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“CAROL DAVILA”, UNIVERSITY OF MEDICINE AND PHARMACY BUCHAREST

SCHOOL OF DOCTORAL STUDIES

MEDICINE

MOLECULAR AND CLINICAL FACTORS INVOLVED IN THE DISEASE EVOLUTION AND TREATMENT RESISTANCE IN MULTIPLE MYELOMA

DOCTORAL THESIS SUMMARY

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CURRENT STAGE OF KNOWLEDGE

The development of multiple myeloma follows a hierarchical process involving the differentiation, proliferation, survival, and dissemination of malignant plasma cells, driven by their interactions with the bone marrow microenvironment. The cell of origin results from the mutations of activated B lymphocytes occurring during their passage through the germinal center. The post-germinal origin of malignant plasma cells is supported by the presence of somatic hypermutations in the immunoglobulin variable region genes and class switching observed in IgG, IgA, and IgD MM types, but not in rare IgM MM cases [1]. The exact causes of monoclonal gammopathies remain unclear. However, the widely accepted oncogenic model involves one of two mutually exclusive categories of founder mutations [2]. The first includes translocations in the immunoglobulin heavy chain (IgH) genes on chromosome 14, most commonly between 14q32 and chromosomes 4, 6, 11, 16, or 20 [3-6]. The second category is hyperdiploidy, frequently involving chromosomes 3, 5, 7, 9, 11, 15, 19, and 21, associated with a favorable prognosis [3-6]. In contrast, IgH translocations with various partner chromosomes are linked to high-risk mutations, such as those enhancing oncogene expression, including Cyclin D1 (11q13 or 6p21), MMSET (4p16), or C-MAF (16q32) [5-7].

MM cells exhibit an intermediate mutational burden, positioned between "simple" malignancies like acute leukemias and "complex" solid tumors [8-11]. A key feature is the intraclonal heterogeneity, evident from the early stages of the disease, which impacts treatment response and clinical progression [9-11]. Patients with monoclonal gammopathy of undetermined significance (MGUS) often have two cellular populations: a "stable" one with a lower mutational burden and an "active" or "progressive" population. Those with a predominance of "stable" cells have a lower risk of progression to active MM, while driver mutations in "progressive" cells result in aggressive disease evolution [8-13]. MM progression follows a Darwinian evolutionary model, with tumor cells becoming heterogeneous under the selection pressure of the tumoral microenvironment, limited nutritional resources, and therapy [12]. However, the genomic evolution is not arbitrary, the acquisition of additional mutations being influenced by the category of the founder mutations [13,14]. For instance, hyperdiploid tumors tend to activate the NRAS signaling pathway, while specific translocations (e.g., t(11;14) or t(4;14)) are associated with mutations in KRAS, IRF4, CCND1, FGFR3, DIS3, and PRKD2 [12-14].

Genomic studies of relapsed MM reveal three distinct evolutionary patterns: stable, linear, and branched [12]. Stable evolution maintains the same mutational spectrum during treatment, linear evolution involves new genetic alterations occurring in pre-existing clones, while branched evolution is characterized by the emergence of new dominant clones, distinct from those present at diagnosis. These patterns are influenced by the depth of response to prior therapies, with deeper responses (e.g., >VGPR) often associated with branched evolution, highlighting the selection pressure driven by the treatment.

The bone marrow microenvironment plays a critical role in MM initiation, treatment resistance, and disease progression [15]. This complex structure includes cellular components (osteoblasts, osteoclasts, stromal cells, endothelial cells, adipocytes), extracellular matrix elements (collagen, fibronectin, laminin, proteoglycans), and soluble factors (cytokines, chemokines, growth factors) [16]. Interactions between malignant plasma cells and the microenvironment, mediated by direct cellular contacts, cytokine secretion, or exosome production, create a bidirectional feedback loop, ultimately driving disease expansion.

