open access journal

hematology reports

ISSN 2038-8322 - eISSN 2038-8330 | www.hematologyreports.org

1st SOHO Italy **Clinical and Biological School of Multiple Myeloma**

Virtual Event, 15 – 17 December 2020

ABSTRACT BOOK

Hematology Reports

ISSN 2038-8322 | eISSN 2038-8330

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15 16 17 Dec. 2020

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Big Faculty:

**Cerchione C, Martinelli G, Lee HC, Mateos MV, Anderson KC,
Bolli N, Bosi A, Cavo M, Derudas D, Di Raimondo F,
Gay F, Lanza F, Larocca A, Martino M, Montefusco V, Olivieri A,
Romano A, Terragna C, Tosi P, Vacca A, Zamagni E**

Day 0

15 Dec 2020

- 18.30 - 18.45 Introduction and presentation of SOHO School project - **SOHO Italy Presidents:** C. Cerchione - G. Martinelli - H. Kantarjian
- 18.45 - 19.00 Presentation of 1st SOHO Clinical and Biological School of Multiple Myeloma - **Scientific Board:** C. Cerchione - G. Martinelli - H.C. Lee - M.V. Mateos - K.C. Anderson
- 19.00 - 19.30 Lecture: How I manage Multiple Myeloma in Europe in 2020 - C. Cerchione
- 19.30 - 20.00 Lecture: How I manage Smoldering Multiple Myeloma in 2020 - M.V. Mateos
- 20.00 - 20.30 Lecture: How I manage relapsed/refractory Multiple Myeloma at MD Anderson Cancer Center - H. Lee
- 20.30 - 21.00 Lecture: Future of Multiple Myeloma - K.C. Anderson

Day 1

16 Dec 2020

Frontal Session

- 08.30-08.50 Opening and introduction to the project:
C. Cerchione - G. Martinelli

Biology and MRD in Multiple Myeloma: N. Bolli - C. Terragna - G. Martinelli

- 08.50 - 09.10 Molecular pathogenesis of multiple myeloma - N. Bolli
- 09.10 - 09.30 Minimal Residual Disease Assessment Within the Bone Marrow of Multiple Myeloma - C. Terragna

- 09.30 – 09.50 Exploring microenvironment – from biology to therapy – **A. Romano**
- 09.50 – 10.10 Importance of imaging for MRD evaluations – **E. Zamagni**
- 10.10 – 10.30 Future perspectives in MRD in Multiple Myeloma: where are we going? – **G. Martinelli**
- 10.30 – 10.50 break

Clinical Session 1 – C. Cerchione – K.C. Anderson – M.V. Mateos – H.C. Lee

- 10.50 – 11.10 Present and future of management of frontline Multiple Myeloma – **M.V. Mateos**
- 11.10 – 11.30 Present and future of management of relapsed/refractory Multiple Myeloma – **C. Cerchione**
- 11.30 – 11.50 Major differences between Europe and USA in the management of Multiple Myeloma – **K.C. Anderson**
- 11.50 – 12.10 Antibody-Drug Conjugates (ADCs) and Bispecific T-cell Engagers (TCEs) in Multiple Myeloma: How are they changing the current panorama – **H. Lee**
- 12.10 – 12.30 Discussion

Clinical Session 2 – A. Bosi – A. Olivieri – V. Montefusco **Frontline transplant-eligible setting**

- 12.30 – 13.00 Transplant-eligible patients: old versus new – **V. Montefusco**
- 13.00 – 13.20 Role of allogeneic stem cell transplantation in MM in novel agents era – **A. Olivieri**
- 13.20 – 13.30 Discussion
- 13.30 – 14.00 Mini lunch break

14.00 - 15.00 **Symposium**

Clinical Session 3 – F. Lanza, P. Tosi, D. Derudas
Transplant-inelegible setting and selected conditions

- 15.00 - 15.20 Transplant-inelegible patients: old and new options
for elderly fit patients – **P. Tosi**
- 15.20 - 15.40 Transplant-inelegible patients: old and new options
for elderly – unfit/frail patients – **A. Larocca**
- 15.40 - 16.00 Extramedullary disease and plasma cell leukemia
D. Derudas

Clinical Session 4 – M. Martino, F. Di Raimondo, A. Vacca
Near future and new perspectives in Multiple Myeloma

- 16.00 - 16.20 CAR-T: lights and shadows in Italy – **M. Martino**
- 16.20 - 16.40 New Drugs on the Horizon – **F. Di Raimondo**
- 16.40 - 17.00 Endothelial cells and dendritic cells as
immunosuppressive agents in patients
with Multiple Myeloma – **A. Vacca**
- 17.00 - 17.30 Discussion – Questions and Answers

Young session

Selected oral presentations with project presentations in different categories

Chairs Big Faculty:

Cerchione C, Martinelli G, Lee HC, Mateos MV, Anderson KC, Bolli N, Bosi A, Cavo M, Derudas D, Di Raimondo F, Gay F, Lanza F, Larocca A, Martino M, Montefusco V, Olivieri A, Romano A, Terragna C, Tosi P, Vacca A, Zamagni E

15 min presentation + 15 min discussion)

Pre-clinical session

- 08.30 - 09.00 New targets and new approaches in Myeloma: extracellular vesicles as liquid biomarkers –
Antonia Reale – Melbourne – Australia
- 09.00 - 09.30 The Myeloma-specific tumor suppressor FAM46C orchestrates plasma cell secretory capacity through its interaction with FNDC3 proteins – Enrico Milan – Milano
- 09.30 - 10.00 Targeting of the cell-adhesion system within the tumor microenvironment blocks angiogenesis, impairs diseases progression and dissemination in multiple myeloma –
Antonio Giovanni Solimando – Bari
- 10.00 - 10.30 Functional role of bone marrow CD8+ tissue-resident memory T-cells in Multiple Myeloma –
Melania Carlisi – Palermo
- 10.30 - 10.45 Questions and answers and discussion for future project/trials
- 10.45-11.00 break

- 11.00 - 11.30 Multiple Myeloma cell-derived MICA+ extracellular vesicles impair NKG2D-mediated NK cell immune surveillance – [Elisabetta Vulpis - Roma](#)
- 11.30 - 12.00 Endothelial cells and dendritic cells as immunosuppressive agents in patients with Multiple Myeloma – [Ilaria Saltarella - Bari](#)
- 12.00 - 12.30 Dissecting Daratumumab mechanisms of resistance at a single-cell level – [Matteo Da Vià - Milano](#)
- 12.30 - 13.00 Predictive parameters at first and second relapse, is there a role for sFLC? A real-life unicentric retrospective study in the era of novel agents – [Uros Markovic - Catania](#)
- 13.00 - 14.00 **Symposium**
Introducing anti-CD38 in daily practice of Multiple Myeloma – [C. Cerchione](#)
- 14.00 - 14.30 Len/dex combination as first-line therapy of frail Multiple Myeloma patients: a monocentric real life study – [Claudia Bellofiore - Catania](#)
- 14.30 - 15.00 Evaluation of treatment approaches and outcome in newly diagnosed Multiple Myeloma patients, autologous stem cell transplant (ASCT) eligible or not, who presented early relapse or were refractory to first-line induction or first-line treatment – [Francesca Fazio - Roma](#)
- 15.00 - 15.30 Combined treatment with doxorubicin and ENPP1 inhibitor for improving anti-Myeloma innate immune response – [Lorenzo Cuollo - Roma](#)
- 15.30 - 16.00 Comparison of different imaging techniques in Multiple Myeloma patients – [Sara Grammatico - Firenze](#)

- 16.00 - 16.30 Addition of BCNU to Melphalan deepens responses without increasing toxicity thereby improving survival before second autologous stem cell transplant for relapsed Multiple Myeloma –
Joshua Thomas - Chicago - USA
- 16.30 - 17.00 Allograft in Multiple Myeloma: Experience of Multiple Myeloma GIMEMA Lazio group –
Federico Vozella – Firenze
- 17.00 - 17.30 Primary Plasma Cell Leukemia experience of 7 years in a Peruvian referral cancer center –
Jule Vasquez - Lima - Perù
- 17.30 - 18.00 Questions and answers and discussion for future projects/trials

