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Melphalan hydrochloride for the treatment of multiple myeloma

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ABSTRACT

Introduction

Multiple myeloma (MM) is an incurable disease characterized by clonal plasma cell proliferation and overproduction of monoclonal paraprotein, hypercalcemia, renal failure, anemia, osteolytic bone lesions, and infections.

Melphalan, a nitrogen mustard, is an alkylating agent synthesized in 1953, and it has been used in multiple myeloma therapy for fifty years. Although novel agents have been introduced in the past few decades improving prognosis of the disease, melphalan still maintains a crucial role in the treatment of MM acting both as cytotoxic agent through damage to DNA, and as immunostimulatory drug by inhibiting Interleukin-6, as well as interaction with dendritic cells, and immunogenic effects in tumor microenvironment.

Areas covered

This review focuses on available data about melphalan pharmacology and its role in clinical practice.

Expert opinion

Melphalan remains crucial in therapy of multiple myeloma because of its good manageability, safety profile, efficacy, and economic sustainability. These characteristics make it pivotal also for new regimens in combination with novel agents.

1. INTRODUCTION

Multiple myeloma (MM) is a B-cell malignancy characterized by clonal plasma cell proliferation and over production of monoclonal paraprotein;^{1,2} it accounts for >10% of hematologic malignancies and ≈1% of all cancers. Typical disease manifestations are: hypercalcemia, renal failure, anemia, osteolytic bone lesions, and infections.³

Nowadays, therapeutic strategy is based on patient's eligibility to autologous stem cell transplantation (ASCT): younger (<65 years), fit patients undergo high-dose therapy (HDT) followed by ASCT, whereas elderly (≥65 years) or unfit patients are treated with conventional chemotherapy.

During the past two decades, the introduction of proteasome inhibitors (PIs), immunomodulatory drugs (IMiDs), and monoclonal antibodies (MoAbs) doubled life expectancy in MM patients.⁴ However, MM remains incurable, with several relapses, and progressively shorter disease free survival (DFS). Therefore, further research to find novel effective drugs is needed. However, the development and administration of new, highly selective, therapeutic agents are associated with elevated economic costs⁵ and not always with a real clinical benefit, due to the high heterogeneity in pathophysiology of MM cells.⁶ In this complex scenario alkylating agents, such as melphalan, still maintain a role, because of proved clinical safety/efficacy and reduced costs.

2. OVERVIEW OF THE MARKET

Currently, melphalan (Alkeran®) is approved by Food and Drug Administration (FDA) and European Medicine Agency (EMA) for the treatment of MM and other hematological and solid cancers. In MM, melphalan is administered either intravenously at the dose of 200 mg/m² (HDM) followed by ASCT in transplant-eligible patients; or orally, in combination with prednisone at various dosages, and usually combined with PIs (such as bortezomib) or IMiDs (such as thalidomide), both in newly diagnosed (ND) and relapsed/refractory (RR) patients, unsuitable for ASCT.

In 2015, melflufen, an alkylating agent consisting of melphalan combined to flufenamide, was approved as orphan drug by both FDA and EMA for the treatment of relapsed/refractory MM.⁷

In 2016, EVOMELA™, propylene glycol-free melphalan was approved by FDA. This formulation incorporates a modified cyclodextrin, Captisol, that increases the solubility and stability of melphalan and provides a safer method of administration.^{8,9}

3. MELPHALAN

3.1. Introduction to the compound

Melphalan, also called L-phenylalanine mustard (L-PAM) or L-sarcolysin, is a phenylalanine derivative of nitrogen mustard with a bifunctional alkylating activity.

Alkeran® is available both orally, in film-coated tablet form containing 2 mg of melphalan, or for intravenous injection, supplied as a sterile, non-pyrogenic, freeze-dried powder. Each single-use vial contains melphalan hydrochloride equivalent to 50 mg melphalan and povidone.⁸

3.2. Chemistry

Melphalan, also known as 4-[bis(2-chloroethyl)amino]-L-phenylalanine or (2S)-2-amino-3-[4-[bis(2-chloroethyl)amino]phenyl]propanoic acid, was synthesized in 1953, substituting the amino acid phenylalanine for the methyl group on nitrogen mustard.¹⁰ The active L-isomer melphalan was preferred for development because the D-isomer was less active against certain animal tumors. Melphalan is almost insoluble in water, soluble in ethanol and propylene glycol, and it has a pK_a of ~2.5. The molecular formula is C₁₃H₁₈Cl₂N₂O₂ and the molecular weight is 305.20 (Drug summary).^{8,11}

