International Journal of *Molecular Sciences*

MDPI

Article

The Effects of GCSF Primary Prophylaxis on Survival Outcomes and Toxicity in Patients with Advanced Non-Small Cell Lung Cancer on First-Line Chemoimmunotherapy: A Sub-Analysis of the Spinnaker Study

Shobana Anpalakhan [1], Prerana Huddar [2], Roya Behrouzi [2], Alessio Signori [3], Judith Cave [4], Charles Comins [5], Alessio Cortellini [6,7], Alfredo Addeo [8], Carles Escriu [9], Hayley McKenzie [4], Gloria Barone [10], Lisa Murray [1], Gagan Bhatnagar [1], David J. Pinato [6,11], Christian Ottensmeier [12], Fabio Gomes [2,†] and Giuseppe Luigi Banna [1,*,†]

1 Portsmouth Hospitals University NHS Trust, Portsmouth PO6 3LY, UK
2 The Christie NHS Foundation Trust, Manchester M20 4BX, UK
3 Section of Biostatistics, Department of Health Sciences University of Genoa, 16126 Genoa, Italy
4 Department of Medical Oncology, University Hospital Southampton NHS Foundation Trust, Southampton SO17 1BJ, UK
5 Bristol Haematology and Oncology Centre, Bristol Royal Infirmary, Bristol BS2 8HW, UK
6 Department of Surgery and Cancer, Imperial College London, Hammersmith Hospital Campus, London SW7 2AZ, UK
7 Medical Oncology, Fondazione Policlinico Universitario Campus Bio-Medico, Via Alvaro del Portillo, 00128 Roma, Italy
8 Oncology Department, HUG—Hopitaux Universitaires de Geneve, 1205 Geneva, Switzerland
9 The Clatterbridge Cancer Centre NHS Foundation Trust, Liverpool L7 8YA, UK
10 University Hospitals of Northamptonshire, Northampton NN1 5BD, UK
11 Division of Oncology, Department of Translational Medicine, University of Piemonte Orientale, 28100 Novara, Italy
12 The Clatterbridge Cancer Centre NHS Foundation Trust, University of Liverpool, Liverpool L7 8YA, UK
* Correspondence: giuseppe.banna@nhs.net; Tel.: +44-023-9228-6000
† These authors contributed equally to this work and are co-last authors.

Abstract: GCSF prophylaxis is recommended in patients on chemotherapy with a >20% risk of febrile neutropenia and is to be considered if there is an intermediate risk of 10–20%. GCSF has been suggested as a possible adjunct to immunotherapy due to increased peripheral neutrophil recruitment and PD-L1 expression on neutrophils with GCSF use and greater tumour volume decrease with higher tumour GCSF expression. However, its potential to increase neutrophil counts and, thus, NLR values, could subsequently confer poorer prognoses on patients with advanced NSCLC. This analysis follows on from the retrospective multicentre observational cohort Spinnaker study on advanced NSCLC patients. The primary endpoints were OS and PFS. The secondary endpoints were the frequency and severity of AEs and irAEs. Patient information, including GCSF use and NLR values, was collected. A secondary comparison with matched follow-up duration was also undertaken. Three hundred and eight patients were included. Median OS was 13.4 months in patients given GCSF and 12.6 months in those not ($p = 0.948$). Median PFS was 7.3 months in patients given GCSF and 8.4 months in those not ($p = 0.369$). A total of 56% of patients receiving GCSF had Grade 1–2 AEs compared to 35% who did not receive GCSF ($p = 0.004$). Following an assessment with matched follow-up, 41% of patients given GCSF experienced Grade 1–2 irAEs compared to 23% of those not given GCSF ($p = 0.023$). GCSF prophylaxis use did not significantly affect overall or progression-free survival. Patients given GCSF prophylaxis were more likely to experience Grade 1–2 adverse effects and Grade 1–2 immunotherapy-related adverse effects.

Keywords: NSCLC; lung cancer; immunotherapy; GCSF; neutropenia; prophylaxis; immune-related toxicity; neutrophil-to-lymphocyte ratio (NLR); neuthrophils; outcome; overall survival

check for updates

Citation: Anpalakhan, S.; Huddar, P.; Behrouzi, R.; Signori, A.; Cave, J.; Comins, C.; Cortellini, A.; Addeo, A.; Escriu, C.; McKenzie, H.; et al. The Effects of GCSF Primary Prophylaxis on Survival Outcomes and Toxicity in Patients with Advanced Non-Small Cell Lung Cancer on First-Line Chemoimmunotherapy: A Sub-Analysis of the Spinnaker Study. *Int. J. Mol. Sci.* **2023**, *24*, 1746. https://doi.org/10.3390/ijms24021746

Academic Editors: Stergios Boussios and Christos Mikropoulos

Received: 26 November 2022
Revised: 3 January 2023
Accepted: 9 January 2023
Published: 16 January 2023

1. Introduction

Granulocyte-colony-stimulating factor (GCSF) prophylaxis is used in patients on chemotherapy based on their risk of developing febrile neutropenia. This risk is determined by factors such as elderly age and neutrophil count. GCSF prophylaxis should be implemented in chemotherapy regimens with a high risk of febrile neutropenia (>20%) and at least considered if there is an intermediate risk (10–20%) [1,2]. During the COVID-19 pandemic, GCSF prophylaxis was used more widely to reduce the risk of adverse outcomes associated with COVID-19 infection [3,4]. There are, however, important safety issues to consider when GCSF is used, including a surge in pulmonary inflammation, macrophage activation and an increase in the neutrophil-to-lymphocyte ratio (NLR), which could subsequently lead to respiratory deterioration in COVID-19-positive patients [5,6].

