Prospective molecular monitoring of minimal residual disease after non-myeloablative allografting in newly diagnosed multiple myeloma

Prospective molecular monitoring of minimal residual disease after non-myeloablative allografting in newly diagnosed multiple myeloma

Marco Ladetto, MD[1,2,3], Simone Ferrero, MD[1,2], Daniela Drandi, PhD[1,2], Moreno Festuccia, MD[1,2], Francesca Patriarca, MD[4], Nicola Mordini, MD[5], Silvia Cena, PhD[1], Roberta Benedetto, PhD[1,2], Guglielmo Guarona, MD[1,2], Federica Ferrando, MD[1], Lucia Brunello, MD[1,2], Paola Ghione, MD[1,2], Viola Boccasavia, PhD[1], Renato Fanin, MD[4], Paola Omedè, PhD[1], Luisa Giaccone, MD[1,2], Antonio Palumbo MD[1,2], Roberto Passera, PhD[6], Mario Boccadoro MD[1,2] , Benedetto Bruno, MD, PhD[1,2]

[1] Division of Hematology, A.O.U. Città della Salute e della Scienza di Torino, University of Torino, Torino, Italy

[2] Department of Molecular Biotechnology and Health Sciences, University of Torino, Torino, Italy

[3] Azienda Ospedaliera SS Antonio e Biagio e Cesare Arrigo, Alessandria, Italy

[4]Division od Hematology, A.O.U, Udine, DISM, University of Udine, Udine, Italy

[5] Division of Hematology, Ospedale "S.Croce e Carlo", Cuneo, Italy

[6] Division of Nuclear Medicine, Statistical Consultant, A.O.U. Città della Salute e della Scienza di Torino, Torino, Italy

Corresponding author:

Benedetto Bruno, M.D., Ph.D.

Division of Hematology,

A.O.U. Città della Salute e della Scienza di Torino and University of Torino,

Via Genova 3,

10126 Torino, Italy

E-mail: benedetto.bruno@unito.it

Phone: +39-011-6334354 Fax: +39-011-6963737

Allografting is potentially curative for myeloma (MM) [1]. Molecular remissions (MR) were reported after myeloablative and reduced-intensity conditionings [2,3]. Before the "new drugs" era, a tandem approach with an autograft after high-dose melphalan (200 mg/m2) followed by a non-myeloablative 200 cGy total body irradiation (TBI)-based allograft was designed in Seattle[4]. At a median follow up of 12.1 years, we report long-term clinical outcomes of minimal residual disease (MRD) kinetics by nested qualitative PCR and real-time quantitative PCR (qPCR) on a cohort of newly diagnosed patients treated with the Seattle approach.

Between December 1999 - July 2009, 26 patients (supplementary Table-S1) with suitable diagnostic bone marrow (BM) specimens for immunoglobulin heavy-chain gene rearrangement (IGH) sequencing were prospectively monitored for MRD. Patients were induced with 2-3 courses of vincristine-adriamycin-dexamethasone (VAD)-based regimens (20/26, 78%) (ClinicalTrial.gov, NCT-00702247), with 3 courses of bortezomib-thalidomide-dexametasone (VTD) (EudraCTNumber:2007-003707-12) (2/26, 8%) or with 4 courses of lenalidomide-dexamethasone (LD) (4/26, 14%) (ClinicalTrial.gov, NCT01264315) followed by the tandem transplant approach [4]. No pre-emptive donor lymphocyte infusions or maintenance/consolidation treatment were allowed except for the 4 patients enrolled in protocol NCT01264315 who started LD maintenance.

