Alma Mater Studiorum – Università di Bologna

Department of Statistical Sciences

Second Cycle degree in Statistical Sciences

SECOND-LINE THERAPY WITH DARATUMUMAB-BORTEZOMIB-DEXAMETHASONE (DaraVD) FOR MULTIPLE MYELOMA (MM) PATIENTS REFRACTORY TO LENALIDOMIDE:

RESULTS OF A MULTICENTER OBSERVATIONAL STUDY

Master Thesis

Tutor:
Chiar.ma Prof.ssa
Angela Montanari

Co-Tutor:
Chiar.ma Prof.ssa
Elena Zamagni
Carolina Terragna

Presented by:
Jose G. Escarraman
Matricula 0001076812

Session I

Academic year 2023/2024

Abstract

The management of lenalidomide-refractory (Len-R) multiple myeloma (MM) patients presents significant clinical challenges, necessitating effective therapeutic strategies. The daratumumab-bortezomib-dexamethasone (DaraVD) has been widely employed in this context. This multicenter observational study aimed to assess the efficacy and safety of DaraVD exclusively in Len-R MM patients. A cohort of 85 patients with relapsed or refractory MM, all previously exposed to lenalidomide, was analyzed. The overall response rate was 84.7%, with 60.0% achieving a very good partial response (VGPR) or better. The regimen demonstrated a manageable safety profile. Median progression-free survival was 14 months, and overall survival was 47 months at a median follow-up of 22 months. Stratified survival analyses highlighted significant associations between key variables such as extramedullary disease, response depth, ECOG performance status, and Urinary proteins level, underscoring their impact on outcomes. These findings support DaraVD as a viable treatment option for Len-R MM patients, warranting its consideration in clinical practice to improve patient outcomes.

KEYWORDS: Multiple myeloma. Lenalidomide refractoriness. Dara-VD · Observational study

List of Content

List of Tables

List of Figures

Dedication

I would like to dedicate this achievement to my grandfather, Amadeo Escarramán, who passed away two years ago.

His memory and desire to honor him has been my main motivation to successfully complete this program.

This work is a tribute to him, his amazing story and the legacy of love, integrity, kindness and inspiration he left behind.

Thank you for being with me on this journey.

Trust the wait.

Embrace the uncertainty.

Enjoy the beauty of becoming.

When nothing is certain, anything is possible

- Mandy Hale

Introduction

Multiple Myeloma

Multiple myeloma (MM) is a complex and heterogeneous hematologic malignancy characterized by the clonal proliferation of malignant plasma cells within the bone marrow microenvironment. These malignant cells produce monoclonal protein, which can be detected in the blood or urine and is associated with various organ dysfunctions. Accounting for approximately 1% of all neoplastic diseases and 10-13% of hematologic cancers (Kazandjian, 2016; Padala et al., 2021), MM predominantly affects older adults, with a median age at diagnosis of around 70 years. The disease manifests through a range of clinical symptoms, including bone pain, pathological fractures, anemia, recurrent infections, hypercalcemia, renal failure, and occasionally, hyperviscosity syndrome and amyloid disease (Palumbo & Anderson, 2011).

Despite its classification as a single disease, MM encompasses a variety of cytogenetically distinct plasma cell malignancies (Firth, 2019). The diagnosis of MM relies on the presence of myeloma-defining events (MDEs) (Cowan et al., 2022; M. A. Dimopoulos, Moreau, et al., 2021) and evidence of clonal plasma cells in the bone marrow or a biopsy-proven plasmacytoma. Key MDEs include CRAB features (hypercalcemia, renal failure, anemia, or lytic bone lesions) and specific biomarkers such as a high serum free light chain ratio and focal lesions on MRI (Kyle et al., 2003).

The survival of MM patients has improved significantly over the past 15 years (Firth, 2019; Thorsteinsdottir et al., 2018), primarily due to advancements in therapeutic options(Cowan et al., 2022). The introduction of autologous stem-cell transplantation, immunomodulatory agents (IMiDs) like thalidomide and lenalidomide, proteasome inhibitors such as bortezomib, and monoclonal antibodies like daratumumab has revolutionized the management of MM (Thorsteinsdottir et al., 2018). Additionally, novel regimens and the concept of minimal residual disease (MRD) as a prognostic indicator are continuously evolving, promising further improvements in patient outcomes. However, the precise etiology of MM remains unclear, with potential factors including radiation, exposure to toxins, and viral infections still under investigation(Cowan et al., 2022).

Epidemiology

Multiple myeloma (MM) is the second most common hematologic malignancy, following lymphoma, and accounts for almost 2% of cancer diagnoses and over 2% of cancer deaths(Kazandjian, 2016; Padala et al., 2021). Although MM is considered rare overall, its incidence is notably higher in developed regions such as North America, Western Europe, and Australasia. This can be attributed to various causes, such as occupational factors, inheritable autosomal mutations, and decreased diagnostic ability in low-income countries compared to high-income countries, which do not necessarily reflect differences in the biology of the disease. (Padala et al., 2021).

The disease predominantly affects older adults, with a median age at diagnosis of around 69 years. Approximately 60% of cases occur in individuals over the age of 65. MM is relatively uncommon in those under 30 years of age, with less than 0.3% of cases occurring in this younger demographic (Firth, 2019).

Over the past few decades, the incidence of MM has increased globally. According to GLOBOCAN 2022 statistics, there were an estimated 188,000 new cases of MM worldwide, representing a 166% increase since 1990 and 18% increase since 2018 (Bray et al., 2018, 2024).

Multiple myeloma exhibits varying incidence rates and survival outcomes across different regions:

- **Italy** (2020): 5,759 new cases (1.5% of all cancers), higher in males (3019) than females (2740). Incidence rates: 11.1 (males) and 7.7 (females) per 100,000. Prevalence: 36,800. 5-year survival: 51% ((AIRTUM & AIOM) 2020).

- **Europe** (2020): Incidence rates: 5.9 (females) and 9.7 (males) per 100,000. Mortality rates: 3.9 (females) and 6.2 (males) per 100,000 (European Cancer Information System. 2020).

- **United States**: Multiple myeloma accounts for 1.8% of new cancer cases. 5-year survival: 53.9%. Median age at diagnosis: 69. Median age at death: 75 (National Cancer Institute of United States 2020).

Diagnosis

The diagnosis of multiple myeloma (MM) is based on the presence of clonal plasma cells in the bone marrow and associated myeloma-defining events. The International Myeloma Working Group criteria outline specific parameters for diagnosing MM and related plasma cell disorders (Kyle et al., 2003).

To diagnose MM, patients must exhibit clonal bone marrow plasma cells comprising at least 10% of the bone marrow or have a biopsy-proven plasmacytoma. Additionally, the presence of one or more myeloma-defining events (MDEs) is required. MDEs include end-organ damage such as hypercalcemia, renal insufficiency, anemia, and bone lesions, collectively known as CRAB criteria (Kyle et al., 2003). Specifically, these are:

- Hypercalcemia: serum calcium >0.25 mmol/L (>1 mg/dL) above the upper limit of normal or >2.75 mmol/L (>11 mg/dL)
- Renal insufficiency: creatinine clearance <40 mL/min or serum creatinine >177 μmol/L (>2 mg/dL)
- Anemia: hemoglobin >2 g/dL below the lower limit of normal or <10 g/dL
- Bone lesions: one or more osteolytic lesions on skeletal radiography, CT, or PET-CT

Furthermore, other biomarkers for MM diagnosis include:

- Clonal bone marrow plasma cell percentage ≥60%
- Involved: uninvolved serum free light chain (FLC) ratio ≥100 (with the involved FLC level being ≥100 mg/L)
- More than one focal lesion on MRI, at least 5 mm in size

A comprehensive diagnosis of multiple myeloma involves several key components. Initially, a detailed medical history and thorough physical examination are conducted to assess symptoms and organ dysfunction. Laboratory tests are then performed, including a complete blood count, chemical analysis, serum and urine protein electrophoresis with immunofixation, and quantification of monoclonal protein levels (Moreau et al., 2017).

For further diagnostic precision, a bone marrow examination is usually conducted, which includes a trephine biopsy and aspirate for cytogenetic analysis or fluorescence in situ hybridization (FISH). Imaging studies are also crucial, with conventional radiography of the spine, skull, chest, pelvis, humeri, and femora to identify bone lesions (Cowan et al., 2022).

Patients with nonsecretory myeloma[1], a rare variant, are diagnosed based on having at least 30% clonal bone marrow plasma cells or a biopsy-proven plasmacytoma, in the absence of detectable monoclonal protein (M. A. Dimopoulos, Moreau, et al., 2021).

Staging and prognosis are essential for treatment planning, commonly utilizing the International Staging System (ISS) or the revised one (R-ISS) (Palumbo et al., 2015) .This system classifies patients into three risk groups based on serum β2-microglobulin and albumin levels. High-risk disease is associated with specific chromosomal abnormalities, such as t(4;14), deletion 17p13, and chromosome 1 abnormalities, as well as high levels of serum β2-microglobulin or elevated lactate dehydrogenase levels. Additionally, genetic profiling, including gene-expression profiling and gene copy-number alterations, provides further prognostic information(Cowan et al., 2022).

Therapy

The treatment of multiple myeloma (MM) has seen significant advancements over recent years, enhancing patient outcomes and prolonging survival(Soekojo & Chng, 2022). While a cure remains elusive, the goal of therapy is to induce remission, manage symptoms, and prolong life. Treatment strategies are tailored based on patient-specific factors such as age, comorbidities, and the presence of high-risk cytogenetic abnormalities.

Initial Treatment: The initial treatment for multiple myeloma generally involves a combination of immunomodulators and steroids, which forms the foundation of therapy. Significant advancements in myeloma treatment have been made with the introduction of thalidomide, lenalidomide, and bortezomib. Bortezomib, a proteasome inhibitor, is also effective but requires careful monitoring due to the risk of peripheral neuropathy.(M. A. Dimopoulos, Moreau, et al., 2021; Moreau et al., 2017; Rajkumar, 2011). Daratumumab, which has become the gold standard in first-line treatment

[1] Unlike typical multiple myeloma, where abnormal plasma cells produce excessive amounts of monoclonal immunoglobulin (also called M protein), nonsecretory myeloma patients do not produce detectable levels of this protein in the blood or urine.

following trials such as MAIA, ALCYONE, and CASSIOPEIA, should also be noted (Facon et al., 2021; Mateos et al., 2018; Moreau et al., 2024)

Relapsed/Refractory Myeloma: Relapse is inevitable for most patients, and treatment is guided by the duration of response to initial therapy, type of initial therapy, patient performance status, and specific relapse factors. In most of the cases, is characterized by the inclusion of monoclonal antibodies like daratumumab, isatuximab, elotuzumab(Moreau, Kumar, et al., 2021):

- Combination Regimens: DRd (Daratumumab, Lenalidomide, Dexamethasone), DVd (Daratumumab, Bortezomib, Dexamethasone), DPd (Daratumumab, Pomalidomide, Dexamethasone), KRd, IRd (Ixazomib, Lenalidomide, Dexamethasone), and ERd (Elotuzumab, Lenalidomide, Dexamethasone), IsaKd (Isatuximab, Carfilzomib, Dexamethasone), IsaPd (Isatuximab, Pomalidomide, Dexamethasone), EloPd (Elotuzumab, Pomalidomide, Dexamethasone), SelVd (Selinexor, Bortezomib, Dexamethasone), and PVd (Pomalidomide, Bortezomib, Dexamethasone) should also be noted are currently used options (Moreau, Kumar, et al., 2021).
- New Agents: Pomalidomide, carfilzomib, panobinostat, and venetoclax are among the newer drugs showing promise (Gao et al., 2023; Richardson et al., 2019).

Ongoing Research and Future Directions: Current research in multiple myeloma encompasses several key areas. First, there is ongoing development of new therapeutic agents such as bispecific antibodies and CAR-T cell therapies(Mikkilineni & Kochenderfer, 2021; Sheykhhasan et al., 2024). Notably, approved therapies like Isatuximab and Teclistamab are already making significant impacts, with others like ide-cel already approved and talquetamab, elranatamab, and cilta-cel anticipated for approval soon(Keam, 2023; Lesokhin et al., 2023; San-Miguel et al., 2023). Second, efforts are focused on refining combination regimens to optimize efficacy and minimize side effects. Lastly, there is a push towards advancing personalized medicine in multiple myeloma, tailoring treatments based on individual genetic profiles and risk assessments(Soekojo & Chng, 2022).

Reason of the study

Using lenalidomide (len) as maintenance therapy after autologous stem cell transplantation (ASCT) or as continuous treatment alongside other agents has become the standard practice for newly diagnosed multiple myeloma (MM) patients (Benboubker et al., 2014; M. A. Dimopoulos et al., 2016; McCarthy et al., 2017; Moreau et al., 2016). However, this widespread use of lenalidomide has led to an increasing number of patients becoming resistant (refractory) to lenalidomide at their first relapse. Managing these len-refractory patients is challenging and requires careful consideration of the available treatment options.

The choice of second-line therapy for patients who relapse while on lenalidomide treatment is still debated (Moreau, Kumar, et al., 2021). Lenalidomide-based treatments, which are usually the most effective at first relapse, are not suitable for len-refractory patients. Consequently, guidelines have recommend switching to treatments based on proteasome inhibitors, like daratumumab (Dara), bortezomib (V), and dexamethasone (D) (DaraVD), which have a different mechanism of action.

DaraVD has been approved for relapsed or refractory multiple myeloma (RRMM) after at least one prior line of therapy. However, there is still limited data on its effectiveness specifically in patients who are refractory to up-front lenalidomide treatment. The CASTOR study , which evaluated DaraVD, included patients who had undergone at least one previous line of therapy, regardless of their lenalidomide exposure(Sonneveld et al., 2023). This study showed promising results, however, it did not specify the outcomes for lenalidomide-refractory patients who had undergone only one previous treatment.

In Italy, according to AIFA (Agenzia Italiana del Farmaco) approval, the currently usable combinations for these patients are DaraVD, KD (carfilzomib and dexamethasone), IsaKd, DaraPD, and PVD (pomalidomide, bortezomib, and dexamethasone) (AGENZIA ITALIANA DEL FARMACO 2023). Among these options, DaraVD can be a good choice, but the specific effectiveness for len-refractory patients at first relapse remains to be fully understood.

Objectives

The aim of this retrospective observational study is to assess the outcomes on the safety and efficacy of the DaraVD combination outside of clinical trials in patients who have relapsed after a first line of therapy and are refractory to lenalidomide.

Primary Objectives:

- Evaluate progression-free survival (PFS) and its risk factors.

Secondary Objectives:

- Evaluate hematologic response rate, including minimal response (MR), partial response (PR), very good partial response (VGPR), complete response (CR), and stringent complete response (sCR), assessed according to the criteria provided by the IMWG.
- Evaluate Overall Survival (OS) and its risk factors
- Evaluate the safety and tolerability of DaraVD

Study design

This retrospective study is non-interventional and observational, drawing data from the medical records of patients diagnosed with multiple myeloma who were refractory to lenalidomide at first line and subsequently treated with DaraVD. The gathered data are compared with existing literature concerning patients experiencing first relapse and refractoriness to lenalidomide, focusing on treatment outcomes with DaraVD and alternative regimens excluding lenalidomide (particularly, but not exclusively, CASTOR, CANDOR, ENDEAVOR, OPTIMISMM, APOLLO and IKEMA studies). Solely existing medical records from patients treated within real-world clinical settings were utilized, with no alteration to ongoing patient therapies.

The study uses data from 9 different centers across Italy, where patients with multiple myeloma refractory to lenalidomide received DaraVD upon first relapse. As a retrospective inquiry, treatment decisions were made irrespective of this study and in accordance with normal clinical practice. Participation in the study did not influence the course of patient care, as treatment decisions were predetermined by attending physicians. Data acquisition was limited to existing medical records from patients treated within real-world clinical contexts, ensuring continuity of ongoing therapies without modification.