There have been reports of GCSF increasing peripheral neutrophil recruitment and programmed death-ligand 1 (PD-L1) expression on neutrophils, as well as a greater decrease in tumour volume in cancers with higher GCSF expression. These mechanisms could result in increased efficacy of immune checkpoint inhibitors (ICIs); therefore, GCSF has been put forward as a potential adjuvant therapy to ICIs [7].

The administration of GCSF can result in both neutrophilia and a reduction in lymphocyte count, thus increasing the NLR [8] and potentially influencing the associated prognosis. The NLR has been shown to be a useful prognostic indicator in patients with non-small-cell lung cancer (NSCLC) as well as a predictor of response to immunotherapy [9–11]. Moreover, in patients with renal cell cancers, variations in the NLR have been shown to influence overall survival (OS) and progression-free survival (PFS) [12].

The Spinnaker retrospective study evaluated efficacy outcomes for patients with advanced NSCLC on first-line chemoimmunotherapy and in doing so, developed the prognostic score 'NHS-Lung score' to allow for risk stratification of these patients regardless of PD-L1 status [13]. This subsequent analysis following on from the Spinnaker study aimed to explore if the use of GCSF could be clinically beneficial in treatment-naïve patients with advanced NSCLC on chemoimmunotherapy either by a synergistic effect with the immunotherapy resulting in prolonged OS and PFS or by reducing the haematologic toxicity and immunosuppression of the chemotherapy. A differential clinical effect of GCSF use according to the baseline NLR level was also investigated with the hypothesis that different outcomes could be observed following the GCSF stimulation in those patients with higher levels of neutrophils to the detriment of lymphocytes.

2. Results

The Spinnaker study included 308 patients of ECOG-PS 0-1 from 7 different centres (16). The characteristics of this patient cohort according to the use of GCSF primary prophylaxis are described in Table 1. As demonstrated, it was noted that patients who received GCSF had a shorter median follow-up duration ($p < 0.001$). The median follow-up duration among those given GCSF prophylaxis was 12.8 months (95% CI: 12.3–13.3 months), while it was 20.7 months (95% CI: 19.0–22.5 months) among those not given any GCSF. The subsequent matched analysis revealed that this difference was not significant ($p = 0.533$). Among the factors that did not differ significantly between patients who received GCSF and those who did not were age, tumour histology, PS, PD-L1 status, pre-treatment steroid use, pre-treatment NLR, pre-treatment SII and the number of metastatic sites. This was confirmed following analysis with matched follow-up. The initial analysis with unmatched follow-up revealed a significant difference in the chemotherapy regimen given to patients on GCSF compared to those not on GCSF ($p = 0.007$). However, when this was repeated with matched follow-up, there was no significant difference found ($p = 0.867$).

Table 1. Association of G-CSF use with patient characteristics and outcomes.

Characteristic	G-CSF (No. 59) No. (%) [Range]	No G-CSF Unmatched (No. 249) No. (%) [Range]	χ^2 Test (Log-Rank) *p*-Value	No G-CSF Matched [a] (No. 114) No. (%) [Range]	χ^2 Test (Log-Rank) *p*-Value
Age					
≥70 years	21 (36)	77 (31)	0.591	40 (35)	0.919
<70 years	38 (64)	172 (69)		74 (65)	
Gender					
Male	31 (53)	140 (56)	0.714	68 (60)	0.463
Female	28 (47)	109 (44)		46 (40)	
Smoking history					
Never	3 (5)	22 (9)	0.494	7 (6)	0.951
Former/Current	56 (95)	227 (91)		107 (94)	
Histology					
Squamous	12 (21)	39 (16)	0.550	27 (25)	0.643
Adenocarcinoma	46 (79)	200 (84)		80 (75)	
Other	1 (2)	10 (4)		7 (6)	
ECOG PS					
0	28 (47)	99 (40)	0.351	39 (35)	0.161
1	31 (53)	150 (60)		72 (65)	
Stage					
IIIB/IVA	22 (37)	115 (46)	0.275	48 (42)	0.654
IVB	37 (63)	134 (54)		66 (58)	
BMI					
Underweight/Normal	27 (46)	135 (54)	0.306	57 (50)	0.713
Overweight/Obese	32 (54)	114 (46)		57 (50)	
Number of metastatic sites					
<3	41 (69)	164 (66)	0.706	80 (70)	0.935
≥3	18 (31)	85 (34)		34 (30)	
Brain metastases	6 (19)	25 (10)	0.833	13 (11)	0.992
Liver metastases	4 (7)	33 (13)	0.249	14 (12)	0.389
PD-L1 IHC Ab [b]					
Negative	34 (61)	131 (55)	0.495	58 (53)	0.416
Positive	22 (39)	109 (45)		52 (47)	
NA	3 (5)	9 (4)		4 (4)	
Pre-treatment steroids	6 (10)	27 (11)	0.933	13 (11)	0.992
Pre-treatment NLR ≥ 4	34 (58)	130 (52)	0.545	59 (52)	0.566
Pre-treatment SII ≥ 1440	30 (51)	124 (50)	1.000	58 (51)	0.875
Type of chemotherapy					
Cisplatin–Pemetrexed	3 (5)	21 (8)	**0.007**	4 (4)	0.867
Carboplatin–Pemetrexed	45 (76)	195 (78)		87 (76)	
Carboplatin–Paclitaxel	11 (19)	33 (13)		23 (20)	
Best response [c]					
CR/PR	37 (65)	161 (68)	0.744	68 (64)	0.706
SD	12 (21)	40 (17)		19 (18)	
PD	8 (14)	37 (16)		20 (19)	
NA	1 (2)	11 (4)			
G1/2 AE–G1/2 irAE	33 (56)–24 (41)	86 (35)–76 (31)	**0.004**–0.199	29 (25)–26 (23)	**<0.001–0.023**
G3/4 AE–G3/4 irAE	14 (24)–9 (15)	48 (19)–40 (16)	0.537–0.993	18 (16)–17 (15)	0.285–0.869
Treatment discontinuation	14 (24)	58 (23)	0.920	19 ()17)	0.359
Follow-up, median, mo. [95%]	12.8 [12.3–13.3]	20.7 [19.0–22.5]	**(<0.001)**	13.9 [9.9–17.8]	0.533

