Allogeneic stem cell transplantation in multiple myeloma: immunotherapy and new drugs.

Allogeneic Stem Cell Transplantation in Multiple Myeloma: Immunotherapy and New Drugs

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Abbreviations

Allo= Allogeneic
ASO= allele-specific oligonucleotide
Auto= Autologous
BOR= Bortezomib
CAR= Chimeric antigen receptor
CR= Complete remission
DLI=donor lymphocyte infusion
DFS= Disease-free survival
EBMT= European Group for Blood and Marrow Transplantation
EFS= event-free survival
GvHD= graft-vs.-host disease
GvM= graft-vs.-myeloma
IMiDs= immunomodulatory drugs
IMWG= International Myeloma Working Group
IR= immunophenotypic responses
Len= Lenalifomide
MAC= myeloablative conditioning
MFC= multiparameter flow cytometry
MM= multiple myeloma
MRD= minimal residual disease
NGS= next-generation sequencing
NRM= Not relapsed mortality
ORR= overall response rate
OS= overall survival
PCR= polymerase chain reaction
PFS= Progression-free survival
SCT= stem cell transplantation
Thal= Thalidomide
TRM= transplant related mortality
RIC=reduced intensity conditioning

ABSTRACT

Introduction: Autologous (Auto) stem cell transplantation (SCT) and the development of new drugs with potent anti-tumor activity have considerably improved the survival of multiple myeloma (MM) patients. By contrast, though potentially curative, the use of allogeneic (Allo)-SCT is controversial

Areas covered: A review has been conducted to examine the current evidence for the use of allo-SCT in MM patients. Moreover, we have examined novel cell therapies that may be exploited to induce myeloma-specific immune responses including the new promising frontier of chimeric antigen receptor (CAR) -T and -NK cells.

Expert Opinion: one of the major controversies facing researchers in exploring the allogeneic approach is the remarkable recent treatment improvement observed with second- and third-generation proteasome inhibitors and immunomodulatory drugs, monoclonal antibodies and deacetylase inhibitors. However, despite these great advances, the disease remains incurable and allo-SCT may still play a role in the cure of MM. Importantly, patient risk stratification will be of paramount importance. We think that allo-SCT conserves a role in MM and its curative potential in high risk patients should be explored in the setting of control clinical trials. Moreover, novel cell therapies such as CAR technologies may open new avenues of research toward a potential cure. Data from currently ongoing prospective studies will be helpful to clarify pending clinical questions.

INTRODUCTION

The treatment of multiple myeloma (MM) has remarkably changed in the past 10 years, and subsequently the role of allogeneic stem cell transplantation (allo-SCT) has been amended over time.

Despite the dramatic recent advances, allo-SCT is still regarded as the only potential cure on account of its well-documented graft-vs.-myeloma (GvM) **(1-4)**. However, the introduction of new compounds capable of inducing high response rates with limited side effects **(5-7)**, has greatly challenged its role, although recent retrospective reports have shown that their use and GvM effect are not mutually exclusive and a strong synergistic effect may be established **(8)**. What is, therefore, the actual role of allo-SCT in MM? Currents efforts aim at defining its role in combination with new drugs in the light of patient risk factors and their impact on clinical outcomes. Clinical trials are in progress in newly diagnosed high risk patients and in early relapses.

Future perspectives are challenging: firstly, the worldwide introduction of new generation immunomodulatory drugs (IMiDs) and proteasome inhibitors may help consolidate disease response after allo-SCT and, secondly, even prevent graft-vs.-host disease (GvHD). In this report, we will examine the latest observations on novel agents such as Thalidomide (Thal), Bortezomib (Bor) and Lenalidomide (Len) in GvHD prevention and treatment. Then, given the growing interest in the evaluation of minimal residual disease (MRD) in haematological malignancies, we will overview its role after allo-SCT as a prognostic factor. Finally, we will explore the new promising frontier of chimeric antigen receptor (CAR) -T and -NK cells where the post-allo-SCT micro-environment may be attractive for adoptive immunotherapies.

ALLOGRAFTING IN MULTIPLE MYELOMA

The GvM effect has been well documented in the past two decades, primarily by the capability of donor lymphocyte infusions (DLI) of inducing remission in MM patients relapsed after an allograft (**2**, **3, 9, 10)**. Indirect evidence of GvM effects was also provided by Bjorkstrand et al. who showed a higher relapse/progression rate in auto-SCT as compared with allo-SCT recipients in a series of 189 patients **(11)**. Furthermore, though similar the intensity of the conditioning regimen, molecular remissions appear to be more frequent after myeloablative allo-SCT than after auto-SCT, predicting better overall survival (OS) and event-free survival (EFS) **(12, 13)**.

Recently, Binsfeld et al. **(14)** described the establishment of a reliable GvM model using allo-SCT in immunocompetent tumor-bearing mice. In this model, effector memory CD4 and CD8 T cells were shown to affect the in vivo GvM effect, with CD8 T cells playing a pivotal role. Within CD4 and CD8 subsets, the Authors identified overlapping TCR-Vb families that reacted against both myeloma cells (anti-tumor) and Balb/cJ cells (alloreactive), underlining the relationship between anti-tumor responses and GvHD. However, some TCR-Vb families within CD4 T cells specifically reacted either against myeloma or host alloantigens. This model may help to investigate in future studies mechanism of IMiDs and their impact on the balance between GvM and GvHost effects.

Despite the curative potential, myeloablative allo-SCT is hampered by a remarkable incidence of transplant related mortality (TRM), the most controversial aspect on its role in MM. To lower TRM, reduced intensity conditioning (RIC) regimens, more immunosuppressive rather than cytoreductive, were explored, primarily in the light of the Seattle experience **(15, 16, 17) (***Figure 1)*. Bensinger et al. recently reported the long-term outcomes of 278 patients treated with allo-SCT after myeloablative conditioning (MAC) (N=144) or RIC/non myeloablative (N=134) regimens. The latter resulted in significantly lower overall mortality (hazard ratio [HR] 0.40, p<0.0001) with far lower TRM (HR 0.22, p< 0.0001) and improved progression free survival (PFS) (HR 0.55, p=

0.0002). Interestingly, the risk of relapse/progression was not significantly different in Cox models between MAC and RIC/non myeloablative conditionings **(18).**

Given the importance of pre-allo-SCT cytoreduction had been widely recognized, the combination of auto followed by allo-SCT with RIC was also investigated in newly diagnosed patients. Recent results reported a TRM of 11% at 1 year, and, after a median follow-up of at least 5 years, a median OS and PFS not reached and of 35 months, respectively **(19, 20)**.

