 4, 8, 24, 48, 72, 96 hours after dosing in a subset of 10 patients. Th1 biomarkers (IFN-γ, CXCL10/IP10, IFN- γ-induced protein 10) were measured using qualified multiplexed electrochemiluminescence immunosorbent assay (ECLIA) with U-plex Biomarker group 1 kits (MesoScale Discovery. Rockville, MD). The lower limits for IFN-γ and CXCL10 were 7 and 11 pg/mL, respectively.

Statistical Analysis

A clinically-relevant ORR of at least 30% was assumed for the combination of efti plus pembrolizumab. Based on historical data of pembrolizumab monotherapy from the KEYNOTE-012 and KEYNOTE-040 trials (7,9), the minimum rate of 15% was

deemed acceptable. Thus, if the ORR was lower than 15%, the adopted treatment might not warrant further investigation.

Based on the above conditions for the maximum and minimum required level of efficacy, Simon's optimal two-stage testing procedure for the sample size calculation with a one-sided type I error of 0.05, and a power of 70% supported that 18 subjects were to be enrolled in the first stage and if at least two responses were reported, a further 19 were to be recruited in the second stage.

The safety population consisted of 39 enrolled patients who received at least one dose of the trial treatment. The modified intention-to-treat (mITT) population included all patients who were enrolled except for two patients who died from fatal COVID-19 infection prior to the first post-baseline staging. mITT was used for baseline disease characteristics and efficacy analyses. The evaluable population included patients who had a post-baseline assessment.

All parameters were evaluated in an explorative or descriptive manner. Quantitative and continuous data were described with the following items: frequency, mean, standard deviations, 95% CIs of the mean, median, first and third quartiles, minimum and maximum values. Qualitative or ordinal data were summarized by frequency, percentages of each modality. The 95% CIs for proportions were calculated. Time-to-event data was analyzed using Kaplan Meier survival analysis methods and figures were created using GraphPad (RRID:SCR_002798). Biomarkers between group comparison was performed by nonparametric rank-sum 2-sided Wilcoxon test. In-between group comparison of posttreatment values to baseline value was tested using matched-paired rank-signed Wilcoxon test using SAS JMP version 12.0.1 software.

Data Availability Statement

The data generated in this trial are not publicly available due to patient privacy restrictions but are available from the corresponding author pursuant to reasonable request and approval from trial Sponsor according to available guidelines at time of request.

Ethics Approval and Consent to Participate

The TACTI-002 trial was conducted in accordance with the Declaration of Helsinki and was approved by all applicable ethics committees and institutional review boards. This trial is registered with ClinicalTrials.gov number NCT03625323.

All patients provided written informed consent to participate in this trial.

RESULTS

Patient Population

Between March 2019 – January 2021, 58 patients were screened across 9 sites in Australia, Spain, United Kingdom, and United States. A total of 40 patients were included in the trial, while 39 patients were enrolled and also received at least one dose of efti and/or pembrolizumab. One patient received no trial treatment after failing the eligibility criteria and was excluded from analyses. Two patients were excluded from efficacy analyses due to fatal COVID-19 prior to their first post-baseline scan as per the Statistical Analysis Plan (**Figure S2**). As of the data cut-off date (March 31, 2023), patients from the mITT population had a median follow-up of 38.8 months (range: 0.3–44.5).

Median age was 63 years (range: 48–84years), 89.2% of the patients were male and almost two-thirds (64.9%) had an ECOG 1. The primary tumor locations were oropharynx (35.1%), oral cavity (29.7%), hypopharynx (18.9%) and larynx (16.2%). Five of the 13 (38.5%) patients with oropharyngeal squamous cell carcinoma were known to be HPV positive. All patients had received prior chemotherapy in the first-line setting, a large proportion (40.5%) also receiving cetuximab. Of the patients with PD-L1-evaluable tumor tissue (32 patients; 86.5%), the majority (53.1%) had a CPS <20, including 7 (21.9%) with CPS <1, and 15 patients (46.9%) had CPS ≥20 (**Table 1**). Nine (9) patients (24.3%) completed 1 year of combined treatment of efti and pembrolizumab and continued with the pembrolizumab monotherapy phase, and 4 patients (10.8%) completed the maximum protocol-specified treatment duration (**Figure S2**).

Primary Objective

Unconfirmed ORR by iRECIST of PD-L1 unselected in the mITT population was 29.7% and DCR was 37.8%, comprising five (13.5%) patients with complete responses (CRs) and six (16.2%) with partial responses (PRs). All but one response was confirmed (27.0%). Results for ORR and DCR by RECIST 1.1 were comparable (**Table 2**). The depth of responses is shown in the waterfall plot in **Figure 1c**. Six patients had no evaluable response assessments due to missing on-trial post-baseline tumor assessment for any reason, including four serious adverse events (SAEs) that were unrelated to trial treatment and clinical progression. Within the population of evaluable patients, unconfirmed ORR by iRECIST was 35.5% (**Table 2**).