Table 1. *Cont.*

Characteristic	G-CSF (No. 59) No. (%) [Range]	No G-CSF Unmatched (No. 249) No. (%) [Range]	χ^2 Test (Log-Rank) *p*-Value	No G-CSF Matched [a] (No. 114) No. (%) [Range]	χ^2 Test (Log-Rank) *p*-Value
Deaths	29 (49)	151 (61)	0.143	56 (49)	0.875
OS, median, mo. [95% CI] 1 yr OS [95% CI] 2 yr OS [95% CI]	13.4 [10.4–16.5] 53.9 [50.2–57.8] 14.4 [12.9–16.4]	12.6 [9.7–15.4] 51.3 [49.6–53.1] 28.1 [27.2–29.2]	(0.948)	11.3 [9.0–13.5] 44.7 [42.3–47.3] 18.2 [16.1–20.9]	0.357
PFS, median, mo. [95% CI]	7.3 [4.6–10.0]	8.4 [7.5–9.3]	(0.369)	7.5 [5.8–9.2]	0.832
COVID-19-positive	2 (3)	8 (3)	0.734	3 (3)	0.844
COVID-19 deaths					
Death rate	1 (3)	7 (5)	0.836	2 (4)	0.555
Positive rate	1 (50)	7 (88)	0.843	1 (33)	0.576

Abbreviations: Ab, antibody; AE, adverse events; BMI, body mass index; CI, confidence interval; CR, complete response; ECOG PS, Eastern Cooperative Oncology Group Performance Status; G, grade; IHC, immunohistochemistry; mo., months; No. number; NA, not assessable; NLR, neutrophil-to-lymphocyte ratio; OS, overall survival; PD, progressive disease; PD-L1, programmed cell death-ligand-1; PFS, progression-free survival; PR, partial response; SD; stable disease; SII, systemic immune-inflammatory index; yr, year. [a] Matched by follow-up time (cut-off date for non-GCSF cohort 25.11.19). [b] Negative, TPS > 1%; positive, TPS 1−49%; high, TPS ≥ 50%. [c] By RECIST version 1.1 criteria. Statistically significant values in bold.

Patients who received GCSF primary prophylaxis were more likely to experience Grade 1–2 AEs ($p = 0.004$), and this was confirmed following analysis with matched follow-up. A total of 56% percent of patients receiving GCSF had Grade 1–2 AEs compared to 35% who did not receive GCSF. There was no significant difference in the proportion of patients who experienced Grade 3–4 AEs ($p = 0.537$). A total of 24% percent of patients on GCSF and 19% of patients not on GCSF experienced Grade 3–4 AEs. There were no significant differences in the proportion of patients experiencing irAEs of any grade in the unmatched follow-up analysis. Repeat assessment with matched follow-up in fact revealed a significant difference in Grade 1–2 irAEs, which were more frequent in patients on GCSF ($p = 0.023$). A total of 41% percent of patients on GCSF experienced Grade 1–2 irAEs compared to 23% of patients not on GCSF.

As demonstrated in Figure 1, the use of GCSF primary prophylaxis had no significant impact on median OS. This was 13.4 months (95% CI: 10.4–16.5 months) in patients given GCSF and 12.6 months (95% CI: 9.7–15.4 months) in patients not given GCSF ($p = 0.948$). It also yielded no significant difference in median PFS between those given GCSF with a median PFS of 7.3 months (95% CI: 4.6–10.0 months) and those not given GCSF with a median PFS of 8.4 months (95% CI: 7.5–9.3 months) ($p = 0.369$).

