Prognostic impact of the immune microenvironment in multiple myeloma: a meta-analysis of current studies

Multiple myeloma (MM) is a hematologic malignancy characterized by the accumulation of plasma cells in the bone marrow, which provides a supportive microenvironment. This allows myeloma cells to evade immune surveillance while altering the protective immune network in the marrow. Understanding the complex interactions between myeloma cells and the immune microenvironment is key to developing effective therapeutic strategies.

We performed a systematic review and meta-analysis to synthesize current evidence on the prognostic roles of specific immune cell types in the myeloma bone marrow microenvironment.

This systematic review and meta-analysis have been conducted following the guidelines reported in the PRISMA statement. Relevant studies were identified by systematically searching PubMed, Embase, and Web of Science electronic databases up to May 2024. Search terms included "multiple myeloma", in combination with terms for key immune cell populations - including e. g. "T cells", "CD8", "CD4", etc.

Studies were included if they met the following criteria: (i) evaluated patients with MM diagnosis, (ii) reported intratumoral immune cell quantification in the bone marrow, (iii) quantitatively assessed the correlation between immune cell levels and clinical outcomes (overall survival [OS]). Reviews, editorials, case reports, and studies lacking survival data were excluded. Two reviewers independently screened titles/abstracts and assessed full texts for eligibility. Disagreements were resolved by consensus.

Data extracted are shown in the *Online Supplementary Table S1*.

The methodological quality of the included studies was evaluated using the Newcastle-Ottawa Scale (NOS) for observational studies. The outcomes have been reported according to AHRQ standards (good, fair, or poor quality).

Pooled hazard ratios (HR) and 95% confidence intervals (CI) were calculated using random effects models to account for heterogeneity across studies. Heterogeneity was assessed using the I^2 statistic. Publication bias assessment with funnel plot visual inspection and Egger's test, both limited by the paucity of the studies included. All analyses were performed using Comprehensive Meta-Analysis (CMA) software v3.

This study was approved by the Ethics Committee of the University of Bari Medical School (study: 2022ZKKWLW, protocol no. 1300).

The literature search identified 3,713 potentially relevant articles; 179 articles were examined at a full-text level, of which 11 studies met eligibility criteria and were included in the meta-analysis (Figure 1).[1-11]

The 11 studies comprised a total of 3,317 patients. Methodological quality was generally high, with all the studies having good quality on the NOS.

Danziger *et al*. analyzed 286 patients, uncovering the association between intratumoral $CD8^+$ T cells and survival outcomes.[2] The analysis showed significantly worse OS (HR=1.84; 95% CI: 1.21-2.78) for patients with high $CD8^+$ infiltration (Figure 2).

Two studies (329 patients)[1,2] examined CD4 T-cell levels and found a significant correlation with worse OS (HR=5.62; 95% CI: 1.49-21.14; I^2=0%) (Figures 2 and 3).

One study[3] comprising 126 patients evaluated PDL1 expression, which was correlated with worse OS (HR=3.14; 95% CI: 1.65-5.99) (Figure 2).

