# Neutrophil-to-Eosinophil Ratio Predicts the Efficacy of Avelumab in Patients With Advanced Urothelial Carcinoma Enrolled in the MALVA Study (Meet-URO 25)

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## Abstract

**Our retrospective analysis from MALVA Meet-URO 25 study reports progression-free survival (PFS) and overall survival (OS) by neutrophil-to-eosinophil ratio (NER) during avelumab treatment for advanced urothelial cancer (aUC). NER <median may be predictive of PFS, and prognostic for OS regardless of treatment. Prospective studies are warranted to validate NER as reproducible laboratory-biomarker for efficacy outcomes of avelumab in aUC.**

**Background:** Neutrophil-to-eosinophil ratio (NER) has been described to be associated with outcomes to immune checkpoint inhibitors (ICI) in several tumor types, but less is known about its role of in the response to avelumab in advanced urothelial cancer (aUC). Thus, we reported outcomes by NER of aUC patients treated with avelumab as maintenance after initial response to platinum-based chemotherapy and enrolled in the Maintenance with AVeLumAb ([MALVA] in advanced urothelial neoplasms in response to first-line chemotherapy: an observational retrospective study)

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study (Meet-URO 25). **Patients and Methods:** Median NER at baseline and after 3 cycles of avelumab were calculated. Progression-free survival (PFS) and overall survival (OS) by NER were reported. **Results:** At the cutoff date (April 15, 2023), a total of 109 patients were included. The median NER was 28.05 at baseline and 24.46 after 3 cycles of avelumab, respectively. Median PFS was not reached for patients with baseline NER less than the median (<median) compared to 5.1 months for patients with baseline NER greater than the median (≥median) ($P = .0005$). Median OS was significantly longer for patients with baseline NER <median compared with patients with baseline NER ≥median (not reached vs. 11.7 months, respectively; $P = .0016$). Significantly better PFS and OS were confirmed for NER after 3 cycles of avelumab <median compared with NER ≥median at the same timepoint. **Conclusion:** NER <median may be predictive of PFS in aUC patients treated with avelumab, and prognostic for OS regardless of treatment. Prospective studies are warranted to validate NER as a readily available and reproducible laboratory-biomarker for efficacy outcomes of avelumab in aUC.

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## Introduction

Bladder cancer (BC) is the 10th most common cancer type worldwide and the second most common malignancy of the genitourinary tract following prostate cancer, with urothelial carcinoma (UC) that represents the main histological subtype, accounting for 90% of all BCs.[1] About three-fourths of patients with BC present with a non–muscle-invasive BC at the diagnosis.[1] Ten to fifteen percent of patients with muscle-invasive BC have metastasis at the time of diagnosis and about half of muscle-invasive BC patients treated with radical intent by cystectomy will relapse.[1] Platinum-based chemotherapy (CT) is the current standard of care for first-line treatment of advanced UC (aUC)[2]; however nearly all patients progress despite the initial response. The advent of immune checkpoint inhibitors (ICI) has revolutionized the management of several solid tumors, including aUC[2,3]. The introduction of avelumab, a programmed-death ligand-1 (PD-L1) inhibitor, in aUC patients who did not progress after first line platinum-based CT, has been shown to significantly prolong progression-free survival (PFS) and overall survival (OS) compared with best supportive care alone, with a median OS of 23.8 months versus 15.0 months, respectively.[4] Thus, avelumab maintenance treatment after first line platinum-based CT is now the standard of care for aUC. Despite the impact, only a minority of patients derive long-term benefit. In this context prognostic and predictive biomarkers which can assist in patient selection are needed. While several candidate biomarkers have been evaluated, none have yet been validated for routine clinical use in aUC. Prior studies have reported eosinophils count as a biomarker for improved response to immunotherapy in melanoma,[5-8] renal cell cancer (RCC)[9,10] and non–small-cell lung cancer (NSCLC),[11,12] suggesting a potential role for baseline eosinophil count as a predictive biomarker. Eosinophils can invade the tumor microenvironment (TME) and may enhance antitumor responses via degranulation with direct cytotoxic effects on neoplastic cells.[13] This appears to occur in higher numbers in some patients, perhaps because they generate more eosinophils due to their activated immune system and lymphocyte T helper 2 production of interleukin (IL)-5.[13] Furthermore, cytokines released from eosinophil degranulation are involved in the activation of dendritic cells, recruitment of T cells and alteration of the TME vasculature. At last, eosinophils may play a role in the enhancement of tumor surveillance.[13-16]

No data about eosinophils are available in patients with aUC treated with avelumab. Therefore, we performed a retrospective evaluation of the incidence of eosinophilia in aUC patients treated with avelumab, aiming to explore the relationship between the development of eosinophilia and disease outcomes in a real-world setting. We investigated the relevance of absolute eosinophil count (AEC), neutrophil-to-eosinophil ratio (NER) and eosinophil-to-lymphocyte ratio (ELR) on PFS and OS among patients with aUC treated with avelumab as maintenance after response to platinum-based regimens enrolled in the Maintenance with AVeLumAb ([MALVA] in advanced urothelial neoplasms in response to first-line CT: an observational retrospective study) study (Meet-URO 25).

## Patients and Methods

Patients with aUC treated with avelumab as maintenance after response to platinum-based regimens at 9 referral Italian centers were eligible for the current retrospective analysis.

