TELOMERE LENGTH IDENTIFIES TWO DIFFERENT PROGNOSTIC SUBGROUPS AMONG VH-UNMUTATED B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA (B-CLL) PATIENTS

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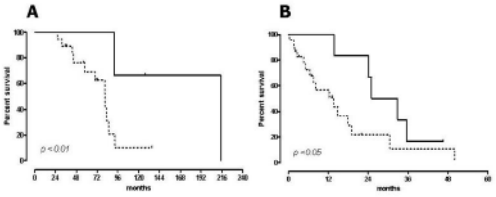
Background. Telomere restriction fragments (TRF) length has a prognostic impact in B-CLL. Some studies suggest that this is a mere reflection of its association to VH-mutational status (VH-MS). However, the relative value of these two parameters has not been clearly defined, particularly in cases in which they are discordant. *Aim.* To compare TRF length and VH-MS in a large population of B-CLL patients, in terms of overall survival (OS), time to first treatment (TTFT) and progression free survival (PFS). *Patients and Methods*. 201 B-CLL patients have been analyzed for TRF length and VH-MS. All samples were taken before treatment start. Males were 124, females 77. Median age was 62 years (range 33-93). According to Binet staging system, 121 were stage A, 39 B and 41 C. Cytogenetics, CD38 and ZAP-70 expression were available in 80% of patients. Median follow-up was 28 months (range 6-281). Eighty-seven patients have been already treated. TRF length was evaluated by Southern blot and VH-MS by direct sequencing. The standard cut-off of 2% deviation from any germ line VH sequence was employed to define VH-MS. Survival analyses were performed using the Kaplan-Meier method. *Results.* Median TRF length was 6014bp (range 1465-16762bp). There was no correlation between TRF length and patient age, sex or stage. TRF length had a major impact on prognosis with best results observed with a cut-off of 4250bp. Patients with TL<4250bp had a worse outcome than patients with TL>4250bp median OS: 83 vs 281 months, $p<0.0001$; median TTFT: 21 vs 60 months, $p<0.0001$; median PFS: 12 vs 49 months, $p<0.0001$). VH-MS analysis was successful in 91%. Overall, discordance between VH-MS and TRF length was observed in 14% of patients. Discordance was common among VH-unmutated patients (41%) but rare among VH-mutated patients (6%). Discordant and concordant patients could not be distinguished based on VH usage or degree of homology (H) to the germline IgH sequence (i.e. H=100% vs H<100% and >99% vs H<99% and >98%). In addition they could not be distinguished based on stage, cytogenetics, CD38 and ZAP70 expression.



The 27 discordant patients with VH-unmutated status and TRF length>4250bp had a clinical outcome that was significantly different from VH-unmutated patients with TRF length<4250bp (median OS: 83 vs 214 months, $p<0.01$ and median PFS: 12 vs 29 months, $p<0.05$) and similar to that of VH-mutated patients (median OS 281 months and median PFS 54 months, p=n.s.) (Figure, panel A -OS- and panel B -PFS-). *Conclusions*. Our data demonstrate that: 1) TRF length effectively predicts outcome in terms of OS, TTFT and PFS; 2) among VH-unmutated patients, those who have TRF length>4250bp have a better clinical outcome compared to VH-unmutated patients with TRF length<4250bp.

TARGETING THE MICROENVIRONMENT IN CLL: EFFICACY OF LENALIDOMIDE (REVLIMID®) IN PATIENTS (PTS) WITH RELAPSED/REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

