

POSTERS

NESTED DUPLEX PCR BCR/ABL (B2A2, B3A2) IN COMPLETE AUTOMATIZED SYSTEM

F. Culurgioni, S. Lilliu, G. Pala, D. Cardia, P. Soro, E. Usala, E. Angelucci

Unità Operativa di Ematologia e Centro Trapianti Midollo Osseo, Ospedale Oncologico "A.Businco" Asl 8 Cagliari, Italy

Introduction. Gene expert is a new complete system in real time pcr for Rna extraction, Cdna and nested real time duplex PCR. Once hematology diagnosis by quantification of P210 (b2a2, b3A2 isoforms) of BCR/ABL gene in chronic myeloid leukaemia was utilized. **Objective.** i) To characterize BCR/ABL rearrangements for chronic myeloid leukemia diagnosis. ii) To utilize at diagnosis, follow-up and in minimal residual disease evaluation. Peripheral blood, bone marrow and crio persevered with standardize protocol. iii) The understanding of pathology process in chronic myeloid leukemia diagnosis and in all myeloproliferative disease for development of other new target therapy in specific target therapy resistance. **Materials and Methods.** Were analyzed 209 cases; 6 CML at diagnosis, 192 CML in follow up, 6 myeloid acute leukemia at diagnosis, 9 post BMT follow up and 29 control with negative BCR/ABL gene transcripts . Of this case/control, 188 (78,9%) were peripheral blood and 50 (21%) were bone marrow samples. Ten samples were cryopreserved cells from peripheral blood obtained by ficoll separation, 200 of peripheral blood, or 200 microliter of bone marrow with 1:20 dilution or 200 microliter of cryopreserved cell in PBS dilution within 48 hours were used. In cartridges were performed lysis , sds- tween and guanidium salt, bind to column, wash for elute pcr inhibitor and water DEPC elution. were performed RT-pcr- nested and duplex pcr. As endogenous control Abelson gene was used. Results were validates with ABL end point fluorescence value (≥ 200) and BCR ABL end-point fluorescence value (≤ 40). **Results and conclusions.** Excellent result was obtained with 200 microliter of peripheral blood, but if we use bone marrow samples of 50 of these 10(20%) were aborted for pump problems. If we use 1: 40, PBS 1x dilution all samples were evaluated. Oresults were obtained with cry preserved cell from wbc (by dry pellet diluted in 1 mL PB 1x at 1:40 dilution before 1 week of cry preservation. Gene expert is easy and highly sensitive, Patients in imatinib mesylate therapy with CML require in clinical laboratory new rapid equipment for level transcript monitoring. Gene expert is easy, speed, with 4 way for fluorescence revelation, utilize micro fluidic cartridge minimize all contamination risks in nested duplex PCR real time reaction.

NEW DIAGNOSTIC MARKERS IN CHRONIC LYMPHOID LYMPHOCYtic LEUKEMIA

F. Culurgioni,¹ S. Lilliu,¹ G. Pala,¹ D. Cardia,¹ A. Murgia,² M. Murtas,² E. Angelucci¹

¹Unità Operativa di Ematologia e Centro Trapianti Midollo Osseo, Ospedale Oncologico "A.Businco" Asl 8 Cagliari; ²Unità Operativa di Anatomia patologica, Ospedale Oncologico "A.Businco" Asl 8 Cagliari, ItalyStudio eseguito con il contributo dell'Assessorato alla Sanità della Regione Sardegna