3.3. Pharmacodynamics

Melphalan, as all nitrogen mustards, acts as non-specific DNA alkylating agent creating bifunctional adducts with cross-links in DNA. These covalent bindings deform the double helix of DNA and are responsible for the cytotoxic action interfering with the polymerases function. Moreover, these adducts can carry out deletions, strand scissions and the formation of open rings leading to cell apoptosis.¹²

Since phenylalanine is an intrinsic part of its structure, melphalan is actively transported into cells by the high-affinity L-amino acid transport system.¹³ Once melphalan gets into intracellular environment, it forms inter-strand or intra-strand DNA cross-links or DNA-protein cross-links by the two chlorethyl groups of the molecule (alkylation). In aqueous solutions, the chlorethyl group can spontaneously lose the chloride ion, leading to a positively charged and highly reactive cation (aziridinium) able to react with negatively charged, electron-rich nucleophilic sites on biologic molecules.¹⁴ The N7 position of guanine is the only biologically relevant attack site, because the other purine basic nitrogen sites are structurally inaccessible to aziridinium (Figure 2).¹⁵

Reparative events of DNA covalent crosslinks are considered a mechanism of clinical resistance to the drug.¹⁶ Although alkylation is the main mechanism of action, melphalan also inhibits malignant cell growth through the deepest reduction in Interleukin-6 (IL-6) protein expression compared with several other chemotherapeutic agents. Indeed, IL-6 is secreted by bone marrow stromal cells and osteoblasts, and it is one of the major cytokines involved in survival and proliferation of myeloma cells through the binding of IL-6-Receptor- α , particularly highly expressed in MM cells.^{17,18} Moreover, melphalan treatment could create an inflammatory milieu, able to activate an immune response against neoplastic cells.¹⁹ Indeed, calreticulin (CRT) surfacing from endoplasmic reticulum (ER) of MM cells represents an “eat-me” signal for dendritic cells (DCs). Furthermore, the high-mobility group box 1 (HMGB1), released by dying tumor cells, could act upon Toll-like Receptor 4 (TLR4) on DCs, and finally, adenosine triphosphate (ATP) released by dying malignant cells can trigger purinergic P2RX7 receptors on DCs inducing the production of pro-inflammatory cytokine IL-1 β through the activation of NLRP3 inflammasome.²⁰

3.4. Pharmacokinetics and metabolism

The pharmacokinetic behavior of melphalan is difficult to determine because the drug undergoes spontaneous hydrolysis in aqueous media, is extensively bound to plasma proteins, and is not significantly metabolized.²¹

3.4.1. Absorption and distribution

Studies of HDM administered orally confirmed that absorption is variable between patients, showing remarkable inter-patient differences in pharmacokinetic parameters with respect to both the time to first appearance of the drug in plasma (range: 0-6 hours) and peak plasma concentration (C_{max}). The average absolute bioavailability of melphalan is also highly variable (range: 56-93%).⁸

Since the elimination half-life ($t_{1/2\beta}$) and the time to peak plasma level are similar, the variability in absorption may be due to effects of prior doses and/or other chemotherapeutic agents on the intestinal mucosa, or to saturation of the energy-dependent amino acid transport system used by melphalan because of the high doses.²² There is some evidence of a saturable mechanism for melphalan absorption. Since there is no appreciable active metabolism in vivo, it is possible that the increase in peak plasma level and area under the concentration/time curve (AUC) might be due to changes in drug transport.²³ In addition, absorption may be affected by food intake with a median reduction of AUC of 39% and a reduction in bioavailability from 85% to 58% when oral melphalan is administered after a meal.²⁴

After intravenous administration, melphalan rapidly disappears from the plasma and is distributed in whole body water ($V_d = 0.66$ L/kg).²⁵ In several studies, the distribution of melphalan shows a biphasic, open, two-compartment model. The distribution half-life ($t_{1/2\alpha}$) is short, ranging between 5 and 15 minutes, and proportions of the $t_{1/2\beta}$ vary five-fold, ranging from 17 to 75 minutes at doses of 140-180 mg/m². The short half-life of HDM allows for the reinfusion of hematopoietic stem cells within 8 to 24 hours by melphalan administration.¹⁴

3.4.2. Metabolism and excretion

Several studies have shown that spontaneous degradation is the primary route for melphalan elimination. The compound seems to be rapidly hydrolyzed in plasma. Almost 90% of drug is bound to plasma proteins. Serum

albumin is the major binding protein, accounting for 40-60% of the plasma protein binding, while α_1 -acid glycoprotein accounts for another 20%. Melphalan is eliminated from plasma primarily by chemical hydrolysis to the non-cytotoxic monohydroxy- and dihydroxy- metabolites.²⁵

The small amount of compound observed in urine suggests that renal function is not important in the excretion of intravenously administered melphalan. However, an increase in non-hematological toxicity and mortality has been reported when HDM was administered in MM patients with renal failure.^{26,27} Therefore, in clinical practice both oral and intravenous melphalan dose reductions, based on glomerular filtration rates, are recommended.²⁸⁻³⁰

Drug interactions with melphalan are unknown since degradation does not involve hepatic metabolism.