The centralized MRD laboratory staff were blinded to clinical findings. BM samples were collected at diagnosis, after the autograft, at 1, 3, 6 months after the allograft and then every 6 months or as clinically indicated. Patient-specific IGH rearrangements were amplified and direct sequenced using consensus sense primers derived from the framework region (FR) FR1 or FR2 and a consensus anti-sense primer derived from FR4, as previously published [5-8]. Qualitative nested-PCR for the IGH rearrangement was performed on genomic DNA as previously described and its sensitivity was $3.3x10^{-6}$ [5-8]. qPCR analysis was performed in all nested-PCR positive cases, when DNA leftovers were available, according to the Euro-MRD criteria [6]. Molecular remission (MR) was defined as 2 consecutive negative MRD results by nested-PCR or, if nested-PCR was positive, as 2 consecutive negative MRD results by qPCR with minimal sensitivity of $5x10^{-5}$ (supplementary data). Overall, a molecular marker was found in 19/26 patients (73%) and 151 nested-PCR determinations on BM samples were performed (median per patient: 8; range, 4-12).

At the time of the allograft, 8/26 (31%) and 4/26 (15%) were in clinical complete remission (CR) and partial remission (PR) respectively (supplementary data). Twelve additional patients (46%) achieved CR and 1 patient PR after the allograft (overall response 96%). At a median follow-up of 12.1 (6.52-15.2) years from diagnosis and 11.1 (5.5-14.2) years from the allograft, median overall survival (OS) was not reached and event-free survival (EFS) was 4 years. Cumulative

incidence of relapse was 3.8% at 1, 30.8% at 2 and 34.6% at 5 years. Interestingly, 5/26 (19%) patients relapsed with extra-medullary disease. Cumulative incidence of grade II-IV GVHD and chronic GVHD were 26.9% and 65.4%. MRD studies showed that, after the autograft, 3/19 patients (16%) were nested-PCR negative. After the allograft, the rate of PCR negativity remained low at month 1 and 3 (5/19, 26% and 3/19, 16%, respectively); then increased up to 44% (8/18) at month 6 and 47% (7/15) at one year post-transplant (Table 1). Among the 7 patients with persistent nested-PCR negativity, only one clinical relapse was observed (10 months after the last MRD determination), while 7/12 patients who did not reach MR relapsed (Figure 1). By qPCR analysis, overall tumor shrinkage throughout treatment resulted in 13.80 ln and always remarkable was tumor reduction after the autograft, the allograft and post-transplant *graft-vs-myeloma* ($p<0.001$) (Table 1). A median tumor burden reduction of 4.59 ln was seen after the autograft and a further decrease of 4.83 ln 1 month after the allograft. At 3 months, MRD levels were similar to those observed right after the allograft (+0.22 ln), whereas a further tumor reduction of 4.61 ln was observed at six months post-transplant. This finding was stable over time suggesting ongoing *graft-vs-myeloma*. At most time-points, patients in continuous CR showed a lower median tumor burden as compared with those who relapsed ($p<0.001$).

An important prognostic role of MR was observed. Overall MR occurred in 12/19 patients (63%). All patients in MR were also in CR. Median time to MR was 6 months (range: 1-18 months) and MR had a median duration of 27 months (range: 3-102 months) with 7 patients in continuous MR and clinical CR. Patients who achieved MR showed a significantly lower incidence of relapse (27.1% vs 71.4% at median follow-up, $p=0.016$) and better median EFS and OS (not reached vs 17.5 months, $p=0.010$; not reached vs 40 months, $p=0.027$, respectively) as compared with those who did not achieve MR (Figure 1, supplementary Figure S1).

Our study shows that our tandem approach induced high rates of prolonged MR by both qPCR and nested-PCR (63% and 47% respectively). After a remarkable median follow up of 12 years (range 6.5-15) from diagnosis, the achievement of MR by nested-PCR was significantly associated with better long-term OS and EFS, median durations of which had not yet been reached (Figure 1). Whether long-term persistence of MRD negativity coincides with disease eradication remains a matter of debate though MR of several years may cautiously suggest cure. With “new drugs” survival has dramatically improved especially in good prognosis patients [9]. However, the vast majority of our patients (78%) were treated on protocols which predated the “new drugs” era and never received them either as induction or maintenance. Lenalidomide did not affect the achievement of MR in the 2 only patients with a molecular marker who received post-transplant

maintenance on protocol NCT01264315. One did not achieve MR and progressed 3 months after the start of lenalidomide and the other reached MR before starting it and died of multi-organ failure 2 months later. These findings are consistent with persistent *graft-vs-myeloma,* potentially curative in a subset of patients. Moreover, there was no correlation between MRD status and chronic GVHD. Most patients had completely withdrawn the immuno-suppression that allowed high quality of life. At the time of this report, only 2 patients had remained on low dose steroids to treat limited chronic GVHD. Overall, NRM was 15% at 5 years. This underlines that, as for all other treatments, the lack of long-term disease control remains the principal cause of treatment failure after an allograft.