Introduction. Chronic lymphocytic leukaemia (CLL) is a B-cell chronic lymphocytic leukemic condition occurring in a premature stage of maturation, when immunoglobuline types IgM and/or IgD on surface are scarcely expressed. Several epidemiological factors may contribute to its occurrence, including familiar predisposition, level of industrialization (higher in Western Countries), age (50-70 years), and gender (M/W 2: 1). For the study (of CLL), clinical and laboratory parameters are used: LDH, $\beta 2$ -microglobulin, clinical stage, bone marrow infiltration type, lymphocytic doubling time as well as others biochemical parameters such as IgVH gene somatic mutations, chromosomal alterations (i.e. deletions on chromosomes 13, 11, and trisomy 12), and CD38 expression. Molecular markers include ZAP-70, CLLU1, the first gene-specific marker in Chronic lymphocytic leukaemia with elevating pattern of expression, which represents a predictive index of CLL risk. Additionally, MUM1 is a recent factor whose expression levels that prognosticates the form of CLLU1 with a favorable clinical course and long survival. **Aims of the study.** i) to characterize the molecular alterations, mutational states and gene rearrangements, associated with this disease; ii) to find biological markers of specific stages of the disease. These prognostics parameters should allow a more correct therapy and a better estimation of the prognosis for patients in the high risk category (Binet stage B-C). **Materials and methods.** 23 cases with a diagnosis of CLL were analyzed. The study was performed between 2006 and 2008 at the "A. Businco" Cancer Hospital in Cagliari, Sardinia, (Italy). Case age, 42-88 years (W/M 39% /61%, ratio M/F 2:1). DNA and total RNA from mononucleated cells obtained from bone marrow and peripheral blood were extracted. serum surveying, measurement of prognostic indicators by real time Polimerase Chain reaction, immunohistochemical and immunocytometrical analysis, and study of trascripts by sequence analisys were performed. **Results.** 30% of the patients showed an aggressive clinical course, while 70% of them had a good clinical course. The CD79b immunologic level resulted immeasurable or very weak in all cases (91%), except in two (9%) where it was high. CD38 was positive in 43% of the cases, while it remained negative in 57%. Mutational status of immunoglobuline IgVH and CD38 correlation: 5% were ZAP-70 +/ IgVH unmutated, 69% were ZAP-70 -/ IgVH mutated, with absence of correlation in 26% of

cases. Histopathological analysis was performed using standard markers (CD5, CD79a, CD20, CD23) in order to differentiate between classic CLL and its variants. The mutation status of IgVH was performed by RT-PCR using 3 sets of primers specific for VHL, VHF, and VHD families. IgVH extension molecular analysis show: 89% mutated, and 11% not mutated. For the IgVH carrying somatic mutations, we studied also the specific interested families: 19% VH1, 52% VH3, 29% VH4. VH3-21 family was absent (unique index negative prognosticates in IgVH with somatic mutation). ZAP-70 and CLLU1 has been performed through RT-PCR by ABI-PRISM 7000 and relative quantification expressed as $(2^{-\Delta\Delta CT})$. From real time evaluation: Positive ZAP-70: 26%, Negatives ZAP-70: 74%; CLLU1 and IgVH not any correlation Expression of MUM1 was high in all ZAP-70 negative cases. *Conclusions.* In the IgVH analysis we found discordant data with respect to the European guidelines, in particular the absence of VH3-21 expression family. The ZAP-70 values performed by molecular biology techniques remain the more important index of the IgVH mutational status. The high expression of MUM1 may be indicative of patients with favorable clinical course and longer survival expectancy. CLLU1, CD38, and ZAP-70 correlation have characterized groups of patients with a better or a worse prognosis. Because of the limited number of cases (23 patients diagnosed) analyzed, our results should in future be extended to investigate the prognostic factors, disease clinical stage study on mutational state of the IgVH, expression of the polymorphism of IL1B and IL6 genes, doubling time of the lymphocyte study CD38 and ZAP-70 quantitation expression.

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JAK2V617F MUTATION IN ESSENTIAL THROMBOCYTHEMIA: CLINICAL CORRELATIONS AND LABORATORY FEATURES AT DIAGNOSIS AND DURING FOLLOW UP IN 115 PATIENTS

A. Belotti, E. Elli, M. Parma, F. Colnaghi, A. Colombo, E. Lanzi, P. Pioltelli, E.M. Pogliani

Division of Hematology, Ospedale San Gerardo, Università degli Studi Milano Bicocca, Monza, Italy