After presentations, all projects will be started to be defined, with their teams, objectives and timepoints

- 18.00 - 18.30 Special Lecture
Present and future of European Myeloma Network (EMN) Clinical Trials – F. Gay
- 18.30 - 19.00 Conclusions and next steps
C. Cerchione, G. Martinelli

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PRE-CLINICAL SESSION

SINGLE-CELL CHARACTERIZATION OF THE BONE MARROW IMMUNE MICROENVIRONMENT IDENTIFIES CD27-NEGATIVE T CELLS AS POTENTIAL SOURCE OF TUMOR-REACTIVE LYMPHOCYTES

C. Botta¹, C. Pérez², I. Goicoechea², N. Puig³, M.T. Cedena⁴, L. Cordon⁵, A. Zabaleta², L. Burgos², C. Maia², S. Rodríguez², D. Alignani², I. Rodríguez², S. Sarvide², A. Vilas-Zornoza², E. Lorenzo-Vivas², L. Rosiñol⁶, A. Oriol⁷, M-J. Blanchard⁸, R. Ríos⁹, A. Sureda¹⁰, R. Martínez¹¹, J. Martín¹², J. Bargay¹³, J. de la Rubia⁵, D.M. Papetti¹⁴, M. Nobile¹⁵, D. Besozzi¹⁴, M. Rossi¹⁶, P. Tassone¹⁶, M. Gentile¹, J. Merino², F. Prósper², A. Orfao¹⁷, M-V. Mateos¹⁸, J-J. Lahuerta⁴, J. Bladé⁶, J. San Miguel², B. Paiva²

¹Department of Oncohematology, "Annunziata" Hospital, Cosenza, Italy; ²Clínica Universidad de Navarra, Pamplona; ³Instituto de Investigación Biomédica de Salamanca, Salamanca; ⁴Hospital Universitario 12 de Octubre, Madrid; ⁵Hematology Research Group, Instituto de Investigación Sanitaria La Fe, Valencia, Spain; ⁶Hospital Clinic i Provincial; ⁷Institut Català d'Oncologia, Barcelona; ⁸Hospital Ramón y Cajal, Madrid; ⁹Hospital Universitario Virgen de las Nieves de Granada, Granada; ¹⁰Hospital de la Santa Creu i Sant Pau, Barcelona; ¹¹Hospital Clínico San Carlos, Madrid; ¹²Hospital Universitario Virgen del Rocío, Sevilla; ¹³Hospital Son Llatzer, Palma de Mallorca; ¹⁴Department of Informatics, Systems and Communication, University of Milano-Bicocca, Milano, Italy; ¹⁵Department of Industrial Engineering & Innovation Sciences, Eindhoven University of Technology, 5612 AZ Eindhoven, The Netherlands; ¹⁶Magna Graecia University of Catanzaro, Catanzaro, Italy; ¹⁷Universidad de Salamanca, ¹⁸Hospital Universitario de Salamanca, Salamanca, Spain

Introduction: The broad use of immunomodulatory drugs (IMiDs) and the breakthrough of novel immunotherapies in MM, urge the optimization of immune monitoring to help tailoring treatment based on better prediction of patients' response according to their immune status. Here we characterized the MM immune microenvironment at the single-cell (sc) level to identify clinically relevant subsets for effective immune monitoring.

Methods: We performed deep characterization of T cells by using combined sc-RNA/TCR sequencing (10xGenomics). We used a novel semi-automated pipeline to unveil full cellular diversity based on unbiased clustering, in a large flow cytometry dataset of 76 MM patients enrolled in the PETHEMA/GEM2012 MENOS65 clinical trial, including immune monitoring at different time points. Immunophenotyping was performed using the first 8-color combination (CD19, CD27, CD38, CD45, CD56, CD81, CD117, CD138) of the next-generation flow (NGF) panel for MRD assessment. Results were then validated in additional 155 patients enrolled in the same trial. Finally, we built a machine learning model based on recurrent neural network to predict the interaction between neoantigens and TCR.

Results: To characterize the T cell response to MM, we performed paired scRNA and TCRseq in 44,969 lymphocytes from 9 MM patients. Simultaneous scTCRseq revealed a total of 90 different clonotypes (median of 12 per patient) which were almost exclusively distributed within the CD8+ population. By compar-

ing clonotypic vs non-clonotypic lymphocytes transcriptomes, we observed that the first presented very low levels of CD27 and CCR7 (confirmed by flow-cytometry). Additionally, we observed a linear relationship between CD27- T cell abundance and the amount of clonotypic T cells. To investigate the clinical relevance of this latter point we performed through our bioinformatic pipeline, a simultaneous analysis of the fcs files from 76 patients at 4 timepoints and identified 25 cell clusters (including 9 myeloid and 13 lymphocytes subsets, either CD27- or CD27+) in the MM immune microenvironment. Afterwards, we correlated those variables with a total of 20 clinical parameters including the International Staging System (ISS) and FISH cytogenetics. Twelve variables had significant impact in progression-free survival (PFS) and the ratio between CD27- vs CD27+ T (CD27R) cells emerged as an independent prognostic factor (HR:0.09, p=0.04) together with the ISS in a Cox regression model. The 3-year PFS rates of patients with high vs low CD27R were 94% vs 71% (p=0.02), respectively; these findings being confirmed in the validation dataset. Thus, we observed in the entire cohort (n=231) that a prognostic score including the CD27R (HR:0.21, p=0.013) and ISS (HR:1.41, p=0.015) outperformed each parameter alone (HR:0.06, p=0.007). In 2 patients from this cohort, we performed exome- and RNA-sequencing of tumor cells and analyzed their HLA profile. By using the neT-MHC Prediction tool, we selected all the "neo-epitopes" predicted to bind at least one of the class I MHC molecules on tumor cells and, thus, potentially recognized by autologous clonotypic CD27- T cells. Our deep learning model, trained on the VDJ-DB database gave us an estimation of the strength of the association between each peptide and TCRs. Interestingly, all the identified "myeloma reactive" clonotypes presented sign of exhaustion/senescence.

Conclusion: We show for the first time that potential MM-reactive T cells are CD27-negative and that their abundances prognostic, possibly due to their reactivation after treatment with IMiDs and autologous transplant. Because NGF is broadly used, these results are readily applicable for effective T cell immune monitoring.

NEW TARGETS AND NEW APPROACHES IN MYELOMA: EXTRACELLULAR VESICLES AS LIQUID BIOMARKERS

A. Reale¹, I. Carmichael², R. Xu^{3,4}, S. Mithraprabhu¹, T. Khong¹, M. Chen¹, H. Fang⁵, I. Savvidou¹, M. Ramachandran¹, N. Bingham¹, R.J. Simpson⁴, D.W. Greening^{4,5}, A. Spencer^{1,6}

¹Myeloma Research Group, Australian Centre for Blood Diseases, Central Clinical School, Monash University/Alfred Health, Melbourne, VIC, Australia; ²Monash Micro Imaging-AMREP, Monash University, Melbourne, VIC, Australia; ³Nanobiotechnology Laboratory, Australian Centre for Blood Diseases, Central Clinical School, Monash University, Melbourne, Victoria, Australia; ⁴Department of Biochemistry and Genetics, La Trobe Institute for Molecular Science, La Trobe University, Melbourne, VIC, Australia; ⁵Molecular Proteomics, Baker Heart and Diabetes Institute, Melbourne, VIC, Australia; ⁶Malignant Haematology and Stem Cell Transplantation, The Alfred Hospital, and Department of Clinical Haematology, Monash University, Melbourne, VIC, Australia

Introduction: Circulating small extracellular vesicles (sEV) represent promising non-invasive biomarkers that may aid in the diagnosis and risk-stratification, monitoring and treatment of multiple myeloma (MM), an incurable blood cancer, given the opti-

mization and validity of their isolation/purification method.