Clinically, MM evolves from MGUS, a premalignant condition defined by a plasma cell infiltrate <10%, serum monoclonal protein <3 g/dL, urinary monoclonal protein <500 mg/24 hours, and absence of CRAB symptoms (hypercalcemia, renal insufficiency, anemia, or bone lesions) [23-25]. Smoldering MM (SMM) represents an intermediate stage, characterized by a plasma cell infiltrate of 10-60% and absence of CRAB criteria but with certain thresholds for serum monoclonal protein (≥3 g/dL) or free light chain ratio (≥100) [24]. Active MM diagnosis, updated in 2014 by the International Myeloma Working Group (IMWG), includes the SLiM-CRAB criteria, which integrate both traditional CRAB features and newer markers such as bone marrow plasma cell infiltration >60% and focal bone lesions on MRI [24,26].

Therapeutic advancements in the past two decades have significantly improved five-year survival rates from <30% in the 2000s to >70% today [27]. Early therapies included alkylating agents, steroids, and autologous stem cell transplantation. The introduction of proteasome inhibitors and immunomodulatory agents marked the first major milestones, enabling the development of triple therapy and maintenance strategies. The development of anti-CD38 monoclonal antibodies further revolutionized treatment, incorporating quadruple therapy into the management of newly diagnosed cases. In recent years, T-cell-based therapies, such as bispecific antibodies and CAR-T cells, have shown remarkable efficacy in heavily pretreated patients.

Despite these advances, MM remains incurable. Persistent disease and relapse are attributed to factors such as cytogenetic abnormalities, clonal evolution, tumor heterogeneity, the protective role of the microenvironment, and the immune suppression associated with the disease. Additionally, therapeutic efficacy is influenced by factors like disease stage, prior treatment exposure, patient age, comorbidities, and treatment adherence.
To this end, this thesis aims to deepen the understanding of mechanisms underlying MM persistence and explore novel strategies for optimizing therapeutic outcomes.

PERSONAL CONTRIBUTION

1. Work hypothesis and general objectives

The first part of this thesis presents a review of the literature on the mechanisms involved in the development and progression of multiple myeloma, the classification criteria for monoclonal gammopathies, as well as the main therapeutic classes and general mechanisms of treatment resistance.

The second part explores, through a multi-layered and multidisciplinary evaluation, previously unexplored factors influencing MM progression and treatment response. The methodology integrates fundamental research, clinical research, and patient surveys, addressing aspects of public health, molecular biology, and clinical hematology, with the goal of developing new strategies for optimizing personalized therapy in MM. To identify new factors affecting treatment response, we pursued four general objectives, each assigned to an independent research study.

Objective 1 focuses on evaluating the role of the surface marker CD38 in MM biology. Although CD38 is uniformly and highly expressed on the surface of tumor cells, its impact on disease progression remains poorly understood. The methodology involved fundamental research techniques such as cell cultures, metabolomics, and animal studies, utilizing preclinical in-vitro and in-vivo models to analyze tumor cell behavior in the presence or absence of CD38 [28]. The findings provide the first evidence of the role of CD38 in cellular metabolism, hypoxic adaptation, and malignant plasma cell tumorigenesis [28].

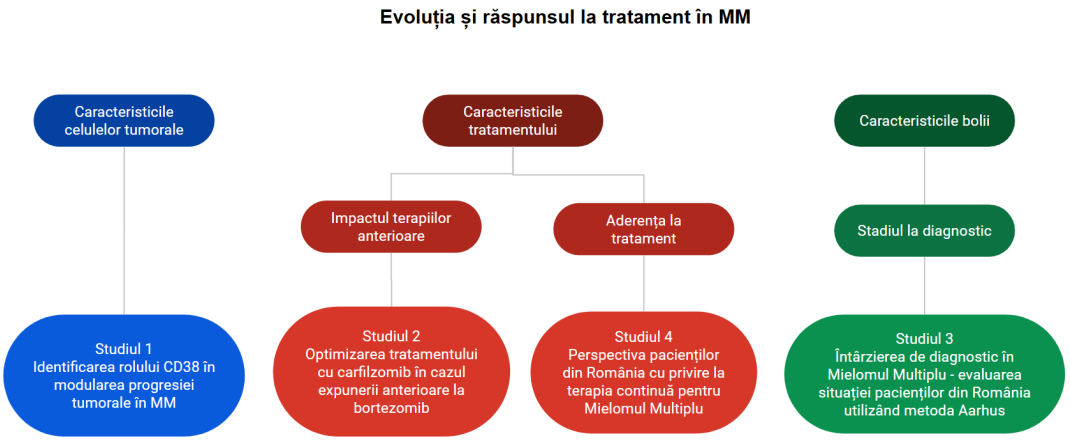
Objective 2 investigates the impact of bortezomib resistance on the efficacy of the second-generation proteasome inhibitor carfilzomib. While carfilzomib is considered suboptimal in patients with prior resistance to bortezomib, the available data on its effectiveness in this context remain limited [29]. Using a clinical research-based methodology, we aimed to define the profile of patients who might benefit from carfilzomib-based combination therapies despite prior resistance to first-generation proteasome inhibitors [29].