Background. Essential thrombocythemia (ET) is thought to derive from the transformation of a multipotent hematopoietic stem cell; acquired mutation of JAK2V617F can be found in approximately 50-60% of ET. The impact of this mutation on its molecular pathogenesis and clinical phenotype is still debated. We investigated the frequency of JAK2 mutation and its possible impact and correlation with clinical and laboratory features at diagnosis and during follow-up in ET patients (pts) referred to our division between 1987 and 2008. *Methods and patients.* Allele-specific PCR for JAK2V617F mutational status was performed on genomic DNA from bone marrow cells or peripheral blood granulocytes in 115 ET (49 males, 66 females) pts. Clinical and laboratory features were evaluated at diagnosis and during follow-up, comparing ET pts harbouring JAK2 mutation versus pts harbouring wildtype (WT) gene. For statistical analysis, data were processed using the Graph Pad PRISM 5 DEMO Software performing non-parametric methods: univariate analysis was performed to evaluate differences in proportions by the chi-square and Fisher's exact tests for categorical nominal variables and the Mann-Whitney rank test for ordinal variables. Continuous variables have been categorized using median values. The log-rank (Mantel-Cox) test applied to Kaplan-Meier method was employed to estimate thrombotic risk and thrombotic event-free survival (TEFS). *Results.* 66 ET pts (57,4%) were JAK2V617F positive. JAK2 mutated pts were older (median age 59 vs 48 years, $p=0.0016$) and presented at diagnosis significantly higher hemoglobin levels (Hb 14,3 g/dL vs. 13,3 g/dL, $p=0,0027$), higher hematocrit (Ht 43% vs. 39,8%, $p<0.0001$) and lower PLT count (PLT 725 vs. $841 \times 10^9/L$, $p=0.005$) respect to WT group. These PV-like features have also maintained during the course of disease, with statistical significance. No difference was observed in term of gender, white blood count, LDH, progression or entity of splenomegaly, and need of cytoreductive therapy between JAK2 mutated and WT ET pts. A highly significant increase of thrombotic complications was registered for JAK2 positive pts. Considering JAK2 WT ET as reference group, the relative risk (RR) of primary thrombotic event was 2,143 (95% CI: 1.053-4.360, $p= 0,035$) for JAK2 mutated pts, with a TEFS lower than wildtype pts (Figure 1).

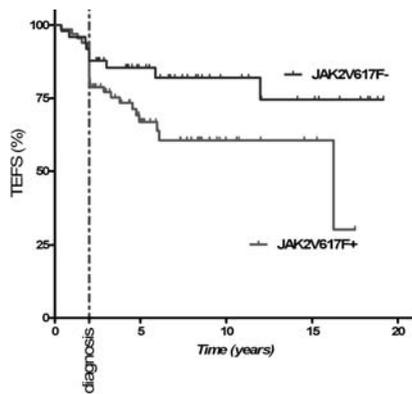


Figure 1. Thrombotic event-free survival (Kaplan-Meier method) in JAK2 positive and negative patients.

Arterial events were more frequent than venous events without statistical difference between two ET groups. A trend of higher risk of recurrent thrombosis ($p=0,056$) was also shown for JAK2 mutated respect to WT ET pts. There was no difference about the risk of haemorrhagic complications, time and incidence of evolution in myelofibrosis between JAK2 mutated or WT ET pts.

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R-FC-R COMBINATION CHEMIO-IMMUNOTHERAPY IN PATIENTS WITH B-CHRONIC LYMPHOCYTIC LEUKEMIA IN PROGRESSIVE DISEASE

C. Minotto, G. Bertoldero, E. Balducci, A. Porcellini, O. Vinante

Department of Medical Sciences, Hemato-oncology unit, Mirano (Ve) Italy

Combination treatment with Fludarabine and Cyclophosphamide (F/C) is the treatment of choice for advanced, relapsed or progressive disease (PD)]^{1,8,11} in B-chronic lymphocytic leukemia (B-CLL). Several studies would indicate that addition of Rituximab (R) to F/C regimen may improve both partial and complete response in B-CLL patients in PD.^{3,4,5,6} From April 2003 to December 2008 we treated 39 LLC-B patients (25 males, 14 females) with advanced or progressive disease. The combination chemio-immunotherapy consisted of two administrations of Rituximab 375 mg/m² on days 1 and 5, Fludarabine 30 mg/m² days 2-4 and Cyclophosphamide 300 mg/m² on days 2-4. Median age was 68.3 years (range 45-81), 5 pts were in RAI stage I, 16 in stage II, 8 in stage III and 10 in stage IV. Fifteen patients had cytogenetic analysis, 11 of which had a normal karyotype, 2 a trisomy 12 and 2 patients had a complex karyotype. Mean administered cycles were 1.9 (range 1-7). 18 patients received 1 cycle only, 13 patients received 2 cycles 3 patients were treated with 3 cycles and 4 with 4 cycles; one patient was treated with 7 successive cycles. Toxicity was acceptable, the most relevant side effect being neutropenia that occurred in 36/39 pts: G I in 8 patients, G II in 5 GIII in 8 and G IV in 15. Fever >38°C occurred in only 2/36 neutropenic patients, 1/36 had HZ and 1/36 had invasive Candida. 8/39 patients died: 2 for invasive aspergillosis, 6 for PD while one patient was lost at follow-up. Seventeen patients are in PR and 12 in clinical CR and off therapy. The mean follow-up is 60.3 months (range 1-73 months); estimated progression free survival (PFS) at 5 years was 72.7%. **Conclusions.** Rituximab, a chimeric anti-CD20 antibody^{2,7,10,12}, increases the activity of cytotoxic agents in several resistant cell lines. Prolonged neutropenia occurred in about 92% of the treated patients; however, serious infections only occurred in a few cases. This chemio-immunologic combination employing 2 successive doses of Rituximab proved to be safe, well tolerated and efficacious.