Methods: sEV isolated from human MM cell lines (HMCL) were purified using a density gradient ultracentrifugation approach (DG-UC). sEV were isolated from peripheral blood plasma (PBPL) isolated from healthy individuals (HD), the premalignant condition MGUS (monoclonal gammopathy of undetermined significance), smouldering MM (SMM) and newly diagnosed MM (NDMM) patients, using commercially-available kits. Pre-analytical conditions (collection tube types, storage conditions) were assessed for sEV yield and EV-marker enrichment. Proteomic profiling (nLC and high-resolution mass spectrometry) and functional studies were also performed.

Results: Highly purified HMCL-sEV were comprehensively characterized by specific EV-marker enrichment and morphology. Importantly, we demonstrated that HMCL-sEV are readily taken up by stromal cells to functionally modulate proliferation. Higher purity/recovery, evaluated by immunoblotting, nanoparticle tracking analysis and electron microscopy, were observed for PBPL-sEV isolated using a resin-based kit. Fresh PBPL samples and Streck-RNA collection tubes provided sEV with higher purity vs frozen PBPL and EDTA tubes. Functionally, PBPL-sEV were shown to regulate stromal cell proliferation and migration. In turn, pre-educated stromal cells favour HMCL adhesion. Interestingly, PBPL-sEV isolated from patients with both pre-malignant plasma cell disorders (MGUS; SMM) and NDMM have a similar ability to promote cell migration and adhesion, suggesting a role for both malignant and pre-malignant PBPL-sEV in disease progression. Proteomic profiling of MM-sEV (339 proteins) revealed enrichment of factors implicated in cell migration and adhesion, in comparison to HD-sEV.

Conclusions: The characterization and proteomic profiling of disease-specific circulating EVs as a biomarker discovery strategy may provide translational applications in MM. Pre-analytical and isolation/purification steps are of critical importance for the characterization of PBPL-derived EVs. Importantly, MM-sEV may play an important role in disease progression by re-programming the tumour microenvironment. Altogether our findings may serve as a guide to choose EV purification and characterization methods for MM studies.

THE MYELOMA-SPECIFIC TUMOR SUPPRESSOR FAM46C ORCHESTRATES PLASMA CELL SECRETORY CAPACITY THROUGH ITS INTERACTION WITH FNDC3 PROTEINS

E. Milan¹, C. Fucci², M. Resnati², E. Riva², T. Perini¹, E. Ruggieri, U. Orfanelli², F. Paradiso², F. Cremasco², A. Raimondi³, E. Pasqualetto⁴, M. Nuvoletone⁵, L. Rampoldi⁴, S. Cenci¹

¹Age Related Diseases Unit, Division of Genetics and Cell Biology, San Raffaele Scientific Institute and University Vita-Salute San Raffaele, Milano, Italy; ²Age Related Diseases Unit, Division of Genetics and Cell Biology, San Raffaele Scientific Institute, Milano, Italy; ³Experimental Imaging Center, Advanced Light and Electron Microscopy BioImaging Center, San Raffaele Scientific Institute, Milano, Italy; ⁴Molecular Genetics of Renal Disorders Unit, Division of Genetics and Cell Biology, San Raffaele Scientific Institute, Milano, Italy; ⁵Amyloidosis Research and Treatment Center, Foundation IRCCS Policlinico San Matteo, Pavia, Italy

Introduction: Multiple myeloma (MM) is a still incurable hematological cancer accounting for ~2% of all cancer deaths. Recently, the FAM46C locus at chromosome 1p12 has been

described to be frequently and uniquely mutated or deleted in up to 20% MM patients, implying an as yet poorly understood plasma cell (PC)-specific tumor suppressor function. A non-canonical poly(A) polymerase, FAM46C has very recently been reported to stabilize mRNAs encoding immunoglobulins and certain endoplasmic reticulum (ER) proteins, but the underlying mechanisms and impact on PC biology are unknown.

Methods: To clarify the role of FAM46C in normal and malignant PCs, we silenced or over-expressed FAM46C in MM cells and dissected its activity and molecular regulation by combining targeted and unbiased proteomic studies of FAM46C interactome and effects with electron microscopy, immunofluorescence, mRNA stability, targeted mutagenesis, and functional assays.

Results: We disclosed that FAM46C is induced by PRDM1 during PC differentiation to selectively and concertedly stabilize mRNAs encoding for proteins targeted to the ER and ER-Golgi intermediate compartment (ERGIC). As a consequence, FAM46C potently and selectively boosts the expression of proteins involved in immunoglobulin folding and trafficking along the secretory pathway, thereby maximizing antibody production. The specific effect exerted by FAM46C on the secretory apparatus derives from its localization on the ER through its interaction with the ER transmembrane proteins FNDC3A and FNDC3B. Indeed, we demonstrated that the presence of at least one member of the FNDC3 family is required for FAM46C activity. Notably, FAM46C-mediated effects on antibody production are beyond sustainability for MM cells, since its overexpression induces ROS accumulation and ATP reduction, followed by apoptotic death and a rapid counterselection for FAM46C low expressing cells. In line with this crucial biological role, we found that FAM46C localization and abundance are tightly regulated by the combined action of the proteasome and autophagy through a previously unrecognized association with the ZZ-domain of the autophagic receptor p62. Indeed, FAM46C is constantly and rapidly degraded by the ubiquitin-proteasome system or sequestered away from the ER in p62-positive aggregates impeding its interaction with FNDC3 proteins.

Conclusions: Our study: i) defines a novel mechanism regulating the biogenesis and function of the secretory apparatus in PCs; and ii) clarifies the onco-suppressive role of FAM46C in MM. Overall, our findings indicate that malignant PCs may benefit from FAM46C inactivation to reduce antibody production to sustainable levels, moderating stress and allocating energy to tumor growth. Altogether, our data provide a novel FAM46C/FNDC3/p62 circuit as a specific tuner of the secretory capacity that may unveil new therapeutic opportunities against PC dyscrasias.

TARGETING OF THE CELL-ADHESION SYSTEM WITHIN THE TUMOR MICROENVIRONMENT BLOCKS ANGIOGENESIS, IMPAIRS DISEASES PROGRESSION AND DISSEMINATION IN MULTIPLE MYELOMA

A.G. Solimando^{1,2}, M.C. Da Viá³, A. Brandl^{4,5}, P. Leone¹, R. Ria¹, N. Bolli^{3,6}, H. Einsele^{4,5}, V. Racanelli¹, A. Vacca¹, A. Beilhack^{4,5}

¹Department of Biomedical Sciences and Human Oncology, Section of Internal Medicine "G. Baccelli", University of Bari Aldo Moro Medical School, Bari, Italy; ²National Cancer Research Centre, IRCCS Istituto Tumori "Giovanni Paolo II", Bari, Italy; ³Department of Oncology and Hemato-Oncology, University of Milan, Milan, Italy; ⁴Department of Medicine II, University Hospital of Würzburg, Würzburg, Germany; ⁵Interdisciplinary

Center for Clinical Research Laboratory, University Hospital of Würzburg, Würzburg, Germany; ⁶Hematology Unit, Fondazione IRCCS Ca Granda Ospedale Maggiore Policlinico, Milan, Italy

Interactions of multiple myeloma (MM) plasma cells (MMPCs) with the microenvironment control MM-cell growth, survival, drug-resistance and dissemination. As MM parallels an increased microvascular density in the bone marrow (BM), we will investigate whether BM MM endothelial cells (MMECs) control disease progression via the adhesion molecules. MM is characterized by dissemination of MM tumor cells throughout the BM. MM progression depends on interactions with the local microenvironment. This project aims to dissect the interaction of MMPCs with other neighbouring cells within their niche. MMPCs in contact with bystander stromal cells and extracellular matrix (ECM) components escape the effects of therapy through cell-adhesion mediated drug resistance (CAM-DR) which is one of the major challenges in treating MM. It still remains elusive what mechanisms or interactions within the MM-BM-compartment encourage one or several malignant clones to leave their initial niche and start disseminating, often in a context-dependent fashion.

