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Efficacy of new drugs as induction treatment before single or tandem autologous stem cell transplantation in newly diagnosed multiple myeloma patients: a single centre experience

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Introduction

Multiple myeloma (MM) is one of the haematological malignant tumour among a heterogeneous group of diseases called plasma cell dyscrasias, characterized by the expansion of monoclonal plasma cells in the bone marrow that, in about 97% of cases, produce a monoclonal immunoglobulin called paraprotein or monoclonal component (M-protein). Such paraprotein, found in serum and / or urine, generally migrates to position γ at the electrophoresis and can be considered as a marker of such disease. Myeloma proteins have an altered structure due to mutations in immunoglobulin genes; sometimes the molecule breaks down and myeloma cells produce only light chains (Bence Jones proteinuria), or only heavy chains (causing a rare condition as "heavy chain disease") or eventually molecular fragments. In general, the distribution of monoclonal gammopathies, depending on the type of immunoglobulin (Ig) involved, is so divided: IgGs are the most common ones, affecting 60% of cases, followed by IgM in 18% and IgA in 11% of cases; rare are light chain and biclonal or IgD and IgE (Figure 1) (Kyle et al, 2006).

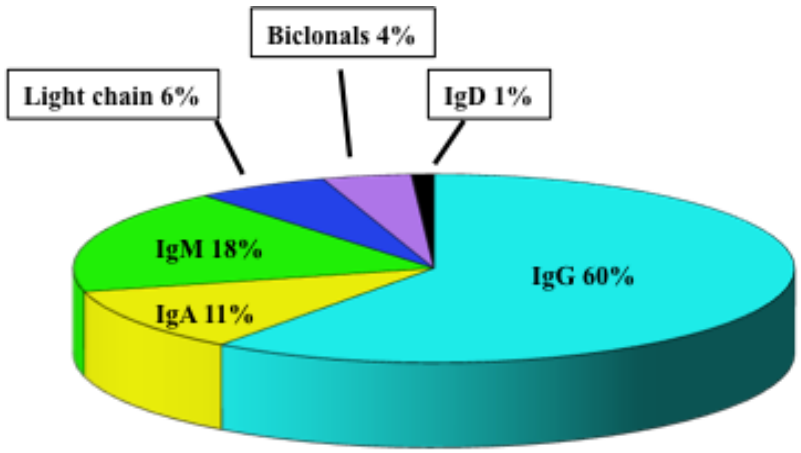


Figure 1: Distribution of monoclonal gammopathies.

Usually the paraprotein is defective, resulting in loss of normal antibody function. Therefore, MM is characterized by an altered immune response due to the proliferation of neoplastic cells with suppression of normal plasmocytes, resulting in lowering normal immunoglobulin levels.

In rare cases (<3%), myeloma cells do not produce any immunoglobulin and therefore it is called non-secretory myeloma, without evidence of M-protein neither in the serum nor in the urine. Sometimes plasma cells can proliferate also extramedullary, providing the so-called plasmacytoma. In some cases, plasma cells proliferation is located at one isolated extramedullary localization or in a single tissue outside the bone, without the bone marrow involvement, leading to the solitary bone plasmacytoma or solitary extramedullary plasmacytoma, respectively.

However, despite the fact that MM is usually characterized by the presence of the M-protein, such marker is not always synonymous of neoplasia. In fact, we must differentiate patients with malignant conditions such as symptomatic MM, solitary plasmacytoma and plasma cell leukaemia, which need to be treated immediately, and benign forms such as monoclonal gammopathies of undetermined significance (MGUS) and smouldering or asymptomatic multiple myeloma (SMM), which need just observation to assess the possible evolution of the disease. The diagnostic criteria for these entities are listed below. The distinction is very important considering the different clinical and therapeutic approaches that these different entities need. Kyle et al evaluated the progression of a large group of patients with SMM or MGUS and reported the evolution into symptomatic MM or lymphoma in about 30% of cases, while most patients (52%) died for reasons unrelated to the gammopathy, 10% had an increase in M-protein ≥ 3 g / dl which, however, remained stable without any need for therapy and 12% did not increase M-protein (Kyle et al, 2002).

Nowadays, there are some clinical trials that evaluate the role of new agents, for example lenalidomide or monoclonal antibodies as elotuzumab or daratumumab, for the treatment of SMM (Mateos et al, 2016; Ghobrial et al, 2016). These trials provide good results in terms of percentage of evolution in

MM, however outside the clinical trials, the current approach for SMM in the real practice is still observation.

Epidemiology:

MM can be considered a tumour of the elderly, affecting > 50% of cases patients older than 60-65 years with a male / female ratio of 3: 2. The median age at diagnosis is 69 years; 28.6% of patients are aged between 65 and 74 years, and 33% are older than 75 years. Only 14% of patients are diagnosed while younger than 55 years (National Cancer Institute 2016).

Etiopathogenesis:

The aetiology of MM is largely unknown, although it is possible to suggest the role of some genetic factors (some studies have speculated an association between MM and certain histocompatibility antigens such as B5, CW5 and CW2), environmental (radiation, toxic agents) and some pathological conditions (infections, autoimmune or dysmetabolic diseases) in the pathogenesis of the disease (Patel, 1999).

Biology:

From the morphological and functional point of view, the tumour cell responsible of MM is the plasma cell, the mature cell of B lymphocyte, which is incapable of supporting tumour proliferation. Numerous studies have suggested an involvement of more immature B cells than plasma cells, also present in peripheral blood (Pilarski et al, 1985; Berenson et al, 1987). This hypothesis is also supported by data

relating to the phenotype (Corradini et al, 1993). Pre-B cell expansion is considered to be a crucial step in the pathogenesis of MM, with the addition of genetic alterations that cause the development of the disease.

Cytokines:

Another controversy, not yet clarified, is the role of interleukin-6 (IL-6), which is considered to be the most important growth factor for the development of MM. Although initial studies suggest that this cytokine is self-produced by plasma cells (Kawano et al, 1988), other researchers support paracrine production of this molecule that stimulates the growth of plasma cells (Klein et al, 1989). In addition, the expression and production of IL-6 by less mature cells has been clearly demonstrated (Hata et al, 1993) by evaluating IL-6 gene expression in purified plasma cells. More mature cells lose expression of IL-6 and hence the ability to respond to this cytokine, as also demonstrated by the loss of IL-6 receptor expression. This cytokine can promote neoplastic proliferation, but also make the myeloma cells resistant to the action of certain drugs such as cortisone, interfering with the mechanisms that control the phenomenon of apoptosis (Hardin et al, 1994). IL-6 also plays a key role in the pathogenesis of osteolytic lesions; in fact, together with its soluble receptor and IL1-α, induces the activation of osteoclasts by causing bone resorption (Carte et al, 1990). Other cytokines, such as the granulocyte-colony stimulating factor (G-CSF), interferon alfa (IFN-α), IL-10 and IL-11 that exert their action through the same IL-6 receptor, play role in MM.

In addition, the increase in osteoclast activity observed in MM patients is due to an imbalance between the receptor activator of nuclear factor kB (RANK) and osteoprotegerin (OPG) due in turn to an increase in production of RANK ligand (RANKL) and a reduced production of OPG. Stromal damage is so important that bone reconstruction is rarely observed even in patients with complete remission

(Roodman et al, 2009).

Cytogenetics:

The mitotic activity of plasma cells in MM is higher than that in normal subjects and varies depending on the state of the disease. It is certainly higher in patients with a resistant disease than those at onset of disease, but it is far less than that of other neoplastic diseases. However, despite the difficulties of this low proliferative activity, cytogenetic studies have demonstrated the presence of karyotype alterations in 40% of cases, with a lower frequency in diagnosed patients than those evaluated at advanced disease. In addition, the fluorescent in situ hybridization (FISH) allows to overcome this limitation and in Table 1 are reported the most frequently encountered abnormalities in MM and their prognostic significance.

Translocations	Risk
del(13q)	High risk? No uniform consensus. For some authors if associated with high risk IgH translocations, particularly t(4;14)
t(4;14)(p16;q32),	High risk
del(17p)	High risk
(1q21)gain	High risk? No uniform consensus
t(14;16)(q32;q23)	High risk? No uniform consensus
t(11;14)	Standard risk
Hyperdiploidy	Standard risk

Table 1. The most frequently abnormalities detected in multiple myeloma and their prognostic significance. Del: deletion; t: translocation; IgH: immunoglobulin heavy chains.

Some chromosomal regions are frequently involved in these aberrations, revealing the role of some oncogenes in the pathogenesis of MM. Among these oncogenes, an important role is attributable to the altered regulation of proteins such as ras, bcl-2 and c- Myc and the loss of protein products of suppressor genes such as retinoblastoma and p53, which are the basis of molecular mechanisms that induce resistance to drugs and radiation in MM.

Primary and secondary genetic abnormalities (predominantly translocations) are recognized. Primary translocations involve 40-50% of patients in the 14q32 region (IgH) of immunoglobulins and are common to MM and MGUS (Hideshima et al, 2007). These injuries are therefore indispensable to the development of gammopathy, while a "second hit" is required for neoplastic evolution. Thus, secondary lesions appear with the progression of the disease and include loss of chromosome 13, NRAS and KRAS oncogenes activating mutations, inactivating mutations or deletions of p53 and phosphatase and tensin homolog (PTEN) inactivation (Kuehl et al, 2012). In fact, the pathogenesis of MGUS and MM can be considered as occurring in three phases:

1. Early, partially overlapping genetic events common to MGUS and MM include at a minimum primary IgH translocations, hyperdiploidy, and deletion of the chromosome 13 that lead directly or indirectly to dysregulation of a *Cyclin D* family (CCND) genes.

2. Second, the transition from MGUS to MM is associated with increased MYC expression and sometimes KRAS mutations, but can also include deletion of the chromosome 13 in t(11;14) tumours.

3. Finally, further progression of the MM seems to be associated with other events. For example, increased proliferation and genomic instability, and decreased dependence on the bone marrow microenvironment, sometimes including extramedullary spread of disease, can be associated with late MYC rearrangements that often involve an Ig locus, activating mutations of the

nuclear factor of kappa light polypeptide gene enhancer in B cells (NF-κB) pathway, deletion or mutation of TP53, and inactivation of genes as p18INK4c or RB1 (Kuehl et al, 2012; Chesi et al, 2013; Furukawa et al, 2015).

Patients with MM may be subdivided into groups that exhibit hyperdiploid and non hyperdiploid plasma cells clones. Based on hyper- or ipodiploids and chromosomal translocations involving the 14q32 region, different subgroups of patients with different prognoses can be identified (Avet-Loiseau et al, 2007). The chromosomal / genomic characteristics of patients can therefore be used as prognostic parameters. Conventionally deletion of the chromosome 17, translocation t(4;14)(p16;q32), or translocation t(14;16)(q32;q23) are considered high risks (Palumbo et al, 2015); no uniform consensus for other abnormalities (deletion or gain of chromosome 1, deletion of chromosome 13 alone or with other alterations….) (Table 1). However, to date, the chromosomal characterization of the disease does not correspond to a specific targeted therapeutic approach.

The concept of the progressive acquisition of different mutations giving a selective advantage to the neoplastic clone, has been questioned by genetic studies conducted on monoclonal plasma cells at different stages of the disease. According to another theory, clones of genotypically different plasma cells would co-exist in the same patient, and the balance of these different clones will be responsible for the natural history of the disease (Bahlis NJ, 2012).

More recently, Manier et al, stated that MM is a genetically complex and heterogeneous disease, in which a combination of primary events, secondary events and marked clonal heterogeneity lead to tumour development and progression from MGUS to late-stage MM. Most likely, several driver events need to coincide for the development and progression of the disease (Manier et al, 2017) (Figure 2).

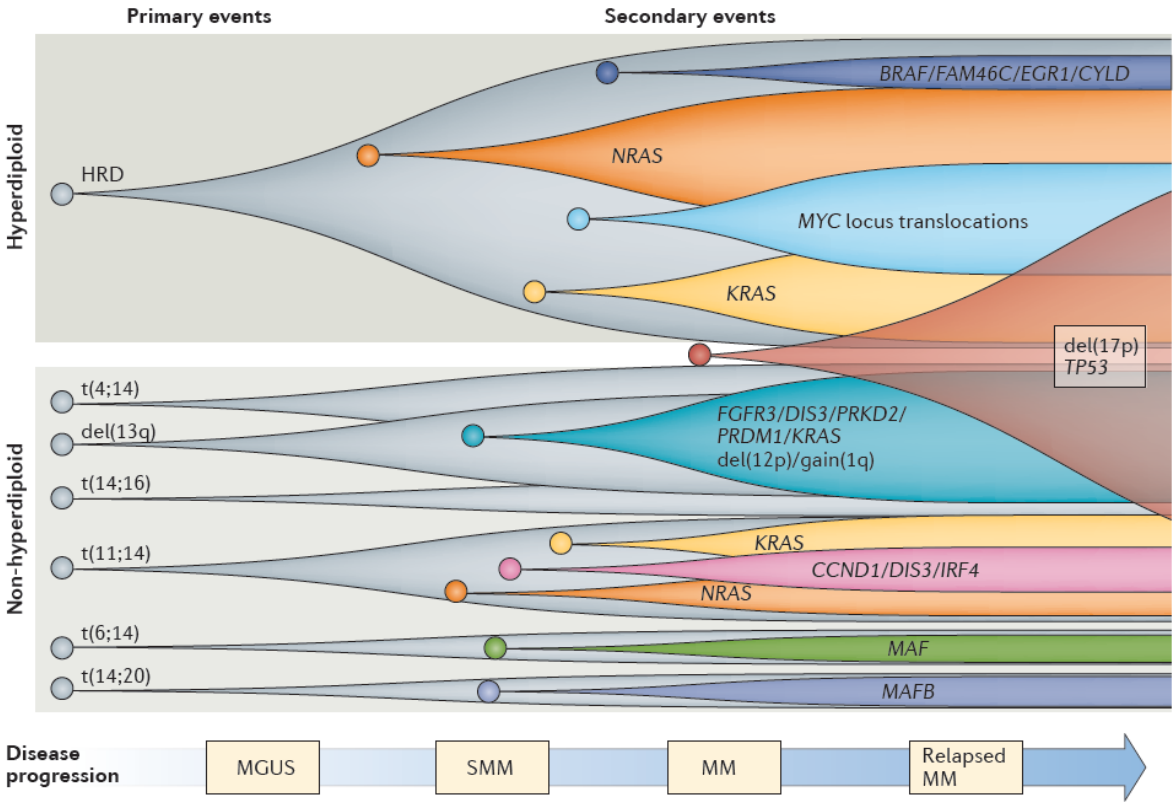


Figure 2. From Manier et al. The primary genomic events affecting certain clones (represented in grey) include hyperdiploid tumours (HRD), translocations in the genes encoding the immunoglobulin heavy chains, and 13q deletion. Secondary events (coloured clones) are represented on the background of the main primary events with which they are associated. Translocations involving MYC can still be detected in some individuals harbouring translocations affecting IgH. Moreover, del(17p) can arise in any multiple myeloma clones, and has been represented at the intersection of HRD tumours and non-HRD tumours. Del: deletion; t: translocation; MGUS: monoclonal gammopathies of undetermined significance; SMM: smouldering multiple myeloma; MM: multiple myeloma.

