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Extramedullary disease in multiple myeloma: clinical and biological features with a comparative analysis of phenotypic differences

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Introduction

The clinical spectrum of multiple myeloma and extramedullary disease definition

Multiple myeloma (MM) is a hematological malignancy characterized by the accumulation in the bone marrow (BM) of terminally differentiated, immunoglobulin-producing plasma cells (PCs).
This mature B-cell neoplasm is defined by either the detection of ≥ 10% clonal PCs in the BM or a biopsy-proven plasmacytoma and by the presence of organ damage related signs summarized by the acronym CRAB (anemia, hypercalcemia, renal impairment, and lytic bone lesions) [1]. Recently, the diagnostic criteria for MM have been updated with the introduction of the so-called SLiM CRAB criteria, which identify myeloma defining events associated with an approximately 80% or higher risk of developing myeloma-related organ damage within two years. In the absence of organ damage according to the CRAB criteria, the presence of at least one of SLiM CRAB markers (serum involved-to-uninvolved free light chain ratio equal to or greater than 100, presence of more than one focal lesion equal to or greater than 5 mm detected by magnetic resonance imaging and ≥ 60% PCs in the BM) identifies an active or symptomatic MM[2].
Extramedullary MM (EMM) is a clinical manifestation of MM, characterized by a plasma cell proliferation outside of the bone marrow. EMM is described in the literature as a very heterogeneous group and includes different clinical variants[3]. Recently a new categorization has been proposed and includes bone-related plasmacytoma (EM-B), extramedullary extraosseous plasmacytoma (EM-E) and plasma cell leukemia (PCL). EM-B plasmacytoma represents a plasma cell proliferation that disrupts the cortical bone and grows contiguously to a segment of axial (ribs, vertebrae, skull, sternum, and pelvis) and, less frequently, appendicular skeleton. EM-E disease refers to localized plasma cell tumors or a plasma cell invasion, *via* blood vessels, arising in tissues of an anatomical district distant from bone marrow (most frequently soft tissues, liver, lymph nodes, skin, central nervous system). PCL is a rare and aggressive form of EMM defined by the presence in the peripheral

blood (PB) of a number of clonal plasma cells > 20% and/or an absolute value >2x10^3/mmc[4]. However, recent studies show that a number of PCs ≥5% at the PB smear in a patient with MM confers a prognostic risk that can be similar to that of the classical PCL definition[5]. Although some authors recognized PCL as an entity distinct from EMM, a concomitant extramedullary involvement (liver, spleen, lymph-nodes and pleural effusion) is very frequent[3,4]. Therefore, PCL can be included in the broader category of EMM when these clinical features are present[6]. Solitary plasmacytoma (SP) is characterized by a localized proliferation and accumulation of clonal plasma cells in the absence of evidence of systemic involvement or other symptoms that are not directly related to the lesion. Extramedullary spread in other organs without proximity to a skeletal segment is described, but as the criteria for MM are not met and the clinical outcome is overall better, SP is not included in the EMM group[7].

Before the introduction of novel agents in the treatment of MM, the pathologic findings from autopsy studies in end-stage MM patients revealed a high incidence of EM-B (61,5%-67%) and of EM-E (63,5%) with spleen, kidney and liver reported as the most frequent involved anatomical sites[8,9]. The high incidence of extramedullary disease findings *post-mortem* could reflect the natural history of MM suggesting that this clinical feature represents a natural evolution of the disease.

In a longitudinal study that analyzed the incidence of EMM in 1003 MM patients, treated between 1971 and 2007, 7% of patients developed EMM at diagnosis and 6% at relapse[10].

EM-E incidence was less frequent than EM-B (15% and 85% of total respectively) and a significant increase of EMM incidence was reported over the period between 2000 and 2007 compared with 1971-1999 period[11]. In a European Society for Blood and Marrow Transplantation (EBMT) Working Party study investigating a cohort of 3744 newly diagnosed and transplant eligible MM patients the global incidence of EMM was 18,2% and the incidence of EM-E was 3,7%[12]. In a retrospective study involving 329 MM patients, diagnosed between 2000 and 2010, the incidence of total extramedullary relapse was 28% with 74% of EM-E cases[13].

Genetic alterations and pathogenesis of extramedullary disease

MM pathogenesis is a multi-step transformation process that depends both on genetic changes inside the PCs clones and reciprocal tumor-microenvironment interactions[14].

Genomic instability is the hallmark of MM biology and, at chromosome level, two main oncogenic pathways are described that drive disease evolution from pre-malignant monoclonal gammopathy of undetermined significance (MGUS) to MM. Hyperdiploidy (HRD) specifically affects odd-numbered chromosomes (chromosomes 3, 5, 7, 9, 11, 15, 19, and 21) and is identified in almost half of MM cases with a favorable prognostic impact. Non-hyperdiploid (NHRD) tumors have fewer than 48 or more than 75 chromosomes and frequently carry a primary IgH translocation which recurrently affects the Cyclin D (CCND) family, the MAF family, and MMSET/FGFR3 genes[15]. The most frequent translocation is t(11;14), which leads to hyperexpression of cyclin D1 and is associated to a standard prognostic risk. Translocations t(4;14) that deregulates FGFR3 expression, t(14;16) that deregulates cMAF expression and t(14;20) that deregulates MAFB expression are associated with an unfavorable prognosis. In addition to these primary events, chromosome gains, such as 1q21 gain, and deletions (del), such as del(17p) involving P53 gene, and del(1p32) involving CDKN2C gene, are more common during MM progression and associated to worse prognosis[15,16]. Furthermore, a very high-risk subgroup of MM patients was defined by bi-allelic TP53 inactivation or amplification (≥4 copies) of 1q21 (amp1q21) in the context of stage III according to International Staging System (ISS)[17].

Recently, next generation sequencing and whole-exome sequencing studies showed a high intra-tumor heterogeneity with several mutations. These mutations most frequently involved genes of MAPK pathway and the NF-κB pathway, harbored by MM cells at clonal and subclonal levels[18-20]. According to a Darwinian model, new mutations can be acquired during the disease course and under treatment selective pressure, resulting in a dynamic change of clonal composition. Different clonal

evolution patterns have been reported such as linear clonal shift, branching clonal shift and stability of the clonal composition[21].

In the branching clonal evolution model, new clones, harboring mutations different from diagnosis, emerge at subsequent time points through divergent mutational dynamics[21].

Moreover, a recent multi-region sequencing performed on BM from focal lesions in different sites of the skeleton, revealed a significant divergence in the clonal architecture[22]. In this model a clone or a subclonal fraction can acquire driver genomic aberrations such as *MYC* translocations, gain(1q), or *RAS* mutations, which promote the tumor growth of fitter MM cells in specific regional BM niches overcoming the restrictions induced by the microenvironment and other less fit clones.

This model is based on an evolutionary pressure and competition for the different BM niches and could explain the selection of clones that eventually lose their dependence on the BM microenvironment and support the growth of extramedullary disease[22].

In a small cohort of EM-E patients the most frequent cytogenetic abnormalities reported in BM PCs at diagnosis were the adverse alterations t(4,14) and del(17p), identified in 57% of the patients analyzed[23]. In another series, such high-risk chromosome aberrations, del(17p) and t(4;14), were also observed in 58% of EM-E patients, whose samples were collected directly at the EM site [24]. A high incidence of del17p in EMM compared to non EMM patients (36% vs 12%) was confirmed in a large cohort of 834 cases[25]. In a retrospective study involving 41 EMM patients the incidence of amp(1q21) was higher compared to a cohort of no EMD patients (55% vs 32%)[26].

Usmani *et al* reported an increased incidence of EM-E in patients with baseline high risk prognostic features, MF (*MAF* overexpression) and PR (also called the "Proliferation" subtype, characterized by the overexpression of pro-proliferative genes) subgroups, based on 70-gene expression profile (GEP) risk model[27]. MF molecular subgroup includes t(14;16) and t(14;20) translocations resulting in activation of c-*MAF* and *MAFB* proto-oncogenes while the PR molecular subgroup represents an highly proliferative disease[28]. A DNA sequencing study in 14 patients with matched BM and extramedullary disease localizations showed a high incidence of activating *RAS* mutations in 67%

BM samples and 64% extramedullary samples[29]. The frequency of *RAS* mutations in this series was higher compared to what was previously reported in newly diagnosed and relapsed MM[29]. In three of six patients with identical IgH sequences in medullary and extramedullary plasma cells, *RAS* mutations were only observed in plasma cells from extramedullary sites, thus suggesting a role of RAS mutations in transition to EMM [37]. Moreover, the activating *BRAF* V600E mutation has been reported to be increased in patients with EMM compared to MM without extramedullary disease[30]. Genetic characterization by sequencing the exomes of triple-matched BM, extramedullary plasmacytomas, and circulating tumor cells (CTCs), showed that 68% of total mutations were simultaneously present in CTCs, BM tumor cells and extramedullary plasmacytomas.

The frequency of mutations found in both extramedullary plasmacytomas and CTCs was 15,2% while the mutations exclusively found in extramedullary plasmacytomas was 3.2%[31]. The similar genetic profile between the three groups suggests that, despite the spatial heterogeneity, dissemination in the extramedullary sites is not induced by specific genetic alterations and reflects a constant dynamic migration of PCs outside of the BM[31].

The transcriptional state of matched BM PCs and CTCs was investigated in 32 MM patients. CTCs overexpressed genes involved in migration, adhesion, inflammation and hypoxia, whereas proliferation genes were less expressed in CTCs compared to BM PCs[32].

A single-cell RNA-seq study on EMM cells samples was performed on 15 patients and revealed a transcriptional upregulation in genes involved in cell-cycle progression, glycolysis, oxidative phosphorylation compared to matched BM samples. EMM cells overexpressed CCL3, IL6 and IL6R, suggesting a potential role of cytokine-induced signals, through a paracrine and autocrine way, in EMM spread. A trajectory analysis of paired BM and EMM samples showed a branched evolution[33].

Metastasis Associated Lung Adenocarcinoma 1 (*MALAT1*) is a long non-coding RNA molecule (lncRNA) involved in cancer metastatic process. *MALAT1* expression was significantly up-regulated in PCs of EMM patients in comparison to PCs of MM patients. Despite the fact that *MALAT1*

localization on chromosome 11, it did not result in up-regulation for MM patients with trisomy 11 or t(11,14)[34]. Furthermore, another study reported as the microRNA miR-130a is significantly expressed in PCs of extramedullary plasmacytomas compared to BM PCs[35].

The use of novel agents such as proteasome inhibitors (PIs) and immunomodulatory drugs (IMiDS) has been hypothesized as a risk factor for the development of EMD. However, recent studies showed no evidence of a strong association. Varga *et al* reported no differences in the incidence of EMD relapse between two group of newly diagnosed patients treated with bortezomib (PI) and lenalidomide (IMiD) combinations or lenalidomide based combination without PIs[36].

Furthermore, Mangiacavalli *et al* reported an increased incidence of EMD relapse in patients with a treatment duration ≥6 months and with >2 previous lines[13]. Collectively this clinical data suggests that the prolonged survival reached after novel agents introduction, therapy length and its total burden may favor a clonal evolution with PCs migration outside BM, otherwise a clear association with a specific class of drug is lacking.

Role of adhesion molecules in dissemination

EMM is a clinical manifestation of MM in which the clonal PCs lost their dependence on the BM microenvironment. In a *seed and soil* model of disease bone marrow stromal cells (BMSCs), endothelial cells, immune system components, osteoblasts, osteoclasts enhance MM cells growth, survival and tumor progression via cell-cell interaction and production of soluble molecules. Different adhesion molecules that mediate BM homing have been described and the modulation of their expression supports clonal PCs migration outside the BM and their dissemination through the bloodstream.

The $\alpha_5\beta_1$ integrin (also called CD49e or VLA-5) is expressed by normal PCs and primary MM samples and interacts with fibronectin (FN), a component of extracellular bone marrow matrix (EBM). VLA-5 is significantly down-regulated in PCs from extramedullary disease localizations and in human myeloma cell lines (HMCLs)[37]. The $\alpha_4\beta_1$ integrin (also designated as CD49d or VLA-4), is expressed by neoplastic PCs and mediates a strong interaction with FN and vascular cell–adhesion molecule 1 (VCAM-1), expressed by BMSCs [38]. The chemokine CXCL-12 (also known as SDF-1) is produced by BMSCs and binds to the chemokine receptor CXCR4 expressed by MM cells. This binding can modulate the strength of the VLA-4-VCAM-1 interaction and enhances BM homing[39]. Roccaro *et al* demonstrated that extramedullary-prone syngeneic HMCLs showed a significant enrichment in epithelial-mesenchymal transition genes as well as higher levels of cell surface CXCR4 expression compared to parental cells[40].

CD44 is a receptor for hyaluronan, which is a component of extracellular matrix, and it is expressed by MM cells in different isoforms [41]. Furthermore, CD44 is involved in neoplastic PCs transendothelial migration through the anchorage with endothelial cells[42]. CD44 is an important mediator of adhesion to BM and CD44 knockdown in HMCLs resulted in a significant reduction in migration in response to SDF-1α and adhesion to fibronectin[32].

CD56 (also designated as NCAM) is aberrantly expressed by neoplastic PCs compared to normal counterparts and regulates an homotypic interaction with osteoblasts in the BM niche[43].

CD56 is down-regulated on BM PCs of EMM patients and in neoplastic plasma cells of PCL patients, suggesting a potential correlation between the lack of this marker and dissemination capacity[4,37].

CD38 is a surface marker that plays the dual function of adhesion molecule and ectoenzyme[44]. CD38 can mediate the interaction with endothelial cells and between PCs themselves through an heterotypic binding with CD31 [45]. It is reported as universally expressed in MM cells, although its intensity is lower when compared to normal PCs[46,47].

Dahl *et al* reported the data of CD56 and CD44 expression on PCs collected simultaneously from BM and extramedullary lesions in seven patients. The clonality between the PCs from different sites was confirmed by heavy chain immunoglobulin gene sequencing. In all extramedullary lesions CD56 was negative. Conversely, BM CD56 expression was heterogeneous across all the patients, with five cases with different levels of expression, one patient with strong positivity and one patient, who subsequently developed a PCL, was CD56 negative. Moreover, a trend towards an increased CD44 up-regulation in extramedullary sites was described[48].

Rasche *et al* described CD56 negativity in 6 out of 10 extramedullary lesions[23]. Weinstock et al. analyzed 11 extramedullary specimens. CD56 was negative in 65% of cases while CD44 was highly expressed in 92% of cases. CXCR4 was positive in 38% of cases[49]. Furthermore, the integrin VLA4 genes resulted up-regulated in EMM samples underlining a potential role of adhesion molecules in MM growth outside of the BM[33].

A phenotypic study on matched PB and BM samples revealed that CTCs displayed a down-regulation of surface integrins (CD49d/CD49e) and other adhesion molecules (CD56, CD38) compared to BM PCs while CD44 and CXCR4 resulted overexpressed[50].

