FULL TEXTS

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Determination of T Lymphocyte Subgroups in the Bone Marrow Micro Environment in Multiple Myeloma: Can Tumor-Directed T Cells Play a Role in Fighting Cancer?

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Aim:
Interactions between the bone marrow microenvironment and plasma cells cause serious expansion of humoral and liver immunity to cells of the immunosuppressive environment. Diffuse in T lymphocyte subgroup distribution includes a role in the pathogenesis of Multiple Myeloma (MM). NKT cells, on the other hand, exert potent anti-tumor effects in MM either through direct cytotoxicity or the release of proinflammatory cytokines, including IFN-γ.

In this regard, many immunotherapy applications are being looked at. Tumor-infiltrating T-lymphocyte (TIL) therapy as an independent, adaptive T-cell therapy.
In this situation; It is planned to determine the T lymphocyte subgroups (T8+, T4, NKT, Th1, Th2, Th17, Treg, Th22) around the bone marrow tumor in MM patients by flow cytometry method.

Method:
Twenty MM patients aged between 18-70 years who applied to Erciyes University Hematology Outpatient Clinic were included in the study. Ten subjects with normal bone marrow were selected as the control group. Two different studies, superficial and intracellular, were performed for flow cytometric analysis. Among the patients diagnosed with MM and in the control group; The ratio and number of CD8+ T lymphocytes (CD3+ CD8+), NKT cells (CD3+ CD56+), Th17 (CD3+ CD8− IL-17A+) cells in the bone marrow were determined by Beckman Coulter Navios flow cytometry device. The distribution of T lymphocytes was compared according to the plasma cell ratios in the bone marrow.

Results:
When the bone marrow of MM patients was examined by flow cytometry; It was observed that T lymphocytes were directed to the tumor periphery according to the plasma cell density. In the Bone Marrow; T lymphocyte subgroups (T4, T8, CD3, NKT, TH17) cells were found in different proportions in patients with Plasma Cell ratio above 60%, 10-60% and below 10%.

Discussion:
While some T Lymphocytes (T-reg) in the bone marrow microenvironment help the development of Tumor cells, some T Lymphocytes are thought to be involved in the fight against Myeloma Cell. High levels of IL-6, TGF-β and IL-1β in the bone marrow microenvironment favor Th17 cell aggregation that produces IL-17.Th17 cells secrete high levels of IL-17, which promotes MM plasma cell growth and inhibits the immune system. Thus, the data obtained will contribute to a more accurate understanding of the pathogenesis of the disease and to the creation of new immunotherapy strategies.

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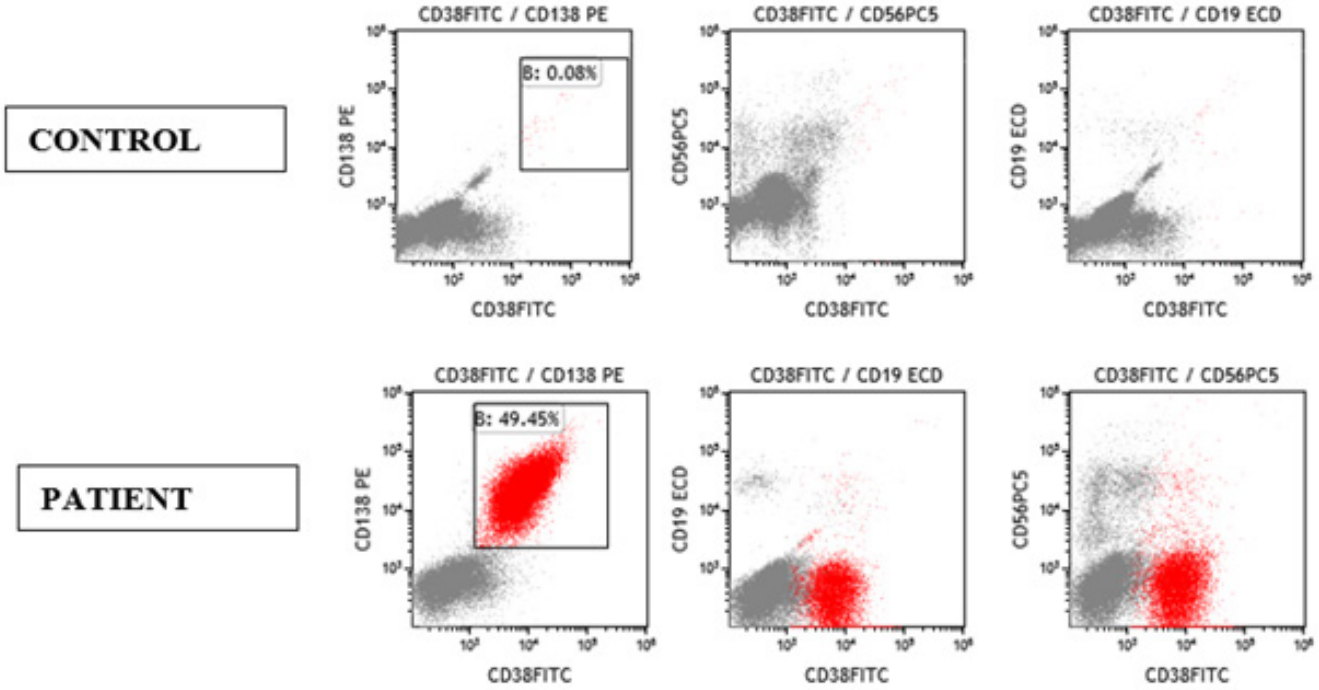