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GENETIC POLYMORPHISM OF P53 GENE AT CODON 72: AN IMPACT ON SUSCEPTIBILITY AND PROGRESSION OF CHRONIC MYELOID LEUKEMIA PATIENTS TREATED WITH IMATINIB IN INDIAN

A.B. Rashid Mir, S. Sudha, B. Bohra, C. Rekha, Y. Tanveer, M. Pravas, S. Alpana, S. Renu

Head, Department of Hematology, All India Institute of Medical Sciences, New Delhi, India

Background. TP53 is a major gene involved in the determination of proliferation or growth arrest at the cellular level. The tumor suppressor p53 pathway plays a crucial role in preventing carcinogenesis through its ability to impose cell cycle arrest and apoptosis following DNA damage or oncogene activation. The genotype (Arg/Arg, Arg/Pro, Pro/Pro) distribution of p53 codon 72 polymorphism has reported to be significantly different among ethnic groups. *Objective.* The specific aim of this study was to investigate whether the p53 codon 72 polymorphism is associated with individual susceptibility and progression of Chronic myeloid leukemia treated with imatinib in Indian patients. *Materials and methods.* Two hundred consecutive patients who presented with a haematological diagnosis of CML had blood examined by

RT-PCR for BCR-ABL transcripts. Among 200 CML patients 100 were in CP-CML, 30 late-CP-CML, 20 AP-CML and 50 BC-CML were selected who were treated with imatinib at 400-1000 mg/day. The allelic distributions of the three genotypes (Arg/Arg, Arg/Pro, Pro/Pro) in patients with susceptibility and progression of Chronic myeloid leukemia patients. DNA was extracted by DNA extraction Kit from Bioserve India. We used (PCR-RFLP) Polymerase chain reaction-restriction fragment length polymorphism analysis to assay the allelic frequencies (Arg/Arg, Arg/Pro, Pro/Pro) in 200 Chronic myeloid leukemia patients treated with imatinib. *Results.* The frequency of the polymorphism was examined in 200 CML patients. The polymorphism analysis was performed by amplifying exon 4 of p53 and digesting the products with restriction enzyme. The frequencies of genotypes: Arg/Arg, Arg/Pro and Pro/Pro were (50/200), (90/200) and (20/200), respectively, in the cases with Chronic myeloid leukemia treated with imatinib and (05/20), (08/20) and (05/20), respectively, in the healthy controls. Statistically, there was a significant difference in the frequency of the genotypes when the healthy controls were compared to the patients with Chronic myeloid leukemia treated with imatinib. The specific allele frequencies showed borderline significance). Our findings suggest that the p53 Pro72 variant is associated with an increased susceptibility and progression of Chronic myeloid leukemia in Indian populations. *Conclusion.* Patients with the *Pro/Pro* genotype tended to have poorer prognosis than those with the *Arg/Pro* genotype ($p=0.05$, by the log-rank test). Our data suggested that *TP53 A1* allele may represent a risk factor for both development of CML and primary molecular resistance to treatment with imatinib. Leukemic cells expressing the Pro72 protein may be more resistant to the apoptosis induced by imatinib than cells expressing exclusively the Arg72 protein. The detection of this p53 polymorphism may be a useful tool for screening of early progression of Chronic myeloid leukemia.