Despite the introduction of the so called "new drugs", single or double auto-SCT remains part of standard upfront treatment in patients up to the age of 65 **(21-24)**, whereas the upfront use of RIC allo-SCT has practically been abandoned and remains undefined.

Only few randomized trials, based on donor availability, compared tandem auto-SCT versus tandem auto/RIC allo-SCT. Importantly, all these trials were designed before "new drugs" became readily available.

An Italian randomized trial showed improvement in PFS and OS in the auto/allo-SCT group **(8, 25)**. After a median follow-up of 7 years, median OS was not reached (p= 0.02) and EFS was 39 months (p= 0.02) in the 58 patients who received tandem auto/allo-SCT, whereas OS of 5.3 years and EFS of 33 months were observed in the 46 who received two auto-SCT. The plateau in OS curve suggests a possible curative effect of RIC allo-SCT in a subgroup of patients.

By contrast, the randomized BMT CTN 0102 phase III trial enrolled 710 patients from Centers across the United States (**26)**. Patients with a HLA-identical sibling received a RIC allo-SCT as part of planned tandem auto/allo-SCT. The study showed no difference in OS (77% versus 80%) or PFS (43% versus 46%) between the auto/allo-SCT group and the tandem auto-SCT group at three years.

Gahrton et al **(27, 28)** published an update, at a median follow-up of 96 months, of the European Group for Blood and Marrow Transplantation (EBMT)-NMAM-2000 study that prospectively compared tandem auto/RIC allo-SCT versus single auto-SCT. Three-hundred-fifty-seven patients up to age 69 years were enrolled. Patients with a HLA-identical sibling were allocated to the auto/RIC allo arm and those without to the single auto-SCT arm. Progression-free survival and OS

were 22% and 49% versus 12% (p= .027) and 36% (p= .030) in the auto/RIC allo and in the auto-SCT groups, respectively. Corresponding relapse/progression rates were 60% versus 82% (p= .0002). Transplant related mortality at 36 months was 13% vs 3% (p= .0004). The importance of long-term follow-up for the correct evaluation of auto/RIC allo versus auto-SCT studies was clearly demonstrated in this study as survival curves between the 2 groups started separating about 3 years post-transplant.

Michallet et al. (**29)** recently presented an EBMT registry study on allo-SCT in MM in 7333 patients who were transplanted at a median age of 51 years between January 1990 and December 2012. Sixty-four % (4726) had been transplanted after the year 2004. The upfront use of an allograft was observed to gradually decrease after the year 2000 to the current 12%. An allo-SCT was more commonly used in recent years and, in 2012, most allo-SCT were performed in patients who relapsed after a first auto-SCT. This suggests a preference in using an allo-SCT at first relapse after a standard auto-SCT. The 1588 patients who received an allo-SCT after a single auto-SCT showed a 5 year PFS and OS of 26% and 33%, whereas the 930 who received it after failing a double auto-SCT showed a 5 years PFS and OS of 24% and 29%, and the 296 transplanted with an allo after at least 3 auto-SCT a 5 years PFS and OS of 15% and 23%, respectively.

Recently, Sahebi et al. **(30)** reported OS of 60%, PFS of 31% and relapse incidence of 59% at 7 years in 60 patients treated with a RIC allo-SCT after a median follow-up of 9.8 years. Relapses as late as 11.5 years post-transplant occurred in 10% of patients. Vekemans et al. **(31)** reported on long-term clinical outcomes of 42 consecutive patients who underwent an allo-SCT after a MAC or RIC regimen. This study confirmed previous observations that more than 10% of patients experienced late progression/relapse at a median follow up 6.8 years. Neither chronic GvHD nor achievement of CR after allo-SCT were significantly associated with improved OS or longer PFS.

Lokhorst et al. **(32)** reported a donor versus no donor analysis in newly diagnosed stage II-III patients who were enrolled in the prospective HOVON50 trial. Patients without a donor received high dose melphalan and auto-SCT followed by maintenance therapy, whereas those with a donor

were intended to receive a planned auto/allo-SCT following a non-myeloablative conditioning. In a first intention to treat analysis, donor availability was not associated with better clinical outcomes and there was no benefit in patients treated with upfront tandem auto/allo-SCT. However, given that a considerable number of patients with a donor were not eventually transplanted, the analysis may have underestimated the potential benefit of the allograft. The same study group **(33)** recently expanded the statistical analyses by evaluating the allo-SCT as a time dependent variable and by carrying out a landmark analysis. By comparing the different methods, it was shown that the efficacy of allo-SCT was underestimated by a donor versus no donor analysis, whereas the use of both the allo-SCT as a time dependent analysis and a landmark analysis may more reliably evaluate its impact in newly diagnosed patients. In the experience on high risk patients by the Intergroup Françophone du Myélome (IFM), after a median follow-up of 56 months, no differences in EFS were observed and there was a trend toward a better OS in the double auto-SCT group as compared with the auto/allo-SCT group (48 vs. 34 months, p= 0.07) **(34, 35)**. In a Spanish trial, only patients who failed to achieve complete remission (CR) or near CR after the first auto-SCT were eligible for the second transplant. Authors showed a trend toward a better PFS (p= 0.08) in 25 patients treated with tandem auto/allo as compared with 85 patients who received double auto-SCT, with a plateau of PFS in patients in CR after tandem auto/allo-SCT. However, this did not translate into a significant advantage in OS after a relatively short follow-up **(36)**.