Importantly, our group also recently reported long-term outcomes of the GIMEMA-VEL-03-096 trial [10]. A cohort of 39 patients treated with bortezomib-thalidomide-dexamethasone (VTD) after an autograft were monitored for MRD with both nested-PCR and qPCR as in the present study. At a median follow-up of 8 years, OS was 72% for patients in MR response versus 48% for those with MRD persistence (p=0.041). Moreover, 26 (67%) patients who achieved MR showed good disease control with median time-to-next therapy (TNT) of 42 months whereas TNT in patients with MRD reappearance and MRD persistence were 9 and 10 months respectively (p=0.706). Importantly, both studies were carried out in the same facility by the same laboratory staff who were blinded to clinical data.

Thought results were controversial, most prospective comparative studies on autografting versus allografting were conducted before the era of new drugs [11-14]. The annual reports of the EBMT activities have however shown a steady increase of the use of allografting in plasma cell dyscrasias. The role of the combination of “new drugs” with *graft-vs-myeloma* has not yet been explored in well designed prospective studies [1]. Interestingly, one comparative study showed higher response rates to salvage therapies in the allograft patients and significantly longer OS from relapse after the allograft than after a second autograft [12]. These findings support a strong synergy between donor T cells and new drugs.

In one Phase II clinical trial the feasibility of bortezomib within a reduced-intensity conditioning and given as maintenance was evaluated [15]. Sixteen high-risk patients relapsed after an autograft were enrolled. Nine/16 (56%) and 5/16 (31%) achieved CR and partial remission respectively. Three-year cumulative incidence of NRM, relapse and OS were 25%, 54% and 41% respectively. For the first time, this trial showed safety and efficacy of an intensified conditioning with a “new drug” in poor prognosis patients. The concept of maintenance treatment after an allograft was also introduced. These findings led to the design of a prospective multi-center trial

through the European Myeloma Network. The trial aims at optimizing clinical outcomes by reducing the risk of relapse and the incidence of GVHD with the integration of bortezomib and lenalidomide in the treatment schema. Candidates are high-risk myeloma patients, younger than 70 years, with early relapse after first-line treatment with new drugs and autografting. Preliminary data will be available shortly (Perez-Simon, personal communication).

Graft-vs-myeloma after non-myeloablative allografting determined prolonged rates of MR similar to those described after myeloablative allografting and higher than those recently reported after a planned treatment combination of an autograft with VTD consolidation [10]. In the light of our and of others' results, it may become ethical to evaluate in newly designed clinical trials the combination of *graft-vs-myeloma* with novel agents in young high-risk and/or early relapsed patients where life expectancy is poor also in the era of new drugs.

Supplementary information is available at *Leukemia's* website

ACKNOWLEDGMENTS

This work was supported by Progetto di Rilevante Interesse Nazionale (PRIN 2009) from Ministero Italiano dell'Università e della Ricerca (MIUR), Roma, Italy (code: 7.07.02.60 AE01); Progetti di Ricerca Finalizzata 2008, (head unit: IRCCS Centro di Riferimento Oncologico della Basilicata (CROB), Rionero in Vulture (Potenza), Italy) (code: 7.07.08.60 P49), Progetto di Ricerca Sanitaria Finalizzata 2008, (head unit: Divisione di Ematologia, A. O. S. Maurizio, Bolzano/Bozen, Italy) (code: 7.07.08.60 P51), Progetto di Ricerca Sanitaria Finalizzata 2008 (code: RF-PIE-2008-1206999), Progetto di Ricerca Sanitaria Finalizzata 2009 (code: RF-2009-1491359), Progetto di Ricerca Sanitaria Finalizzata 2009, (head unit: Divisione di Ematologia, A. O. S. Maurizio, Bolzano/Bozen, Italy) (code: RF-2009-1469205), Progetto di Ricerca Sanitaria Finalizzata 2010, (head unit: Divisione di Ematologia, A. O. S. Maurizio, Bolzano/Bozen, Italy) (code: RF-2010-2307262), Fondi di Ricerca Locale, Università degli Studi di Torino, Torino, Italy and by Fondazione Neoplasie del sangue (FO.NE. SA),Torino, Italy.