4. CLINICAL EFFICACY

The first report of melphalan in anti-myeloma therapy dates back to 1958 by Blokhin et al, who treated 6 patients and obtained a considerable reduction in the tumor size in 3 of them.³¹ Since then, melphalan therapeutic utilization evolved along two parallel lines: oral administration in combination with steroids and high-dose intravenous infusion as preparative regimen for autologous transplantation. Additionally, a melphalan derivative prodrug, melflufen, has been recently developed and is under clinical evaluation.

4.1 Oral melphalan

The oral combination of melphalan and prednisone (MP) against MM was first described by Alexanian et al in 1969.³² In clinical trials, MP showed an overall response rate (ORR) of 35-55% with a median survival of 2-3 years. Subsequently it was introduced as standard treatment for elderly MM patients,³³ and was considered the reference approach for more than 30 years.³⁴ A data review of 27 randomized clinical trials carried out by The Myeloma Trialists' Collaborative Group in 1998 found no difference in terms of survival between MP and melphalan in combination with procarbazine, carmustine, cyclophosphamide, methyl-CCNU, vincristine, doxorubicin, peptichemio, or dexamethasone.³⁵ Recently, the introduction of IMiDs, such as thalidomide or lenalidomide, and the PI bortezomib, has changed the treatment paradigm of MM and extended survival. A number of phase 3 trials have demonstrated the efficacy of novel agent combinations including MP as backbone for the treatment of elderly NDMM patients.

Six randomized studies showed superiority of MP-thalidomide (MPT) combination vs standard MP: higher responses translated into a longer progression-free survival (PFS). However, the effect on overall survival (OS) varied across trials.³⁶⁻⁴² A meta-analysis of these studies, including 1685 patients, integrated the existing efficacy data and showed a 5-month increase in median PFS and also a 6-month longer median overall survival (OS) in favor of MPT (Table 1).⁴³

The phase 3, randomized, multicenter, VISTA trial, randomly assigned 682 patients to receive nine 6-week cycles of melphalan (at a dose of 9 mg/m²) and prednisone (at a dose of 60 mg/m²) on days 1 to 4, either alone or with bortezomib (VMP) at a dose of 1.3 mg/m² on days 1, 4, 8, 11, 22, 25, 29, and 32 during cycles 1 to 4 and on days 1, 8, 22, and 29 during cycles 5 to 9. ORR in bortezomib group almost doubled the MP control group (74 vs 39%, P<0.001), and median progression-free survival (PFS) significantly differed in favor of bortezomib group (22 vs 15 months, P<0.001).⁴⁴ The median overall survival (OS) was not reached in the VMP arm vs 43 months in the MP group, and hazard ratio (HR) also favored the bortezomib-containing group (HR 0.653, P <0.001) (Table 1).⁴⁵

The MM-015 trial was a double-blind phase 3 trial in which 459 patients were randomly assigned to receive MP alone, or in combination with lenalidomide (MPR) with or without lenalidomide maintenance (MPR-R). Primary endpoint was PFS and MPR-R resulted superior than MPR and MP (31 vs 14 vs 13 months, respectively). The major benefit on PFS was associated with lenalidomide maintenance therapy. Also ORR was higher in MPR-R group (77% vs 68% vs 50%, respectively). Median 3-years OS did not differ significantly (Table 1).⁴⁶

In a subsequent phase 3 trial, MPT was compared to MPR but not significant differences were showed in ORRs, PFS, or OS (Table 1).⁴⁷

More recently, results of a phase 3 trial, comparing fixed duration of carfilzomib plus MP (KMP) vs VMP for 9 induction cycles were presented.⁴⁸ The primary endpoint of KMP superiority in median PFS was not met (22 months for both KMP and VMP, HR 0.91, 95% CI, 0.75 - 1.10). The ORR was 84% with KMP vs 79% with VMP. The HR for OS (KMP versus VMP) was 1.21 (95% CI, 0.90 - 1.64)⁴⁹ (Table 1).