Patients included for analysis were required to begin treatment avelumab between the years 2021 and 2023. The cutoff date for data collection was April 15, 2023, for all patients. Patient demographics, tumor characteristics, and treatment information were collected via IRB-approved retrospective review of electronic medical records at all institutions. Patient demographics included age, race, and sex; treatment information included prior cystectomy, prior systemic therapy, baseline complete blood count with differential, disease control rate disease control rate ([DCR], percentage of patients with a complete or partial response or a stable disease using response evaluation criteria (RECIST) version 1.1[17] in solid tumors and immune-related RECIST (iRECIST),[18] overall response rate (ORR), (percentage of patients with a complete or partial response using iRECIST), PFS (defined by time from treatment initiation until death, radiographic or clinical progression), and OS (defined as time from treatment initiation until death) were collected. Patients without progression or death at the study cutoff date or date of last follow up were censored for analysis. Responder patients were defined as patients with SD, PR, or CR as best overall response

according to iRECIST. On the other hand, nonresponders were patients with PD as best overall response according to iRECIST. The NER was calculated by the absolute neutrophil count (number of cells × 103/μL) divided by AEC (number of cells × 103/μL). To allow the NER to be calculated for patients with an AEC of zero (ie, to avoid zero in the denominator), the AEC for these patients was adjusted to 0.01 × 103/μL (the lowest baseline AEC recorded from patients from both laboratories). The ELR was calculated by the absolute eosinophil count (AEC, number of cells × 103/μL) divided by the absolute lymphocyte count (number of cells × 103/μL). The neutrophil-to-lymphocyte ratio and platelets-to-lymphocyte ratio were also calculated by the absolute neutrophil count (absolute neutrophil count, number of cells × 103/μL) and the absolute platelet count (number of cells × 103/μL), respectively, divided by the absolute lymphocyte count (number of cells × 103/μL).

Primary endpoints were OS and PFS. Secondary endpoints included the ORR and the DCR.

All statistical analyses were conducted using the R software v4.3.1[19] and the packages survival v3.5-7,[20] and dplyr v1.1.4.[21]

Categorical data were reported as counts and percentages, while continuous data were reported as median and range. When necessary, dichotomization of continuous data was obtained using the median as cut-off value.

Correlation between categorical variables was estimated with a chi-squared test. Correlation between categorical and continuous variables was estimated with the Welch t-test or the Wilcoxon Rank-sum test based on the distribution of the data. Differences in continuous data in the same sample but different times were estimated with a paired samples t-test. When appropriate, *P* values were corrected for multiple hypothesis testing with the Bonferroni method. Survival rate between different groups was estimated with the Kaplan–Meier method and log-rank test. Multivariate Cox analysis was used to compare the influence of multiple parameters on the survival rate.

## Results

### *Baseline Patient Characteristics*

A total of 109 patients (pts) with aUC treated with avelumab were identified and all were included for analysis. Baseline characteristics of the overall cohort are shown in Table 1. Median age was 72 years and 82% were male. Median follow-up time was 9.1 months (0.5-24.6). First-line platinum-based chemotherapy comprised carboplatin + gemcitabine in 62 pts (56.9%), cisplatin + gemcitabine in 47 (43.1%); 63 pts (59.6%) received 4 cycles of chemotherapy, 46 (40.4%) received 6 cycles. Median time was between last cycle of chemotherapy and first dose of Avelumab was 43 days. Median Avelumab duration of treatment was 2.8 months, with a median number of 4 cycles administered. The median PFS (mPFS) for the entire cohort was 7.1 months [95% confidence interval (CI) 5.5-15.2] and the median OS (mOS) was 12.9 [95% CI 11.1-not reached (NR)]. ORR was 22.9% and DCR 61.5%.

### *Clinical Outcomes When Dividing Patients by the AEC at Baseline and After 3 Cycles of Avelumab*

The mPFS was NR for patients with baseline AEC greater than the median (≥median) compared to 5.1 months for patients with baseline AEC less than the group median (<median) ($P = .0012$), as well mOS was NR for patients with high AEC compared to 12.9 months for patients with low baseline AEC ($P = .035$) (Figure 1A). Similarly, mOS and mPFS were significantly improved in patients with high AEC after 3 cycles of treatment (Figure 1B). No statistically significant difference in AEC fold change (baseline to 3 cycles) was detected in responders versus nonresponder comparison (Figure 1C). Similarly, paired comparisons of AEC between baseline and after 3 cycles of avelumab showed no significant results both in responders and nonresponders (Figure 1D).

### *Clinical Outcomes When Dividing Patients by the Median NER at Baseline and After 3 Cycles of Avelumab*

The median NER was 28.1 at baseline and 24.5 at 6-week, respectively (Table 2). Median PFS was NR for patients with baseline NER <median compared to 5.1 months for patients with baseline NER ≥median ($P = .0005$; Figure 2). Median OS was significantly longer for patients with baseline NER <median compared with patients with baseline NER ≥median (NR vs. 11.7 months, respectively; $P = .0016$; Figure 2B). Significantly better PFS and OS were also confirmed for patients with NER <median after 3 cycles of avelumab compared with those with NER ≥median at the same timepoint (Figure 2C). In addition, low NER after 3 cycles of treatment trended toward better DCR ($P = .0599$). By univariate and multivariate analysis, baseline NER <median was confirmed to be associated with improved PFS and OS, as was NER <median after 3 cycles of treatment (Tables 3 and 4). No statistically significant difference in NER fold change (baseline to 3 cycles) was detected in responders versus nonresponder comparison. Similarly, paired comparisons of NER between baseline and after 3 cycles of avelumab showed no significant results both in responders and nonresponders (Figure 2B).

### *Clinical Outcomes When Dividing Patients by the Median ELR at Baseline and After 3 Cycles of Avelumab*

The median ELR was 0.08 at baseline and 0.10 after 3 cycles of avelumab, respectively (Table 2). Median OS was significantly longer for patients with baseline ELR ≥median compared with patients with baseline ELR <median (NR vs. 11.7 months, respectively; $P = .013$; Figure 3A). In addition, baseline ELR ≥median trended toward better PFS ($P = .059$; Figure 3A).

Significantly better PFS and OS were also confirmed for patients with ELR ≥median after 3 cycles of avelumab compared with those with ELR <median at the same timepoint (Figure 3B). No statistically significant difference in ELR fold change (baseline to 3 cycles) was detected between responders and nonresponders (Figure 3C). On the other hand, a statistically significant difference in ELR between baseline and after 3 cycles of avelumab was detected in the paired comparison for the nonresponder group (Figure 3C).