The cell-cell and cell-stroma interactions in the MM milieu have been recognized as a major mechanism of MM cell survival and the development of drug resistance and therefore became a promising target in MM treatment. Recent publications from our group have demonstrated that tight junction molecules, including junctional adhesion molecule (JAM)-family members, are crucial components of signaling pathways that regulate cell polarity and vascular permeability, and have a huge potential to both halt and boost cancer progression. We recognized members of the JAM-family as promising candidates to target MM-milieu/endothelium-interactions. JAMs molecules feed into a vicious cycle between MM and the endothelium, that behaves as a gatekeeper for both the MM and the stroma counterpart. Thus, the dynamics of these molecules leads to divergent signal transduction within heterogeneous subclones. To test this hypothesis, we will visualize MM cell interactions with the BM vascular/environment regarding the involvement of cell-adhesion molecules using state-of-the-art readout systems. Collectively, our preliminary findings support the cell-adhesion system as an important mediator of MM progression through facilitating MM-associated vasculogenesis. Therefore, we propose JAM-molecules as promising tumor biomarkers and an attractive pharmacological target in MM patients.

FUNCTIONAL ROLE OF BONE MARROW CD8+ TISSUE-RESIDENT MEMORY T-CELLS IN MULTIPLE MYELOMA

M. Carlisi¹, S. Mancuso², M.P. La Manna³, V. Orlando⁴, N. Caccamo⁵, F. Dieli⁶, S. Siragusa⁷

¹Health Promotion Sciences, Maternal and Infant Care, Internal Medicine and Medical Specialties (PROMISE) Department, University of Palermo, Italy; ²Health Promotion Sciences, Maternal and Infant Care, Internal Medicine and Medical Specialties (PROMISE) Department, University of Palermo; ³Central Laboratory for Advanced Diagnosis and Biomedical Research (CLADIBIOR)- and Department Bi.N.D., University of Palermo; ⁴Central Laboratory for Advanced Diagnosis and Biomedical Research (CLADIBIOR)- and Department Bi.N.D., University of Palermo; ⁵Central Laboratory for Advanced Diagnosis and Biomedical Research (CLADIBIOR)- and

Department Bi.N.D., University of Palermo; ⁶Central Laboratory for Advanced Diagnosis and Biomedical Research (CLADIBIOR)- and Department Bi.N.D., University of Palermo; ⁷Health Promotion Sciences, Maternal and Infant Care, Internal Medicine and Medical Specialties (PROMISE) Department, University of Palermo, Italy

Background: Naïve T-cells, once activated by an antigen, undergo an enormous clonal expansion and at the same time differentiate into a short-lived effector and in long-lived memory cells. Within T-cell memory pool, we can distinguish two cellular subgroups: central memory T-cells (TCM) and effector-memory T-cells (TEM). Was also described a group of non-circulating resident T cells (TRM) which live permanently in some peripheral tissues where they survive in a state of quiescence. Recent studies have shown that also in the BM is possible to observe the presence of TRM cells that may contribute to the defence of the host in the neoplastic diseases. In relation to this last topic, we evaluated the frequency, the phenotype and the homeostatic maintenance of T CD8+ memory cells residing in BM of subjects with a diagnosis of MM (Multiple Myeloma) but our next aim is to evaluate their possible functional activity against pathological plasma cells, evaluating the CD107a/b and NKG2D expression on BM CD8+ TRM. Methods: we analyzed 21 patients with a new diagnosis of MM. At the time of the bone marrow assessment, we collected a sample for the flow cytometry analysis and in vitro cell culture. The ex vivo evaluation of CD8+ TRM frequency and phenotype in BM samples was performed using anti-human mAbs to CD3, CD103, CD69, CD45, CD8, CD45RA and CCR7 (CD197). Moreover, we performed in vitro experiments of BM-derived mononucleate cells of MM patients cultured in the presence of homeostatic cytokines in maintaining these cells for a long time (IL15, IL7 and TGF- β). In order to evaluate the possible cytotoxic immune function of BM CD8+ TRM against cancer cells, our next step will be to evaluate the CD107a/b and NKG2D expression on their cell surface after stimulation with activation molecules.

Results: Cytofluorimetric analysis performed on bone marrow blood samples showed that the ex vivo average frequency of CD8+ TRM was of 0.48% and the phenotype was represented mainly by TEM followed by TEMRA, naïve cells and TCM. We maintained BM-derived mononucleate cells from MM patients for 4 days in presence of homeostatic cytokines, IL-15, IL-7 plus IL-15 and IL-7 together with IL-15 and TGF- β . The result showed an increase of the percentage of CD8+ TRM in all conditions tested, especially in presence of all cytokines, with a strongest proliferative input from IL-15.

Conclusions: We evaluated the frequency and the phenotype of CD8 TRM in BM of MM patients and we want to continue our study performing a functional analysis of these cells. In MM patients, the increase of CD8+ TRM cells could have an anti-tumor role in the control of MM. The next step is to investigate the cytotoxic activity of these cells, using the expression of CD107 and NkG2D. BM CD8+ TRM cells could in fact represent an important therapeutic target, with the possibility of further improving the results of immunotherapy with the hope of increasing the response rate to the treatment of patients.

MULTIPLE MYELOMA CELL-DERIVED MICA+ EXTRACELLULAR VESICLES IMPAIR NKG2D-MEDIATED NK CELL IMMUNESURVEILLANCE

E. Vulpis¹, L. Loconte¹, A. Peri¹, R. Molfetta¹, L. Masuelli², G. Caracciolo¹, M.T. Petrucci³, R. Paolini¹, A. Soriani¹, M. Cippitelli¹, A. Santoni¹, A. Zingoni¹

¹Department of Molecular Medicine, Istituto Pasteur-Fondazione Cenci Bolognietti, Sapienza University of Rome, Italy; ²Department of Experimental Medicine, Sapienza University of Rome, Italy; ³Department of Cellular Biotechnologies and Hematology, Sapienza University of Rome, Italy

Introduction: Natural Killer (NK) cells represent a subset of innate lymphoid cells that play a key role in the immunosurveillance of Multiple Myeloma (MM) thanks to their ability to directly recognize and kill MM cells. MM is intrinsically linked with the immune system and its development can induce relevant NK cell alterations resulting in poor disease control. NKG2D is an NK cell activating receptor that binds to ligands (i.e. MICA/B and ULBPs) typically expressed on cancer cells including MM cells. The release of NKG2D ligands by cancer cells in the extracellular milieu through protease-mediated cleavage or by extracellular vesicle (EV) secretion is a mode to control their cell surface expression and escape from NK cell-mediated immunosurveillance.

Methods: EV isolation from plasma of bone marrow (BM) and peripheral blood (PB) of MM patients or from conditioned supernatant of cell culture was performed by ultracentrifugation. Their size was measured by electron microscope analysis and EV markers were evaluated by Western blot and FACS. The investigation of the MICA+ EVs immunomodulatory properties on NK cells was performed using a model of MM cell line transfected with MICA. NKG2D and MICA expression levels were evaluated by immunofluorescence and FACS analysis. NK cell cytotoxicity was assessed through 7AAD and degranulation assay.

Results: Interestingly, we found that MICA+ extracellular vesicles are primarily found in the BM of MM patients and are mainly derived from malignant plasma cells suggesting that they might have a crucial immunomodulatory action in the tumor microenvironment. Moreover, our findings show that persistent stimulation mediated by EV-associated MICA leads to NKG2D downmodulation related to a strong reduction of NKG2D-mediated functions. Moreover, we observed that MICA associated to EVs can be transferred to the surface of NK cells inducing NK cell fratricide.

Conclusions: All together, these findings suggest a novel mechanism of tumor immune evasion based on the impairment of NKG2D-mediated immunosurveillance and the induction of NK cell death by MICA+ extracellular vesicles.