Median OS and PFS among patients with a high NLR did not differ significantly whether GCSF was used or not ($p = 0.954$ and $p = 0.358$, respectively). In patients with a high NLR, the median OS was 13.4 months (95% CI: 8.0–18.9 months) in those given GCSF and 11.8 months (95% CI: 9.1–14.5 months) in those not given GCSF. Median PFS in this same sub-cohort with a high NLR was 5.5 months (95% CI: 4.0–7.0 months) when given GCSF and 6.9 months (95% CI: 5.0–8.8 months) when not given GCSF prophylaxis. Patients with a low NLR also showed no significant difference in median OS or PFS ($p = 0.924$ and $p = 0.883$, respectively) with or without the use of GCSF. Median OS was 13.5 months (95% CI: 11.1–15.9 months) in patients given GCSF and 16.0 months (95% CI: 12.4–19.7 months) in patients not given GCSF. Median PFS was 8.0 months (95% CI: 4.4–11.5 months) for patients on GCSF and 9.0 months (95% CI: 7.3–10.7 months) in patients not given GCSF (Figure S1). In the following analysis with matched follow-up, the differences in OS and PFS among patients with high ($p = 0.357$ and $p = 0.832$, respectively) and low ($p = 0.932$ and $p = 0.417$, respectively) NLR values remained non-significant.

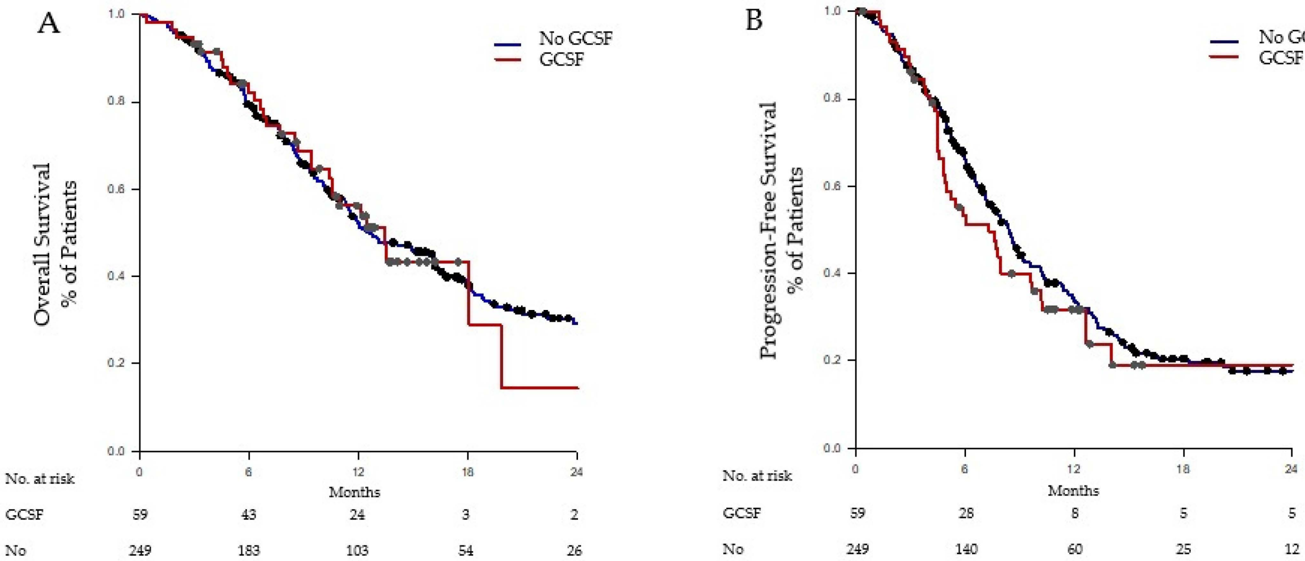

Figure letter (N)	Variable [N - %]	Median OS mo. (95% CI)	1-yr OS % (95% CI)	2-yr OS % (95% CI)	p-value (log-rank)	Median PFS mo. (95% CI)	p-value (log-rank)
A/B (N= 308)	GCSF [59 – 19]	13.4 (10.4-16.5)	53.9 (50.2-57.8)	14.4 (12.9-16.4)	0.948	7.3 (4.6-10.0)	0.369
	No GCSF [249 – 81]	12.6 (9.7-15.4)	51.3 (49.6-53.1)	28.1 (27.2-29.2)		8.4 (7.5-9.3)	
Abbreviations: aNSCLC, advanced non-small-cell-lung cancer; CI, confidence interval; OS, overall survival; PFS, progression-free survival							

Figure 1. OS (**A**) and PFS (**B**) by GCSF use in patients with aNSCLC treated with chemoimmunotherapy.

3. Discussion

The results of this analysis have shown that the use of GCSF prophylaxis does not affect the survival outcomes of patients with advanced NSCLC treated with chemoimmunotherapy. It has shown, following analysis with matched follow-up, that patients given GCSF prophylaxis were more likely to experience Grade 1–2 AEs and Grade1–2 irAEs. The key limitations of this analysis include its retrospective nature, the lack of experimental verification and detailed information about the type of GCSF used and timing of GCSF administration, the dynamics and characterisation of the peripheral and tumour-infiltrating immune cells, and NLR changes following the GCSF stimulation and initial unmatched follow-up among the two groups of patients, which introduced a potential time bias, although this was accounted for with a subsequent analysis with matched follow-up. Furthermore, a specific effect of GCSF with immunotherapy could be masked by concomitant chemotherapy. On the other hand, the multicentre and real-life nature of this study allows for the generalisability of its results.