Knop et al. presented results of a German study which investigated the impact of del(13q) **(37)**. Patients received either a planned double auto-SCT or an upfront auto followed by an allo-SCT. The study included 199 patients with a median age of 53 years. Seventy-three patients received the double auto-SCT, whereas the remaining 126 received tandem auto/allo-SCT. The study showed that patients with del(13q) may benefit from the auto/allo-SCT approach. After a median follow-up up of 7.6 years, auto/allo-SCT group had significantly longer median PFS than the double auto-SCT group (34.5 and 21.8 months, respectively). The benefit in PFS was independent of donor type (matched related or matched unrelated donor). Median OS did not significantly differ between the

two treatment groups (71.8 months for double auto-SCT and 70.2 months for auto/allo-SCT patients). Two-year NRM was 12% in the auto/allo and 4% in the double auto-SCT group. Hofmann et al. reported a retrospective analysis on 95 allo-SCT patients treated between 1994 and 2013 at Ulm University Hospital **(38)**. Cytogenetic abnormalities did not affect PFS and OS. Overall survival was significantly better in patients who did not develop acute GvHD, whereas chronic GvHD had no impact on clinical outcomes. Patients who received a myeloablative conditioning showed a trend for lower relapse as compared with those who received a RIC. Transplant related mortality did not differ. Donato et al. reported on a single Center experience at Hackensack University Medical Center, NJ **(39**). Overall, 57 patients received an allo-SCT. Twenty-six patients underwent allo-SCT as consolidation after a response to their first auto-SCT, while 30 patients received an allo-SCT as salvage therapy. The median follow-up was 52 months. At 5 years, 49.2% of all patients were in CR. The 5-year OS for patients who received transplantation as salvage was substantially lower than in those who received it as consolidation. Acute GvHD was associated with poorer OS and PFS, whereas chronic GvHD with better clinical outcomes suggesting an important role for the GvM effect.

Overall, results of comparative studies have been rather conflicting, probably due to small numbers and heterogeneity in patients characteristics. Recent meta-analyses that included studies on tandem double auto and tandem auto/allo-SCT in newly diagnosed patients were published. One study included 1538 patients. Despite higher CR rates, the meta-analysis did not show any improvement in OS and EFS with the auto/allo-SCT. The Authors suggested the use of an allo-SCT only in the context of clinical trials **(40)**. Importantly, at the time these studies were designed, no risk assessment at diagnosis was routinely possible. In another meta-analysis, **(41)**, the Authors included 6 trials for a total of 1192 patients in the double auto and 630 in the auto/allo SCT group. Patients in the latter group had a higher chance of reaching CR that did not however translated in better OS at 3 years from treatment assignment because of higher TRM. An updated meta-analysis with longer follow up is in progress.

NEW DRUGS AND ALLOGRAFTING IN MULTIPLE MYELOMA

The advances in the understanding of the pathophysiology of MM culminated in the development of smart drugs that target specific intracellular pathways and the crosstalk between clonal plasma cells and the microenvironment. Clinical trials showed potent activity in first-line and in salvage treatments in relapse/refractory disease **(5-7, 42-46)**.

In the light of these findings, further strategies have been developed to explore feasibility and efficacy of the combination of an allograft with new drugs. Their incorporation in induction regimens before auto-SCT has demonstrated to increase the post-transplant CR rate **(7, 48).** El-Cheich et al. compared clinical outcomes of 45 patients transplanted between 1999 and 2006 who had not received neither novel agents prior to allo-SCT (group 1), with 34 patients transplanted after 2006 treated with Len and/or Bor before the allo-SCT (group 2). There was a trend for better 2-year disease-free survival (DFS) in group 2 (65% vs. 45%, p= 0.18) **(49)**. Hough prospective data are lacking, with higher pre-transplant tumour reduction, higher CR rates and better outcomes should be expected after allo-SCT because the potential ongoing GvM effects.

Is there any evidence of a synergy between donor T cells and new drugs?

Allografting and "new drugs" as maintenance

To improve clinical outcomes of allo-SCT as part of first-line treatment, the role of Len maintenance was investigated. The first study reported on 30 patients, who started Len from 1 to 6 months after a non-myeloablative allo-SCT. Lenalidomide was administered on a 10 mg daily schedule 21 out of 28 day cycles. Responses were observed in 37% of patients, but the main limit was drug discontinuation due to flare of GvHD in 43% (13) patients. Furthermore, 5 patients (17%) stopped Len maintenance because of other adverse events and 5 after disease progression **(50)**. Conversely, with a different schedule, Kroger et al. suggested feasibility and efficacy of Len maintenance after allo-SCT, with a 3-year estimated probability of PFS and OS of 52% and 79%

respectively **(51)**. Preliminary results of a dose finding study conducted on 24 patients showed that Len 5 mg daily started between day 100 and 180 post-transplant is the maximum tolerated dose to avoid excessive toxicity **(52)**.

In another recent trial **(53)**, the use of Len as maintenance was primarily evaluated as an immunomodulatory agent for additional disease control and to allow time for a more potent GvM effect to be established. Lenalidomide at 10 mg daily (3-weeks on and 1 off) started approximately at day 100 post-transplant was feasible. Although a 38% incidence of acute GvHD was reported, survival outcomes were promising in this cohort of high-risk patients. Despite others studies that associated post-transplant Len given early after transplant with unacceptable toxicity **(50)**, this study by Alsina et al. demonstrated that if Len therapy is scheduled later after transplant, similar to that used in the post auto-SCT setting, clinical advantages may be observed.

At least 3 studies, where new drugs are evaluated as maintenance in high risk MM, are in progress: one study with Len plus Sirolimus (NCT01303965); one study on safety and efficacy of Len after tandem auto/allo-SCT in newly diagnosed MM by the Gruppo Italiano Malattie EMatologiche dell'Adulto (GIMEMA) (NCT01264315); a phase II multicenter study of the new proteasome inhibitor MLN9708 (NCT2168101). Finally, a phase II prospective safety study on Len maintenance in high risk population was recently closed (NCT00847639) and results will be available soon.