The evaluation of galectin-9 (gal-9) expression stratified ac-

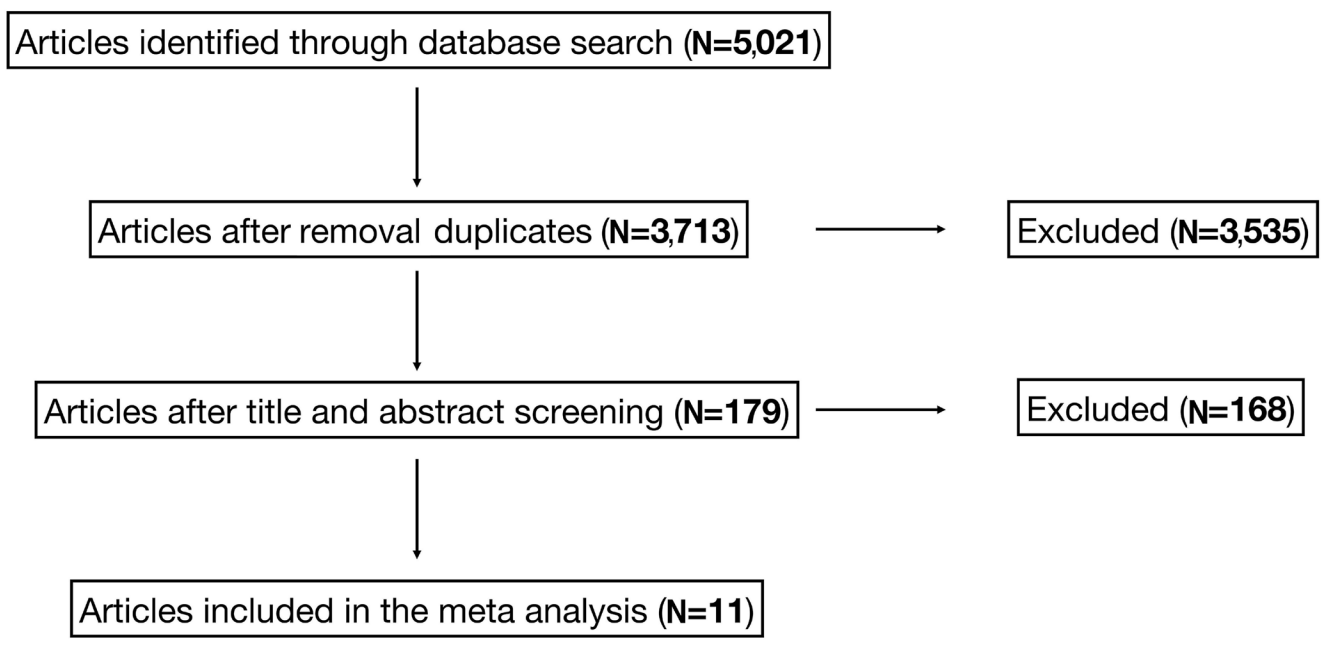


Figure 1. PRISMA flow-chart.

cording to the PDL1 expression in 109 subjects[9] uncovered a worse OS in PDL1-high patients (HR=5.25; 95% CI: 1.20-22.90) (Figure 2).

One study[4] (62 patients) examined CD200 expression. Higher expression was associated with worse OS (HR=2.30; 95% CI: 1.20-4.20) (Figure 2).

Mutsaers *et al.*[11] evaluated data from 1,654 patients, scrutinizing V-domain Ig suppressor of T-cell activation (VISTA) expression, finding higher levels to be associated with better OS (HR=0.76; 95% CI: 0.67-0.86) (Figure 2).

In the same study[11] the authors examined the CD40 expression. Higher levels were associated with better OS (HR=0.81; 95% CI: 0.64-1.00) (Figure 2).

Memory B-cell levels were also investigated in a cohort of 98 individuals.[8] Higher counts correlated significantly with worse OS (HR=1.2; 95% CI: 1.10-1.27) (Figure 2).

Three studies[2,6,8] assessed M2 ($CD68^{+}CD163^{+}$) macrophages. Increased levels correlated with poorer OS (HR=2.75; 95% CI: 1.07-7.08; I^2=92%) (Figures 2 and 3).