Phenotype:

The use of monoclonal antibodies allows to identify the phenotype of tumour cells from normal plasma cells. Myeloma plasma cells show a significant antigenic heterogeneity, also due to the different antibody expression that varies in relation to the maturation status of pathological plasma cells. The strong expression of the CD38 antigen allows the distinction of plasma cells from other haemopoietic cells, while the distinction between normal and pathological would be based on the study of other antigens such as VLA4, VLA5, MPC1, CD44, but especially CD19 which is positive in normal plasma cells while CD56, CD20, CD24 and CD10 are negative. Although it has not yet clearly demonstrated the role of the CD56, this antigen, in association with CD19 expression, makes it possible to clearly distinguish pathological plasma cells from the normal ones. The latter are CD56- and CD19 +, and the most mature in the majority are CD56 + and CD19- and a small minority of CD56- CD19- and CD56 + and CD19 +. More recently, another antigen, CD138 (syndecan-1) has been identified on the surface of myeloma plasma cells; this antigen, which is part of the adhesion molecule group, promotes and maintains the aggregation of myeloma cells at the bone marrow level, while it is lost by the plasma cells that are found in peripheral blood in the case of plasma cell leukaemia (Raja et al, 2010).

The probability of detecting probable specific antigens of this disease by flow cytometry could be particularly useful in evaluating the minimum residual disease (MRD).

Prognostic factors:

Prognosis depends on either the number of neoplastic cells or specific characteristics of the disease, including β2-microglobulin, protein C reactive (CRP), serum creatinine and patient age at diagnosis.

β2-microglobulin, which is the light chain component of classical human leukocyte antigen (HLA)

antigens present on the surface of cells, is a low molecular weight protein (11,800 dalton). It can be increased in the serum and urine of patients with inflammatory and malignant diseases. In MM patients its serum levels correlate with tumour mass when evaluated prior to therapy. Furthermore, it has been shown that high levels of β2-microglobulin are associated with a significantly reduced survival and that its measurement can provide information similar to those obtained from M-protein levels in seriated monitoring of such patients.

CRP is directly related to the activity of IL-6, which, as mentioned, is the most important growth factor of the plasma cells. High CRP values are often present in the serum of patients undergoing diagnosis and during the relapse of the disease, while they are low in the remission phase.

Serum creatinine is a very important parameter of renal function evaluation, which is particularly important for MM patients, considering the frequent impairment of this organ.

The age of the patient to diagnosis is another prognostic factor that plays a key role in the therapeutic choice.

Staging:

Despite the numerous attempts to evaluate parameters that are able to classify this tumour differently, the Durie and Salmon system reported in 1975 still remains valid. This system correlates the most important clinical parameters such as M-protein levels, haemoglobin and calcium levels, presence of osteolytic lesions and renal function (Figure 3).

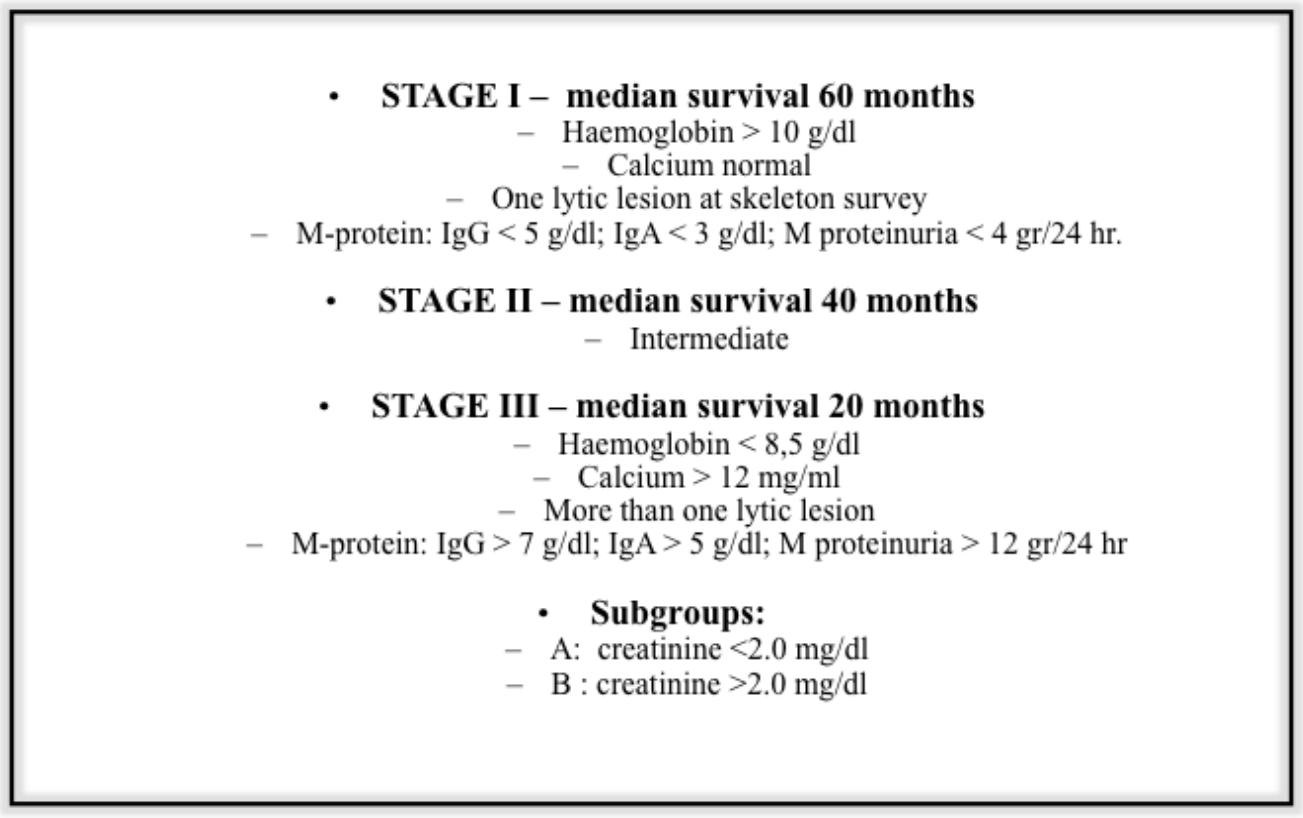


Figure 3: Durie and Salmon staging system.

Subsequently, Durie presented a new staging system, which, however, did not replace the old classification and is based on two parameters β2-microglobulin (cut-off 4 μg / ml) and labelling index (cut-off 0.4%). Patients according to the values of these two parameters are divided into three stages: low, intermediate and high risk.

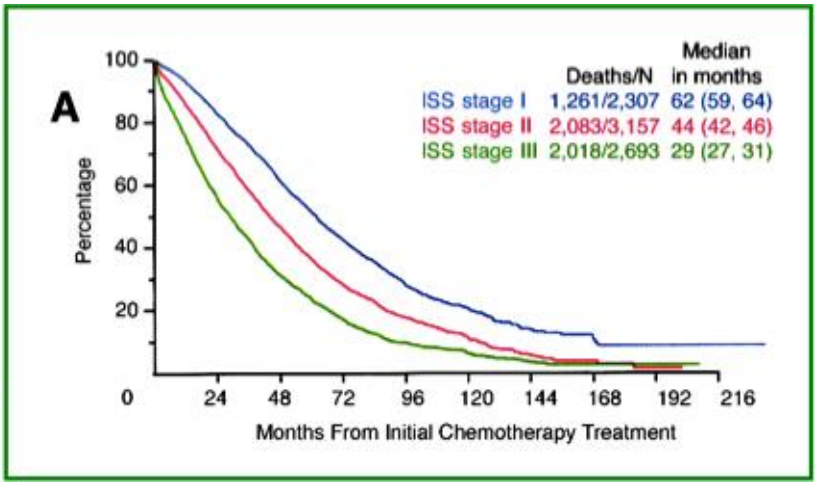
In 1992 Battaille proposed another staging system by dividing patients relative to β2-microglobulin and CRP values. Using an identical cut-off for the two parameters (6 mg / l), according to this classification patients are divided into three risk groups: low, intermediate and high (Battaille et al, 1992).

Numerous other prognostic factors are considered, including molecular alterations such as oncogenic and anti-oncogenic mutations, which, however, require very expensive examinations such as FISH;

thus these alterations are still not always routinely evaluated in clinical practice outside experimental trials.

The International Staging System (ISS) is a more cost-effective alternative to the Durie-Salmon staging system (Greipp et al, 2005). The ISS is based on the assessment of two blood test results: β2-microglobulin and albumin (Figure 4A). This evaluation has shown the greatest prognostic power for MM, and it has already been proven more sensitive in discriminating between three stages of MM. These stages indicate different levels of projected survival rates (Figure 4B).

Stage I	**β_2M <3.5 and ALB ≥3.5**
Stage II	**ALB <3.5 and β2M <3.5 or β2M 3.5–<5.5* * Indip from ALB**
Stage III	**β2M ≥5.5**



4A 4B

Figure 4A. ISS stage based on β2-microglobulin and albumin levels. 4B Overall survival according to ISS stages. B2M: β2-microglobulin (g/dl); ALB: albumin (g/dl); ISS: International Staging System.

More recently, the Revised International Staging System (R-ISS) has combined elements of tumour burden (ISS) and disease biology (presence of high-risk cytogenetic abnormalities or elevated lactate

dehydrogenase level) to create a unified prognostic index that helps in clinical care as well as in comparison of clinical trial data (Palumbo et al, 2015).

Diagnosis:

The suspected diagnosis of MM must be investigated in case of a serum protein electrophoresis and / or urine protein alteration, that sometimes is randomly detected during routine investigations.

In the presence of such alteration, laboratory and instrumental examinations will be required to distinguish the various types of plasma cell dyscrasia and to evaluate the need for any treatment (Table 2).

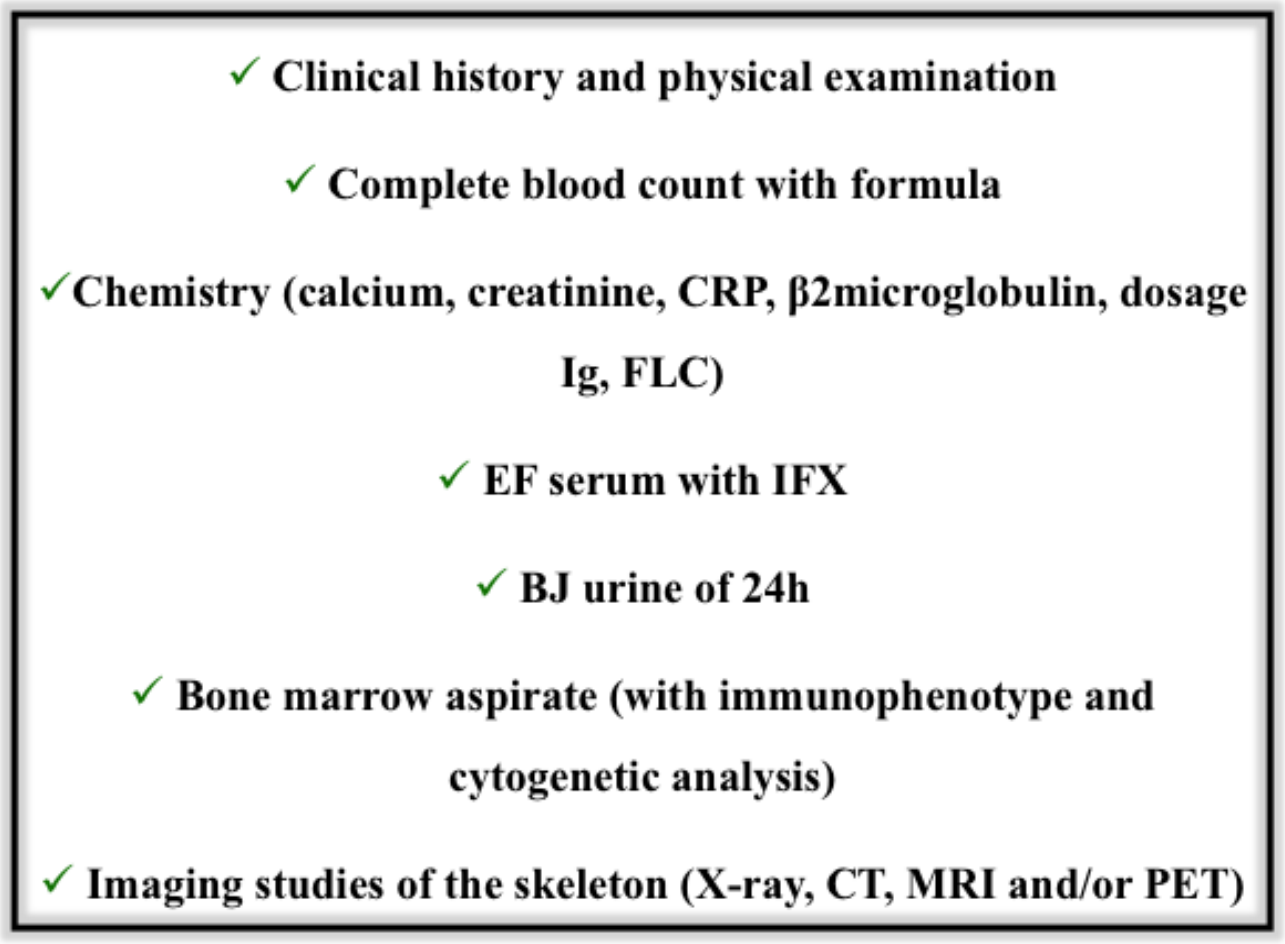


Table 2. Laboratory and instrumental examinations necessary for the diagnosis of multiple myeloma. CRP: protein C reactive; Ig: immunoglobulins; FLC: Free light chain; EF: electrophoresis; IFX: immunofixation; BJ: Bence Jones; h: hours; CT: computed tomography; MRI: magnetic resonance imaging; PET: positron emission tomography.