For patients with high PD-L1 expression (CPS ≥20), an ORR of 60.0% (per iRECIST) was reported. There were two additional responders (ORR, 11.8%) with CPS <20 (one CR with negative CPS and one PR with absolute CPS 1) (**Table 2**, **Figure 1c**).

Secondary Efficacy Endpoints

Median PFS according to iRECIST was 2.1 months (95% CI, 2.0–4.3) based on 31 (83.7%) events in the mITT. The proportion of patients who were progression-free at 6 months was 32.4%. Results according to RECIST 1.1 were comparable (**Figure S3a–b**).

Patients with PD-L1 CPS ≥20 showed an increased median PFS of 13.6 months compared to patients with CPS ≥1 who had a median of 2.0 months (**Figure S3c**).
At the time of data cut-off (March 31, 2023), median DoR was not reached. Eight of the ten (80.0%) confirmed responders were still responding after 12 months of treatment and 6 (60.0%) continued to respond after 24 months (**Figure 1b**).
The median OS in the mITT was 8.7 months (95% CI, 4.8–15.6), based on 31 (83.7%) events, and the proportion of surviving patients at 12- and 24-months were 46.0% and 27.0%, respectively. In patients with PD-L1 CPS ≥20, the treatment resulted in a median OS of 15.5 months (95% CI, 4.9–31.1) (**Figure 2**).

Exploratory Analysis - Pharmacodynamics

Exploratory analyses were conducted to investigate pharmacodynamic parameters and their potential link to clinical response.

An early and sustained ALC increase was seen in responder patients (who experienced a best objective response [BOR] of CR or PR), while an ALC decrease was observed in non-responders (those with progressive disease as their BOR and early dropout patients) (**Figure 3a**). All but one patient (93.8%) with $<0.2 \times 10^9$ lymphocytes/L increase within 13 weeks (i.e., after 4 cycles) was considered a non-responder to the treatment (Fisher's exact test for responders greater in the subgroup with ALC change ≥0.2, $P = 0.004$). In contrast, 90.0% (9/10) of responders showed an ALC increase at least $\geq 0.2 \times 10^9$ lymphocytes/L before the fifth cycle (**Figure 3b**). In the responder subgroup, the ALC increase was maintained for more than one year (**Figure S4**).

The subset of patients with ALC increase ($\geq 0.2 \times 10^9$ Lymphocytes/L) within 13 weeks had a significantly decreased risk of death (HR, 0.47; 95% CI, 0.05–0.21; $P = 0.026$) and disease progression (HR, 0.41; 95% CI, 0.18–0.91; $P = 0.015$) compared with patients with decrease or minimal increase in ALC. Median PFS (per iRECIST) was 8.5 versus 2.0 months and median OS was 18.8 versus 5.5 months, respectively (**Figure 3c–d**).

Low Neutrophil-to-Lymphocyte ratio (NLR) (i.e., below the median) at baseline was associated with a lower risk of death (HR, 0.47; 95% CI, 0.22–0.97; $P = 0.02$), but not significantly associated with a lower risk of progression (HR, 0.77; 95% CI, 0.37–1.56; $P = 0.23$).

Soluble Th1 biomarkers were increased significantly shortly after dosing, with maximal fold changes of about 5-fold for IFN-γ and 2-fold for CXCL10 as shown in **Figure 4a**. Minimal residual effects i.e. pre-dose at Cycle 5 and Cycle 9 showed maintained increase for both parameters (**Figure 4b**).

Safety

Six (15.4%) patients experienced any treatment-emergent adverse event (TEAE) leading to discontinuation of the trial treatment; for two subjects (5.1%), it was deemed at least possibly related to efti and/or pembrolizumab – specifically fatigue and arthralgia (each grade 2) and pneumonitis (grade 3). In total, 28 patients (71.8%)

encountered at least one TEAE grade ≥3, with five (12.8%) patients experiencing a treatment-related AE grade ≥3. 22 (56.4%) patients had at least one SAE, including 3 (7.7%) patients who experienced a treatment-related SAE. The most common AEs (incidence ≥15%) were hypothyroidism (20.5%), asthenia (20.5%), anemia (17.9%), cough (17.9%), weight decrease (17.9%), and fatigue (15.4%) (**Table S1**). The most frequent TEAEs at least possibly related to trial treatment (efti and/or pembrolizumab) were hypothyroidism (20.5%), fatigue (10.3%) and pruritus (10.3%).

DISCUSSION

The present trial shows that combining the soluble LAG-3 protein efti with anti-PD-1 therapy pembrolizumab yielded an encouraging tumor response in R/M HNSCC patients in the second-line setting (platinum failed and anti-PD-1 naïve), irrespective of PD-L1 status. An ORR of 29.7%, including 5 complete responses (13.5%), was achieved, demonstrating an approximately two-fold increase compared to ORRs observed with ICI monotherapies such as pembrolizumab (14.6%; 1.6% complete responses), and nivolumab (13.3%; 7.2% complete responses) (9,23). The higher number of complete responders is noteworthy. Because the DCR did not reach 50%, median OS and PFS were not improved compared to the historical data, however, patients who had initial benefit (PR or CR) were shown to have a long-term benefit with responses that were both deep and durable, reflected by a median DoR that was not reached at median follow up of 39 months and DoR rates of 80.0% and 60.0% at 12- and 24-months, respectively. This falls within a similar range to what has been observed for anti-PD-1 alone (median DoR of 18.4 months) (9) and is in line with previous data showing improved efficacy of ICIs after adding APC activator efti in patients with metastatic melanoma, NSCLC, and other solid tumors (22,24). These results contrast, however, to what has been observed when chemotherapy has been combined with anti-PD-1 where the ORR also increased but median DoR decreased from 22.6 months to 4.2 months (25).