Allografting and "new drugs" as salvage therapy (Table 1)

The long term follow up of a prospective Italian study comparing double auto vs. auto/allo-SCT in newly diagnosed patients allowed to formulate suggestive hypothesis. Among relapsed patients, the "allo-group" rescued with Thal, Len or Bor showed a significantly better median OS from the start of salvage therapy as compared with the "double auto-group" (not reached vs. 1.7 years, p= 0.01) **(8)**. This finding may partly be explained firstly by a synergy between the high percentage of donor T cells, usually seen at relapse after a non-myeloablative allo-SCT, and IMiDs that may help

to restore GvM, and secondly, that donor T cells may favour the anti-MM effect of new drugs in the marrow milieu.

Other groups also reported interesting data. The efficacy and safety of Thal in relapsed/refractory patients after allo-SCT was retrospectively evaluated only in few small cohorts without very promising results **(54-57)**. An interesting experience comes from the IFM group. An overall response rate (ORR) of 29% without CR was reported in 31 patients enrolled in a multi-center trial. Thal was discontinued in 19% because of neurological toxicity **(54)**.

Bortezomib was employed in more trials. In a retrospective cohort of 37 patients, peripheral neuropathy, mild thrombocytopenia and fatigue were the most frequent adverse events. However, 27/37 patients (73%; 95% CI, 59-87%) achieved an objective response. The estimated OS was 65% at 18 months from Bor initiation at a median follow-up of 9 months **(58)**. Other similar experiences confirmed these promising results with a warning on a possible enhance of neurotoxicity if a prolonged cyclosporine treatment is associated **(59-60)**.

The role of Len as salvage was more extensively investigated. Len has been associated with the ability to stimulate the cytotoxic activity of mononuclear cells and to inhibit regulatory T cells **(61)**. Montefusco et al. reported a multi-center case-matched analysis on Len plus dexamethasone after auto or allo-SCT in 2 cohorts of 40 patients each. At a median follow-up of 22 months, median PFS was 8.9 in the auto-group vs. 13.1 months in allo-group (p= 0.03). This advantage also translated into a significantly better median OS (22.1 vs. 51.3 months, p= 0.04) **(62)**. Interestingly, in line with previous observations **(8)**, a benefit was also described in patients with stable disease suggesting activity even in patient with persistent disease after allo-SCT. Unexpected toxicities were not observed and there were no differences between the 2 cohorts. Of note, initiation of Len relatively late after allo-SCT (median time 32 months) appeared to prevent the flare of acute GvHD previously described by the HOVON group where Len was started at a median of only 3 months from allo-SCT **(50)**. Other experiences were reported. Coman et al. assessed the efficacy of Len as salvage therapy, alone or associated with dexamethasone, in a retrospective cohort of 52 heavily

pre-treated MM patients after allo-SCT. The ORR was 83%, including 29% of CR. Interestingly, previous refractoriness to IMiDs did not impact on response. After a median follow up of 16.3 months (range, 3.7- 49.6), median PFS and OS were 18 and 30.5 months, respectively. In this study, commonly described adverse events led to dose adaptation and drug withdrawal in 44% and 17% of patients respectively. As reported by the HOVON group, the risk of developing *de novo* or exacerbation of acute GvHD was related to early Len introduction after allo-SCT **(63)**. Results of other reports on smaller patient series confirmed these data **(64-66**).

Overall, despite the limitation of retrospective studies, Len appears to be the most promising drug as salvage after allo-SCT enforcing the hypothesis that Len enhances GvM by interacting with donor T cells **(67-69)**. Unfortunately, the potential survival benefit may be outweighed by its side effects. In particular, the risk of GvHD should be considered as a major issue if Len is administered early after allo-SCT.

"NEW DRUGS" AND GRAFT-VS.-HOST DISEASE

Graft-vs.-host disease remains one of the most significant causes of TRM after allo-SCT. The pathophysiology of acute GvHD can be briefly resumed in 3 phases: first, the damage of host tissues associated with the conditioning regimen; second, the donor T-cells recognition of allo-antigens presented by host antigen-presenting cells (APCs); third, apoptosis mediated by cellular effectors and inflammatory cytokines (*Figure 3).* By contrast, the pathological model of chronic GvHD is poorly understood. Several studies showed the important role of B cells and T helper-2 cells together with abnormalities in regulatory T cells that combine to lose immune-tolerance and form the basis for cellular reactions against tissues **(70)**. Both acute and, in particular, chronic GvHD are though to be triggered by the same allo-reactive T cells of donor origin that underline the curative potential of GvM effect. In fact, efforts to reduce the incidence of GvHD by *in vivo* or pre-infusion T cell depletions have also been associated with diminished responses and higher relapse

rates. The role of IMiDs and proteasome inhibitors in prevention and treatment of GvHD, while sparing GvM effect, has been investigated with some interesting results summarized below.

Thalidomide

Preclinical studies demonstrated the Thal is characterized by many immunological effects such as the reduction of TNF-alpha levels, inhibition of IL-1, IL-6, IL-12 production, co-stimulation of IL-2 and IFN-gamma producing T cells, and down-regulation of adhesion molecules involved in leukocyte migration and angiogenesis **(71, 72)**. Thal was also employed in few studies for the treatment of chronic GvHD. The ORR varied and primarily observed in oral and skin GvHD **(73-76)**. However, in some experiences, a remarkable drug discontinuation rate up to 90% was reported due to haematological and neurological toxicities **(77, 78)**. Thal is currently only rarely used in refractory chronic GvHD and specifically in muco-cutaneous manifestations **(72)**.

Lenalidomide and pomalidomide

As previously described, some issues on Len feasibility, used as maintenance/consolidation therapy after allo-SCT, have emerged regarding its early administration post-transplant and excessive flare of GvHD **(50, 51)**. Linhares et al. assert that some trials may also have been conducted but not reported because of negative results, determining a publication bias **(79)**.

The novel IMiD pomalidomide (Pom), 100-fold more potent than its parent compound, was evaluated in a small pilot study on steroid refractory cGvHD. Only 3/8 patients completed the 6 month treatment. Two/3 achieved complete and 1/3 partial responses. The remaining 5/8 patients discontinued the drug for absence of response or toxicity **(80)**. A larger randomized phase II trial of Pom in refractory cGvHD patients is currently in progress (NCT01688466).