CONCOMITANT DIAGNOSIS OF ADULT ONSET STILL'S DISEASE AND CHRONIC MYELOID LEUKAEMIA: SUCCESSFULLY TREATMENT WITH TIROSIN-KINASE INHIBITORS FOR BOTH PATHOLOGIES

G. Monaco, S. Iaccarino, E. Attingenti, M.L. Vigliotti, A. Abbadessa

U.O.C. Oncoematologia, AORN Sant'Anna e San Sebastiano, Caserta, Italy

Background. We describe a case of a young man affected by Adult Onset Still's Disease (AODS) and Chronic Myeloid Leukaemia (CML), both responsive to tyrosin-kinase inhibitors (TKIs). *Case report.* A twenty years old man in October 2004 presents intermittent fever with peak of 40°C, macular-papular rash associated to fever, arthralgias with migrant joint swellings, lateral cervical lymphadenopathies, splenomegaly. Blood test shows: neutrophilic leucocytosis (white blood cells $20.5 \times 10^9/L$) and trombocytosis (platelets $799 \times 10^9/L$), hypertransaminasemia, elevated inflammatory index (ESR 120 mm/L,

CRP 299 ng/L, ferritin 1200 ng/dL). Diagnosis is AODS for presence of four major diagnostic criteria (fever, arthralgias, typical rash, leucocytosis) and two minor criteria (liver dysfunction, lymphadenopathy with splenomegaly) (Yagamuchi *et al.* J Rheumatol 1992;19(3):424-30). Patient begins therapy with steroids and methotrexate. After one month with this therapy, fever disappears with employment of general conditions. After other four months lymphadenopathies, rash, arthralgias are present yet. For persisting leucocytosis, patient comes to our attention. Blood smear analysis shows immature myeloid elements (neutrophils 61%, eosinophils 2%, basophils 1%, lymphocytes 16%, monocytes 8%, promyelocytes 2%, myelocytes 4%, metamyelocytes 6%). Bone marrow aspirate shows a granuloblastic hyperplasia. Cytogenetic and molecular analysis allows to make diagnosis of CML. Therapy with imatinib (400 mg/die) is started with interruption of therapy with steroids and methotrexate. AODS's symptomatology disappears after one week. At third month of follow up, a suboptimal molecular response is present. At 31st month molecular analysis shows one-log increase of leukemic mass. Therapy with dasatinib 100 mg/die is started. Three months later, complete molecular response is reached. Forty-six months since diagnosis, after therapy with TKIs, CML's complete remission and AODS's regression persist. *Discussion.* AODS is defined as a systemic inflammatory disorder that typically afflicts young adults. Characteristic manifestations include quotidian fevers, typical rash, pharyngitis, arthralgias, arthritis, hepatosplenomegaly, polyserositis, leukocytosis, and negative serological tests for antinuclear antibodies (ANA) and rheumatoid factor (RF). Its clinical course is marked by systemic exacerbations and/or chronic arthritis, frequently with disease-free intervals. AODS can cause serious damages to the joints and also impair the function of the heart and lungs. The goal of treatment for AODS is to control the symptoms of arthritis. Nonsteroidal anti-inflammatory drugs are usually the first treatment. Steroids may be used for more severe cases. If the disease becomes chronic, drugs that suppress the immune system might be needed such as methotrexate or tumor necrosis factor (TNF) antagonists. Recently, possible use of TKIs, fundamental in treatment of CML, has been studied in rheumatic diseases. Imatinib is able to block pathways involved in their pathogenesis such as mast cell c-kit signalling, TNF release, macrophage c-Fms activation, cytokine production, fibroblast PDGFR signalling and proliferation (Paniagua *et al.*, Nature Clinical Practice Rheumatology 2007;3:190-191). Described case shows an association between AODS and CML, diagnosed with an interval of five months. Thanks to imatinib and then dasatinib, concomitant molecular remission for CML and disappearance of AODS's evidence have been obtained, without immunosuppressive therapy. *Conclusions.* Inhibition of tyrosinase's pathways in rheumatic diseases, through imatinib and other TKIs, can become a valid therapeutic approach for these pathologies, especially in cases refractory to conventional treatment.

IS EARLY INTENSIFICATION USEFUL FOR

RELAPSED OR REFRACTORY HL PATIENTS?