ENDOTHELIAL CELLS AND DENDRITIC CELLS AS IMMUNOSUPPRESSIVE AGENTS IN PATIENTS WITH MULTIPLE MYELOMA

A. Vacca¹, A.G. Solimando^{1,2}, V. Racanelli¹, A. Frassanito¹, A. Melaccio¹, R. Ria¹

¹Department of Biomedical Sciences and Human Oncology, Section of Internal Medicine 'G. Baccelli', University of Bari Medical School, Bari, Italy; ²Istituto di Ricovero e Cura a Carattere Scientifico-IRCCS Istituto Tumori "Giovanni Paolo II" of Bari, Italy

Multiple myeloma bone marrow-derived endothelial cells (MMECs) contour the milieu microvascular environment and are in neighbouring interaction with CD8+ T cells that come and go across the permeable capillaries. MMECs are characterized by distinct "gene/molecular fingerprint" driving vascular mechanisms boosting the malignant progression from quiescent monoclonal gammopathy of undetermined significance. Moreover, given the close interactions between MMECs and the immune niche, we investigated the capacity of MMECs to act gatekeepers, influencing CD8+ T cell activation and proliferation within the myeloma (MM) niche. We uncovered MMECs as semi-professional antigen-presenting cells (APC), expressing low concentrations of the co-stimulatory molecules CD40, CD80 and CD86, and of the inducible co-stimulator ligand (ICOSL). Additionally, they do not undertake the durable switch from immunoproteasome to standard proteasome subunit expression which is characteristic of mature APC. MMECs can trap and present antigen to CD8+ T cells, enhancing the activation of a central memory CD8+ T cell population, expressing Foxp3 and producing enhanced amounts of IL-10 and TGF- β . Conversely, another CD8+ T cell population is stimulated by professional APC, secretes IFN- γ , and being operative in antitumor activity. Therefore, two distinct CD8+ T cell populations cohabit in the MM niche: the first one is sustained by MMECs, expresses Foxp3, sheds IL-10 and TGF- β , and create a pro-tumour activity by negatively regulating the second population. Our findings uncover the role of MMEC as a pivotal player in MM biology and point toward an additional immune regulatory mechanism that inhibits the development of antitumor immunity and may impair the success of cancer immunotherapy. In line with the phenotypic behaviour the distinct MMECs gene expression profiles and vascular phenotypes detected in this study prompt the targeting of the bone marrow microenvironment in patients with active disease. Importantly, we uncovered a novel immune regulatory role for endothelial cells in multiple myeloma biology and shows an additional mechanism of tumour immune evasion. In particular, it describes how bone marrow endothelial cells stimulate a subset of central memory CD8+ T cells with a strong capacity to suppress the proliferation of plasma cell tumour-specific effector memory CD8+ T cells.

DISSECTING DARATUMUMAB MECHANISMS OF RESISTANCE AT A SINGLE-CELL LEVEL

M.C. Da Vià¹, M. Lionetti¹, A.G. Solimando^{2,4}, A. Maeda¹, L. Baldini^{1,3}, A. Neri^{1,3}, N. Bolli^{1,3}

¹Department of Oncology and Hemto-Oncology, University of Milan. Milan, Italy; ²Department of Biomedical Sciences and Human Oncology, Section of Internal Medicine "G. Baccelli", University of Bari Medical School, Bari, Italy; ³Hematology Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy; ⁴IRCCS Istituto Tumori "Giovanni Paolo II", Bari, Italy

Multiple Myeloma (MM) still represent and incurable disease. It is clinically and biologically highly heterogenous, characterized by a huge clonal, subclonal and spatial heterogeneity. Since the introduction of immunomodulatory drugs (IMiDs) and proteasome inhibitors (PIs) the overall survival improves significantly. Nevertheless, other most powerful drugs came into the routine clinical practice like the monoclonal antibody Daratumumab (Dara) with whom MM enter in the field of immunotherapy. The survival rates upon the Dara introduction improves as like single-agent, as in combination with IMiDs or PIs. Despite these huge improvements, MM is still an incurable disease and patients continue to relapse. In particular, several clinical trials have reported a different progression-free survival (PFS) rates depending also from the number of prior therapies to whom the patients have undergone. CAS-TOR study has showed a 40% of patients relapsed at one year. Also in the POLLUX one the patients relapsed in the first year was almost 20%. Given that patients still relapsed upon Dara treatment the central hypothesis of this project is to dissect at a single-cell(sc) level the tumor-intrinsic and -extrinsic molecular mechanisms underlying Dara resistance development.

I will enroll patients, who relapse after the first or second line of therapy and who will submit to Dara-containing regimens. The patients will be biopsied before starting treatment and after the disease relapse. The samples will be analyzed by a single-cell approach (10X Genomics platform). I will study the tumor heterogeneity in the two different phases of the disease applying a scRNAseq approach associated with a study of the immunoglobulin rearrangements to better define the peculiar clone responsible for the disease. Given the usual CD38 downregulation upon Dara treatment, I will integrate the molecular dissection applying an ATACseq approach to study epigenetic modification. Finally, I will also depict the immune microenvironment modifications implemented by the study of the TCR, studying possible clonal expansion and later exhaustion of different T cell populations.

PREDICTIVE PARAMETERS AT FIRST AND SECOND RELAPSE, IS THERE A ROLE FOR sFLC? A REAL-LIFE UNICENTRIC RETROSPECTIVE STUDY IN THE ERA OF NOVEL AGENTS

U. Markovic, A. Romano, C. Bellofiore, A. Bulla, V. Del Fabro, F. Di Raimondo, C. Conticello
Division of Hematology, Azienda Policlinico-OVE, University of Catania, Italy

Introduction: In multiple myeloma due to the overproduction of only one type of monoclonal FLC there is an imbalance between serum FLCs with an increase of involved/uninvolved FLCs ratio (iFLCr). Although there are different assays with polyclonal and monoclonal antibodies, in the updated IMWG diagnostic criteria the only recognized sFLC assay was Freelite. Involved FLCr was demonstrated to represent a prognostic variable at myeloma diagnosis independent from ISS or other prognostic parameters. In line with this, it has been pointed out that normalization of iFLCr during treatment is a favorable sign and it is related to a longer PFS. Thus, SFCLs are a pivotal tool not only for diagnosis but also for monitoring MM patients, especially oligosecretory and nonsecretory.

Methods: In this real-life survey a total of 130 RRMM patients treated at our center from 2000 until 2020 were investigated as retrospective cohort. Data was collected from medical records of the patients treated with at least three lines of therapy including novel agents. In this work, we have focused on the predictive role of sFLC ratio and involved sFLC in MM patients beyond the first line of treatment, at first and second relapse. In addition to sFLC, we retrospectively analyzed different parameters to find any correlation between biochemical or clinical relapse and these variables.

Figure 1.

Results: Median age at diagnosis was 64 years (range 31-80 years), with more than half of patients being male. All patients had secretory disease. About 80% of evaluated MM patients had standard risk cytogenetic alterations, while rest of them were high risk. Median number of treatment lines was 4 (range 3-8); 45% of patients were exposed to more than four lines of treatment. Out of 90 normosecretory patients around 25% of them had switched to oligosecretory MM, including micromolecular disease, mainly in the first three lines of treatment. Aggressive disease was present in around 10-20% of disease relapses throughout the different treatment lines. As for the clinical presentation EMD together with renal failure was present in more than 30% of patients with aggressive presentation. Around 60-70% of the patients had increased sFLC values at disease relapse. Predictive role of involved sFLC on PFS3 was demonstrated with value greater than 100 mg/ml. Therefore,

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we performed a roc analysis and evaluated the prognostic significance of a iFLC and sFLC ratio. The analysis showed that both involved sFLC <250 and ratio <25 are associated with longer PFS3. **Conclusions:** In this work evaluation of sFLC assay demonstrated that both involved sFLC and sFLC ratio significantly correlate with outcome at second relapse as well as biochemical or clinical presentation. Although limited in numbers, data are significant. Unfortunately, the majority of exams were performed with Siemens, that is not indicated by IMWG guidelines. Further evaluations need to be performed with more data and Binding Site method of sFLC detection.