Within this PD-L1 all-comer trial, 46.9% of patients were high PD-L1 expressors (CPS ≥20), a factor that has been already linked to improved responses. Although comprising relatively small subset of patients, an ORR of 60.0% for the high PD-L1 expressors is remarkable compared to what has been previously reported in KEYNOTE-040 with an ORR of 21.9%. In other studies, high PD-L1-expressing R/M HNSCC patients (CPS ≥20 or tumor proportion score [TPS] of 50% or higher) benefited more from pembrolizumab monotherapy than patients with low expression as well as the overall population in both first and second line settings (9,10,25). Patient numbers were too small to interrogate further with respect to other clinical factors such as smoking or HPV status.

Although much effort has been made to identify and investigate predictive factors within the tumor environment, noninvasive and easy-to-collect circulating biomarkers capable of predicting the clinical benefit of ICI therapy are of particular interest (26,27). Reports of ALC as a predictive biomarker for ICI therapies have been limited with findings primarily centered on patients with advanced melanoma. Martens et al

reported that an early increase of ALC in patients within 8 weeks after starting ipilimumab correlated with prolonged OS and response/benefit rate (28). In another study of HNSCC patients treated with anti-PD-1 therapy, an increased in ALC at week 6 was associated to a longer median PFS, however, the absolute change from baseline failed to be predictive (29). Here, we report an early and sustained increase of ALC from baseline in patients with a good response to treatment (**Figure 3a–d**). This change from baseline was correlated with an improved response rate and longer median OS and PFS and is consistent with prior observations of efti's pharmacodynamics as an APC activator in persistently boosting T-cell responses. This phenomenon was indeed previously reported in efti-treated subjects in a randomized double-blinded phase 2b AIPAC trial in metastatic breast cancer (MBC) patients treated with efti plus paclitaxel versus placebo plus paclitaxel, where an early and lasting increase in ALC was found to be predictive for survival in a post hoc analysis (20,30). In this double-blinded study, which tested the pharmacodynamics of a single immuno-oncology product, the increase in ALC was explained by an increase in circulating CD4 and CD8 subsets (30).

The initial increase (after first dose of efti) and late increase (2 weeks after sixth or twelfth dose) of circulating biomarkers levels of Th1 cytokines IFN-γ and CXCL10 are indicative of extended pharmacodynamic activity. Some moderate and transient increases of IFN-γ following PD-1/PD-L1 blockade were reported in advanced or metastatic solid tumors or hematological malignancies (11,31). As with the increase in ALC, a Th1 biomarker (CXCL10) was significantly increased by efti compared to placebo (in the absence of anti-PD-1 therapy) in the randomized double-blinded AIPAC trial in MBC (30). These biomarker findings warrant further investigation.

This trial was limited by its small sample size and heterogenous patient population that contained a large proportion treated with SOCs that are no longer relevant. Due to the shift in treatment landscape, the anti-PD-1 naïve second-line population no longer exists, with most patients exposed to anti-PD-(L)1 in the first-line setting. A further constraint was the lack of a pembrolizumab-only control arm, hindering understanding of the contributory effects of efti.

Combining two immunotherapies in second-line HNSCC has been attempted before where anti-PD-(L)1 antibodies were combined with anti-CTLA-4 in the EAGLE and CHECKMATE-714 studies, ultimately leading to inferior OS or ORR for the combination arms compared to mono anti-PD(L)1 therapy, respectively (32,33). One of the reasons could have been the greater toxicity usually observed from treatment with anti-CTLA-4. Besides efti's orthogonal mechanism of action in activating APCs and not directly targeting T cells may be beneficial for tumors poorly infiltrated by T cells, and its safety profile may have contributed to the low discontinuation rates in the trial.

The strategy of adding an APC activator to an anti-PD-1 therapy demonstrates enhanced tumor responsiveness to immune checkpoint inhibition and demands further exploration in randomized controlled trials. A randomized phase 2b trial is currently underway to assess the potency of combining efti plus pembrolizumab in

first-line metastatic HNSCC (NCT04811027). Results may determine the potential role of efti in addition to anti-PD-1 therapies in HNSCC.

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Funding acquisition: FT.

Investigation: all authors.

Visualization: FT, CM, CB.

Methodology: FT, CM, CB.

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Writing, reviewing, and editing: All authors.

Acknowledgments

We thank the patients, their supporters, and the investigators at the various trial centers. Medical writing and editorial assistance were provided, under the direction of the author Maria Loose, of IKF, supported by Immutep.