### *Univariate and Multivariate Analysis of Clinical and Pathological Factors for Clinical Outcomes*

Results of univariate and multivariate analysis of clinicopathological and biomolecular variables related to PFS and OS are shown in Tables 3 and 4. In the univariate analysis, baseline NER <median group had significantly longer PFS and OS than the high one [PFS

**Table 1** Baseline Characteristics of Patients with aUC Treated With Avelumab in the MALVA Study (Meet-URO 25)

| Patient Demographics and Characteristics | | |
|---|---|---|
| **Characteristic** | | ***N* = 109** |
| Median age (range) y | | 72 (54 -7) |
| Age | <75 y | 69 (63%) |
| | ≥75 y | 40 (37%) |
| Sex | Male | 89 (82%) |
| | Female | 20 (18%) |
| Histology | Adenocarcinoma | 1 (1%) |
| | Squamous | 5 (5%) |
| | Small cells | 2 (2%) |
| | Pure urothelial | 101 (92%) |
| Median avelumab duration of treatment (range) mo | | 2.8 (0.5-20.0) |
| Median number of cycles (range) | | 4 (3-6) |
| Median G8 score[a] (range) | | 13 (8-17) |
| G8 score[a] | ≤14 | 47 (92%) |
| | >14 | 4 (8%) |
| ECOG performance status[b] | 0 | 66 (61%) |
| | 1 | 39 (36%) |
| | 2 | 4 (3%) |
| Prior platinum-based therapy in neo-/adjuvant setting | Yes | 2 (2%) |
| | No | 107 (98%) |
| Metastases at diagnosis | Yes | 44 (41%) |
| | No | 64 (59%) |
| First line best objective response according RECIST 1.1 criteria | PR | 53 (49%) |
| | CR | 5 (5%) |
| | SD | 51 (46%) |
| Avelumab beyond progression | Yes | 13 (14%) |
| | No | 78 (86%) |
| Radiotherapy during Avelumab | Yes | 18 (18%) |
| | No | 81 (82%) |

Abbreviations: aUC = advanced urothelial cancer; CR = complete response; ECOG = Eastern Cooperative Oncology Group; MALVA = maintenance with AVeLumAb; PR = partial response; RECIST = response evaluation criteria; SD = stable disease.
[a] Geriatric screening tool lying between 0 and 17, with higher score indicates better health status.
[b] Eastern Cooperative Oncology Group classification ranging from 1 to 5, with lower scores indicating better functionality.

**Table 2** Peripheral Blood Immune Cell Subsets

| | Median Baseline (Range) | Median After 3 Cycles of Avelumab (Range) |
|---|---|---|
| AEC | 0.11 (0.00-2.00) | 0.18 (0.00-1.68) |
| ELR | 0.08 (0.00-2.86) | 0.10 (0.00-0.74) |
| NER | 28.05 (1.00-334.00) | 24.46 (2.93-539.00) |
| PLR | 153.84 (0.17-545.87) | 142.31 (36.26-790.11) |
| NLR | 2.41 (0.00-12.43) | 2.52 (0.95-19.17) |

Abbreviations: AEC = absolute eosinophil count; ELR = eosinophil-to-lymphocyte ratio; NER = neutrophil-to-eosinophil ratio; NLR = neutrophil-to-lymphocyte ratio; PLR = platelet-to-lymphocyte ratio.

hazard ratio (HR) = 1.006, 95%CI 1.002-1.01, $P = .0014$; OS HR = 1.007, 95%CI 1.002-1.013, $P = .021$]. A significant positive impact both in PFS and OS was also reported in the univariate analysis for NER <median after 3 cycles of avelumab (PFS HR 1.006, 95%CI 1.003-1.009, $P < .0001$; OS HR = 1.005, 95%CI 1.002-1.008, $P = .0003$), duration of therapy with avelumab (PFS HR = 0.72, 95%CI 0.63-0.82, $P < .0001$; OS HR = 0.7, 95%CI 0.59-0.84, $P = .0001$), and PS ECOG ≤ 1 (PFS HR = 1.7, 95%CI 1.2-3.2, $P = .008$; OS HR = 2, 95%CI 2.6-23, $P = .0003$).

After adjustment for confounding factors, NER <median after 3 cycles of avelumab was associated with longer PFS and OS (PFS HR = 1.04, 95%CI 1.01-1.08, $P = .017$; OS HR = 1.03, 95%CI 1-1.06, $P = .027$) in multivariate analysis. Finally, also duration of therapy with avelumab resulted to

**Figure 1** (A) PFS and OS difference between patients with baseline AEC <median (low) and ≥median (high). (B) PFS and OS difference between patients with AEC after 3 cycles of avelumab <median (low) and ≥median (high). (C) Fold change of AEC after 3 cycles of avelumab treatment and baseline in responders and nonresponder patients. (D) Paired comparisons of AEC baseline to on-avelumab (after 3 cycles of treatment) in responders and nonresponder patients. Abbreviations: AEC = absolute eosinophil count; OS = overall survival; PFS = progression free survival.