LEN/DEX COMBINATION AS FIRST-LINE THERAPY OF FRAIL MULTIPLE MYELOMA PATIENTS: AN UNICENTRIC REAL LIFE STUDY

C. Bellofiore, V. Leotta, A. Romano, V. Del Fabro, F. Di Raimondo, C. Conticello

Division of Hematology, Azienda Policlinico-OVE, University of Catania, Italy

Introduction: Lenalidomide plus dexamethasone (Rd), has recently become a new standard of care for newly diagnosed multiple myeloma (NDMM) patients. The FIRST phase 3 trial showed that continuous therapy with Rd is superior in PFS and OS compared to fixed therapy or triplet including melphalan, thalidomide and prednisone, leading to approval of Rd for NDMM. However, little is known about safety and efficacy of Rd in frail and ultra-frail patients in the real life setting.

Methods: From March 2017 up to now, 77 consecutive frail NDMM patients were treated with Rd regimen, according to the FIRST schedule. Lenalidomide dosage was reduced in patients with low platelets count and/or renal failure according to manufacturer's guidelines. We evaluated safety and efficacy of Rd combination and the prognostic significance of several parameters on PFS in 77 myeloma patients treated with Rd in first line, according to routine clinical practice of our Institution.

Results: Median age was 76 years (range 41-92), and 58 (75%) patients had an ECOG higher or equal to 2. Creatinine clearance was lower than 50 ml/min in 31 patients (57%). 41 patients (52.6%) had an evaluable FISH, 12 (29.3%) were classified as high risk for the presence of del(17p) or t(4;14) or t(14;16), and 26 (63.4%) carried acq1q. Nine patients have an extramedullary disease. Median duration of treatment was 19.4 months; 62 (91.9%) patients received more than 2 cycles and 22 (28.6%) patients are still on treatment. Hematological adverse events were reported in 25 patients, grade 4 anemia was reported only in one patient treated with blood transfusion. Grade 2 neutropenia and anemia were treated with granulocyte growth factors and/or erythropoietin, according to local policy. Grade 2 thrombocytopenia was managed by delaying next cycle. Non hematological toxicity was reported in 39 patients. Grade 3 infections, thromboembolic events and pneumonia were more frequent events (N=10, 8 and 3 patients respectively) and induced treatment interruption. Among patients who received at least 2 cycles, the overall response rate was 91.7% (N=57), including 2 (3%) complete response, 17 (22%) very good partial response (VGPR) and 20 (26%) partial response (PR). Overall, median PFS was 32 months; 32 (41.5%) patients had biochemical or clinical relapse during the treatment and 26 (34%) patients had clinical response to therapy (defined as no further need of transfusion, stable disease, regression of pain). Univariate analysis showed that clearance creatinine <50 ml/min, age <75 years and the development of anemia were strong negative predictors of overall survival. While acq1q detected by FISH, LDH >200 U/L and ISS 3 stage were not associated to inferior outcome in our series.

Conclusion: These real-world data confirm the efficacy of Len/Dex combination in frail patients; more importantly, it is demonstrated for the first time outside of a clinical trial that high ECOG or age >75 years do not contraindicate treatment.

EVALUATION OF TREATMENT APPROACHES AND OUTCOME IN NEWLY MULTIPLE MYELOMA PATIENTS, AUTOLOGOUS STEM CELL TRANSPLANT ELIGIBLE OR NOT, WHO PRESENTED EARLY RELAPSE OR WERE REFRACTORY TO FIRST-LINE INDUCTION OR FIRST-LINE TREATMENT

F. Fazio¹, M.L. De Luca², S. Rosati³, F. Vozella², M.T. Petrucci⁴

¹Department of Traslational and Precision Medicine - Sapienza University of Rome, Italy; ²Hematology, San Giovanni di Dio Hospital, Florence, Italy; ³Hematology Department, F. Spaziani Hospital, Frosinone, Italy; ⁴Hematology, Department of Translational and Precision Medicine, Azienda Ospedaliera Policlinico Umberto I, Sapienza University of Rome, Rome, Italy

Introduction: The treatment options in MM has changed over the last years and the therapy armamentarium of effective anti-myeloma drugs, used both at diagnosis and at relapse, has been expanded with the introduction of several novel drugs, including proteasome inhibitors, immunomodulatory drugs and monoclonal antibodies, that represent the standard of care. Novel drugs incorporated into induction therapy before high-dose therapy and ASCT have significantly improved OS in MM patients. Despite improvements in the MM outcome and in the depth and duration of response, MM remains an incurable disease whose natural history is characterized by an alternation of phases of remission followed by relapses. The duration of remission phases tends to progressively reduce at every subsequent relapse until MM becomes refractory to all available drugs. Nevertheless, several randomized clinical trial demonstrated a prolonged PFS in RRMM patients thanks to the introduction of newly combinations therapy based on the novel drugs. Monocentric experiences revealed about 20% of early relapse (ER) after ASCT, regardless of the depth of response, suggesting that biological mechanisms are related to resistant clone's selection. Additional studies demonstrated that ER is an independent prognostic factor for OS and ER patients present a higher rate of high risk cytogenetic abnormalities compared to other patients.

Methods: This is a multicenter, retrospective and prospective observational study aimed to describe the different treatment approaches among the Italian Hematology Centers; the biological, clinical characteristics and outcome of MM patients, ASCT eligible or not, who presented early relapse (within 18 months) or who were refractory to first-line therapy. A longitudinal survey will be carried out by collecting data of patients with newly diagnosed of MM from 01.01.2010 until 30.06.2019, in order to enroll the last RRMM patients within 31.12.2020. 102 patients represented the minimum sample size, according to the results of previous studies, in order to observe an OS rate of 60% at 12 months, with a confidence interval of 95% (two-tailed test).

Results: The primary end-point of this study is OS. The secondary end-points is to estimate PFS, PFS2, ORR, DOR, TTNT; safety of conventional treatment options in terms of hematological and non-hematological toxicity (especially, peripheral neuropathy, thromboembolic events, infections, cardiovascular events and infusion related toxicity); survival outcomes in order to evaluate changes in treatment approaches according to lines of therapy and survival outcomes according to clinical and biological characteristics.

Conclusions: The aim of this observational study is to evaluate

the efficacy of the different available combination therapy, excluding patient enrolled in clinical trials, for MM patients who have been shown early relapse or were refractory to first-line therapy, according to clinical and biological characteristics.

COMBINED TREATMENT WITH DOXORUBICIN AND ENPP1 INHIBITOR FOR IMPROVING ANTI-MYELOMA INNATE IMMUNE RESPONSE

L. Cuollo, A. Zingoni, M. Cippitelli, C. Fionda, M.T. Petrucci, A. Santoni, A. Soriani

Università degli Studi di Roma "La Sapienza"; Istituto Italiano di Tecnologia, Italy

Introduction: The tumor microenvironment created by multiple myeloma within the bone marrow is characterized by profound immunosuppression that affects several immune cell types, including NK cells. This loss of immune surveillance is thought to contribute to the progression from MGUS to MM and to the high frequency of relapse. The partial success obtained with immunomodulatory drugs and monoclonal antibodies confirm the importance of restoring the immune function for achieving longer disease-free survival. STING agonists, such as the endogenously produced 2'3'-cGAMP, demonstrated remarkable anti-tumor activity in vivo models of several types of cancer; these compounds can broadly activate immune effector functions, presumably by increasing the production of Type 1 Interferons and other activating cytokines. Moreover, interestingly, multiple myeloma cells (and lymphocytes in general) seem to undergo apoptosis when challenged with STING agonists, at least in mouse model. 2'3'-cGAMP has been recently demonstrated to be secreted in the extracellular space by tumor cells; its secretion is enhanced by ionizing radiation and contributes to a protective immune response. The ectoenzyme ENPP1, originally identified on the surface of plasma cells, is so far the only known enzyme able to degrade cGAMP; for this property, it is considered a possible therapeutic target in cancer immunotherapy.