Literature Review

General Principles and Current First-Line treatment Regime

Over the past several decades, the treatment landscape for multiple myeloma (MM) has undergone remarkable transformation, leading to significant improvements in patient outcomes and survival rates(Thorsteinsdottir et al., 2018). Historically considered an incurable disease, advancements in MM therapy have now enabled many patients to achieve prolonged remission and improved quality of life. The development and integration of novel agents, particularly immunomodulatory drugs and proteasome inhibitors have been pivotal in this progress(Rajkumar, 2019).

Every line of treatment for myeloma typically involves several stages, which are fundamentally *induction, consolidation and Maintenance*: First, induction therapy aims to reduce the number of myeloma cells in the body, often using a combination of drugs such as proteasome inhibitors, immunomodulators, and steroids. This is followed by consolidation therapy to further decrease the number of cancer cells and achieve deeper responses. Consolidation may involve high-dose chemotherapy with stem cell transplantation (ASCT) for eligible patients. Maintenance therapy then helps to keep the disease under control, usually with lower doses of the drugs used in induction therapy(M. A. Dimopoulos, Moreau, et al., 2021; Moreau et al., 2017).

Throughout treatment, doctors monitor the patient's response and adjust the therapy as needed, and if the patient relapses to the therapy (reappearance of the cancer), the procedure is usually to stop current drug regimen and a star another line of therapy with a new drug combination.

Currently, many classes of drugs are used (see table 1), including new options like proteasome inhibitors, immunomodulatory drugs, monoclonal antibodies, antibody-drug conjugates, bispecific T-cell engagers, CAR-T cell therapy, peptide-drug conjugates, selective inhibitors of nuclear export, and small-molecule targeted therapies (Rajkumar, 2019).

Table 1- Classes of drugs currently used in Multiple Myeloma Treatment[2]

Name of the Agent	Common Drugs	Brief Description
Alkylating agents	• Melphalan • Cyclophosphamide • Melflufen	Drugs that interfere with the DNA of cancer cells, preventing their replication.
Steroids	• Dexamethasone • Prednisone	Drugs that reduce inflammation and suppress the immune system.
Proteasome inhibitors	• Bortezomib • Carfilzomib • Ixazomib	Drugs that block the action of proteasomes, leading to the buildup of proteins and cell death in cancer cells.
Immunomodulators	• Thalidomide • Lenalidomide • Pomalidomide	Drugs that modify the immune response, often used to enhance the body's ability to fight cancer.
Histone deacetylase inhibitors	• Panobinostat	Drugs that modify gene expression by affecting histone acetylation, potentially leading to cancer cell death.
Monoclonal antibodies	• Daratumumab • Isatuximab • Elotuzumab	Laboratory-produced antibodies that target specific proteins on cancer cells, triggering their destruction by the immune system.
Chimeric antigen receptor T cells (CAR-T)	• Ide-cel • Ciltacel	Immunotherapy that involves genetically engineering a patient's T cells to express chimeric antigen receptors, enabling them to recognize and kill cancer cells expressing specific antigens.
Bispecific antibodies	• Teclistamab • Talquetamab • Elranatamab	Antibodies designed to bind to two different types of antigens simultaneously, enhancing targeting of cancer cells.

[2] Author's analysis based on (M. A. Dimopoulos, Moreau, et al., 2021; Paul et al., 2019; Rajkumar, 2011, 2019; Soekojo & Chng, 2022)

For the **induction stage** of treating of MM, prior Autologous Stem Cell Transplant for eligible patients, drugs like proteasome inhibitors (PIs) and immunomodulatory drugs (IMiDs) have been key over the past decade. Combining a PI with an IMiD, like lenalidomide, and steroids has been a common induction therapy, with bortezomib-lenalidomide-dexamethasone (VRd) being standard in many places. Daratumumab, an anti-CD38 antibody, is also becoming a primary treatment for MM, its use has expanded for both newly-diagnosed and relapsed/refractory MM, and for patients eligible or ineligible for transplant(Bazarbachi et al., 2022; Paul et al., 2019).

After the first stage, Autologous Stem Cell Transplant (ASCT), that is usually considered for patients up to 70 years old (and sometimes older, depending on their overall health), is the standard treatment in the **consolidation** therapy for eligible multiple myeloma (MM) patients(M. A. Dimopoulos, Moreau, et al., 2021; Moreau et al., 2017), and it determines following drug regimes. This procedure involves administering high-dose chemotherapy followed by the patient's own stem cells to restore bone marrow function. A second ASCT can be performed if the patient relapses. When MM is initially diagnosed, determining a patient's eligibility for a clinical trial or for high-dose therapy and ASCT is a priority. Upfront transplants should be offered to all eligible patients, although a delayed initial stem cell transplant might be considered for certain individuals.

For transplant-eligible patients, proteasome inhibitors (PIs) and immunomodulatory drugs (IMiDs) have been crucial in multiple myeloma (MM) treatment over the past decade. Although the VRd regimen (bortezomib-lenalidomide-dexamethasone) is a standard of care in many places (Rajkumar, 2011), it is not approved in Italy. In Italy, the new standard is Dara-VTd (daratumumab, bortezomib, thalidomide, and dexamethasone), following the publication of the CASSIOPEIA protocol. (Moreau et al., 2024; Rajkumar, 2011, 2019).

In the case of transplant-ineligible patients, the FIRST (Facon et al., 2018) study compared two drug regimens (MPT and lenalidomide-dexamethasone) in patients who couldn’t have stem cell transplants. Lenalidomide-dexamethasone significantly improved how long patients lived without the disease worsening and their overall survival. The SWOG S0777 study added bortezomib to lenalidomide-dexamethasone, which further improved patient outcomes (Benboubker et al., 2014; Durie et al., 2020). In Italy, the recommended therapies for transplant-ineligible patients are Dara-Rd (daratumumab, lenalidomide, and dexamethasone) as per the MAIA protocol(Facon et al.,

2021), Dara-VMP (daratumumab, bortezomib, melphalan, and prednisone) as per the ALCYONE protocol(Mateos et al., 2018), or VRd (bortezomib, lenalidomide, and dexamethasone).

After the consolidation procedures, for both transplant-eligible and transplant-ineligible patients, **maintenance therapy** is the following standard of care. which goal is to prolong the duration of remission, delay disease progression, and improve overall survival. Maintenance therapy is particularly important after autologous stem cell transplantation (ASCT), but it can also be used in patients who are not candidates for transplant. Lenalidomide is also one of the most commonly used drugs for maintenance therapy in multiple myeloma, it has been shown to improve progression-free and overall survival. Bortezomib is considered for high-risk patients as part of maintenance therapy (McCarthy et al., 2017).

Taking into account all stages of therapy for MM patients, it can be noticed that lenalidomide (Len), a potent immunomodulatory agent, stands out as a cornerstone in the first-line treatment of multiple myeloma (MM). Its efficacy, particularly in combination with dexamethasone (Len-Dex), is well-documented (Benboubker et al., 2014; M. A. Dimopoulos et al., 2016; McCarthy et al., 2017), significantly improving survival in both transplant-eligible and transplant-ineligible patients. Len's effectiveness is due to its multifaceted mechanism of action, which includes direct antitumor effects, modulation of the tumor microenvironment, and enhancement of the immune system. Clinical trials, such as the FIRST trial, have demonstrated the superiority of lenalidomide-based regimens over traditional therapies like melphalan and prednisone, solidifying its role in frontline therapy(Facon et al., 2018).

Effectiveness assessment

During all the line of treatment the doctors measure, several times, the response of the therapy, typically using standardized like the International Uniform Response Criteria for Multiple Myeloma (Durie et al., 2006)(See table 2). The classification is assessed through a combination of laboratory tests (such as serum protein electrophoresis, immunofixation, serum free light chain assay), imaging studies (such as skeletal survey, MRI, PET-CT), and clinical evaluations (including assessment of symptoms and bone marrow biopsy) (M. A. Dimopoulos, Moreau, et al., 2021; Moreau et al., 2017).

One of the key indicators of the effectiveness of treatment is the so called “best response”, which is the most favorable or optimal response achieved by a patient during a treatment regimen.

Table 2- Response classifications based on the International Uniform Response Criteria for Multiple Myeloma[3]

Response Category	Description
No Response (NR)	Lack of any detectable response to treatment.
Progressive Disease (PD)	Worsening of disease status despite treatment, characterized by an increase in tumor burden or the appearance of new lesions.
Stable Disease (SD)	Disease status remains unchanged with no significant reduction or increase in tumor burden.
Minimal Response (MR)	A modest reduction in tumor burden that falls short of meeting the criteria for PR.
Partial Response (PR)	Reduction in tumor burden by at least 50%, as measured by laboratory tests, imaging studies, and clinical evaluations.
Very Good Partial Response (VGPR)	More substantial reduction in tumor burden compared to PR, typically at least 90% reduction in serum M-protein levels or bone marrow plasma cells.
Complete Response (CR)	Complete disappearance of all signs and symptoms of myeloma, including normal levels of serum and urine M-protein, absence of bone marrow plasma cells, and resolution of related symptoms.
Stringent Complete Response (sCR)	A more stringent form of CR, requiring additional criteria such as normal levels of serum free light chains (FLC) and absence of clonal plasma cells by immunohistochemistry or immunofluorescence.

Another two important measures used in clinical trials and oncology to assess the effectiveness of cancer treatments are **Progression-Free Survival (PFS)** and **Overall Survival (OS).**

[3] Author's Analysis based on (Durie et al., 2006)

PFS refers to the length of time during and after treatment that a patient lives with the disease without it getting worse. It measures the time from the start of treatment until the disease progresses or the patient dies from any cause, whichever comes first. OS in the other hand refers to the length of time from either the date of diagnosis or the start of treatment that patients are still alive. It measures the time from the start of treatment until death from any cause. Both metrics are crucial for understanding the impact of a treatment on cancer control and patient longevity. (Delgado & Guddati, 2021)

Treatment for Relapsed/Refractory Patients (RRMM)

The increase of treatment's arsenal for Multiple Myeloma have changed both the clinical course of the disease and Significant improved relatively survival, overall response (ORR)[4] and progression-free survival (PFS) rates for myeloma patients(Thorsteinsdottir et al., 2018). However, most patients who achieve a prolonged response following initial therapy ultimately relapse and/or become refractory to treatment (RRMM).

For refractory multiple myeloma (MM), the term refers to a disease that has not shown at least a partial response after three or more cycles of myeloma therapy or has progressed within 60 days of the last treatment. Primary refractory MM includes patients who have undergone multiple lines of therapy but have never achieved at least a partial response to any of them. Relapsed MM refers to previously treated MM that requires new salvage therapy after a partial or complete remission of at least 60 days (Sonneveld & Broijl, 2016). The definition of disease relapse follows the criteria set by the International Myeloma Working Group (IMWG) (Kyle et al., 2003).

The objective of second-line treatment is to target myeloma cells differently and overcome any resistance that may have developed during initial treatment. For example, certain drugs used in first-line treatment, such as lenalidomide, may be excluded from second-line therapy because myeloma cells can become resistant to these agents over time. This necessitates the use of alternative therapies to effectively combat the disease.

[4] Overall response (ORR) is calculated by adding the percentages of patients who achieve partial response (PR) or better.

The choice of treatment program for relapse should consider several factors: the duration and quality of response to previous therapy, clinical characteristics of high-risk relapse (such as symptoms, organ damage, and elevated LDH), high-risk cytogenetic abnormalities (like chromosome 17 deletion), residual toxicity from prior therapy (including peripheral neuropathy and thrombosis), and the patient's comorbidities, among others (Sonneveld & Broijl, 2016).

In most cases of relapse after initial treatment, the primary concern is whether the patient received lenalidomide as part of their initial treatment regimen or not. This because of the extensive use of it as a maintenance therapy or in combination with dexamethasone as an induction treatment, which is increasingly leading to the encounter of first-relapsed patients who have been exposed to, or more importantly, become refractory to Len. Another increasingly significant factor to consider is if the disease is advancing despite receiving front-line therapies containing daratumumab (Moreau, Kumar, et al., 2021).

It is recommended that patients that took Lenalidomide in the first line should not use it in subsequent lines of treatment, as it is understood that they have developed resistance to the drug and it would lead to suboptimal results. Consequently, guidelines recommend switching to treatments based on proteasome inhibitors, like daratumumab (Dara), bortezomib (V), and dexamethasone (D) (DaraVD), which have a different mechanism of action (Moreau, Kumar, et al., 2021).

In contrast with the consensus on lenalidomide-based regimens for initial treatment, there is considerable variability in second-line therapies. Some of the most used options for refractory patients at the first line are, for patients not refractory to Lenalidomide:

- DRd=daratumumab plus lenalidomide plus dexamethasone.
- DVd=daratumumab plus bortezomib plus dexamethasone
- KRd=carfilzomib plus lenalidomide plus dexamethasone
- EloRd= Elotuzumab, Lenalidomide, and Dexamethasone

For Patients refractory to Lenalidomide:

- PVd=pomalidomide plus bortezomib plus dexamethasone.
- DKd=daratumumab plus carfilzomib plus dexamethasone.
- IsaKd= Isatuximab, Carfilzomib, and Dexamethasone.
- DaraPd=Daratumumab, Pomalidomide, and Dexamethasone
- DVd=daratumumab plus bortezomib plus dexamethasone

(Chim et al., 2018; Lonial et al., 2015; Moreau, Kumar, et al., 2021; Sonneveld & Broijl, 2016)

For patients not refractory to lenalidomide experiencing their first relapse, treatment choices depend on previous therapies. Lenalidomide-based combinations with dexamethasone are preferred. Options include carfilzomib, daratumumab, ixazomib, or elotuzumab with lenalidomide and dexamethasone, all showing better outcomes than lenalidomide plus dexamethasone (Stewart et al., 2015). Daratumumab plus lenalidomide plus dexamethasone offers one of the most effective option, significantly prolonging progression-free survival (M. A. Dimopoulos et al., 2016). Patients previously treated with a proteasome inhibitor may benefit from retreatment with a proteasome inhibitor-based regimen, as shown in trials like ENDEAVOR and CASTOR (M. A. Dimopoulos et al., 2017; Sonneveld et al., 2023). These findings suggest various effective treatments for first relapse, providing both efficacy and potential survival advantages.

In the case of patients who are refractory to lenalidomide, the current standard for treating relapsed or refractory multiple myeloma is still debated. Several second-line therapies have been explored to address this unmet need. Combinations like Daratumumab-Bortezomib-Dexamethasone (DaraVD or DVd), Pomalidomide, Bortezomib, and Dexamethasone (PVd), and Carfilzomib-Dexamethasone (KD) have shown promise, demonstrating substantial activity in relapsed and refractory MM. Additionally, combinations such as Isatuximab-Carfilzomib-Dexamethasone (IsaKd) as per the IKEMA study(Moreau, Dimopoulos, et al., 2021), and Daratumumab-Pomalidomide-Dexamethasone (DaraPd) as per the APOLLO study (M. A. Dimopoulos, Terpos, et al., 2021) are also available and have shown significant efficacy. Daratumumab, a monoclonal antibody targeting CD38, has been particularly effective when combined with proteasome inhibitors and immunomodulatory drugs, offering a novel mechanism of action that complements

existing therapies. Carfilzomib, a second-generation proteasome inhibitor, has also exhibited high efficacy, especially in patients with high-risk cytogenetics (Nooka et al., 2019).

Several phase 3 trials have evaluated proteasome inhibitor-based combinations, primarily comparing them with bortezomib plus dexamethasone. For instance, in the ENDEAVOR trial, carfilzomib plus dexamethasone demonstrated superior progression-free survival and overall survival compared to bortezomib plus dexamethasone, though patients with lenalidomide-refractory disease showed relatively short progression-free survival. Similarly, in the CASTOR trial, the addition of daratumumab to bortezomib plus dexamethasone showed significantly longer progression-free survival, especially in patients with lenalidomide-refractory disease (Sonneveld et al., 2023).

Other promising combinations include pomalidomide plus bortezomib plus dexamethasone, which in the OPTIMISMM study has shown improved progression-free survival compared to bortezomib plus dexamethasone alone, particularly in patients with lenalidomide-refractory disease. Also, the addition of daratumumab to pomalidomide and dexamethasone (DaraPd), and the combination of isatuximab with carfilzomib and dexamethasone (IsaKd), explored in the APOLLO and IKEMA trials respectively, showed promising results in enhancing progression-free survival (M. A. Dimopoulos, Terpos, et al., 2021; Moreau, Dimopoulos, et al., 2021; Richardson et al., 2019).