Moreover, a study comparing median fluorescence intensity (MFI) of healthy donor BM PCs to BM MM cells and MM CTCs showed a decreasing expression of CD38, CD49d and CD56 [51]. Overall, this data highlights the existence of phenotypic differences between CTCs, extramedullary PCs and

BM counterpart justifying a decreased dependence on the bone marrow microenvironment and a greater tendency to metastasize.

Clinical features and diagnostic tools in EMM

EMM, particularly, the extra-skeletal and soft tissue variant, is associated with poor prognostic features, such as high LDH level[52]. In a series of 24 patients with EM-E LDH level was increased above the normal limit in about 80% of cases[23]. Avivi *et al* reported in a retrospective cohort of 127 EM-E patients an increased LDH levels in 59%[53]. Noteworthy, LDH and high-risk cytogenetic features, frequently reported findings in EMM, are now incorporated in a new revised MM international staging system and associated to worse prognosis[53]. LDH increase is also associated to early progressive disease (≤ 18 months) in 926 newly diagnosed MM patients from the CoMMpass study[54].

Regarding the myeloma subtype, EM-E patients showed more frequently a light chain disease and a worse renal function compared to MM patients and a light chain escape from intact immunoglobulin has been described during extramedullary relapse[12,55]. Furthermore, a high frequency of EMM has been reported in IgD subtype[56]. The light chain secretory and non secretory disease can be considered as a sign of de-differentiation and cell immaturity, often associated with plasmablastic morphology[57]. Another feature described in EMM is the dissociation between the treatment response in BM and extramedullary localizations. Indeed, extramedullary relapse without BM involvement has been reported suggesting a different sensitivity to the anti-myeloma drugs or stem cell transplantation[58]. EMM can appear at relapse despite clearance of BM PCs below the sensitivity of 0,01% in a flow cytometry essay, suggesting that sensitive imaging methods are necessary to monitor the disease response during follow up[59].

Recently, an International Myeloma Working Group (IMWG) consensus updated the imaging recommendations of MM diagnosis and monitoring by incorporating greater sensitivity imaging techniques such as low-dose whole-body computed tomography (LD-CT), positron emission tomography/computed tomography (PET/CT), and whole-body magnetic resonance imaging (WBMRI) for the detection of bone or extramedullary lesions which represent a

symptomatic MM defining event[60].

As compared with conventional X-ray, LD-CT can assess more accurately the extent of bone destruction, detecting also small lytic lesions (<5 mm) that are usually below the X-ray resolution threshold. LD-CT can also identify the presence of associated extra-osseous disease, although it has a lower ability to diagnose visceral involvement[61]. ^{18}F-FDG PET/CT combines functional imaging assessed by PET with morphological evaluation analyzed by CT. The most significant advantages are the assessment of disease burden in the whole body, including extra-medullary disease, and the ability to distinguish between metabolically active and metabolically inactive lesions[62].

The incidence of extramedullary disease detected by PET/CT in newly diagnosed, transplant eligible MM was 6-10% and it was associated with a worse prognosis in multivariate analysis [63,64].

In a PET/CT study involving newly diagnosed and relapsed/refractory (RRMM) EMM patients a multifocal involvement was detected in 71% of cases and the involvement of some anatomic sites such as liver, lungs, and muscles was associated with a shorter survival *[65]*.

Due to its higher resolution for soft tissue, MRI offers a good characterization of EMM particularly in paravertebral sites with spine compression and in intracranial space. In one study, bone-related lesions were hypointense to isointense on T1-weighted images and hyperintense on T2-weighted images while lesions non-contiguous to the bone were more often hypointense on T2-weighted images. MRI was helpful in radiation therapy and surgical treatment planning in 60% of patients[66].

Therapy and prognosis of EMM

EMM is an extraordinarily heterogeneous disease and patient management can be particularly challenging. Furthermore, EMM is relatively infrequent disease and patients with nonsecretory EMM, PCL, and CNS myeloma are often excluded from clinical trials, thus information regarding treatment is derived from retrospective series and a standard therapy has not been established yet.

In a large University of Pavia cohort, 74% of MM patients with and without extramedullary disease (EMD) were treated with cyclophosphamide, thalidomide, and dexamethasone while 26% were treated with novel agents. Thirty-five percent of all patients underwent autologous stem cell transplantation. In a multivariate analysis, EMM conferred a negative prognostic impact on overall survival (OS) and progression free survival (PFS) compared to MM without extramedullary lesions. The use of novel agents such as bortezomib, thalidomide and lenalidomide or autologous stem cell transplantation was not associated with increased risk of EMM at relapse[10]. In the whole patient population of a three-arm PETHEMA trial comparing conventional chemotherapy versus thalidomide/dexamethasone, versus bortezomib/thalidomide/dexamethasone (VTD), the incidence of EMM was 18%. Overall, the progressive disease rate during the induction phase was significantly higher in patients with extramedullary involvement (27% *vs* 12%) and the lowest disease progression rate was observed with VTD[67]. In another study comparing the outcome of newly diagnosed MM patients with and without EMD, EMD group treated with chemotherapy had significantly worse OS compared to those without EMD. High-dose therapy (HDT) followed by autologous stem cell transplantation was associated with a significantly improved OS in both groups and overcame the negative prognostic impact of EMD[68].

Usmani *et al* reported a significant reduction of 5 year survival (31% versus 59%) in newly diagnosed patients with soft tissue EM-E compared to patients without EMM treated with different therapies including Total Therapy Protocol[27].

In a metanalysis of eight Fonesa Onlus and Hovon Foundation clinical trials, including 2,332 newly diagnosed MM patients mainly treated with lenalidomide as a first line of therapy, the outcome of EMM patients was analyzed. Most EMM patients presented bone-related plasmacytomas (91%). Interestingly, the median PFS was superimposable to that of non-EMM patients (25.3 vs 25.2 months) while the OS was significantly different (63.5 vs 79 months), suggesting a detrimental effect of this feature in the later disease phases. The OS was not influenced by treatment type that included novel agents such as bortezomib and lenalidomide and HDT[69].

A large study from an EBMT registry compared the outcome of EM-E, EM-B and no EMM patients after high dose therapy. EM-E patients had significantly inferior 3-year PFS (39,9% vs 50% vs 47.9%) and 3-year OS (58% vs 77,7% vs 80%) in comparison to MM and EM-B patients. No significant differences where observed in the outcome between EM-B and no EMM patients[12].

A retrospective multicenter study evaluated newly diagnosed and relapsed EMM patients and ISS stage (I vs II and III), time of EMM diagnosis (initial diagnosis vs relapse) and type of extramedullary involvement (EM-B vs EM-E) were associated with a better OS in a multivariate analysis [70].

In another retrospective study the median OS from extramedullary relapse was very poor (5 months) and the median OS from the initial MM diagnosis in patients with EMD was significantly decreased compared to those without extramedullary relapse (38 vs. 59 months)[71].

In a cohort of 226 RRMM, previously treated mainly with novel agents, EMM incidence at relapse was 24% and in about 50% of patients the EMD appeared at first relapse. A significant difference in the prognosis between EM-B and EM-E was observed with an OS of 12 versus 5 months from extramedullary diagnosis respectively, demonstrating a potential different biological behavior among the two entities, with a more aggressive clinical course in EM-E patients[72].

Regarding the use of proteasome inhibitors (PIs) in EMM treatment the results of first in class proteasome inhibitor bortezomib are conflicting. Rosiñol *et al* reported that bortezomib treatment induced plasmacytoma disappearance in three out four RRMM patients with extramedullary

plasmacytoma[73]. In contrast other reports underlined disease progression and bortezomib resistance in this specific setting[74].

Carfilzomib, a second generation irreversible proteasome inhibitor, showed a limited efficacy in RRMM patients with EMD. Zhou *et al* reported a biochemical overall response rate (ORR) of 57% in 45 patients with extramedullary RRMM treated with carfilzomib- based combinations. Interestingly, the response on extramedullary lesions, evaluated by imaging techniques, was only 27% suggesting an intra-tumor heterogeneity. The median PFS was 5 months and the median OS was 10 months with worse PFS and OS in EM-E compared to EM-B[75]. In a multicenter retrospective study investigating carfilzomib in RRMM the ORR was 40% vs 49% in EMM and no EMM patients respectively and a significant reduction of duration of response was observed in EMM group (3,9 months vs 9,3 months)[76].

Results about the use of of immunomodulatory drugs (IMiDS) in EMM are also available. Thalidomide has been reported as poor effective in RRMM patients with an ORR of 0% compared to ORR of 59% in patients without EMD. These results suggests that the efficacy and the anti-angiogenic properties of the drug are dependent on tumor-associated microenvironment[77].

Lenalidomide and pomalidomide are second and third generation IMiDS that compared to thalidomide induced a direct apoptosis on MM cells and is a stronger enhancer of NK and T cells[78]. In a serie of 18 RRMM patients with extramedullary lesions treated with lenalidomide the ORR was 61% with a complete disappearance of plasmacytoma in about 40% of patients. The median OS and PFS were 14.6 and 9.8 months, respectively[79].

Short *et al* reported a response of 30% in EMM patients treated with third generation IMiD pomalidomide, despite a reduced survival in EMM patients compared to others was reported[80].

In contrast in a spanish retrospective study only 9% of 21 RRMM patients with extramedullary lesions obtained a response in terms of plasmacytoma reduction. No patients with EM-E responded to pomalidomide based treatment. The median PFS from was 1.7 months and the median OS was 4.5 months[81].

Among alkylating agents, melflufen, a first-in-class peptide-drug conjugate, was investigated in a prospective study in RRMM patients heavily pre-treated with previous therapies including daratumumab or pomalidomide. The response rate among EMM patients was 24% and the median PFS 2.8 months while the ORR was 29% and PFS 4.2 months in the entire population suggesting a consistent efficacy also in this patient subset[82].

Selinexor, a first-in-class oral Selective Inhibitor of Nuclear Export (SINE), was investigated in 122 heavily pretreated RRMM showing an ORR of 26%[83]. In a subgroup of EMM patients with plasmacytoma response assessment the ORR was 18.5% suggesting the efficacy of this drug despite refractoriness to Daratumumab, PIs and IMiDs[84].

The efficacy of monoclonal antibodies anti-CD38 daratumumab and isatuximab) and anti SLAM7 (elotuzumab) in EMM is poorly investigated. The pooled analysis from two trials which investigated daratumumab as single agent in RRMM showed an ORR of 31% in the entire population, while the response in patients with extramedullary disease was inferior (16.7%)[85].

Isatuximab in association to pomalidomide and dexamethasone was investigated in a phase 3 trial including RRMM who had received at least two prior lines of therapy. The ORR (60.4% vs 35.3%) and PFS (11.5 months vs 6.5 months) were superior in the experimental arm compared to the control arm including pomalidomide and dexamethasone[86]. In EMM patients included in the study, despite the trend in efficacy was preserved (response rate 50% vs 10%), the PFS of isatuximab arm was shorter compared the entire population (4.57 months)[87].

Elotuzumab based combination therapy was investigated in a retrospective study including 15 RRMM patients with extramedullary lesions with a median of 4 previous therapy lines.

A biochemical response was observed in 40% of cases, but only 27% with imaging follow-up obtained a plasmacytoma reduction. Interestingly, both before and after elotuzumab therapy, BM and EMD samples showed a strong SLAM7 expression, suggesting other mechanisms other than target loss or reduction involved in the antibody resistance[88].

In a study evaluating the efficacy reduced-intensity conditioning (RIC) allogenic stem cell

transplantation (allo-SCT) in 70 MM patients, the incidence of relapse with extramedullary lesions was 37% indicating that graft *versus* myeloma effect mediated by donor T lymphocytes is less effective in sites outside the BM[89]. Furthermore, in other study EMD before allo-SCT is associated in a multivariate analysis with shorter OS and PFS[90].

Aim of the study

Extramedullary disease is defined by the presence of clonal plasma cells in a site outside of the bone marrow in a patient with multiple myeloma. This entity is uncommon at diagnosis and represents a high risk disease feature conferring a poor prognosis and treatment resistance.

A variable expression of adhesion molecules, including CD44 and CD56, has been hypothesized in the pathophysiology of the extramedullary spread. CD38 is a transmembrane glycoprotein, highly and uniformly expressed by bone marrow plasma cells, which plays a dual role as a receptor and ectoenzyme. CD38 is considered a hallmark of MM cells however its expression by extramedullary plasma cells is still unknown. Recently, anti-CD38 targeted monoclonal antibodies, such as Daratumumab, have been included in the therapeutic armamentarium of multiple myeloma and the lack of CD38 by multiple myeloma cells may confer resistance to an anti-CD38 antibody-based approach[91].

The aims of this thesis is define the expression profile, including CD38, in the extramedullary disease of 22 multiple myeloma patients, treated from 1999 to 2020, affected by plasma cell dyscrasia and presenting a biopsy proven extramedullary extraosseous plasmacytoma.

A comparative analysis of phenotypic differences was performed in order to disclose antigenic changes that may contribute to extramedullary disease onset.

Patients, materials, and methods

This is a retrospective, single center study conducted at Hematology Unit, Azienda Ospedaliero-Universitaria di Parma.

Three-hundred and sixty-nine adult (≥18 years) consecutive patients with newly diagnosed MM and PCL between February 1999 and December 2019 where analyzed for the presence of a biopsy proven diagnosis of EM-E at any time of follow up. Only patients with soft tissue or solid organ, other than bone, biopsy proven plasmocytoma were included. Patients with only skeletal paraosseous plasmacytomas were excluded from the analysis.

Disease stage at diagnosis was determined according to the International Staging System (ISS; I-III). Response to therapy and remission were defined according to standard International Myeloma Working Group (IMWG) criteria[92]. Time to progression to EM-E was calculated from the date of diagnosis of MM until the date of disease progression with soft tissue plasmacytoma appearance. Clinical data included, type of disease, sex, age at the time of diagnosis and at the time of EMD, myeloma type, ISS stage, cytogenetic abnormalities, number and types of therapies, response, and OS. OS survival from MM diagnosis and from EMD diagnosis was analyzed using the Kaplan-Meier method; subgroups of patients were compared with the log-rank test.

The diagnosis of plasma cell neoplasm was made on tissue sections as part of routine clinical practice in accordance with the 2008 World Health Organization Classification system.

BM aspirates and bone marrow biopsies were obtained from the iliac crest after informed consent according to the Declaration of Helsinki. Study protocol was approved by the University of Parma Institutional Review Board (Parma, Italy).

Patient bone biopsies were fixed in formalin at 10%. The samples were embedded in paraffin , so as to allow the cut to the microtome thin sections (3 μm). Bone biopsy sections were incubated with the following primary antibodies: CD38 (clone SP149, ready to use, Ventana/Roche), CD56 (clone

MRQ-42, ready to use, Ventana/Roche), CD44 (clone SP37, ready to use, Ventana/Roche), CD138(clone B-A38, ready to use, Ventana/Roche). The sections were immunostained in automatic immunostaining Benchmark Ultra- Roche with HRP polymeric system Ultraview DAB Detection Kit (Ventana/Roche) in accordance with the manufacture's specifications. Negative controls consisted of substituting normal serum for the primary antibody.