Results: Our preliminary results demonstrate that 2'3'-cGAMP can directly enhance cytotoxic functions of primary NK cells from healthy donors, possibly by increasing their perforin content. Bone marrow mesenchymal stromal cells (BMSCs) derived from MM patients upregulate the expression of IFN- α , IFN- β , CXCL10 and IL-15 in response to stimulation with cGAMP. Multiple myeloma cell lines treated with low doses of doxorubicin secrete relevant amounts of cGAMP in the conditioned medium. Inhibition of the ectoenzyme ENPP1 can further increase the concentration of extracellular cGAMP. Currently we are investigating whether the treatment with doxorubicin combined with ENPP1 inhibitor could increase the cytotoxicity of NK cells against MM cells and enhance the secretory activity of BMSCs.

COMPARISON OF DIFFERENT IMAGING TECHNIQUES IN MULTIPLE MYELOMA PATIENTS

F. Vozella¹, S. Grammatico², M. Staderini³, G. Fontanelli⁴, F. Innocenti¹, L. Mannelli², R. Giachetti³, M. Vigliotta⁴, M. Rocco¹, M. De Luca¹, S. Birtolo³, M. Pirrotta⁴, S. Moretti¹, S. Santini²

¹*Ematologia Clinica ed Oncoematologia Ospedale Santo Stefano, Prato;* ²*Ematologia Clinica ed Oncoematologia, Ospedale San Giovanni di Dio, Firenze;* ³*Ematologia Clinica ed Oncoematologia Ospedale San Jacopo, Pistoia;* ⁴*Ematologia Clinica ed Oncoematologia Ospedale San Giuseppe, Empoli, Italy*

Introduction: In the last years numerous studies reported higher efficacy of the novel imaging methods as ¹⁸F-fluorodeoxyglucose positron emission tomography/computed tomography (¹⁸F-FDG PET/CT), whole-body computed tomography (WBLDCT), whole-body magnetic resonance imaging (WBMRI), respect to whole-body x-ray (WBXR) (1). The most recent International Myeloma Working Group (IMWG) guidelines, published in 2014, updated the diagnostic criteria for Multiple Myeloma (MM); the novel definition of bone lesions were: the presence of at least one lytic lesion detected not only by WBXR but also by one of the novel morphologic imaging techniques, such as CT, WBLDCT, or PET/CT; and the presence of more than one FL on MRI (2). Last year, given the multiple options available for the detection of bone and bone marrow lesions, the IMWG established guidelines on optimal use of imaging methods at different disease stages to help standardization of imaging worldwide and to allow the comparison of results and the unification of treatment approaches for MM (3). There are numerous studies that compare the novel morphologic imaging techniques, but they were heterogenous and lack an independent reference standard (4).

Methods: We designed a prospective study aimed at the evaluation of the novel imaging techniques in young patients (<65-70 years, eligible for transplant) newly diagnosed active MM patients and in smouldering MM patients. ¹⁸F-FDG PET/CT, WBLDCT and WBMRI will be performed at the onset of the disease, after autologous stem cell transplantation (MM), after one year of observation (SMM) and at the time of biochemical and clinical progression. The imaging findings will be compared to the results of bone marrow aspiration.

Aims: Our aim is the evaluation the specificity and sensitivity of the three different methods in MM patients and the concordance between the different methods. We start our analysis from a restricted group of young patients newly diagnosed active MM patients and smouldering MM patients. Moreover, we want to evaluate if one technique is more suitable than the other in case of different involvement of the disease (for example: spinal compression, extramedullary masses, osteolytic lesion of the skull vs lesion of the spine, etc).

ADDITION OF BCNU TO MELPHALAN DEEPENS RESPONSES WITHOUT INCREASING TOXICITY THEREBY IMPROVING SURVIVAL BEFORE SECOND AUTOLOGOUS STEM CELL TRANSPLANT FOR RELAPSED MULTIPLE MYELOMA

J. Thomas¹, M. Hussain², A. Seddon^{2,3}, E. Tollkuci^{2,4}, R. Myers², A. Varma², N. Hilal², T. Cozzi², J. Baptista², K. Cavazos², M. Weber², A. Reimer², G. Yassa², H. Yun², A. Paner², P. Venugopal², S. Nathan², C. Ustun²

¹*Department of Internal Medicine, Rush University Medical Center;* ²*Division of Hematology, Oncology and Cellular Therapy, Rush University Medical Center;* ³*Department of Pharmacy Practice, Midwestern University College of Pharmacy;* ⁴*College of Pharmacy, Rosalind Franklin University of Medicine and Science*

Introduction: High-dose chemotherapy (HDT) followed by autologous stem cell transplant (ASCT) is a standard frontline therapy for newly diagnosed multiple myeloma (MM). Single agent melphalan (Mel) is the most commonly used conditioning regimen prior to ASCT. However, in relapsed MM, there is no consensus regarding the best conditioning regimen prior to second autologous stem cell transplant (ASCT2). We analyzed the outcomes of carmustine (BCNU) with Mel, as a conditioning regimen prior to ASCT2.

Methods: Between 2010-2019, 58 patients with relapsed MM underwent ASCT2 at Rush University Medical Center (RUMC). All patients received single agent Mel at initial ASCT. Patients were divided into two cohorts: cohort 1 (n=31) received BCNU/Mel, while cohort 2 (n=27) received other Mel based conditioning (single agent Mel (n=19), Mel and Bortezomib (n=7), and BCNU, Etoposide, Cytarabine, and Mel, BEAM, (n=1)) prior to ASCT2. All patients received peripheral blood stem cell autograft. Kaplan-Meier methods were used to estimate overall survival (OS) and progression free survival (PFS). Patient-, disease-, and ASCT-characteristics were compared with Chi-square, Fischer's Exact test, or t-test.

Results: Both cohorts were similar regarding age at the time of ASCT2, sex, BMI, duration between ASCT and ASCT2, and disease status prior to ASCT2 ($p>0.05$ for all). Cohort 1 had more advanced stage MM per Durie-Salmon staging (DSS) (93% stage 3 vs 70% stage 3 respectively, $p=0.039$). Median duration of febrile neutropenia (4 vs 3 days), length of hospitalization (17 vs 17 days), and time to absolute neutrophil (13 vs 13 days) and platelet engraftment (18 vs 17 days) after ASCT2 were comparable between both groups. Use of IMiD-based maintenance was similar in both groups (54.8% vs 33.3%, $p=0.10$). There were no differences in secondary cancers after ASCT2 (3.2% vs 3.7%, $p=0.92$). More patients (83.8%) in cohort 1 achieved complete response (CR) or very good partial response (VGPR) on day 100 after ASCT2 compared with cohort 2 (59.2%) ($p=0.036$). Median PFS was longer for cohort 1 (29 months) compared with cohort 2 (21 months), but not statistically significant ($p=0.318$). Median OS was 41 months for cohort 1 vs 38 months for cohort 2 ($p=0.034$) with median follow up for living patients 45 months in cohort 1 vs 80 months for cohort 2. Regardless of conditioning, greater than one-half of patients were alive and about one-third of patients had no progression 6 years after ASCT2 (Figure 1).

Conclusion: This retrospective analysis shows that the addition of BCNU to melphalan in the conditioning regimen prior to ASCT2 deepens the response at day 100, without increased short or long-

term toxicities and thus improves survival. Prospective, larger studies are warranted. Regardless of the addition of BCNU, ASCT2 provides long-term survival.

Figure 1A and 1B. PFS and OS after ASCT2: BCNU/melphalan vs Other Conditioning.