The haemocromocytometric examination allows to detect the possible anaemia, that is usually macrocitic, and other potential peripheral cytopenias. The quantitative and qualitative study of serum M-protein and / or proteinuria of Bence Jones are evaluated by electrophoresis, immunofixation and dosing of serum and urinary immunoglobulins. Biochemical examinations including calcium, creatinine, albumin, β2-microglobulin and CRP assays allow the evaluation of serum calcium levels, renal function and disease activity. In addition, the free light chain (FLCs) assay, that provides quantification of kappa and lambda chains not bound to intact immunoglobulin molecules and allows the determination of clonality based on the involved and uninvolved light chains ratio (serum FLC ratio ≥100) (Dispenzieri et al, 2009), define more precisely the diagnosis of MM.

Bone marrow aspiration, with morphologic, immunophenotipic and cytogenetic analysis is essential for diagnosis of MM. Bone marrow biopsy is mandatory in case of non-secretory MM (without evidence of M-protein in serum or urine) and in case of solitary plasmacytoma to exclude the bone marrow involvement. The presence of monoclonal plasma cells can be accurately evaluated using anti-CD138 antibodies. In addition, preferably on purified plasma cells, FISH with probes including the 17p13, t (4; 14), and (14; 16) chromosomes lead to the identification of chromosomal aberrations that can have prognostic implications (Avet-Loiseau H, 2007b).

Possible bone lesions should studied with radiological examinations such as, at least standard radiography of the entire skeleton and/or computed tomography (CT), magnetic resonance imaging (MRI) or 18 Fluorodeoxyglucose positron emission tomography (FDG PET). Bone scintigraphy, is not

recommended for many false negatives due to the failure to activate osteoblasts in response to bone destruction by activated osteoclasts. In the case of negative radiographies, it may be necessary to carry out an MRI, which is also useful for highlighting not only bone marrow involvement but also possible spinal compression (Terpos et al, 2011). The role of FDG PET is still under definition in MM. This method is very useful for the identification of extramedullary disease, whereas, for the bone marrow study, MRI has shown greater sensitivity to the identification of bone myeloma lesions compared to FDG PET. However, in recent years, great attention has been paid to the use of this method for identifying predictive factors for long-term therapy and survival, similar to those occurring for other neoplastic diseases (e.g. lymphomas). Analysis of the metabolic activity of the disease by FDG PET has been shown to be a statistically significant prognostic factor both at diagnosis and for monitoring the response obtained by therapy (Zamagni et al, 2007; Zamagni et al, 2011). An extended and high capture intensity in terms of Standardized Uptake Value (SUV) and the presence of extramedullary disease at diagnosis are unfavourable prognostic factors. Concerning the evaluation of response to therapy, the signal suppression correlates with the biochemical response obtained after chemotherapy: the persistence of FDG PET positivity is significantly associated with a lower survival comparing with FDG PET negative patients.

The classic criteria for diagnosis of MM are based on the presence of bone marrow plasmocytosis and the presence of M-protein, as well as the typical symptoms: hypercalcemia, renal insufficiency, anaemia and/or bone lesions (called CRAB manifestation). SMM is considered an asymptomatic MM; however some studies reported specific cases of SMM with a very high risk of development into symptomatic MM. Therefore, in 2014, new MM diagnostic criteria had been revised by the International Myeloma Working Group (IMWG), extending the diagnosis of active MM to SMM with

> 60% of plasma cells bone marrow invasion, FLCs ratio> 100, > 1 focal lesion identified by MRI (high risk of progression) (Rajkumar et al, 2014) (Figure 5).

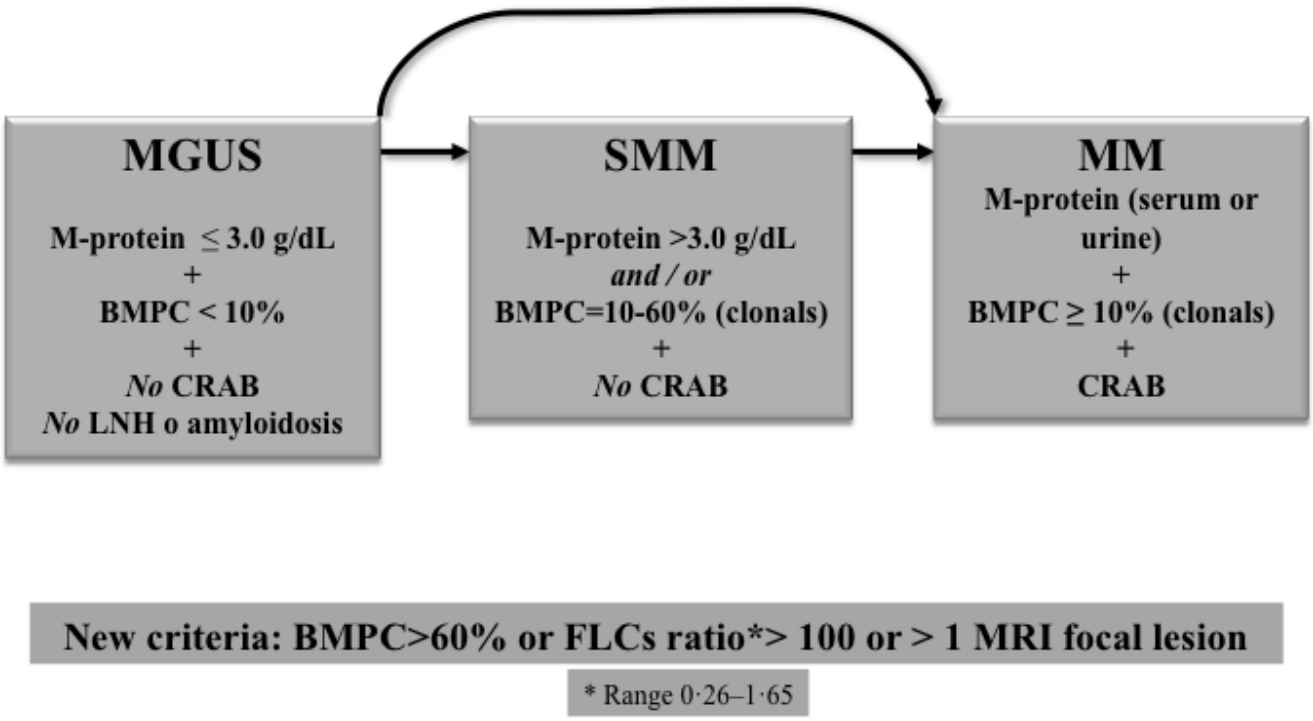


Figure 5. Diagnostic criteria. MGUS: monoclonal gammopathies of undetermined significance; SMM: smouldering multiple myeloma; MM: multiple myeloma; LNH: Non-Hodgkin Lymphoma; BMPC: bone marrow plasma cells; FLCs: free light chains; MRI: magnetic resonance imaging.

According to the new criteria, it is defined as:

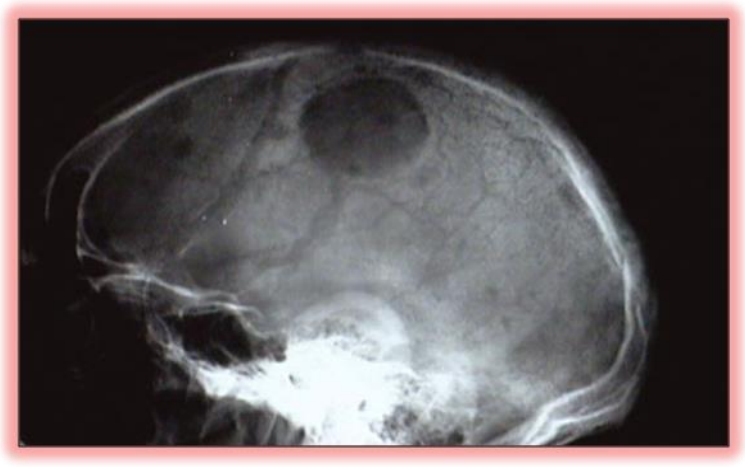
-MGUS: IgG or IgA monoclonal component <3 g / dl (<0.5 g / day if urinary component) with a clonal plasma cells in the bone marrow <10% and in the absence of CRAB symptoms;

SMM: IgG or IgA monoclonal component ≥ 3 g / dl (≥ 0.5 g / dl if urinary component) or a 10-60% clonal plasma cells in the bone marrow, without any CRAB symptoms;

MM: ≥ 10% clonal plasma cells in the bone marrow and CRAB symptoms or > 60% of medullary monoclonal plasma cells or FLCs ratio > 100 mg / dl or > 1 focal lesions highlighted by MRI.

Signs and symptoms:

Bone involvement: severe osteoporosis or osteolytic lesions are often responsible for pathological fractures, spinal cord compression, hypercalcemia and bone pain. These lesions can affect any skeletal segment. Bone pains are often very severe and are the cause of an unacceptable quality of life. Bone destruction is related to increased bone resorption with reduction in the formation of new bone matrix. Excessive osteoclast activity is generally a very early process in this disease, while inhibition of osteoblast activity appears later. In figure 6A and 6B some example of osteolytic lesions are represented.



6A

6B

Figure 6A. Osteolytic lesion of the skull detected by conventional X-rays; 6B. Osteolytic lesion of the clavicola detected by three-dimensional computed tomography

Hypercalcemia: directly related to bone remodeling. It may be symptomatic (anorexia, polyuria and polydipsia, up to vomiting and dehydration, nausea and ultimately signs of encephalopathy in severe cases) and may, in some cases, aggravated by concomitant renal failure.

Renal insufficiency: the pathogenesis is multifactorial, with a primary role played by the excess of light monoclonal chains in plasma, filtered at the glomerular level and then reabsorbed and catabolized at tubular level. During these processes, light chains may precipitate at intratubular level, depositing at the basal membrane of tubules or glomeruli or causing direct or mediated cellular damage. The most frequent morphological and functional aspect is myeloma kidney, whose most common clinical

manifestation is chronic kidney failure (an acute renal failure framework is rarer and generally occurs for other precipitating factors such as hyperuricaemia, dehydration, e.g.). Also light chain disease and amyloidosis can cause renal failure.

Bone marrow insufficiency: the inability to produce normal blood cells due to the proliferation of myeloma cells leading to the progressive replacement of normal cellular lineages. Paleos, asthenia, tachycardia, stress or rest disorder are symptoms that affect about 40% of patients who are just anaemic. Anaemia in many patients is also due to a haemodilution, that is due to the presence and concentration of M-protein and also to a possible endogenous erythropoietin production deficiency that may be related to renal impairment. Neutropenia and thrombocytopenia are less common symptoms in these patients but associated with other factors such as reduced production of normal immunoglobulins and M-protein interference with coagulation factors, in some cases contributing to increased susceptibility to infections and haemorrhagic symptoms that are usually limited to the skin, but may also be due to mucous membranes and parenchymas.

In addition to the aforementioned haemodilution and haemorrhagic symptoms due to the interference with other plasma proteins such as coagulation factors (I, II, V, VII, VIII) and platelet membrane proteins, M-protein may be responsible of increased blood viscosity. This may be due to the physical characteristics of M-protein, which is more pronounced in myeloma IgA (dimer) and IgM (pentamerum) than IgG. Symptoms can vary from modest headaches and dizziness to a true hyperviscosity syndrome. The latter causes, at the microcirculation level, a slowing down of the flow with distension and tortuosity of vessels easily evaluable with the examination of the ocular fund. It may cause congestive heart failure due to increased cardiac work as a result of increased flow

resistance. Peripheral symptomatology is characterized by haemorrhagic syndrome and circulatory insufficiency especially in the lower limbs, while at the cerebral level there is reduction of visus due to retinal microhaemorrhages and neurological symptoms such as headache, ear arousal, difficulty concentrating, drowsiness, regeneration of the sensory until to coma. Even M-protein can directly cause functional damage by depositing itself in the various organs. This is particularly common in the kidneys at both tubule and glomerular levels, causing a progressive picture of chronic renal failure.