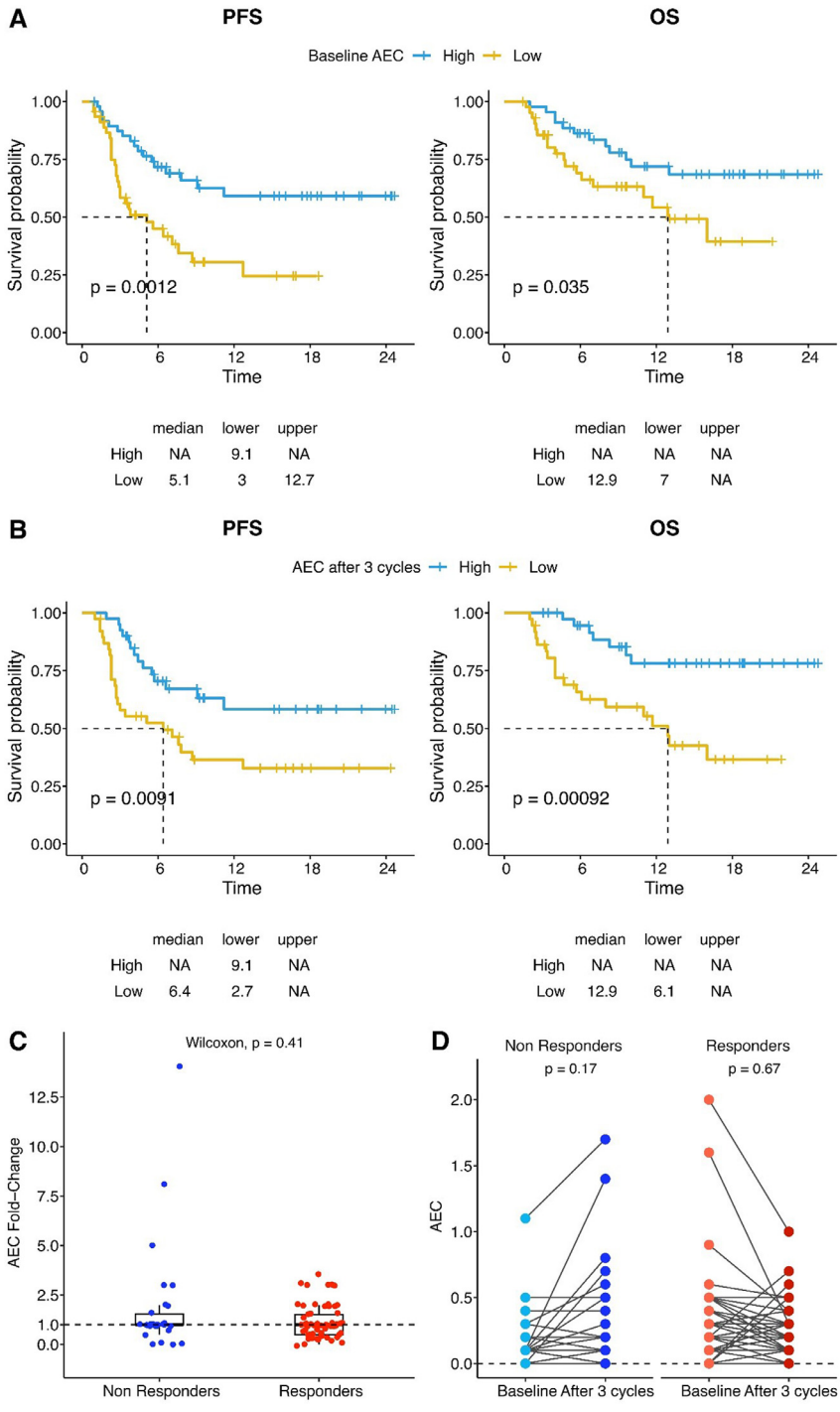


**Figure 2** (A) PFS and OS difference between patients with baseline NER <median (low) and ≥median (high). (B) PFS and OS difference between patients with baseline NER after 3 cycles of avelumab <median (low) and ≥median (high). (C) Fold change and paired comparison of NER after 3 cycles avelumab treatment and baseline in responders and nonresponder patients. (D) Paired comparisons of NER baseline to on-avelumab (after 3 cycles of treatment) in responders and nonresponder patients. Abbreviations: NER = neutrophil-to-eosinophil ratio; OS = overall survival; PFS = progression free survival.