Figure 1. Plasma Cell Detection in Flow cytometry.

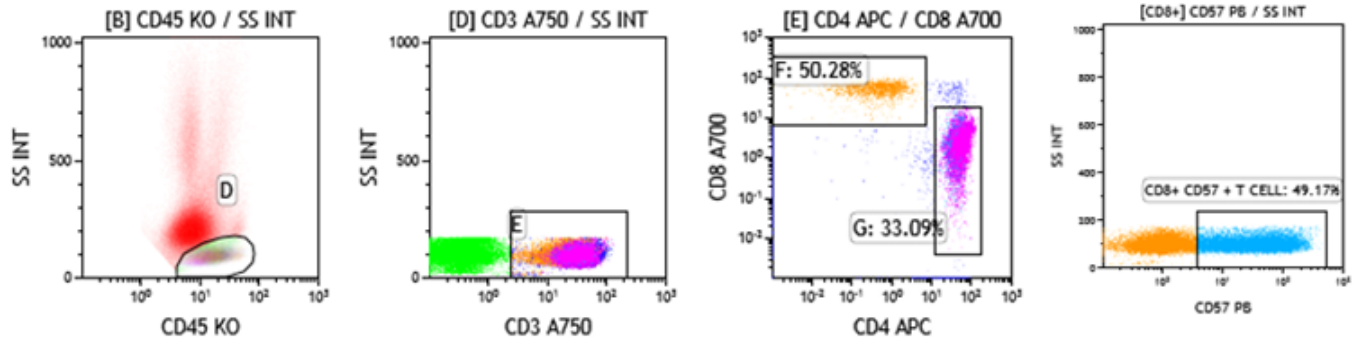

Figure 2. T4, T8, T3, NKT cells oriented around the Plasma Cell in the Bone Marrow.

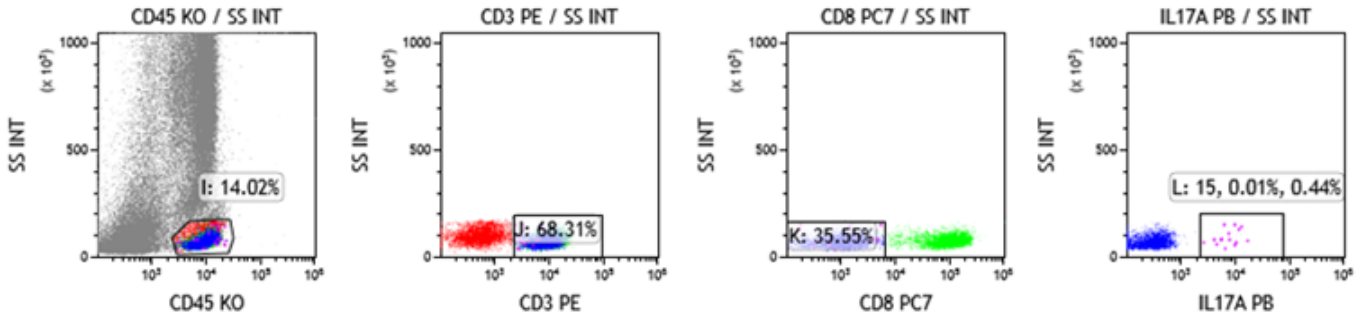

Figure 3. TH17 cells directed to the Plasma Cell region.