Main clinical Trial Studies on Second Line

The exploration of effective second-line therapies in MM has led to the conduction of several pivotal clinical trials, including CASTOR, CANDOR, ENDEAVOR, OPTIMISMM, APOLLO and IKEMA, each contributing valuable insights into the management of relapsed or refractory multiple myeloma (RRMM).

CASTOR: The CASTOR trial evaluated the efficacy of adding daratumumab to the standard regimen of bortezomib and dexamethasone (D-Vd) in patients with RRMM. It selected patients with ≥ 1 line of prior therapy that were randomly assigned to Vd (up to eight cycles) with or without daratumumab (until disease progression). At a median follow-up of 72.6 months, the addition of daratumumab significantly prolonged overall survival (OS) compared to bortezomib and dexamethasone alone. The hazard ratio (HR) for OS was 0.74, indicating a 26% reduction in

the risk of death with D-Vd. Median OS was 49.6 months with D-Vd versus 38.5 months with Vd. The study highlighted the greatest OS benefit in patients with one prior line of therapy and demonstrated improved outcomes across various subgroups, including older patients, those with high-risk cytogenetic abnormalities, and those previously treated with bortezomib. In this study 24% of the patients were refractory to lenalidomide, regardless of previous lines of therapy. (Sonneveld et al., 2023).

CANDOR: The CANDOR trial compared the combination (KdD) against (Kd) alone in patients with RRMM. Patients aged 18 and older with relapsed or refractory multiple myeloma and at least a partial response to one to three prior therapies were recruited globally and randomly assigned to receive either KdD (carfilzomib, daratumumab, and dexamethasone) or Kd (carfilzomib and dexamethasone). The study found that KdD significantly prolonged progression-free survival (PFS) compared to Kd. After a median follow-up of approximately 17 months, median PFS was not reached in the KdD group versus 15.8 months in the Kd group, with an HR of 0.63. The trial also showed a favorable benefit-risk profile for KdD, with a similar frequency of adverse events leading to treatment discontinuation between both groups. In this study 21% of the patients were refractory to lenalidomide, regardless of previous lines of therapy (M. Dimopoulos et al., 2020).

OPTIMISMM: The OPTIMISMM trial focused on patients with RRMM who were previously treated with lenalidomide with 71% of them being refractory to Len. This study assessed the efficacy of pomalidomide, bortezomib, and dexamethasone (PVd) compared to bortezomib and dexamethasone (Vd) alone. With a median follow-up of 15.9 months, the results indicated that PVd significantly improved PFS compared to Vd, with median PFS of 11.20 months versus 7.10 months, respectively (HR 0.61). The study supports PVd as a standard treatment option for patients with RRMM who have previously received lenalidomide (Richardson et al., 2019).

ENDEAVOR: The ENDEAVOR trial was a head-to-head comparison of carfilzomib and dexamethasone (Kd) versus bortezomib and dexamethasone (Vd) in patients with RRMM. Median follow-up was 37.5 months. The interim analysis showed that Kd provided a significant improvement in OS compared to Vd, with median OS of 47.6 months versus 40.0 months (HR 0.791). In this study 21% of the patients were previously exposed to lenalidomide, regardless of previous lines of therapy (M. A. Dimopoulos et al., 2017).

APOLLO: The APOLLO trial, an open-label, randomized, aimed to assess the efficacy of adding daratumumab to pomalidomide and dexamethasone compared to pomalidomide and dexamethasone alone in patients with relapsed or refractory multiple myeloma. A total of 304 patients, who had previously received treatment with lenalidomide and a proteasome inhibitor, were enrolled. Eligible patients had at least one previous line of therapy, including lenalidomide and a proteasome inhibitor and were refractory to lenalidomide if only one previous line of therapy was received. The primary endpoint, progression-free survival, significantly favored the daratumumab combination group, with a median of 12.4 months compared to 6.9 months in the control group, at a median follow-up of 16.9 months. These findings suggest that daratumumab plus pomalidomide and dexamethasone represents a substantial advancement in the treatment landscape for relapsed or refractory multiple myeloma, offering improved outcomes and potentially becoming a new standard of care in this patient population(M. A. Dimopoulos, Terpos, et al., 2021).

IKEMA: The IKEMA trial, a multicentre, open-label, randomised phase 3 study, evaluated the efficacy of adding isatuximab to carfilzomib-dexamethasone compared to carfilzomib-dexamethasone alone in patients with relapsed multiple myeloma. Conducted across 69 study centres in 16 countries, the trial enrolled 302 patients who had received one to three previous lines of therapy. The primary endpoint, progression-free survival, showed significant improvement with median not reached in the isatuximab group compared to 19.15 months in the control group (hazard ratio 0.53, one-sided p=0.0007). Adverse events were common but manageable, with higher rates of severe events in the isatuximab group. This study establishes isatuximab plus carfilzomib-dexamethasone as a new standard of care for relapsed multiple myeloma, offering improved outcomes and depth of response(Moreau, Dimopoulos, et al., 2021)**.**

Table 3- Results of main 3 Phase Clinical Trials related to Second Line therapy in Multiple Myeloma[5]

Name of the Study	Combination of Drug Evaluated	Median Follow-Up	Median OS (Overall Survival)	Median PFS (Progression-Free Survival)
CASTOR	D-Vd vs Vd	72.6 months	49.6 months (D-Vd) vs 38.5 months (Vd); HR 0.74	37.7 (D-Vd) vs 19.9 months (Vd); HR 0.63
CANDOR	KdD vs Kd	17 months	Not Reached both	Not reached (KdD) vs 15.8 months (Kd); HR 0.63
OPTIMISMM	PVd vs Vd	15.9 months	40 months (Pvd) vs 30 months (Vd)	11.20 months (PVd) vs 7.10 months (Vd); HR 0.61
ENDEAVOR	Kd vs Vd	37.5 months	47.6 months (Kd) vs 40.0 months (Vd); HR 0.791	-
APOLLO	DaraPd vs Pd	16.9 Month	-	12.4 months (DaraPd) vs 6.9 months (Pd); HR 0·63
IKEMA	IsaKd vs Kd	44 months	-	Not reached (IsaKd) vs 19.2 (Kd); HR 0.53

These findings set the stage for discussing the main clinical trials on second-line therapies. These studies have provided crucial insights into the efficacy of various treatment combinations in relapsed and refractory multiple myeloma. However, they were not primarily focused on lenalidomide-refractory patients. Excluding OPTIMISMM and APOLLO, they included limited numbers of lenalidomide-refractory patients, thereby restricting the generalizability of their

[5] Author's Analysis based on (M. Dimopoulos et al., 2020; M. A. Dimopoulos et al., 2017; M. A. Dimopoulos, Terpos, et al., 2021; Moreau, Dimopoulos, et al., 2021; Richardson et al., 2019; Sonneveld et al., 2023)

findings to this subgroup. The heterogeneity of multiple myeloma and the diverse patient population further complicate the establishment of a standardized second-line treatment approach.

DaraVD represents one of the main therapeutic options currently available for patients at the first relapse refractory to lenalidomide, but this combination regimen was studied within the CASTOR trial, which was not specifically designed to analyze this subgroup of patients. In contrast, the OPTIMISMM study for PVD and the APOLLO study with DaraPd represents the only phase III trials in the early relapse stages specifically designed to evaluate the population of patients exposed to lenalidomide.

Methods and Statistical Analysis

This section provides a detailed account of the design, patient selection criteria, data collection procedures, and statistical methods utilized in this retrospective observational study assessing the outcomes of the DaraVD combination in multiple myeloma patients refractory to lenalidomide.

This study is a retrospective, non-interventional, observational analysis conducted across nine centers in Italy with a total of 85 patients. The primary aim is to evaluate the safety and efficacy of the DaraVD combination in patients with multiple myeloma who have become refractory to lenalidomide following their first line of therapy. The data were sourced from existing medical records, and the study did not influence patient care or treatment decisions, ensuring that the therapies administered were consistent with normal clinical practice.

Patient Selection and Data Collection

Patients who met the following criteria were enrolled in this study:

- Age 18 years or older.
- Diagnosis of symptomatic multiple myeloma (according to CRAB criteria and myeloma-defining events) (Moreau et al., 2017) that relapsed after one line of therapy including lenalidomide and treated in the second line with daratumumab combined with bortezomib and dexamethasone for at least two cycles (or for just one cycle in the subgroup of patients analyzed in this thesis).
- Patients refractory to lenalidomide, defined as disease progression during treatment or within 60 days of stopping the therapy.

Exclusion Criteria

The following patients were excluded from the study:

- Patients with AL amyloidosis, any monoclonal gammopathy of clinical significance (MGCS), including monoclonal gammopathy of renal significance (MGRS), or plasma cell leukemia.

Data Collection and variables

Data were collected using a case report form (CRF) in accordance with the guidelines for data collection in clinical drug studies, Guidelines of July 24, 2008) (GPDP 2008). For the purposes of the study, no assessments outside of normal clinical practice were required, so if a parameter requested by the CRF was not collected, the field was left blank.

The main parameters investigated and evaluated were as follows:

Patient Demographics and Disease Characteristics

- Date of birth
- Gender.
- Date of multiple myeloma diagnosis.
- Type of multiple myeloma.
- Biochemical, hematologic, and cytogenetic evaluations at diagnosis and before treatment with DaraVD: Isotype, light chain type, beta-2 microglobulin, LDH, creatinine, creatinine clearance, serum monoclonal component, urine protein, urinary monoclonal component, % of bone marrow plasma cells, FISH (fluorescence in situ hybridization) diagnosis, hemoglobin, platelets, calcium, CRP (C-reactive protein), among others.
- Characteristics of the myeloma (risk factors, stage) at diagnosis and before treatment with DaraVD (if available): ISS, R-ISS.
- ECOG performance status[6] at diagnosis and before treatment (if available).
- Combination of drugs received as the first line of treatment and at each relapse after therapy with DaraVD.

Treatments Outside of DaraVD

- Start date.
- Combination of drugs administered.
- Any performance of ASCT.

[6] ECOG performance status, developed by the Eastern Cooperative Oncology Group, is a scale used by healthcare providers to assess a patient's level of functioning and ability to perform daily activities. It ranges from 0 to 5, with higher numbers indicating greater disability (Mischel & Rosielle, 2022)

- End date of treatment.

Treatment with DaraVD

- Start date.
- Planned dosage and treatment schedule (drug dosages, number of cycles).
- Administration schedule.
- Treatment response evaluation.
- Number of dose reductions performed.
- Biochemical, hematologic, and cytogenetic evaluations after treatment with DaraVD: Isotype, light chain type, beta-2 microglobulin, LDH, creatinine, creatinine clearance, serum monoclonal component, urine protein, urinary monoclonal component, % of bone marrow plasma cells, FISH (fluorescence in situ hybridization) diagnosis, hemoglobin, platelets, calcium, CRP (C-reactive protein), among others.
- Toxicity and grade.
- End date of treatment.
- Reasons for treatment discontinuation (if applicable).
- Best Response to Each Line of Treatment
- Date of Last Follow-Up
- Disease status, survival status, possible cause of death, and corresponding date (if applicable).

There were several missing values in the original dataset, particularly for the FISH diagnostic variables, where more than 60% of the observations were missing for some variables. However, demographic and date information were complete.

Statistical Methods

Descriptive analysis

The initial phase of the project involved a descriptive and exploratory analysis to examine the distribution and key statistics of each variable within the analyzed population.

Several Biochemical, hematologic *and clinical* variables were dichotomized by using the reference values established by the International Myeloma Working Group as cutoffs(Kyle et al., 2003). This approach was taken to simplify the analysis and interpretation of the data. For instance, levels of beta-2 microglobulin, LDH, creatinine, and calcium, among others, were categorized into binary groups, distinguishing between normal and abnormal values according to the specified guidelines. By converting these continuous variables into dichotomous ones, we aimed to enhance the clarity of the comparisons and highlight clinically significant differences in the patient population.

Next, the dataset was divided into categorical and numerical variables. For instance:

- **Numerical variables**: age, LDH level, CRP, MRI lesions, pre-ASCT cycles, Dara cycles, time of exposure to lenalidomide, etc.
- **Categorical variables**: best response value, patients older than 70, type of light chain, ISS and R-ISS stages, hemoglobin greater than 10.5 g/dL, creatinine greater than 1.2 mg/dL, LDH greater than 248 U/L, etc.

Following this categorization, basic statistics (mean, median, standard deviation, minimum, maximum) were calculated for the continuous variables, while frequency distributions were computed for the categorical variables.

Correlational analysis

To further explore and assess the relationship between demographic/clinical characteristics and the best response to DaraVD, a correlational analysis was conducted using correlation coefficients. Initially, a general analysis was performed, comparing every variable against each other (all-against-all) to identify the most significant associations based on the p-value.

Following this, three dichotomous variables based on the best response to the DaraVD treatment were selected for individual comparison with demographic and clinical characteristics. These were: 1) best response ≥ CR (complete response), 2) best response ≥ VGPR (very good partial response), and 3) best response ≤ SD (stable disease).

The correlation coefficients/tests used were:

- *Kruskal-Wallis Test* for categorical vs numerical variables: This non-parametric test compares the medians of three or more groups to determine if they come from the same distribution(Kruskal & Wallis, 1952).
    - **Formula**:

$$H = \frac{12}{N(N+1)} \sum_{i=1}^{k} \frac{R_i^2}{n_i} - 3(N+1)$$

1

- Where H is the Kruskal-Walli's statistic, N is the total number of observations, R_i is the sum of ranks for the i-th group, and n_i is the number of observations in the i-th group.

- *Fisher's Exact Test* for categorical vs categorical variables: This test is used to determine if there are nonrandom associations between two categorical variables, especially in small sample sizes. It is calculated based on the hypergeometric distribution of the observed values in a contingency table, testing the null of independence of rows and columns.(Lury & Fisher, 1972) (Agresti, 1990)

- *Spearman's Rank Correlation Coefficient* for numerical vs numerical variables: This non-parametric measure assesses how well the relationship between two numerical variables can be described using a monotonic function. (Spearman, 1904)
  - **Formula**:

$$\rho = 1 - \frac{6 \sum d_i^2}{n(n^2 - 1)}$$

2

- Where ρ is the Spearman's rank correlation coefficient, $d_i$is the difference between the ranks of the corresponding variables, and n is the number of observations.

Univariate Survival analysis

The survival analysis was conducted on two survival outcomes. Primary objective: Progression-Free Survival (PFS) and secondary objective: Overall Survival (OS).

- **Progression-Free Survival (PFS):** The event for PFS was either progression or death of the patients. The start time was the beginning of the DaraVD therapy.
- **Overall Survival (OS):** The event for OS was the death of the patient. The start time was the beginning of the DaraVD therapy.

The univariate analyses focused on:

1. *Kaplan-Meier Estimation*: The Kaplan-Meier method was used to estimate the survival function. The Kaplan-Meier estimator is given by:

$$\hat{S}(t) = \prod_{t_i \leq t} \left(1 - \frac{d_i}{n_i}\right)$$

3

where:

- t_i is the time of the i-th event,
- d_i is the number of events (e.g., deaths) at time t_i
- n_i is the number of individuals at risk just before time t_i.

The Kaplan-Meier survival function $\hat{S}(t)$ is defined as the probability that an individual will survive from the time origin (e.g., start of treatment) to a specified future time t. This method provides a way to visualize the survival experience of a cohort over time. Individuals who are censored [7]are considered at risk up to the point of censoring. Their data contribute to the denominator (n_i) but not the numerator (d_i) after the time of censoring.(D'Arrigo et al., 2021; Kaplan & Meier, 1958)

2. *Univariate Cox Proportional Hazards Model***:** The Cox model was used to evaluate the individual significance of each variable to PFS and OS. The Cox proportional hazards model is defined as:

$$h(t|X) = h_0(t)\exp(\beta X)$$

4

where:

- $h(t|X)$ is the hazard function at time t for a given covariate vector X ,
- $h_0(t)$ is the baseline hazard function,
- β is the vector of coefficients.