Histone deacetylase inhibitors and Carfilzomib

Interesting preclinical data are emerging on Histone deacetylase inhibitors (HDACis) and GvHD. The Food and Drug Administration (FDA) approved Vorinostat showed efficacy in GvHD treatment in numerous mouse models **(81, 82)**. This led to the incorporation of Vorinostat in GvHD prophylaxis in the clinical setting. Choi et al. recently reported the results of a phase I/II prospective trial of Vorinostat plus tacrolimus and mycophenolate in 50 patients transplanted for various hematological malignancies. The cumulative incidence of grade 2-4 acute GvHD at 100 days was 22%. The limit of this study was the single-arm design **(83)**. Data from other prospective trials are still pending (NCT01789255; NCT01790568). Surprisingly, Wang et al. found that the other very recently approved HDACis LBH589 (Panobinostat) accelerates rather than alleviates GvHD in mice **(84)**. At our knowledge, no data on Carfilzomib in GvHD treatment or prophylaxis are available at the moment.

Bortezomib

Among the so called new drugs, Bor is by far the most appealing agent for prevention of GvHD. In preclinical studies, Sun et al. demonstrated that Bor significantly inhibits the *in vitro* proliferation and promotes the apoptosis of allo-reactive T-cells **(85-87)**. Furthermore, through the stabilization of the NF-kB inhibitor IkB, Bor reduces the NF-kB expression **(88, 89)** in activated T cells and affects T cell mediated immune response (*Figure 3).*

In the clinical setting, preliminary studies showed the ability of Bor to improve GvHD control. Conversely to Len, no acute GvHD exacerbation was observed with reasonably low toxicity **(60)**. A phase I prospective trial evaluated the potential benefit of the combination of Bor, tacrolimus and methotrexate for GvHD control in 23 patients with haematological malignancies. Grade II-IV acute GvHD and chronic GvHD at 1 year occurred in only 13% and 41% of patients respectively **(90)**.

The efficacy of Bor in chronic GvHD treatment was confirmed in a small series of MM patients by Mateos-Mazon et al. **(60)**. The Authors described a remarkable drug activity in four patients with extensive refractory chronic GvHD **(91)**. A phase II prospective trial of Bor plus prednisone for initial therapy of newly diagnosed chronic GvHD has been recently completed. Results are not yet available (NCT00815919).

In summary, preliminary data suggest a possible role of new drugs other than Len in GvHD prevention or second line treatment.

Allografting and minimal residual disease

There is growing interest in MRDto evaluate depth of response in hematological malignancies, especially in B-cell disorders. Minimal residual disease has become a relevant tool to predict patient outcome and potentially modify therapeutic approaches **(92-95)**. In MM, the impact on long-term outcomes of achieving clinical CR, or, more recently, MRD by molecular technology, has been very well documented **(96).** In MM, the clonally rearranged immunoglobulin heavy chain (Ig-H) gene is a sensitive tumor marker and has been used to assess the MRD status after treatment. Techniques available for its evaluation include polymerase chain reaction (PCR) and, more recently, next-generation sequencing (NGS). Moreover, the detection of aberrant expression of cell surface antigens by multiparameter flow cytometry (MFC) is a useful tool. Both flow-cytometry and molecular biology techniques are potent tools but the lack of a standard limits for now their widespread use.

The importance of complete immunophenotypic responses (IR) byMFC was recently recognized by a consensus panel of the IMWG **(97)**. With a technical success rate greater than 95% and a sensitivity of at least 1 x 10^{-3}, it appears a promising tool **(97-100).** Despite the high, favorable prognostic value associated with MFC remission after auto-SCT, data after allo-SCT are lacking. Recently, Giaccone et al. documented for the first time the impact of MFC remission following

allo-SCT. After a median follow up of 69 months, median OS and EFS in patients who achieved IR were 96 and 55 months vs. 36 and 7 months in those who did not ($p< 0.001$) **(101)**.

Stronger evidence on the role of molecular MRD evaluation after allo-SCT has been reported. Corradini et al. **(12)** initially reported data on qualitative PCR based approaches using allele-specific oligonucleotide (ASO) primers. This method has a sensitivity of at least 1×10^{-5}, but requires the sequencing of individual patient clones and the development of specific reagents. Furthermore this technique can be successful in less than 80% of patients **(12, 13, 102, 103)**. In this study, the Authors showed, in 15 auto and 14 allo-SCT patients with a molecular marker, a higher proportion of PCR-negative remissions after allo-SCT rather than auto-SCT (50 vs. 7%) that translated into better clinical outcomes. Interestingly, one patient obtained molecular CR several years after transplant suggesting that the chemo-resistance of MM cells may be overcome by the allogeneic T-cell anti-tumor effect. The findings by Corradini et al. were confirmed shortly after by Martinelli et al. **(77)**. Fifty % of the patients who achieved molecular CR, defined as PCR negativity on 2 consecutive samples, remained in continuous clinical CR for a median follow-up of 72 months.

The prognostic role of molecular remission after allo-SCT was further investigated in a retrospective multi-center EBMT study on 70 MM patients in clinical CR **(13)**. In this group, a patient specific marker was generated in 48 (69%) of the patients. The relapse risk for MRD positive patients (13/40, median follow-up 23 months) was significantly higher than mixed pattern (19/48, median follow-up 46 months) or persistently MRD negative patients (16/48, median follow-up 36 months) with a 3 year cumulative risk of relapse of 61%, 14% and 0%, respectively. In line with these findings, Galimberti et al. described the statistically significant impact of MRD negativity on OS after allo-SCT in a cohort of 20 patients. Twenty months OS from allo-SCT was 76% vs. 34% ($p= 0.03$) in 12 PCR negative vs. 8 positive patients respectively **(105)**.

More recently, with the aim of overcoming the limitations of PCR, Ladetto et al **(102)** employed the LymphoSIGHT NGS method for MRD assessment in 10 MM patients **(106)**. The procedure was at least as efficient as ASO-PCR in IgH-based clono-types detection allowing the identification of a

patient specific molecular marker in 8/10 of patients. Interestingly, the simultaneous use of PCR and the NGS allowed to identify at least one MRD marker in all patients. Minimal residual disease studies after allo-SCT are summarized in *Table 2.*

In summary, MRD assessment seems to have a role in predicting outcome after MM standard treatment as well as Allo-SCT and its evaluation should be included in future prospective trials.