Images were captured by DP22 digital camera (Olympus; Hamburg, Germany) and analyzed with the OLYMPUS Stream software, adjusting tone and contrast to ensure the best image quality.

All the immunostains were scored using a semiquantitative evaluation of the percentage of CD38, CD44, CD56 on tumor cells on a 5-tiered scale (Immunohistochemical scores: score 0, < 5% positive tumor cells; score 1, 5% to 24% positive tumor cells; score 2, 25% to 49% positive tumor cells; score 3, 50% to 75% positive tumor cells; score 4, > 75% positive tumor cells.

All examinations were revised by two hemopathologists. In case of discrepancy, the mean of the two values was reported.

Bone marrow samples were analyzed using FACSCanto II flow cytometer and FACS Diva Version 6.1.3. software (Becton Dickinson). To identify neoplastic plasma cells the following fluorochrome conjugated monoclonal antibodies were included: CD19-PE-Cy7 (BD), CD45-APC7 (BD), CD56-APC (BD), CD38-FITC (BD), CD138-PE (BD). The absence of CD45 and CD19, and the acquired expression of CD56 were used to identify an aberrant phenotype. The percentage of positive cells expressing specific antigens as well as the MFI of expression on the neoplastic PCs population were analyzed with Graph Pad Prism for Windows, version 5 (Graph Pad Software Inc., San Diego, CA, 2005)

Fresh $CD138^+$ plasma cells were purified from isolated mononuclear cells with immunomagnetic method using anti-CD138 monoclonal antibody-coated microbeads (MACS, Miltenyi Biotec., Bergisch-Gladbach, Germany).

Fluorescence in situ hybridization analysis was performed on fresh $CD138^+$ plasma cells, testing the presence of: del(13q) (D13 S319SO/CEP 12SG, Metasystems, Altlussheim, Germany); del(17p)

(LSI ATM SG/p53SO, Metasystems); hyperdiploidy (ON9RED/15GREEN, Kreatech, Diagnostics, Durham, NC, USA); amp(1q21); del(1p32) (XL1p32SG/1q21SO, Metasystems) and chromosome 14 translocation (14 BREAK-APART, Metasystems). t(4;14) (FGFR3SO/IGHSG, Abbott Laboratories, Abbott Park, IL, USA), t(11;14) (LSI IGH/CCND1XT, Abbott Laboratories) and t(14;16) (IGH/MAF, Abbott Laboratories) were performed in $CD138^{+}$ cells carrying chromosome 14 translocation. Patients were divided into prognostic groups: those with either amp(1q21), del(1p32) del(17p), t(4;14), t(14;16), t(14;20) were considered to be high risk while others were considered to be standard risk.

Results

Clinical characteristics

A total of 22 EM-E MM patients met the predetermined criteria for the inclusion in the study. Twenty patients presented an initial diagnosis of MM while two patients were affected by primary PCL. Noteworthy, one MM patient showed neoplastic PCs in the peripheral blood during diagnostic process but he does not meet the criteria for PCL definition as the PCs were inferior to 20% of the white blood cell count and less than 2000/mmc. Other two patient developed secondary PCL at the time of relapse. Median age at the time of the diagnosis was 67 years old (range 47-76). Men outnumbered the women and were the 60% of the entire population.

Four patients presented EM-E at diagnosis while 18 patients at relapse.

The median time to extramedullary disease appearance during relapse phase was 29 months (range 9-201 months). The most frequent MM subtype was light chain MM (41%) followed by IgG and IgA. IgM and IgD subtypes were absent.

Thirty-five percent of the patients presented a high stage (ISS III) a median LDH value, available in 14 patients, was slightly elevated and equal to 517 (normal value < 500 U/L) with about half of the patients with a value above the normal range (Table 1).

The most frequent CRAB criteria was anemia (73%) followed by bone disease with lytic lesions (64%), renal failure (27%) and hypercalcemia (14%).

In the study population a high BM tumor burden was documented with a median BM monoclonal plasma cell infiltration equal to 60% (range 0 – 90%).

FISH analysis was available for 14 patients with diagnosis BM sample and for 5 patients at the time of MM relapse. In relapsed phase FISH analysis was performed in peripheral blood neoplastic PCs in one case, in PCs from extramedullary plasmacytoma in one case, and in BM PCs in the remaining cases.

Overall 14 of 19 (79%) patients with available FISH at any time point considered, showed two or more cytogenetic alterations suggesting and high clonal heterogeneity in this subgroup of MM patients. Fourteen of 19 (73%) patients were included in a high risk prognostic group.
The abnormalities of chromosome arm 1q21, including gain (53%, 10 patients) and amplification (21%, 4 patients), were the most frequent cytogenetic alterations reported (74%, 14 patients), followed by del(13q) (58%, 11 patients), del(1p32) (42%, 8 patients), hyperdiploidy (21%, 4 patients) and del(17p) (11%, 2 patients).
The 14q32 disruption was reported in 6 patients, t(11;14) was identified in 2 patients (11%), t(4;14) in 2 patients (11%), while t(14;16) and t(14;20) were found in single cases (Figure 1).

Features and phenotypic analysis of extramedullary plasmacytomas

Overall, 12 of 22 (55%) patients with EM-E developed multiple plasmacytomas.
The most common site was soft tissue (muscle, subcutaneous fat) and liver/spleen which represents the 42%, of the total extramedullary localizations, 21% and 21% respectively, followed by lymph nodes (15% of the total number), upper respiratory tract, including oro-maxillary space, (11% of the total number), testes (7% of the total number), stomach and bowel (7% of the total number), skin (7% of the total number), lung and pleura (5% of the total number), pancreas (2% of the total number), kidney (2% of the total number), central nervous system (2% of the total number) (Figure 2). Interestingly, in 8 patients (36%) the extramedullary lesions, at biopsy time point, were dissociated from BM without evidence of intramedullary monoclonal plasmacytosis in sample from iliac crest biopsy. To identify phenotypic differences, an immunohistochemical analysis, including, CD38, CD56, CD44 immunostains, was performed on BM samples and extramedullary disease (EMD) samples. Whenever possible extramedullary a patient specific sample was compared with matched BM sample obtained at the same time point during the course of the disease. The

extramedullary cases without a matched BM sample available at the same time point were compared with diagnosis BM sample.

An evaluation of the expression was performed applying a score as reported in the Methods section. The CD38, CD56 and CD44 scores on BM and EMD samples are reported in Table 2.

CD38 immunohistochemical score was analyzed in 19 BM samples and in 25 EMD samples.

In BM samples, high immunohistochemical CD38 score (3-4) was reported in 15 out of 19 samples (79%) while 2 samples were negative (score 0). In EMD samples, CD38 was negative (score 0) in 3 out of 22 samples (14%) and showed a low score (1-2) in 5 of 22 (23%) patients (Table 2).

A comparative analysis, regarding CD38 expression on BM and EMD samples was conducted in 19 patients. In 11 patients the expression was concordant: 10 patients with high scores both in EMD an BM and 1 patient negative in BM and EMD. In 8 patients the expression was discordant. Five patients with discordant CD38 expression (26%) showed a down-regulation of CD38 in the EMD samples compared to BM, 2 patients with score 0 and 3 with score 1-2. Three discordant patients (16%) showed a slight up-regulation of CD38 in BM compared to EMD (Table 3).

CD56 immunohistochemical score was analyzed in 18 BM samples and in 22 EMD samples.

In BM samples, high immunohistochemical CD56 score (3-4) was reported in 8 out of 18 samples (44%) while 9 samples (50%) were negative (score 0). In EMD samples, CD56 was negative (score 0) in 14 out out of 22 samples (64%) and showed a high score (3-4) in 5 of 22 (23%) patients (Table 2). A comparative analysis of CD56 expression on BM and EMD samples was conducted in 18 patients. In 13 patients (72%) the expression was concordant, 5 patients (28%) with high scores both in EMD an BM and 8 patients (44%) negative in BM and EMD (table 4).

In 5 patients the expression was discordant. Three patients with discordant CD56 expression (17%) showed a strong down-regulation of CD56 in the EMD samples compared to BM (Table 4).

CD44 immunohistochemical score was analyzed in 16 BM samples and in 20 EMD samples.

In BM samples, high immunohistochemical CD44 score (3-4) was reported in 8 out of 16 samples (50%) while 3 samples were negative (score 0). In EMD samples, CD44 was negative (score 0) in 3 out of 20 samples (15%) and showed a high score (3-4) in 14 out of 20 (70%) patients (Table 2).
A comparative analysis of CD44 expression on BM and EMD samples was conducted in 16 patients. In 8 patients (50%) the expression was concordant, 6 patients (38%) with high scores both in EMD an BM and 2 patients (12%) with low score (1-2) both in BM and EMD (table 5).
In 8 patients the expression was discordant. Five patients with discordant CD44 expression (31%) showed an up-regulation of CD44 in the EMD samples compared to BM (Table 5).
A down-regulation of CD38 antigen in a patient subset with EMD was observed. A flow cytometry analysis was performed on BM PCs in order to identify if CD38 median fluorescent intensity (MFI) could be associated to a low immunohistochemical score in EMD samples. CD38 expression levels were analyzed in 10 patients with a high immunohistochemical score in EMD samples and in 7 patients with a low immunohistocehmical score in EMD samples.
CD38 antigen was expressed by neoplastic PCs in all the BM samples of the patients analyzed. However, a substantial heterogeneity in the intensity of CD38 expression was observed.
Patients with a low EMD immunohistochemical score in EMD had lower baseline CD38 expression levels on BM PCs compared with patients with a high immunohistochemical score (Mean Log_{10} MFI CD38: 4,3 *vs* 3,7; *p* .004) (Figure 5).

Prior therapies, EMD treatment and response

Prior therapies were analyzed in 18 patients with RRMM of the study population.
RRMM patients prior to extramedullary appearance received a median of two (range 1-6) therapy lines. About a quarter of patients underwent high-dose chemotherapy followed by autologous hematopoietic stem cell transplantation (SCT) (n = 5; 26%). All but one patients had received treatment with proteasome inhibitors (PIs) bortezomib, carfilzomib and ixazomib and 78% had been exposed to immunomodulatory drugs (IMiDs) thalidomide, lenalidomide and pomalidomide.

The exposition to IMiDs and PIs does not significantly differ in the group of patients with low CD38 immuhistochemical score in EMD compared to the group with high CD38 score.

Most of patients developed EMD during the course of last line of therapy (n = 14; 78%) while the remaining developed EMD after a period of treatment-free remission (n = 4; 22%) (Table 6).

At the time of EMD relapse the majority of patients were on PIs based treatment (n = 9), followed by IMiDs based treatment (n = 7). Among PIs, bortezomib (n = 5) was the drug more frequently employed followed by carfilzomib (n = 3) and ixazomib (n = 1). Among IMiDs, lenalidomide (n = 6) was the drug more used followed by pomalidomide (n = 1). In two patients EMD occurred during the exposition to the association of carfilzomib, lenalidomide, dexamethasone (n = 2; 14% and other 2 two patients (n = 2; 14%) were on daratumumab treatment during EMD development.

EMD treatment in the study population was extremely heterogeneous.

Twenty patients received an active treatment for EMD. Surgery, radiation therapy, conventional chemotherapy, novel agent-based therapies, autologous SCT and allogeneic SCT were employed. Two patients did not receive an active treatment of EMD because double refractoriness to bortezomib and lenalidomide and a poor performance status. Radiation therapy as local therapy was delivered in two patients mainly with a disease control intent, rather than curative. Orchiectomy, a surgical procedure, was performed in two patients with an isolated extramedullary relapse in the testes. Conventional chemotherapy like CVAD (hyper- fractionated cyclophosphamide with conventional vincristine, doxorubicin, and dexamethasone) and PACE (continuous-infusion cisplatin, doxorubicin, cyclophosphamide, and etoposide) was administered in three young (age ≤ 70) patients (n = 3, 15%). Among novel agents PIs were the drugs more frequently used (n = 8, 40%) mainly in association with alkylating agents (melphalan, cyclophosphamide, bendamustine) or anthracycline (doxorubicin) (n = 4, 20%). IMiDs were administered with different therapeutic schemes mostly in patients already exposed to PIs (n = 7; 35%). Anti-CD38 monoclonal antibody daratumumab was used in one patient in association to conventional chemotherapy.

High dose chemotherapy followed by autologous SCT was performed 4 out of 20 (20%) patients while allogenic-SCT was performed in only one patient.

The biochemical response was evaluated according IMWG criteria. Response based on extramedullary tumor reduction wherever possible was evaluated by sensitive imaging techniques (PET/CT, CT, MRI). In the study population the ORR (≥ partial response) to treatments was reported in 7 out of 20 patients (35%), mainly treated with chemotherapy regimen, PIs based combination and autologous-SCT. Three patients (15%) achieved a complete response (CR), 2 with EMD at diagnosis and one with a localized form of extramedullary relapse of the testes. The majority of patients, 13 out of 20 (65%), were considered refractory to the treatment. Three patients with multiple extramedullary lesions achieved a mixed response with resolution or reduction of some tumor masses and increase in dimension or occurrence of others. None of the patients treated with lenalidomide or pomalidomide without PIs achieved an objective response (Table 7).

The median OS from MM diagnosis of the entire study population was 49,3 months (95 % CI: 30,97 – NA) (Figure 6). The median OS from EMD diagnosis for the entire population was 13,1 months (95% CI: 6,8 – 25,3) (Figure 7). The median OS from EMD diagnosis of patients with low CD38 immunohistochemical score was reduced compared to the median OS of patients with high CD38 immunohistochemical score (7,3 vs 18,05 months) (Figure 8).

Discussion and conclusion

Soft tissue extra-osseous EMD is considered a rare MM feature at diagnosis (incidence 1,7%-4,5%) while the frequency increases at relapse (incidence 3,4% - 10%). The real incidence, that in the past years may be underestimated, has increased in the last decade following the more frequent employment of sensitive imagining techniques (PET/CT, WBMRI) during disease staging at diagnosis and relapse[93]. EM-E patients compared to MM without EMD showed a high incidence of unfavorable factors such as increased LDH, elevated ISS and R-ISS and high-risk chromosomal abnormalities[6]. In this retrospective study involving 22 patients with a biopsy-proven EM-E a high incidence of adverse prognostic factors was described according to previous reports[23,53,94].
To be more specific, the majority of patients (46%) at diagnosis presented and ISS III and the median LDH value was above normal range (517, normal value < 500 U/L). Furthermore, three patients, including two patients with overt PCL, presented more than 5% of neoplastic PCs in peripheral blood that predicts a poor prognosis[5]. About 70% of patients with available FISH were classified in high risk group. According to other study, gain and amplification of chromosome arm 1q21 were the most frequent chromosomal aberration found in our cohort and often associated to other high risk chromosomal abnormalities[26]. Copy number of 1q21 are secondary chromosomal events, often present at sub-clonal level, with a frequency that increases across the different disease phases ranging from 30–50% of newly diagnosed MM to 50–80% of RRMM[95]. Moreover, two patients presented chromosomal translocations, t(14;16) and t(14;20), included in the high risk MF molecular subgroup, whose incidence is reported as over-represented in in patients with EM-E[27]. In contrast to other works, that reported the lack of t(11;14) in EM-E and argued a protective role of this standard risk translocation in the EMD development, two patients in this study showed this cytogenetic alteration[36,96]. In addition, in this analysis the incidences of del(17p) and t(4;14) were relatively low, both 11%, in opposition with other reports[24,26].