A particular picture due to the deposition of M-protein is secondary amyloidosis, which is due to a clinical picture very similar to primary amyloidosis and affects 10% of patients with MM. For its diagnosis it is indispensable to demonstrate the deposition of the amyloid substance in the various organs (subcutaneous tissue, intestinal mucosa, gums, etc.), highlighted with red congo coloration of histological preparations which, when positive, give a typical green fluorescence with polarized light. The most typical sign of amyloidosis is organomegaly; typical, in fact, is the volumetric increase of the tongue, liver and heart, with the possibility of functional hepatic and cardiac deficiency. It is possible to have chronic renal failure due to kidney involvement, congestive heart failure and rhythm disturbances for heart failure, distal sensory neuropathies and malabsorption syndrome, for peripheral and intestinal nerves involvement, respectively. Functional reduction of wrist and finger joint movements may occur in the case of carpal tunnel syndrome due to the deposition of M-protein.

Treatment approaches:

The outcome of MM patients has markedly improved over the past decade, both in young and elderly patients. In the past years, conventional therapy, such as melphalan plus prednisone (MP), was the only active treatment against MM. Since the 1980s, high doses of chemotherapy plus autologous stem cell transplantation (ASCT) proved to be the most suitable option for young newly diagnosed MM (YNDMM) patients (Attal *et al*, 1996; Child *et al*, 2003). More recently, new active classes of drugs, such as proteasome inhibitors-PIs (e.g., bortezomib) and immunomodulatory drugs-IMiDs (e.g., thalidomide and lenalidomide) became the standard of care, alone or in association with the old agents, either for first-line therapy in the transplant and non-transplant settings, or for the treatment of relapsed disease.

Three-drugs combinations, including at least bortezomib or lenalidomide in combination with dexamethasone, are now routinely used as induction therapy for YNDMM patients. In addition, consolidation with high-dose chemotherapy and ASCT is the standard care for these patients. Novel agents have also shown efficacy as maintenance treatment post-ASCT, even if only thalidomide and lenalidomide are allowed outside clinical trials.

Novel agents are also used as induction therapy for elderly patients not eligible for ASCT. Combinations of melphalan, prednisone with thalidomide (MPT) (Fayers et al, 2011) or bortezomib (VMP) (San Miguel et al, 2008) have shown improved progression-free survival (PFS) and overall survival (OS) compared with MP alone. Recently, lenalidomide plus dexamethasone (Rd) has also been licensed for the treatment of non-ASCT eligible patients (Facon et al, 2016).

Treatment for relapse/refractory patients include combinations of new-generation agents, such as carfilzomib (Stewart KA et al, 2015), ixazomib (Moreau et al, 2016a, b) and elotuzumab (Lonial et al, 2016), combined with Rd.

Moreover, patients previously treated with bortezomib and lenalidomide can benefit from

pomalidomide or daratumumab alone or in combination with bortezomib and lenalidomide (Lonial et al, 2016b).

Pomalidomide is the third- generation IMiD, that can be used also in patients with renal failure. Carfilzomib, a second-generation PI used as single agent or in association with lenalidomide, is effective in relapsed myeloma, with a reduced neurotoxicity compared to bortezomib. However, it must be used carefully in patients with severe hypertension or cardiac disease. Ixazomib, another new PI, used in association with lenalidomide, has the advantage of oral administration, but can cause rash and diarrhoea.

The monoclonal antibodody elotuzumab (anti-SLAMF7) plus Rd, has been recently approved for relapsed refractory MM. Daratumumab (anti-CD38) is licensed alone or in association with lenalidomide or bortezomib. Thanks to their good tolerability, these drugs can be especially indicated in the elderly. In particular, daratumumab seems to be the most effective drugs for heavily pre-treated patients, with surprising result as salvage treatment; however, patients with pulmonary disease must be strictly monitored for infusion reaction (bronchospasm, dyspnoea, cough, dizziness e.g.) that can appear during the administration.

In the relapse setting, for young, fit and high-risks patients, reduced-intensity allogeneic transplantation after ASCT can also be considered, if a suitable donor is available.

The choice of the best treatment for relapsed/refractory MM patients depends on:

-compliance, age, comorbidities,

-number and type of previous treatments, type of response, depth and length of response, side effects.

-aggressive disease, high-risks characteristics, plasma cells leukaemia or extramedullary involvement.

The response to treatment is evaluated according to the IMWG criteria. Response criteria are based on serum and urine monoclonal proteins evaluation and bone marrow assessment. However, given the high rates of complete response (CR) seen in patients with new treatment approaches, new response

categories have been recently defined for the identification of deeper and more sensitive responses. In the modern era of the MRD assessment by flow cytometry or gene sequencing and more sensitive imaging techniques, the IMWG has defined new response categories of MRD negativity, with or without imaging-based absence of extramedullary disease (Kumar et al, 2016).

	Response criteria*
IMWG MRD criteria (requires a complete response as defined below)	
Sustained MRD-negative	MRD negativity in the marrow (NGF or NGS, or both) and by imaging as defined below, confirmed minimum of 1 year apart. Subsequent evaluations can be used to further specify the duration of negativity (eg, MRD-negative at 5 years)†
Flow MRD-negative	Absence of phenotypically aberrant clonal plasma cells by NGF‡ on bone marrow aspirates using the EuroFlow standard operation procedure for MRD detection in multiple myeloma (or validated equivalent method) with a minimum sensitivity of 1 in 10^5 nucleated cells or higher
Sequencing MRD-negative	Absence of clonal plasma cells by NGS on bone marrow aspirate in which presence of a clone is defined as less than two identical sequencing reads obtained after DNA sequencing of bone marrow aspirates using the LymphoSIGHT platform (or validated equivalent method) with a minimum sensitivity of 1 in 10^5 nucleated cells§ or higher
Imaging-positive MRD-negative	MRD negativity as defined by NGF or NGS plus disappearance of every area of increased tracer uptake found at baseline or a preceding PET/CT or decrease to less mediastinal blood pool SUV or decrease to less than that of surrounding normal tissue¶
Standard IMWG response criteria‖	
Stringent complete response	Complete response as defined below plus normal FLC ratio** and absence of clonal cells in bone marrow biopsy by immunohistochemistry (κ/λ ratio ≤4:1 or ≥1:2 for κ and λ patients, respectively, after counting ≥100 plasma cells)††
Complete response	Negative immunofixation on the serum and urine and disappearance of any soft tissue plasmacytomas and <5% plasma cells in bone marrow aspirates
Very good partial response	Serum and urine M-protein detectable by immunofixation but not on electrophoresis or ≥90% reduction in serum M-protein plus urine M-protein level <100 mg per 24 h
Partial response	≥50% reduction of serum M-protein plus reduction in 24 h urinary M-protein by ≥90% or to <200 mg per 24 h; If the serum and urine M-protein are unmeasurable, a ≥50% decrease in the difference between involved and uninvolved FLC levels is required in place of the M-protein criteria; If serum and urine M-protein are unmeasurable, and serum-free light assay is also unmeasurable, ≥50% reduction in plasma cells is required in place of M-protein, provided baseline bone marrow plasma-cell percentage was ≥30%. In addition to these criteria, if present at baseline, a ≥50% reduction in the size (SPD)§§ of soft tissue plasmacytomas is also required
Minimal response	≥25% but ≤49% reduction of serum M-protein and reduction in 24-h urine M-protein by 50–89%. In addition to the above listed criteria, if present at baseline, a ≥50% reduction in the size (SPD)§§ of soft tissue plasmacytomas is also required
Stable disease	Not recommended for use as an indicator of response; stability of disease is best described by providing the time-to-progression estimates. Not meeting criteria for complete response, very good partial response, partial response, minimal response, or progressive disease
Progressive disease ¶¶,‖‖	Any one or more of the following criteria: Increase of 25% from lowest confirmed response value in one or more of the following criteria: Serum M-protein (absolute increase must be ≥0·5 g/dL); Serum M-protein increase ≥1 g/dL, if the lowest M component was ≥5 g/dL; Urine M-protein (absolute increase must be ≥200 mg/24 h); In patients without measurable serum and urine M-protein levels, the difference between involved and uninvolved FLC levels (absolute increase must be >10 mg/dL); In patients without measurable serum and urine M-protein levels and without measurable involved FLC levels, bone marrow plasma-cell percentage irrespective of baseline status (absolute increase must be ≥10%); Appearance of a new lesion(s), ≥50% increase from nadir in SPD§§ of >1 lesion, or ≥50% increase in the longest diameter of a previous lesion >1 cm in short axis; ≥50% increase in circulating plasma cells (minimum of 200 cells per μL) if this is the only measure of disease

(Table 4 and footnotes continue on the next page)

Figure 7. New IMWG response criteria from Kumar et al, 2016. MRD: minimal residual disease; NGF: next-generation flow; NGS: next-generation sequencing; PET: positron emission tomography; CT: computed tomography; SUV: Standardized Uptake Value; SPD: sum of the products of the maximal perpendicular diameters of measured lesions; FLC: free light chain; IMWG: International Myeloma Working Group.

Treatment in young newly diagnosed Multiple Myeloma patients:

Three-drugs combinations, including at least bortezomib or lenalidomide in combination with dexamethasone, are now routinely used as induction therapy for YNDMM patients.

Novel agents showed better responses than the old conventional drugs in numerous studies. The French group reported that newly diagnosed patients receiving induction therapy with bortezomib and dexamethasone (VD) versus (vs) vincristine, doxorubicin and dexamethasone (VAD) obtained a marked improvement in overall responses either after induction or after ASCT. After induction, CR and near CR (nCR) rate were 14.8% in the VD arm and 6.4% in the VAD arm; at least very good partial response (VGPR) rate was 37.7% vs 15.1%, and overall response was 78.5% vs 62.8%. CR/nCR and at least VGPR rates were higher regardless of disease stage or adverse cytogenetic abnormalities. After the first ASCT, CR and nCR rate were 35% in the VD arm, compared with 18.4% in the VAD arm; the VGPR rate was 54.3% of patients of the VD arm and in 37.2% of patients of the VAD arm. The PFS was 36 vs 29.7 months ($p = 0.064$) with VD vs VAD and was longer in patients achieving at least VGPR after ASCT (Harousseau *et al*, 2008; Harousseau *et al*, 2010).

Sonneveld et al reported the experience of 827 eligible young patients with newly diagnosed MM that were randomly assigned to receive VAD or bortezomib, doxorubicin, and dexamethasone (PAD) followed by high-dose melphalan and ASCT and a maintenance treatment with thalidomide 50 mg in the VAD arm or bortezomib every 2 weeks for 2 years in the PAD arm. CR and nCR were superior after PAD induction (15% v 31%; $p < 0.001$). After a median follow-up of 41 months, PFS was superior in the PAD arm (median of 28 months vs 35 month); in multivariate analysis, OS was better in the PAD arm. Moreover, in high-risk patients with increased creatinine or with deletion 17p13, bortezomib significantly improved PFS and OS (Sonneveld *et al*, 2012).

In the light of these results, nowadays, novel agents have become the standard treatment for young MM patients. Different combinations with novel agents as induction therapy for ASCT have been explored.

Doublet therapies combining either an IMiD or bortezomib with dexamethasone (eg, thalidomide and dexamethasone-TD or lenalidomide and dexamethasone-Rd or VD) affected higher overall response rates than traditional treatments (Cavo *et al,* 2005; Macro *et al,* 2006, Cavo *et al,* 2009, Rajkumar *et al,* 2008, Harousseau *et al,* 2008; Harousseau *et al,* 2010). For example, in comparison with VAD, thalidomide plus dexamethasone resulted in a significantly higher response rate (52% vs 76%, respectively; $p < 0.001$) (Cavo *et al,* 2005; Macro *et al,* 2006, Cavo *et al,* 2009).

However, triplet induction regimens, in particular, bortezomib plus thalidomide and dexamethasone (VTD), further increased the rate of CR and/or at least VGPR, both before and after autotransplantation (Cavo *et al,* 2010; Rosinol *et al,* 2010; Rosinol *et al,* 2012).

Cavo et al. analysed 480 YNDMM patients randomly assigned to receive VTD or TD plus double ASCT followed by two cycles of their assigned drug regimen as consolidation therapy. After induction therapy, CR or nCR were significantly higher in the VTD arm after induction therapy, after the first ASCT and after the second ASCT, with an overall CR/nCR rate of 71% in the VTD arm and 54% in the TD arm ($p < 0.0001$). Grade 3 or 4 adverse events were recorded in a significantly higher number of patients on VTD with a higher occurrence of peripheral neuropathy but almost reversible (Cavo *et al*, 2010).

Also the Spanish Group confirmed the superiority of VTD respect to TD and to conventional chemotherapy. The CR rate was significantly higher with VTD than with TD (35% vs 14%, $p = 0.001$) or with chemotherapy (35% vs 21%, $p = 0.01$). The median PFS was significantly longer with VTD (56.2 vs 28.2 vs 35.5 months, $p = 0.01$). The post-ASCT CR rate was higher with VTD than with TD (46% vs 24%, $p = 0.004$) (Rosinol *et al,* 2012). Even reduced doses of bortezomib (vTD), compared with VD, permitted the achievement of higher VGPR rates (Moreau *et al,* 2011a).

Several newer induction treatments, such as RVD (lenalidomide, bortezomib and dexamethasone), VCD (bortezomib plus cyclophosphamide and dexamethasone), PAD and Rd, have been also included

as a category 1 recommendation in the United States National Comprehensive Cancer Network Clinical Practice Guidelines in Oncology for Multiple Myeloma Version 1.2011.23. (Kumar et al, 2008; NCCN; Cavo *et al,* 2011; Moreau *et al,* 2011b; Sonneveld *et al,* 2012). For example, Richardson et al showed that RVD had favourable tolerability and was highly effective with an estimated 18-month PFS and OS for the combination treatment with/without transplantation of 75% and 97%, respectively (Richardson *et al,* 2010). Recently the French group, with the same regimen, observed 58% of CR rate and 68% of MRD negativity by flow cytometry. The most common toxicities were neurological and haematological with grade 3 to 4 neutropenia (35%), and thrombocytopenia (13%) (Russell *et al,* 2014). Also VCD can be used, however studies comparing VTD and VCD showed higher responses and less toxicity with VTD regimen (Cavo *et al,* 2015; Moreau *et al,* 2016c).