CHIMERIC ANTIGEN RECEPTORS IMMUNOTHERAPY in MM

The potential efficacy of cellular therapies in MM is highlighted by the observation that allo-SCT and donor lymphocyte infusions had been associated with disease response due to GVM effect. Novel strategies designed to obtain a specific immune response decreasing the immune-mediated toxicity to normal tissues are under development. Investigators have pursued MM vaccine models in an effort to elicit tumor-specific immunity. Due to the results in preclinical experiences, vaccination with autologous and allogeneic dendritic cells (DC) and with DC/MM fusions cells was investigated. Results of small phase I clinical trials are promising, but we are still far away from a wide clinical diffusion **(107, 108)**. Lavenga et al. applied for the first time DC vaccines after allo-SCT in only 6 patients with limited toxicity **(109)**. Moreover, responses to WT1-specific cytotoxic T cells had been reported in allo-SCT setting opening a new field of vaccine research **(110)**.

In recent years, biological anticancer agents as monoclonal antibodies, bispecific T cells engaging molecules orCAR cells have been developed and tested in phase I clinical trials. Chimeric antigen receptors are synthetic proteins composed of an antigen recognition portion and an intracellular activation domain. The incorporation of this receptor on T cell and, more recently, NK cell surfaces allows to achieve a strong combination of antibody specificity and a direct cytotoxic power. This strategy of adoptive immunotherapy may even bypass the immune-escape ability of cancer cells **(111)**. In fact, initial observations from preclinical and clinical studies revealed a surprising CAR-mediated anticancer activity **(112)**.

Overall, most encouraging clinical observations have been achieved in patients with $CD19^+$ haematological malignancies, especially in chronic and acute lymphoid leukemias **(113-115)**. A

phase I trial of anti-CD19 CAR-T cells in MM is mow recruiting patients (NCT02735406). Unfortunately, a specific and well determined target antigen in MM is still lacking. Molecules such as CD20, CD22, CD30 and CD33 are being evaluated as potential novel targets. Preclinical studies also evaluated the activity of CAR-T cells against MM cell lines carrying antigens such as Lewis Y, B Cell Maturation Antigen (BMCA), CD38 and CS1. In all studies, high cytotoxic activity *in vitro* and *in vivo* in murine models was observed **(116-118)**. Unlike CD19, MM specific cells targets such as CD38, CD56 and CD138 are co-expressed in other tissues with potential side toxicity.

An Italian group has recently demonstrated the efficacy of anti-CD44v6 CAR-T cells that co-expressed a suicide gene. This may allow a rapid pharmacological ablation of CAR cells and reduce their side effects **(119)**. Chimeric antigen receptors-NK cells were also investigated. In the current few reports, NK cells appear characterized by a safer toxicity profile in terms of risks of cytokine storm and/or tumor lysis syndrome risk and, importantly, in GvHD induction in allo-SCT setting **(120-122)**.

Some other clinical studies on the use of CAR-T cells as salvage therapy in MM are in progress. A phase I study investigates the use of CAR-T cells against the kappa light chain of the immunoglobulins (NCT00881920). One Chinese study evaluates the efficacy and safety of anti-CD138 CAR-T cells (NCT01886976); and another phase I study the role of Lewis Y CAR-T cells in MM, acute myeloid leukemia (AML) and high risk myelodysplastic syndromes (NCT01716364) **(123)**. Recent trials have also incorporated patient immuno-regulatory cells depletion that has shown a parallel increase in the anti-tumor activity of CAR-T cells **(124, 125)**.

Though promising, CAR T and NK cells technologies and their application in clinical trails are still at an investigational stage. Modality of CAR cells expansion, their timing of infusion and their integration into therapeutic algorithms are aspects that will have to be carefully evaluated as potentially lethal severe adverse events were reported **(117,124)**.

Finally, another interesting target in MM cells with the potential of improve outcome of immunotherapies is the programmed death receptor-1 (PD1)/PD ligand-1(PD-L1) pathway. The

inhibitory signal mediated by PD1 seems to contribute to impair T cells anti-tumor control **(125)**. Kearl et al. recently reported that lymphodepletion and PD-L1 blockade synergize to eradicate MM in 40% of murine models **(126)**. In the other hand, a preclinical experience in allo-SCT murine model raised concerns about increased GvHD related to PD-L1 blockade, raising new question for preclinical investigators **(127)**.

Expert opinion

The growing impetus for developing modern cell immuno-therapies largely stems from clinical observations that allo-SCT is curative in a variety of hematological diseases. Initially conceived as a mere rescue strategy after myeloablative doses of chemotherapy, it is now clear that the donor immune system transferred into the recipient may highly contribute to disease eradication given its immunological driven anti-tumor effects.

In MM, high TRM and limited efficacy in long-term disease control have so far prevented the demonstration of a clear benefit of all-SCT **(17)**. While RIC regimens have lowered toxicity and early TRM, and published data demonstrate that allo-SCT for MM can lead to sustained complete remissions, even in patients with relapsed or refractory disease, the incidence of acute and chronic GvHD, as well as late disease relapse, remain an issue. Moreover, one of the major controversies facing researchers in exploring the allogeneic approach is the remarkable recent treatment improvement observed with second- and third-generation proteasome inhibitors and IMiDs, monoclonal antibodies and HDACis. However, despite these great advances, the disease remains incurable and allo-SCT may still play a role in the cure of MM. Importantly, patient risk stratification will be of paramount importance. The dissection of the complex genomics and proteomics of the disease have substantially contributed to our understanding of its molecular biology and, in turn, our ability to estimate prognosis. Risk-adapted strategies tailored to biological parameters are now commonly applied for treatment decision in daily practice and may help indicate the appropriateness of an allo-SCT. A rationale for future prospective studies is the

integration of novel therapies into the allo-SCT paradigm in subsets of patients with high risk disease [plasma cell leukemias, MM with del(17p), t(4;14), t(14;16) or 1p/q abnormalities] where both auto-SCT and new drugs are poorly effective. New avenues of research should focus on the combination of new drugs and GvM effects to both prevent GvHD prevention and to establish long-term disease control.