Objective 3 evaluates diagnostic pathways and the causes of diagnostic delays in MM patients in Romania. Disease stage at diagnosis significantly affects treatment response and overall survival. Available data suggest that a considerable proportion of MM patients in Romania face diagnostic errors or significant delays in diagnosis [30]. Using clinical and epidemiological research methods, this study provides novel insights into the diagnostic pathways and delays faced by MM patients in Romania [30].

Objective 4 analyzes patient perspectives on continuous therapy for MM, which directly influences treatment adherence and, consequently, therapeutic efficacy. In the context of current recommendations for continuous treatment until disease progression or significant toxicity, this study offers innovative insights into how patients perceive the impact of therapy on their lives and identifies patient subgroups who might benefit from finite-duration treatment [31].

A schematic representation of the research studies is shown in Figure 1, with complete study details are provided in the following chapters.

Figure 1.1 Schematic representation of the research studies



2. Identification of the role of CD38 in modulating the tumoral evolution in MM

2.1 Introduction

CD38 was first identified in 1980 by the team led by Reinherz and Schlossman during their studies focused on characterizing the T-cell receptor [32]. CD38 is highly expressed on the surface of plasma cells, as well as activated B and T lymphocytes, and natural killer (NK) cells [33,34]. Initially described as a surface receptor, CD38 was later discovered to possess ecto- and endoenzymatic catalytic activity [35,36]. Its primary intracellular enzymatic role involves NAD+ catabolism and the synthesis of ADPR and cyclic ADPR, that regulate calcium release from the endoplasmic reticulum [36]. Additionally, CD38 modulates extracellular adenosine levels through NAD+ degradation via a non-canonical pathway [35,37,38]. Adenosine serves as a key regulator of the immune system due to its potent immunosuppressive effects, that include, among others, the inhibition of NK cells, dendritic cells, macrophages, and helper or cytotoxic T cells [39-41].

The administration of the monoclonal antibody daratumumab is associated with a rapid decrease in CD38 expression on malignant plasma cells [42]. However, following treatment discontinuation or as the disease progresses, CD38 expression returns to pre-treatment levels, suggesting a potential role for CD38 in MM progression [42].

2.2 Materials and Methods

CD38 expression was knocked out in human and murine cell lines using CRISPR-Cas9. Immunocompetent Balb/c mice and immunodeficient NSG mice were subcutaneously injected with either unmodified (NT) or CD38 knockout (KO) J558 cells. Stromal adhesion was assessed using labeled NT and KO cells with OP-9 murine stromal cells. NAD+ levels were quantified using the Promega Glo Assay, and mitochondria were isolated with the Mitochondria Isolation Kit (Thermo Scientific). Oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) were measured using Seahorse Assay. Cellular response to hypoxia was evaluated in a modular hypoxic chamber, and cell cycle distribution was quantified using propidium iodide staining.

2.3 Results

To investigate the effect of CD38 on tumor growth in an immunocompetent model, we used Balb/c mice injected with syngeneic J558 plasmacytoma cells, either CD38-positive (NT) or CD38-negative (KO). Despite uniform in-vitro growth, CD38 KO tumors exhibited significantly smaller volumes compared to NT tumors in-vivo (235.08 mm^3 vs. 1173.34 mm^3, p=0.001). Subsequently, we assessed whether CD38 effect on promoting tumoral growth persisted independently of its potential immunomodulatory role by using immunodeficient NSG mice. Similarly to the previous experiment, despite similar in-vitro growth, CD38 KO tumors showed significantly reduced expansion compared to NT tumors (707.88 mm^3 vs. 1591.81 mm^3, p=0.02).