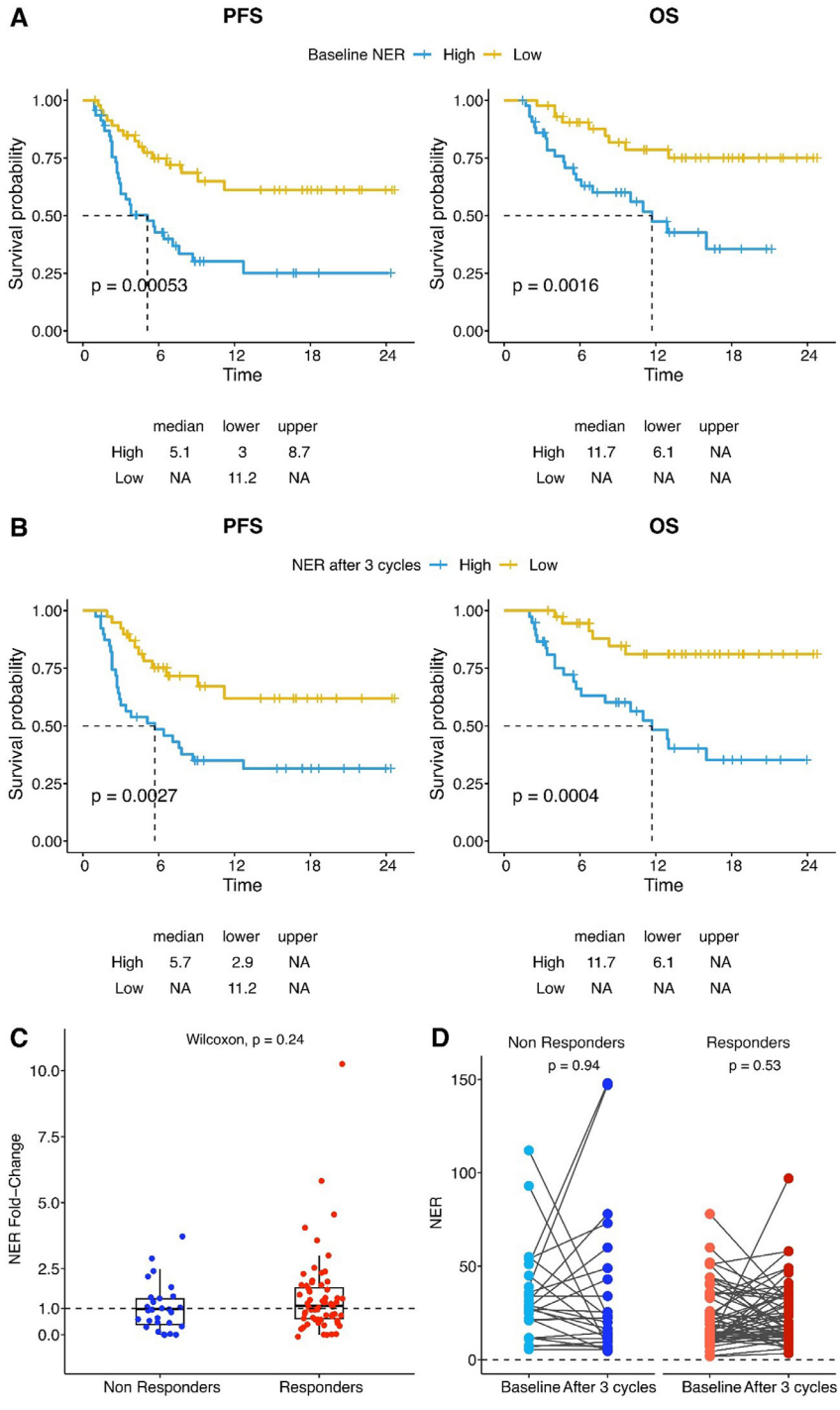


**Table 3** Univariate and Multivariate Analysis for PFS

| Covariate | | Univariate | | Multivariate | |
|---|---|---|---|---|---|
| | | HR (95%CI) | *P*-Value | HR (95%CI) | *P*-Value |
| Age | | 1 (0.96-1) | .79 | | |
| Sex | | 1.7 (0.82-3.6) | .15 | | |
| ECOG | 1 | 1.7 (1.2-3.2) | **.008**[a] | 3.87 (1.23-12.2) | **.021** |
| | 2 | 1.7 (1.8-15) | **.002**[a] | 3.34 (0.764-14.6) | .11 |
| G8 score | | 0.91 (0.75-1.1) | .32 | | |
| Metastases at diagnosis | | 1.3 (0.79-2.1) | .31 | | |
| Avelumab beyond PD | | 1.5 (0.81-2.8) | .2 | | |
| RT during avelumab | | 1.2 (0.7-2.2) | .46 | | |
| Avelumab DOT | | 0.72 (0.63-0.82) | **<.0001**[a] | 0.569 (0.409-0.791) | **.0008** |
| Number of cycles | | 0.87 (0.62-1.2) | .43 | | |
| NLR | | 1.2 (0.99-1.4) | **.06** | 0.918 (0.662-1.27) | .61 |
| PLR | | 1 (1-1) | .77 | | |
| AEC | | 0.61 (0.21-1.8) | .36 | | |
| NER | | 1 (1.002-1.01) | **.0014**[a] | 1.02 (1-1.05) | .054 |
| ELR | | 0.44 (0.07-2.9) | .4 | | |
| NLR after 3 cycles | | 1.1 (1.1-1.2) | **.0014**[a] | 0.677 (0.448-1.02) | .062 |
| PLR after 3 cycles | | 1.004 (1.001-1.006) | **.0008**[a] | 1.01 (1-1.02) | **.04** |
| AEC after 3 cycles | | 0.53 (0.13-2.1) | .36 | | |
| NER after 3 cycles | | 1.006 (1.003-1.009) | **<.0001**[a] | 1.04 (1.01-1.08) | **.017** |
| ELR after 3 cycles | | 0.12 (0.011-1.3) | **.08** | 2.56 (0.0543-121) | .63 |
| Eosinophils FC | | 1 (0.84-1.2) | .81 | | |
| NER FC | | 1 (0.85-1.2) | .81 | | |

Abbreviations: AEC = absolute eosinophil count; DOT = duration of treatment; ECOG = Eastern Cooperative Oncology Group; ELR = eosinophil-to-lymphocyte ratio; FC = fold change; NER = neutrophil-to-eosinophil ratio; NLR = neutrophil-to-lymphocyte ratio; PD = progressive disease according RECIST 1.1 criteria; PFS = progression free survival; PLR = platelet-to-lymphocyte ratio; RT = radiotherapy.
The boldface is to underline the statistically significance of the *p*-value.
[a] Indicates statistical significance of $P < .05$.

be independent prognostic factors for longer survival outcomes (Tables 3 and 4).

## Discussion

MALVA study evaluated the feasibility of eosinophils count as well as ratios with neutrophils and lymphocytes (NER and ELR, respectively) as biomarkers to forecast outcomes in aUC upon initiation of avelumab. Our analysis has demonstrated an association of elevated NER with worsened survival outcomes at the start of immunotherapy. Thus, median NER may be a feasible low-cost, clinical laboratory-based biomarker to prognosticate aUC patient outcomes after avelumab treatment. There is no optimal cutpoint of NER. One previously published work calculated the optimal cutpoint for NER in RCC separately using the method of Contal and O'Quigley, which uses the log-rank test statistic to estimate the cutpoint to assess the accuracy of the median of use compared to the optimal cutpoint for NER.[10] Nevertheless, there is not a validated cutpoint of NER that can be used for all tumors, including UC. Thus, we used median NER to divide patients with NER <median or ≥median.