Four-drugs induction regimens have been also investigated, but toxicities appeared too high respect to a real advantage in terms of efficacy (Kumar *et al,* 2012a; Ludwig *et al,* 2013).

In summary, latest 2015 IMWG recommendation provided the use of a three-drug regimen based on bortezomib/dexamethasone, with the addition of a third agent as cyclophosphamide, adriamycin, thalidomide, or lenalidomide (Cavo *et al,* 2011).

In the absence of randomized studies comparing different induction regimens, it is difficult to recommend one induction regimen over another. However, biological and clinical characteristics of patients at the onset may influence the choice. For example, high-risk patients with t(4;14), translocation or deletion of chromosome 17p could benefit from bortezomib-based regimen. In the presence of patients with comorbidities, for example acute renal failure or thromboembolic disease, bortezomib-based regimens should be used, while patients with pre-existing neuropathy could be treated with lenalidomide-based regimens.

Novel agents translate into higher frequencies of CR or at least VGPR, before and after single or double ASCT; moreover, the achievement of deeper responses translates into a significant prognostic

factor for the outcome of these patients resulting in improvement of PFS and OS.

Not at least, better results after ASCT must be interpreted considering also the improvements in supportive care strategies.

However, in the era of novel agents, numerous issues arise. One is the length of induction treatment: the choice of giving 3 to 6 cycles of induction therapy depends on the depth of response and toxicity observed. However, especially in patients treated with lenalidomide-based regimens, peripheral blood stem cells should be collected early, after 4 to 6 cycles of induction therapy.

In addition, also the role of chemotherapy mobilization is under debate. In fact, given the impressive results of the newer agents, maybe chemotherapy mobilization, with potential side effects, could become unnecessary and growth factors alone could be sufficient.

Another question is the number of transplants and the timing of transplantation. Some retrospective trials analysed the role of early vs delayed ASCT after novel agents and PFS and OS were similar (Kumar *et al,* 2012b; Dunavin *et al,* 2013). However, there are few prospective trials that compare novel agents vs ASCT; data seem to be in favour of ASCT (Attal *et al,* 2015; Gay *et al,* 2015). Palumbo et al. recently reported that ASCT, compared with MPR (melphalan, prednisone and lenalidomide) consolidation, significantly prolonged PFS and OS (Palumbo *et al*, 2014). Therefore, until further clinical trials will definitively answer to this question, ASCT, performed after induction with novel agents, still remain the treatment of choice.

Autologous transplantation may be single or tandem (a planned second course of ASCT within 6 months of the first). Studies performed in the old era, showed improvement in OS with tandem ASCT (Attal *et al,* 2003); this is confirmed particularly in patients who failed to achieve at least a VGPR (Cavo *et al,* 2007; Harousseau *et al,* 2009). Also with novel agents, some groups strongly suggested tandem ASCT in case of MRD positivity after induction therapy. Moreover, recent studies suggest that there is a role for tandem ASCT in the presence of adverse cytogenetic abnormalities (Cavo *et al,* 2013;

Martino *et al*, 2016).

The future question in the "modern era" is about the role and the timing of ASCT in the light of the high efficacy of the new generation IMiDs, PIs and monoclonal antibodies.

Regarding the role of IMiDs and PIs that are now routinely used in the clinical practice, we wanted to collect the results obtained in YNDMM patients in a single Centre experience and compare the actual induction regimens with alkylating agents and conventional chemotherapy used in the past. In addition, we wanted to analyse the role of single and tandem ASCT in the different regimens (old vs novel agents).

Materials and methods

Patients and treatments:

We retrospectively analysed a cohort of YNDMM patients treated in our Centre between October 1988 and November 2014. At the onset, serological testing, bone marrow aspiration and skeletal survey were performed for all patients. Bone marrow biopsy with morphological and immunohistochemical analysis was performed only in case of not evaluable bone marrow aspiration. Flow cytometry was not routinely performed. FLC determination, CT, FDG PET or other diagnostic procedures were introduced only in the recent years, so were not included in the analysis. Diagnosis was made according to the IMWG standard criteria used at the time of the diagnosis. All patients received old or novel agents followed by ASCT (single or tandem) as first line treatment. The chemotherapy regimens included MP, VAD, VTD, VCD, TD and Rd. Tandem ASCT was usually performed sequentially 3–6 months after the first transplant. All patients received primary induction chemotherapy, followed by stem cell mobilization with high dose cyclophosphamide (4 gr/m^2 day) on day 0 and filgrastim at a dose of 10 μg/kg body weight per day from day +5. Aphereses were performed trying to collect a minimum of 4 x 10^6 CD34-positive cells per kilogram body weight. Subsequently stem cells were cryopreserved and thawed immediately before re-transfusion. Conditioning chemotherapy consisted of melphalan at a dose of 200 mg/m^2, given in two doses at days –3 and –2 or as single dose at day –2 before stem cell transplantation. Patients with reduced performance score, organ dysfunctions, renal insufficiency or age over 65 years received a reduced dose in the range of 100–140 mg/m2 melphalan. Patients received filgrastim from day +5 on or pegfilgrastim 3 μg/kg/day once on day +1 after ASCT to shorten time to engraftment. The primary end-point was OS; secondary end-point was PFS.

Response criteria:

Response assessment was based on the European Group for Blood and Marrow Transplant (EBMT) criteria, proposed in 1998 (Blade et al, 1998) (Figure 8), applicable for all our patients (diagnosis from 1988 to 2014). Assessment of disease was chiefly based on serological testing because bone marrow biopsy after transplantation was omitted in most of our patients. MRD was only recently introduced in clinical practice therefore new IMWG response criteria were not applicable.

RESPONSE TO TREATMENT	EBMT CRITERIA FOR COMMON TYPE	EBMTCRIERIA FOR LIGHT CHAIN
Stable Disease **(SD)**	Less than 25% ↓ of Monoclonal Protein (MP) in the blood	
Minimal Response **(MR)**	Between 25 and 49% ↓ of Monoclonal Protein (MP) in the blood + 50-89% reduction in 24h urinary light chain excretion (monoclonal proteinuria>200 mg/d)	50-89% reduction in 24h urinary light chain excretion and monoclonal proteinuria > 200 mg/d
Partial Response **(PR)**	Over 50% ↓ of serum MP + > 90% reduction in 24h urinary light chain excretion or M proteinuria < 200mg/d	> 90% reduction in 24h urinary light chain excretion or monoclonal proteinuria < 200mg/d
near Complete Response (**nCR**)	Serum MP = 0 but Serum IF > 0	
Complete Response (**CR**)	No Monoclonal Protein (MP) in the blood + No serum/urine MP by Immunofixation (IF < 0) + < 5% plasma cells in bone marrow aspirate	**Partial Response Criteria** + No serum/urine MP by Immunofixation (IF < 0) + < 5% plasma cells in bone marrow aspirate

Figure 8. The EBMT response criteria from Blade et al, 1998. EBMT: European Group for Blood and Marrow Transplant.

Statistics:

Non-parametric tests were applied, in univariate analyses, for comparisons between groups (Chi-Squared and Fisher Exact test for difference in terms of categorical variables or response rate, Mann-Whitney and Kruskal-Wallis test for difference in terms of continuous variables). OS, PFS, Progression-free Survival 2 (PFS2) were estimated using Kaplan-Meier curves and, when appropriate, differences were evaluated with Log-Rank test in univariate analysis and by means of Cox regression model in multivariate analyses, after assessment of proportionality of hazards. OS was calculated from the start of first line treatment until death for any reason. PFS was calculated from the start of first line treatment to objective disease progression, or death from any cause, whichever occurs first. PFS2 was calculated from the start of first line treatment to second objective disease progression, or death from any cause, whichever first. The confidence intervals were calculated at 95%, all tests were two-sided and a P-value $p<0.05$ was considered statistically significant. All statistical tests were performed using the SAS statistics software (release 9.4).

Results

Patient's characteristics:

We retrospectively analysed 258 YNDMM patients treated in our Centre between October 1988 and November 2014. The median age was 54 years (range, 18-69). Even if young patients are usually considered ≤ 65 years, 4 patients > 65 years were treated as young patients because evaluated particularly fit. Totally, 137 were men and 121 were female.

Patient's characteristics are reported in Table 3A and 3B.

	n	*%*
Sex		
Female	121	46.90
Male	137	53.10
type of heavy chain		
0	34	13.18
IgA	59	22.87
IgD	6	2.32
IgG	159	61.63
type of light chain		
Kappa	168	65.12
Lambda	90	34.88
bone lesions		
0	73	28.29
1	32	12.41
1-3	25	9.69
>3	128	49.61
plasmacytoma		
Yes	38	14.73
No	220	85.27
ISS stage		
I	100	58.48
II	49	28.65
III	22	12.87

Table 3A. Patient's characteristics at the onset. Sex, heavy and light chain, myeloma subtype, presence of bone lesions or plasmacytoma and ISS were reported; ISS stage was evaluable in 171 patients. N: number; ISS: International Staging System.

	Median	***n***	***Min***	***Max***
age	54.98	258	18.92	69.43
CM gr/dl	3.50	257	0.00	12.85
BJ gr/24hr	0.32	227	0.00	20.40
%Plasm BM	46.00	237	1.00	95.00
Hb	11.25	254	4.60	16.30
calcium	9.43	250	2.20	15.20
creatinine	1.00	254	0.30	15.00

Table 3B. Patients' characteristics at the onset. N: number; Min: minimum; Max: maximum; CM= M-protein expressed in gr/dl; BJ= Bence Jones expressed in gr/24 hours; %Plasm BM= percentage of plasmacells in bone marrow aspiration; Hb= haemoglobin level expressed in g/dl; calcium and creatinine level expressed in mg/dl.

At the serum immunofixation the IgG was the most frequent heavy chain type (61.36%). Frequency of IgA and IgD isotype disease was 22.87% and 2.32%, respectively. Few patients (13.18%) had only light chain components. Among all patients, kappa light chain expression was more frequent than lambda light chain expression (65.12% vs 34.88%). Extramedullary plasmacytoma at the time of diagnosis occurred in 38 patients (14.73%).

Between August 1989 and May 2014, all 258 YNDMM patients underwent ASCT. As induction treatment, between October 1988 and October 2008, 173/258 patients received old drugs, i.e. VAD (n=167) or MP (n=6), while 85/258 patients, between February 2005 and November 2013, were treated with novel agents, i.e. bortezomib-based (n=67) or IMiD-based regimens (n=18). Among patients treated with bortezomib-based regimens, 36 patients received VTD, 25 patients VCD and 6 patients VD. Among patients treated with IMiD-based regimens, 12 patients received Rd and 6 TD (Table 4).

	Old drugs (number)	**New drugs (number)**
Total Patients (258)	**173**	**85**
VAD	**167**	**-**
MP	**6**	**-**
bortezomib- based regimens (VTD, VCD, VD)	**-**	**67 (36, 25, 6)**
IMiDs-based regimens (Rd, TD)	**-**	**18 (12, 6)**

Table 4. Induction regimens used for all 258 patients. VAD: vincristine, doxorubicin and dexamethasone; MP: melphalan and prednisone; VTD: bortezomib, thalidomide and dexamethasone; VCD: bortezomib, cyclophosphamide and dexamethasone; VD: bortezomib and dexamethasone; Rd: lenalidomide and dexamethasone; TD: thalidomide and dexamethasone.

All 258 patients received high doses melphalan 200 mg/m^2 as conditioning regimen and a single (n=153) or tandem (n=105) ASCT.

The median time from diagnosis to ASCT was 6 months (range, 4-36 months).

Treatment responses:

We evaluated the association between responses and patients' characteristics. Only the use of novel agents vs old agents had a significant impact on the responses obtained. No differences were observed between patients treated with IMiDs-based regimens and bortezomib-based regimens in terms of responses (Table 5A and 5B).

Responses	*CR/NCR/VGPR*		*other*		*All patients*	
	n	***%***	***n***	***%***	***n***	***p-value***
All patients	67	25.97	191	74.03	258	
sex						0.49
female	29	23.97	92	76.03	121	
male	38	27.74	99	72.26	137	
novel agents						**<.0001**
no	31	17.92	142	82.08	173	
yes	36	42.35	49	57.65	85	
type of heavy chain						0.05
0	15	44.12	19	55.88	34	
IgA	11	18.64	48	81.36	59	
IgD	1	16.67	5	83.33	6	
IgG	40	25.16	119	74.84	159	
type of light chain						0.85
Kappa	43	25.60	125	74.40	168	
Lambda	24	26.67	66	73.33	90	
bone lesions						0.10
<3	28	21.54	102	78.46	130	
>3	39	30.47	89	69.53	128	
plasmacytoma						0.21
yes	13	34.21	25	65.79	38	
no	54	24.55	166	75.45	220	
type of drugs						0.77
IMiDs-based	7	38.89	11	61.11	18	
Bortezomib-based	29	436.28	38	56.72	67	
tandem ASCT						0.71
no	41	26.79	112	73.20	153	
yes	26	24.77	79	74.53	105	
ISS stage						0.15
I-II	32	23.53	104	76.47	136	
III	8	38.10	13	61.90	21	

Table 5A. Association between responses and patient's characteristics at the onset. Only the use of novel agents vs old agents had a significant impact on the response obtained ($p < 0.0001$). CR: complete response; nCR: near complete response; VGPR: very good partial response; IMiDs: immunomodulatory drugs; ISS: International Staging System.