In this analysis the predominant locations of extramedullary lesions were soft tissue, liver and spleen followed by limph-nodes and most patients presented multiple lesions supporting the proposed pathophysiologic mechanism of haematogenous dissemination[94].

The pathogenesis of the extramedullary spread remains poorly elucidated. Some studies reported a different expression of receptors and adhesion molecules in extramedullary PCs compared to BM counterpart that may be involved in the dissemination. CD56 that can mediate the adhesion to BM osteoblastic niche is frequently described as down-regulated while CD44, a receptor involved in transendothelial migration, resulted up-regulated[23,48,49]. This different pattern of expression could reflect a spatial clonal evolution which characterize MM both at diagnosis and during the course of the disease[22]. According to previous reports, in this study CD56 was negative in 64% of extramedullary specimens and three patients with matched samples showed a lower immunohistochemical score in EMD compared to BM[23,48,49]. Furthermore, CD44 resulted highly expressed in 70% of extramedullary specimens and five patients with matched samples showed a higher immunohistochemical score in EMD compared to BM, confirming the role of this antigen in dissemination capacity outside of the BM[32,48].

CD38 is a transmembrane glycoprotein expressed by MM cells and recently has been recognized as an important therapeutic target for antibody based treatment modalities. Anti-CD38 monoclonal antibodies like daratumumab and isatuximab are now considered a new backbone in MM therapy both at diagnosis and relapse settings[97].

The immunohistochemistry analysis showed that 36% of total extramedullary samples were characterized by a low CD38 immunohistochemical score with a percentage of positive PCs inferior to 49%. Noteworthy, in about a quarter of patients with matched BM and EMD biopsies available a reduction of CD38 expression on neoplastic PCs was observed in extramedullary samples compared to BM. This observation is in accordance with the notion that CD38 MFI is higher in BM PCs , compared to CTCs, which are characterized by a weak dependence from BM microenvironment, high clonogenic potential and tendency to egress in peripheral blood[50].

In other blood disease such as acute myeloid leukemia the CD38 expression enables leukemic cells to be confined in the BM through adhesion to hyaluronate[98]. CD38 is universally expressed by MM cells but the intensity of expression is reported as highly heterogeneous among different MM patients and lower compared to normal PCs[46,47,99]. Some case reports described the lack of CD38 on MM cells both at diagnosis and relapse[100-102]. Interestingly, Ise *et al* reported the CD38 loss in one RRMM patient with extramedullary subcutaneous localizations of disease[102]. The exposure to anti-CD38 monoclonal antibody daratumumab induced a reduction of CD38 surface expression on MM cells in all treated patients and this observation could be explained by the shedding of CD38-daratumumab complex from MM cells membrane and by trogocytosis through the action of some effector cell subsets[103]. The modulation of CD38 expression can be influenced by microenvironment factors and by drugs. The interleukin-6 binding on MM cells activated JAK-STAT3 pathway, resulting in a downregulation of CD38 expression[104]. All-trans retinoic acid (ATRA) augments CD38 expression targeting retinoic acid responsive element located in the first intron of CD38 gene[105]. Furthermore, histone deacetylase inhibitors (HDACi), panobinostat and ricolinostat, selectively increased CD38 expression on MM cells[106,107]. IMiDs treatment up-regulated CD38 on neoplastic PCs with a molecular mechanism involving Ikaros and Aiolos degradation[108].

This study, for the first time, described the lack or reduced expression of CD38 in a consistent percentage of EMD samples with a potential therapeutic impact involving the efficacy of anti-CD38 monoclonal antibodies in MM patients with extramedullary lesions. This hypothesis is in line with two observations: a) clinical trials, with few EMM patients enrolled, disclosed that daratumumab and isatuximab are less effective in EMM patients compared to MM patients without extramedullary disease[85,87]; b) CD38 higher MFI on BM PCs is reported as predictor of response to daratumumab therapy[99]. Clinical data regarding the efficacy of daratumumab in EMM are needed and prospective Phase II trial of daratumumab combined with bortezomib, cyclophosphamide and dexamethasone in patients with EMM at diagnosis and first relapse is

ongoing (EMN19 study, NCT 04166565)[94].

However, the results should be interpreted with caution due to several limitations.

Particularly, this is a retrospective study conducted on a relative small number of patients and immunohistochemical analysis, performed as study technique, is lesser sensitive than other techniques such as flow cytometry or gene expression profiling.

This study involved mainly heavily pre-treated MM patients with a median of two previous lines. At the moment of EMD relapse only two out of 18 patients were on daratumumab treatment, one with high immunohistochemical score on extramedullary sample and one with low immunohistochemical score both in extramedullary sample and in BM sample of diagnosis.

The ORR to the treatment was about 30% with a short duration and the survival from EMD diagnosis was low (13 months) confirming the poor outcome of this MM clinical feature as reported by other studies[23,71,72,87]. Interestingly, two patients with testicular extramedullary relapse, one after auto-ASCT and one during lenalidomide maintenance, without other evidence of BM or systemic involvement, were treated with orchiectomy and are still alive after 17 and 21 months respectively suggesting that this localization, when isolated, may confer a better outcome[109].

Lastly, the OS according to CD38 immunohistochemical score in EMD was worse in the group with low CD38 score compared to the group with high score. This observation is in line with other study that showed how CD38 low $CD45^+$ $CD81^+$ phenotypic profile identified a group of MM patients with poor outcome, however in this study the patient number is low and this result does not take into account potential confounders such as heterogeneous treatment, ISS, cytogenetic risk, and age[110].

Figures and tables

Legend

Table 1. Patient Characteristics at MM Diagnosis.

Abbreviations: M: male; F: female; MM: multiple myeloma; PCL: plasma cell leukemia; LC: light chain, BM: bone marrow, PB: peripheral blood, EM: extramedullary plasmacytoma.

Table 2. CD38, CD44, CD56 Immunohistochemistry (IHC) Score

Abbreviations: ND: newly diagnosed; NA: not available; Pol PCs: polyclonal PCs; BM: bone marrow; EMD: extramedullary disease.

IHC scores: score 0, < 5% positive tumor cells; score 1, 5% to 24% positive tumor cells; score 2, 25% to 49% positive tumor cells; score 3, 50% to 75% positive tumor cells; score 4, > 75% positive tumor cells.

Table 3. CD38 IHC Score of Matched BM and EMD

Table 4. CD56 IHC Score of Matched BM and EMD

Table 5. CD44 IHC Score of Matched BM and EMD

Table 6. Prior Therapies

Abbreviations: EMD: extramedullary disease ; IMiDs: immunomodulatory agents; SCT: stem cell transportation

Table 7. Summary of previous myeloma treatments and treatment modality for EMD

Abbrevoations: EMD: extramedullary disease; RR: relapsed refractory; ND: newly diagnosed; RT: radiotherapy; CVAD: cyclophosphamide, vincristine, doxorubicin, and dexamethasone; ASCT: autologous stem cell transplantation; Dara: Daratumumab; PACE: dexamethasone, cisplatin, doxorubicin, cyclophosphamide, and etoposide; PD: progressive disease; VGPR: very good partial response; PR: partial response; CR: complete response

Figure 1. Interphase fluorescence in situ hybridization analysis (FISH) and incidence of incidence of chromosomal abnormalities in neoplastic plasma cells from 19 cases. Hy: hyperdiploidy.

Figure 2. Frequency of extramedullary localizations.

CNS: Central Nervous System

Figure 3. CD38 protein expression by plasma cells assessed by immunohistochemistry on bone marrow.

(A) Immunohistochemical score 0 (rare CD38 positive cells, <5%).

(B) Immunohistochemical score 1 (CD38 positive cells, 5% to 24%)

(C) Immunohistochemical score 3 (CD38 positive cells, 25% to 49%)

(D) Immunohistochemical score 4 (CD38 positive cells >75%)

Figure 4. CD38 protein expression by plasma cells assessed by immunohistochemistry on extra medullary lesions.

(A) Soft tissue sample, immunohistochemical score 0 (rare CD38 positive cells, <5%).

(B) Soft tissue sample, immunohistochemical score 1 (CD38 positive cells, 5% to 24%)

(C) Liver sample, immunohistochemical score 3 (CD38 positive cells, 25% to 49%)

(D) Bowel sample, immunohistochemical score 4 (CD38 positive cells >75%)

Figure 5. CD38 expression levels on bone marrow plasma cell and correlation with extramedullary immunohistochemical score

Figure 6. Probability of OS from multiple myeloma diagnosis.

Figure 7. Probability of OS from extramedullary diagnosis.

Figure 8. Probability of OS from extramedullary diagnosis according to immuhistochemical CD38 score in extramedullary lesions.

Table 1

Patient Characteristics at MM Diagnosis		
Characteristics	**Number of Cases**	**Number (%) or Median (range)**
Gender (M/F)	22	13/9 (60/40%)
Median age	22	67 (47-76)
EM-E Diagnosis/Relapse	22	4/19
Disease (MM/pPCL)	22	20/2
Isotype (LC, IgA, IgG)	22	9/7/6 (41%/32%/27%)
Light Chain (kappa/lambda)	22	10/12 (45%/55%)
ISS (I/II/III)	22	6/6/10 (27%/27%/46%)
LDH (median)	14	517 (277-1145)

Table 2: BM and EMD Immunohistochemical Characteristics

Patient	Status	CD38 EMD	CD56 EMD	CD44 EMD	CD38 BM	CD56 BM	CD44 BM	Dissociated EMD
1	R/R	4	0	4	4	0	2	no
2	R/R	4	4	4	4	4	4	no
3	ND	4	0	0	4	0	4	no
4	R/R	4	0	3	2	0	1	no
5	R/R	4	0	4	4	0	4	no
6	ND	4	0	4	Pol. PCs	Pol. PCs	Pol. PCs	yes
7	R/R	0	0	4	3	0	NA	yes
8	R/R	4	2	4	4	0	4	no
9	R/R	4	0	4	4	0	4	no
10	R/R	2	0	1	4	1	1	no
11	R/R	2	4	0	4	4	3	no
12	ND	0	0	2	4	0	0	yes
13	R/R	4	0	4	Pol. PCs	Pol. PCs	Pol. PCs	yes
14	ND	2	0	0	0	0	1	no
15	R/R	4	4	4	3	4	4	yes
16	R/R	4	1	NA	Pol. PCs	Pol. PCs	Pol. PCs	yes
17	R/R	2	1	4	0	NA	NA	yes
18	R/R	4	4	4	4	4	0	no
19	R/R	4	0	NA	4	4	NA	no
20	R/R	2	0	4	4	4	0	no
21	R/R	0	0	4	0	4	4	no
22	R/R	4	4	1	4	4	1	yes

Table 3

EMD CD38+ Score	BM CD38+ Score: Score 0	Score 1-2	Score 3-4	Total
Score 0	1		2	
Score 1-2	2		3	
Score 3-4		1	10	
Total	3	1	15	**19**

Table 4

EMD CD56+ Score	BM CD56+ Score: Score 0	Score 1-2	Score 3-4	Total
Score 0	8	1	3	
Score 1-2	1			
Score 3-4			5	
Total	9	1	8	**18**

Table 5

EMD CD44+ Score	BM CD44+ Score: Score 0	Score 1-2	Score 3-4	Total
Score 0		1	2	
Score 1-2	1	2		
Score 3-4	2	2	6	
Total	3	5	8	**16**

Table 6

Prior Lines of Therapy before EMD, *n* 22	**Patients, *n* (%)**
0	4 (18%)
1	6 (27%)
2	7 (32%)
≥3	5 (23%)
Treatment Status, *n* 18	
On Treatment	14 (78%)
Treatment Free Remission	4 (22%)
Prior Treatments, *n* 18	
Proteasome Inhibitors	17 (94%)
IMiDs	14 (78%)
Alkylating agents	8 (44%)
Autologous SCT	5 (28%)

Table 7

	Status at EMD	Previous Lines	Age at EMD Presentation	EMD Site	Ongoing Therapy during EMD Presentation	EMD Treatment	Best Response
EMD CD38 low score	ND	0	53	Liver, Kidney, Soft Tissue	EMD at diagnosis	Cyclophosphamide, Bortezomib, and Dexamethasone	PD
	RR	3	77	Soft Tissue	Lenalidomide/Dexamethasone	Best supportive care	PD
	RR	2	55	Skin, Soft Tissue, Lymph Nodes, Liver	Cyclophosphamide, Bortezomib, and Dexamethasone	RT + Dexamethasone	PD
	RR	1	70	Skin, Soft Tissue, Lymph Nodes, Testes, CNS	Carfilzomib/Dexamethasone	CVAD + RT + ASCT	VGPR
	RR	1	78	Soft Tissue	Ixazomib	Cyclophosphamide/Lenalidomide/Dexamethasone	SD
	RR	3	69	Spleen	Treatment free remission	EMD detection post-mortem	/
	RR	2	37	Upper Respiratory Tract	Daratumumab/Bortezomib/Dexamethasone	CVAD	Mixed Response
	ND	0	62	Pleura and Lung	EMD at diagnosis	Bortezomib/Dexamethasone	PR
EMD CD38 high score	RR	2	78	Upper Respiratory Tract	Cyclophosphamide, Bortezomib, and Dexamethasone	Melphalan/Lenalidomide/Dexamethasone	PD
	RR	2	48	Pleura, Liver, Soft Tissue, Limph Nodes	Carfilzomib/Lenalidomide/Dexamethasone	CVAD + Dara PACE + ASCT	Mixed Response
	RR	3	79	Soft Tissues	Daratumumab/Bortezomib/Dexamethasone	Bortezomib/Doxorubicin/Dexamethasone	Mixed Response
	RR	2	49	Upper Respiratory Tract	Carfilzomib/Lenalidomide/Dexamethasone	Allo-SCT	PD
	RR	6	75	Soft Tissue	Lenalidomide/Dexamethasone	Pomalidomide/Dexamethasone	SD
	RR	1	67	Liver, Lymph Nodes	Treatment free remission	Bortezomib/Dexamethasone + ASCT	PD
	RR	2	79	Skin, Liver, Soft Tissue	Cyclophosphamide, Bortezomib, and Dexamethasone	Best supportive care	PD
	RR	1	58	Soft Tissue, Lymph Nodes, Spleen	Treatment free remission	Carfilzomib/Lenalidomide/Dexamethasone + allo SCT	VGPR
	RR	3	72	Upper Respiratory Tract	Pomalidomide/Dexamethasone/Cyclophosphamide	Bendamustine/Bortezomib/Dexamethasone	PD
	RR	2	74	Liver and Spleen	Lenalidomide/Dexamethasone	Pomalidomide/Dexamethasone	SD
	ND	0	64	Lymph Nodes	EMD at diagnosis	Bortezomib/Thalidomide/Dexamethasone + ASCT	CR
	ND	0	71	Upper Respiratory Tract	EMD at diagnosis	Melphalan/Bortezomib/Prednsione	CR
	RR	1	78	Testes	Lenalidomide	Surgery	CR
	RR	1	74	Testes	Treatment free remission	Surgery + Bortezomib/Dexamethasone	VGPR