Further investigations into the mechanisms underlying this proliferation advantage revealed that CD38 influences stromal adhesion. Using murine J558 plasmacytoma cells and OP-9 stromal cells, we confirmed that CD38-positive cells exhibited twice the adhesion capacity compared to CD38-negative cells, aligning with existing data on daratumumab-induced loss of adhesion [43].

Given the variable levels of hypoxia associated with the tumor microenvironment, we next assessed cellular behavior under normoxic and hypoxic conditions. Under prolonged hypoxia, CD38 KO cells exhibited significantly reduced proliferation compared to NT cells, and cell cycle analysis revealed that hypoxia induced a marked G0-G1 phase arrest in CD38 KO cells relative to NT cells.

We also explored the enzymatic activity of CD38 and found that CD38 KO cells had up to twice the NAD+ levels of NT cells, consistent with literature data. Given NAD+'s role in mitochondrial biosynthesis, we observed that CD38 loss correlated with a significant increase in cellular mitochondrial content. Further metabolic analysis using Seahorse XF assays revealed that CD38 KO cells exhibited a twofold increase in oxidative phosphorylation (OXPHOS) compared to CD38-positive cells. Moreover, the OCR/ECAR ratio (indicating OXPHOS vs. glycolysis metabolic preference) was 2-3 times higher in KO cells, suggesting a shift towards an oxidative metabolism.

Under hypoxic conditions, most eukaryotic cells can adjust their metabolic strategy, with hypoxia-inducible factor 1-alpha (HIF-1α) serving as the primary regulator of this adaptive response. Given the preference of CD38 KO cells for oxidative phosphorylation metabolism, we aimed to assess HIF-1α expression in these cell lines. Using Western blot analysis following hypoxic exposure, we demonstrated that HIF-1α levels were significantly lower in CD38 KO cells compared to NT cells.

Furthermore, in these cell types exposed to anaerobic growth conditions, but not in those grown in normoxia , we observed a significant difference in the number of colonies formed by CD38 KO cells versus WT cells. Notably, when serially replated, CD38 KO cells previously exposed to hypoxia retained their advantage in colony formation compared to CD38 NT cells, exhibiting more aggressive behavior and superior self-renewal and clonogenic capacity.

Finally, we examined the effect of chronic adenosine exposure. While acute exposure to NECA induced apoptosis, chronic exposure resulted in tolerance, dependence, and an increase in the clonogenic potential of malignant plasma cells.

2.4 Discussion

This study provides the first comprehensive characterization of the CD38 role in MM development and persistence. Loss of CD38 expression reduces tumor proliferation potential in-vivo but not in-vitro, highlighting a context-dependent role involving metabolic reprogramming and diminished stromal adhesion. Furthermore, CD38 loss under hypoxia promotes an aggressive, stem-like behavior in malignant plasma cells. We also demonstrated that chronic adenosine stimulation drives dependence and amplifies the clonogenic potential of MM cells, linking CD38 enzymatic activity to tumor progression.

3. Optimizing carfilzomib treatment in patients with previous bortezomib exposure

3.1. Introduction

The development of the first-generation proteasome inhibitor bortezomib marked a pivotal advancement in MM treatment. Approved in 2003, this agent revolutionized therapy for MM patients, increasing the five-year survival rate to over 50% [44]. For patients with primary or secondary resistance to bortezomib, current guidelines recommend anti-CD38 monoclonal antibodies as the next line of therapy, while second-generation proteasome inhibitor carfilzomib is considered suboptimal [45,46]. Following the introduction of the updated 2021 ESMO guidelines, the daratumumab–bortezomib combination has been widely used as first-line treatment. This has limited subsequent therapeutic options for patients who develop resistance to the daratumumab–bortezomib combination. Although carfilzomib-based regimens have demonstrated a remarkable efficacy in clinical trials, there

is insufficient real-world or clinical trials data to clarify the feasibility of using these combinations in patients with prior bortezomib resistance.