		Response		*All patients*	
		CR/NCR/VGPR	***other***		**p-value**
age	*Median*	55.73	54.75	54.98	0.23
	Min	37.35	18.92	18.92	
	Max	69.43	68.77	69.43	
CM gr/dl	*Median*	2.95	3.60	3.50	0.27
	Min	0.00	0.00	0.00	
	Max	12.50	12.85	12.85	
BJ gr/24hr	*Median*	0.25	0.36	0.32	0.89
	Min	0.00	0.00	0.00	
	Max	11.20	20.40	20.40	
%Plasm BM	*Median*	48.00	44.50	46.00	0.27
	Min	5.00	1.00	1.00	
	Max	91.00	95.00	95.00	
Hb	*Median*	11.45	11.20	11.25	0.98
	Min	4.60	6.60	4.60	
	Max	16.30	15.90	16.30	
calcium	*Median*	9.38	9.45	9.43	0.61
	Min	7.50	2.20	2.20	
	Max	15.20	15.20	15.20	
creatinine	*Median*	1.00	1.00	1.00	0.07
	Min	0.50	0.30	0.30	
	Max	15.00	10.40	15.00	

Table 5B. Association between responses and patients' characteristics at the onset. CR: complete response; nCR: near complete response; VGPR: very good partial response; CM= M-protein expressed in gr/dl; BJ= Bence Jones expressed in gr/24 hours; %Plasm BM= percentage of plasmacells in bone marrow aspiration; Hb= haemoglobin level expressed in g/dl; calcium and creatinine level expressed in mg/dl.

Overall, after induction, 67 patients (25.9%) achieved CR, nCR or VGPR. Among patients treated with new drugs, a CR/nCR/VGPR was observed in 36/85 patients (42.3%) after induction, in 36/85 patients (42.3%) after single ASCT and in 27/50 patients (54%) after tandem ASCT. No differences were observed in terms of response and survival between IMiDs or bortezomib-based regimens (data not shown). For patients treated with old drugs, a CR/nCR/VGPR was recorded in 31/173 (17.9%) after induction, in 50/173 patients (28.9%) after single ASCT and in 19/55 patients (34.5%) after tandem ASCT (Tab 6).

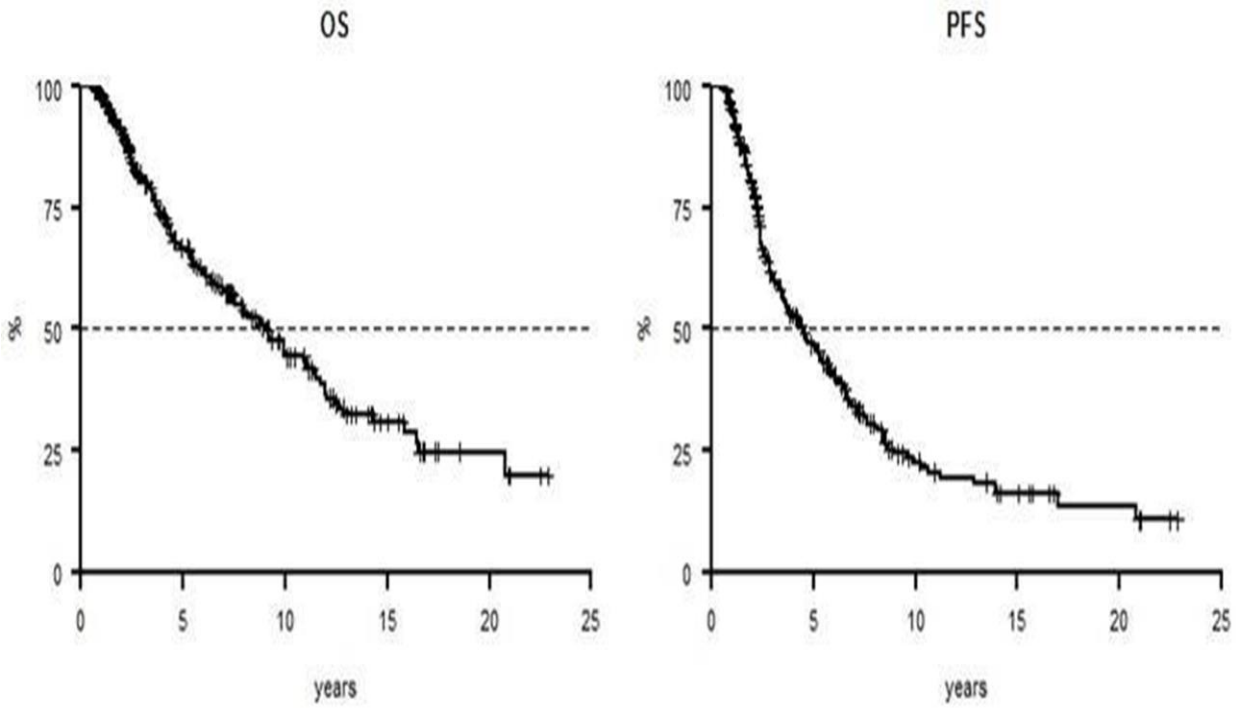
	Old drugs (CR/nCR/VGPR %)	**New drugs (CR/nCR/VGPR %)**
Post induction	**17,9**	**42,3**
After single ASCT	**28,9**	**42,3**
After tandem ASCT	**34,5**	**54**

Table 6. Responses ≥ VGPR after induction, after a single ASCT and after tandem ASCT in patients treated with old drugs and with novel agents. CR: complete response; nCR: near complete response; VGPR: very good partial response; ASCT: autologous stem cell transplantation.

Out of 105 patients who received tandem ASCT, CR/nCR/VGPR was recorded in 33/105 (31.4%) after the first ASCT and 46/105 (43.8%) after the second ASCT, p=<.0001. The contribution of the second ASCT was statistically significant both among patients treated with new and old drugs (p=0.001 and p=0.005, respectively).

Overall survival and Progression-free Survival:

OS at 10 years, for all 258 patients, was 44.4% (median 8.9 years; IC 95% 37.4-52.6) (Fig 9A). PFS at 10 years was 22.5%, (median 4.5 years; IC 95% 16.8-30.1) (Fig 9B).



9A 9B

Figure 9A and 9B. OS and PFS for all 258 patients. OS: overall survival; PFS: progression-free survival.

OS was better for patients in CR/nCR/VGPR after induction than patients who obtained a partial response (PR) or showed a stable disease (SD). In fact, OS at 10 years was 62.1% (IC 95%: 49.6-77.7) for patients in CR/nCR/VGPR and 40.7% (IC 95%: 33.1-50.0) for patients who obtained a PR or SD (p = 0.0632) (Fig 10A). Median OS were 16.5 vs 8.3 years, respectively.

Also PFS was better for patients in CR/nCR/VGPR after induction than patients who obtained a PR or SD. In fact, PFS at 10 years was 36.2% (IC 95%: 23-57.0) for patients in CR/nCR/VGPR and 17.2% (IC 95%: 12-24.6) for patients who obtained a PR or SD (p = 0.0685) (Fig 10B). Median PFS were 6.6 vs 3.8 years, respectively.

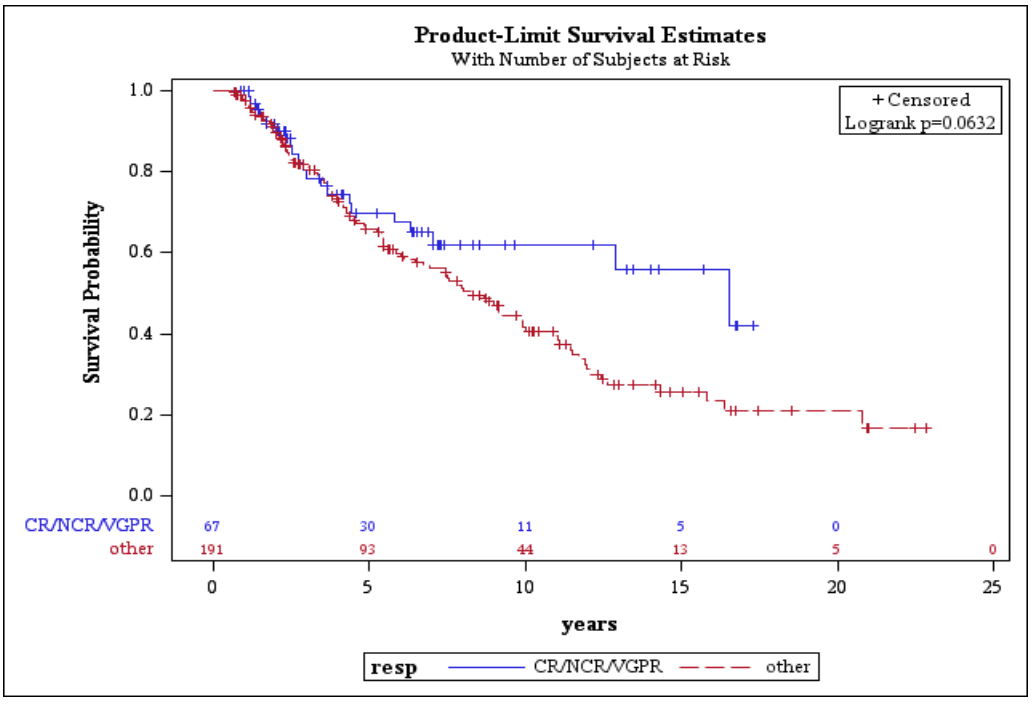


Figure 10A. OS of patients that achieved at least a VGPR after induction (blue line) and of patients that obtained other responses, that is partial responses or stable disease (red line). Resp: responses; CR: complete response; nCR: near complete response; VGPR: very good partial response; OS: overall survival.

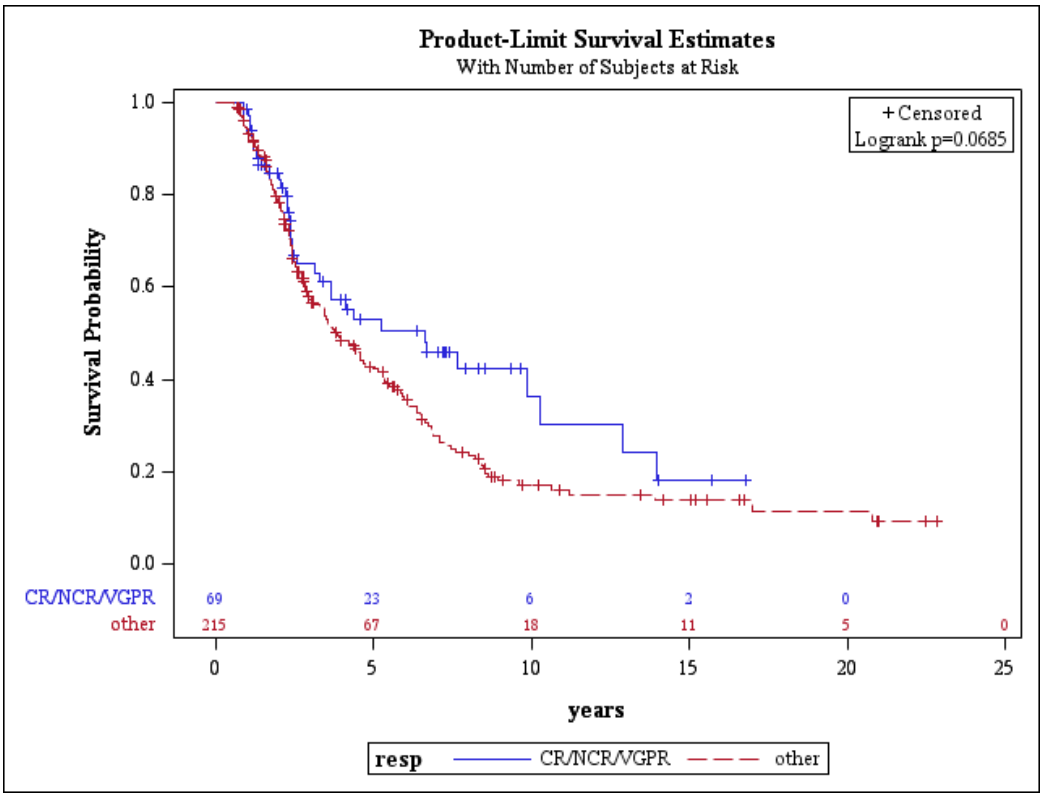


Figure 10B. PFS of patients that achieved at least a VGPR after induction (blue line) and of patients that obtained other responses, that is partial responses or stable disease (red line). Resp: responses; CR: complete response; nCR: near complete response; VGPR: very good partial response; PFS: progression-free survival.

OS was slightly better for patients treated with new drugs than those treated with old drugs. In fact, OS at 8 years was 66.1% (IC 95%: 53.5-81.7) for the first group vs 51.5% (IC 95%: 44.3-60.0) for the second group, respectively (p = 0.26 n.s.) (Fig 11A). Median OS was not reached for patients treated with novel agents and 8.7 years for patients treated with old drugs.

However, PFS was significantly improved for patients treated with new drugs than those treated with old drugs. PFS at 8 years was 55% (IC 95%: 41.2-73.4) for the first group vs 25.3%, (IC 95%: 19.4-33.0) for the second group, respectively (p = 0.0047) (Fig 11B). Median PFS was not reached for patients treated with novel agents and 3.7 years for patients treated with old drugs.