4.2 High-dose intravenous melphalan

The earliest attempts of HDT with melphalan 80-140 mg/m² infusion were not followed by ASCT.⁵⁰⁻⁵⁶ Barlogie et al found that the myelotoxicity of HDM was reduced in patients who underwent ASCT.^{57,58} Various trials were performed to compare HDT-ASCT with conventional chemotherapy, but only two of them, conducted by Attal and Child et al, provided strong evidence for survival benefit (Table 2).⁵⁹⁻⁶⁴ The superiority of HDT-ASCT as upfront approach in NDMM patients ≤65 years of age, also in comparison to chemotherapeutic regimens incorporating novel agents, such as lenalidomide and bortezomib, has been recently confirmed (Table 2).⁶⁵⁻⁶⁷

Melphalan 200 mg/m² is the optimal myelo-preparative conditioning regimen for ASCT in MM. Higher doses, or association with total body irradiation (TBI) or with other chemotherapeutic drugs, such as busulfan, failed to improve efficacy and worsened toxicity profile.⁶⁸⁻⁷⁰ On the other side, dose reductions, despite being associated with a better safety profile, showed lower efficacy, and should be considered only in patients with comorbidities or at higher risk for transplant-related complications.^{39,71}

4.3 Melflufen

Melflufen is a novel melphalan containing prodrug with Flufenamide that enables the agent to easily get into cells.

Preclinical in vitro and in vivo studies showed higher anti-MM activity compared to melphalan, as well as synergistic effects in combination with bortezomib, lenalidomide or dexamethasone.⁷²⁻⁷⁵ NCT01897714 is an ongoing Phase 1/2a study evaluating melflufen in combination with dexamethasone for RRMM patients. The maximum tolerated dose (MTD) of melflufen was determined to be 40 mg intravenously every 3 weeks in combination with low-dose dexamethasone. In 23 patients evaluable for response, the ORR was 48%.^{76,77}

4.4 Evomela

The new formulation of melphalan with Captisol EVOMELA,⁹ was tested in MM patients undergoing ASCT. It was administered as 2 doses of 100 mg/m² in 61 patients. The ORR reported prior of ASCT was 79% and increased to 100% after ASCT with a CR rate of 21%.⁷⁸

5. POST MARKETING SURVEILLANCE

The Regulatory Authorities (RA) have developed post marketing surveillance programs to monitor the safety profile of new marketed drugs.^{7,79-81} FDA has introduced the use of black boxed warnings to advise users on the risk of serious adverse drug reactions (ADRs).⁸² Recently, an analysis of boxed warnings issued for all MM drugs highlighted that, after melphalan approval in 1964, four different warning revisions have been published (Table 3).⁸³ A significant change in reporting rates for melphalan-associated leukemia, bone marrow suppression and chromosomal aberrations has been detected after the introduction of boxed warnings.⁸⁴ The

notification of ADRs to the FDA Adverse Event Reporting System (FAERS)^{79,80} increased the statistical association measured with the Empirical Bayes Geometric Mean (EBGM) for melphalan-associated bone marrow suppression and chromosomal aberrations (Table 3).

A Japanese study reviewed the reports submitted to FAERS to assess taste dysfunction induced by the administration of MM drugs, covering the period from 1997 to 2014.⁸⁵ Signals were detected via quantitative data mining algorithms specific for the terms: ageusia, dysgeusia and hypogeusia. The highest signal score related to melphalan was associated to hypogeusia.

6. SAFETY and TOLERABILITY

6.1 Oral melphalan

MP showed good safety profile: grade (G) ≥ 3 hematological toxicities were about 15%, whereas G ≥ 3 non-hematological adverse events (AEs) were rare (<5%), and the majority of them were infections.^{86,87} MPT combination was associated with higher toxicity compared with MP: the six randomized studies included in the meta-analysis by Fayer et al showed that AE rates in MPT arm were almost double as compared with MP, and the most frequent ones were polyneuropathy, constipation, infections, thrombosis, and exanthema.³⁶⁻⁴³ As for VMP combination, peripheral sensory neuropathy, G ≥ 3 gastrointestinal AEs, and incidence of Herpes Zoster were more frequent in bortezomib group, whereas VMP and MP did not differ significantly in death rates during treatment (5% and 4%, respectively) or treatment-related death (1% and 2%).⁴⁴

MPR showed similar overall toxicity profile compared to MPT; indeed, despite a significant reduction in G ≥ 2 neuropathy (44% in MPT vs 8% in MPR, $P < 0.001$), G ≥ 3 hematologic AEs were significantly more frequent in the lenalidomide group (anemia: 14% vs 5%; thrombocytopenia: 30% vs 8%; neutropenia: 64% vs 27%; all $P < 0.001$), whereas the incidence of G ≥ 2 venous thrombotic events was 8% in both arms.⁴⁷

In the KMP vs VMP trial, rates of G ≥ 3 hypertension, dyspnea, acute renal failure, and cardiac failure were higher in the carfilzomib group compared to the bortezomib one.