Figure 1

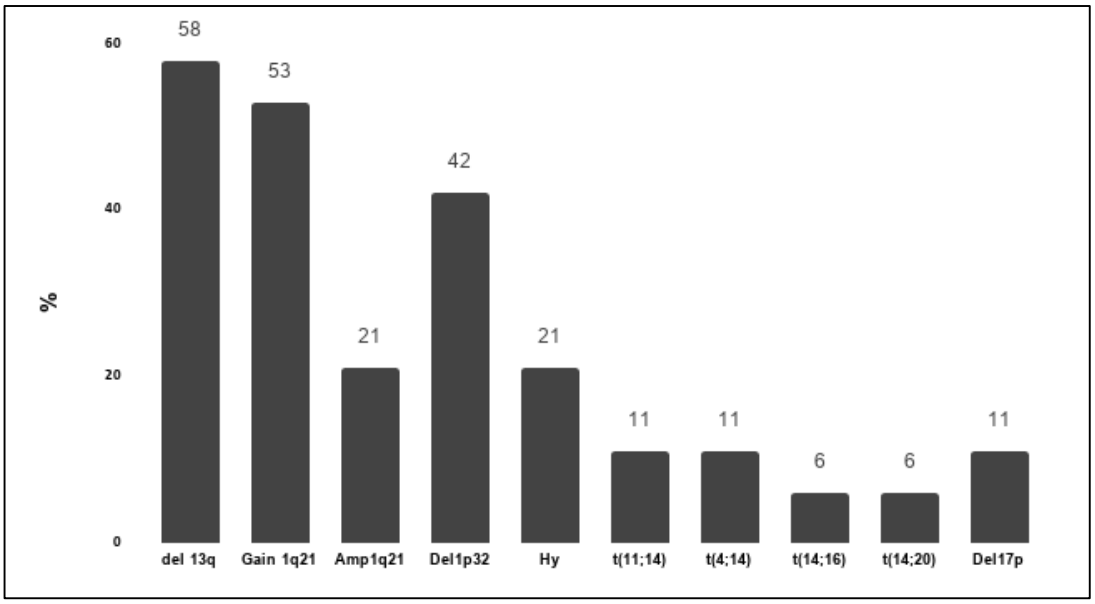


Figure 2

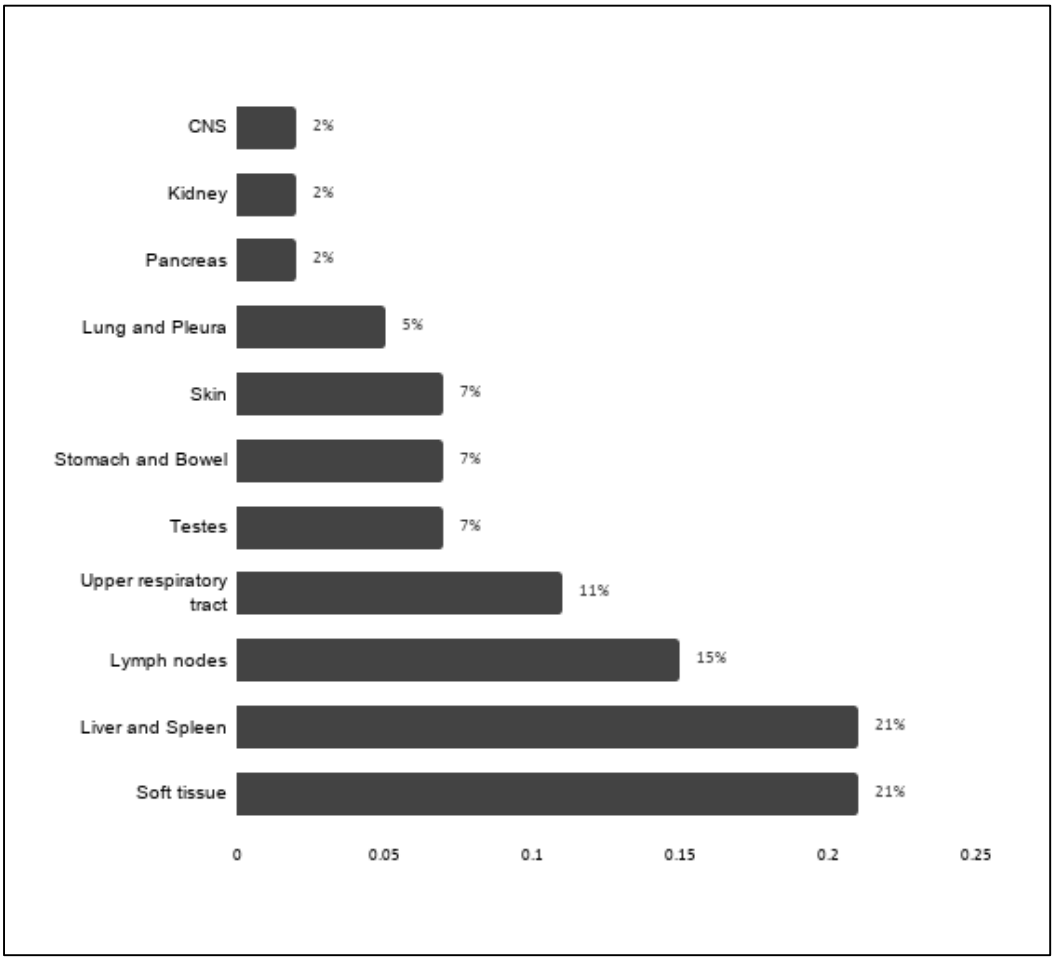


Figure 3

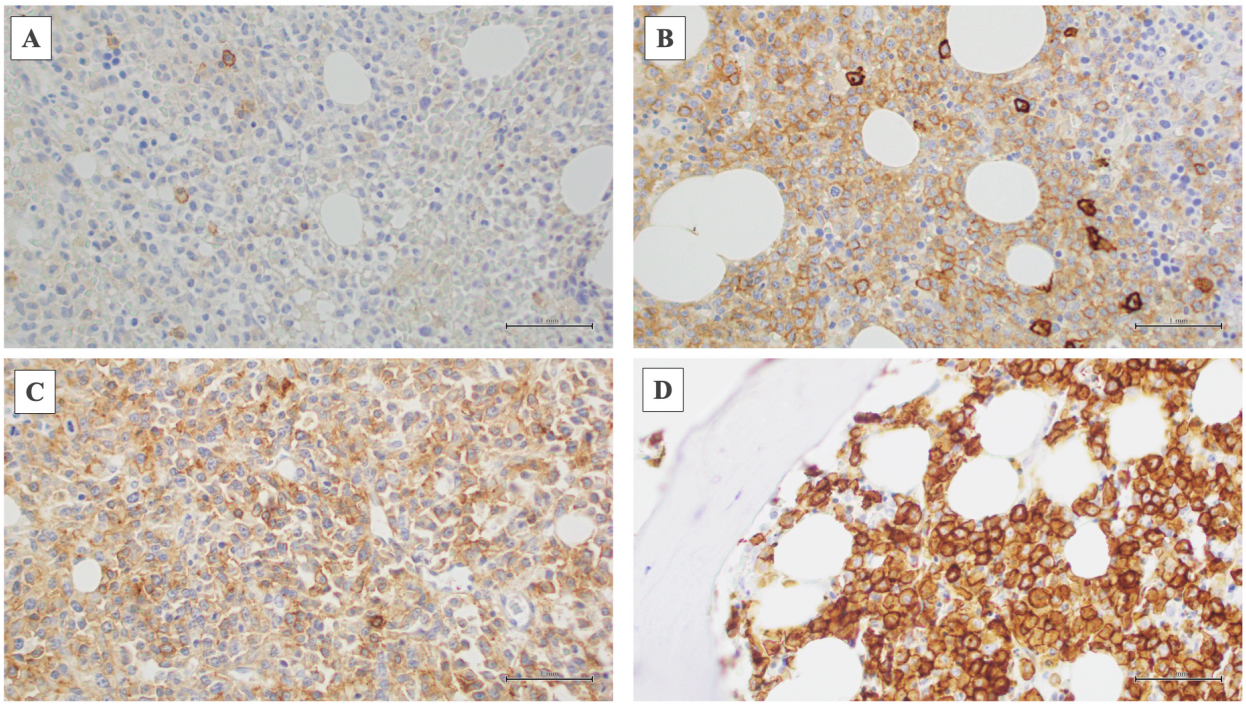


Figure 4

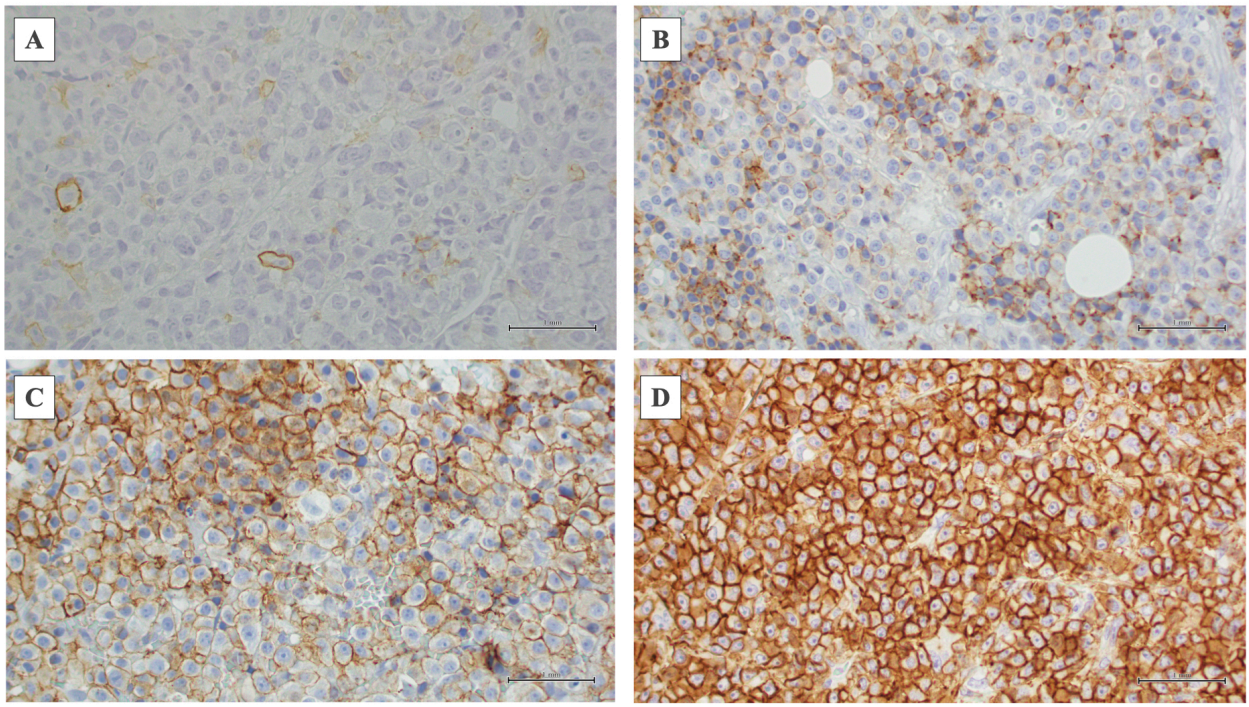


Figure 5

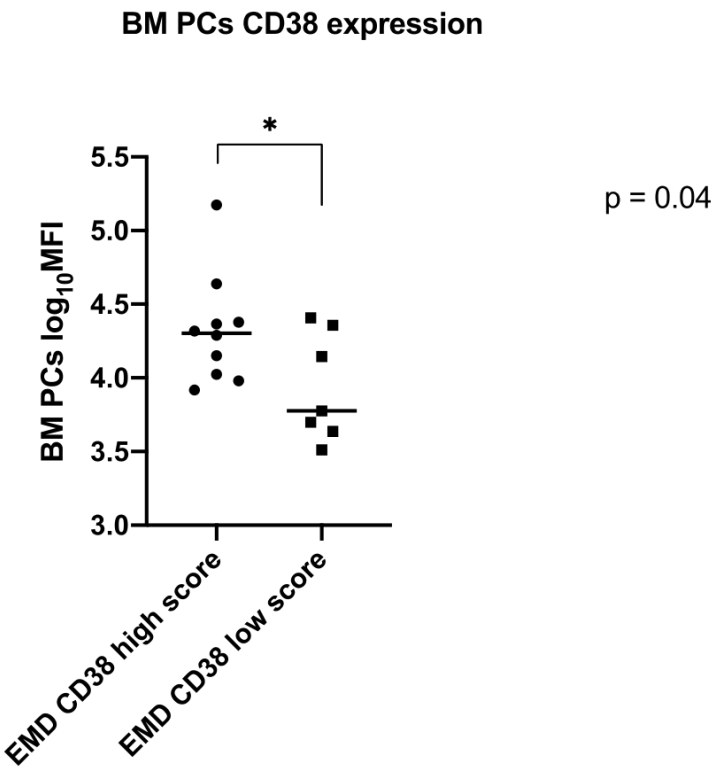

BM PCs CD38 expression
*
5.5
5.0
4.5
4.0
3.5
3.0
BM PCs log10MFI
EMD CD38 high score
EMD CD38 low score
p = 0.04