Eosinophils are known as a minor population of granulocytes that are mostly explored in asthma and allergic disorders.[22] Several studies have demonstrated the central role of eosinophils in tumoral disease progression or metastasis through their action within the TME. Eosinophils' mediators and receptors allows them to contribute to innate and adaptive immunity, such as type 1 and type 2 immunity, and thus remodel TME and affect tumor outcomes. Based on TME cells and cytokines, activated eosinophils drive other immune cells to eventually promote or suppress tumor growth. Eosinophil mediators, such as IL-5, IL-33, granulocyte–macrophage colony-stimulating factor, thymic stromal lymphopoietin, and chemokine ligand (CCL)11 also determine eosinophil behaviour toward tumor cells. Considering these properties, eosinophils could ultimately synergize with ICI therapy to enhance efficacy of immunotherapies.[22] A recent study has successfully tried to elucidate the role of eosinophils in ICI treatment in a nonimmunogenic primary and metastatic breast cancer mouse model.[23] Eosinophils were the only cells that reliably increased after ICI therapy in the primary and metastatic tumor and they were reported as necessary for T cell activation, even in the presence of ICI.[22-25]

Through recognition of distinct tumor-associated molecular markers as well as facilitation by other leukocytes, eosinophils degranulate, and subsequently release TNF-$\alpha$, granzymes, major essential protein, and metalloproteinases with a wide catch-net of effects involving recruitment of other leukocytes, antigen presentation to T cells, and tumor cell destruction.[26-28] The release of ribonucleases and cationic proteins forms a cytotoxic extracellular

**Table 4** Univariate and Multivariate Analysis for OS

| Covariate | | Univariate | | Multivariate | |
|---|---|---|---|---|---|
| | | HR (95%CI) | *P*-Value | HR (95%CI) | *P*-Value |
| Age | | 0.99 (0.95-1) | .60 | | |
| Sex | | 1.3 (0.59-2.7) | .54 | | |
| ECOG | 1 | 2 (1.5-5) | **.001**[a] | 2.16 (0.629-7.42) | .22 |
| | 2 | 2 (2.6-23) | **.0003**[a] | 3.6 (0.757-17.1) | .11 |
| G8 score | | 0.91 (0.72-1.2) | .46 | | |
| Metastases at diagnosis | | 1.2 (0.69-2.2) | .48 | | |
| Avelumab beyond progression | | 1.4 (0.64-3) | .41 | | |
| RT during avelumab | | 1.6 (0.84-3.2) | .15 | | |
| Avelumab DOT | | 0.7 (0.59-0.84) | **.0001**[a] | 0.725 (0.575-0.915) | **.0068** |
| Number of cycles | | 0.89 (0.55-1.4) | .64 | | |
| NLR | | 1.2 (0.99-1.4) | **.061** | 0.772 (0.535-1.11) | .17 |
| PLR | | 1 (1-1) | .63 | | |
| AEC | | 1.2 (0.51-2.8) | .69 | | |
| NER | | 1.007 (1.002-1.013) | **.021**[a] | 1.01 (0.988-1.03) | .4 |
| ELR | | 2 (0.57-7) | .28 | | |
| NLR after 3 cycles | | 1.2 (1.1-1.3) | **.0027**[a] | 0.806 (0.572-1.14) | .22 |
| PLR after 3 cycles | | 1.004 (1.001-1.006) | **.0019**[a] | 1.01 (1-1.02) | **.031** |
| AEC after 3 cycles | | 0.7 (0.16-3) | .63 | | |
| NER after 3 cycles | | 1.005 (1.002-1.008) | **.0003**[a] | 1.03 (1-1.06) | **.027** |
| ELR after 3 cycles | | 0.24 (0.015-3.7) | .3 | | |
| Eosinophils FC | | 1 (0.81-1.2) | .97 | | |
| NER FC | | 1.1 (0.9-1.4) | .35 | | |
| ELR FC | | 0.88 (0.58-1.3) | .54 | | |