Contrarily to other reports which investigated the use of primary GCSF prophylaxis with chemotherapy regimens characterised by a high risk of febrile neutropenia [14,15], the chemotherapy regimen used in the present series does not have a high risk of febrile neutropenia and, therefore, does not necessarily require GSCF prophylaxis [1,2]. Moreover, this analysis has shown that a multitude of factors used in various prognostic tools, such as age, histology, PS, PD-L1 status, pre-treatment steroids, pre-treatment NLR, pre-treatment SII and the number of metastatic sites, did not predict whether patients received GCSF prophylaxis. The Spinnaker study established the NHS-Lung score, uses the number of metastatic sites, tumour histology and the SII score to guide prognostication [13]. It concluded that more metastatic sites, squamous tumour histology and a higher SII score conferred a poorer prognosis. The Lung Cancer Prognostic Index (LCPI) uses a number of factors, including tumour stage and histology, mutation status, PS, weight loss, smoking history, respiratory comorbidity, sex and age, to determine prognosis [16]. Moreover, elderly age was also deemed an important factor in determining the risk of febrile neutropenia [1]. The Lung Immuno-oncology Prognostic Score-3 (LIPS-3) attributes a poor prognosis to patients with a PS of $\geq$2, requiring pre-treatment steroids (indicating possible brain or liver metastases or nutritional issues), and an NLR $\geq$ 4 [17]. PD-L1 expression has also been shown to be a marker for poor prognosis [18,19]. In addition to this, GCSF prophylaxis has been suggested as a potential adjunct to immunotherapy [7]. Despite these reports, these numerous factors were shown not to influence GCSF primary prophylaxis use in this analysis.

Median follow-up was seen to be significantly shorter in the group receiving GCSF. This observation was potentially due to a larger proportion of patients receiving GCSF and commencing follow-up from the start of the COVID-19 pandemic in the United Kingdom in March 2020 compared to other patients who had been followed up since April 2019. Therefore, primary prophylaxis with GCSF was likely adopted as a general precautionary strategy offered during the COVID-19 pandemic.

The administration of GCSF prophylaxis was shown not to affect OS or PFS in this series of patients treated with first-line chemoimmunotherapy for advanced NSCLC. This is in contrast to another study on patients with metastatic pancreatic cancer on FOLFIRINOX chemotherapy that found primary GCSF prophylaxis actually improved OS [20]. The global, randomised ECHELON-1 study on patients with Hodgkin's lymphoma on brentuximab vedotin and doxorubicin as well as vinblastine and dacarbazine also found an improvement in survival outcomes with the use of GCSF through reduced frequency and severity of AEs, treatment delays and episodes of chemotherapy discontinuations [21]. These discrepancies could be attributed to the toxicity variations with different chemotherapy regimens.

The observations in survival outcomes noted in this analysis were consistent when stratified by NLR values as well. Previous reports had suggested the use of GCSF prophylaxis potentially affects the NLR [11] and, thus, prognosis. Further work, including experimental models, needs to be undertaken to assess the dynamics and characterisation of the peripheral and tumour-infiltrating immune cells and changes in the NLR following GCSF administration and the nature and duration of these changes, taking into account that patients tend to be on relatively short courses of GCSF prophylaxis of 5–7 days. While GCSF has been reported to increase PD-L1 expression on neutrophils and contribute to increased tumour shrinkage in cancers with GCSF expression, further research on the effects of GCSF prophylaxis on these and the duration of these effects is required [7].

There were significantly more patients on GCSF experiencing general Grade 1–2 AEs than those not on GCSF. A potential explanation for this is that side effects attributed to treatment regimens may be in fact secondary to GCSF administration, including myalgia, fatigue and skin rash. The matched follow-up analysis revealed a significant difference in Grade 1–2 irAEs as well. This may demonstrate a true increase in mild immunotherapy-related side effects with GCSF use. As GCSF use has been linked to potentially increasing the NLR, this may explain the higher incidence of Grade 1–2 irAEs among patients given GCSF prophylaxis in this analysis. The REISAMIC registry prospective study found that an elevated NLR was associated with more severe immunotherapy-related toxicities [22]. On the other hand, this finding could also be explained by the similar side effects of chemotherapy and immunotherapy or even treatment overlap, but these are being labelled as immunotherapy-related effects.

Nonetheless, there were no significant differences in treatment discontinuation rates between the groups despite more Grade 1–2 AEs in patients receiving GCSF prophylaxis. There were also no significant differences in the proportion of patients experiencing Grade

3–4 AEs of any nature. This could also be explained by the only temporarily increased neutrophil counts following GCSF administration. Pre-treatment with steroids may have contributed to patients not experiencing irAEs, although only approximately 10–11% of patients in each group had been on steroids.