3.2 Materials and Methods

We conducted a retrospective, observational, unicentric study in the Hematology Department of the Fundeni Clinical Institute. Patient data was extracted from the electronic medical records. The study included patients diagnosed with relapsed or refractory MM, according to IMWG criteria, who received at least two complete cycles of carfilzomib-based treatment in our clinic between January 2018 and December 2022. All included patients had prior exposure to bortezomib, at least during the first-line therapy.

3.3 Results

The proportion of patients achieving at least a partial response to carfilzomib-based regimens was significantly higher in bortezomib-responsive patients (Group 1: 95.5%) compared to bortezomib-refractory patients (Group 2: 82.05%) (OR 4.593 [95% CI, 1.259–16.758], p = 0.032).

As expected, the superior response rates in Group 1 translated into an improved progression-free survival. The PFS in bortezomib-sensitive patients was 523 days, significantly longer than the 310 days observed in bortezomib-refractory patients (p = 0.003). Among patients uniformly treated with the KRd combination, the median PFS in Group 1 was 523 days (95% CI: 372–613) compared to 399 days (95% CI: 231–506) in Group 2. For high-risk cytogenetic patients, the median PFS in Group 1 was 523 days (95% CI: 321–863), significantly longer than the 247 days (95% CI: 168–692) in Group 2 (p = 0.012). However, for standard-risk cytogenetic patients, no statistically significant differences in PFS were observed between Groups 1 (482 days) and 2 (399 days) (p = 0.125). In patients with at least two prior lines of therapy, median PFS was 523 days in Group 1 (95% CI: 314–587) versus 310 days in Group 2 (95% CI: 226–453).

We also analyzed the impact of prior exposure to IMiDs on the efficacy of carfilzomib-based therapy, but no significant difference in PFS was observed between the two groups regardless of previous exposure. Among patients with prior IMiD exposure, median PFS was 322 days in Group 1 versus 301 days in Group 2 (p = 0.109). In patients without prior IMiD exposure, median PFS was 535 days in Group 1 versus 544 days in Group 2 (p = 0.446).

Another factor potentially influencing treatment response is the ISS staging. In our study, for patients with ISS stage I or II, Group 1 achieved significantly longer median PFS compared to Group 2 when treated with carfilzomib-based combinations (613 days [95% CI: 392–861] vs. 247 days [95% CI: 160–480], p = 0.002). However, for ISS stage III patients, no significant differences in PFS were observed between the two groups.

Regarding the overall survival, no significant difference was observed between the groups. Median OS was 1258 days for bortezomib-sensitive patients compared to 1411 days for bortezomib-refractory patients (p = 0.632). Similarly, no significant differences were observed when the patients were analyzed based on the cytogenetic risk, number of prior lines of treatment, or ISS stage.

3.4 Discussion

The efficacy of carfilzomib-based combinations in treating relapsed MM is well-established. However, the outcomes in patients refractory to prior bortezomib treatment remain unclear, as most clinical trials exclude this subgroup from enrollment.
Our study demonstrated that patients refractory to prior bortezomib treatment had significantly lower overall response rates compared to bortezomib-sensitive patients. However, we identified a subgroup of patients, characterized by ISS stage III and standard cytogenetic risk, who may benefit from carfilzomib treatment regardless of prior bortezomib response. Conversely, carfilzomib treatment appears suboptimal in patients with high cytogenetic risk, ISS stage I or II, and prior bortezomib resistance.