This is a semi-parametric model used to assess the effect of one or more variables on the time to a specified event. In the univariate Cox model, it is examined the effect of a single variable at a time on the hazard, which represents the instantaneous rate at which events occur, given that the individual has survived up to time t.(Abd Elhafeez et al., 2021)

[7] Censored observations are individuals for whom the event has not occurred by the end of the study or who are lost to follow-up.

X represents the variable being analyzed (e.g., age, LDH level). The coefficient β quantifies the impact of X on the Hazard. The model also provides a hazard ratio (HR)8, which is the exponentiated value of the coefficient: HR = exp(β).

Multivariate Analysis

A multivariate analysis was performed to assess the simultaneous impact of multiple variables on Progression-Free Survival (PFS) and Overall Survival (OS).

The variables for the initial model were selected based on several criteria. Firstly, each variable included in the final model should have at least 50 observations to ensure minimal statistical robustness. Secondly, variables with a p-value ≤ 0.2 from the univariate analysis were considered to capture potential associations with survival outcomes. Lastly, essential demographic variables such as age and sex were included to control for potential confounding effects.

After selecting the initial variables, a backward selection process[9] was performed to iteratively refine the model. This stepwise approach, using the Akaike Information Criterion (AIC) as the criterion, removes variables that do not contribute significantly to the model's explanatory power, ensuring a parsimonious final model that retains only statistically significant predictors.

Model Specification: The Cox proportional hazards model was utilized to estimate the survival probabilities based on the selected variables(Hosmer et al., 2011; Ibrahim et al., 2010). The formula for the Cox proportional hazards mode can be represented as:

$$h(t|X) = h\_0(t) \exp(\beta_1 X_1 + \beta_2 X_2 + \cdots + \beta_n X_n)$$

5

where:

- $h(t|X)$ represents the hazard function at time t given covariates X,
- $h\_0(t)$ is the baseline hazard function,
- $\beta_{1,2\ldots n}$ are the coefficients for each respective variable X_i.

Alternative Models: Several alternative models including Weibull, exponential, gamma, and parametric frailty models were also estimated for comparison purposes. However, based on criteria such as Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC)(Akaike,

[8] An HR greater than 1 suggests an increased risk of the event with higher values of X, while an HR less than 1 suggests a decreased risk.

[9] A backward selection process is a stepwise method used in statistical modeling to select a subset of variables from a larger set for inclusion in the final model. It starts with all candidate variables included and iteratively removes the least significant variables based on a predetermined criterion until a stopping rule is met.(Barbur et al., 1994)

1974; Kass & Raftery, 1995), the Cox proportional hazards model demonstrated the best fit to the data.

Assessment of Proportional Hazards (PH) Assumption: The PH assumption was assessed using graphical evaluation of scaled Schoenfeld residuals. Variables that violated the PH assumption were either adjusted by adding a time interaction term or excluded from the model due to their non-proportional effects over time.

Validation: The validity of the final Cox model was assessed using several key tests:

- *Log-likelihood ratio test:* Evaluates whether the addition of explanatory variables significantly improves the fit of a statistical model compared to a simpler version without those variables. It is used to assess the overall goodness-of-fit of a model by comparing the likelihoods of the full model against a nested model or baseline.(Azzalini, 2017)

- *Log-rank test:* It determines if there is a statistically significant difference between the survival curves of different groups based on observed event times.(Murphy & Kleinbaum, 1997)

- *Concordance index*[10] *(C-index):* quantifies the discriminatory power of a survival model by evaluating its ability to correctly rank order survival times for pairs of individuals.(Hosmer et al., 2011)

- *Wald test:* Assesses the statistical significance of individual predictor variables in regression models, such as the Cox proportional hazards model. It evaluates whether each predictor has a significant impact on the hazard or survival function by testing the null hypothesis that its coefficient is zero. (Hosmer et al., 2011)

These tests collectively evaluate the goodness-of-fit, discriminatory ability, and significance of the model's coefficients in predicting survival outcomes.

Limitations

- The retrospective nature of the study may introduce biases related to patient selection and data completeness.
- The observational design limits causal inferences.
- The relatively modest sample size (85 patients), coupled with missing values, significantly impacts the robustness and generalizability of the model.

[10] It ranges from 0.5 (random predictions) to 1.0 (perfect predictions), with higher values indicating better predictive performance in terms of correctly predicting the relative survival outcomes.

Results

Characteristics of patients and of their first line of treatment

In this study, 85 patients were included, and their main characteristics at diagnosis are summarized in Table 4.

The median age of the patients at onset is 65 years, with an age range of 37 to 84 years. 57.6% of the patients were diagnosed at an age less than or equal to 65 years, while 42.4% were older than 65 years. The gender distribution shows a slight predominance of females, with 56.5% female and 43.5% male patients. Regarding the ISS classification at onset, 45.9% of the patients were ISS I, 32.9% were ISS II, and 18.8% were ISS III. The R-ISS classification at onset was available for 61 patients, with 25.9% classified as R-ISS I, 40.0% as R-ISS II, and 5.9% as R-ISS III.

The isotype of the heavy chain, data was available for 69 patients: 60.0% of the patients had IgG, 17.6% had IgA, and 1.2% had IgD. The median β2-microglobulin level was 2.615 mg/L, with a range of 0.900 to 11.600 mg/L. 28.2% of the patients had β2-microglobulin levels below 3.5 mg/L, and 14.1% had levels of 3.5 mg/L or higher.

When considering the history of autologous stem cell transplant (ASCT), 55.3% of the patients underwent ASCT, while 44.7% did not. The types of induction therapy received by the patients were diverse, with RD (lenalidomide and dexamethasone) being the most common regimen, administered to 36.5% of the patients. Other induction therapies included VTD (bortezomib, thalidomide, and dexamethasone) in 32.9% of the patients, VCD (bortezomib, cyclophosphamide, and dexamethasone) in 11.8%, KRD (carfilzomib, lenalidomide, and dexamethasone) in 7.1%, KCD (carfilzomib, cyclophosphamide, and dexamethasone) in 4.7%, and smaller proportions received Durvalumumab-RD, ELO-RD, R, RCD, and VRD.

Consolidation therapy was documented for 23 patients, with VTD being the most common (8.2%), followed by KRD (5.9%), KCD (4.7%), VRD (3.5%), VD (2.4%), and VMP (2.4%). Maintenance therapy data was available for 50 patients, with the majority (56.5%) receiving R (lenalidomide), while KR and TR regimens were each used by 1.2% of the patients.

The median time patients were exposed to lenalidomide in the first line was 12.5 months, with an exposure range of 2 to 95 months. 48.2% of the patients were exposed to lenalidomide for 12 months or less, while another 48.2% were exposed for more than 12 months.

Table 4- Main descriptive statistics of First Line Therapy[11]

Characteristics	Number (%)	Median (min-max)	NA
Total Number of Patients		**85**	
Age at onset		65 (37-84)	0
≤65	49 (57.6)		
>65	36 (42.4)		
Gender			0
Male	37 (43.5)		
Female	48 (56.5)		
ISS classification at onset			2
1	39 (45.9)		
2	28 (32.9)		
3	16 (18.8)		
R-ISS classification at onset			24
1	22 (25.9)		
2	34 (40.0)		
3	5 (5.9)		
ASCT (Transplant)			0
No	38 (44.7)		
Yes	47 (55.3)		
Type of induction Therapy			0
Durvalumumab-RD	1 (1.2)		
ELO-RD	1 (1.2)		
KCD	4 (4.7)		
KRD	6 (7.1)		
R	1 (1.2)		
RCD	1 (1.2)		
RD	31 (36.5)		
VCD	10 (11.8)		
VRD	2 (2.4)		
VTD	28 (32.9)		
Type of consolidation therapy at first line			62
KCD	4 (4.7)		
KRD	5 (5.9)		
VD	2 (2.4)		
VMP	2 (2.4)		
VRD	3 (3.5)		
VTD	7 (8.2)		
Type of maintenance therapy			35
KR	1 (1.2)		

[11] Author's analysis based on current study

R	48 (56.5)		
TR	1 (1.2)		
Time exposed to Lena at first line (months)		12.5 (2-95)	3
≤12	41 (48.2)		
>12	41 (48.2)		

Characteristics of patients and of their first relapse

The main characteristics of the patients at the beginning of DaraVD treatment are summarized in Table 5.

The median age of the patients at the beginning of DaraVD therapy is 68 years, with an age range of 39 to 85 years. 36.5% of the patients were aged 65 years or younger, while 63.5% were older than 65 years. The performance status, assessed using the ECOG scale, was less than 2 in 85.9% of the patients, with 14.1% having an ECOG score of 2 or higher.

The median LDH level was 188.0 U/L, with a range of 104.0 to 1190.0 U/L. 62.4% of the patients had LDH levels within the normal range (≤248 U/L), while 30.6% had elevated LDH levels. The median creatinine level was 0.85 mg/dL, with 84.7% of the patients having creatinine levels below 1.2 mg/dL and 14.1% having elevated creatinine levels. The median creatinine clearance was 80.00 ml/min, with 90.6% of the patients having a clearance rate of 50 ml/min or higher.

The median serum monoclonal component level was 103.00 mg/dL, with 92.9% of the patients having levels below 3000 mg/dL. Urinary protein levels were measured in 71 patients, with a median of 270.0 mg/day; 88% had levels below 3500 mg/day. The median urinary monoclonal component level was 31.5 mg/day, with 52.9% of the patients having levels below 500 mg/day.

Serum free light chains were evaluated in 76 patients. The median kappa light chain level was 82.75 mg/L, the median lambda light chain level was 12.800 mg/L, and the median kappa/lambda ratio was 9.850. 80.0% of the patients had a kappa/lambda ratio within the normal range (≥0.26 and ≤1.65), while 14.1% had a ratio of 100 or higher.

Cytogenetic abnormalities were evaluated using fluorescence in situ hybridization (FISH). The presence of specific abnormalities and the number of patients tested were as follows: t(4;14) was present in 1.2% of 84 patients, t(14;16) in 1.2% of 84 patients, t(11;14) in 3.5% of 81 patients, del17 in 7.1% of 79 patients, gain1q in 11.8% of 75 patients, and amp1q in 5.9% of 80 patients. Overall, 9.4% of the patients were classified as having high cytogenetic risk.

Bone marrow plasma cells were assessed in 37 patients, with a median percentage of 30%. 32.9% of the patients had less than 60% plasma cells, while 10.6% had 60% or more. PET lesions were assessed in 31 patients, with a median of 2 lesions; 22.4% had fewer than 5 lesions, while 14.1% had 5 or more lesions. MRI lesions were assessed in 27 patients, with a median of 3 lesions; 23.5%

had fewer than 5 lesions, while 8.2% had 5 or more lesions. Extramedullary disease was not present in 81.2% of the patients, while it was detected in 5.9%.

The median hemoglobin level was 11.50 g/dL, with 25.9% of the patients having hemoglobin levels below 10.5 g/dL. The median platelet count was 160.0 x 10^3/µL, with 41.2% of the patients having a platelet count below 150 x 10^3/µL. The median calcium level was 9.120 mg/dL, with 95.3% of the patients having calcium levels below 10.5 mg/dL. The median C-reactive protein (CRP) level was 0.530 mg/dL, with 49.4% of the patients having CRP levels of 0.5 mg/dL or higher.

Regarding the duration of DaraVD therapy, data was available for 56 patients, with a median duration of 7 months. 41.2% of the patients were treated for less than 6 months, while 24.7% were treated for 6 months or more. The median number of DaraVD cycles was 9, with a range of 1 to 73 cycles.

Table 5- Descriptive statistics of patients after first relapse[12]

Characteristics	Number (%)	Median (min-max)	Missing Values
Total number of patients	85		
Age at beginning of DaraVD		68.00 (39.00-85.00)	0
≤65	31 (36.5)		
>65	54 (63.5)		
ECOG			0
<2	73 (85.9)		
≥2	12 (14.1)		
Iso Type Heavychain			16
IgA	15 (17.6)		
IgD	1 (1.2)		
IgG	51 (60.0)		
β2-microglobulin (mg/L)		2.615 (0.900-11.600)	49
<3,5	24 (28.2)		
≥3,5	12 (14.1)		
<5,5	34 (40.0)		
≥5,5	2 (2.4)		
LDH (U/L)		188.0 (104.0-1190.0)	6
≤248	53 (62.4)		
>248	26 (30.6)		
Creatinine (mg/dL)		0.85 (0.04-4.13)	1
<1,2	72 (84.7)		

[12] Author's analysis based on current study

≥1,2	12 (14.1)		
Creatinine Clearance (ml/min)		80.00 (9.00-129.00)	1
<50	7 (8.2)		
≥50	77 (90.6)		
Serum Monoclonal Component (mg/dL)		103.00 (0.00-7526.00)	4
<3000	79 (92.9)		
≥3000	2 (2.4)		
Urinary Proteins (mg/day)		270.0 (30.0-9086.0)	14
<3500	15 (88)		
≥3500	2 (12)		
Urinary Monoclonal Component (mg/day)		31.5 (0.0-6278.4)	31
<500	45 (52.9)		
≥500	9 (10.6)		
Serum Free Light Chains (mg/L)			9
Kappa		82.75 (4.00-5366.00)	
Lambda		12.800 (0.900-13420.980)	
Ratio		9.850 (0.000-2605.000)	
Ratio ≥0,26 e ≤1,65	68 (80.0)		
Ratio <0,26 o >1,65	8 (9.4)		
Ratio <100	64 (75.3)		
Ratio ≥100	12 (14.1)		
Fluorescence In Situ Hybridization (FISH)			
t(4;14) not present	18 (21.2)		66
t(4;14) present	1 (1.2)		66
t(14;16) not present	19 (22.4)		65
t(14;16) presente	1 (1.2)		
t(11;14) no present	18 (21.2)		64
t(11;14) present	3 (3.5)		64
del17 not present	23 (27.1)		56
del17 present	6 (7.1)		56
gain1q not present	15 (17.6)		60
gaing1q present	10 (11.8)		60

amp1q not present	21 (24.7)		59
amp1q present	5 (5.9)		59
Citogenetic risk			62
High	8 (9.4)		
Standard	15 (17.6)		
Bone Marrow Plasma Cells (%)		30 (0.00-100.00)	48
<60%	28 (32.9)		
≥60%	9 (10.6)		
PET Lesions		2 (0.000-10.000)	54
<5	19 (22.4)		
≥5	12 (14.1)		
RMN Lesions		3 (0.000-11.000)	58
<5	20 (23.5)		
≥5	7 (8.2)		
Extramedullary Disease (EMD)			52
Not Present	69 (81.2)		
Present	5 (5.9)		
Hemoglobin (g/dL)		11.50 (7.00-17.70)	0
<10,5	22 (25.9)		
≥10,5	63 (74.1)		
Platelets (x 10^3/µL)		160.0 (24.0-407.0)	0
<150	35 (41.2)		
≥150	50 (58.8)		
Calcium Levels (mg/dL)		9.120 (2.000-11.960)	1
<10,5	81 (95.3)		
≥10,5	3 (3.5)		
C-reactive Protein (PCR)		0.530 (0.000-72.300)	12
<0,5	31 (36.5)		
≥0,5	42 (49.4)		
DaraVD duration in months			29
<6	35 (41.2)	7 (0-43)	
≥6	21 (24.7)		
Cicles of DaraVD		9 (1-73)	1

Best Response to DaraVD

The response characteristics during DaraVD therapy are detailed in Table 6. At a median follow-up of 22 months, the overall response rate observed was 84.7%, with 60.0% of patients achieving a VGPR or better and 24.7% achieving a CR or better. Specifically, 7 patients (8.2%) achieved a sCR, 14 patients (16.5%) achieved a CR, 30 patients (35.3%) achieved a VGPR, and 21 patients (24.7%) achieved a PR. Additionally, 8 patients (9.4%) had a SD, and 2 patients (2.4%) experienced disease progression.