ALLOGRAFT IN MULTIPLE MYELOMA: EXPERIENCE OF MULTIPLE MYELOMA GIMEMA LAZIO GROUP

F. Vozella¹, A.P. Iori¹, R. Cerretti², A. Severino³, P. Chiusolo⁴, A. Piciocchi⁵, L. Cesini¹, E. Mariggiò¹, F. Fazio¹, L. Cudillo², L. Franceschini², T. Za⁴, S. Sica⁴, V. De Stefano⁴, L. De Rosa³, W. Arcese², M.T. Petrucci¹

¹Hematology, Department of Cellular Biotechnologies and Hematology, "Sapienza" University; ²Hematology, Department of Biomedicine and Prevention, Università di Roma "Tor Vergata"; ³Hematology Division, San Camillo Hospital; ⁴Hematology, Department of Hematology, Università Cattolica del Sacro Cuore; ⁵GIMEMA Data Center, Italy

Introduction: In the era of new drugs prognosis of patients (pts) with multiple myeloma (MM) has significantly improved. Nevertheless the majority of pts with MM will relapse or become refractory and the remission duration in relapsed MM decreases with each regimen. If the role of autologous stem cell transplantation has been confirmed by many trials, allogeneic hematopoietic cell transplantation (HCT) is less commonly used due to high treatment related mortality (TRM) and worsening of the quality of life. As a result of this issues, it is still unclear how to best utilize this potent and effective treatment modality. **Methods:** We report the experience of Multiple Myeloma GIMEMA Lazio Group in 70 pts with newly diagnosed (38) or relapsed/refractory (32) MM who underwent HCT between February 1985 and February 2017. The median age was 45.7 years (range, 32.1 - 67.1), 43 men and 27 women. Median age at HCT was 48.6 years (range, 32.6 - 65.8), median time from diagnosis to HCT was 16.8 months (range, 3 - 130.6). As induction treatment 43/70 pts received old drugs, i.e. vincristine, doxorubicin and dexamethasone (VAD; n=33) or melphalan and prednisone (MP; n=10), while 27/70 pts were treated with novel agents, i.e. velcade-based (n=13) or IMiD-based regimens (n=14). Among newly diagnosed MM, HCT was performed as frontline therapy in 24/38 pts and in a tandem autologous/allogeneic in 14/38 pts. No differences in terms of OS and PFS were found between HCT "frontline vs relapse", $p=0.72$ and $p=0.34$ respectively, neither between induction treatment with "old vs new

drugs", $p=0.72$ and $p=0.15$ respectively. After induction, 9 pts (13%) achieved complete response (CR), 8 pts (11.3%) achieved a very good partial response (VGPR), 41 pts (58.6%) achieved partial response (PR), 1 patient (1.4%) maintained a stable disease (SD) and 11 pts (15.7%) performed HCT in progression disease (PD).

Results: Overall, 65 pts (93%) achieved a response (CR; n=32, VGPR; n=2, PR; n=31), 2 pts (2.8%) showed PD during or immediately after HCT, 3 pts (4.2%) died for TRM. More in detail a CR was observed in 7/9 pts who underwent HCT in CR, 4/8 in VGPR, 17/41 in PR and 4/11 in PD. Among 65 pts who obtained a response, 35 pts (54%) presented, a disease recurrence in 48% and 54% at 5 and 10 years, respectively. TRM was 14% and 18% at 5 and 10 years, respectively. Acute and chronic GVHD occurred in 37 and 36 pts respectively. Overall survival (OS) and progression-free survival (PFS) at 10 years was 43.1% (range, 32.3 - 57.4) and 25.1% (range, 16.5 - 38.2) respectively.

Conclusions: Autologous stem cell transplant remains the standard of care for young MM pts but it's not curative. Our retrospective analysis presents some methodological limitations but it shows that the role of HCT is not clear. Physicians should evaluate its combination in prospective trials for young high-risk or relapsed/refractory pts (not beyond the first relapse), in a tandem autologous/allogeneic transplant, with an induction and a possible maintenance based on new drugs.

PRIMARY PLASMA CELL LEUKEMIA EXPERIENCE OF 7 YEARS IN A PERUVIAN REFERRAL CANCER CENTER

J. Vasquez, E. Peña, E. Diaz, E. Poquioma

Instituto Nacional de Enfermedades Neoplasicas, Perú

Introduction: Primary plasma cell leukemia (pPCL) is a rare and aggressive malignancy with worse prognosis than multiple myeloma. Aim: To report the incidence and clinical characteristics of pPCL.

Methods: Review of the database of Department of Epidemiology from 2012 to 2018 at Peruvian National Cancer Institute. Results: 559 with multiple myeloma were diagnosed during the period of study with a median age of 60 years. Of the entire cohort 7 patients (1.2%) had pPCL, the median age at diagnosis was 53 years (range 28-72), women were 71% of the patients. ECOG Performance status was 2 in 85% of patients. Median hemoglobin was 8.4g/dL, median white blood count was $22400 \times 10^9/L$, median platelet was $88000 \times 10^9/L$, median percentage of plasma cell leukemia in peripheral blood was 35%, median albumin was 3.5 g/dl, creatinine was 1.8 mg/dl. Only 1 patient had heavy chain component (IgG), two were unknown, and the remaining 4 (57%) had free light chain only (100% kappa). Six (85%) patients received any treatment (2 VDT-PACE, 2 VAD, 1 CTD, 1 TD), the median of cycles was 3. Three (50%) patients achieved complete response (CR). No patient underwent stem cell transplantation, among the patients with complete response the reason for not transplantation was progressive disease by delayed treatment and one due to religious beliefs. Only two patients are alive, one in CR and the other with active disease. The median of overall survival was 9 months (range 1-30).

Conclusions: The patients with pPCL were younger than multiple myeloma patients. The median overall survival is short in the absence of stem cell transplantation.

TARGETED CHEMOTHERAPY OF THE ADVANCED RELAPSED AND REFRACTORY MULTIPLE MYELOMA

Vasile Musteata

*State University of Medicine and Pharmacy "N. Testemitanu",
Institute of Oncology, Moldavia*

Introduction: Multiple myeloma (MM) is one the most frequent hematological malignancies within the structure of morbidity by blood neoplasms with primary bone marrow (BM) involvement, being characterized in the advanced stages by a recurrent evolution, unfavorable prognosis and negative socio-economic impact. The increased rates of morbidity and disability, frequent relapses and complications, unfavorable socio-economic impact categorize MM as an actual issue of hematooncology and public health.

Methods: We performed the clinico-analytical and descriptive study of two clinical cases. These patients were treated and followed up at the Institute of Oncology from Moldova between 2012-2020. The diagnosis was asserted by cytological, immunohistochemical, immunophenotyping examinations of the BM and blood immunoelectrophoresis. The morphological type of hematological malignancy was identified according to the Revised 2017 WHO Classification of Tumors of Hematopoietic and Lymphoid Tissues. The stage of MM in each case was determined according to the Durie-Salmon Staging System and Revised International Staging System.

Results: Two patients of 62 (male) and 67 (female) years old with stage IIIA MM were included in our study. Both patients had IgG type and disseminated radiological form of MM. The patients experienced the history of anemic syndrome, bone pain and related decreased mobility, with the ECOG performance status 3. X-ray examinations and CT scans detected the multiple osteolytic lesions, predominantly affecting the axial skeleton, and diffuse skeletal osteopenia. Blood analyses revealed moderate anemia, elevated sedimentation rate and hyperproteinemia. The rates of myeloma cells in the BM aspirates ranged between 20-30%, and were represented mostly by proplasmacytes. One patient underwent the first-line treatment with VAD regimen. The older patient with comorbidities received the first-line chemotherapy with ciclofosfamide, vincristine and prednisolone, followed by thalidomide. Radiotherapy was administered in both patients in order to control locally the disease, and led to the increase of mobility due to the temporary relief of the pain syndrome. Bisphosphonate therapy had been used before radiotherapy, with the moderate improvement in pain and patient functioning. Only partial unstable responses were achieved. The patients relapsed and underwent CyBorD and BVD regimens with proteasome inhibitors because of the refractory evolution. The stable long-lasting responses were obtained in both patients. No serious side effects were registered during hospitalizations. The ECOG performance status was recovered up to 1.

Conclusions: The targeted chemotherapy with proteasome inhibitors proved to be efficient in the described advanced relapsed and refractory cases of MM regardless the age, gender, first-line treatments and disease span.