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Introduction. CLL, an incurable hematologic cancer, has limited therapeutic options especially in the relapsed or refractory clinical setting.Investigators have identified the critical role played by tumor microenvironment(ME)in the pathogenesis of CLL. Abnormalities in both the cytokine and cellular ME have been reported. Aberrant expression of various prosurvival cytokines such as VEGF, TNF-α and PDGF delivers growth promoting signals to the CLL cells and contributes to immune evasion of the malignant cells. Traditional therapeutic approaches in CLL have primarily focused on targeting only the malignant. ME as a therapeutic target is a novel concept that is remains to be fully characterized. Lenalidomide (L) is an immunomodulating agent (IMiD®), with ability to modulate in vivo, several cytokines including TNF-α, VEGF, PDGF and IL-6. It is also reported to activate immune effector cells (T & NK cells). Consequently, L is an agent that can potentially be used to target both the cytokine and the cellular ME in CLL. We hypothesized that L modulates CLL cell ME, interrupting the prosurvival signaling mediated through aberrant cytokine production in the malignant milieu and thereby predisposing the malignant CLL cell to undergo apoptosis. Through a phase II clinical study we investigated the clinical efficacy of L in rel/ref CLL pts. Preliminary results of this study are presented here. *Patients and Methods.* All pts with rel/ref CLL requiring therapy as per 1996 Cheson criteria were eligible. Oral L was given at 25 mg/day for 21 out of a 28 day cycle. Clinical response was recorded after each cycle using NCI-WG 1996 criteria. Treatment was continued until molecular complete response (mCR) or progressive disease (PD). Those with PD were then treated with L in combination with rituximab (reported separately). Polymerase chain reaction (PCR) for immunoglobulin heavy chain gene was used to determine molecular remission (mCR). *Results.* Forty one pts (8F, 33M; median age 64 years, range: 47-75) have been enrolled. The median number of prior therapies is 4 (range 1-10). Toxicity is reported on all, while response on 26 evaluable pts. Nine pts are inevaluable (2 withdrew consent and 5 received < 2 months of therapy due to toxicity). Major response was noted in 17 of 26 evaluable pts (65%) with 3 CR (2 mCR) and 14 PR. On an intent-to-treat analysis the response rate was 41%. (Note: some pts, still on therapy are too early for response assessment). *Toxicity.* Most common grade 3/4 adverse effects (AE) were neutropenia (60%) and thrombocytopenia (55%). Another common AE was tumor flare (79%); characterized by tender swelling of lymph nodes, spleen or liver associated with low-grade fever and/or rash. This was usually only seen during the first cycle. *Conclusions*. ME plays an important role in the CLL cell survival, targeting the ME is a novel concept that merits further investigation. This is the first clinical study investigating the role of L in pts with CLL. Our observations demonstrate that L can deliver high ORR including mCR in rel/ref CLL. Hematologic toxicity was the most common AE requiring dose reduction. Overall safety profile was predictable and manageable.

INACTIVATION OF THE BLIMP1 GENE IN NON GERMINAL-CENTER TYPE DIFFUSE LARGE B CELL LYMPHOMA

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PRDM1/BLIMP1 is a zinc finger transcriptional repressor expressed in a subset of germinal center (GC) B cells and in all plasma cells, and required for terminal B cell differentiation. The BLIMP1 locus lies on chromosomal band 6q21-q22.1, a region frequently deleted in B-cell non-Hodgkin lymphoma, suggesting the existence of one or more tumor suppressor loci (Gaidano *et al.*, Blood 80: 1781, 1992). To investigate whether structural alterations of the BLIMP1 gene may be involved in the pathogenesis of Diffuse Large B cell Lymphoma (DLBCL), we performed PCR amplification and direct sequencing of the BLIMP1 coding sequences in 219 samples, including 20 cell lines and 199 primary biopsies. Gene expression profiling analysis using the Affymetrix GeneChip system was used in 170 cases to characterize them as GC B cell-like (GCB, N=78), activated B cell-like (ABC, N=51) and unclassified (N=37). Inactivating mutations of the BLIMP1 gene were found in 20% (N=10/51) ABC-DLBCL, but not in GCB-DLBCL (N=0/78) and only in 1/37 unclassified DLBCL (3%), while 2 additional mutated cases were not profiled. BLIMP1 alterations included gene rearrangements (N=1), nonsense mutations (N=1), frameshift deletions (N=7) and splice site mutations (N=6), which generated aberrant transcripts encoding severely truncated proteins of 41 to 603 aminoacids. Biallelic inactivation of the gene by deletion or mutation of the second allele was demonstrated in all mutated cases studied (N=9), indicating that, in these tumors, BLIMP1 is inactivated by the classic *two-hit* mechanism described for most tumor suppressor genes. Consistently, no BLIMP1 protein expression was detected by immunohistochemistry in these cases. In addition, immunohistochemical analysis of 58 primary biopsies demonstrated complete lack of BLIMP1 protein expression in most non-GCB DLBCL (N=20/26, 77%, including 4/4 unmutated cases), despite the presence of IRF4, a marker of plasmacytic differentiation which, in normal cells, is consistently co-expressed with BLIMP1. Consistent with our previous findings (Pasqualucci *et al.,* J Exp Med 203: 311, 2006), these data indicate that BLIMP1 mutations represent a common and specific genetic alteration in ABC-DLBCL, and suggest that alternative mechanisms, including epigenetic silencing or defects in protein translation or stability, may contribute to BLIMP1 inactivation in an additional fraction of cases. Notably, chromosomal translocations deregulating BCL6, a transcriptional repressor required for GC formation and downregulated in post-GC plasma cells, were only found in 1/12 BLIMP1 mutated cases, and were mostly restricted to the unmutated cases (N=19/139, 14%). Since it has been suggested that downregulation of BCL6 is also required for plasmacytic differentiation, our data suggest that BCL6 deregulation and BLIMP1 inactivation represent alternative pathogenetic mechanisms, which may contribute to lymphomagenesis by blocking terminal differentiation of B cells into plasma cells.