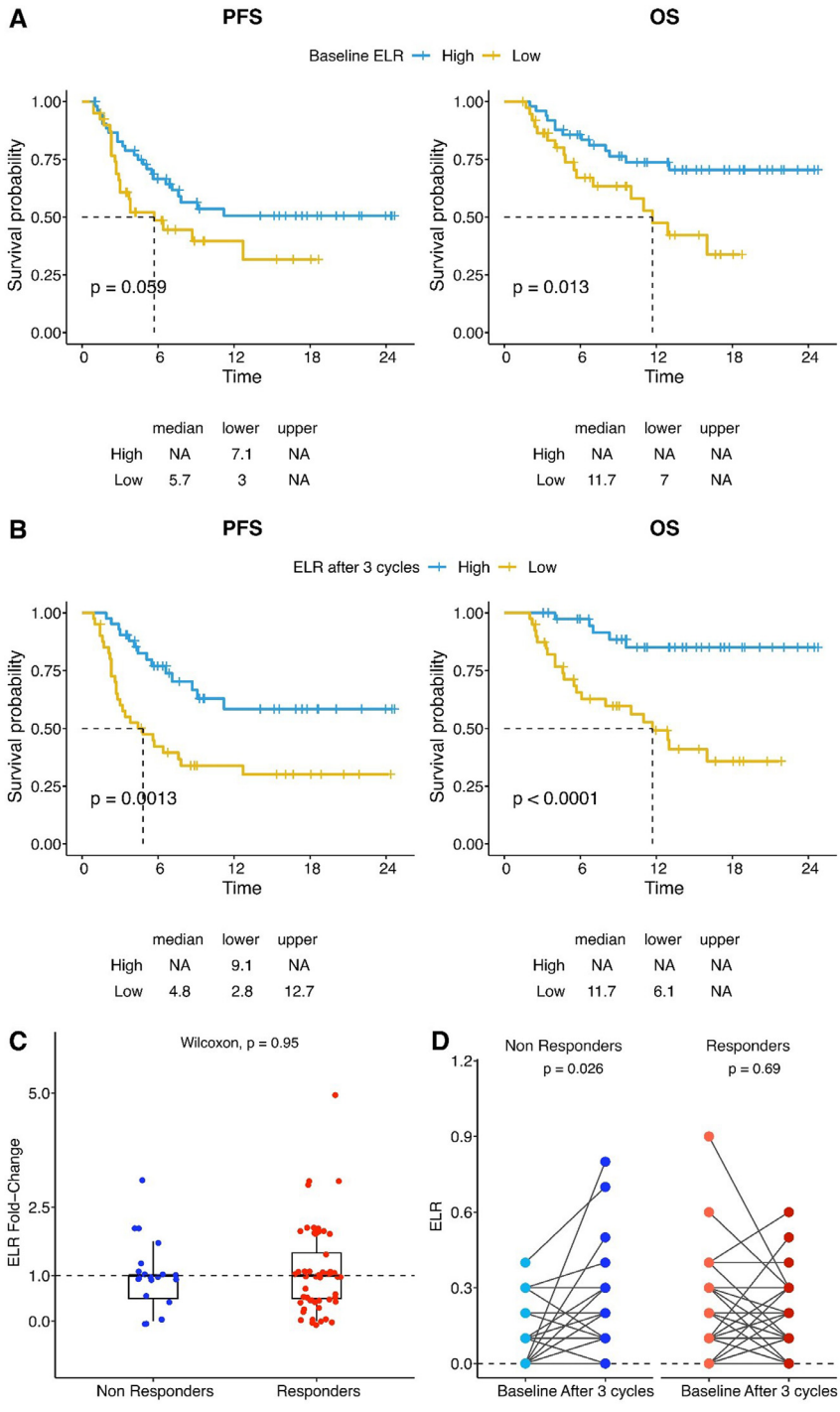
Abbreviations: AEC = absolute eosinophil count; DOT = duration of treatment; ECOG = Eastern Cooperative Oncology Group; ELR = eosinophil-to-lymphocyte ratio; FC = fold change; NER = neutrophil-to-eosinophil ratio; NLR = neutrophil-to-lymphocyte ratio; OS = overall survival; PLR = platelet-to-lymphocyte ratio; RT = radiotherapy.
The boldface is to underline the statistically significance of the *p*-value.
[a] Indicates statistical significance of $P < .05$.

trap driving tumor cell death. Previous in vitro studies demonstrate dead tumor cells recruit eosinophils within melanoma preclinical models.[27,29] Although the biological mechanism is unclear, retrospective clinical studies in melanoma showed a potential association of lower baseline median NER and AEC with improved outcomes at upfront immunotherapy.[26-28] In vivo studies postulated CC-chemokine ligands promote both eosinophil recruitment and subsequent cancer destruction in solid tumors. CCL5, CCL-11, C-X-C motif ligand 9, and C-X-C motif ligand 10 are hypothesized as the main drivers in eosinophil-mediated tumor cell necrosis. Notably, decreased CCL-11 expression is associated with increased tumor burden and absence of eosinophils compared to CCL-11-rich involvement in preclinical murine models.[28,30]

Our work demonstrated concordant outcomes and disease evolution of eosinophilia and NER compared to prior similar studies in aUC mRCC, melanoma, and NSCLC with similar OS, PFS, and clinical benefits.

A retrospective study that evaluated the association of clinical outcomes with post-treatment changes in the NER in patients with aUC treated with pembrolizumab reported a significant difference in the OS between the increased and decreased NER groups at 3 weeks after pembrolizumab ($P < .001$ and .002, respectively).[31] A retrospective analysis of patients with metastatic RCC treated with nivolumab monotherapy showed that a higher baseline eosinophil count (>0.1 k/uL) was associated with a lower risk of progression (HR 0.54, $P = .042$).[32] In addition, a *post hoc* analysis of the JAVELIN 101 study, exploring the clinical benefit of avelumab and axitinib, has shown an association of lower NER with better PFS and ORR.[33] A follow-up study at ASCO 2022 performed a landmark analysis of NER changes at week 6 of nivolumab/ipilimumab.[34] It showed clinical benefit with 68% of patients having decreased NER at week 6. Decreased NER ≥50% was associated with longer OS (adjusted HR 0.38 [0.17-0.85], *P*-value .02) and PFS (adjusted HR 0.55 [0.31-0.95], *P*-value .03).[34] Median pretreatment NER and NER at 1-month post-treatment are recently reported to be associated with improved survival in melanoma patients treated with nivolumab.[8] Lower baseline NER was associated with improved OS (HR: 0.442, 95%CI: 0.288-0.681, $P < .001$, respectively) on univariate testing. After accounting for multiple covariates, multivariate analysis found that lower pretreatment NER was associated with better ORR (by immune-related RECIST) (OR: 2.199, 95%CI: 1.071-4.582, $P = .033$) and improved OS (HR: 0.480, 95%CI: 0.296-0.777, $P = .003$), suggesting baseline NER merits additional investigation as a novel prognostic marker for advanced melanoma patients receiving anti-PD-1-based regimens.[8] Furthermore, baseline high-AEC (≥130/μL) was reported to be associated with a significantly longer PFS and OS than the low-AEC group (mPFS = 7.0 months, 95%CI 5.0-10.0 vs. 2.5 months,

**Figure 3** (A) PFS and OS difference between patients with baseline ELR <median (low) and ≥median (high). (B) PFS and OS difference between patients with ELR after 3 cycles of avelumab <median (low) and ≥median (high). (C) Fold change of ELR after 3 cycles avelumab treatment and baseline in responders and nonresponder patients. (D) Paired comparisons of ELR baseline to on-avelumab (after 3 cycles of treatment) in responders and nonresponder patients. Abbreviations: ELR = eosinophil-to-lymphocyte ratio; OS = overall survival; PFS = progression free survival.