4. Materials and Methods

The Spinnaker study was a retrospective multicentre observational cohort study focusing on real-world patients with histologically confirmed advanced NSCLC, any PD-L1 tumour proportion score (TPS), no actionable genomic alterations and an Eastern Cooperative Oncology Group Performance Status (ECOG PS) ≤ 1, who had been treated with first-line chemotherapy and pembrolizumab and whose outcome results have recently been reported [13]. Patients were treated in six United Kingdom centres and one Swiss centre between March 2018 and April 2021. Among the collected patient information, the use of primary prophylaxis with GCSF was recorded and defined by the use of any non-pegylated drugs for at least five consecutive days (i.e., filgrastim 300–480 mcg or lenograstim 263 mcg subcutaneous daily), or pegylated ones for one day (i.e., pegfilgrastim 6 mg or lipegfilgrastim 6 mg), following each chemoimmunotherapy cycle starting from the first one. The NLR was calculated as the ratio between neutrophils and lymphocytes from the peripheral blood count of a standard blood test performed within 14 days of the treatment start date. A high NLR was considered a value ≥ 4 according to the literature-reported cut-off [17]. The systemic immune-inflammatory index (SII) was calculated as the NLR multiplied by the platelet count with the previously reported cut-off value of ≥ 1440 [13].

The primary endpoint of this analysis was to describe the patients' characteristics and survival outcomes (i.e., OS and PFS) according to the use of GCSF primary prophylaxis. Secondary endpoints included the frequency and severity of adverse events (AEs) and immune-related adverse events (irAEs) as adjudicated by the clinician and graded according to the common toxicity criteria for adverse effects (CTC-AE) version 5.0, and the interaction between GCSF primary prophylaxis and NLR as per the above cut-off in OS and PFS and with PD-L1 status (TPS <1%—negative vs. $\geq$1%—positive) and chemotherapy regimen in OS.

Clinical data were analysed by descriptive statistics, using percentages for the binary variables and medians for the continuous variables, reporting their respective dispersion values. For the comparison of binary variables, the chi-square test with an acceptable significance value of $p < 0.05$ was performed. The OS was calculated from the treatment start date until death or the date of the last follow-up; the PFS was taken from the treatment start date to disease progression or death from any cause. Patients who had not had any events at the time of the analysis were censored. OS and PFS were estimated using the Kaplan–Meier method and reported as medians with confidence limits (95% CIs), and compared using a two-sided log-rank test with an acceptable significance value of $p < 0.05$ [23]. An interaction test between the GCSF primary prophylaxis and the above factors was performed. The statistical analysis was carried out using the SigmaPlot software version 12.5 (Systat Software, San Jose, CA, USA).

Given the significant difference found in the median follow-up time between patients treated with GCSF primary prophylaxis versus those who did not receive it in the study population, a secondary comparison of the study endpoints was performed using a cohort of patients who did not receive GCSF matched according to their follow-up time. The follow-up time was matched by manually searching within the non-GSCF cohort for a treatment start date by which the median follow-up time coincided with the GCSF cohort; this corresponded to the end of November 2019.

5. Conclusions

This analysis has shown that the use of GCSF prophylaxis does not affect OS or PFS in patients with advanced NSCLC on chemoimmunotherapy. There was a higher incidence of

Grade 1–2 general and immunotherapy-related side effects in patients given GCSF. Future prospective research is required to further assess these outcomes.

Supplementary Materials: The supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ijms24021746/s1.

Author Contributions: Conceptualisation, A.A., F.G. and G.L.B.; methodology, A.S. and G.L.B.; software, A.S. and G.L.B.; validation, A.S.; formal analysis, A.S. and G.L.B.; investigation, J.C., C.C., A.C., C.E., G.B. (Gloria Barone), G.B. (Gagan Bhatnagar), D.J.P. and C.O.; resources, L.M.; data curation, P.H., R.B., A.C., A.A., H.M. and G.L.B.; writing—original draft, S.A. and G.L.B.; writing—review and editing, S.A., P.H., R.B., A.S., J.C., C.C., A.C., A.A., C.E., H.M., G.B. (Gloria Barone), L.M., G.B. (Gagan Bhatnagar), D.J.P., C.O., F.G. and G.L.B.; supervision, A.A., C.E. and G.L.B.; project administration, L.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: This study was registered and approved as an audit by the multiple participating sites, with the coordinating centre being Portsmouth Hospital University NHS Trust (UK). Clinical data were anonymised before being shared with the coordinating centre for analysis. The audit procedures were compliant with the Data Protection Act 2018 and the precepts of the Good Clinical Practice guidelines with regard to the collection, storage, processing and disclosure of personal information, and the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations.

Informed Consent Statement: Patients in each Center signed a specific consent form for their data collection and sharing with other Institutions.

Data Availability Statement: The datasets generated and analysed during the current study are not publicly available as they are part of the confidential medical record but are available from the corresponding author on reasonable request.

Acknowledgments: Alessio Cortellini would like to acknowledge the support provided by the National Institute for Health Research (NIHR) Imperial Biomedical Research Centre (BRC). David J Pinato would like to acknowledge the support received from Wellcome Trust Strategic Fund (PS3416) and from the Associazione Italiana per la Ricerca sul Cancro (AIRC MFAG Grant ID 25697). He also acknowledges the support of the NIHR Imperial Biomedical Research Centre (BRC), the Imperial Experimental Cancer Medicine Centre (ECMC) and the Imperial College Tissue Bank.