The use of bortezomib within the conditioning may prevent GvHD, given its ability to eradicate alloreactive T cells, and limit the risk of relapse after RIC allo-SCT **(86, 90, 129-131)**. Bortezomib may potentially be associated also with DLI where, though an extensive application, a CR rate of less than 30% and a close association between anti-myeloma responses and GvHD have been reported **(130)**. Recently, Alsina et al. also showed that a lower dose of Len after allo-SCT is feasible and could represent a strategy of maintenance therapy as similar to that used in the post auto-SCT setting **(53)**. The development of novel immunotherapeutic strategies may further support research programs that target specific disease antigens with cell therapies **(131)**. More recently, MM has offered several appealing targets for the development of vaccines and CAR-based immunotherapies that may potentially be well combined with current treatments.

In conclusion, we think that allo-SCT conserves a role in MM and its curative potential in high risk patients should be explored in the setting of control clinical trials. Moreover, novel cell therapies such as CAR technologies may open new avenues of research toward a potential cure. Data from currently ongoing prospective studies will be helpful to clarify pending clinical questions.

Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents, received or pending, or royalties.

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Table 1. Multiple myeloma treatment with new drugs in allogeneic SCT setting.

	Patients	Median age (range)	Disease status after Allogeneic SCT	Regimen	Median time SCT/ salvage	Overall response (at least PR)	PFS after salvage treatment (follow up)	OS after salvage treatment (follow up)
Montefusco et al 2014 (62)	40	47 (30-63)	Relapse	Len	46 mo	60%	50% (13 mo)	50% (51 mo)
Bensinger et al 2014 (66)	18	55 (33-66)	Relapse	Len	12 mo	56%	NR	NR
Coman et al 2013 (63)	52	48 (32-61)	Relapse	Len (12) Len+Dex (40)	24 mo	83%	50% (18 mo)	50% (31 mo)
El Cheikh et al 2012 (65)	12	56 (46-64)	Relapse/ refractory	Len+ DLI	6 mo	58%	75% (12 mo)	83% (12 mo)
Lioznov et al 2010 (69)	24	59 (37-70)	Relapse	Len	11 mo	66%	50% (10 mo)	50% (20 mo)
Minnema et al 2009 (64)	16	58 (43-67)	Relapse/ refractory	Len (8) Len+Dex (8)	NR	87%	50% (11 mo)	50% (13 mo)
Kroger et al 2009 (133)	25/32	50 (35-68)	No CR after DLI *	Thal (15) Vel (8) Len (2)	NR	59%**	58% vs. 35%*** (56 mo)	90% vs. 62%*** (56 mo)
El-Cheikh et al 2008 (58)	37	49 (27-64)	Relapse/ refractory	Vel (11) Vel+Dex (26)	20 mo	73%	NR	65% (18 mo)
Bruno et al 2006 (59)	23	53 (31-66)	Relapse/ refractory	Vel (9) Vel+Dex (14)	20 mo	61%	50% (6 mo)	NR
van de Donk et al 2006 (57)	21/63	NR	No response to DLI	Vel (7) Thal (9) Vel+Thal (5)	NR	NR§	NR	NR
Mohty et al 2005 (54)	31	54 (39-64)	Relapse/ refractory	Thal	NR	*29%*	*NR*	*50%* ~ 15 mo §
Kroger et al 2004 (56)	18	53 (31-64)	Relapse/ refractory	Thal+DLI	12 mo	*50%*	*84%* (12 mo)	*100%* (12 mo)

Abbreviations: SCT, stem cells transplantation; NR, not reported; PR, partial remission; PFS, progression free survival; OS, overall survival; CR, complete remission; DLI, donor limphocytes infision; SD, stable disease; mo, months.

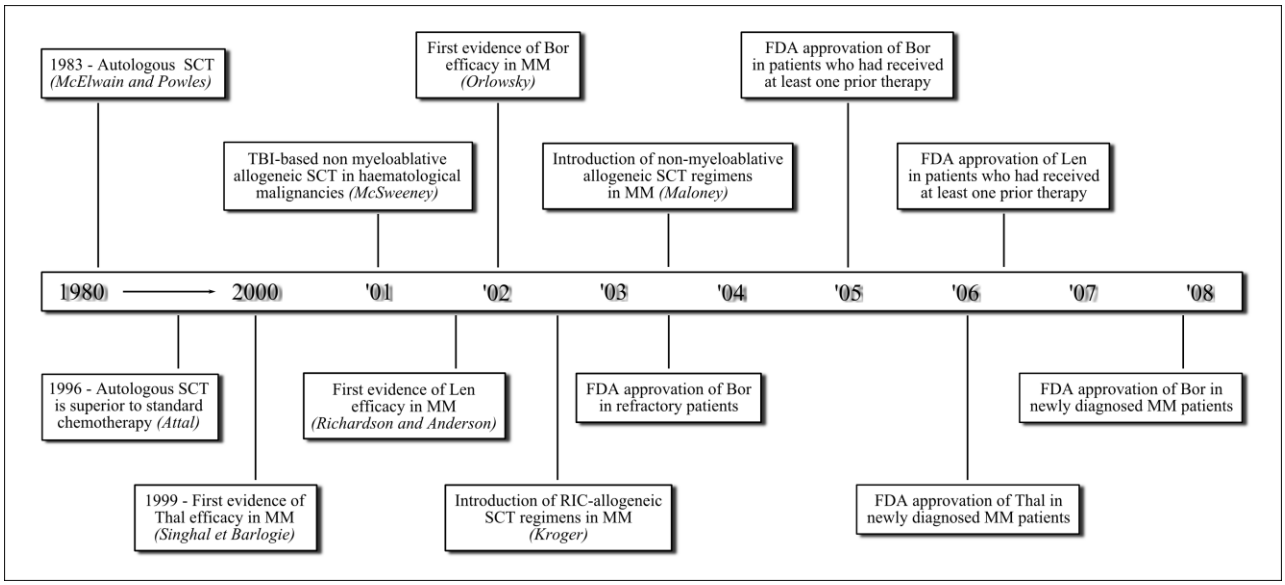
§ see text; * DLI was administrated in 32 patients who achieved only partial remission after allogeneic SCT; ** percentage of CR obtained after DLI and treatment with new drugs; *** patient who achieved CR versus patients who did not achieve CR; ^ obtained after RIC allogeneic SCT; ^^ obtained before RIC allogeneic SCT; ^^^ calculated on all 36 patients.