6.2 High-dose intravenous melphalan

HDM without ASCT led to 8-20% of mortality rate, usually for sepsis, and the median duration of neutropenia and thrombocytopenia was 24-30 and 28-36 days, respectively.^{51-56,88} The introduction of autologous transplant and G-CSF support significantly reduced myelotoxicity,⁵⁶ and advances in support-therapy strongly reduced mortality rate (<2%).^{56,57,67,89} In the last phase 3, multicenter, randomized clinical trial in which HDM was compared to novel agents other frequent AEs related to HDM included infections (20%), mucositis (17%), nausea and vomiting (7%), diarrhea (4%), fatigue (2%).^{67,89} To prevent mucositis, amifostine administration prior to HDM showed some utility in clinical trials.^{90,91} At diagnosis, 15-40% of patients with MM have renal impairment. Although melphalan clearance is renal function-dependent, pharmacokinetic studies showed large inter-individual variations in pharmacokinetic parameters.⁹² For these reasons, melphalan optimal dosing in patients with renal clearance <60 ml/min is a controversial topic. Although a recent retrospective study seems to suggest improved response and longer treatment-free survival in patients with renal clearance <60 ml/min treated with melphalan 200 mg/m²,⁹³ a Consensus Statement on behalf of the International Myeloma Working Group, and the European Myeloma Network Guidelines for the management of MM related complications recommends to reduce melphalan dose to 140 mg/m² in case of renal clearance <60 ml/min.^{92,94}

6.3 Melflufen

Melflufen, in phase 2a trial, was associated mostly with hematologic toxicities (thrombocytopenia: 68%, neutropenia: 55%, anemia: 42%, leukopenia: 32%)⁷⁶.

6.4 Evomela

Safety profile of EVOMELA was shown to be consistent with the established side effect profile of high-dose i.v. melphalan, with a lower rate of mucositis compared (12% to 43%).⁷⁸

7. REGULATORY AFFAIRS

Since melphalan approval in 1964, the standard melphalan formulation used for MM treatment is Alkeran®, a registered trademark of GlaxoSmithKline. Tablet formulation was reviewed by FDA on 24th May 2001. On 1st July 2002, FDA approved Alkeran® (melphalan hydrochloride) for injection. On 22nd March 2017,^{95,96} FDA approved the first bioequivalent of oral Alkeran®, produced by Alvogen Malta. Six different bioequivalent drugs of Alkeran® for injection have been approved since 2009.⁹⁷

In March 2015, a prodrug of melphalan currently evaluated in a phase 1/2a trial, Melflufen, received the Orphan Drug Designation from both FDA and EMA.⁹⁸

A new formulation for injection was approved on 3rd October 2016, called EVOMELA™ (Captisol®-enabled melphalan HCl). It has two indications in MM patients: HDT prior to ASCT and palliative treatment.^{99,100}

8. CONCLUSION

After fifty years of use, the alkylating agent melphalan still maintains a crucial role in MM therapy. In younger, fit patients intravenous HDM is used as preparative regimen for ASCT, whereas in elderly patients oral melphalan is used in combination regimens with PIs, IMiDs, MoAbs and/or prednisone. Oral melphalan, thanks to its limited toxicity and easy management, has also a palliative function in frail patients.

9. EXPERT OPINION

In MM there is growing need for therapeutic strategies that could prolong the survival of patients as much as possible. Indeed, despite the recent advances with novel effective agents, MM patients eventually relapse. In particular, the disease is characterized by multiple relapses, development of drug-resistance, and decreasing DFS, therefore newer agents to be used in subsequent lines of therapy are needed.

Although several novel agents have been introduced in the past few years, regulatory and/or economic issues may be a limitation to their use and could reduce their availability.

In the pharmaco-economic context, when analyzing the economic issues associated with the use of new drugs, three different and highly relevant aspects should be taken into account. First, survival of MM patients has considerably increased (to more than 10 years) in the last fifteen years, thus patients receive effective – and also quite expensive – treatments for a prolonged time. Second, combo-therapy, that is the association of synergistic and highly effective novel agents, has become a common and valid practice, therefore treatments include more than one effective – and again expensive – agents. Third, continuous therapy has become a widely accepted strategy, and most of trials in fact incorporate a maintenance treatment for at least two years or until relapse/intolerance, thus prolonging the time on treatment. All these factors, despite showing the impressive steps forward thanks to the clinical research, have dramatically increased the economic burden to national health systems.