4. Diagnostic delays in Multiple Myeloma – Evaluating diagnostic pathways in Romania using the Aarhus method

4.1 Introduction

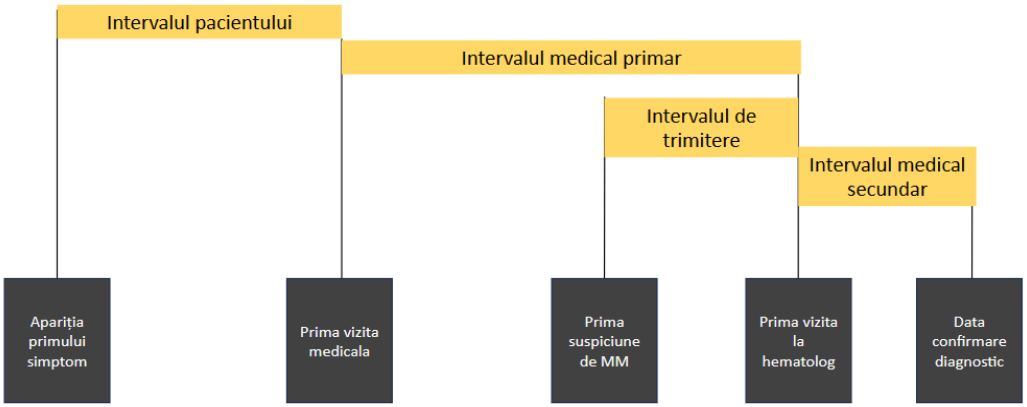
Multiple myeloma is considered to be one of the most challenging malignancies to diagnose [47,48]. Despite the importance of timely and accurate diagnosis for an effective MM management, globally, over one-third of patients are diagnosed at advanced stages, often requiring emergency medical care. Similarly, in Romania, a significant proportion of MM patients are misdiagnosed or diagnosed at advanced stages of the disease [49-51]. Until the

publication of this study, there were no reports on the diagnostic pathways of MM patients in Romania, from symptom onset to diagnosis, or on the factors associated with diagnostic delays.

4.2 Materiale și metode

This prospective, observational, single-center study was conducted in the Hematology Department of Fundeni Clinical Institute between January 2022 and March 2023. The study included all adult patients diagnosed with MM during the study period. To accurately identify causes of diagnostic delays, we used a questionnaire based on the standardized Aarhus method (Fig. 1).

Figure 4.1: Diagnostic intervals according to the Aarhus method



4.3 Results

Diagnostic delays during the patient interval often result from the patient's inability to recognize cancer-associated symptoms. In our study, the median duration of the patient interval was 162 days. The most common symptoms prior to diagnosis were bone pain (78.64% of cases) and fatigue (57.28%). Although over three-quarters of patients experienced bone pain, only 59.02% sought medical care, with a median delay of 191 days between symptom onset and the first medical visit.

The primary care interval reflected the specific characteristics of the Romanian healthcare system, where patients can consult specialists without first seeing a general practitioner. Among the patients included, only 42.71% initially consulted a GP for their symptoms, while over a quarter presented as medical emergencies. On average, patients required 3.7 medical visits (range: 2–16) before MM suspicion was raised, and 18.44% needed five or more consultations before diagnostic suspicion arose. Notably, patients who

initially consulted a GP had a significantly shorter primary care interval compared to those who pursued other diagnostic routes (median 26 days vs. 93 days, p = 0.004).

No significant diagnostic delays were observed during the secondary care interval, as diagnoses were established within 72 hours of hospital admission. Among the study cohort, 31.06% of the patients required emergency care for acute renal failure, hypercalcemia, spinal cord compression syndrome, or severe anemia. Additionally, 60.19% of patients presented with advanced ISS stage III disease, and 15.53% required the initiation of hemodialysis.

Table 4.2. Diagnosis intervals duration according to the Aarhus method

		Median(days)
Patient interval		162
Primary care interval	First medical visit / First time diagnostic suspicion is raised	66
	First time diagnostic suspicion is raised / First hematologist referral	19
Secondary care interval		0

4.4 Discussion

This study provides the first assessment of the diagnostic pathways and subsequent delays for MM patients in Romania. The most significant delays occurred during the patient interval, likely due to the insidious onset of MM, the absence of alarming symptoms, and delays in seeking medical help. The primary care interval also contributed to diagnostic delays, but GPs appear to play a key role in reducing these delays. In our cohort, an initial visit to a GP was associated with a significantly shorter primary care interval compared to patients who first consulted other specialists. This finding highlights the importance of GP involvement in the early identification and referral of MM cases.