Table 2. MRD monitoring in MM after allogeneic SCT.

	Patients	Method	Sensitivity declared	Technical Success Rate	CR Rate	MRD Negativity Rate
Giaccone et al 2013 (ASH abstract 3371)	66	MCF	10^{-4}	100%	36% (24/66)	68% (45/66)
Raab et al 2004 (134)	11	ASO-PCR	10^{-4}-10^{-5}	NR	27% (3/11)	27% (3/11)
Corradini et al 2003 (13)	70	ASO-PCR	10^{-6}	69%	100%	33% (16/48)
Martinelli et al 2000 (104)	68	ASO-PCR	10^{-5}	88%	38% (26/68)	50% (7/14)
Cavo et al 2000 (103)	13	ASO-PCR	10^{-5}-10^{-6}	69%	92% (12/13)	69% (9/13)
Corradini et al 1999 (12)	17	ASO-PCR	10^{-5}-10^{-6}	84%	100%	*50% (7/14)*

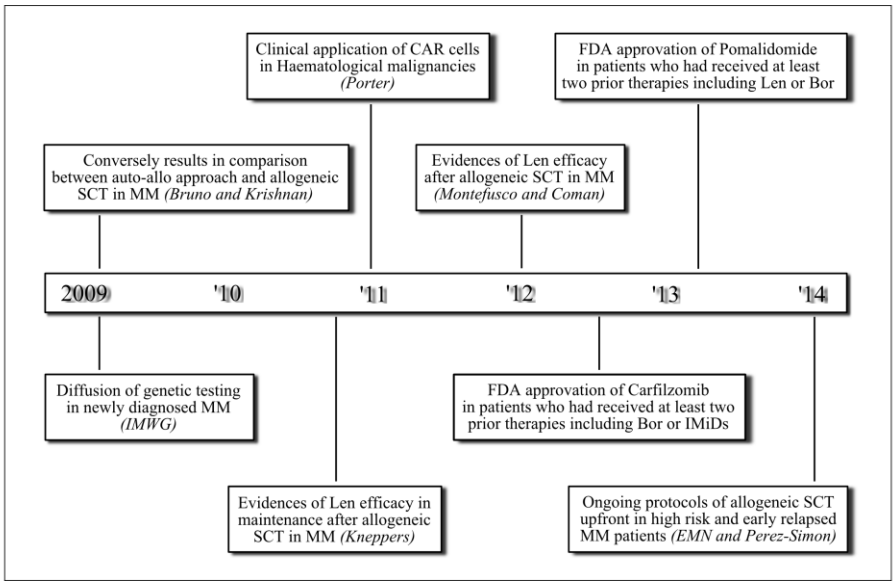
Abbreviations: MRD, minimal residual disease; MM, multiple myeloma; SCT, stem cell transplantation; CR, complete remission; MFC, multiparameter flow cytometry; ASO-PCR, allele-specific oligonucleotide polymerase chain reaction; NR, not reported.

Figure 1. Timeline showing the history of MM treatment.



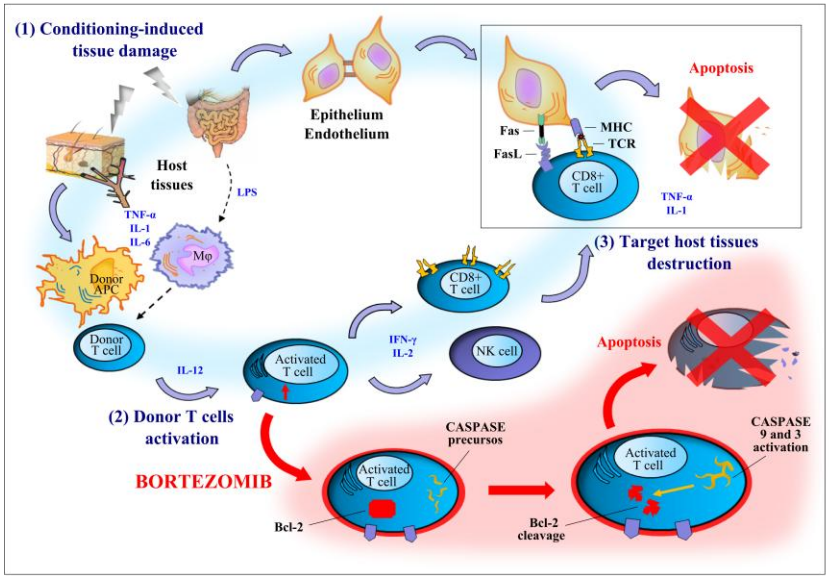
Abbreviations: SCT, stem cell transplantation; Thal, thalidomide; TBI, total body irradiation; Len, lenalidomide; MM, multiple myeloma; Bor, bortezomib; RIC, reduced intensity conditioning; FDA, food and drug administration.

Figure 2. Timeline showing advances in MM treatment.



Abbreviations: CAR, chimeric antigen receptor; IMWG, international myeloma working group; IMiDs, immunomodulatory drugs; SCT, stem cell transplantation; Len, lenalidomide; MM, multiple myeloma; Bor, bortezomib; FDA, food and drug administration.

Figure 3.



Abbreviations: TNF-α, Tumor Necrosis Factor-α; IL, interleukin; APC, antigen presenting cell; MΦ, macrophages; LPS, lipopolysaccharide; NfkB, nuclear factor-kappa B; IFN-γ, interferon-gamma; MHC, major histocompatibility complex; TCR, T cell receptor; FasL, Fas ligand.