Table 6-Characteristics of the obtained responses[13]

Best response DaraVD	Number of patients (%)
PD	2 (2.4)
SD	8 (9.4)
PR	21 (24.7)
VGPR	30 (35.3)
CR	14 (16.5)
sCR	7 (8.2)
≥VGPR	51 (60.0)
≥CR	21 (24.7)
Overall Response rate (≥PR)	**72 (84.7)**

In Table 7, the correlational analysis stratified by best response categories to DaraVD reveals several significant associations. For patients achieving a response of ≥ CR, the number of cycles of DaraVD shows a significant correlation (P = 0.002), alongside creatinine levels (P = 0.011), urinary protein excretion (P = 0.016), serum free light chain ratios (P = 0.020), urinary monoclonal component (P = 0.022), and specific serum free light chain types (kappa: P = 0.033, lambda: P = 0.033) using the Kruskal-Wallis test. Additionally, gender (P = 0.011), ECOG performance status (P = 0.016), high cytogenetic risk (P = 0.025), and C-reactive protein levels (P = 0.029) showed significant correlations using the Fisher Exact Test. Similarly, among patients achieving ≥ VGPR, significant correlations were found for calcium levels (P = 0.009) and bone marrow plasma cell percentages (P = 0.033) using the Kruskal-Wallis test, as well as FISH analysis for del17 (P = 0.008) and hemoglobin levels (P = 0.044) using the Fisher Exact Test. These findings underscore the clinical and biochemical parameters associated with treatment response outcomes in patients undergoing DaraVD therapy.

[13] Author's analysis based on current study

Table 7-Correlational analysis by Best Response category[14]

Variables stratify by: BestResponse to DaraVD ≥ CR		
Variable	**P value**	**Type of test**
Number Of cycles DaraVD	0.002	Kruskal-Wallis test
Creatinine	0.011	Kruskal-Wallis test
Urinary Proteins (mg/day)	0.016	Kruskal-Wallis test
Serum Free Light Chains (mg/L) ratio k/l	0.02	Kruskal-Wallis test
Urinary Monoclonal Component (mg/day)	0.022	Kruskal-Wallis test
Serum Free Light Chains (mg/L) kappa	0.033	Kruskal-Wallis test
Serum Free Light Chains (mg/L) lambda	0.033	Kruskal-Wallis test
Gender	0.011	Fisher Exact Test
ECOG	0.016	Fisher Exact Test
High citogenetic risk	0.025	Fisher Exact Test
PCR	0.029	Fisher Exact Test
Variables stratify by: Bestresponse to DaraVD ≥ VGPR		
Variable	**P value**	**Type of test**
Calcium Level	0.009	Kruskal-Wallis test
Bone Marrow Plasma Cells (%)	0.033	Kruskal-Wallis test
FISH: del17	0.008	Fisher Exact Test
Hemoglobin	0.044	Fisher Exact Test

Safety

Hematological and non-hematological toxicities are summarized in Table 8. Anemia was the most frequent adverse event (AE), occurring in 56% of cases and with grade 3-4 events in 24%% of cases. Thrombocytopenia was reported in 52% of patients (any grade), with 45.9% experiencing grade 3-4. Neutropenia was observed in 8% of patients (grade 1-2) and 18.8% (grade 3-4).

[14] Author's analysis based on current study

Among non-hematological toxicities, peripheral neuropathy was the most prevalent, affecting 54.1% of patients, primarily grade 3-4 (37.6%). Gastrointestinal adverse events were reported in 24.7% of cases, predominantly grade 1-2 (23.5%). Infectious events were noted in 29% of patients, with grade 3-4 infections in 8.2% of cases. Notably, hepatic toxicity and cardiovascular events were rare, occurring in 3.6% and 1.2% of patients, respectively.

Table 8-Hematological and non-hematological toxicities of D-VD[15]

Toxicity	Number (% of the total)	
	Grade 1–2	**Grade 3–4**
Neutropenia	7 (8.2)	16 (18.8)
Anemia	28 (32.9)	20 (23.5)
Thrombocytopenia	5 (5.9)	39 (45.9)
Peripheral neuropathy	14 (16.5)	32 (37.6)
Hepatic	1 (1.2)	2 (2.4)
Gastro-intestinal	20 (23.5)	1 (1.2)
Cardio-vascular	0 (0)	1 (1.2)
Infectious events	18 (21.2)	7 (8.2)

Survival analysis of DaraVD

Survival outcomes were evaluated in the 85 patients, with the start point being the initiation of DaraVD therapy. The median follow-up duration was 22 months, during which median progression-free survival (PFS) (Figure1) was 14 months and median overall survival (OS) (Figure 2) was 47 months.

[15] Author's analysis based on current study

16

Figure 1-Progression free survival (PFS) Kaplan–Meier curves of D-VD treated patients showing survival probability and median survival time.

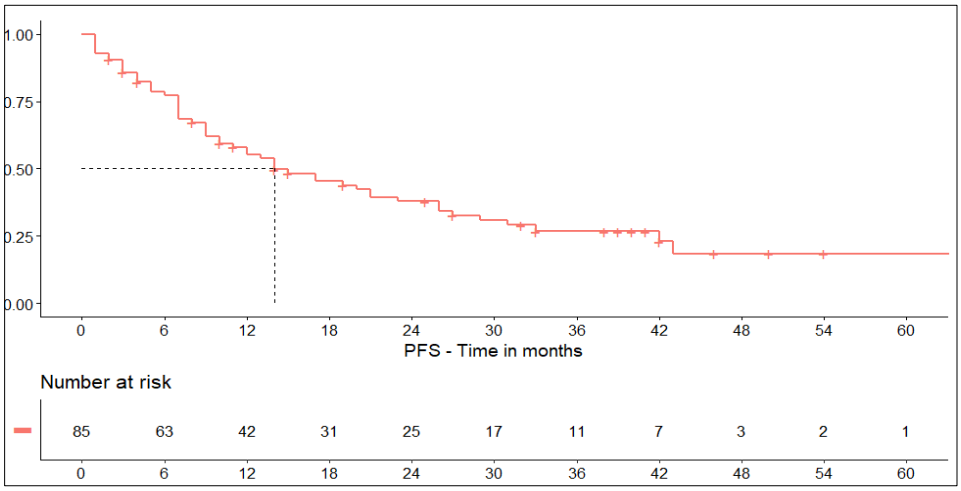
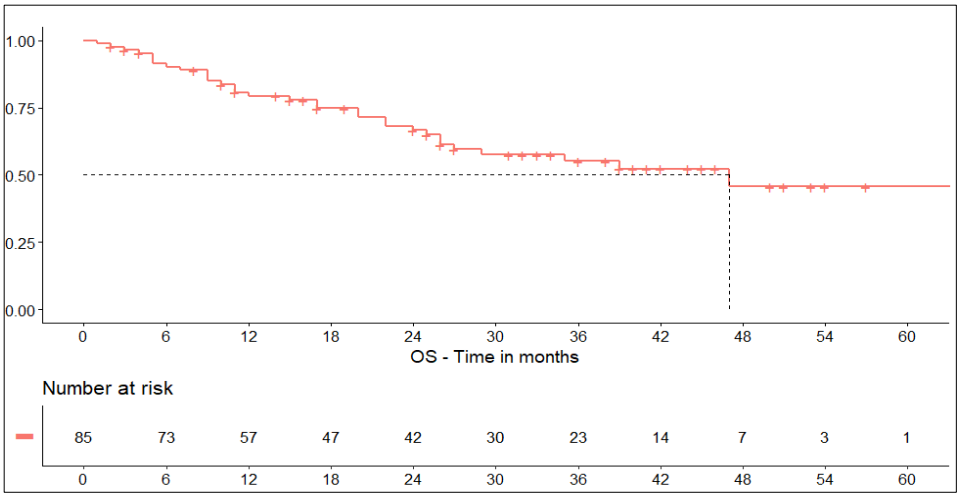


Figure 2-Overall survival (OS) Kaplan–Meier curves of D-VD treated patients showing survival probability and median survival time.



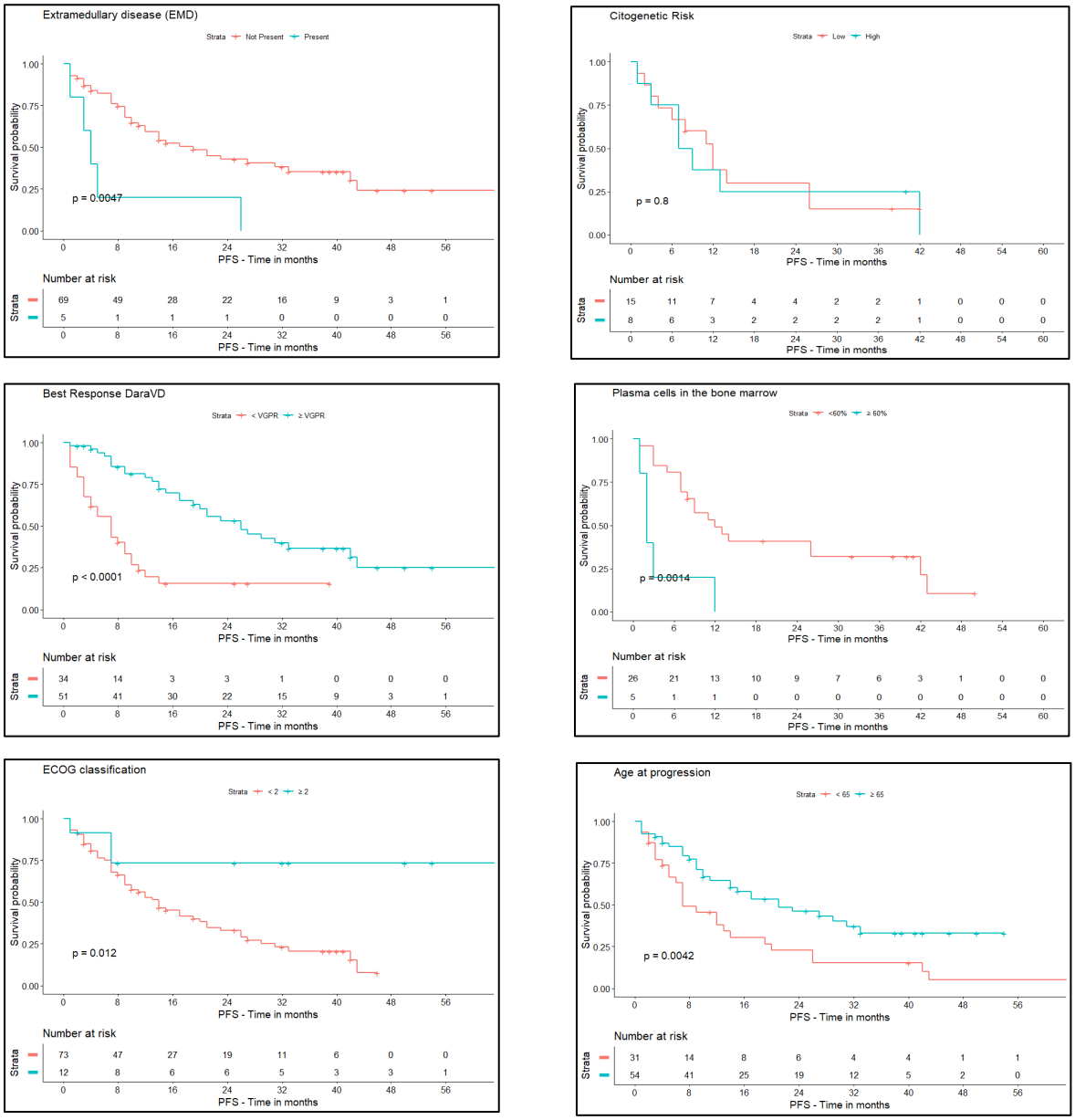
17

Analysis of survival curves stratified by key variables after relapse (Figure 3) revealed significant associations. The presence of extramedullary disease (EMD) was associated with a median survival of 4 months compared to 17 months in its absence ($p = 0.0047$). Achieving a best response $\geq$ VGPR demonstrated significantly prolonged survival with a median of 26 months compared to

[16] Author's analysis based on current study
[17] Author's analysis based on current study

8 months for < VGPR ($p < 0.00001$). Notably, ECOG performance status > 2 was associated with an undefined median survival compared to 15 months for ECOG < 2 ($p = 0.012$). Surprisingly, age at relapse < 65 years was associated with shorter median survival of 8 months compared to 20 months for age ≥ 65 years ($p = 0.0042$), a finding meriting further investigation. Cytogenetic risk stratification showed a median survival of 12 months for low-risk patients compared to 9 months for high-risk patients ($p = 0.8$). Plasma cells in the bone marrow also demonstrated a significant impact on survival, with patients having < 60% plasma cells showing a median survival of 13 months, whereas those with > 60% plasma cells had a median survival of 3 months ($p = 0.0014$).

Figure 3- Progression free survival of D-VD treated patients. Main clinical and biological characteristic.



In the Univariate Cox regression analysis for progression-free survival[19] in D-VD treated patients, variables were selected based on significance at a 5% level. The analysis included variables

[18] Author's analysis based on current study. The showed variables were, from top to bottom and from left to right, Extramedullary Dissease (present or not), Citogenetic Risk (high or low), Best Response to DaraVD (greater or not VGPR), Percentage of plasma cell in bone marrow (greater than 60% or not), ECOG performance (greater than 2 or not), Age at Progression (greater than 65 or not).

[19] This analysis was also performed for OS (see table 11 in the appendix)

assessed after the first relapse, focusing on those showing statistical significance. Achieving ≥ VGPR (HR 0.262, 95% CI 0.149-0.461, p < 0.00001), younger age at progression (< 65 years; HR 0.468, 95% CI 0.276-0.795, p = 0.0049), absence of EMD at progression (HR 3.627, 95% CI 1.407-9.347, p = 0.0076), better ECOG performance status (< 2; HR 0.144, 95% CI 0.034-0.621, p = 0.0093), and lower urinary protein levels (< 1000 mg/day; HR 3.39, 95% CI 1.158-9.923, p = 0.0259) were independently associated with improved outcomes. Additionally, FISH amplification of amp1q at progression showed a significant association with poorer outcomes (HR 3.197, 95% CI 1.039-9.843, p = 0.0428).

Table 9-Univariate Cox regression analysis for PFS in D-VD treated patients[20].

Variable	HR (95% CI)	P-value	NA's
BestResp≥VGPR	0.262 (0.149-0.461)	0.00001	0
Age at progression ≥ 65	0.468 (0.276-0.795)	0.0049	0
EMD at progression	3.627 (1.407-9.347)	0.0076	11
ECOG >2	0.144 (0.034-0.621)	0.0093	0
UrinaryProteins > 1000	3.39 (1.158-9.923)	0.0259	14
FISH_amp1qat progression	3.197 (1.039-9.843)	0.0428	59

Multivariate model

A multivariate model was performed for both Progression-Free Survival (PFS) and Overall Survival (OS) to address confounding factors that could not be observed in the univariate analysis (see table 11). Variables were selected based on their significance in the univariate analysis and the amount of missing data (variables with many missing values were excluded due to robustness and power issues). Age, R-ISS stage, and sex were arbitrarily included in the model to reduce confounding. Following this, a backward selection process was employed, resulting in the inclusion of 7 variables in the final model:

[20] Author's analysis based on current study

$$\text{PFS} = \text{h}_0(\text{t})\exp(\beta_1 ECOG + \beta_2\text{Age} + \beta_3\text{UrProteins} + \beta_4\text{BestResponse} + \beta_5\text{ConsolPeriod} + \beta_6\text{RISS} + \beta_7\text{Gender})$$

5

$$\text{OS} = \text{h\_0(t)}\ \exp(\beta_1 ECOG + \beta_2\text{Age} + \beta_3\text{PCR} + \beta_4\text{BestResponse} + \beta_5\text{ConsolPeriod} + \beta_6\text{RISS} + \beta_7\text{Gender})$$

6

After visual inspection of the Schoenfeld residuals of the Cox model (see figure 4 and 5 in the appendix), it was determined that some variables violated the proportional hazards assumption. These variables were R-ISS stage for the PFS model and gender and consolidation post-ASCT for the OS model. To address this, time interactions were added to these variables in the model. This approach allows the effect of these variables on the hazard function to vary over time, thereby accommodating the time-varying nature of their impact and ensuring a more accurate representation of their influence on survival outcomes.