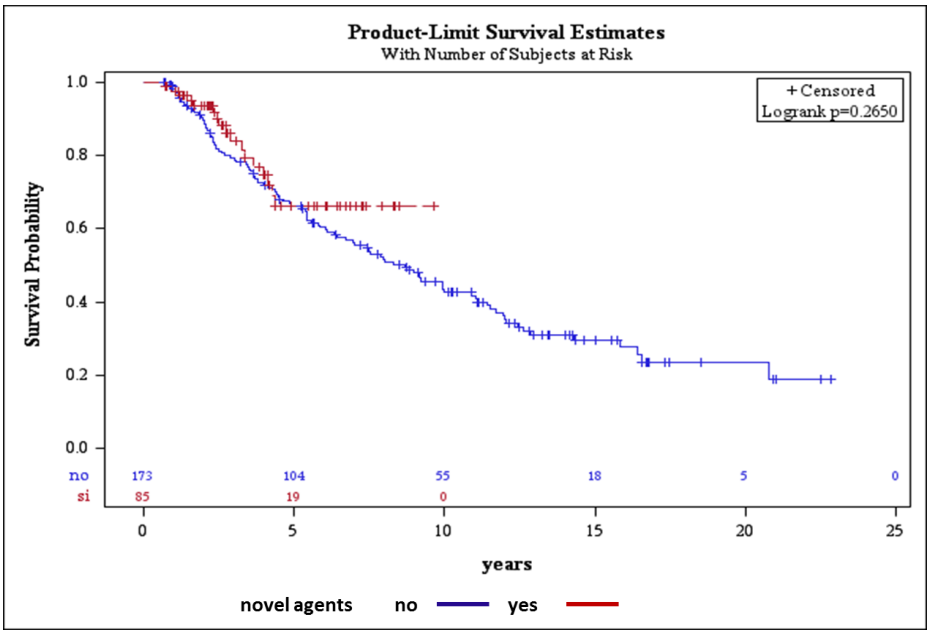


Figure 11A. OS of patients treated with novel agents (red line) and of patients treated with old drugs (blue line). OS: overall survival.

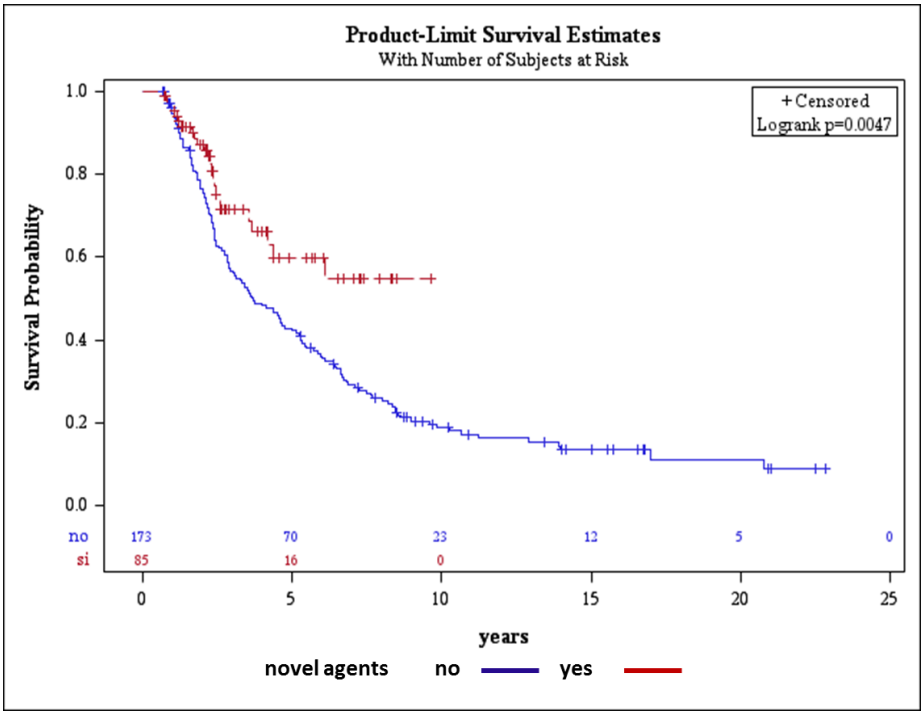


Figure 11B. PFS of patients treated with novel agents (red line) and of patients treated with old drugs (blue line). PFS: progression-free survival.

No differences were observed in terms of OS and PFS if we analysed the group treated with novel agents according to the regimen used, IMiDs- or bortemib- based regimens (Fig 12A and B). However, only few patients were treated with IMiDs- based regimens (n=18).

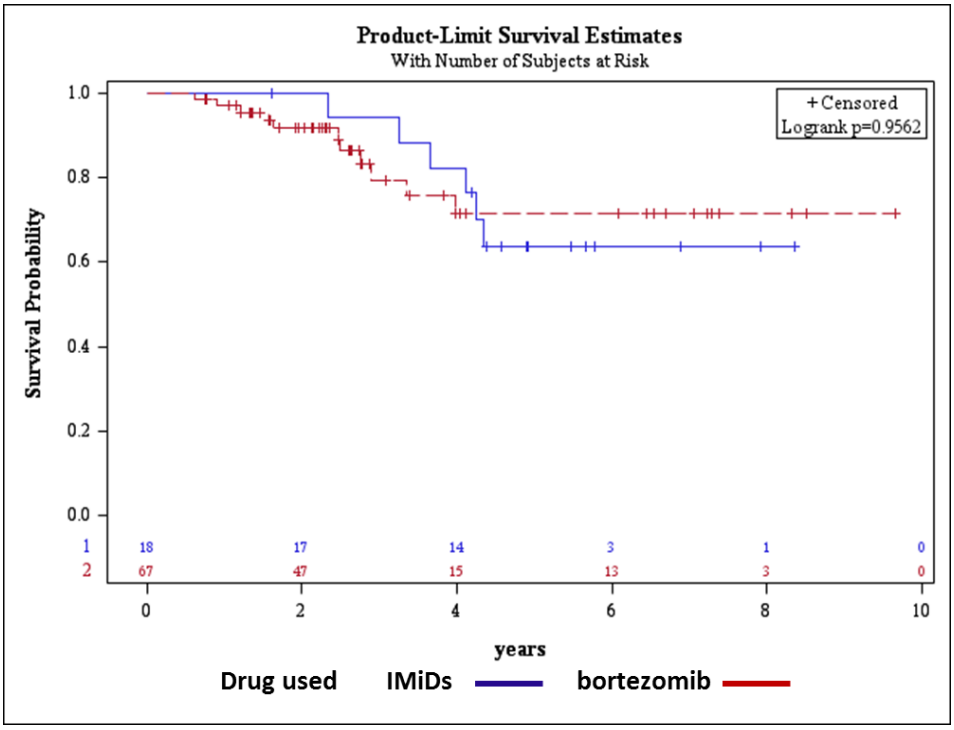


Figure 11A. OS at 8 years was 63% (IC 95%: 44.2-91.8) for patients treated with IMiDs-based regimens and 71.5% (IC 95%: 57.5-89.0) for patients treated with bortezomib-based regimens (p = 0.9562). OS: overall survival. IMiDs: immunomodulatory drugs.

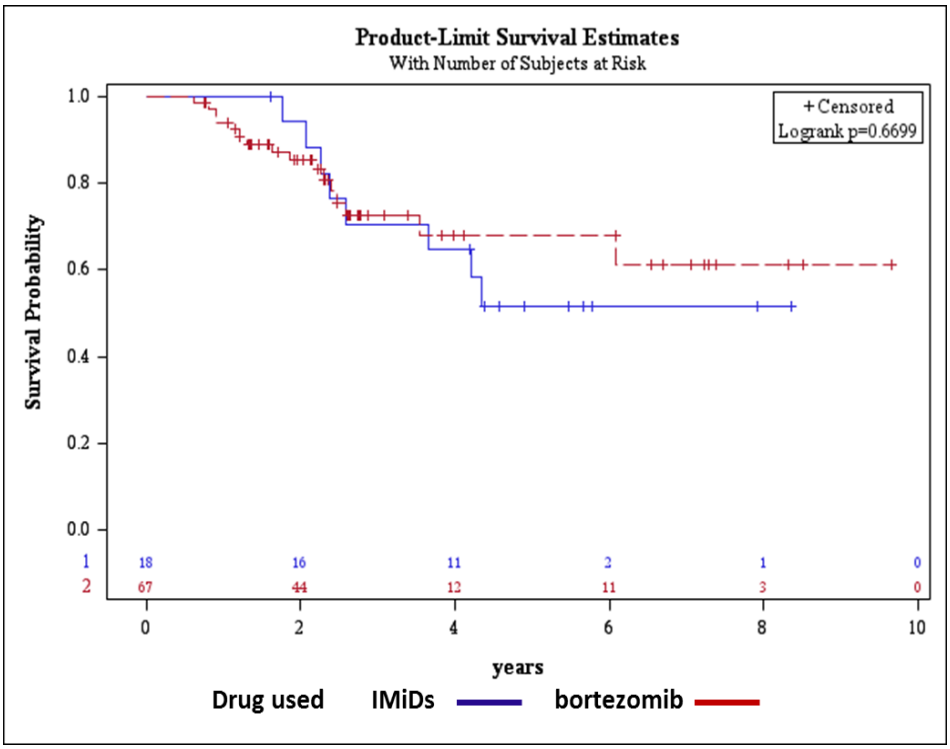


Figure 11B. PFS at 8 years was 51.8% (IC 95%: 32.4-82.7) for patients treated with IMiDs-based regimens and 61.1% (IC 95%: 45.2-82.6) for patients treated with bortezomib-based regimens (p = 0.6699). PFS: progression-free survival. IMiDs: immunomodulatory drugs.

Survival after the first relapse and Progression-free survival 2:

Supposing that the impact on OS was influenced by the salvage treatments used after the progression of disease, we further analysed patients that relapsed. Among our 258 patients, 144 presented a first relapse.

Our cohort of relapsed patients was divided in 4 different groups:

1) 51 patients treated with old agents as first and second line therapy (35.4%);

2) 79 patients treated with old agents for first line therapy and novel agents for second line therapy (54.9%);

3) 2 patients treated with novel agents in induction therapy and subsequent old agents in second line therapy for worsening clinical condition (1.4%);

4) 12 patients treated with novel agents both in first and second line treatment (8.3%).

Our analysis was focused on group 1 and 2. The group 3 was not considered for the small number of patients and because the choice of treatment was based exclusive on clinical worsening condition; the group 4 was not included for the small number of patients and because follow up is too short.

OS at 10 years for patients of group 2 was significantly higher than patients of group 1 (20.4 vs 2.4%; $p < 0.0001$) (Fig 12A). Median OS was 4.1 vs 1.4 years, respectively. Also PFS at 10 years showed better results for patients of group 2 (10% vs 2.3%; $p = 0.02$) (Fig 12B). Median PFS was 1.4 vs 0.7 years, respectively.

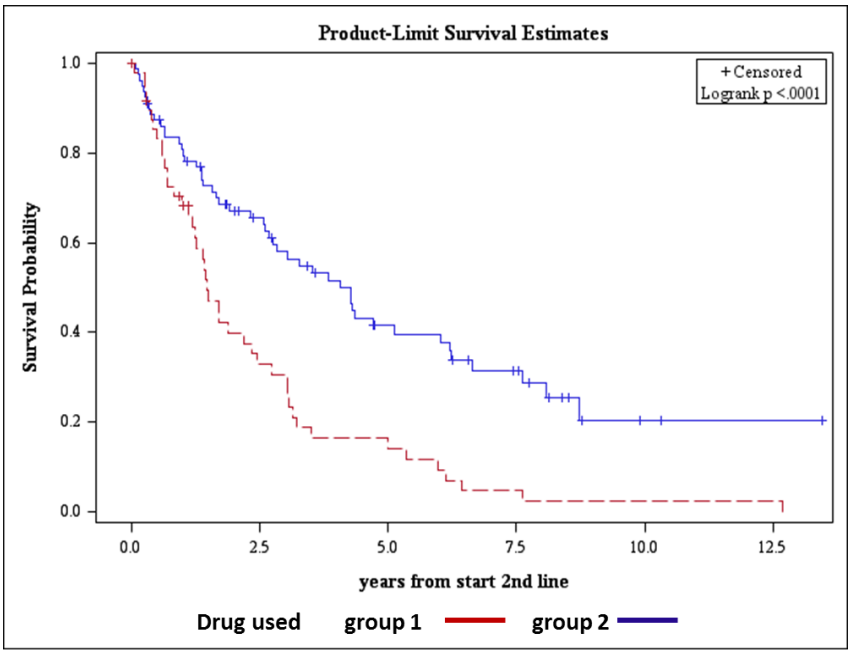


Figure 12A. OS for patients of group 1 (red line) and for patients of group 2 (blue line). OS: overall survival.

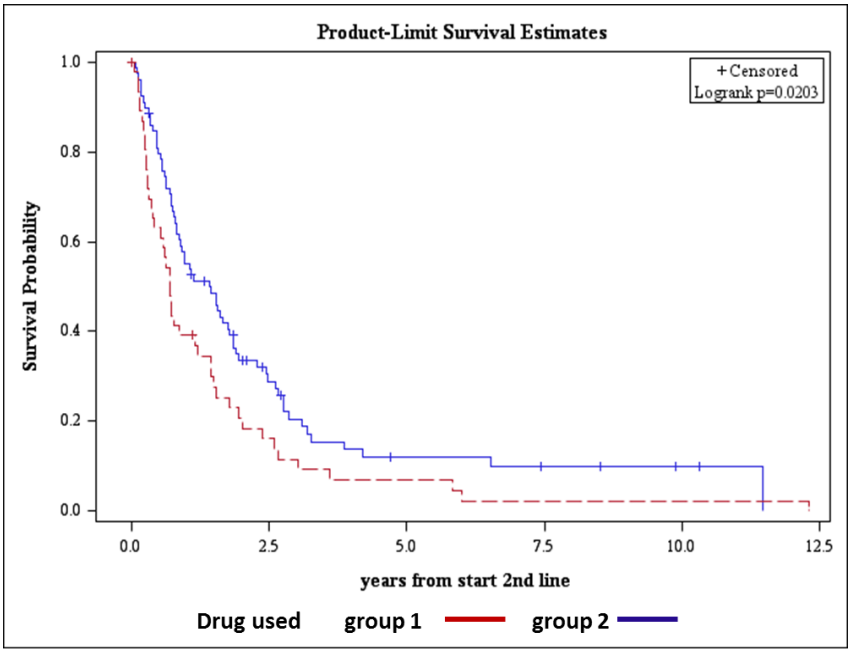


Figure 12B. PFS for patients of group 1 (red line) for patients of group 2 (blue line). PFS: progression-free survival.