95%CI 2.0-4.0, $P = .007$, and mOS = 9.0 months, 95%CI 7.0-15.0 vs. 5.5 months, 95%CI 4.0-8.0, $P = .009$, respectively) in advanced NSCLC patients treated with single-agent anti-PD1/anti-PDL1 monoclonal antibody.[12]

Finally, we have reported no statistically significant difference in fold change and paired AEC and NER both among responders and nonresponders, suggesting that an eosinophil increase might be less relevant in highly immunogenic tumors like UC[35] and underlying the major role played by the absolute value of eosinophils. Intriguingly, we have observed a statistically significant difference in paired ELR after 3 cycles of avelumab among nonresponders, confirming that lymphocytopenia could be represent a worse predictor of response to avelumab. Notably, lymphocytopenia has been yet associated with poor survival in numerous settings, as tumors may induce lymphocyte apoptosis both within the TME and in peripheral circulation as a means of avoiding immune recognition.[35-38]

To our knowledge, our study represents the first evaluation of the NER as widely available and practical laboratory-based biomarker to predict survival and outcome to avelumab in aUC. We have found a significantly improved PFS and OS in patients with low baseline NER. Notably, NER has been consistently shown to be associated with improved survival with anti-Vascular Endothelial Growth Factor (VEGF) Tyrosine Kinase Inhibitors (TKIs), mammalian target of rapamycin (mTOR) inhibition, immunotherapy, and postnephrectomy in RCC supporting the notion that NER could serve as prognostic biomarker in advanced RCC regardless of treatment type.[10] No data are currently available for baseline NER as prognostic biomarker in aUC, thus our study represents the first to hopefully report the prognostic significant of NER in these tumors. Therefore, further prospective validation of the NER as a prognostic biomarker in aUC is warranted.

Our study had several limitations, including being retrospective and largely descriptive in nature without having a nonimmunotherapy agent for comparison, such as an antibody-drug conjugated and the novel combination of immunotherapy and antibody-drug conjugated. In the next future, with the combination of enfortumab vedotin plus anti-PD1 pembrolizumab that has been recently approved by Food and Drug Administration (FDA) as the new first-line standard of care for aUC patients,[39,40] the NER may help to select which patients are most likely to benefit from immunotherapy alone, potentially sparing adverse effects of long term enfortumab vedotin use and lead to a de-intensification treatment with only pembrolizumab after initial OR. Therefore, further prospective validation of the NER as a predictive biomarker for immunotherapy in aUC is warranted. It is expected that with advances in biomarker-tumor profiling and genomic sequencing, future treatments for a UC will become more targeted, personalized, and thus optimized.

Furthermore, given that both neutrophils and eosinophils may be affected by a variety of medications, infections, and autoimmune conditions, our study is further limited in its ability to adequately account for potential influences from these external sources.

Finally, eosinophil expansion was not restricted to responders, but was also observed in a proportion of nonresponders, limiting its potential for clinical decision making. Therefore, increased eosinophils upon avelumab response, combined with the previous reported preclinical proofs of their causal role in ICI response, should be considered as an important lead for the development of immunomodulatory strategies to engage eosinophils rather than a biomarker.

## Conclusion

Our study confirmed that systemic eosinophil expansion is a common feature of ICI response also in aUC. Reporting that low NER is associated with longer PFS and OS with aUC treated with avelumab, our study confirm that NER may have prognostic importance regardless of ICI treatment type, but may have predictive importance for patients most likely to respond to immunotherapy. To our knowledge, our investigation is one of the most comprehensive studies to identify baseline NER as a feasible prognostic low-cost laboratory-based biomarker. This retrospective study is hypothesis-generating. Larger prospective data are needed for further validation of NER as a biomarker. Future prospective studies exploring the predictive utility of the baseline NER should also measure serum levels of eosinophil related cytokines at baseline and on treatment. In addition, an understanding of eosinophils in the aUC TME may be extended with the validation of dynamic changes in NER with histopathologic data in chemokine expression. In light of these evidence, combining translational research on clinical trials with mechanistic research in preclinical models may hopefully be a powerful strategy to unravel mechanisms of ICI response and to identify future targets and mechanisms to increase the chances of patients with high baseline NER to respond to immunotherapy.

### *Clinical Practice Points*

- Systemic eosinophil expansion is a common feature of avelumab response in aUC.
- Low NER is associated with longer PFS and OS with aUC treated with avelumab.
- NER can be used as feasible prognostic and predictive low-cost laboratory-based biomarker.

## Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## CRediT authorship contribution statement

**Elisabetta Gambale:** Data curation, Writing – original draft, Visualization, Writing – review & editing. **Marco Maruzzo:** Data curation. **Carlo Messina:** Data curation. **Irene De Gennaro Aquino:** Data curation. **Ismaela Anna Vascotto:** Data curation. **Virginia Rossi:** Data curation. **Davide Bimbatti:** Data curation. **Nicolò Cavasin:** Data curation. **Marco Messina:** Data curation. **Alessia Mennitto:** Data curation. **Sara Elena Rebuzzi:** Data curation. **Cecilia Nasso:** Data curation. **Chiara Mercinelli:** Data curation. **Brigida Anna Maiorano:** Data curation. **Martina Fanelli:** Data curation. **Mariella Sorarù:** Data curation. **Federico Scolari:** Data curation. **Marinella Micol Mela:** Data curation. **Luca Galli:** Data curation. **Alessia Salfi:** Data curation. **Mimma Rizzo:** Data curation. **Silvia Puglisi:** Data curation. **Valentina**

**Orlando:** Data curation. **Giuseppe Fornarini:** Data curation. **Alessandro Rametta:** Data curation. **Patrizia Giannatempo:** Data curation. **Linda Cerbone:** Data curation. **Laura Doni:** Data curation. **Giandomenico Roviello:** Data curation. **Serena Pillozzi:** Conceptualization, Methodology, Formal analysis, Validation, Writing – review & editing. **Lorenzo Antonuzzo:** Conceptualization, Methodology, Supervision.

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