Table 10-Multivariate Cox Proportional Hazards Models for PFS and OS[21]

	Hazard Ratio (HR)	
	PFS Model	OS Model
ECOG>2	0.31* p = 0.06	0.18** p = 0.05
Age at relapse	0.94*** p = 0.01	0.94 p = 0.11
UrProteins>1000	5.02*** p = 0.004	
PCR>0.5		4.61*** p = 0.005
BestResp>VGPR	0.08*** p = 0.000	0.12*** p = 0.000
ConsolpostASCT	0.50 p = 0.11	
Gender=Male	1.35 p = 0.41	
tt(RISS Stage)	1.04** p = 0.03	
tt(ConsolpostASCT)		0.55** p = 0.02
tt(Gender=Male)		1.45*** p = 0.004
RISS Stage=2		1.96 p = 0.24
RISS Stage=3		7.45 p = 0.11
Observations	59	56
R^2	0.60	0.52
Max. Possible R^2	0.99	0.95
Log Likelihood	-120.51	-64.47
Wald Test	46.91*** (df = 7)	29.31*** (df = 8)
LR Test	54.24*** (df = 7)	41.20*** (df = 8)

[21] Author's analysis based on current study

Score (Logrank) Test	64.58*** (df = 7)	42.49*** (df = 8)
Note:		*p<0.1 **p<0.05 ***p<0.01

In the PFS model, the variable 'UrProteins' was included, while in the OS model, 'PCR' was included instead (this based on the criteria mentioned earlier).

The results indicate that age appears to be a protective factor for PFS (HR = 0.94, p = 0.01), which is unusual and warrants further investigation to understand the underlying reasons. Notably, gender was significant in the OS model, indicating a potential influence on overall survival, but it was not significant in the PFS model. The ECOG performance status > 2 was a significant factor in both models, with a lower hazard ratio indicating better survival outcomes for patients with better performance status (PFS: HR = 0.31, p = 0.06; OS: HR = 0.18, p = 0.05). 'UrProteins > 1000' was a significant risk factor in the PFS model (HR = 5.02, p = 0.004), while 'PCR > 0.5' was a significant risk factor in the OS model (HR = 4.61, p = 0.005). Best response better than VGPR (very good partial response) significantly improved outcomes in both models (PFS: HR = 0.08, p < 0.0001; OS: HR = 0.12, p < 0.0001). 'ConsolPostASCT' showed a non-significant trend towards better outcomes in the PFS model (HR = 0.5, p = 0.11), but it was not included in the OS model without a time interaction. Gender was a significant risk factor in the OS model when accounting for the time interaction (HR = 1.45, p = 0.004) but not in the PFS model (HR = 1.35, p = 0.41). The time interaction term for RISS was significant in the PFS model (HR = 1.04, p = 0.03), and the time interaction terms for 'ConsolPostASCT' and gender were significant in the OS model (HR = 0.55, p = 0.02 and HR = 1.45, p = 0.004, respectively). RISS stage did not reach statistical significance in OS model, though higher stages were associated with worse outcomes.

The models demonstrated good fit and validity, with R^2 values of 0.6 for PFS and 0.52 for OS, indicating a substantial proportion of the variance explained by the models. The Wald, Likelihood Ratio (LR), and Score (Logrank) tests all showed significant results ($p < 0.01$), further confirming the robustness and validity of the models. Different kinds of models, such as parametric models (exponential, Weibull, log-logistic, etc.) and Accelerated Failure Time (AFT) models, were also performed but discarded after an AIC/BIC comparison (see Table 12 and 13 on Appendix).

Discussion

This study provides a comprehensive evaluation of the efficacy and safety of the Daratumumab-Bortezomib-Dexamethasone (DaraVD) regimen as a second-line therapy for multiple myeloma (MM) patients refractory to lenalidomide. Our findings, which include an overall response rate of 84.7% and a median progression-free survival (PFS) of 14 months, align with and extend the existing literature on the management of relapsed and refractory multiple myeloma (RRMM).

The results of our study can be contextualized by comparing them with key clinical trials such as CASTOR, CANDOR, OPTIMISMM, APOLLO, IKEMA and ENDEAVOR. The CASTOR trial demonstrated that adding daratumumab to bortezomib and dexamethasone significantly improves overall survival (OS) and PFS in patients with RRMM (Sonneveld et al., 2023). The median PFS for the daratumumab combination in CASTOR was not reached at the time of analysis (72 months), with significant improvements noted in patients who had received one prior line of therapy. Our study, which specifically focused on lenalidomide-refractory patients, found a median PFS of 14 months and a median OS of 47 months, indicating that the DaraVD regimen is effective even in this subgroup.

Similarly, the CANDOR trial compared carfilzomib, daratumumab, and dexamethasone (KdD) against carfilzomib and dexamethasone (Kd) alone, showing that the triplet regimen significantly improved PFS (Dimopoulos et al., 2020). The median PFS was not reached for the KdD group at a median follow-up of 17 months. Although our study's PFS of 14 months appears shorter, this discrepancy may be attributed to the higher proportion of lenalidomide-refractory patients in our cohort, emphasizing the difficulty in treating this population.

The OPTIMISMM trial evaluated the efficacy of pomalidomide, bortezomib, and dexamethasone (PVd) in lenalidomide-refractory patients, with a reported median PFS of 11.20 months (Richardson et al., 2019). Our findings of a 14-month median PFS with DaraVD suggest that the DaraVD regimen provides a competitive alternative to PVd, offering a viable option for patients who have exhausted lenalidomide-based therapies.

In the ENDEAVOR trial, carfilzomib and dexamethasone (Kd) showed a median PFS of 18.7 months and OS of 47.6 months (Dimopoulos et al., 2017). Although our median PFS was shorter,

the OS of 47 months in our study closely mirrors the ENDEAVOR results, underscoring the efficacy of the DaraVD regimen in extending overall survival, even in lenalidomide-refractory patients.

The APOLLO trial, which evaluated daratumumab in combination with pomalidomide and dexamethasone, found a median PFS of 12.4 months in the daratumumab group compared to 6.9 months in the control group. These results support the substantial benefit of adding daratumumab to different backbone regimens in improving outcomes for relapsed or refractory MM patients. The IKEMA trial further supports the benefit of monoclonal antibody-based combinations. It demonstrated that adding isatuximab to carfilzomib and dexamethasone resulted in a significantly longer median PFS, which was not reached in the isatuximab group compared to 19.15 months in the control group.

Lenalidomide maintenance therapy has become a cornerstone in MM treatment for eligible and non-eligible ASCT patients, but resistance remains a significant hurdle (Benboubker et al., 2014; Dimopoulos et al., 2016; McCarthy et al., 2017; Moreau et al., 2016). Our study specifically addresses this clinical challenge, providing evidence that the DaraVD regimen can achieve high response rates and meaningful survival outcomes in lenalidomide-refractory patients. With 60.0% of patients achieving a very good partial response (VGPR) or better and 24.7% achieving complete response (CR) or better, our findings are comparable to the response rates seen in broader RRMM populations from major trials.

The analysis of survival curves highlighted significant associations. Extramedullary disease (EMD) was linked to a median survival of 4 months versus 17 months without EMD. Achieving a best response ≥ VGPR resulted in a median survival of 26 months compared to 8 months for < VGPR. ECOG performance status > 2 indicated better survival outcomes than ECOG < 2. Age at relapse < 65 years was unexpectedly associated with shorter median survival (8 months) compared to age ≥ 65 years (20 months). Cytogenetic risk showed no significant difference, with 12 months for low-risk and 9 months for high-risk patients (p-value>0.1). Patients with < 60% plasma cells had a median survival of 13 months, while those with > 60% plasma cells had 3 months.

The safety profile observed in our study aligns with known toxicities associated with daratumumab and bortezomib (Dimopoulos et al., 2020; Sonneveld et al., 2023). Hematological toxicities such

as anemia, thrombocytopenia, and neutropenia were common, with grade 3-4 events occurring in 24%, 45.9%, and 18.8% of patients, respectively. Non-hematological toxicities, including peripheral neuropathy (37.6% grade 3-4) and infections (8.2% grade 3-4), were also prevalent but manageable. These adverse events are consistent with those reported in the CASTOR and CANDOR trials, affirming the tolerability of the DaraVD regimen.

The multivariate Cox model analysis revealed that age was a protective factor for progression-free survival (PFS), while gender influenced overall survival (OS). ECOG performance status > 2, higher urinary protein levels, and plasma cell ratio > 0.5 were significant risk factors in both models. Achieving a response better than VGPR significantly improved both PFS and OS. Having a Consolidation period post ASCT showed a trend towards better outcomes in the PFS model and in the OS model. Time interaction terms for RISS, Consolidation period post ASCT and gender were significant in the models. Higher RISS stages were associated with worse outcomes.

The findings from our study suggest that the DaraVD regimen is a robust second-line therapy option for MM patients who are refractory to lenalidomide. Given the comparable efficacy outcomes to those observed in the CASTOR and OPTIMISMM trials, and the manageable safety profile, DaraVD offers a viable alternative in this patient population. Notably, DaraVD is especially beneficial for patients who cannot tolerate more intensive and toxic regimens like IsaKd. These results support the incorporation of DaraVD into treatment guidelines for lenalidomide-refractory MM patients, providing clinicians with an effective option to address the growing challenge of lenalidomide resistance.

Conclusion

In conclusion, the observational study on second-line therapy with Daratumumab-Bortezomib-Dexamethasone (DaraVD) for multiple myeloma patient's refractory to lenalidomide sheds light on effective treatment strategies in a challenging clinical scenario. The study demonstrated promising outcomes with an overall response rate of 84.7%, emphasizing DaraVD's efficacy in this patient cohort. Survival analysis showed a median PFS of 14 months and a median OS of 47 months and underscored the impact of key variables such as extramedullary disease, best response achieved, ECOG performance status, age, cytogenetic risk, and bone marrow plasma cell percentage on patient outcomes.

The results suggest that the Daratumumab-Bortezomib-Dexamethasone (DaraVD) regimen is an effective second-line therapy for multiple myeloma (MM) patient's refractory to lenalidomide. DaraVD is a viable alternative, particularly for patients who cannot tolerate intensive regimens. These findings provide valuable insights into optimizing therapeutic decisions and improving survival outcomes for lenalidomide-refractory multiple myeloma patients.

Further research is needed to refine the optimal sequencing of therapies for MM, particularly for patients refractory to lenalidomide. Prospective randomized control trials studies focusing specifically on lenalidomide-refractory populations would be valuable. Additionally, real-world studies examining long-term outcomes and quality of life in patients receiving these therapies can provide further insights into the best practices for managing RRMM.

References

Abd Elhafeez, S., D'Arrigo, G., Leonardis, D., Fusaro, M., Tripepi, G., & Roumeliotis, S. (2021). Methods to Analyze Time-to-Event Data: The Cox Regression Analysis. *Oxidative Medicine and Cellular Longevity*, *2021*. https://doi.org/10.1155/2021/1302811

Akaike, H. (1974). A New Look at the Statistical Model Identification. *IEEE Transactions on Automatic Control*, *19*(6). https://doi.org/10.1109/TAC.1974.1100705

Azzalini, A. (2017). Statistical inference: Based on the likelihood. In *Statistical Inference: Based on the Likelihood*. https://doi.org/10.1201/9780203738627

Barbur, V. A., Montgomery, D. C., & Peck, E. A. (1994). Introduction to Linear Regression Analysis. *The Statistician*, *43*(2). https://doi.org/10.2307/2348362

Barilà, G., Quaglia, F. M., Furlan, A., Pescosta, N., Bonalumi, A., Marcon, C., Pascarella, A., Tinelli, M., De March, E., Lico, A., Sartori, R., Clissa, C., De Sabbata, G., Nappi, D., Porrazzo, M., De Marchi, R., Pavan, L., Tosetto, A., Gherlinzoni, F., … Zambello, R. (2024). A real-life study of daratumumab-bortezomib-dexamethasone (D-VD) in lenalidomide exposed/refractory multiple myeloma patients: a report from the Triveneto Myeloma Working Group. *Annals of Hematology*, *103*(1), 125–132. https://doi.org/10.1007/s00277-023-05443-8

Bazarbachi, A. H., Al Hamed, R., Malard, F., Bazarbachi, A., Harousseau, J.-L., & Mohty, M. (2022). Induction therapy prior to autologous stem cell transplantation (ASCT) in newly diagnosed multiple myeloma: an update. *Blood Cancer Journal*, *12*(3), 47. https://doi.org/10.1038/s41408-022-00645-1

Benboubker, L., Dimopoulos, M. A., Dispenzieri, A., Catalano, J., Belch, A. R., Cavo, M., Pinto, A., Weisel, K., Ludwig, H., Bahlis, N., Banos, A., Tiab, M., Delforge, M., Cavenagh, J., Geraldes, C., Lee, J.-J., Chen, C., Oriol, A., de la Rubia, J., … Facon, T. (2014). Lenalidomide and Dexamethasone in Transplant-Ineligible Patients with Myeloma. *New England Journal of Medicine*, *371*(10), 906–917. https://doi.org/10.1056/NEJMoa1402551

Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L., Torre, L. A., & Jemal, A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians*, *68*(6), 394–424. https://doi.org/10.3322/caac.21492

Bray, F., Laversanne, M., Sung, H., Ferlay, J., Siegel, R. L., Soerjomataram, I., & Jemal, A. (2024). Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians*, *74*(3), 229–263. https://doi.org/10.3322/caac.21834

Chim, C. S., Kumar, S. K., Orlowski, R. Z., Cook, G., Richardson, P. G., Gertz, M. A., Giralt, S., Mateos, M. V, Leleu, X., & Anderson, K. C. (2018). Management of relapsed and refractory multiple myeloma: novel agents, antibodies, immunotherapies and beyond. *Leukemia*, *32*(2), 252–262. https://doi.org/10.1038/leu.2017.329

Cowan, A. J., Green, D. J., Kwok, M., Lee, S., Coffey, D. G., Holmberg, L. A., Tuazon, S., Gopal, A. K., & Libby, E. N. (2022). Diagnosis and Management of Multiple Myeloma. *JAMA*, *327*(5), 464. https://doi.org/10.1001/jama.2022.0003

D'Arrigo, G., Leonardis, D., Abd Elhafeez, S., Fusaro, M., Tripepi, G., & Roumeliotis, S. (2021). Methods to Analyse Time-to-Event Data: The Kaplan-Meier Survival Curve. In *Oxidative Medicine and Cellular Longevity* (Vol. 2021). https://doi.org/10.1155/2021/2290120

Delgado, A., & Guddati, A. K. (2021). Clinical endpoints in oncology - a primer. *American Journal of Cancer Research*, *11*(4), 1121–1131.