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Investigation of JAK2, CARL, and MPL Gene Mutations in Myeloproliferative Neoplasms

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Introduction

Myeloproliferative neoplasms (MPNs) are clonal diseases of hematopoietic stem cells driven by genetic abnormalities and inflammatory signaling (Braun and Zeiser, 2020; Kang et al., 2016). Myeloproliferative disorders are characterized by pathological changes leading to excessive production of myeloid blood cells and a high risk of progression to acute myeloid leukemia (AML). According to the WHO classification, BCR-ABL-negative myeloproliferative disorders are categorized into three classical subgroups: primary myelofibrosis (PMF), polycythemia vera (PV), and essential thrombocythemia (ET). These disorders are marked by abnormalities in blood production due to clonal defects in hematopoietic cells, overproduction of blood cells, bone marrow dysfunction, and clinical variability (Braun and Zeiser, 2020).

Materials and Methods

Between 2021 and 2024, peripheral blood samples were collected in EDTA tubes from 1,523 patients with a preliminary diagnosis of MPN referred to the Department of Medical Genetics at Erciyes University. Following automated DNA isolation, JAK2 V617F, JAK2 Exon 12, MPL W515L, MPL W515K, MPL W515R, CALR Type 1, and CALR Type 2 mutations were screened using the ipsogen® CALR RGQ PCR, ipsogen JAK2 RGQ PCR Kit, ipsogen MPL W515L/K MutaScreen Kit, and SNP Biotechnology MPN Screening Kit via Real-Time PCR.

Results

Of the 1,523 patients referred to the Department of Medical Genetics at Erciyes University with a preliminary diagnosis of MPN between 2021 and 2024, 121 patients tested positive for the analyzed mutations. Among the 121 BCR-ABL-negative patients, 90% (110 patients) had the JAK2 V617F mutation, 6% (5 patients with Type 1 and 2 patients with Type 2) carried CALR mutations, 2.5% had the MPL W515L mutation, and 1% (1 patient) had the JAK2 Exon 12 mutation.

Discussion

BCR-ABL-negative myeloproliferative disorders represent a significant subset of myeloid-origin hematological diseases. Consistent with the literature, our study demonstrated that the most frequently observed mutation in this disease group is the JAK2 V617F mutation. Following the JAK2 V617F mutation, CALR gene mutations are the next most common in these disorders. The diagnosis of BCR-ABL-negative myeloproliferative neoplasms relies on clinical findings, hematological and biochemical laboratory results, and genetic testing. While the JAK2 V617F mutation is widely used as a first-line genetic diagnostic marker, the frequencies of MPL W515L/K and CALR mutations should not be overlooked. The presence of these mutations has significant implications for treatment strategies in clinical practice. With the advent of next-generation screening kits, it is now possible to simultaneously assess these mutations and more, providing rapid and reliable diagnostic results for patients with a preliminary diagnosis of MPN.

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The Path to the Product with a Two-Way Concept from Bench to Bedside and Vice Versa

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Translational medicine serves as a critical bridge between basic scientific research and clinical application, ensuring that laboratory discoveries rapidly translate into improved patient care. The bidirectional approach, commonly described as "bench to bedside" and "bedside to bench," accelerates the development of innovative therapeutic strategies (Marincola, 2003). The bidirectional model of translational medicine creates a continuous feedback loop where laboratory discoveries and clinical experiences mutually enhance each other, fostering rapid innovation that directly benefits patients (Haeussler & Assmus, 2021).