Figure 6

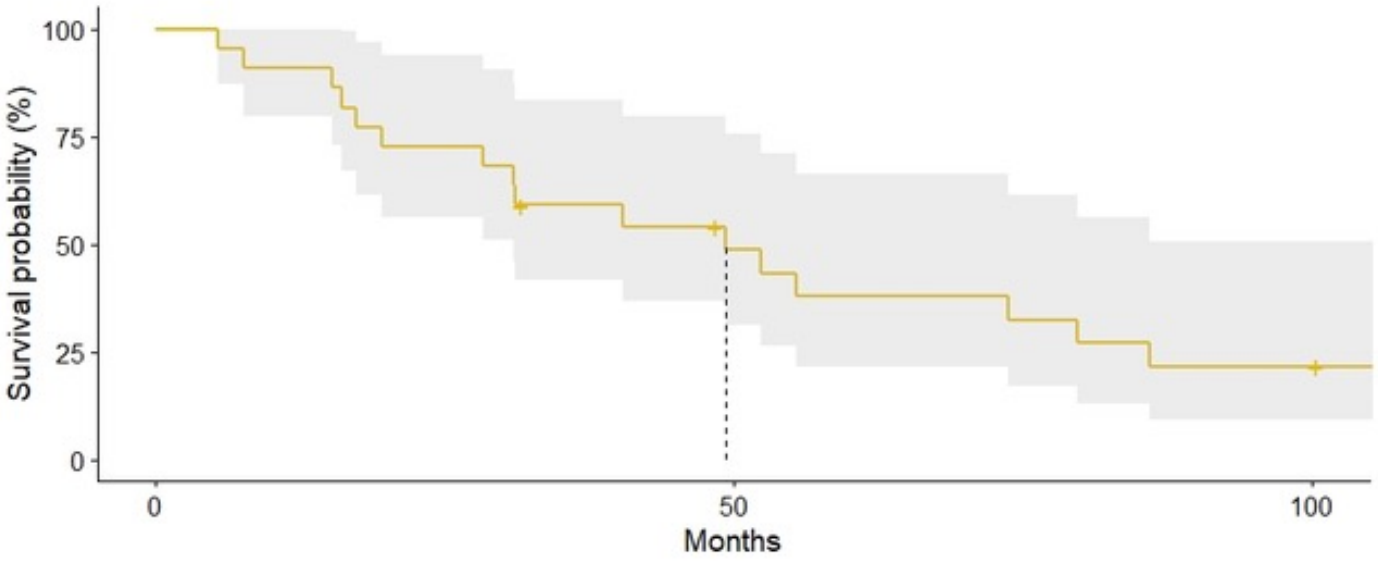


Figure 7

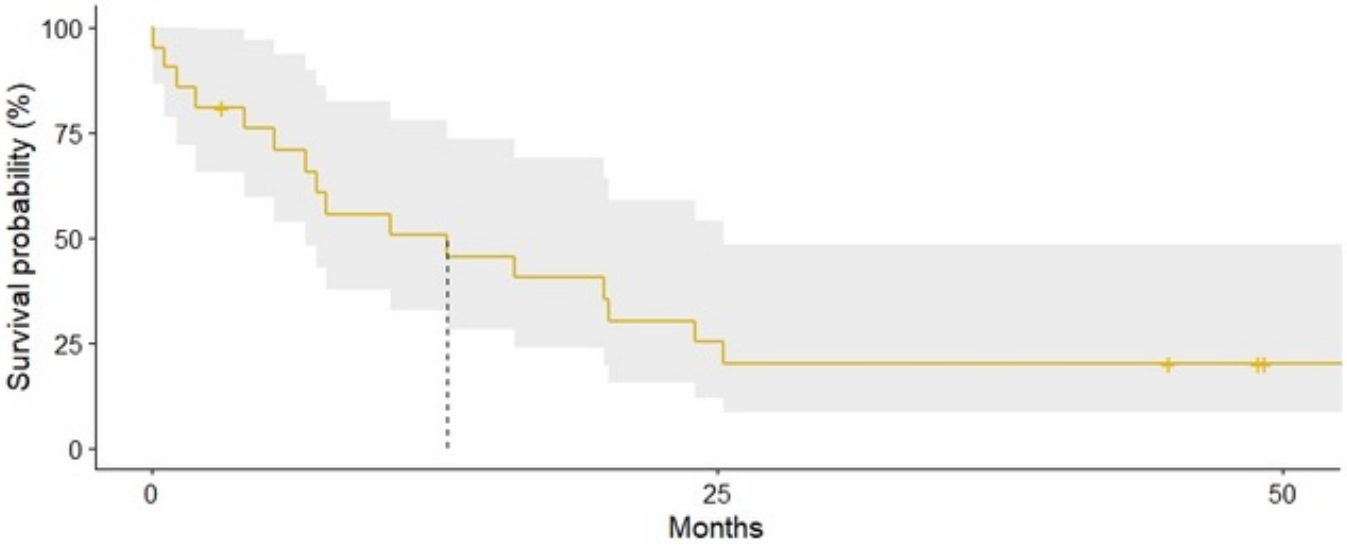
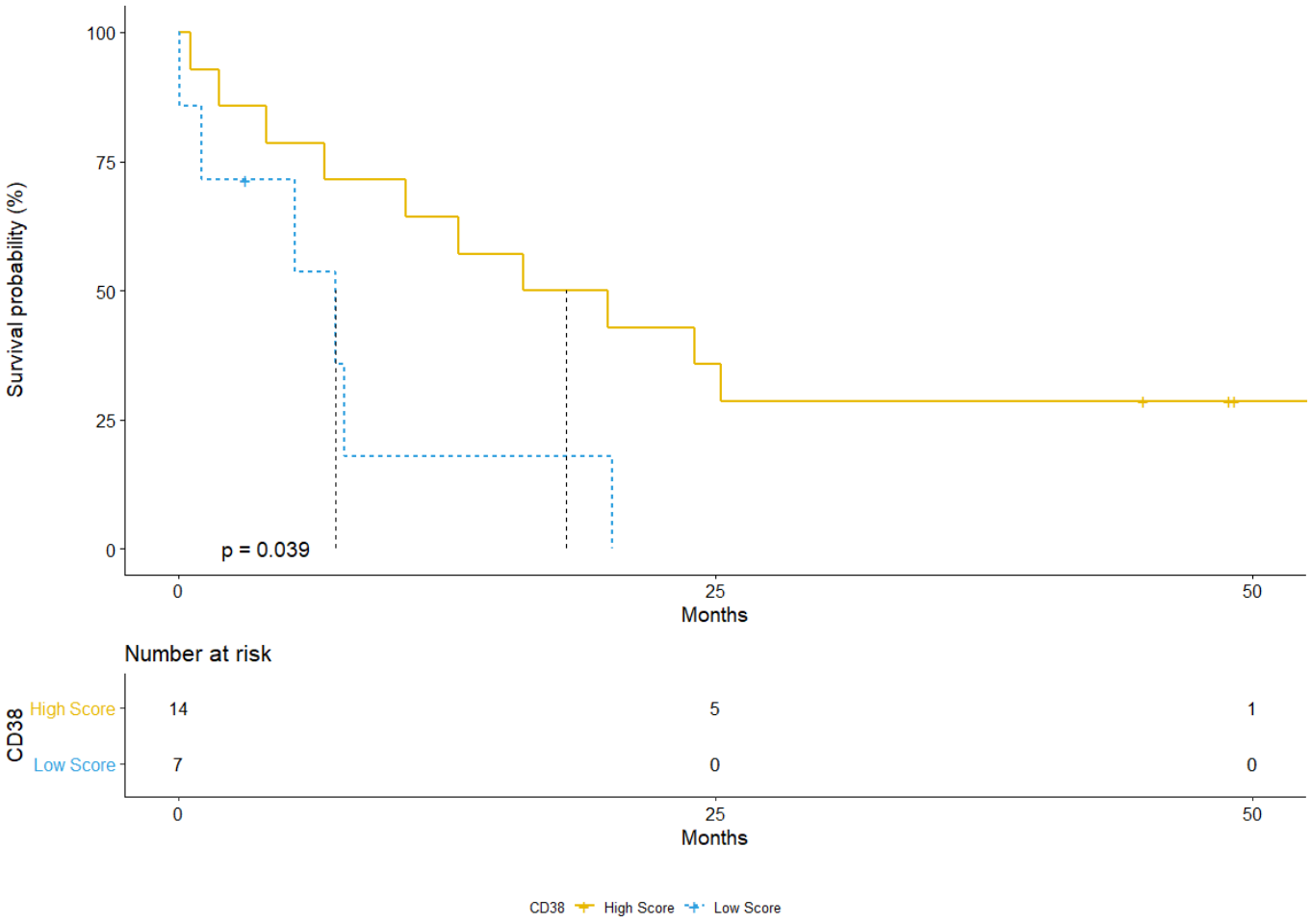


Figure 8


Survival probability (%)
100
75
50
25
0
p = 0.039
0
25
50
Months
Number at risk
CD38
High Score
Low Score
14
5
1
7
0
0
0
25
50
Months
CD38
High Score
Low Score

References

1. Palumbo , A.; Anderson , K. Multiple Myeloma. *N. Engl. J. Med.* **2011**, *364*, 1046-1060, doi:doi:10.1056/NEJMra1011442.
2. Rajkumar, S.V.; Dimopoulos, M.A.; Palumbo, A.; Blade, J.; Merlini, G.; Mateos, M.-V.; Kumar, S.; Hillengass, J.; Kastritis, E.; Richardson, P. International Myeloma Working Group updated criteria for the diagnosis of multiple myeloma. *The Lancet Oncology* **2014**, *15*, e538-e548.
3. Touzeau, C.; Moreau, P. How I treat extramedullary myeloma. *Blood, The Journal of the American Society of Hematology* **2016**, *127*, 971-976.
4. Musto, P.; Simeon, V.; Todoerti, K.; Neri, A. Primary plasma cell leukemia: identity card 2016. *Curr. Treat. Options Oncol.* **2016**, *17*, 19.
5. Granell, M.; Calvo, X.; Garcia-Guiñón, A.; Escoda, L.; Abella, E.; Martínez, C.M.; Teixidó, M.; Gimenez, M.T.; Senín, A.; Sanz, P. Prognostic impact of circulating plasma cells in patients with multiple myeloma: implications for plasma cell leukemia definition. *Haematologica* **2017**, *102*, 1099-1104.
6. Bhutani, M.; Foureau, D.M.; Atrash, S.; Voorhees, P.M.; Usmani, S.Z. Extramedullary multiple myeloma. *Leukemia* **2020**, *34*, 1-20.
7. Caers, J.; Paiva, B.; Zamagni, E.; Leleu, X.; Bladé, J.; Kristinsson, S.; Touzeau, C.; Abildgaard, N.; Terpos, E.; Heusschen, R. Diagnosis, treatment, and response assessment in solitary plasmacytoma: updated recommendations from a European Expert Panel. *J. Hematol. Oncol.* **2018**, *11*, 10.
8. Oshima, K.; Kanda, Y.; Nannya, Y.; Kaneko, M.; Hamaki, T.; Suguro, M.; Yamamoto, R.; Chizuka, A.; Matsuyama, T.; Takezako, N. Clinical and pathologic findings in 52 consecutively autopsied cases with multiple myeloma. *Am. J. Hematol.* **2001**, *67*, 1-5.
9. Kapadia, S.B. Multiple myeloma: a clinicopathologic study of 62 consecutively autopsied cases. *Medicine* **1980**, *59*, 380-392.
10. Varettoni, M.; Corso, A.; Pica, G.; Mangiacavalli, S.; Pascutto, C.; Lazzarino, M. Incidence, presenting features and outcome of extramedullary disease in multiple myeloma: a longitudinal study on 1003 consecutive patients. *Ann. Oncol.* **2010**, *21*, 325-330.
11. Varettoni, M.; Corso, A.; Pica, G.; Mangiacavalli, S.; Pascutto, C.; Lazzarino, M. Incidence, presenting features and outcome of extramedullary disease in multiple myeloma: a longitudinal study on 1003 consecutive patients. *Annals of oncology: official journal of the European Society for Medical Oncology* **2010**, *21*, 325.
12. Gagelmann, N.; Eikema, D.-J.; Iacobelli, S.; Koster, L.; Nahi, H.; Stoppa, A.-M.; Masszi, T.; Caillot, D.; Lenhoff, S.; Udvardy, M. Impact of extramedullary disease in patients with newly diagnosed multiple myeloma undergoing autologous stem cell transplantation: a study from the Chronic Malignancies Working Party of the EBMT. *Haematologica* **2018**, *103*, 890-897.
13. Mangiacavalli, S.; Pompa, A.; Ferretti, V.; Klersy, C.; Cocito, F.; Varettoni, M.; Cartia, C.; Cazzola, M.; Corso, A. The possible role of burden of therapy on the risk of myeloma extramedullary spread. *Ann. Hematol.* **2017**, *96*, 73-80.
14. Kuehl, W.M.; Bergsagel, P.L. Molecular pathogenesis of multiple myeloma and its premalignant precursor. *The Journal of clinical investigation* **2012**, *122*, 3456-3463.
15. Corre, J.; Munshi, N.; Avet-Loiseau, H. Genetics of multiple myeloma: another heterogeneity level? *Blood* **2015**, *125*, 1870-1876.

16. Corre, J.; Munshi, N.C.; Avet-Loiseau, H. Risk factors in multiple myeloma: is it time for a revision? *Blood, The Journal of the American Society of Hematology* **2021**, *137*, 16-19.
17. Walker, B.A.; Mavrommatis, K.; Wardell, C.P.; Ashby, T.C.; Bauer, M.; Davies, F.; Rosenthal, A.; Wang, H.; Qu, P.; Hoering, A. A high-risk, Double-Hit, group of newly diagnosed myeloma identified by genomic analysis. *Leukemia* **2019**, *33*, 159-170.
18. Walker, B.A.; Boyle, E.M.; Wardell, C.P.; Murison, A.; Dahir, N.M.; Proszek, P.Z.; Johnson, D.C.; Kaiser, M.F.; Melchor, L.; Aronson, L.I. Mutational spectrum, copy number changes, and outcome: results of a sequencing study of patients with newly diagnosed myeloma. *J. Clin. Oncol.* **2015**, JCO. 2014.2059. 1503.
19. Bolli, N.; Avet-Loiseau, H.; Wedge, D.C.; Van Loo, P.; Alexandrov, L.B.; Martincorena, I.; Dawson, K.J.; Iorio, F.; Nik-Zainal, S.; Bignell, G.R. Heterogeneity of genomic evolution and mutational profiles in multiple myeloma. *Nature communications* **2014**, *5*.
20. Lohr, J.G.; Stojanov, P.; Carter, S.L.; Cruz-Gordillo, P.; Lawrence, M.S.; Auclair, D.; Sougnez, C.; Knoechel, B.; Gould, J.; Saksena, G. Widespread genetic heterogeneity in multiple myeloma: implications for targeted therapy. *Cancer Cell* **2014**, *25*, 91-101.
21. Manier, S.; Salem, K.Z.; Park, J.; Landau, D.A.; Getz, G.; Ghobrial, I.M. Genomic complexity of multiple myeloma and its clinical implications. *Nature reviews Clinical oncology* **2017**, *14*, 100.
22. Rasche, L.; Chavan, S.; Stephens, O.; Patel, P.; Tytarenko, R.; Ashby, C.; Bauer, M.; Stein, C.; Deshpande, S.; Wardell, C. Spatial genomic heterogeneity in multiple myeloma revealed by multi-region sequencing. *Nature communications* **2017**, *8*, 1-11.
23. Rasche, L.; Bernard, C.; Topp, M.S.; Kapp, M.; Duell, J.; Wesemeier, C.; Haralambieva, E.; Maeder, U.; Einsele, H.; Knop, S. Features of extramedullary myeloma relapse: high proliferation, minimal marrow involvement, adverse cytogenetics: a retrospective single-center study of 24 cases. *Ann. Hematol.* **2012**, *91*, 1031-1037.
24. Billecke, L.; Murga Penas, E.M.; May, A.M.; Engelhardt, M.; Nagler, A.; Leiba, M.; Schiby, G.; Kröger, N.; Zustin, J.; Marx, A. Cytogenetics of extramedullary manifestations in multiple myeloma. *Br. J. Haematol.* **2013**, *161*, 87-94.
25. Deng, S.; Xu, Y.; An, G.; Sui, W.; Zou, D.; Zhao, Y.; Qi, J.; Li, F.; Hao, M.; Qiu, L. Features of extramedullary disease of multiple myeloma: high frequency of p53 deletion and poor survival: a retrospective single-center study of 834 cases. *Clinical Lymphoma Myeloma and Leukemia* **2015**, *15*, 286-291.
26. Qu, X.; Chen, L.; Qiu, H.; Lu, H.; Wu, H.; Qiu, H.; Liu, P.; Guo, R.; Li, J. Extramedullary manifestation in multiple myeloma bears high incidence of poor cytogenetic aberration and novel agents resistance. *BioMed research international* **2015**, *2015*.
27. Usmani, S.Z.; Heuck, C.; Mitchell, A.; Szymonifka, J.; Nair, B.; Hoering, A.; Alsayed, Y.; Waheed, S.; Haider, S.; Restrepo, A. Extramedullary disease portends poor prognosis in multiple myeloma and is over-represented in high-risk disease even in the era of novel agents. *Haematologica* **2012**, *97*, 1761-1767.
28. Zhan, F.; Huang, Y.; Colla, S.; Stewart, J.P.; Hanamura, I.; Gupta, S.; Epstein, J.; Yaccoby, S.; Sawyer, J.; Burington, B. The molecular classification of multiple myeloma. *Blood* **2006**, *108*, 2020-2028.
29. De Haart, S.; Willems, S.; Mutis, T.; Koudijs, M.; Van Blokland, M.; Lokhorst, H.; De Weger, R.; Minnema, M. Comparison of intramedullary myeloma and corresponding extramedullary soft tissue plasmacytomas using genetic mutational panel analyses. *Blood cancer journal* **2016**, *6*, e426-e426.