The PFS2 at 10 years, considered as the interval from the start of the first line treatment to progression after second-line therapy or death from any cause, showed better results for patients of group 2 than patients of group 1 (25.7% vs 9.2%; p = 0.0002) (Fig 13). Median PFS2 was 6.8 vs 3.7 years, respectively.

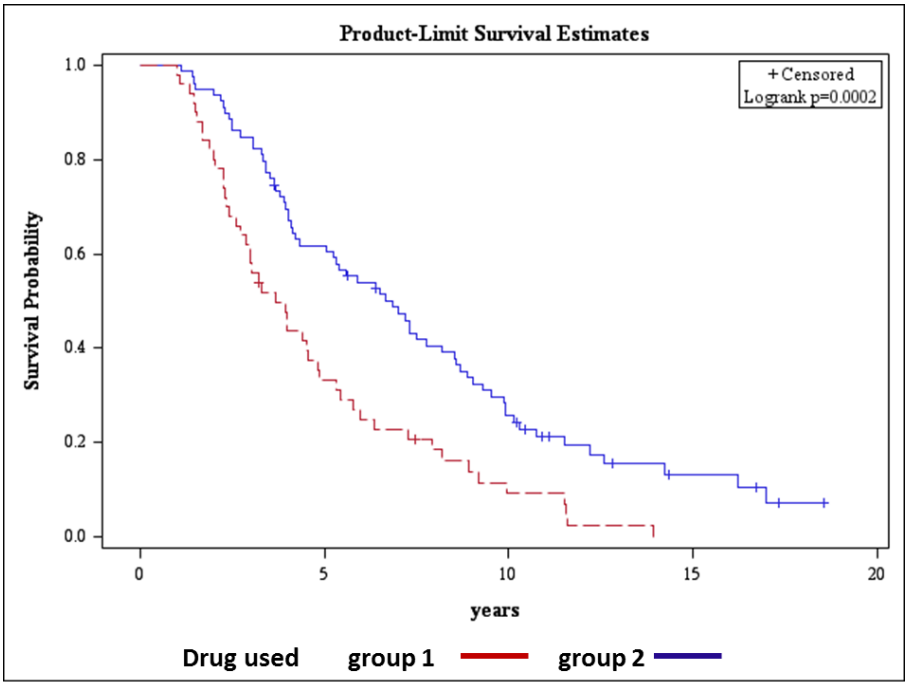


Figure 13. PFS2 was better for patients of group 1 (red line) respect to patients of group 2 (blue line). PFS2: progression-free survival 2.

Conclusions

Novel agents, such as bortezomib and lenalidomide, are currently used as standard treatment in the frontline setting, either in young or elderly patients. However, also in the era of these drugs, ASCT remain the optimal care for YNDMM patients. Therefore, IMWG recommends a three-drug regimen based on bortezomib/dexamethasone, with the addition of a third agent as cyclophosphamide, adriamycin, thalidomide, or lenalidomide as induction treatment before ASCT.

The role of ASCT in the novel agent era was recently investigated in the phase III EMN02/HO95. This study was designed to compare VMP vs high-dose melphalan followed by ASCT after 3-4 cycles of VCD as induction therapy. A second randomization to consolidation therapy vs no consolidation was performed after intensification therapy, followed by lenalidomide maintenance until progression or toxicity in both arms. In centres with a policy of tandem ASCT, patients were randomized in a 1:1:1 ratio to either VMP or single ASCT or double ASCT in order to prospectively compare single vs tandem, as an additional study objective.

Totally, 1510 patients were enrolled and 1192 of these were eligible for the first randomization. Focusing the analysis of VMP patients (n=497 patients) vs single or tandem ASCT patients (n=695 patients), median PFS was 44 months in the VMP arm and was not yet reached in the ASCT arm; 3-year estimates of PFS were 57.5% and 66%, respectively (HR=0.73; 95% CI=0.59-0.90; P=0.003). PFS benefit with ASCT was retained across predefined subgroups, including patients with ISS stage I, revised ISS stage II, revised ISS stage III, standard-risk cytogenetics and a high-risk cytogenetic profile. The probability of achieving at least a VGPR was 85.5% in the ASCT group vs 74% in the VMP group ($p < 0.001$). In a multivariate analysis stratified by ISS, randomization to ASCT and absence of high-risk cytogenetic abnormalities were the leading independent predictors of prolonged PFS. OS was not yet mature and no difference between the two treatment groups was evident. This

study supported the conclusion that upfront ASCT still continues to be the reference treatment for fit patients with YNDMM, even in the novel agent era (Cavo *et al*, 2016a). In addition, this study evaluated of the role single vs tandem ASCT as a supplemental study objective. More in detail, 614 eligible patients received the diagnosis of MM in centres with a double intensification policy and they were randomly assigned to VMP (n=199) or single ASCT (n=208) or tandem ASCT (n=207). On an intention-to-treat basis, the median PFS was 45 months in the single ASCT arm and was not yet reached for patients in the tandem ASCT; 3-year estimates of PFS were 60% and 73%, respectively (HR=0.66; 95% CI=0.45-0.96; P=0.030). PFS benefit with tandem ASCT was retained across predefined subgroups, including patients with β2-microglobulin >3.5 mg/L, bone marrow plasma cells >60%, LDH values above the upper limits, revised ISS stage II and high-risk cytogenetic. In a multivariate analysis ISS stage, randomization to tandem ASCT and high-risk cytogenetic were the leading independent predictors of PFS. OS was not yet mature and no difference between the two treatment groups was evident. Therefore, the authors concluded that upfront tandem ASCT after bortezomib-based induction therapy for newly diagnosed MM was superior over a single ASCT in terms of prolonged PFS (Cavo *et al*, 2016b).

Thus, the European Myeloma Network (EMN) still consider tandem ASCT, whenever it is possible to be performed, the optimal choice for YNDMM patients after induction regimen with a three drug combination, as recommended.

However, there is not uniform consensus about the role of tandem ASCT. Recently, Stadtmauer et al, reported the results of a phase III on 758 transplant-eligible MM that compared single ASCT plus consolidation with RVD (254 patients), vs tandem ASCT (247 patients), vs single ASCT (257 patients). With a median follow up of 38 months, no significant differences in terms of PFS (57% vs 56% vs 52%), OS (86% vs 82% vs 83%) and disease-free Survival (DFS) (42% vs 42% vs 47%) were observed between the three different groups (Stadtmauer *et al*, 2016).

We wanted to retrospectively evaluate our Centre experience regarding the results obtained in the last 25 years comparing old drugs vs novel agents and performing an additional analysis on single vs tandem ASCT. Two-hundred and fifty-eight YNDMM patients, treated in our Centre between October 1988 and November 2014, have been analysed. Between August 1989 and May 2014, all 258 newly diagnosed MM patients underwent ASCT. As induction treatment, between October 1988 and October 2008, 173/258 patients received old drugs, i.e. VAD (n=167) or MP (n=6), while 85/258 patients, between February 2005 and November 2013, were treated with novel agents, i.e. bortezomib-based (n=67) or IMiD-based regimens (n=18). All 258 patients received high doses melphalan 200 mg/m^2 as conditioning regimen and a single (n=153) or tandem (n=105) ASCT.

Our experience confirmed that novel agents, such as bortezomib and lenalidomide, provided better results in terms of depth of responses, and as a consequence, in terms of OS and PFS.

In fact, patients treated with new drugs obtained a CR/nCR/VGPR in 42.3% of cases after induction, in 42.3% after single ASCT and in 54% after tandem ASCT. Patients treated with old drugs, obtained a CR/nCR/VGPR in 17.9% of cases after induction, in 28.9% of cases after single ASCT and in 34.5% of cases after tandem ASCT.

According to our experience the contribution of the second ASCT was statistically significant both among patients treated with new and old drugs (p=0.001 and p=0.005, respectively).

In addition, OS at 10 years was 62.1% for patients in CR/nCR/VGPR and 40.7% for patients who obtained a PR or SD (p = 0.0632). PFS at 10 years was 36.2% for patients in CR/nCR/VGPR and 17.2% for patients who obtained a PR or SD (p = 0.0685).

Moreover, OS was slightly better for patients treated with new drugs than those treated with old drugs,

even if differences was not statistically significant (66.1% vs 51.5%, p = 0.26), but PFS was significantly improved for patients treated with new drugs than those treated with old drugs (55% vs 25.3%, p = 0.0047).

No differences, among patients treated with novel agents, were observed in terms of responses, OS and PFS according to the regimen used, IMiDs- or bortemib- based regimens.

Novel agents have also a significant impact in the subsequent lines of treatments. Among our 258 patients, 144 presented a first relapse. Overall survival at 10 years for patients treated with old agents as first line therapy and novel agents as second line therapy was significantly higher than patients treated with old agents as first and second line therapy (20.4 vs 2.4%; p < 0.0001) and PFS at 10 years showed better results for the first ones (10% vs 2.3%; p = 0.02). The PFS2 at 10 years, showed better results for the former group than the latter (25.7% vs 9.2%; p = 0.0002).

In the light of the newer IMiDs and PIs, the challenge is to assess the exact role of ASCT in the modern setting. Zimmermann et al designed a phase 2 study to assess carfilzomib, lenalidomide and dexamethasone (KRd) plus ASCT. Patients received 4 cycles of KRd followed by stem cell collection, high-doses melphalan and ASCT, and 4 cycles of KRd as consolidation. After these, patients received maintenance KRd for an additional 10 cycles as maintenance and then single agent lenalidomide was recommended for prosecution.

Seventy-six patients were enrolled. Response rates at the end of cycle 8 were: 96% VGPR, 73% CR, and 69% stringent complete remission (sCR). The rate of sCR has been improving during the post-transplant phase of the KRd, from 20% post-ASCT to 69% after 4 cycles of KRd consolidation, and to 82% after 10 additional cycles of KRd maintenance. After a median follow up of 17.5 months, 2-years PFS was 97% and 2- years OS was 99%, respectively. For MRD-negative patients at the end of cycle 8, 2- years PFS/OS was 100% and for MRD-positive/unknown PFS was 93% and OS 98%. For MRD-

negative patients at the end of consolidation, 2- years PFS/OS was 100% and for MRD-positive/unknown PFS was 93% and OS 98%. For high-risk disease, 2- years PFS was 96%. KRd-related adverse events (AEs) were generally grade 1 and 2. Most common grade 3/4 AEs were lymphopenia (28%), neutropenia (18%), and infections (8%). Two of 71 patients evaluated pre-transplant had asymptomatic decrease of ejection fraction 45-50%. These results showed that KRd plus ASCT results in high rates of sCR and MRD-negative disease in both standard and high-risk disease, which correspond to high rates of PFS and OS (Zimmermann *et al*, 2016). Similar results were obtained by the French group (Roussel *et al*, 2016).

Carfilzomib, thalidomide and dexamethasone (KTD) was given as induction and consolidation treatment in 111 YNDMM patients. Induction was followed by ASCT followed by 4 cycles of consolidation. Overall response rate for all cohorts was 95%. Complete response /sCR was 31% after ASCT and 64% after consolidation, respectively. No differences in terms of CR/sCR rate were seen between standard-risk and high-risk defined as t(4;14) and/or del17p and/or add1q and/or ISS III. OS at 30 months was comparable between standard risk and high-risk patients: 91% versus 90%. PFS at 30 months for standard risk and high-risk was 79% and 62%, respectively (p=0.02; HR=2.3, 95% CI=1.1-4.5) (Wester et al, 2016).

Also the ixazomib-lenalidomide-dexamethasone (IRd) combination was assessed in a phase 2 study. The all-oral triplet combination IRd administered as induction prior to, and as consolidation following ASCT, appeared safe, convenient, and effective, leading to 80% VGPR and 44% CR before maintenance (Moreau et al, 2016b).

However, for all these studies, longer follow up are necessary for data on OS, PFS, safety and tolerability.

Our next step will be the assessment of the role of ASCT in our single Centre experience also in the modern era of new PIs and IMiDs as frontline treatment. In this context, our Centre took part in the

FORTE trial, a multicentre randomized open label phase III study, that compared carfilzomib-cyclophosphamide-dexamethasone (KCd) vs KRd in YNDMM transplant-eligible patients. Patients were randomized (1:1:1; stratification ISS and age) to: 4 28-day KCd cycles (carfilzomib:20/36 mg/m2 IV d 1, 2, 8, 9, 15, 16; cyclophosphamide 300 mg/m2 d 1, 8, 15; dexamethasone: 20 mg d 1, 2, 8, 9, 15, 16) followed by high-dose melphalan and ASCT and consolidation with 4 KCd cycles; or 4 28-day KRd cycles (carfilzomib and dexamethasone as above; lenalidomide:25 mg d 1-21) followed by ASCT and 4 KRd cycles; or 12 KRd cycles. After the 4th induction cycle, all patients received cyclophosphamide 2 g/m2, followed by peripheral blood stem cell collection. A total of 281 patients were evaluated (94 assigned to KCd treatment and 187 to KRd treatment); our Centre enrolled 20 patients.

In the KCd vs KRd arms, 99% vs 95% (p=0.44) of patients mobilized stem cells (median number of stem cells collected: 9 vs 6x10^6CD34/Kg with KCd vs KRd). Plerixafor was required in 10% vs 24% (p=0.01), respectively. At least a VGPR was reported in 61% of patients receiving KCd vs 74% receiving KRd (p=0.05). Results are preliminary. The enrolment stopped on April 2016 and analysis for further results is still on going.

Our data confirmed that novel agents, such as bortezomib and lenalidomide, have replaced the old drugs in the current treatment of MM leading to deeper responses and better OS and PFS; however, ASCT is still the optimal treatment for YNDMM. Moreover, whenever it is possible, tandem ASCT should be performed.

Our next effort will be the evaluation of YNDMM patients treated with the newer agents (carfilzomib and others) in terms of outcome and safety, and the role of ASCT even in this setting, in a larger cohort of patients and with a appropriate follow up.

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