5. The perspective of Romanian patients on continuous Multiple Myeloma treatment

5.1 Introduction

The prognosis of multiple myeloma patients has improved significantly in the recent decade due to the introduction of new treatment classes [52-54]. The enhanced efficacy and

favorable safety profile of these therapies have enabled their prolonged use. Current therapeutic strategies predominantly involve the continuous administration of combination regimens until disease progression or occurrence of significant toxicities. However, data regarding patients' perspectives on continuous therapy in MM remain limited.

5.2 Materials and Methods

This study included the adult MM patients receiving continuous therapy at the Hematology Department of the Fundeni Clinical Institute during the study period, from March to July 2023. Eligible patients received treatment for at least six months, either in outpatient settings or through short hospital stays (<72 hours). To assess the impact of continuous therapy on various aspects of patients' lives, we developed a questionnaire incorporating both qualitative and quantitative tools. The questionnaire was based on validated scales (EORTC QLQ-C30, EORTC QLQ-MY20, Generalized Anxiety Disorder 7-item Scale) and the questions were translated and adapted to accommodate patients with diverse educational backgrounds.

5.3 Results

When questioned about the impact of continuous therapy on their quality of life, 74.83% of patients reported a negative effect. Given a choice between high-intensity fixed-duration therapy and continuous therapy, only 28.26% would choose fixed-duration therapy if it were less effective than continuous treatment. Additionally, 73.54% expressed fear of discontinuing continuous therapy, primarily due to concerns about a more rapid or aggressive relapse.

Only 18.06% of patients reported experiencing high or very high levels of anxiety associated with hospital visits, while 43.87% reported that frequent hospital visits provided a high or very high level of psychological comfort.

Regarding the financial impact, 36.12% of patients reported that continuous therapy had a high or very high impact on their economic status. From a personal perspective, 25% of patients reported that continuous therapy had a significant negative impact on their family life, while 31.61% believed that the negative effects extended to their family members. Regarding the symptoms they experienced, fatigue and insomnia were identified as the most burdensome side effects, with 54.19% and 42.58% of patients, respectively, reporting being significantly or very significantly affected.

5.4 Discussion

Our study highlights the significant personal, social, financial, and professional burden continuous therapy poses for a substantial proportion of MM patients. Three-quarters of respondents felt that treatment negatively affected their quality of life, and if offered the choice, nearly 60% would prefer fixed-duration therapy if it provided equivalent efficacy. However, despite these challenges, most patients expressed reluctance to discontinue therapy, likely driven by fear of relapse and value the psychological comfort derived from their relationship with medical staff. In conclusion, choosing between continuous and fixed-duration therapy depends on balancing optimal disease control with minimizing the impact on patients' quality of life and daily activities.

6. Conclusions and personal contributions

The past decades brought significant progress in the treatment of multiple myeloma with the introduction of the novel therapeutic classes, including proteasome inhibitors, immunomodulatory agents, monoclonal antibodies, bispecific antibodies, and CAR-T cell therapy. These advancements have shifted the therapeutic goals from merely achieving a treatment response to implementing personalized strategies that integrate clinical and biological factors known to modulate treatment outcomes. In this context, identifying novel determinants of treatment response is essential to optimize therapeutic regimens, enhancing both efficacy and patient survival.

This doctoral research aimed to contribute to the understanding of mechanisms influencing disease progression and treatment response. The objectives were achieved through a multidisciplinary approach combining fundamental research, clinical studies, and patient perspective analysis via questionnaires. The methodology addressed public health, molecular biology, and clinical hematology, offering a comprehensive perspective. The findings reflect original and relevant contributions aligned with the research objectives.

1. **Confirmation of the role of the surface marker CD38 in the MM tumor biology, providing the first evidence of the association between CD38 expression and a context-dependent proliferative advantage (Chapter 5).**

1.1 Loss of CD38 correlates with reduced tumoral proliferation potential in-vivo and the emergence of an aggressive, stem-like cell population.