30. Andrulis, M.; Lehners, N.; Capper, D.; Penzel, R.; Heining, C.; Huellein, J.; Zenz, T.; von Deimling, A.; Schirmacher, P.; Ho, A.D. Targeting the BRAF V600E mutation in multiple myeloma. *Cancer Discov.* **2013**, *3*, 862-869.
31. Garcés, J.-J.; Bretones, G.; Burgos, L.; Valdes-Mas, R.; Puig, N.; Cedena, M.-T.; Alignani, D.; Rodriguez, I.; Puente, D.A.; Álvarez, M.-G. Circulating tumor cells for comprehensive and multiregional non-invasive genetic characterization of multiple myeloma. *Leukemia* **2020**, *34*, 3007-3018.
32. Garcés, J.-J.; Simicek, M.; Vicari, M.; Brozova, L.; Burgos, L.; Bezdekova, R.; Alignani, D.; Calasanz, M.-J.; Growkova, K.; Goicoechea, I. Transcriptional profiling of circulating tumor cells in multiple myeloma: a new model to understand disease dissemination. *Leukemia* **2020**, *34*, 589-603.
33. Ryu, D.; Kim, S.J.; Hong, Y.; Jo, A.; Kim, N.; Kim, H.-J.; Lee, H.-O.; Kim, K.; Park, W.-Y. Alterations in the transcriptional programs of myeloma cells and the microenvironment during extramedullary progression affect proliferation and immune evasion. *Clin. Cancer Res.* **2020**, *26*, 935-944.
34. Handa, H.; Kuroda, Y.; Kimura, K.; Masuda, Y.; Hattori, H.; Alkebsi, L.; Matsumoto, M.; Kasamatsu, T.; Kobayashi, N.; Tahara, K.i. Long non-coding RNA MALAT 1 is an inducible stress response gene associated with extramedullary spread and poor prognosis of multiple myeloma. *Br. J. Haematol.* **2017**, *179*, 449-460.
35. Besse, L.; Sedlarikova, L.; Kryukov, F.; Nekvindova, J.; Radova, L.; Slaby, O.; Kuglik, P.; Almasi, M.; Penka, M.; Krejci, M. Circulating serum MicroRNA-130a as a novel putative marker of extramedullary myeloma. *PLoS One* **2015**, *10*, e0137294.
36. Varga, C.; Xie, W.; Laubach, J.; Ghobrial, I.M.; O'Donnell, E.K.; Weinstock, M.; Paba-Prada, C.; Warren, D.; Maglio, M.E.; Schlossman, R. Development of extramedullary myeloma in the era of novel agents: no evidence of increased risk with lenalidomide–bortezomib combinations. *Br. J. Haematol.* **2015**, *169*, 843-850.
37. Pellat-Deceunynck, C.; Barillé, S.; Puthier, D.; Rapp, M.-J.; Harousseau, J.-L.; Bataille, R.; Amiot, M. Adhesion molecules on human myeloma cells: significant changes in expression related to malignancy, tumor spreading, and immortalization. *Cancer Res.* **1995**, *55*, 3647-3653.
38. Sanz-Rodríguez, F.; Ruiz-Velasco, N.; Pascual-Salcedo, D.; Teixidó, J. Characterization of VLA-4-dependent myeloma cell adhesion to fibronectin and VCAM-1. *Br. J. Haematol.* **1999**, *107*, 825-834.
39. Alsayed, Y.; Ngo, H.; Runnels, J.; Leleu, X.; Singha, U.K.; Pitsillides, C.M.; Spencer, J.A.; Kimlinger, T.; Ghobrial, J.M.; Jia, X. Mechanisms of regulation of CXCR4/SDF-1 (CXCL12)–dependent migration and homing in multiple myeloma. *Blood* **2007**, *109*, 2708-2717.
40. Roccaro, A.M.; Mishima, Y.; Sacco, A.; Moschetta, M.; Tai, Y.-T.; Shi, J.; Zhang, Y.; Reagan, M.R.; Huynh, D.; Kawano, Y. CXCR4 regulates extra-medullary myeloma through epithelial-mesenchymal-transition-like transcriptional activation. *Cell reports* **2015**, *12*, 622-635.
41. Van Driel, M.; Günthert, U.; Stauder, R.; Joling, P.; Lokhorst, H.; Bloem, A. CD44 isoforms distinguish between bone marrow plasma cells from normal individuals and patients with multiple myeloma at different stages of disease. *Leukemia* **1998**, *12*, 1821-1828.
42. Okada, T.; Hawley, R.G.; Kodaka, M.; Okuno, H. Significance of VLA-4–VCAM-1 interaction and CD44 for transendothelial invasion in a bone marrow metastatic myeloma model. *Clin. Exp. Metastasis* **1999**, *17*, 623-629.
43. Barille, S.; Collette, M.; Bataille, R.; Amiot, M. Myeloma cells upregulate interleukin-6 secretion in osteoblastic cells through cell-to-cell contact but downregulate osteocalcin. **1995**.

44. Horenstein, A.L.; Faini, A.C.; Morandi, F.; Bracci, C.; Lanza, F.; Giuliani, N.; Paulus, A.; Malavasi, F. The Circular Life of Human CD38: From Basic Science to Clinics and Back. *Molecules* **2020**, *25*, 4844.
45. Vallario, A.; Chilosi, M.; Adami, F.; Montagna, L.; Deaglio, S.; Malavasi, F.; Caligaris-Cappio, F. Human myeloma cells express the CD38 ligand CD31. *Br. J. Haematol.* **1999**, *105*, 441-444.
46. Bataille, R.; Jégo, G.; Robillard, N.; Barillé-Nion, S.; Harousseau, J.-L.; Moreau, P.; Amiot, M.; Pellat-Deceunynck, C. The phenotype of normal, reactive and malignant plasma cells. Identification of" many and multiple myelomas" and of new targets for myeloma therapy. *Haematologica* **2006**, *91*, 1234-1240.
47. Arroz, M.; Came, N.; Lin, P.; Chen, W.; Yuan, C.; Lagoo, A.; Monreal, M.; de Tute, R.; Vergilio, J.A.; Rawstron, A.C. Consensus guidelines on plasma cell myeloma minimal residual disease analysis and reporting. *Cytometry Part B: Clinical Cytometry* **2016**, *90*, 31-39.
48. Dahl, I.M.S.; Rasmussen, T.; Kauric, G.; Husebekk, A. Differential expression of CD56 and CD44 in the evolution of extramedullary myeloma. *Br. J. Haematol.* **2002**, *116*, 273-277.
49. Weinstock, M.; Aljawai, Y.; Morgan, E.A.; Laubach, J.; Gannon, M.; Roccaro, A.M.; Varga, C.; Mitsiades, C.S.; Paba-Prada, C.; Schlossman, R. Incidence and clinical features of extramedullary multiple myeloma in patients who underwent stem cell transplantation. *Br. J. Haematol.* **2015**, *169*, 851-858.
50. Paiva, B.; Paino, T.; Sayagues, J.-M.; Garayoa, M.; San-Segundo, L.; Martín, M.; Mota, I.; Sanchez, M.-L.; Bárcena, P.; Aires-Mejia, I. Detailed characterization of multiple myeloma circulating tumor cells shows unique phenotypic, cytogenetic, functional, and circadian distribution profile. *Blood, The Journal of the American Society of Hematology* **2013**, *122*, 3591-3598.
51. Klimienė, I.; Radzevičius, M.; Matuzevičienė, R.; Sinkevič-Belliot, K.; Kučinskienė, Z.A.; Pečeliūnas, V. Adhesion molecule immunophenotype of bone marrow multiple myeloma plasma cells impacts the presence of malignant circulating plasma cells in peripheral blood. *Int. J. Lab. Hematol.* **2020**.
52. Barlogie, B.; Smallwood, L.; Smith, T.; Alexanian, R. High serum levels of lactic dehydrogenase identify a high-grade lymphoma-like myeloma. *Ann. Intern. Med.* **1989**, *110*, 521-525.
53. Avivi, I.; Cohen, Y.C.; Suska, A.; Shragai, T.; Mikala, G.; Garderet, L.; Seny, G.M.; Glickman, S.; Jayabalan, D.S.; Niesvizky, R. Hematogenous extramedullary relapse in multiple myeloma-a multicenter retrospective study in 127 patients. *Am. J. Hematol.* **2019**, *94*, 1132-1140.
54. D'Agostino, M.; Zaccaria, G.M.; Ziccheddu, B.; Rustad, E.H.; Genuardi, E.; Capra, A.; Oliva, S.; Auclair, D.; Yesil, J.; Colucci, P. Early relapse risk in patients with newly diagnosed multiple myeloma characterized by next-generation sequencing. *Clin. Cancer Res.* **2020**, *26*, 4832-4841.
55. Dawson, M.A.; Patil, S.; Spencer, A. Extramedullary relapse of multiple myeloma associated with a shift in secretion from intact immunoglobulin to light chains. *Haematologica* **2007**, *92*, 143-144.
56. Bladé, J.; Lust, J.A.; Kyle, R.A. Immunoglobulin D multiple myeloma: presenting features, response to therapy, and survival in a series of 53 cases. *J. Clin. Oncol.* **1994**, *12*, 2398-2404.
57. Weinstock, M.; Ghobrial, I.M. Extramedullary multiple myeloma. *Leuk. Lymphoma* **2013**, *54*, 1135-1141.

58. López-Anglada, L.; Gutiérrez, N.C.; García, J.L.; Mateos, M.V.; Flores, T.; San Miguel, J.F. P53 deletion may drive the clinical evolution and treatment response in multiple myeloma. *Eur. J. Haematol.* **2010**, *84*, 359-361.
59. Came, N.; Nguyen, V.; Westerman, D.; Harrison, S. Aggressive and extramedullary plasma cell myeloma evade bone marrow flow cytometric minimal residual disease detection. *Br. J. Haematol.* **2016**, *173*, 947-949.
60. Hillengass, J.; Usmani, S.; Rajkumar, S.V.; Durie, B.G.; Mateos, M.-V.; Lonial, S.; Joao, C.; Anderson, K.C.; García-Sanz, R.; Riva, E. International myeloma working group consensus recommendations on imaging in monoclonal plasma cell disorders. *The Lancet Oncology* **2019**, *20*, e302-e312.
61. Gleeson, T.; Moriarty, J.; Shortt, C.; Gleeson, J.; Fitzpatrick, P.; Byrne, B.; McHugh, J.; O'Connell, M.; O'Gorman, P.; Eustace, S. Accuracy of whole-body low-dose multidetector CT (WBLDCT) versus skeletal survey in the detection of myelomatous lesions, and correlation of disease distribution with whole-body MRI (WBMRI). *Skeletal Radiol.* **2009**, *38*, 225-236.
62. Cavo, M.; Terpos, E.; Nanni, C.; Moreau, P.; Lentzsch, S.; Zweegman, S.; Hillengass, J.; Engelhardt, M.; Usmani, S.Z.; Vesole, D.H. Role of 18 F-FDG PET/CT in the diagnosis and management of multiple myeloma and other plasma cell disorders: a consensus statement by the International Myeloma Working Group. *The Lancet Oncology* **2017**, *18*, e206-e217.
63. Zamagni, E.; Patriarca, F.; Nanni, C.; Zannetti, B.; Englaro, E.; Pezzi, A.; Tacchetti, P.; Buttignol, S.; Perrone, G.; Brioli, A. Prognostic relevance of 18-F FDG PET/CT in newly diagnosed multiple myeloma patients treated with up-front autologous transplantation. *Blood* **2011**, *118*, 5989-5995.
64. Moreau, P.; Attal, M.; Caillot, D.; Macro, M.; Karlin, L.; Garderet, L.; Facon, T.; Benboubker, L.; Escoffre-Barbe, M.; Stoppa, A.-M. Prospective evaluation of magnetic resonance imaging and [18F] fluorodeoxyglucose positron emission tomography-computed tomography at diagnosis and before maintenance therapy in symptomatic patients with multiple myeloma included in the IFM/DFCI 2009 trial: results of the IMAJEM study. *J. Clin. Oncol.* **2017**, *35*, 2911.
65. Tirumani, S.H.; Sakellis, C.; Jacene, H.; Shinagare, A.B.; Munshi, N.C.; Ramaiya, N.H.; Van den Abbeele, A.D. Role of FDG-PET/CT in extramedullary multiple myeloma: correlation of FDG-PET/CT findings with clinical outcome. *Clin. Nucl. Med.* **2016**, *41*, e7-e13.
66. Tirumani, S.H.; Shinagare, A.B.; Jagannathan, J.P.; Krajewski, K.M.; Munshi, N.C.; Ramaiya, N.H. MRI features of extramedullary myeloma. *American Journal of Roentgenology* **2014**, *202*, 803-810.
67. Rosiñol, L.; Oriol, A.; Teruel, A.I.; Hernández, D.; López-Jiménez, J.; de la Rubia, J.; Granell, M.; Besalduch, J.; Palomera, L.; González, Y. Superiority of bortezomib, thalidomide, and dexamethasone (VTD) as induction pretransplantation therapy in multiple myeloma: a randomized phase 3 PETHEMA/GEM study. *Blood* **2012**, *120*, 1589-1596.
68. Wu, P.; Davies, F.E.; Boyd, K.; Thomas, K.; Dines, S.; Saso, R.M.; Potter, M.N.; Ethell, M.E.; Shaw, B.E.; Morgan, G.J. The impact of extramedullary disease at presentation on the outcome of myeloma. *Leuk. Lymphoma* **2009**, *50*, 230-235.
69. Montefusco, V.; Gay, F.; Spada, S.; De Paoli, L.; Di Raimondo, F.; Ribolla, R.; Musolino, C.; Patriarca, F.; Musto, P.; Galieni, P. Outcome of paraosseous extra-medullary disease in newly diagnosed multiple myeloma patients treated with new drugs. *Haematologica* **2020**, *105*, 193.
70. Beksac, M.; Seval, G.C.; Kanellias, N.; Coriu, D.; Rosiñol, L.; Ozet, G.; Goranova-Marinova, V.; Unal, A.; Bila, J.; Ozsan, H. A real world multicenter retrospective study on