Conflicts of Interest: G.L.B. has received grant consultancies from Astrazeneca and Astellas Pharma. A.C. has received speaker fees and grant consultancies from Astrazeneca, MSD, IQVIA, OncoC4 and EISAI. A.A. has received consulting fees from BMS, Astrazeneca, Boehringer-Ingelheim, Roche, MSD, Pfizer, Eli Lilly and Astellas and speaker fees from Eli Lilly and Astrazeneca. D.J.P. has received lecture fees from ViiV Healthcare, Bayer Healthcare, BMS, Roche, EISAI and Falk Foundation; travel expenses from BMS and Bayer Healthcare; consulting fees from Mina Therapeutics, EISAI, Roche, Avamune, Exact Sciences, Mursla, DaVolterra and Astra Zeneca; and research funding (to institution) from MSD and BMS. G.B. has received travel expenses from Novartis. CO reports personal fees from BMS. All other authors declare no competing interest.

Glossary

AEs	adverse events
NSCLC	non-small-cell lung cancer
ECOG	PS Eastern Cooperative Oncology Group performance status
ICI	immune checkpoint inhibitors
irAEs	immune-related AEs
LCPI	Lung Cancer Prognostic Index
LIPS	Lung Immuno-oncology Prognostic Score
NHS-Lung score	N = number of metastatic sites (cut-off $\geq$ 3) H = histology (i.e., squamous) S = SII ($\geq$1440)
NLR	neutrophils-to-lymphocytes ratio
OS	overall survival

SII	systemic immune-inflammatory index
PD-L1	programmed death ligand-1
PFS	progression-free survival
TPS	tumour proportion score

References

1. Smith, T.J.; Bohlke, K.; Lyman, G.H.; Carson, K.R.; Crawford, J.; Cross, S.J.; Goldberg, J.M.; Khatcheressian, J.L.; Leighl, N.B.; Perkins, C.L.; et al. Recommendations for the Use of WBC Growth Factors: American Society of Clinical Oncology Clinical Practice Guideline Update. *J. Clin. Oncol.* **2015**, *33*, 3199–3212. [CrossRef]
2. Aapro, M.S.; Bohlius, J.; Cameron, D.A.; Dal Lago, L.; Donnelly, J.P.; Kearney, N.; Lyman, G.H.; Pettengell, R.; Tjan-Heijnen, V.C.; Walewski, J.; et al. 2010 update of EORTC guidelines for the use of granulocyte-colony stimulating factor to reduce the incidence of chemotherapy-induced febrile neutropenia in adult patients with lymphoproliferative disorders and solid tumours. *Eur. J. Cancer* **2011**, *47*, 8–32. [CrossRef] [PubMed]
3. Passaro, A.; Addeo, A.; Von Garnier, C.; Blackhall, F.; Planchard, D.; Felip, E.; Dziadziuszko, R.; de Marinis, F.; Reck, M.; Bouchaab, H.; et al. ESMO Management and treatment adapted recommendations in the COVID-19 era: Lung cancer. *ESMO Open* **2020**, *5*, e000820. [CrossRef] [PubMed]
4. Banna, G.; Curioni-Fontecedro, A.; Friedlaender, A.; Addeo, A. How we treat patients with lung cancer during the SARS-CoV-2 pandemic: Primum non nocere. *ESMO Open* **2019**, *4*, e000765. [CrossRef] [PubMed]
5. Nawar, T.; Morjaria, S.; Kaltsas, A.; Patel, D.; Perez-Johnston, R.; Daniyan, A.F.; Mailankody, S.; Parameswaran, R. Granulocyte-colony stimulating factor in COVID-19: Is it stimulating more than just the bone marrow? *Am. J. Hematol.* **2020**, *95*, E210–E213. [CrossRef] [PubMed]
6. Lasagna, A.; Zuccaro, V.; Ferraris, E.; Rizzo, G.; Tancredi, R.J.; Pedrazzoli, P. How to Use Prophylactic G-CSF in the Time of COVID-19. *JCO Oncol. Pract.* **2020**, *16*, 771–772. [CrossRef]
7. Kaisar-Iluz, N.; Arpinati, L.; Shaul, M.E.; Mahroum, S.; Qaisi, M.; Tidhar, E.; Fridlender, Z.G. The Bilateral Interplay between Cancer Immunotherapies and Neutrophils' Phenotypes and Sub-Populations. *Cells* **2022**, *11*, 783. [CrossRef]
8. Tavakkoli, M.; Wilkins, C.R.; Mones, J.V.; Mauro, M.J. A Novel Paradigm Between Leukocytosis, G-CSF Secretion, Neutrophil-to-Lymphocyte Ratio, Myeloid-Derived Suppressor Cells, and Prognosis in Non-small Cell Lung Cancer. *Front. Oncol.* **2019**, *9*, 295. [CrossRef]
9. Zer, A.; Sung, M.R.; Walia, P.; Khoja, L.; Maganti, M.; Labbe, C.; Shepherd, F.A.; Bradbury, P.A.; Feld, R.; Liu, G.; et al. Correlation of Neutrophil to Lymphocyte Ratio and Absolute Neutrophil Count with Outcomes with PD-1 Axis Inhibitors in Patients with Advanced Non-Small-Cell Lung Cancer. *Clin. Lung Cancer* **2018**, *19*, 426–434. [CrossRef]
10. Takeda, T.; Takeuchi, M.; Saitoh, M.; Takeda, S. Neutrophil-to-lymphocyte ratio after four weeks of nivolumab administration as a predictive marker in patients with pretreated non-small-cell lung cancer. *Thorac. Cancer* **2018**, *9*, 1291–1299. [CrossRef]
11. Suh, K.J.; Kim, S.H.; Kim, Y.J.; Kim, M.; Keam, B.; Kim, T.M.; Kim, D.-W.; Seog Heo, D.S.; Lee, J.S. Post-treatment neutrophil-to-lymphocyte ratio at week 6 is prognostic in patients with advanced non-small cell lung cancers treated with anti-PD-1 antibody. *Cancer Immunol. Immunother.* **2018**, *67*, 459–470. [CrossRef] [PubMed]
12. Rebuzzi, S.E.; Signori, A.; Stellato, M.; Santini, D.; Maruzzo, M.; De Giorgi, U.; Pedrazzoli, P.; Galli, L.; Zucali, P.A.; Fantinel, E.; et al. The prognostic value of baseline and early variations of peripheral blood inflammatory ratios and their cellular components in patients with metastatic renal cell carcinoma treated with nivolumab: The Δ-Meet-URO analysis. *Front. Oncol.* **2022**, *12*, 955501. [CrossRef] [PubMed]
13. Giuseppe, L.; Banna, O.C.; Muthuramalingam, S.; Cave, J.; Comins, C.; Cortellini, A.; Addeo, A.; Signori, A.; McKenzie, H.; Escriu, C.; et al. Efficacy outcomes and prognostic factors from real-world patients with advanced non-small-cell lung cancer treated with first-line chemoimmunotherapy: The Spinnaker retrospective study. *Int. Immunopharmacol.* **2022**, *110*, 108985.
14. Fujita, A.; Ohkubo, T.; Hoshino, H.; Takabatake, H.; Tagaki, S.; Sekine, K.; Abe, S. Phase II study of cisplatin, ifosfamide, and irinotecan with rhG-CSF support in patients with stage IIIb and IV non-small-cell lung cancer. *Br. J. Cancer* **2003**, *89*, 1008–1012. [CrossRef] [PubMed]
15. Hejna, M.; Kornek, G.V.; Raderer, M.; Ulrich-Pur, H.; Fiebiger, W.C.; Marosi, L.; Schneeweiss, B.; Greul, R.; Scheithauer, W. Treatment of patients with advanced nonsmall cell lung carcinoma using docetaxel and gemcitabine plus granulocyte-colony stimulating factor. *Cancer* **2000**, *89*, 516–522. [CrossRef]
16. Alexander, M.; Wolfe, R.; Ball, D.; Conron, M.; Stirling, R.G.; Solomon, B.; MacManus, M.; Officer, A.; Karnam, S.; Burbury, K.; et al. Lung cancer prognostic index: A risk score to predict overall survival after the diagnosis of non-small-cell lung cancer. *Br. J. Cancer* **2017**, *117*, 744–751. [CrossRef]
17. Banna, G.L.; Cortellini, A.; Cortinovis, D.L.; Tiseo, M.; Aerts, J.G.J.V.; Barbieri, F.; Giusti, R.; Bria, E.; Grossi, F.; Pizzutilo, P.; et al. The lung immuno-oncology prognostic score (LIPS-3): A prognostic classification of patients receiving first-line pembrolizumab for PD-L1 $\geq$ 50% advanced non-small-cell lung cancer. *ESMO Open* **2021**, *6*, 100078. [CrossRef]
18. Zhao, Y.; Shi, F.; Zhou, Q.; Li, Y.; Wu, J.; Wang, R.; Song, Q. Prognostic significance of PD-L1 in advanced non-small cell lung carcinoma. *Medicine* **2020**, *99*, e23172. [CrossRef]
19. Zhou, Z.J.; Zhan, P.; Song, Y. PD-L1 over-expression and survival in patients with non-small cell lung cancer: A meta-analysis. *Transl. Lung Cancer Res.* **2015**, *4*, 203–208. [CrossRef]