This trial is in collaboration with Merck Sharp & Dohme LLC, a subsidiary of Merck & Co., Inc., Rahway, NJ, USA, who supplied the pembrolizumab.

Martin Forster is supported by the UCL/UCLH National Institute for Health Research (NIHR) Biomedical Research Centre and runs early phase studies in the NIHR UCLH Clinical Research Facility supported by the UCL Experimental Cancer Medicine Centre (ECMC). Matthew Krebs acknowledges support from NIHR Manchester Biomedical Research Centre, NIHR Manchester Clinical Research Facility at The Christie and Manchester Experimental Cancer Medicine Centre (Manchester, UK).

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TABLE LEGEND

Table 1. Baseline patient and disease characteristics in the modified ITT population

CPS, combined positive score; ECOG PS, Eastern Cooperative Oncology Group Performance Status; mITT, modified intent to treat population; PD-L1, programmed death ligand 1.
[1]Central assessment of PD-L1 CPS using Dako IHC 22C3 pharmDx (N=32).

Table 2. Objective Response Rate and Duration of Response

Objective response rate and duration of response for the mITT population (N=37), evaluable population* (N=31), and by PD-L1 CPS status.
*All patients with ≥1 on-trial post-baseline tumor staging; [1]calculated using Clopper-Pearson method; [2]calculated using Kaplan-Meier survival analysis method.
CI, confidence interval; CPS, combined positive score; iRECIST, immune Response Evaluation Criteria in Solid Tumors; mITT, modified intent-to-treat; PD-L1, programmed death ligand 1; RECIST 1.1, Response Evaluation Criteria in Solid Tumors version 1.1.

Table 1. Baseline patient and disease characteristics in the modified ITT population

n (%)	**mITT (*n* = 37)**
Age, years	
Median (range)	63 (48–84)
Sex	
Male	33 (89.2)
Female	4 (10.8)
ECOG PS	
0	13 (35.1)
1	24 (64.9)
Primary tumor location	
Oropharynx	13 (35.1)
Oral cavity	11 (29.7)
Hypopharynx	7 (18.9)
Larynx	6 (16.2)
HPV status	
Positive	5 (38.5)
Negative	5 (38.5)
Not tested	3 (23.1)
Smoking status	
Current	10 (27.0)
Ex-smoker	22 (59.5)
Non-smoker	5 (13.5)

Metastatic disease	
No	3 (8.1)
Yes	34 (91.9)
Localization of lesions:	
Lymph node	27 (73.0)
Lung	17 (45.9)
Oropharynx	13 (35.1)
Oral cavity	11 (29.7)
Bone	6 (16.2)
Larynx	6 (16.2)
Liver	4 (10.8)
Last therapy prior to enrolment	
Platinum-based chemotherapy	37 (100)
Platinum-based chemotherapy plus cetuximab	15 (40.5)
PD-L1 status (centrally assessed)[1]	
CPS <1	7 (21.9)
CPS 1-19	10 (31.3)
CPS ≥20	15 (46.9)

Table 2. Objective Response Rate and Duration of Response

	Overall population (mITT) (*n* = 37)				**PD-L1 CPS ≥1 (*n* = 25)**	**PD-L1 CPS ≥20 (*n* = 15)**
	iRECIST		**RECIST 1.1**		**iRECIST**	**iRECIST**
n (%)	**unconfirmed**	**confirmed**	**unconfirmed**	**confirmed**	**unconfirmed**	**unconfirmed**
Complete response	5 (13.5)	4 (10.8)	5 (13.5)	4 (10.8)	4 (16.0)	4 (26.7)
Partial response	6 (16.2)	6 (16.2)	5 (13.5)	6 (16.2)	6 (24.0)	5 (33.3)
Stable disease	3 (8.1)	4 (10.8)	3 (8.1)	3 (8.1)	2 (8.0)	0
Progressive disease	17 (46.0)	17 (46.0)	18 (48.6)	18 (48.6)	8 (32.0)	5 (33.3)
Not evaluable	6 (16.2)	6 (16.2)	6 (16.2)	6 (16.2)	5 (20.0)	1 (6.7)
Objective response rate [95% CI][1]	11 (29.7) [15.9-47.0]	10 (27.0) [13.8-44.1]	10 (27.0) [13.8-44.1]	10 (27.0) [13.8-44.1]	10 (40.0) [21.1-61.3]	9 (60.0) [32.3-83.7]
Disease control rate [95% CI][1]	14 (37.8) [22.5-55.2]	14 (37.8) [22.5-55.2]	13 (35.1) [20.2-52.5]	13 (35.1) [20.2-52.5]	12 (48.0) [27.8-68.7]	9 (60.0) [32.3-83.7]
	Evaluable population* (*n* = 31)				**PD-L1 CPS ≥1 (*n* = 25)**	**PD-L1 CPS ≥20 (*n* = 15)**
	iRECIST		**RECIST 1.1**		**iRECIST**	**iRECIST**
n/N (%)	**unconfirmed**	**confirmed**	**unconfirmed**	**confirmed**	**unconfirmed**	**unconfirmed**
Objective response rate – evaluable patients* [95% CI][1]	11/31 (35.5) [19.2-54.6]	10/31 (32.3) [16.7-51.4]	10/31 (32.3) [16.7-51.4]	10/31 (32.3) [16.7-51.4]	10/20 (50.0) [27.2-72.8]	9/14 (64.3) [35.1-87.2]
n (%)	**iRECIST (*n* = 10)**		**RECIST 1.1 (*n* = 10)**		**PD-L1 CPS ≥1 iRECIST (*n* = 9)**	**PD-L1 CPS ≥20 iRECIST (*n* = 8)**
Duration of response, months [95% CI][2]	Not reached		Not reached		Not reached	Not reached