1.2 Absence of CD38 decreases adhesion to stromal cells
1.3 CD38 loss leads to reduced proliferation under hypoxic conditions, driven by increased reliance on mitochondrial respiration and oxidative phosphorylation, similarly to healthy cells.
1.4 Hypoxia adaptation is impaired in CD38-deficient cells, as evidenced by decreased HIF-1α expression.
1.5 Despite their proliferative disadvantage in hypoxia, CD38 KO cells exhibit higher long-term viability and superior clonogenic and self-renewal capacities compared to CD38-positive counterparts.
1.6 Chronic adenosine exposure induces tolerance and dependence via adenosine receptor signaling (A2A).
1.7 Chronic adenosine exposure enhances the clonogenic potential of malignant plasma cells.

2. Defining the patient profile that benefits from carfilzomib-based therapy in the context of prior bortezomib resistance (Chapter 6)
2.1 Patients with advanced disease and standard cytogenetic risk demonstrate favorable responses to carfilzomib, regardless of prior bortezomib exposure.
2.2 High cytogenetic risk or ISS stage I/II patients exhibit suboptimal responses to carfilzomib when prior primary or secondary bortezomib resistance is present.

3. The analysis of diagnostic pathways for MM patients in Romania revealed significant delays that negatively impact disease stage at the time of diagnosis and, consequently, treatment response (Chapter 7).
3.1 The longest delays occurred during the patient interval, likely due to low symptom awareness, lack of routine check-ups, insidious disease onset, and non-specific symptoms.
3.2 On average, patients required five medical consultations before diagnosis.
3.3 General practitioners played a crucial role in reducing diagnostic delays.

4. Patients' perspectives on continuous therapy in MM influence treatment adherence and, consequently, therapeutic efficacy (Chapter 8).
4.1 Continuous therapy negatively impacts the quality of life for most patients, though the majority fear stopping treatment due to concerns about rapid or aggressive relapse.
4.2 Frequent hospital visits provide psychological comfort for many patients, improving treatment adherence.

4.3 Continuous therapy affects professional and social life and imposes financial burdens, particularly for working patients, highlighting the need for tailored approaches, such as fixed-duration therapies for specific subpopulations.
4.4 Integrating patient perspectives into therapeutic decision-making is essential, focusing on balancing treatment efficacy with the impact on daily life.

Overall, the factors identified in this research open new possibilities for optimizing therapeutic strategies in MM, and emphasize a personalized approach. Thus, we consider that the research aims have been fulfilled. However, there are certain limitations that need to be acknowledged. Studies II, III, and IV were single-center, limiting generalizability, and the small patient cohorts reduced statistical power. Regarding the first study, additional experiments are needed to assess the role of CD38 loss in treatment response and to develop therapeutic combinations that include specific enzymatic inhibitors.

Optimizing MM therapy offers significant benefits, directly impacting survival and quality of life. Personalized approaches that include factors influencing therapeutic efficacy can extend overall survival and progression-free intervals while reducing symptom burden. Emerging therapeutic regimens, including monoclonal antibodies, proteasome inhibitors, immunomodulatory agents, and cellular therapies, show promise for improving long-term outcomes while economically, the optimized therapeutic interventions can reduce the costs associated with frequent hospitalizations and additional treatments for relapse. However, challenges such as the high costs of modern therapies and ensuring equitable access persist. Despite these hurdles, the survival and quality-of-life benefits justify these investments, marking a substantial advancement in managing an incurable disease.

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1. **Irimia R,** Badelita SN, Barbu S, Zidaru L, Carlan IL, Coriu D. The Efficacy of Carfilzomib Treatment in Bortezomib-Refractory Patients-Real Life Experience in a Tertiary Romanian Hospital. J Clin Med. 2024 Apr 9;13(8):2171. doi: 10.3390/jcm13082171. PMID: 38673444; PMCID: PMC11050610.

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2. **Irimia R**, Badelita S, Barbu S, Cirlan IL, Zidaru L, Coriu D. Determining diagnostic delays in Romanian multiple myeloma patients using the Aarhus statement. Front

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3. **Irimia R,** Badelita SN, Barbu S, Zidaru L, Carlan IL, Coriu D. The perspective of Romanian patients on continuous therapy for Multiple Myeloma

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Published abstracts:

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