REPETITIVE HIGH DOSE THERAPY FOLLOWED BY AUTOLOGOUS STEM CELL TRANSPLANTATION (MEGACHOEP) AS PRIMARY TREATMENT FOR ALK NEGATIVE MATURE T-NHL: RESULTS OF 3 CONSECUTIVE PHASE II TRIALS

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Mature (peripheral) T-cell lymphoma (T-NHL) patients have a poor prognosis. The only entity with favorable outcome is anaplastic lymphoma kinase (ALK) positive anaplastic large cell lymphoma (ALCL). One major problem of mature T-NHL is the high rate of progressive disease during first line treatment. The German high grade lymphoma study group (DSHNHL) conducted 3 consecutive phase II trials consisting of dose escalated CHOP plus etoposide followed by 3 courses of high dose chemotherapy and autologous stem cell transplantation for younger patients (<60) with high risk Non Hodgkin's Lymphoma (NHL) defined by elevated LDH at diagnosis. Treatment course and doses were as described by Schmitz *et al.*, (Cancer 2006; 106(1):136) and Glass *et al.*, (Blood 2006;107(8):3058). From January 1997 to February 2003, 312 patients with aggressive NHL were enrolled. From 170 patients eligible for this analysis, 24 had ALK negative ALCL or non-ALCL mature T-NHL and 146 aggressive B-NHL, respectively. The T-NHL group consisted of 11 ALK- ALCL, 7 peripheral T-NHL unspecified, 3 angioimmunoblastic lymphoma (AIL), 2 NK-/ cytotoxic T-NHL and 1 T-NHL unspecified. 9 patients with ALK+ (6) or ALK-unknown (3) ALCL were not included into this analysis. The B-NHL group consisted mostly of diffuse large B cell lymphoma (83%). At diagnosis, the T-NHL patients had slightly higher IPI scores and more extranodal involvement (not statistically significant) and the B-NHL patients had a higher rate of bulky disease (69.7 vs. 24.2, $p<0.001$). During chemotherapy the T-NHL patients had a higher rate of primary progressive disease (27.3 vs. 15.8%); thus less patients received all the planned therapy (69.7 vs. 84.2%). There was a trend towards worse complete remission (CR) rate (51.5% vs. 67.1%) and worse overall survival (48.5 vs. 63.2, $p=0.074$), but these results were statistically not significant, probably due to low patient numbers. Even for T-NHL patients achieving a CR, the relapse rate was high (58%). The event free survival was significantly worse for the T-NHL patients than for B-NHL patients (25.0 vs. 58.8%, $p=0.0007$). This was confirmed by a multivariate Cox analysis. The MegaCHOEP protocol did not reduce the rate of primary progressive disease in this group of mature T-NHL patients and the relapse rate was high. Therefore, new strategies are needed to increase remission rates and reduce relapse rates. Primary allogeneic transplantation will be compared to HDT and autologous transplantation in an DSHNHL/EBMT trial planned to start early next year.

CHEMOIMMUNETHERAPY WITH CHOP SUPPLEMENTED BY CAMPATH-1H (CHOP-C) IN UNTREATED PTCL PATIENTS: PRELIMINARY RESULTS OF A PHASE-II MULTICENTER CLINICAL TRIALON BEHALF OF GITIL.