FIGURE LEGEND

Figure 1. Tumor responses according to iRECIST

(a) Timeline of percentage change of sum of target lesion diameters from baseline for each evaluable patient by PD-L1 expression status (N=31). Patients without any evaluable post-baseline staging were excluded. (**b)** Kaplan-Meier curve of the duration of response per iRECIST for patients with a confirmed response (N=10). (**c)** Waterfall plot of best percentage change from baseline of the sum of target lesion diameters for each evaluable patient (N=31) by PD-L1 expression status. There were a total of 11 responses. One patient experienced a -54.5% reduction in sum of target lesion diameters, however due to progression in non-target lesions and the presence of new lesions, no response was recorded. PD-L1 was centrally assessed with Dako IHC 22C3 pharmDx.

CI, confidence interval; CPS, combine positive score; CR, complete response; H, hypopharynx; iRECIST, immune Response Evaluation Criteria In Solid Tumors; L, larynx; NC, not calculated; NR, not reached; OC, oral cavity; PD, progressive disease; PD-L1, programmed cell death ligand 1, PR, partial response; SD, stable disease.

Figure 2. Overall survival

Kaplan-Meier curves of OS **(a)** within the overall modified intent-to-treat population, irrespective of PD-L1 expression (N=37) and **(b)** within the subpopulations with PD-L1 CPS ≥1 (N=25) and ≥20 (N=15).

CI, confidence interval; CPS, combined positive score.

Figure 3. Association between change of ALC from baseline and clinical outcomes

(a) Mean (±SEM) of change from baseline of ALC tested pre-dose before each cycle by BOR per iRECIST. Timepoints with ≥10 subjects per subgroup are displayed. Two-sided rank-sum Wilcoxon *P* values are indicated. **(b)** Individual maximal ALC change from baseline obtained up to week 13 per confirmed BOR response, quartile boxes, with minimum, maximum, median (white line) and mean (black line) are shown. Two-sided rank-sum Wilcoxon p values are indicated. **(c)** Kaplan-Meier curve of OS and **(d)** PFS (per iRECIST) of subgroups determined by ALC change from baseline before week 13 (cut-off, 0.2×10^9/L of blood).

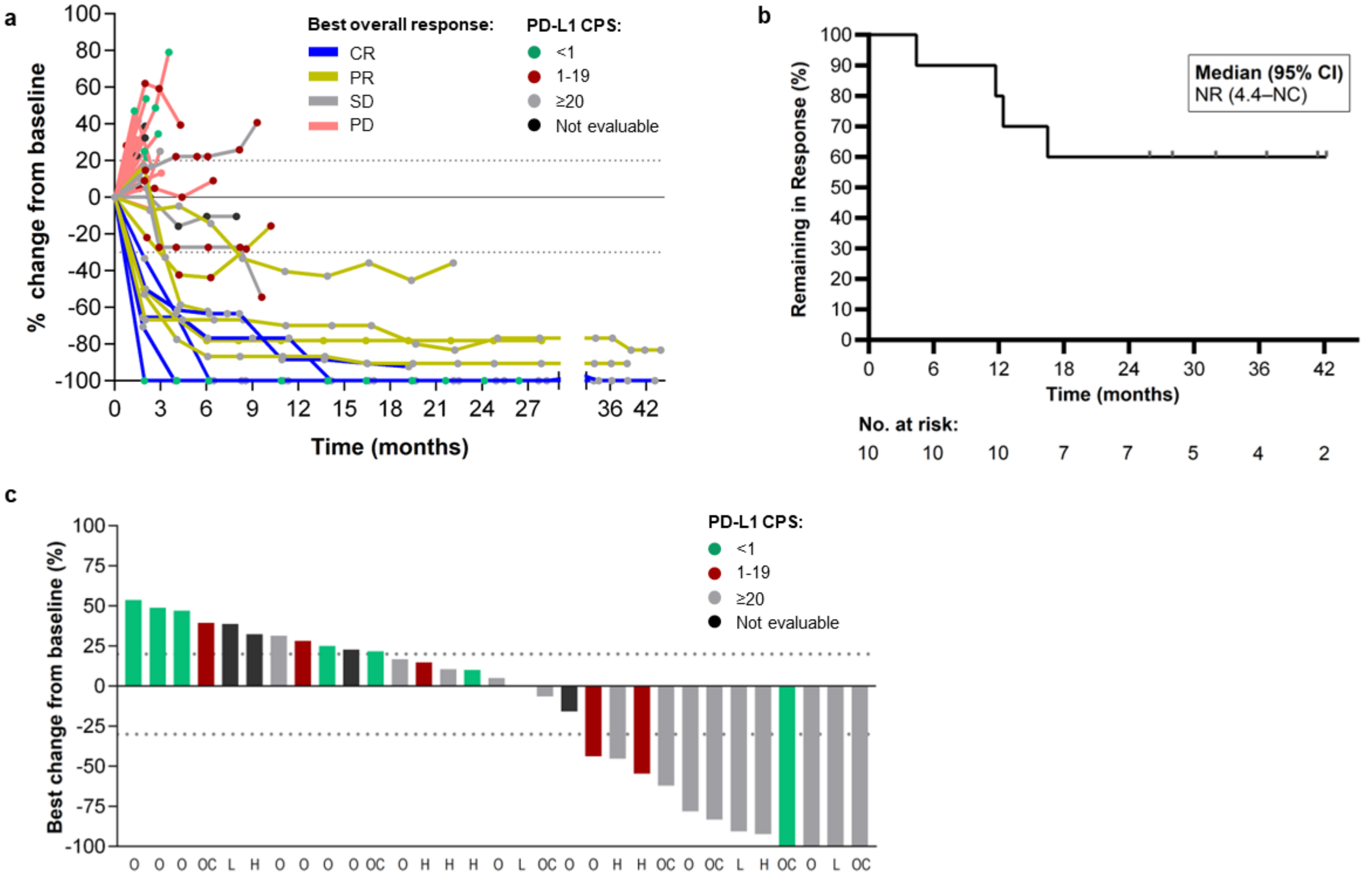
ALC, absolute lymphocyte count; BOR, best objective response; CI, confidence interval; iRECIST, immune Response Evaluation Criteria In Solid Tumors; OS, overall survival; PFS, progression-free survival, W, week.