The authors would like to thank Maria Josè Fornaro, Antonella Fiorillo and Franca Trotto Gatta for excellent secretarial support.

AUTHOR DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

A.P. has received consultancy and honoraria from Amgen, Bristol-Myers Squibb, Celgene, Janssen, Millenium, Onyx; M.B. has received research support, consultancy and scientific advisory board for

Celgene and Janssen-Cilag; M.L. has received research support from Janssen-Cilag, Amgen, Roche, and Italfarmaco, and honoraria from Celgene, Roche, Bayer, Amgen, and Mundipharma; S.F. has received speakers honoraria from Celgene and Mundipharma; B.B. has received honoraria from Gilead, Pfizer, Celgene, Hospira and research support form Celgene, Pierre Fabre, ADIENNE, Hospira Italia, MSD Italia. F.P. has received honararia from Celgene, Janssen, MSD Italia; Advisory Board for Mundipharma, Bristol MSD, Janssen The remaining authors declare that they have no conflicts of interest.

REFERENCES

1. Festuccia M, Martino M, Ferrando F, Messica G, Moscato T, Fedele R, et al. Allogeneic stem cell transplantation in multiple myeloma: immunotherapy and new drugs. *Expert Opin Biol Ther* 2015;**15**:857-72.
2. Corradini P, Cavo M, Lokhorst H, Martinelli G, Terragna C, Majolino I, et al. Molecular remission after myeloablative allogeneic stem cell transplantation predicts a better relapse-free survival in patients with multiple myeloma. Blood 2003;**102**:1927-1929.
3. Kröger N, Badbaran A, Zabelina T, Ayuk F, Wolschke C, Alchalby H, et al. Impact of high-risk cytogenetics and achievement of molecular remission on long-term freedom from disease after autologous-allogeneic tandem transplantation in patients with multiple myeloma. Biol Blood Marrow Transplant. 2013;**19**:398-404.
4. Maloney DG, Molina AJ, Sahebi F, Stockerl-Goldstein KE, Sandmaier BM, Bensinger W, et al. Allografting with nonmyeloablative conditioning following cytoreductive autografts for the treatment of patients with multiple myeloma. *Blood* 2003;**102**:3447-3454.
5. Voena C, Ladetto M, Astolfi M, Provan D, Gribben JG, Boccadoro M, et al. A novel nested-PCR strategy for the detection of rearranged immunoglobulin heavy-chain genes in B cell tumors. *Leukemia* 1997;**11**:1793-1798.
6. Van der Velden VH, Cazzaniga G, Schrauder A, Hancock J, Bader P, Panzer-Grumayer ER, et al. Analysis of minimal residual disease by Ig/TCR gene rearrangements: guidelines for interpretation of real-time quantitative PCR data. *Leukemia* 2007;**21**:604-611.
7. Ladetto M, Donovan JW, Harig S, Trojan A, Poor C, Schlossnan R, et al. Real-Time polymerase chain reaction of immunoglobulin rearrangements for quantitative evaluation of minimal residual disease in multiple myeloma. *Biol Blood Marrow Transplant* 2000;**6**:241-253.
8. Ladetto M, Pagliano G, Ferrero S, Cavallo F, Drandi D, Santo L, et al. Major tumor shrinking and persistent molecular remissions after consolidation with bortezomib, thalidomide, and dexamethasone in patients with autografted myeloma. *J Clin Oncol* 2010;**28**:2077-2084.
9. Bruno B, Giaccone L, Rotta M, Anderson K, Boccadoro M. Novel targeted drugs for the treatment of multiple myeloma: from bench to bedside. *Leukemia* 2005;**19**:1729-1738.
10. Ferrero S, Ladetto M, Drandi D, Cavallo F, Genuardi E, Urbano M, et al. Long-term results of the GIMEMA VEL-03-096 trial in MM patients receiving VTD consolidation after ASCT: MRD kinetics' impact on survival. *Leukemia* 2015;**29**:689-95.
11. Bruno B, Rotta M, Patriarca F, Rotta M, Sorasio R, Allione B, et al. A comparison of allografting with autografting for newly-diagnosed myeloma. *N Engl J Med* 2007;**356**:1110-1120.
12. Giaccone L, Storer B, Patriarca F, Rotta M, Sorasio R, Allione B, et al. Long-term follow-up of a comparison of nonmyeloablative allografting with autografting for newly diagnosed myeloma. Blood. 2011;**117**:6721-6727.
13. Krishnan A, Pasquini MC, Logan B, Stadtmauer EA, Vesole DH, Alyea E 3rd, et al. Tandem autologous versus single autologous transplantation followed by allogeneic hematopoietic cell transplantation for patients with multiple myeloma: results from the blood and marrow transplant clinical trials network (BMT CTN) 0102 trial. *Lancet Oncol* 2011;12:1195-03.
14. Gahrton G, Iacobelli S, Bjorkstrand B, Hegenbart U, Gruber A, Greinix H, et al. Autologous/reduced-intensity conditioning allogeneic stem cell transplantation versus autologous transplantation in multiple myeloma: long term results of the EBMT-NMAM2000 study. *Blood* 2013;**121**:5055-63.
15. Caballero-Velázquez T, López-Corral L, Encinas C, Gaspar HB, Cesaro S, Dreger P, et al.