Increased neutrophils levels in 286 individuals correlated

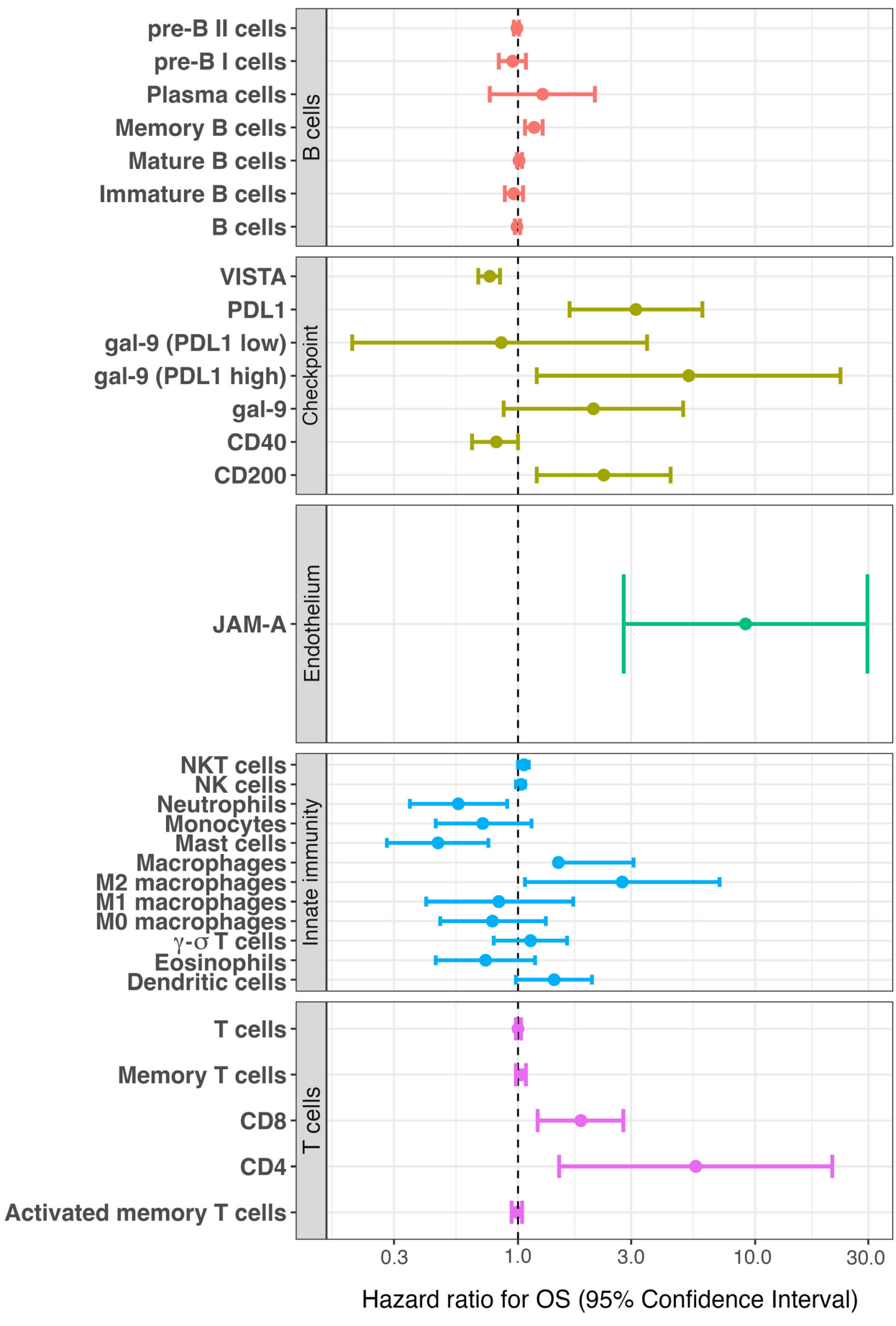
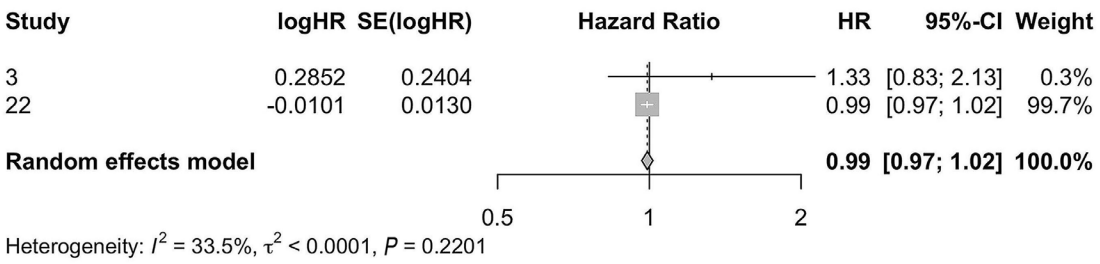
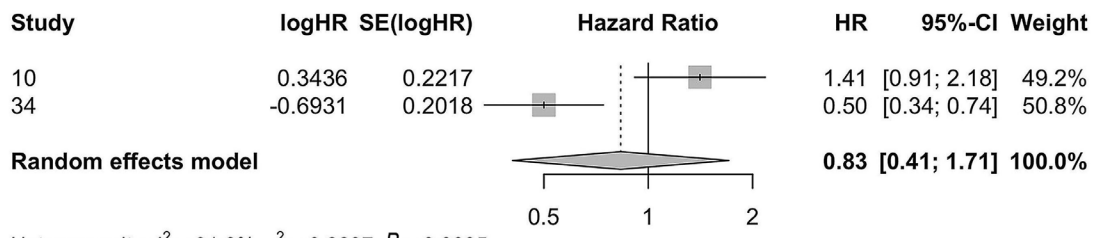


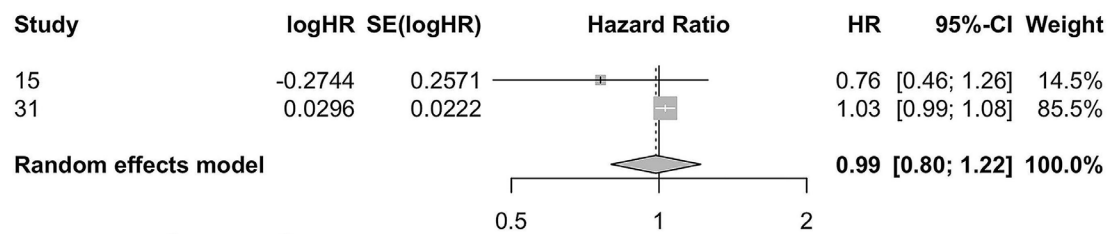
Figure 2. Forest plot for hazard ratios for overall survival of the single cell types, with 95% confidence interval. OS: overall survival; NK: natural killer.