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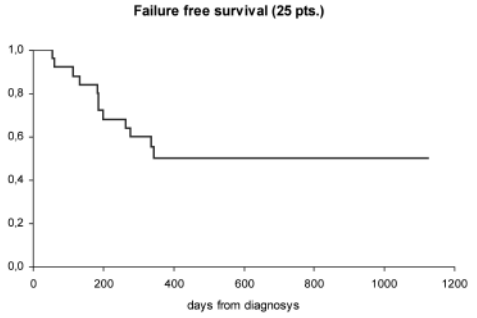
Introduction. The prognosis of PTCL is very poor, with a 5-year overall survival ranging between 25 and 40%. Promising results have been reported with Alentuzumab (Campat ®) in the therapy of neoplasms of T cell lineage, although the expression of CD 52 is variable in these disorders. Here we report the preliminary results of standard CHOP therapy plus Campath (CHOP-C) in a cohort of untreated PTCL patients (pts.). *Patients and Methods*. Starting from January 2003, 25 consecutive PTCL pts. were treated by CHOP for 8 courses, preceded on day -1 by Campath 30 mg. s.c. before the courses 1-4, for the first 4 pts. (Cohort-1), and before all the 8 courses for the remaining pts. (Cohort-2). The diagnosis was PTCL-U in 16 pts., AILD-T in 6, and ALCL Alk- in 3. Histopathology was centrally reviewed in all cases. CD 52 expression was checked on immunohistochemical grounds on paraffin-embedded material. The clinical characteristics of the 25 patients were: mean age 51.2 years (28-69), stage III-IV in 22, Bulky disease in 3, Bone marrow involvement in 11, high LDH

values in 13, IPI age-adjusted 0 in 5 pts., 1 in 8 pts., 2 in 9 pts., and 3 in 3 pts. 25/25 pts. completed the therapy and are therefore valuable for the analysis. *Results*. after a mean follow-up of 436 days (105-1124), 14/25 pts. are still alive and 11/25 have died mostly of progressive disease (10/11). 17/25 pts. achieved CR 1 PR and 1 MR; 6 pts. showed progression. 6/6 pts in Pro, 1 pt in RP, and 1 pt. in MR and 2 pt. in CR have died despite salvage treatment; one patient died +198 d. in CR for pneumonia. Three pts. relapsed +62, +119 and +145 days after CR. The mean duration of response was 273 days. The 2-y. OS and FFS were 45% and 50% days, respectively (Fig. 1). The CD 52 expression was available in 16/25 patients: in 11 progressing/relapsing pts. CD 52 was positive in 5, negative in 2 and not available in 4; in 14 responding pts. It was positive in 7, negative in 2 and not available in 5. *Conclusions*. C-CHOP seems a feasible chemoimmunotherapy regimen: the efficacy of this therapy seems fairly good (17/25 CR); however 6/25 pts. showed disease progression during therapy, most frequently after the third C-CHOP course. CD 52 was expressed on most of them (12/16), without any relationship with treatment outcome.



COLOGNE HIGH-DOSE SEQUENTIAL CHEMOTHERAPY IN RELAPSED AND REFRACTORY HODGKIN LYMPHOMA ≥40 MONTHS FOLLOW UP AND FIRST RESULTS FROM THE HDR-2 TRIAL OF THE GHSG/EORTC/EBMT

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Purpose. Combination chemotherapy can cure patients (pts) with Hodgkin lymphoma (HD), but those with treatment failure or relapse still have a poor prognosis. We thus, designed a dose- and time-intensified high-dose sequential chemotherapy regimen with a final myeloablative course. *Patients and Methods.* Eligibility criteria included age 18-65 years, histologically proven primary progressive (PD) or relapsed HD. Treatment consists of two cycles DHAP (dexamethasone 40 mg d1-4, high-dose cytarabin 2 g/m^2 12q d2, cisplatinum 100 mg/m^2 d1); pts with partial (PR) or complete remission (CR) received cyclophosphamide 4g/m^2, followed by peripheral blood stem cell (PBSC) harvest; methotrexate 8 g/m^2 plus vincristine 1,4 mg/m^2; and etoposide 2 g/m^2. The final myeloblative course was BEAM followed by PBSCT. *Results.* 102 pts (median age 34 years, range 18-64) were enrolled. The response rate (RR) at the final evaluation (100 days posttransplantation) was 80% (72% CR, 8% PR). PBSC harvest was succesful in 96% of pts. Toxicity was tolerable. With a median follow-up of 40 months (range 3-84 months) freedom from second failure (FF2F) and overall survival (OS) were 52% and 73% for all patients, respectively. FF2F and OS for patients with early relapse were 61% and 80%, for late relapse 62% and 81%; for PD: 41% and 45% and for multiple relapse 39% and 44%, respectively. In multivariate analysis response after 2 cycles of DHAP (p<0.0001) and duration of first remission (PD and multiple relapse vs. early and late relapse; p =0.0127) were prognostic factors for FF2F. Response after DHAP (p<0.0081), duration of first remission (p=0.0017) and anemia (p=0.019) were identified as prognostic factors for OS. *Conclusions*. We conclude that this regimen is feasible, tolerable and highly effective in poor risk patients with relapsed and refractory HD. Based on these results a prospective randomized european intergoup study was started comparing this intensified regimen with two courses of DHAP followed by BEAM (HD-R2 protocol). First results of the third interim analysis of this study will be presented.