Figure 4. Th1 biomarkers increase

(a) Mean (±SEM) fold change from baseline of IFN-γ and CXCL10 tested 1, 2, 4, 8, 24, 48, 72, 96 hours post-efti dosing in a subset of patients (N=10). The maximal values obtained post-dose 1 are displayed. **(b)** Mean (±SEM) fold change from

baseline of IFN-γ and CXCL10 in samples collected prior to the efti dose 7 (Week 13, N=18) and dose 13 (week 25, Cycle 9, N=15). Significant two-sided matched paired signed rank Wilcoxon *P* values are indicated.

Figure 1


a
Best overall response:
CR
PR
SD
PD
PD-L1 CPS:
<1
1-19
≥20
Not evaluable
% change from baseline
Time (months)
b
Remaining in Response (%)
Median (95% CI)
NR (4.4–NC)
Time (months)
No. at risk:
10 10 10 7 7 5 4 2
c
PD-L1 CPS:
<1
1-19
≥20
Not evaluable
Best change from baseline (%)
O O O OC L H O O O O OC O H H H O L OC O O H H OC O OC L H OC O L OC

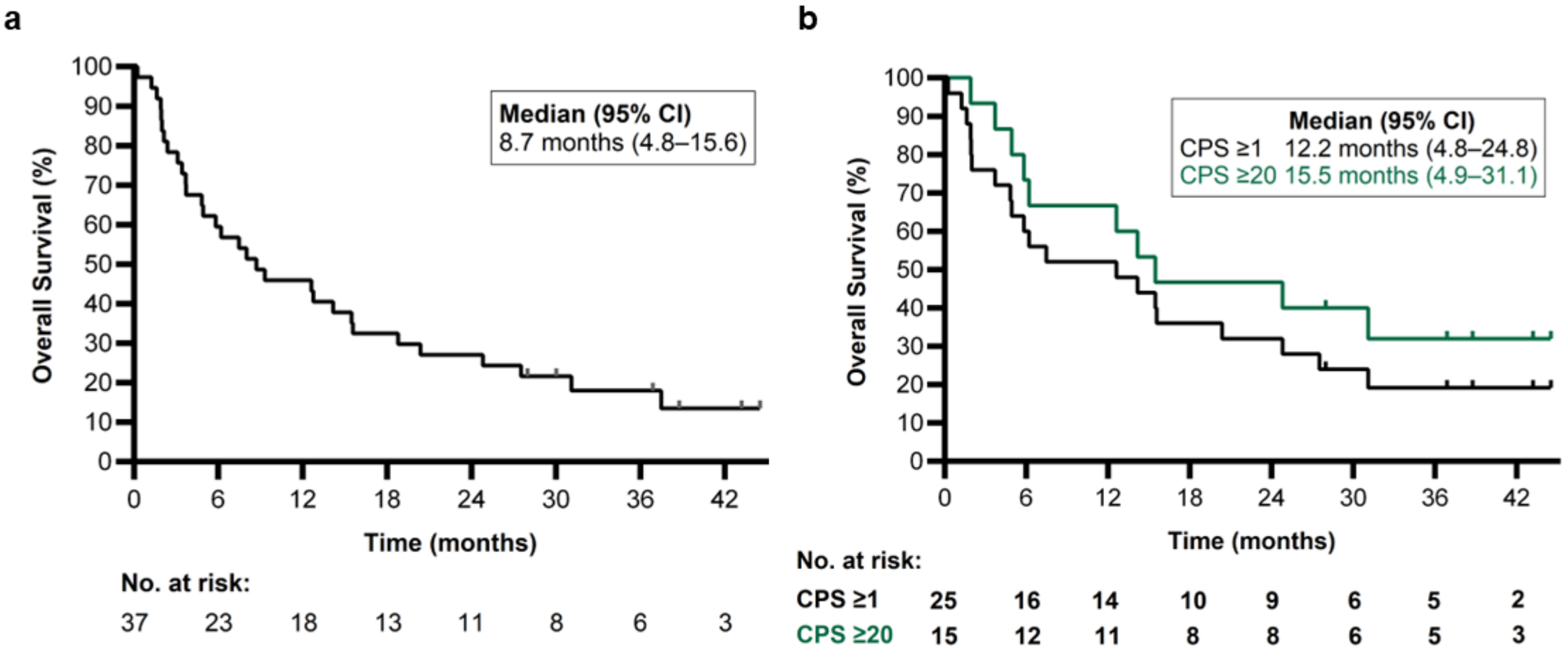

a
Overall Survival (%)
100
90
80
70
60
50
40
30
20
10
0
Median (95% CI)
8.7 months (4.8–15.6)
0
6
12
18
24
30
36
42
Time (months)
No. at risk:
37 23 18 13 11 8 6 3
b
Overall Survival (%)
Median (95% CI)
CPS ≥1 12.2 months (4.8–24.8)
CPS ≥20 15.5 months (4.9–31.1)
Time (months)
No. at risk:
CPS ≥1 25 16 14 10 9 6 5 2
CPS ≥20 15 12 11 8 8 6 5 3

Figure 3

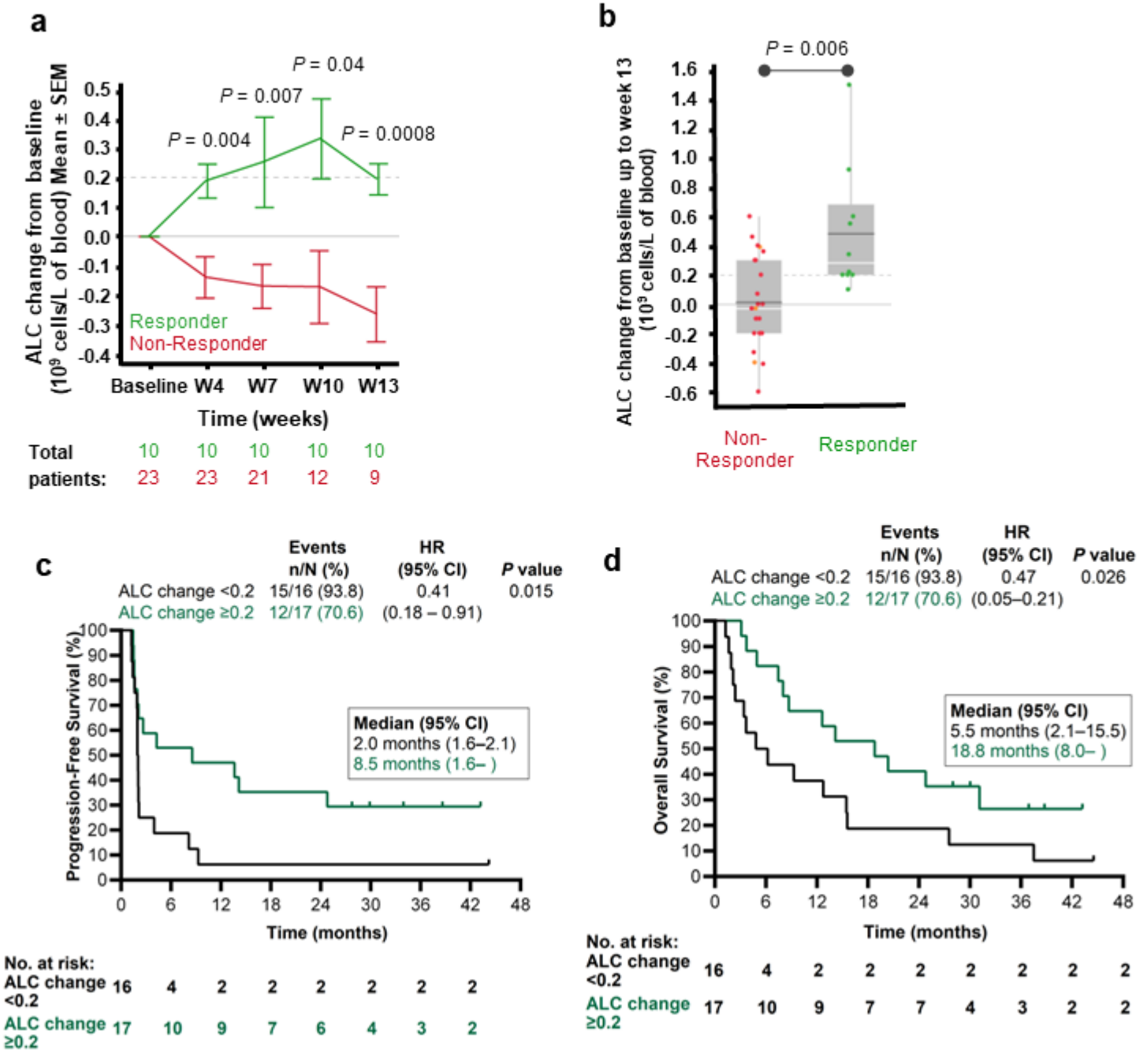
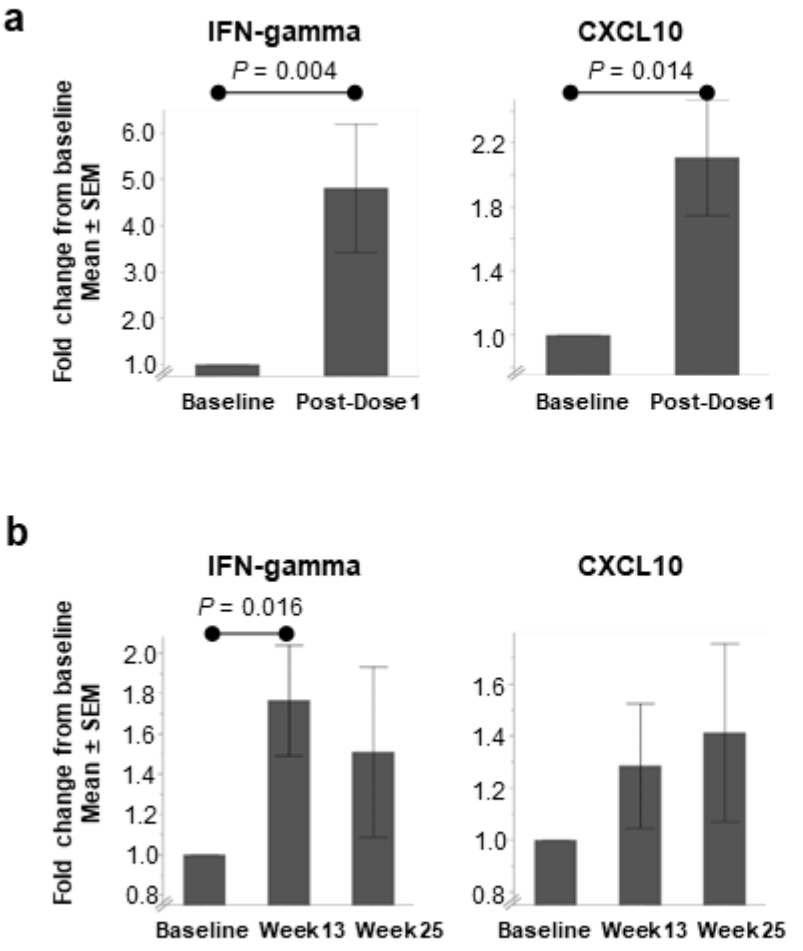