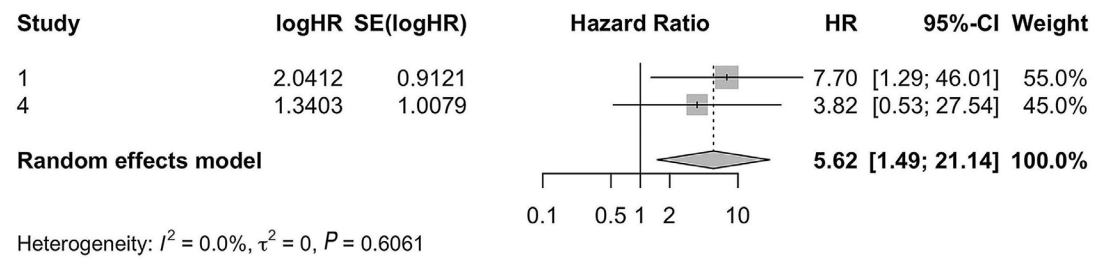
B cells



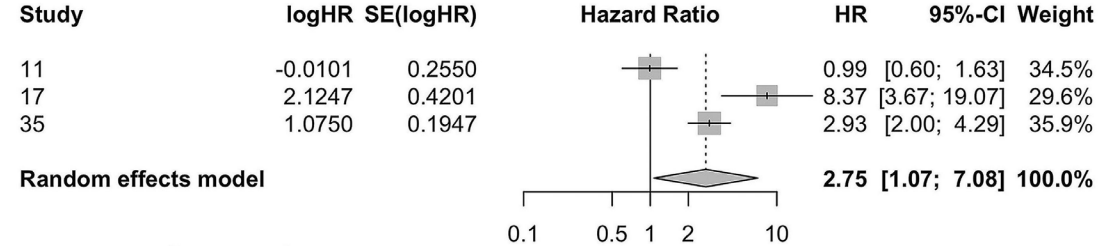
M1 macrophages



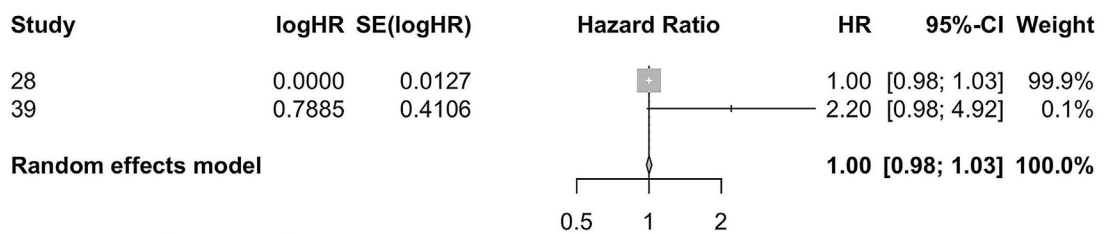
NK cells



CD4 cells



M2 Macrophages



T cells

Figure 3. Forest plot for the meta-analyses for the cell types whose hazard ratio for overall survival has been reported in more than one study. HR: hazard ratio; OS: overall survival; CI: confidence interval; NK: natural killer.

with an improved OS outcome (HR=0.56; 95% CI: 0.35-0.90) (Figure 2). Mast cell level paralleled this behavior, predicting an improved survival in the same patients' cohort[2] (HR=0.46; 95% CI: 0.28-0.75) (Figure 2).

Conversely, higher NKT cells in 98 patients significantly correlated with worse OS (HR=1.06; 95% CI: 1.00-1.11)[7] (Figure 2).

Finally, data obtained from 312 patients uncovered junctional adhesion molecule-A (JAM-A) expression in the bone marrow endothelial cells as a predictor for worse OS (HR=9.11; 95% CI: 2.79-29.76)[5] (Figure 2).

Funnel plots showed some asymmetry suggesting potential publication bias in M2 macrophages. However, Egger's test was not significant (*P* value =0.76).