AN ITALIAN MULTICENTER CONTROLLED STUDY OF HCV-RELATED MALIGNANCIES: ROLE OF THE HLA CLASS II

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Background. HCV is associated with chronic liver diseases and lymphoproliferative disorders. Increasing evidences indicate an important role of CD4+T cells responses for HCV clearance. Information regarding HLA II molecules restricted to specific HCV diseases are relevant to known pathogenetic mechanisms involved in such malignant processes. *Methods.* HCV-infected patients homogeneously distributed from different regions of Italy are considered. HLA class II DRB1 and DQB1 alleles were determined at high resolution by PCR amplification and sequencing. 116 subjects have a type II mixed cryoglobulinemic syndrome (MC+), 59 a NHL with MC, 60 a NHL without MC, and 58 an HCC. Data obtained were then compared with a control group of 14.923 and 4.575 italian bone marrow donors for HLA DRB1 and HLA DQB1, respectively. Results are then evaluated using the in silico conformational and functional characteristics of the HLA molecules supertype classification Results: DRB1 genotype analysis, evidences a consistent homozigosity in all the HCV-related malignancy , although the difference was marked in association with MC. Data of DRB1 and DQB1 in silico cluster-phenotype analysis: DR5 supertype is more associated to MC while DQ3 to NHL-Mcpos. By converse DR5 and DQ3 are protective for HCC. *Conclusions.* A different pathogenetic mechanism was evidenced in NHL from HCV patients with autoimmunity with respect to that without a previous or a concomitant MC autoimmune syndrome. An opposite HLA distribution pattern was found for HCC patients. HLA supertypes clustering offer an approach to better understand the HCCV-related mechanisms inducing different malignancies.
Controls. N=14923 (%) MCN=116 (%) NHL-McPosN=59 (%) NHL-McNegN=60 (%) HCCN=58 (%) DR1 8043 (53.90) DR1-DR1 2121 (14.21) DR1-DR2 2921 (19.57) DR1-DR7 3001 (20.10) 49 (42,24)11 (9,48)25 (21,55)13 (11,21) 27 (45,76)9 (15,25)8 (13,56)10 (16,95) 43 (71,66)18 (30,00)14 (23,33)11 (18,33) 44 (75,86)4 (6,90)26 (44,83)14 (24,14)
DR3 2318 (15.53) 21 (18,10) 9 (15,25) 3 (5,00) 10 (17,24)
DR4 4054 (27.16) 29 (25,00) 17 (28,81) 14 (23,33) 7 (12,07)
DR5 13020 (87.25) 113 (97,41) 56 (94,91) 50 (83,33) 45 (77,58)
N=4575 (%) N=116 (%) N=59 (%) N=60 (%) N=58 (%)
DQ1 3086 (67.45) 65 (56,44) 37 (63,16) 38 (63,33) 36 (62,07)
DQ2 1539 (33,63) 22 (26,73) 12 (21,05) 12 (20,00) 20 (34,48)
DQ3 3536 (77,29) 67 (79,20) 51 (85,96) 47 (78,33) 26 (44,83).