Phase II clinical trial for the evaluation of bortezomib within the reduced intensity conditioning regimen (RIC) and post-allogeneic transplantation for high-risk myeloma patients. *Br J Haematol* 2013;**162**:474-82.

Table 1. Molecular evaluation of MRD. Rates of nested-PCR negativity at each time-point (Table2-A) and tumour burden shrinkage, reported as observed marginal medians of ln qPCR results (Table2-B).

Figure 1. Long-term clinical outcomes after tandem auto-allo transplantation and according

to molecular remission status

Probability of overall survival **(A)**, event free survival **(B)** and relapse **(C)** of the study cohort (26 patients) and of patients who reached molecular remission (MR) either by nested qualitative PCR or real-time quantitative PCR (qPCR) (no. 12) *versus* those who had persistent disease (no. 7). Median follow up was 12 years from diagnosis and 11 years after the allograft.

265

266

267

268

Table 1. Molecular evaluation of MRD. Rates of nested-PCR negativity at each time-point (Table2-A) and tumour burden shrinkage, reported as observed marginal medians of ln qPCR results (Table2-B).

Table 1-A

Number of PCR negative patients	**Post autograft**	**Post allograft**	**3 months FU**	**6 months FU**	**12 months FU**
Nested-PCR	16% (3/19)	26% (5/19)	16% (3/19)	44% (8/18)	47% (7/15)
qPCR	16% (3/19)	37% (7/19)	37% (7/19)	44% (8/18)	53% (8/15)

Table 1-B

MRD burden (median value of ln qPCRresults)	**Post autograft**	**Post allograft**	**3 months FU**	**6 months FU**	**12 months FU**
Overall	6.91	2.08	2.30	-2.30	-2.30
ContinuosClinical CR	5.64	1.10	1.10	-2.30	-2.30
Relapsed	9.01	7.30	6.43	3.26	1.10

Abbreviations: q-PCR, real time quantitative polymerase chain reaction; ln, natural logarithm; FU, follow-up; MRD, minimal residual disease; CR, complete remission.