In more detail, patients who receive induction, transplantation and maintenance, cost about 100.000\$ per year.⁵ The introduction of novel agents increase costs to 300.000\$ per year.¹⁰¹ Recently, Niphadkar et al compared cost between novel agents and ASCT. The study showed that induction with lenalidomide and dexamethasone, followed by ASCT and lenalidomide maintenance is efficacious for the treatment of newly diagnosed MM, and the cost associated with the induction and transplant represents only 22% of the total cost, whereas lenalidomide maintenance makes up 78%. Moreover, the study showed that most of the novel agents used in combination regimens are more expensive than ASCT, despite non clear data of significant superiority in terms of efficacy..¹⁰²

Melphalan, approved in 1964, is a well-known alkylating agent that is still pivotal in MM therapy, due to its demonstrated efficacy, good safety profile, and limited costs thanks to several bioequivalent drugs already marketed. Moreover, oncologists and hematologists have been using this drug for about 50 years in clinical practice, which makes them quite confident with melphalan administration. The good manageability of this alkylating agent is confirmed by the fact that HDM followed by ASCT in accurately selected American and Canadian patients is safely performed also in outpatient setting, with great patient satisfaction.¹⁰³

Of note, because of its versatility, melphalan can be easily combined with novel agents, enhancing their efficacy.

Topicality of melphalan is reinforced by the new discoveries of its prodrug melflufen and the new formulation EVOMELA. Melflufen showed ORRs of $\approx 40\%$ in heavily pre-treated, pluri-refractory MM patients. This was possible thanks to both the intrinsic property of melflufen to reach higher concentration in MM cells than its parental drug, and because of its mechanism of action capable to overcome heterogeneity in neoplastic clones. EVOMELA combines the Captisol technology, also used in 6 other drugs approved by FDA (Vfen, Nexterone, Geodon, Abifly, Kyprolis and Noxaifl). This formulation avoids the use of propylene glycol than can contribute to the onset of metabolic and renal dysfunction mainly. Results showed the bioequivalence of the two melphalan formulations, with consistent safety and efficacy profile of conventional melphalan formulation.⁷⁸

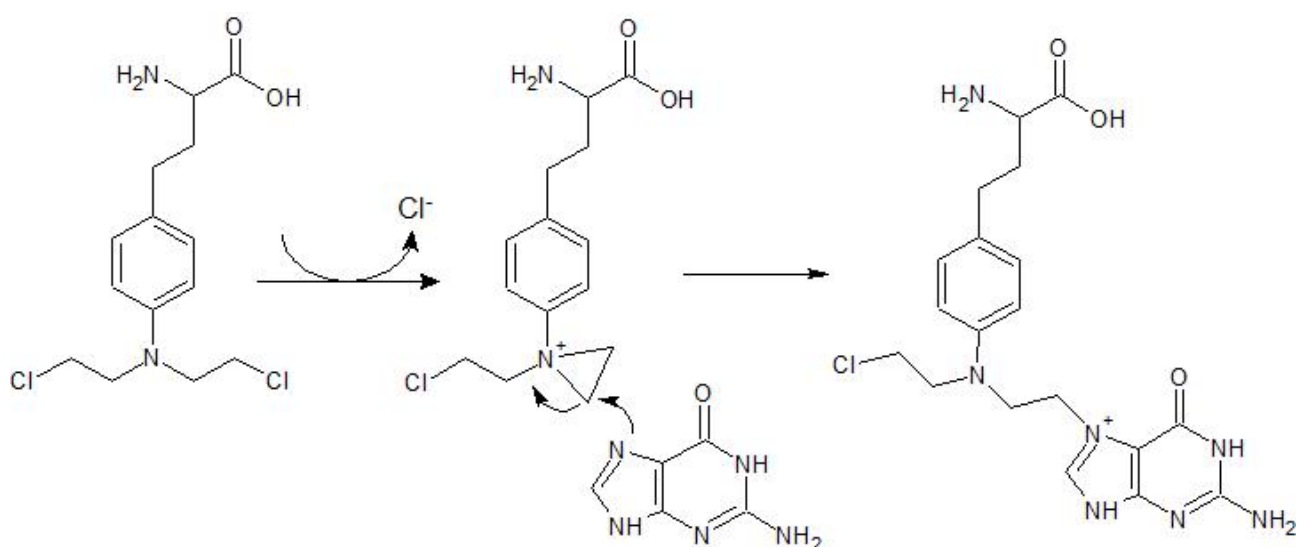
In conclusion, melphalan in MM therapy remains crucial because of its easy-management, safety, efficacy profile, and economic sustainability. These characteristics make it pivotal also for new regimens in combination with novel agents.