HEPATITIS C-VIRUS POSITIVE SPLENIC MARGINAL ZONE LYMPHOMA PRESENTING FINDINGS AND OUTCOME IN A SERIES OF 17 PATIENTS

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Background. Case-control and epidemiological studies have shown an association between HCV infection and some histotypes of non Hodgkin lymphomas. Recently, therapy with interferon and ribavirin has been reported to produce good quality and sustained remissions in HCV-positive SMZL patients, after the loss of HCV-rna. These results have raised the idea that HCV may play an etiological role or at least may act as a cofactor in the development of SMZL. However, there are only few data about the presenting features and the clinical outcome of HCV-positive SMZL patients who do not undergo an antiviral treatment. We have retrospectively analysed our series of 120 SMZL patients. *Results.* Serology for hepatitis viruses was available in 98 cases. Seventeen patients (17.3%) were HCV positive, eight (9%) were HbsAg positive, and two patients had both infections. The median age of HCV positive patients was 61 (range 47-77) and nine patients were male (53%). Except for three patient (17%), who complained of B-symptoms (weight loss or night sweats), the performance status was good. Moderate anemia (Hb< 11 gr/dL) and lymphocytosis (>5000) were recorded in 30% and 65% of cases, respectively. Small peripheral lymphnodes were recorded in four patients and three of them had also abdominal adenopathies. Five patients (29%) had a small monoclonal (<2 gr/dL) component (MC) in the peripheral blood (4 IgM-K; 1 IgG-K). Crioglobulins were detected in 3 out nine screened patients; of them, one suffered of secondary recurrent vascular purpura. According to the Intergruppo Italiano Linfomi score system, 35% of patients fell in the low-risk group, 35% in the intermediate-risk, and 30% in the high-risk group. Eleven patients (65%) were followed-up without therapy (watch and wait), four received monotherapy with chlorambucil. Two patients received an HCV-oriented therapy (1-IFN; 1-IFN+Ribavirin). With a median follow-up time of 15 months (range, 4-81) only one patient died of progressive lymphoma. Comparing clinical features and outcome of HCV-positive SMZL cases to those of the 103 HCV-negative patients, only the incidence rate of hepatomegaly (47% vs 20%; p=.009) and hypertransaminasemia (65% vs 49%; p=.000) proved significantly different. *Conclusions.* HCV-positive SMZL patients display presenting features similar to those of HCV-negative cases, except for data associated to the liver disease. In this series, HCV-positive patients showed an indolent clinical course that allowedthe adoption of a watch-and-see policy in 53% of cases. Furthemore, 93% of patients are projected to be alive at four years from diagnosis.

PRELIMINARY RESULTS OF A PHASE II STUDY OF LENALIDOMIDE ORAL MONOTHERAPY IN RELAPSED/REFRACTORY AGGRESSIVE NON-HODGKIN'S LYMPHOMA

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Background. Lenalidomide (Revlimid®), an immunomodulatory drug (IMiD®), was recently approved in the US for treatment of relapsed/refractory multiple myeloma and myelodysplastic syndromes associated with a deletion 5q[31] cytogenetic abnormality. Lenalidomide also has demonstrated activity in chronic lymphocytic leukemia and cutaneous T-cell lymphoma while thalidomide, a less potent IMiD®, has activity in non-Hodgkin's lymphoma as both monotherapy and in combination with rituximab. Aim: To assess the safety and activity of lenalidomide monotherapy in subjects with relapsed/refractory aggressive non-Hodgkin's lymphoma (NHL). *Methods*. Subjects with relapsed/refractory aggressive NHL following at least 1 prior treatment regimen with measurable disease are eligible. Subjects receive 25 mg lenalidomide orally once daily on Days 1-21 every 28 days and continue therapy for 52 weeks as tolerated or until disease progression. Response and progression are evaluated using the IWLRC methodology. *Results.* Twenty-five subjects of a planned 40 have enrolled and 22 have received drug. Sixteen subjects are currently evaluable for tumor response. The median age of the 16 response-evaluable subjects is 63 (45-80) and 8 are female. Histology is diffuse large B-cell lymphoma [DLBCL] (n=8), follicular center lymphoma grade 3 [FL] (n=2), mantle cell lymphoma [MCL] (n=3), transformed [TSF] (n=1) and unknown (n=2). Median time from diagnosis to lenalidomide monotherapy is 2.3 (1-8) years and median number of prior treatment regimens per subject is 2.5 (1-6). Five of 16 subjects (31%) exhibited an objective response (2 complete responses unconfirmed (CRu) and 3 partial responses (PR)), 4 had stable disease (SD) for a tumor control rate (TCR) of 56% and 7 progressive disease (PD). Lenalidomide produced a response in each of the aggressive histologic subtypes studied: DLBCL (3/8), FL (1/2), MCL (1/3). Median time-to-response was 2 months. Grade 3 or 4 adverse events occurred in 10 of 22 (45%) subjects receiving drug. These were predominantly Grade 3 hematological events (neutropenia, thrombocytopenia) with only 3 subjects (14%) experiencing a Grade 4 adverse reaction. *Conclusions*. Preliminary results indicate that lenalidomide oral monotherapy is producing a 31% response rate and manageable side effects in relapsed/refractory aggressive NHL. Further investigation of lenalidomide in NHL, both as monotherapy and in combination with other agents, is warranted. *Keywords.* Lenalidomide, Monotherapy, Phase II, Non-Hodgkin's Lymphoma