a
P = 0.04
P = 0.007
P = 0.004
P = 0.0008
ALC change from baseline (10⁹ cells/L of blood) Mean ± SEM
Responder
Non-Responder
Baseline W4 W7 W10 W13
Time (weeks)
Total patients: 10 10 10 10 10 / 23 23 21 12 9
b
P = 0.006
ALC change from baseline up to week 13 (10³ cells/L of blood)
Non-Responder
Responder
c
Events n/N (%)
HR (95% CI)
P value
ALC change <0.2 15/16 (93.8) 0.41 0.015
ALC change ≥0.2 12/17 (70.6) (0.18 – 0.91)
Progression-Free Survival (%)
Median (95% CI)
2.0 months (1.6–2.1)
8.5 months (1.6–)
Time (months)
No. at risk:
ALC change <0.2 16 4 2 2 2 2 2 2
ALC change ≥0.2 17 10 9 7 6 4 3 2
d
Events n/N (%)
HR (95% CI)
P value
ALC change <0.2 15/16 (93.8) 0.47 0.026
ALC change ≥0.2 12/17 (70.6) (0.05–0.21)
Overall Survival (%)
Median (95% CI)
5.5 months (2.1–15.5)
18.8 months (8.0–)
Time (months)
No. at risk:
ALC change <0.2 16 4 2 2 2 2 2 2 2
ALC change ≥0.2 17 10 9 7 7 4 3 2 2

Figure 4


a
IFN-gamma
P = 0.004
Fold change from baseline
Mean ± SEM
6.0
5.0
4.0
3.0
2.0
1.0
Baseline
Post-Dose 1
CXCL10
P = 0.014
2.2
1.8
1.4
1.0
Baseline
Post-Dose 1
b
IFN-gamma
P = 0.016
Fold change from baseline
Mean ± SEM
2.0
1.8
1.6
1.4
1.2
1.0
0.8
Baseline
Week 13
Week 25
CXCL10
1.6
1.4
1.2
1.0
0.8
Baseline
Week 13
Week 25