Declaration of Interest: Mario Boccadoro has received honoraria from Sanofi, Celgene, Amgen, Janssen, Novartis, Abbvie, BMS; research funding from Celgene, Janssen, Amgen, BMS, Mundipharma, Novartis, Sanofi; Alessandra Larocca has received honoraria from Amgen, BMS, Celgene and Janssen-Cilag. The other authors have no conflicts of interest

Figure Legend.

Figure 1. Melphalan alkylating mechanism

Figure 1.



The chloroethylamine moiety forms an aziridinium cation which reacts with DNA bases such as N7 position of guanine, producing an alkylated purine. The alkylation of a second guanine residue leads to cross-linking of DNA strands

Table 1: efficacy results of phase 3, randomized, multicenter clinical trials combining novel agents to melphalan and prednisone as upfront therapy in MM patients ineligible for autologous transplantation.

STUDY	TARGET	N° OF PATIENTS	MEDIAN AGE (RANGE) (y)	TREATMENT SCHEME	ORR (%)		MEDIAN PFS (m)		OS	
					Mel + NA	CG	Mel + NA	CG	Mel + NA	CG
Meta-analysis by Fayers et al ⁴³	NDMM ineligible for ASCT	1685	>55	MPT vs MP	r 57-76	r 35-48	20	15	39 m	33 m
VISTA ⁴⁴	NDMM ineligible for ASCT	682	71 (r 48-91)	VMP vs MP	74 (CR 33)	39 (CR 4)	22	15	NR	43 m
Palumbo et al ⁴⁶	Elderly NDMM ineligible for ASCT	459	71 (r 65-91)	MPR-R vs MPR vs MP	MPR-R 77 (CR 10) MPR 68 (CR 3)	50 (CR 3)	MPR-R 31 MPR 14	13	3-y OS: MPR-R 70%† MPR 62%†	3-y OS: 66%†
Zweegman et al ⁴⁷	NDMM ineligible for ASCT	637	72 (r 60-91)	MPR vs MPT	84 (CR 13)†	81 (CR 10)†	23†	20†	4-y OS: 56%†	4-y OS: 52%†
NCT01818752 ⁴⁹	NDMM ineligible for ASCT	955	72	KMP vs VMP	84†	79†	22†	22†	HR 1.21 for KMP group (95% CI, 0.90 - 1.64)	

Legend: y: years; ORR: overall response rate (\geq PR); PR: partial response; PFS: progression-free survival; m: months; OS: overall survival; Mel: melphalan per os; NA: novel agent; CG: control group; NDMM: newly diagnosed multiple myeloma; ASCT: autologous stem cell transplantation; MPT: melphalan, prednisone, thalidomide; MP: melphalan, prednisone; r: range; VMP: bortezomib, melphalan, prednisone; CR: complete response; NR: not reached; MPR: melphalan,

prednisone, lenalidomide; MPR-R: melphalan, prednisone, lenalidomide, followed by lenalidomide maintenance; MPT-T: melphalan, prednisone, thalidomide, followed by thalidomide maintenance; †: results not significantly different; KMP: carfilzomib, melphalan, prednisone; HR: hazard ratio.

Table 2: efficacy results of phase 3, randomized, multicenter clinical trials in favor of high dose melphalan followed by autologous transplantation as upfront therapy in transplant eligible MM patients.

STUDY	N° OF PATIENTS	TREATMENT SCHEME	ORR (%)		MEDIAN PFS (m)		OS	
			HDT-ASCT	CC	HDT-ASCT	CC	HDT-ASCT	CC
Attal et al ⁵⁹	204	MEL140 + TBI (8 Gy) vs VMCP/BVAP → IFN- α maintenance until relapse	81 (CR 22)	57 (CR 5)	27*	18*	Median OS: NR	Median OS: 37 m
Child et al ⁶¹	407	VCAM + MEL200 vs BCAM	86 (CR 44)	48 (CR 8)	32	20	Median OS: 54 m	Median OS: 42 m
Palumbo et al ⁸⁹	302	Rd → Tandem MEL200 vs 6 MPR → R maintenance until relapse	93 (CR 23)†	91 (CR 18)†	43	22	4-year OS: 82%	4-year OS: 65%
Gay et al ¹⁰⁴	389	Rd → Tandem MEL200 vs 6 CDR → Rp maintenance until relapse	91 (CR 13)†	89 (CR 12)†	43	29	4-year OS: 86%	4-year OS: 73%
Cavo et al ⁶⁶	1503	VCD → MEL200 vs 4 VMP → 2 VRD consolidation vs no consolidation → R maintenance until relapse	VGPR 84	VGPR 74	PFS prolonged in HDT-ASCT arm (HR=0.76; 95% CI=0.61-0.94; P=0.010)		NP	NP
Attal et al ⁶⁷	707	VRD → MEL200 + 2 VRD vs 5 VRD → R maintenance until relapse	98 (CR 59)	97 (CR 48)	50	36	4-year OS: 81%	4-year OS: 82%