Intratumoral natural killer T (NKT) cells significantly correlated with worse OS outcomes. NKT is a hybrid type of leukocyte that shows adaptive-immunity characteristics and NK cell membrane markers. Several NKT-cell phenotypes have been described in the literature: a Th1-like polarization, which has an immune cell-activating role, and a Th2-like polarization, which shows immune-regulatory characteristics. Unfortunately, the examined studies do not show such an in-depth characterization of the intratumoral NKT.

The available evidence pinpoints PDL1 to be expressed by tumor cells and, by recognition of his cognate receptor on T cells (PD1), exerts a T-cell inhibitory effect. Concordantly, we found that its expression in the bone marrow is correlated with worse OS in myeloma patients. Gal-9 also has a T-cell inhibitory effect, which may explain the association with worse OS found in the present study. CD200 behaves in much the same way and MM represents no exception, being expressed on dendritic cells, B and T lymphocytes, and its interaction with its cognate receptor (CD200R) causes inhibition of T-cell response. VISTA is a homolog of PDL1 and halts T-cell activity. Counterintuitively, the study reported in this meta-analysis showed its association with a better OS in myeloma patients. We speculate that this could be due to increased VISTA expression as negative feedback after initial T-cell activation against the tumor.[11] On the other hand, CD40 expression on antigen-presenting cells is associated with germinal center development, immunoglobulin class-switching, and memory B-cell differentiation. Its expression in the bone marrow correlated to better OS.[11]

M2 macrophages, associated with an anti-inflammatory milieu, correlate with worse prognosis and may promote tumor progression. In contrast, evidence is present that an M1-predominant macrophage profile appears clinically beneficial, by promoting an anti-tumor immune response.

Neutrophils and mast cells were found to be positively associated with OS. Both these cells have been reported to infiltrate many kinds of tumors, with either a tumor-promoting or a tumor-halting activity.

The study from Stork *et al*.[8] found memory B cells to be associated with worse OS. This is an unexpected finding, given that other works uncovered conflicting results. This could be due to the different timing of bone marrow examination, or to the fact that memory B cells could harbor a clonogenic potential and could thus evolve into myeloma cells in a dynamic and context dependent fashion.

CD8 and CD4 T cells were found to be associated with worse prognosis in myeloma patients in this meta-analysis. T cells have been shown to take heterogeneous phenotypes. Therefore, it should be acknowledged that a deeper level of complexity and multimodal methodologies are needed to crunch the immune landscape of multiple myeloma, also informed by recent single cell approaches.[12]

Curbing the immune dysfunction through checkpoint blockade or other strategies may help overcome immune evasion and unleash destructive immunity against myeloma and overcome the vicious cycles existing within the cancer microenvironment.[13] Further mechanistic studies are warranted to identify new targets modulating immune effector/regulatory crosstalk in the myeloma niche.

Some limitations exist. Unadjusted confounding by clinical factors and time and geogpraphical regions diversities may have introduced bias, immune heterogeneity between cohorts precluded separate analyses by disease stage or risk, and expression data from purified cells alone may not fully capture bone marrow microenvironmental immunity. These results pave the way for potential therapeutic interventions. The JAM-A-mediated and the other loops with pro angiogenic activities are fueled by interleukin (IL)-6 signaling. IL-6 promotes granulocyte macrophage colony-stimuating factor (GM-CSF) from MM cells, enhancing macrophage recruitment/angiogenesis via paracrine pathways. Combined anti-IL6/GM-CSF therapy could synergistically inhibit MM-macrophage crosstalk.[14]

Nicotinamide riboside may also counteract IL-6 in MM by inducing suppressor of cytokine signaling 3, attenuating downstream GM-CSF and HB-EGF.

A multi-targeted strategy blocking IL-6, GM-CSF and elevating NAD^+ with nicotinamide riboside warrants study to comprehensively disrupt pathogenic MM-macrophage signaling limiting pro-tumor impacts.[15]

In conclusion, this comprehensive meta-analysis underscores the prognostic value of intratumoral immune cells in multiple myeloma. Regulatory subsets impair disease control and careful subtyping is crucial to draw prognostic implications. Modulating this immune balance represents a promising strategy, which needs prospective clinical evaluation through RCT.

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Disclosures

No conflicts of interest to disclose.

Contributions

NS and AGS performed research. NS, PB, CB and AGS performed data analysis. NS and AGS wrote the manuscript. PB, CB and AGS supervised the study.

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Data-sharing statement

Full data is available in the *Online Supplementary Appendix*.

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