Dimopoulos, M. A., Goldschmidt, H., Niesvizky, R., Joshua, D., Chng, W.-J., Oriol, A., Orlowski, R. Z., Ludwig, H., Facon, T., Hajek, R., Weisel, K., Hungria, V., Minuk, L., Feng, S., Zahlten-Kumeli, A., Kimball, A. S., & Moreau, P. (2017). Carfilzomib or bortezomib in relapsed or refractory multiple myeloma (ENDEAVOR): an interim overall survival analysis of an open-label, randomised, phase 3 trial. *The Lancet Oncology*, *18*(10), 1327–1337. https://doi.org/10.1016/S1470-2045(17)30578-8

Dimopoulos, M. A., Moreau, P., Terpos, E., Mateos, M. V., Zweegman, S., Cook, G., Delforge, M., Hájek, R., Schjesvold, F., Cavo, M., Goldschmidt, H., Facon, T., Einsele, H., Boccadoro, M., San-Miguel, J., Sonneveld, P., & Mey, U. (2021). Multiple myeloma: EHA-ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up†. *Annals of Oncology*, *32*(3), 309–322. https://doi.org/10.1016/j.annonc.2020.11.014

Dimopoulos, M. A., Oriol, A., Nahi, H., San-Miguel, J., Bahlis, N. J., Usmani, S. Z., Rabin, N., Orlowski, R. Z., Komarnicki, M., Suzuki, K., Plesner, T., Yoon, S.-S., Ben Yehuda, D., Richardson, P. G., Goldschmidt, H., Reece, D., Lisby, S., Khokhar, N. Z., O'Rourke, L., … Moreau, P. (2016). Daratumumab, Lenalidomide, and Dexamethasone for Multiple Myeloma. *New England Journal of Medicine*, *375*(14), 1319–1331. https://doi.org/10.1056/NEJMoa1607751

Dimopoulos, M. A., Terpos, E., Boccadoro, M., Delimpasi, S., Beksac, M., Katodritou, E., Moreau, P., Baldini, L., Symeonidis, A., Bila, J., Oriol, A., Mateos, M.-V., Einsele, H., Orfanidis, I., Ahmadi, T., Ukropec, J., Kampfenkel, T., Schecter, J. M., Qiu, Y., … Zweegman, S. (2021). Daratumumab plus pomalidomide and dexamethasone versus pomalidomide and dexamethasone alone in previously treated multiple myeloma (APOLLO): an open-label, randomised, phase 3 trial. *The Lancet Oncology*, *22*(6), 801–812. https://doi.org/10.1016/S1470-2045(21)00128-5

Dimopoulos, M., Quach, H., Mateos, M.-V., Landgren, O., Leleu, X., Siegel, D., Weisel, K., Yang, H., Klippel, Z., Zahlten-Kumeli, A., & Usmani, S. Z. (2020). Carfilzomib, dexamethasone, and daratumumab versus carfilzomib and dexamethasone for patients with relapsed or refractory multiple myeloma (CANDOR): results from a randomised, multicentre, open-label, phase 3 study. *The Lancet*, *396*(10245), 186–197. https://doi.org/10.1016/S0140-6736(20)30734-0

Durie, B. G. M., Harousseau, J.-L., Miguel, J. S., Bladé, J., Barlogie, B., Anderson, K., Gertz, M., Dimopoulos, M., Westin, J., Sonneveld, P., Ludwig, H., Gahrton, G., Beksac, M., Crowley, J., Belch, A., Boccadaro, M., Turesson, I., Joshua, D., Vesole, D., … Rajkumar, S. V. (2006). International uniform response criteria for multiple myeloma. *Leukemia*, *20*(9), 1467–1473. https://doi.org/10.1038/sj.leu.2404284

Durie, B. G. M., Hoering, A., Sexton, R., Abidi, M. H., Epstein, J., Rajkumar, S. V., Dispenzieri, A., Kahanic, S. P., Thakuri, M. C., Reu, F. J., Reynolds, C. M., Orlowski, R. Z., & Barlogie, B. (2020). Longer term follow-up of the randomized phase III trial SWOG S0777: bortezomib, lenalidomide and dexamethasone vs. lenalidomide and dexamethasone in patients (Pts) with previously untreated multiple myeloma without an intent for immediate autologous stem cell transplant (ASCT). *Blood Cancer Journal*, *10*(5), 53. https://doi.org/10.1038/s41408-020-0311-8

Facon, T., Dimopoulos, M. A., Dispenzieri, A., Catalano, J. V., Belch, A., Cavo, M., Pinto, A., Weisel, K., Ludwig, H., Bahlis, N. J., Banos, A., Tiab, M., Delforge, M., Cavenagh, J. D., Geraldes, C., Lee, J.-J., Chen, C.,

Oriol, A., De La Rubia, J., … Hulin, C. (2018). Final analysis of survival outcomes in the phase 3 FIRST trial of up-front treatment for multiple myeloma. *Blood*, *131*(3), 301–310. https://doi.org/10.1182/blood-2017-07-795047

Facon, T., Kumar, S. K., Plesner, T., Orlowski, R. Z., Moreau, P., Bahlis, N., Basu, S., Nahi, H., Hulin, C., Quach, H., Goldschmidt, H., O'Dwyer, M., Perrot, A., Venner, C. P., Weisel, K., Mace, J. R., Raje, N., Tiab, M., Macro, M., … Usmani, S. Z. (2021). Daratumumab, lenalidomide, and dexamethasone versus lenalidomide and dexamethasone alone in newly diagnosed multiple myeloma (MAIA): overall survival results from a randomised, open-label, phase 3 trial. *The Lancet Oncology*, *22*(11), 1582–1596. https://doi.org/10.1016/S1470-2045(21)00466-6

Firth, J. (2019). Haematology: multiple myeloma. *Clinical Medicine*, *19*(1), 57–60. https://doi.org/10.7861/clinmedicine.19-1-58

Gao, X., Zeng, H., Zhao, X., Wu, H., Yan, M., Li, Y., Zhang, G., & Sun, F. (2023). Efficacy and safety of venetoclax in patients with relapsed/refractory multiple myeloma: a meta-analysis. *BMC Cancer*, *23*(1), 1058. https://doi.org/10.1186/s12885-023-11553-3

Hosmer, D. W., Lemeshow, S., & May, S. (2011). Applied Survival Analysis: Regression Modeling of Time to Event Data: Second Edition. In *Applied Survival Analysis: Regression Modeling of Time to Event Data: Second Edition*. https://doi.org/10.1002/9780470258019

Ibrahim, J. G., Chu, H., & Chen, L. M. (2010). Basic concepts and methods for joint models of longitudinal and survival data. In *Journal of Clinical Oncology* (Vol. 28, Issue 16). https://doi.org/10.1200/JCO.2009.25.0654

Kaplan, E. L., & Meier, P. (1958). Nonparametric Estimation from Incomplete Observations. *Journal of the American Statistical Association*, *53*(282). https://doi.org/10.1080/01621459.1958.10501452

Kass, R. E., & Raftery, A. E. (1995). Bayes factors. *Journal of the American Statistical Association*, *90*(430). https://doi.org/10.1080/01621459.1995.10476572

Kazandjian, D. (2016). Multiple myeloma epidemiology and survival: A unique malignancy. *Seminars in Oncology*, *43*(6), 676–681. https://doi.org/10.1053/j.seminoncol.2016.11.004

Keam, S. J. (2023). Talquetamab: First Approval. *Drugs*, *83*(15), 1439–1445. https://doi.org/10.1007/s40265-023-01945-x

Kruskal, W. H., & Wallis, W. A. (1952). Use of Ranks in One-Criterion Variance Analysis. *Journal of the American Statistical Association*, *47*(260). https://doi.org/10.1080/01621459.1952.10483441

Kyle, R. A., Child, J. A., Anderson, K., Barlogie, B., Bataille, R., Bensinger, W., Bladé, J., Boccadoro, M., Dalton, W., Dimopoulos, M., Djulbegovic, B., Drayson, M., Durie, B., Facon, T., Fonseca, R., Gahrton, G., Greipp, P., Harousseau, J. L., Harrington, D., … Wheatley, K. (2003). Criteria for the classification of monoclonal gammopathies, multiple myeloma and related disorders: A report of the International Myeloma Working Group. In *British Journal of Haematology* (Vol. 121, Issue 5). https://doi.org/10.1046/j.1365-2141.2003.04355.x

Lesokhin, A. M., Tomasson, M. H., Arnulf, B., Bahlis, N. J., Miles Prince, H., Niesvizky, R., Rodríguez-Otero, P., Martinez-Lopez, J., Koehne, G., Touzeau, C., Jethava, Y., Quach, H., Depaus, J., Yokoyama, H., Gabayan, A. E., Stevens, D. A., Nooka, A. K., Manier, S., Raje, N., … Mohty, M. (2023). Elranatamab in relapsed or refractory multiple myeloma: phase 2 MagnetisMM-3 trial results. *Nature Medicine*, *29*(9), 2259–2267. https://doi.org/10.1038/s41591-023-02528-9

Lonial, S., Dimopoulos, M., Palumbo, A., White, D., Grosicki, S., Spicka, I., Walter-Croneck, A., Moreau, P., Mateos, M.-V., Magen, H., Belch, A., Reece, D., Beksac, M., Spencer, A., Oakervee, H., Orlowski, R. Z., Taniwaki, M., Röllig, C., Einsele, H., … Richardson, P. (2015). Elotuzumab Therapy for Relapsed or Refractory Multiple Myeloma. *New England Journal of Medicine*, *373*(7), 621–631. https://doi.org/10.1056/NEJMoa1505654

Lury, D. A., & Fisher, R. A. (1972). Statistical Methods for Research Workers. *The Statistician*, *21*(3). https://doi.org/10.2307/2986695

Mateos, M.-V., Dimopoulos, M. A., Cavo, M., Suzuki, K., Jakubowiak, A., Knop, S., Doyen, C., Lucio, P., Nagy, Z., Kaplan, P., Pour, L., Cook, M., Grosicki, S., Crepaldi, A., Liberati, A. M., Campbell, P., Shelekhova, T., Yoon, S.-S., Iosava, G., … San-Miguel, J. (2018). Daratumumab plus Bortezomib, Melphalan, and Prednisone for Untreated Myeloma. *New England Journal of Medicine*, *378*(6), 518–528. https://doi.org/10.1056/NEJMoa1714678

McCarthy, P. L., Holstein, S. A., Petrucci, M. T., Richardson, P. G., Hulin, C., Tosi, P., Bringhen, S., Musto, P., Anderson, K. C., Caillot, D., Gay, F., Moreau, P., Marit, G., Jung, S.-H., Yu, Z., Winograd, B., Knight, R. D., Palumbo, A., & Attal, M. (2017). Lenalidomide Maintenance After Autologous Stem-Cell Transplantation in Newly Diagnosed Multiple Myeloma: A Meta-Analysis. *Journal of Clinical Oncology*, *35*(29), 3279–3289. https://doi.org/10.1200/JCO.2017.72.6679

Mikkilineni, L., & Kochenderfer, J. N. (2021). CAR T cell therapies for patients with multiple myeloma. *Nature Reviews Clinical Oncology*, *18*(2), 71–84. https://doi.org/10.1038/s41571-020-0427-6

Mischel, A. M., & Rosielle, D. A. (2022). Eastern Cooperative Oncology Group Performance Status #434. *Journal of Palliative Medicine*, *25*(3). https://doi.org/10.1089/jpm.2021.0599

Moreau, P., Dimopoulos, M.-A., Mikhael, J., Yong, K., Capra, M., Facon, T., Hajek, R., Špička, I., Baker, R., Kim, K., Martinez, G., Min, C.-K., Pour, L., Leleu, X., Oriol, A., Koh, Y., Suzuki, K., Risse, M.-L., Asset, G., … Ozkalemkas, F. (2021). Isatuximab, carfilzomib, and dexamethasone in relapsed multiple myeloma (IKEMA): a multicentre, open-label, randomised phase 3 trial. *The Lancet*, *397*(10292), 2361–2371. https://doi.org/10.1016/S0140-6736(21)00592-4

Moreau, P., Hulin, C., Perrot, A., Arnulf, B., Belhadj, K., Benboubker, L., Zweegman, S., Caillon, H., Caillot, D., Avet-Loiseau, H., Delforge, M., Dejoie, T., Facon, T., Sonntag, C., Fontan, J., Mohty, M., Jie, K.-S., Karlin, L., Kuhnowski, F., … Sonneveld, P. (2024). Bortezomib, thalidomide, and dexamethasone with or without daratumumab and followed by daratumumab maintenance or observation in transplant-eligible newly diagnosed multiple myeloma: long-term follow-up of the CASSIOPEIA randomised controlled phase 3 trial. *The Lancet Oncology*. https://doi.org/10.1016/S1470-2045(24)00282-1

Moreau, P., Kumar, S. K., San Miguel, J., Davies, F., Zamagni, E., Bahlis, N., Ludwig, H., Mikhael, J., Terpos, E., Schjesvold, F., Martin, T., Yong, K., Durie, B. G. M., Facon, T., Jurczyszyn, A., Sidana, S., Raje, N., van de Donk, N., Lonial, S., … Richardson, P. G. (2021). Treatment of relapsed and refractory multiple myeloma: recommendations from the International Myeloma Working Group. *The Lancet Oncology*, *22*(3), e105–e118. https://doi.org/10.1016/S1470-2045(20)30756-7

Moreau, P., Masszi, T., Grzasko, N., Bahlis, N. J., Hansson, M., Pour, L., Sandhu, I., Ganly, P., Baker, B. W., Jackson, S. R., Stoppa, A.-M., Simpson, D. R., Gimsing, P., Palumbo, A., Garderet, L., Cavo, M., Kumar, S., Touzeau, C., Buadi, F. K., … Richardson, P. G. (2016). Oral Ixazomib, Lenalidomide, and Dexamethasone for Multiple Myeloma. *New England Journal of Medicine*, *374*(17), 1621–1634. https://doi.org/10.1056/NEJMoa1516282

Moreau, P., San Miguel, J., Sonneveld, P., Mateos, M. V., Zamagni, E., Avet-Loiseau, H., Hajek, R., Dimopoulos, M. A., Ludwig, H., Einsele, H., Zweegman, S., Facon, T., Cavo, M., Terpos, E., Goldschmidt, H., Attal, M., & Buske, C. (2017). Multiple myeloma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Annals of Oncology*, *28*, iv52–iv61. https://doi.org/10.1093/annonc/mdx096

Murphy, J. R., & Kleinbaum, D. G. (1997). Survival Analysis: A Self-Learning Text. *Journal of the American Statistical Association*, *92*(438). https://doi.org/10.2307/2965741

Nooka, A. K., Kaufman, J. L., Hofmeister, C. C., Joseph, N. S., Heffner, T. L., Gupta, V. A., Sullivan, H. C., Neish, A. S., Dhodapkar, M. V., & Lonial, S. (2019). Daratumumab in multiple myeloma. *Cancer*, *125*(14), 2364–2382. https://doi.org/10.1002/cncr.32065

Padala, S. A., Barsouk, A., Barsouk, A., Rawla, P., Vakiti, A., Kolhe, R., Kota, V., & Ajebo, G. H. (2021). Epidemiology, Staging, and Management of Multiple Myeloma. *Medical Sciences*, *9*(1), 3. https://doi.org/10.3390/medsci9010003

Palumbo, A., & Anderson, K. (2011). Multiple Myeloma. *New England Journal of Medicine*, *364*(11), 1046–1060. https://doi.org/10.1056/NEJMra1011442

Palumbo, A., Avet-Loiseau, H., Oliva, S., Lokhorst, H. M., Goldschmidt, H., Rosinol, L., Richardson, P., Caltagirone, S., Lahuerta, J. J., Facon, T., Bringhen, S., Gay, F., Attal, M., Passera, R., Spencer, A., Offidani, M., Kumar, S., Musto, P., Lonial, S., … Moreau, P. (2015). Revised International Staging System for Multiple Myeloma: A Report From International Myeloma Working Group. *Journal of Clinical Oncology*, *33*(26), 2863–2869. https://doi.org/10.1200/JCO.2015.61.2267

Paul, B., Lipe, B., Ocio, E. M., & Usmani, S. Z. (2019). Induction Therapy for Newly Diagnosed Multiple Myeloma. *American Society of Clinical Oncology Educational Book*, *39*, e176–e186. https://doi.org/10.1200/EDBK_238527

Rajkumar, S. V. (2011). Treatment of multiple myeloma. *Nature Reviews Clinical Oncology*, *8*(8), 479–491. https://doi.org/10.1038/nrclinonc.2011.63

Rajkumar, S. V. (2019). Multiple myeloma: Every year a new standard? *Hematological Oncology*, *37*(S1), 62–65. https://doi.org/10.1002/hon.2586

Richardson, P. G., Oriol, A., Beksac, M., Liberati, A. M., Galli, M., Schjesvold, F., Lindsay, J., Weisel, K., White, D., Facon, T., San Miguel, J., Sunami, K., O'Gorman, P., Sonneveld, P., Robak, P., Semochkin, S., Schey, S., Yu, X., Doerr, T., … Yee, A. (2019). Pomalidomide, bortezomib, and dexamethasone for patients with relapsed or refractory multiple myeloma previously treated with lenalidomide (OPTIMISMM): a randomised, open-label, phase 3 trial. *The Lancet Oncology*, *20*(6), 781–794. https://doi.org/10.1016/S1470-2045(19)30152-4