The "bench to bedside" pathway is the cornerstone of translational medicine (Marincola, 2003). It concentrates on transforming fundamental scientific findings into clinical applications, including biomarkers, 3D bioprinting, regenerative medicine, targeted therapies, and sophisticated diagnostic tools, among others. The process begins with a comprehensive understanding of disease mechanisms, which involves identifying molecular pathways, genetic factors, epigenetic modifications, and protein alterations, cellular interactions that contribute to disease progression (Bustin & Jellinger, 2023). Once potential therapeutic targets are identified, experimental models, including *in vitro* systems and *in vivo* models, are used to validate these targets. Clinical trials in various phases evaluate the safety, dosage, and efficacy of treatments, leading to regulatory approvals from bodies like the Food and Drug Administration (FDA) or the European Medicines Agency (EMA) upon successful completion (Kandi & Vadakedath, 2023) (Umscheid

et al., 2011) (Joppi et al., 2020).

While the "bench to bedside" approach focuses on translating laboratory findings into clinical practice, the "bedside to bench" pathway emphasizes the role of clinical observations in guiding research. It can be observation-driven hypothesis generation, mechanistic insights, real-world data integration (Marincola, 2003) (Swift et al., 2018). Clinicians at the front lines may notice patterns or anomalies in patient responses to treatments or natural disease progression and these observations can serve as the initial spark for formulating hypotheses that can be tested in more controlled, experimental settings (Vandenbroucke & Pearce, 2018). Additionally, utilizing real-world data from electronic health records and patient registries unlocks a wealth of information that can uncover trends and relationships not evident in controlled clinical settings, thereby generating fresh hypotheses for further laboratory research.

Successful translational medicine hinges on effective collaboration between a diverse array of stakeholders, including academic institutions, industry partners, healthcare providers, and regulatory agencies (Austin, 2021). Academic researchers are at the forefront of basic scientific discovery and they contribute essential knowledge, conduct preclinical studies, and develop innovative methodologies (Salman, Sevgi, Kurnaz, & Sart, 2019). By leveraging advanced research facilities and fostering interdisciplinary collaboration, academic institutions play a pivotal role in translating basic research findings into clinical applications. Industry partners bring the expertise and resources necessary for scaling up promising therapies, and their role encompasses drug development, clinical trial management, manufacturing, and commercialization (Hartl et al., 2021). Companies often provide funding and technological platforms that academic researchers may lack, thus enabling the transition from bench to bedside. Moreover, clinicians and healthcare providers are integral to translational medicine as they identify unmet clinical needs and contribute to the design and execution of clinical trials. Also, regulatory bodies, such as the FDA and the EMA, ensure that new medical products are safe and effective before reaching the market (Joppi et al., 2020). Early engagement with these agencies during the research and development process can help streamline regulatory approval, ensuring timely patient access to innovative treatments.

Despite its potential, translational medicine faces several significant challenges; infrastructure development, career development for young investigators, regulatory hurdles, funding constraints and collaborative barriers (Freeman & Dervan, 2014). Building and maintaining research infrastructure is costly and requires long-term investment. Specialized facilities, such as biobanks, advanced imaging centers, and clinical trial units, are essential for conducting translational research. Without adequate infrastructure, it becomes difficult to bridge the gap between laboratory discoveries and clinical implementation. Moreover, supporting young investigators through mentorship, training, and funding is vital for their career development, yet many struggle to secure stable positions and funding (Salman, Sevgi, Kurnaz, & Sart, 2019). Furthermore, compliance with stringent regulatory requirements is essential but time-consuming, and delays in regulatory approvals can hinder the progress of high-potential projects (Salman, Sevgi, Kurnaz, & Sart, 2019). Securing funding for translational research is highly competitive, so many promising projects fail to advance due to insufficient financial support. While government grants are valuable, there is a growing need for alternative funding mechanisms, such as venture capital, crowdfunding, and industry collaborations.

In conclusion, translational medicine effectively connects scientific research with clinical applications via its dual "bench to bedside" and "bedside to bench" approach, driven by clinical demands to optimize patient care. Despite facing obstacles such as infrastructure, regulatory complexities, and funding challenges, collaborative efforts among academia, industry, and healthcare sectors help overcome these barriers.

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