FIGURE 1

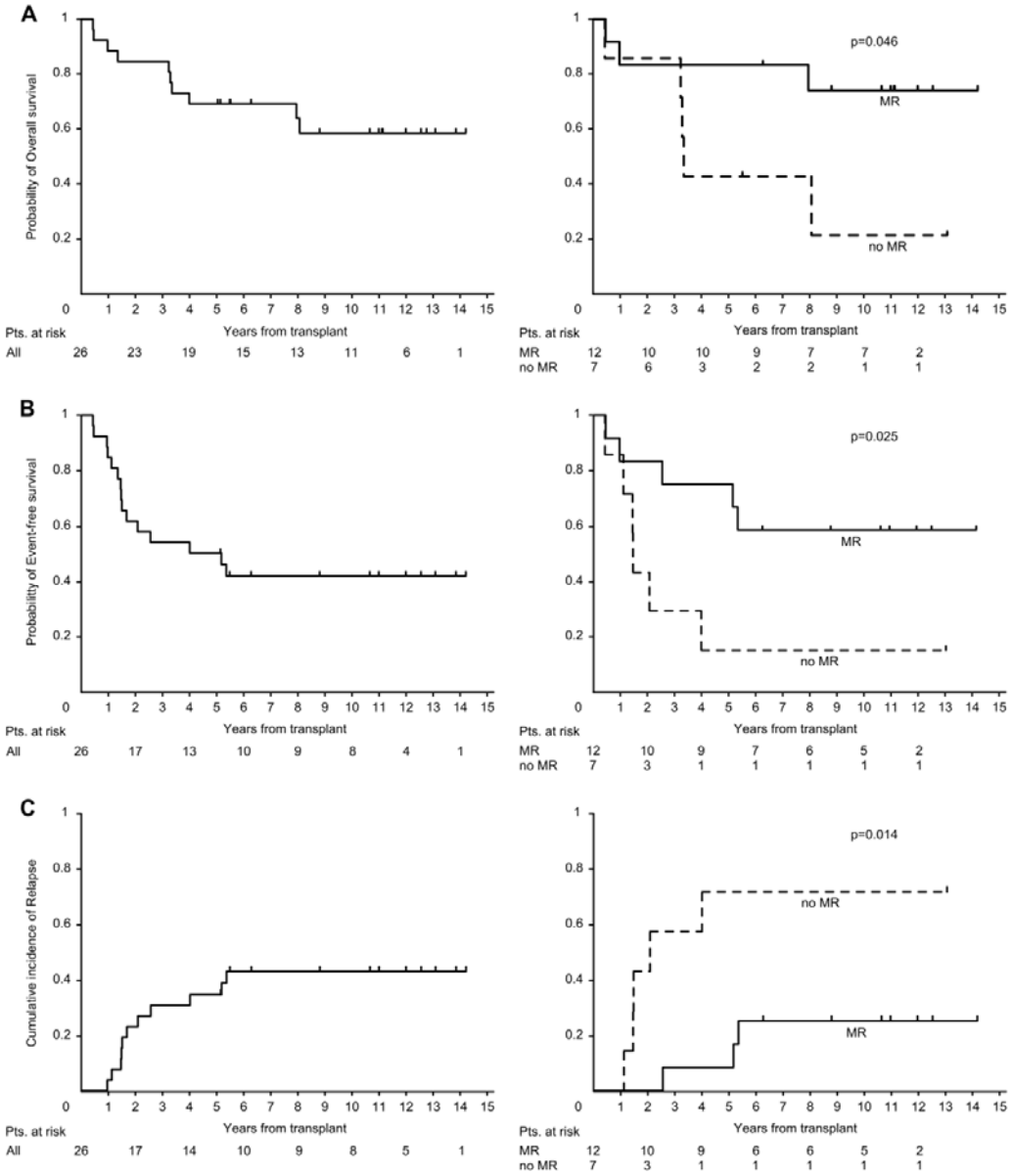

Figure 1. Long-term clinical outcomes after tandem auto-allo transplantation and according to molecular remission status

Probability of overall survival **(A)**, event free survival **(B)** and relapse **(C)** of the study cohort (26 patients) and of patients who reached molecular remission (MR) either by nested qualitative PCR or real-time quantitative PCR (qPCR) (no. 12) *versus* those who had persistent disease (no. 7). Median follow up was 12 years from diagnosis and 11 years after the allograft.