San-Miguel, J., Dhakal, B., Yong, K., Spencer, A., Anguille, S., Mateos, M.-V., Fernández de Larrea, C., Martínez-López, J., Moreau, P., Touzeau, C., Leleu, X., Avivi, I., Cavo, M., Ishida, T., Kim, S. J., Roeloffzen, W., van de Donk, N. W. C. J., Dytfeld, D., Sidana, S., … Einsele, H. (2023). Cilta-cel or Standard Care in Lenalidomide-Refractory Multiple Myeloma. *New England Journal of Medicine*, *389*(4), 335–347. https://doi.org/10.1056/NEJMoa2303379

Sheykhhasan, M., Ahmadieh-Yazdi, A., Vicidomini, R., Poondla, N., Tanzadehpanah, H., Dirbaziyan, A., Mahaki, H., Manoochehri, H., Kalhor, N., & Dama, P. (2024). CAR T therapies in multiple myeloma: unleashing the future. *Cancer Gene Therapy*, *31*(5), 667–686. https://doi.org/10.1038/s41417-024-00750-2

Soekojo, C. Y., & Chng, W. J. (2022). Treatment horizon in multiple myeloma. *European Journal of Haematology*, *109*(5), 425–440. https://doi.org/10.1111/ejh.13840

Sonneveld, P., & Broijl, A. (2016). Treatment of relapsed and refractory multiple myeloma. *Haematologica*, *101*(4), 396–406. https://doi.org/10.3324/haematol.2015.129189

Sonneveld, P., Chanan-Khan, A., Weisel, K., Nooka, A. K., Masszi, T., Beksac, M., Spicka, I., Hungria, V., Munder, M., Mateos, M.-V., Mark, T. M., Levin, M.-D., Ahmadi, T., Qin, X., Garvin Mayo, W., Gai, X., Carey, J., Carson, R., & Spencer, A. (2023). Overall Survival With Daratumumab, Bortezomib, and Dexamethasone in Previously Treated Multiple Myeloma (CASTOR): A Randomized, Open-Label, Phase III Trial. *Journal of Clinical Oncology*, *41*(8), 1600–1609. https://doi.org/10.1200/JCO.21.02734

Spearman, C. (1904). The Proof and Measurement of Association between Two Things. *The American Journal of Psychology*, *15*(1). https://doi.org/10.2307/1412159

Stewart, A. K., Rajkumar, S. V., Dimopoulos, M. A., Masszi, T., Špička, I., Oriol, A., Hájek, R., Rosiñol, L., Siegel, D. S., Mihaylov, G. G., Goranova-Marinova, V., Rajnics, P., Suvorov, A., Niesvizky, R., Jakubowiak, A. J., San-Miguel, J. F., Ludwig, H., Wang, M., Maisnar, V., … Palumbo, A. (2015). Carfilzomib, Lenalidomide, and Dexamethasone for Relapsed Multiple Myeloma. *New England Journal of Medicine*, *372*(2), 142–152. https://doi.org/10.1056/NEJMoa1411321

Thorsteinsdottir, S., Dickman, P. W., Landgren, O., Blimark, C., Hultcrantz, M., Turesson, I., Björkholm, M., & Kristinsson, S. Y. (2018). Dramatically improved survival in multiple myeloma patients in the recent decade: results from a Swedish population-based study. *Haematologica*, *103*(9), e412–e415. https://doi.org/10.3324/haematol.2017.183475

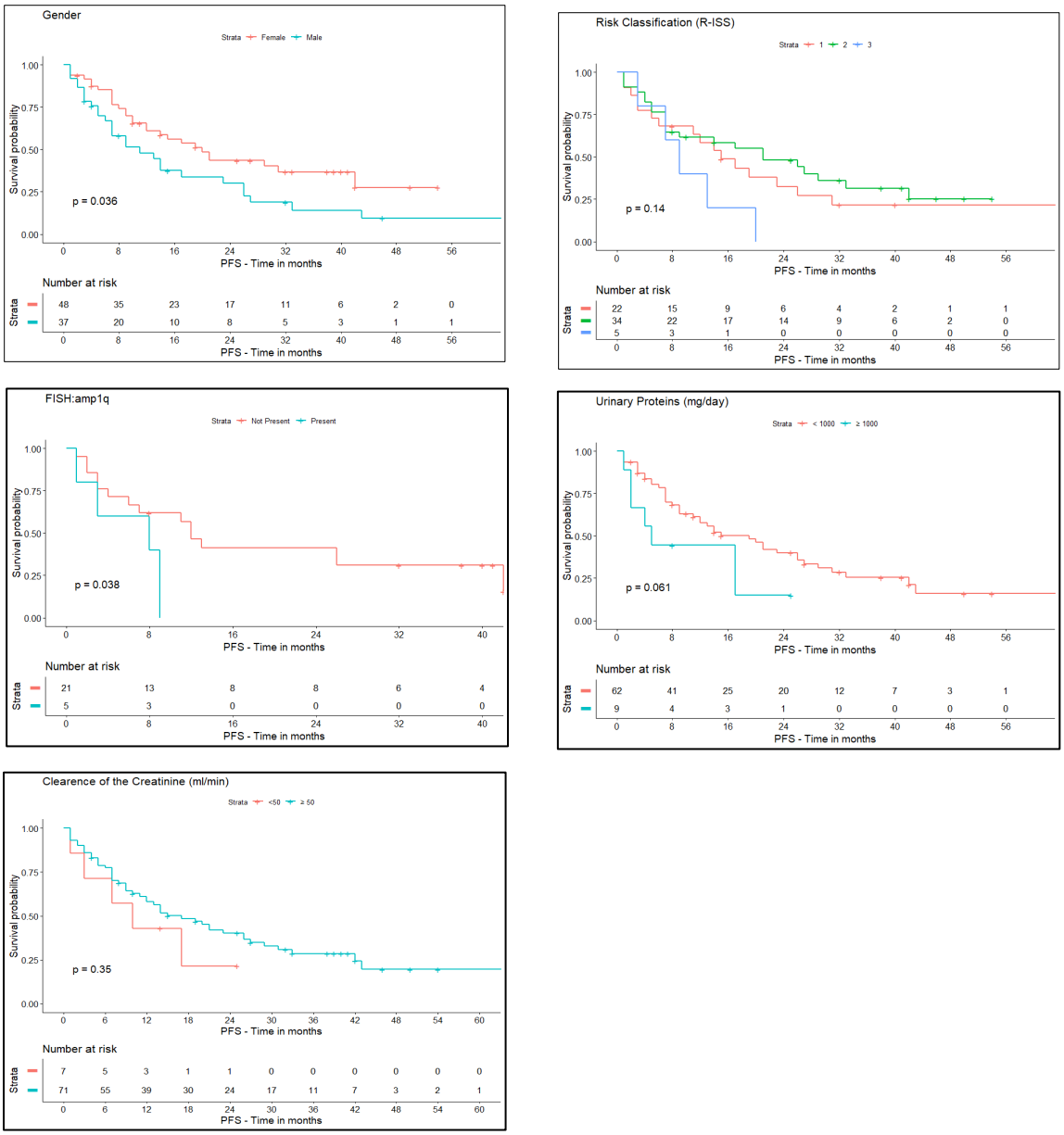
Appendix

Table 11-Univariate Cox regression analysis for OS in D-VD treated[22]

Variable	HR (95% CI)	p-value	NA
BestResp≥VGPR	0.262 (0.149-0.461)	0	0
Age at progression ≥ 65	0.468 (0.276-0.795)	0.0049	0
EMD at progression	3.627 (1.407-9.347)	0.0076	11
ECOG at onset	0.144 (0.034-0.621)	0.0093	0
Urinary Proteins > 3500	3.39 (1.158-9.923)	0.0259	14
FISH_amp1q at progression	3.197 (1.039-9.843)	0.0428	59
FISH_t1416 at onset	30.439 (3.024-306.412)	0.0037	29
FISH_Del17 at onset	3.558 (1.628-7.775)	0.0015	23

[22] Author's Analysis based on Current study

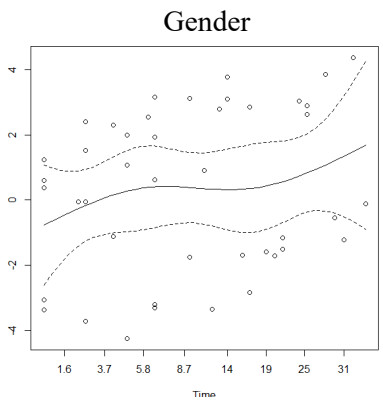
Figure 4-Other relevant Progression free survival (PFS) Kaplan–Meier curves of D-VD treated

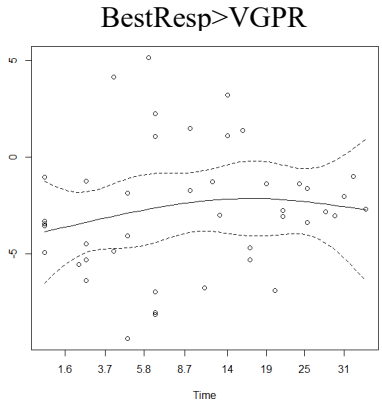


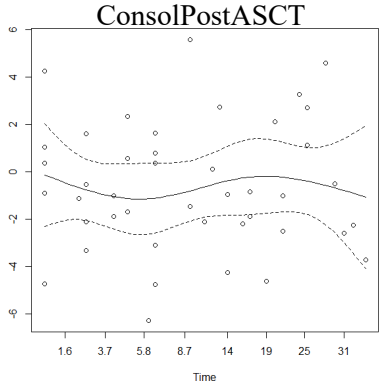
23

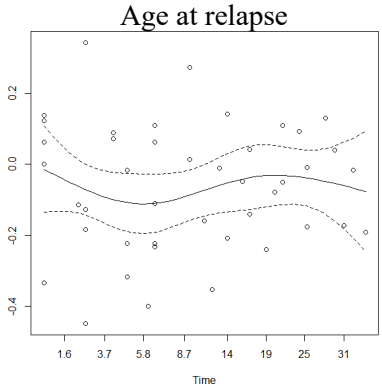
[23] Author analysis based on current study. The selected variables are, from top to bottom and from left to right, Gender (Male or not), Risk classification R-ISS (I, II or III), Fish amp1q (present or not), Urinary Proteins (greater than 1000 mg/day or not), clearence of the creatinine (Greater than 50 ml/min or not)

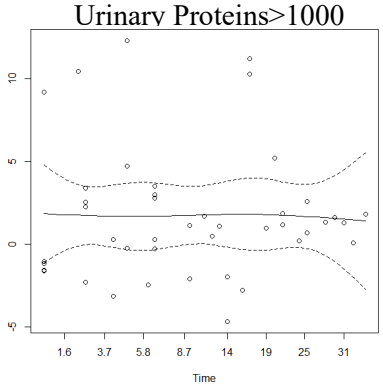
Figure 5- Scaled Schoenfeld residuals Plots with Proportional Hazard Test Values (PFS model)

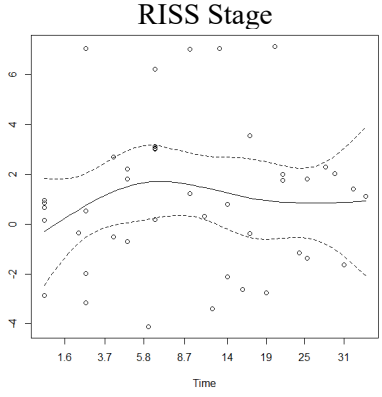


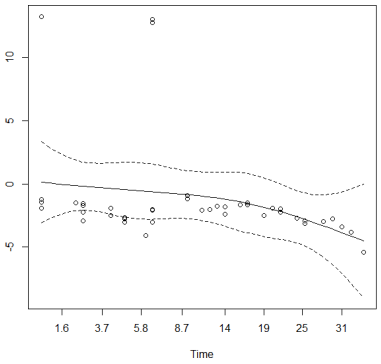












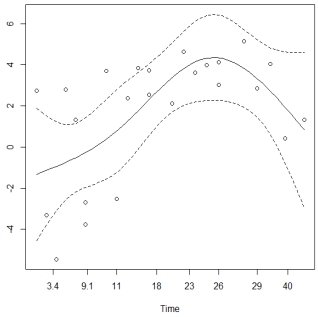
Proportional hazard test

	χ^2	DF	P-value
Gender	1.562	1	0.211
Best Response>VGPR	2.769	1	0.096
ConsolPostASCT	0.1192	1	0.730
Age at relapse	0.017	1	0.897
Urinary Proteins>1000	0.245	1	0.621
RISS stage	7.504	2	0.023
ECOG>2	3.432	1	0.064

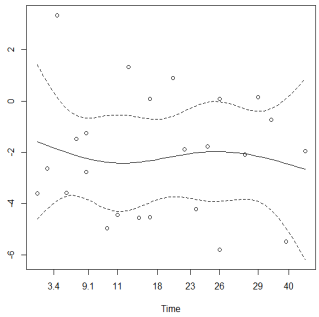
[24] Author's analysis based on current study.

Figure 6-Scaled Schoenfeld residuals Plots with Proportional Hazard Test Values (OS model)

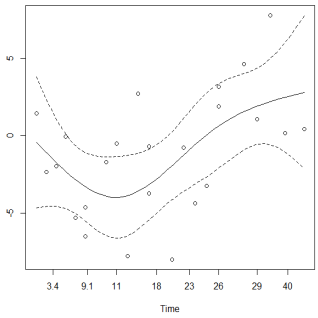
Gender



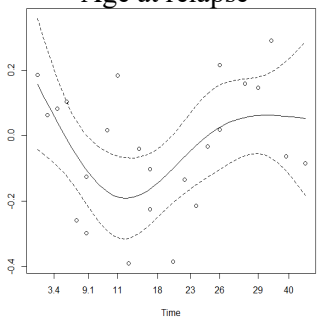
BestResp>VGPR



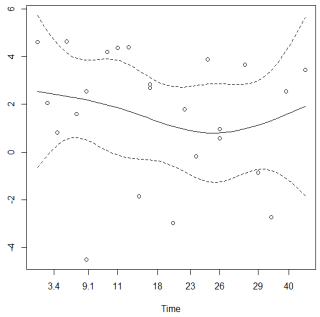
ConsolPostASCT



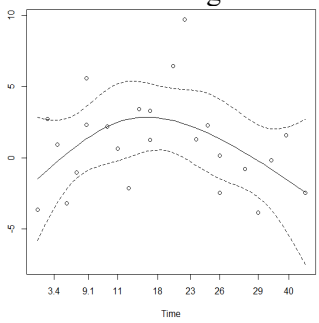
Age at relapse



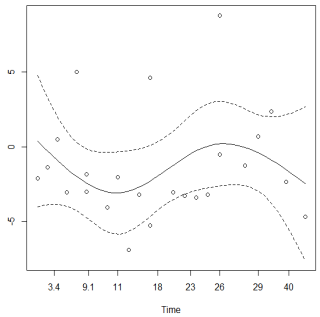
Urinary Proteins>1000



RISS Stage



ECOG>2



Proportional hazard test

	χ^2	DF	P-value
Gender	7.59	1	0.006
Best Response>VGPR	0.159	1	0.689
ConsolPostASCT	4.324	1	0.038
Age at relapse	0.704	1	0.401
PCR>0.5	1.204	1	0.272
RISS stage	0.4875	2	0.7837
ECOG>2	0.052	1	0.819

[25]Author analysis based on current study

Table 12-AIC and BIC model comparison for PFS[26]

Model	AIC	BIC
Exponential	327.9842	346.682
Log-Normal	318.9257	339.7011
Log-Logistic	318.9253	339.7007
Weibull	316.4468	337.2221
COXph	255.398	269.4876

Table 13-AIC and BIC model comparison for OS[27]

Model	AIC	BIC
Exponential	232.7175	250.9457
Log-Normal	228.2281	248.4817
Log-Logistic	227.6169	247.8704
Weibull	227.0842	247.3377
COXph	145.1522	154.5766

[26] Author's analysis based on current study
[27] Author's analysis based on current study