R. Sancetta,¹ L. Rigacci,² B. Puccini,² P. Pregno,³ U. Vitolo,³ E. Brusamolino,⁴ M. Gotti,⁴ M. Magagnoli,⁵ M. Balzarotti,⁵ T. Chisesi¹

¹U.O. di Ematologia, Osp. Dell'Angelo, Venezia-Mestre; ²SOD Ematologia, Azienda Ospedaliera Universitaria Careggi, Firenze; ³S.C.D.O. Ematologia 2, A.O.U. S.Giovanni Battista, Torino; ⁴Clinica Ematologica, Fondazione IRCCS Policlinico San Matteo, Università di Pavia, Pavia; ⁵Dipartimento di Oncologia medica ed Ematologia, Istituto Clinico Humanitas-Rozzano (MI); Italy

Introduction. Forty pts, coming from different Italian Hematologic centres, with relapsed or refractory Hodgkin Lymphoma (HL) were analyzed retrospectively, to evaluate the outcome according to Time to Treatment. *Material and methods.* Patients' characteristics: 16 M and 24 F, median age 37 yrs (range 20-77), 10 stage I-IIA and 30 stage IIB-IV; 19 pts had bulky disease. 9 pts (22%) have already undergone ASCT during 1st line or immediately after its end ("early") and 5 pts (12%) will undergo "early" ASCT for presence of active disease documented by PET2+. 9 pts (22%) underwent ASCT after at least 3 months from the end of therapy; 17 pts (44%) did not receive any intensification of therapy. *Results.* all the 9 pts who have already received "early" ASCT are alive (66% CR, 34% PD). 9 pts were transplanted as salvage therapy: 22% are in CR, 11% are in PR, 11% are in MR, 22% are in PD, 34% died in PD. The 17 pts who did not have an intensification of therapy are alive: 76% are in CR, 6% are in PR, 18% are in PD. *Conclusions.* Our preliminary data suggest that the "early" transplant has an advantage in terms of achievement of remission.

IS AUTOLOGOUS HAEMATOPOIETIC STEM CELL TRANSPLANTATION IN ELDERLY PATIENTS A HIGH RISK PROCEDURE?

E. Trabacchi, C.F. Moroni, C. Bosi, A. Arcari, P. Bernuzzi, A. Lazzaro, L. Cappucciati, D. Vallisa

Hematology and Transplant Unit, Ospedale Civile G. Saliceto, Piacenza, Italy

High dose chemotherapy followed by autologous previously collected stem cell transplantation has shown to significantly improve the outcome in several haematopoietic malignancies. In advanced Aggressive Non Hodgkin Lymphomas high dose chemotherapy followed by autotransplantation is a widely applied policy. In Multiple Myeloma one or two autologous transplant are considered the golden standard in patients <65 years and several clinical trials have shown that responses are higher and overall survival is better in auto transplanted young patients than in non auto transplanted ones. The role of autologous stem cell transplantation in Acute Myeloid Leukemias is still a concern; several authors suggest that high dose chemotherapy and reinfusion of patient stem cells can be the best consolidation treatment in first remission low risk leukemias. Few studies have so far analyzed the toxicity, feasibility and response in elderly patients. We performed a retrospective analysis on a cohort of consecutive patients affected by haematologic malignancies, submitted to autologous stem cells transplantation at age >65 years in our Institution from 2005 to 2008. The study includes 30 procedures in 26 patients (Multiple Myeloma: n. 12; Non Hodgkin Lymphoma: n. 10; Acute Leukemias: n. 4). The mean age at transplant was 68 years (median 68, range: 65-75 years). They received 1 transplant (n. 22) or 2 transplants (n. 4) depending on stage of disease and diagnosis. Transplant policy was included in first line therapy in patients (median age). Conditioning regimens were BEAM in 8, Alkeran alone in 18, BuCy in 3, Bu Mel in 1. Drug dosages were adjusted considering clinical conditions and previous therapy lines. Mean dosage administered was 75% of that scheduled in adult patients. Early transplant mortality (<1 month) was registered in only 1 patient, transplanted in progression for aggressive refractory non Hodgkin Lymphoma. Response to treatment depended on stage of disease. Hematologic recovery was not affected by the age of the patient at transplant. Toxicity was not different from that registered in younger patients who underwent the same procedures.

We conclude that patients >65 years can be submitted to autologous stem cell transplantation especially when autotransplantation is planned in first or second remission.