extramedullary disease from Balkan Myeloma Study Group and Barcelona University: analysis of parameters that improve outcome. *Haematologica* **2020**, *105*, 201-208.
71. Papanikolaou, X.; Repousis, P.; Tzenou, T.; Maltezas, D.; Kotsopoulou, M.; Megalakaki, K.; Angelopoulou, M.; Dimitrakoloulou, E.; Koulieris, E.; Bartzis, V. Incidence, clinical features, laboratory findings and outcome of patients with multiple myeloma presenting with extramedullary relapse. *Leuk. Lymphoma* **2013**, *54*, 1459-1464.
72. Pour, L.; Sevcikova, S.; Greslikova, H.; Kupska, R.; Majkova, P.; Zahradova, L.; Sandecka, V.; Adam, Z.; Krejci, M.; Kuglik, P. Soft-tissue extramedullary multiple myeloma prognosis is significantly worse in comparison to bone-related extramedullary relapse. *Haematologica* **2014**, *99*, 360-364.
73. Laura, R.; Cibeira, M.T.; Uriburu, C.; Yantorno, S.; Salamero, O.; Bladé, J.; Montserrat, E. Bortezomib: an effective agent in extramedullary disease in multiple myeloma. *Eur. J. Haematol.* **2006**, *76*, 405-408.
74. Ali, R.; Ozkalemkas, F.; Ozkan, A.; Ozkocaman, V.; Ozcelik, T.; Ozan, U.; Kurt, M.; Tunali, A. Bortezomib and extramedullary disease in multiple myeloma: the shine and dark side of the moon. *Leuk. Res.* **2006**, *31*, 1153-1155.
75. Zhou, X.; Flüchter, P.; Nickel, K.; Meckel, K.; Messerschmidt, J.; Böckle, D.; Knorz, S.; Steinhardt, M.J.; Krummenast, F.; Danhof, S. Carfilzomib based treatment strategies in the management of relapsed/refractory multiple myeloma with extramedullary disease. *Cancers (Basel)* **2020**, *12*, 1035.
76. Muchtar, E.; Gatt, M.E.; Rouvio, O.; Ganzel, C.; Chubar, E.; Suriu, C.; Tadmor, T.; Shevetz, O.; Lavi, N.; Shochat, T. Efficacy and safety of salvage therapy using Carfilzomib for relapsed or refractory multiple myeloma patients: a multicentre retrospective observational study. *Br. J. Haematol.* **2016**, *172*, 89-96.
77. Rosiñol, L.; Cibeira, M.T.; Bladé, J.; Esteve, J.; Aymerich, M.; Rozman, M.; Segarra, M.; Cid, M.C.; Filella, X.; Montserrat, E. Extramedullary multiple myeloma escapes the effect of thalidomide. *Haematologica* **2004**, *89*, 832-836.
78. Zhu, Y.X.; Kortuem, K.M.; Stewart, A.K. Molecular mechanism of action of immune-modulatory drugs thalidomide, lenalidomide and pomalidomide in multiple myeloma. *Leuk. Lymphoma* **2013**, *54*, 683-687.
79. Calvo-Villas, J.M.; Alegre, A.; Calle, C.; Hernández, M.T.; García-Sánchez, R.; Ramírez, G.; GEM-PETHEMA/Spanish Myeloma Group, S. Lenalidomide is effective for extramedullary disease in relapsed or refractory multiple myeloma. *Eur. J. Haematol.* **2011**, *87*, 281-284.
80. Short, K.D.; Rajkumar, S.V.; Larson, D.; Buadi, F.; Hayman, S.; Dispenzieri, A.; Gertz, M.; Kumar, S.; Mikhael, J.; Roy, V. Incidence of extramedullary disease in patients with multiple myeloma in the era of novel therapy, and the activity of pomalidomide on extramedullary myeloma. *Leukemia* **2011**, *25*, 906-908.
81. Jiménez-Segura, R.; Granell, M.; Gironella, M.; Abella, E.; García-Guiñón, A.; Oriol, A.; Cabezudo, E.; Clapés, V.; Soler, J.A.; Escoda, L. Pomalidomide-dexamethasone for treatment of soft-tissue plasmacytomas in patients with relapsed/refractory multiple myeloma. *Eur. J. Haematol.* **2019**, *102*, 389-394.
82. Richardson, P.G.; Oriol, A.; Larocca, A.; Bladé, J.; Cavo, M.; Rodriguez-Otero, P.; Leleu, X.; Nadeem, O.; Hiemenz, J.W.; Hassoun, H. Melflufen and dexamethasone in heavily pretreated relapsed and refractory multiple myeloma. *J. Clin. Oncol.* **2021**, *39*, 757-767.
83. Chari, A.; Vogl, D.T.; Gavriatopoulou, M.; Nooka, A.K.; Yee, A.J.; Huff, C.A.; Moreau, P.; Dingli, D.; Cole, C.; Lonial, S. Oral selinexor–dexamethasone for triple-class refractory multiple myeloma. *N. Engl. J. Med.* **2019**, *381*, 727-738.

84. Yee, A.J.; Huff, C.A.; Chari, A.; Vogl, D.T.; Gavriatopoulou, M.; Nooka, A.K.; Moreau, P.; Dingli, D.; Cole, C.E.; Lonial, S. Response to therapy and the effectiveness of treatment with selinexor and dexamethasone in patients with penta-exposed triple-class refractory myeloma who had plasmacytomas. American Society of Hematology Washington, DC: 2019.
85. Usmani, S.Z.; Weiss, B.M.; Plesner, T.; Bahlis, N.J.; Belch, A.; Lonial, S.; Lokhorst, H.M.; Voorhees, P.M.; Richardson, P.G.; Chari, A. Clinical efficacy of daratumumab monotherapy in patients with heavily pretreated relapsed or refractory multiple myeloma. *Blood* **2016**, *128*, 37-44.
86. Attal, M.; Richardson, P.G.; Rajkumar, S.V.; San-Miguel, J.; Beksac, M.; Spicka, I.; Leleu, X.; Schjesvold, F.; Moreau, P.; Dimopoulos, M.A. Isatuximab plus pomalidomide and low-dose dexamethasone versus pomalidomide and low-dose dexamethasone in patients with relapsed and refractory multiple myeloma (ICARIA-MM): a randomised, multicentre, open-label, phase 3 study. *The Lancet* **2019**, *394*, 2096-2107.
87. Beksac, M.; Richardson, P.; Unal, A.; Corradini, P.; DeLimpasi, S.; Gulbas, Z. Isatuximab plus pomalidomide and dexamethasone in patients with relapsed/refractory multiple myeloma and soft-tissue plasmacytomas: ICARIA-MM subgroup analysis. In Proceedings of European Hematology Association Congress (EHA 25), Virtual.
88. Danhof, S.; Rasche, L.; Mottok, A.; Steinmüller, T.; Zhou, X.; Schreder, M.; Kilian, T.; Strifler, S.; Rosenwald, A.; Hudecek, M. Elotuzumab for the treatment of extramedullary myeloma: a retrospective analysis of clinical efficacy and SLAMF7 expression patterns. *Ann. Hematol.* **2021**, 1-10.
89. Perez-Simon, J.; Sureda, A.; Fernandez-Aviles, F.; Sampol, A.; Cabrera, J.; Caballero, D.; Martino, R.; Petit, J.; Tomas, J.; Moraleda, J. Reduced-intensity conditioning allogeneic transplantation is associated with a high incidence of extramedullary relapses in multiple myeloma patients. *Leukemia* **2006**, *20*, 542-545.
90. Rasche, L.; Röllig, C.; Stuhler, G.; Danhof, S.; Mielke, S.; Grigoleit, G.U.; Dissen, L.; Schemmel, L.; Middeke, J.M.; Rücker, V. Allogeneic hematopoietic cell transplantation in multiple myeloma: focus on longitudinal assessment of donor chimerism, extramedullary disease, and high-risk cytogenetic features. *Biol. Blood Marrow Transplant.* **2016**, *22*, 1988-1996.
91. Van De Donk, N.W.; Usmani, S.Z. CD38 antibodies in multiple myeloma: mechanisms of action and modes of resistance. *Front. Immunol.* **2018**, *9*, 2134.
92. Kumar, S.; Paiva, B.; Anderson, K.C.; Durie, B.; Landgren, O.; Moreau, P.; Munshi, N.; Lonial, S.; Bladé, J.; Mateos, M.-V. International Myeloma Working Group consensus criteria for response and minimal residual disease assessment in multiple myeloma. *The Lancet Oncology* **2016**, *17*, e328-e346.
93. Zamagni, E.; Tacchetti, P.; Cavo, M. Imaging in multiple myeloma: How? When? *Blood* **2019**, *133*, 644-651.
94. Rosiñol, L.; Beksac, M.; Zamagni, E.; Van de Donk, N.W.; Anderson, K.C.; Badros, A.; Caers, J.; Cavo, M.; Dimopoulos, M.A.; Dispenzieri, A. Expert review on soft-tissue plasmacytomas in multiple myeloma: definition, disease assessment and treatment considerations. *Br. J. Haematol.* **2021**.
95. Hanamura, I. Gain/Amplification of Chromosome Arm 1q21 in Multiple Myeloma. *Cancers (Basel)* **2021**, *13*, 256.
96. Bink, K.; Haralambieva, E.; Kremer, M.; Ott, G.; Beham-Schmid, C.; de Leval, L.; Peh, S.C.; Laeng, H.R.; Jütting, U.; Hutzler, P. Primary extramedullary plasmacytoma: similarities with

and differences from multiple myeloma revealed by interphase cytogenetics. *Haematologica* **2008**, *93*, 623-626.
97. van de Donk, N.W.; Janmaat, M.L.; Mutis, T.; Lammerts van Bueren, J.J.; Ahmadi, T.; Sasser, A.K.; Lokhorst, H.M.; Parren, P.W. Monoclonal antibodies targeting CD 38 in hematological malignancies and beyond. *Immunol. Rev.* **2016**, *270*, 95-112.
98. Gallay, N.; Anani, L.; Lopez, A.; Colombat, P.; Binet, C.; Domenech, J.; Weksler, B.B.; Malavasi, F.; Herault, O. The Role of Platelet/Endothelial Cell Adhesion Molecule–1 (CD31) and CD38 Antigens in Marrow Microenvironmental Retention of Acute Myelogenous Leukemia Cells. *Cancer Res.* **2007**, *67*, 8624-8632.
99. Nijhof, I.S.; Casneuf, T.; Van Velzen, J.; van Kessel, B.; Axel, A.E.; Syed, K.; Groen, R.W.; van Duin, M.; Sonneveld, P.; Minnema, M.C. CD38 expression and complement inhibitors affect response and resistance to daratumumab therapy in myeloma. *Blood* **2016**, *128*, 959-970.
100. Tembhare, P.; Yuan, C.; Korde, N.; Maric, I.; Landgren, O. Antigenic drift in relapsed extramedullary multiple myeloma: plasma cells without CD38 expression. *Leuk. Lymphoma* **2012**, *53*, 721-724.
101. Singh, N.; Agrawal, N.; Mehta, A.; Panaych, A.; Sekhri, R. CD38—Negative Myeloma with Anaplastic Morphology at Presentation: A Case Report. *Indian Journal of Hematology and Blood Transfusion* **2018**, *34*, 362-364.
102. Ise, M.; Matsubayashi, K.; Tsujimura, H.; Kumagai, K. Loss of CD38 expression in relapsed refractory multiple myeloma. *Clinical Lymphoma, Myeloma and Leukemia* **2016**, *16*, e59-e64.
103. Plesner, T.; van de Donk, N.; Richardson, P.G. Controversy in the use of CD38 antibody for treatment of myeloma: is high CD38 expression good or bad? *Cells* **2020**, *9*, 378.
104. Ogiya, D.; Liu, J.; Ohguchi, H.; Kurata, K.; Samur, M.K.; Tai, Y.-T.; Adamia, S.; Ando, K.; Hideshima, T.; Anderson, K.C. The JAK-STAT pathway regulates CD38 on myeloma cells in the bone marrow microenvironment: therapeutic implications. *Blood, The Journal of the American Society of Hematology* **2020**, *136*, 2334-2345.
105. Kishimoto, H.; Hoshino, S.-i.; Ohori, M.; Kontani, K.; Nishina, H.; Suzawa, M.; Kato, S.; Katada, T. Molecular mechanism of human CD38 gene expression by retinoic acid: identification of retinoic acid response element in the first intron. *J. Biol. Chem.* **1998**, *273*, 15429-15434.
106. García-Guerrero, E.; Gogishvili, T.; Danhof, S.; Schreder, M.; Pallaud, C.; Pérez-Simón, J.A.; Einsele, H.; Hudecek, M. Panobinostat induces CD38 upregulation and augments the antimyeloma efficacy of daratumumab. *Blood, The Journal of the American Society of Hematology* **2017**, *129*, 3386-3388.
107. García-Guerrero, E.; Götz, R.; Doose, S.; Sauer, M.; Rodríguez-Gil, A.; Nerreter, T.; Kortüm, K.M.; Pérez-Simón, J.A.; Einsele, H.; Hudecek, M. Upregulation of CD38 expression on multiple myeloma cells by novel HDAC6 inhibitors is a class effect and augments the efficacy of daratumumab. *Leukemia* **2021**, *35*, 201-214.
108. Fedele, P.L.; Willis, S.N.; Liao, Y.; Low, M.S.; Rautela, J.; Segal, D.H.; Gong, J.-N.; Huntington, N.D.; Shi, W.; Huang, D. IMiDs prime myeloma cells for daratumumab-mediated cytotoxicity through loss of Ikaros and Aiolos. *Blood* **2018**, *132*, 2166-2178.
109. Muchtar, E.; Bladé, J.; Gertz, M.A. Testicular plasmacytoma: unique location or circumstantial presentation? *Leuk. Lymphoma* **2018**, *59*, 1769-1771.
110. Arana, P.; Paiva, B.; Cedena, M.-T.; Puig, N.; Cordon, L.; Vidriales, M.-B.; Gutierrez, N.C.; Chiodi, F.; Burgos, L.; Anglada, L.-L. Prognostic value of antigen expression in multiple myeloma: a PETHEMA/GEM study on 1265 patients enrolled in four consecutive clinical trials. *Leukemia* **2018**, *32*, 971-978.