20. Jung, J.H.; Shin, D.W.; Kim, J.; Lee, J.C.; Hwang, J.H. Primary Granulocyte Colony-Stimulating Factor Prophylaxis in Metastatic Pancreatic Cancer Patients Treated with FOLFIRINOX as the First-Line Treatment. *Cancers* **2020**, *12*, 3137. [CrossRef]
21. Straus, D.; Collins, G.; Walewski, J.; Zinzani, P.L.; Grigg, A.; Sureda, A.; Illes, A.; Kim, T.M.; Alekseev, S.; Specht, L.; et al. Primary prophylaxis with G-CSF may improve outcomes in patients with newly diagnosed stage III/IV Hodgkin lymphoma treated with brentuximab vedotin plus chemotherapy. *Leuk. Lymphoma* **2020**, *61*, 2931–2938. [CrossRef] [PubMed]
22. Ruste, V.; Goldschmidt, V.; Laparra, A.; Messayke, S.; Danlos, F.-X.; Romano-Martin, P.; Champiat, S.; Voisin, A.-L.; Baldini, C.; Massard, C.; et al. The determinants of very severe immune-related adverse events associated with immune checkpoint inhibitors: A prospective study of the French REISAMIC registry. *Eur. J. Cancer* **2021**, *158*, 217–224. [CrossRef] [PubMed]
23. Kaplan, E.L.; Meier, P. Nonparametric Estimation from Incomplete Observations. *J. Am. Stat. Assoc.* **1958**, *53*, 457–481. [CrossRef]