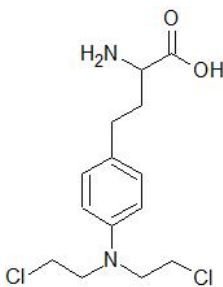
Legend: ORR: overall response rate (\geq PR); PR: partial response; CR: complete response; PFS: progression-free survival; m: months; OS: overall survival; HDT-ASCT: high-dose chemotherapy followed by autologous stem cell transplant; CC: conventional chemotherapy; MEL140: melphalan 140 mg/m² followed by ASCT; TBI: total body irradiation; MEL200: melphalan 200 mg/m² followed by ASCT; VMCP: vincristine, melphalan, cyclophosphamide, prednisone; BVAP: vincristine, carmustine, doxorubicin, prednisone; *: in this trial survival parameter was event-free survival; NR: not reached; VCAM: vincristine, cyclophosphamide, doxorubicin, metil-prednisolone; BCAM: carmustine, cyclophosphamide, doxorubicin, melphalan; Rd: lenalidomide, dexamethasone; MPR: melphalan, prednisone, lenalidomide; †: results not significantly different; CDR: cyclophosphamide, dexamethasone, lenalidomide; Rp: lenalidomide, prednisone; ‡: interim analysis; VCD: bortezomib, cyclophosphamide, dexamethasone; VMP: bortezomib, melphalan, prednisone; VRD: bortezomib, lenalidomide, dexamethasone; VGPR: very good partial response; NP: not published; VRD: bortezomib, lenalidomide, dexamethasone.

Table 3: Melphalan associated FDA boxed warnings and related EBGMs

Melphalan FDA approval 1 Jan 1964	Date	ADR added	Number of reports before a boxed warning	Number of reports after a boxed warning	EBGM before a boxed warning	EBGM after a boxed warning
First boxed warning	16 June 1980	Leukemia	90	380	23.89	6.21
First revision	22 September 1986	Chromosomal aberrations	0	46	0.00	13.19
Second Revision	25 August 1994	Bone marrow suppression	24	368	5.93	7.84
Third Revision	14 May 2003	Hypersensitivity	12	41	0.40	0.42

Legend: ADR: adverse event reaction, EBGM: Empirical Bayes Geometric Mean,

Drug Summary

DRUG SUMMARY	
Drug name	Melphalan (Alkeran ®, GlaxoSmithKline)
Phase	Oral formulation approved by FDA in 1964. On 2002 FDA approval of Alkeran® for injection. Currently in Phase IV (Post marketing surveillance)
Indication	Oral formulation: treatment of MM patients ineligible for ASCT. Intravenous HDM: preparative regimen for ASCT
Pharmacology description	Nitrogen mustard with a bi-functional non-specific alkylating activity towards DNA. IL-6 expression reduction in bone marrow environment. Immunogenic cell death.
Route of administration	Oral; Intravenous
Chemical structure	 <chem>NC(CCC1=CC=C(C=C1)N(CCCl)CCl)C(=O)O</chem>
Pivotal trial(s)	Alexanian et al ³² ; San Miguel et al ⁴⁴ ; Fayers et al ⁴³ ; Attal et al ⁵⁹ ; Child et al ⁶¹ ; Palumbo et al ⁸⁹ ;

Legend: MM: multiple myeloma, ASCT: autologous stem cell transplantation; HDM: high-dose-melphalan; IL-6: interleukin-6.

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Reference annotations:

Reference 2: *Major review article on multiple myeloma.

Reference 11: *Important work on the alkylating mechanism of melphalan.

Reference 14: ** Major review article on pharmacology of melphalan.

Reference 17: * Relevant work on the mechanism of action of melphalan.

Reference 32: *Important clinical study including melphalan

Reference 43: ** Important meta-analysis of the different clinical studies with melphalan plus prednisone and thalidomide.

Reference 44: *Important clinical study including melphalan

Reference 59: *Important clinical study including melphalan

Reference 61: *Important clinical study including melphalan

Reference 65: *Important clinical study including melphalan

Reference 84: * Revision of post-marketing surveillance on multiple myeloma drugs, including melphalan.