THE VALUE OF RITUXIMAB IN CLEARING MINIMAL RESIDUAL DISEASE (MRD) IN MOLECULAR RELAPSES OF MANTLE CELL LYMPHOMA.

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Introduction. Mantle Cell Lymphoma (MCL) is one of the worst subtypes of B-cell lymphoma. In MCL molecular remission (MR) predicts improved outcome (Pott *et al.*, Blood 2006). If MR is lost patients are at high-risk of relapse. Treatment with Rituximab, in MCL, seems to be beneficial in combination either with conventional chemotherapy or with autologous stem cell transplantation (ASCT). Especially the latter treatment showed excellent results and one important observation was the frequent achievement of MR, as opposed to patients treated with Rituximab-free autografting regimens. However, the value of this drug is not exhaustively established. We studied the molecular and clinical follow-up of four patients with post-autograft molecular relapse (M-rel) treated with Rituximab. Methods. M-rel was defined as the occurrence of PCR-positivity in two consecutive bone marrow samples in a patient, with previous MR and ongoing clinical complete remission (CR). Our patients relapsed at 3, 6, 39 and 52 months, respectively. M-rels were confirmed by direct sequencing of the t(11;14) for two patients and IgH rearrangement for the other two. Monitoring of minimal residual disease (MRD) was performed by qualitative and Real-time quantitative PCR. All patients received four Rituximab courses at the dosage of 375 mg/sqm, with two additional infusions if MR was not reinduced. Results. CR was observed in all patients throughout the whole follow-up, that is respectively 18, 55, 71 and 72 months from transplantation. All the M-rels had the same rearrangement seen

at diagnosis. Rituximab treatment was effective on all the four M-rels as all patients reverted to a PCR-negativity following four or six Rituximab courses. This status is currently maintained in three patients at 3, 6 and 18 months. Only one patient experienced a second molecular relapse 24 months later, again sensitive to the drug. Interestingly, Real-time PCR showed stable or increasing tumor burden before Rituximab delivery and a progressive clearance of tumor cells after treatment. This reduction resulted in a re-achievement of MR after four courses in two patients and after six courses in the other two. *Discussion.* For the first time, our study proved that Rituximab can reinduce MR in MCL patients experiencing M-rel following intensified Rituximab-containing autografting regimen. None of the patients, re-entering MR, experienced clinical relapse. This showed the value of Rituximab in inducing effective clearance of residual MCL cells and suggests that molecularly tailored maintenance therapy needs to be investigated in clinical trials.

THE HISTONE DEACETYLASE INHIBITOR ITF2357 INDUCES APOPTOSIS OF LEUKEMIA CELLS AND INHIBITS IL-6 AND VEGF PRODUCTION BY BONE MARROW MESENCHYMAL STROMAL CELLS

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Several studies demonstrate that aberrant acetylation/deacetylation of histone proteins is an integral part of growth arrest, differentiation and apoptosis in many tumours. Various agents such as the Histone Deacetylase Inhibitors (HDACIs) modify histone status and are being examined for their potential therapeutic use in the treatment of numerous malignancies and in particular of multiple myeloma (MM) and acute myeloid leukemia (AML). We investigated the activity of ITF2357 (Italfarmaco, Italia), a novel hydroxamate HDAC inhibitor (HDACi), on MM and AML cells. ITF2357 induced apoptosis in 8/9 MM and 6/7 AML cell lines, with a mean IC50 of 0.17 μM and 0.23 μM compared to 0.95 μM and 0.83 μM for SAHA, the prototypic HDACi. ITF2357 had potent cytotoxic activity also against freshly isolated purified MM and AML cells, with a mean IC50 of 0.23 μM and 0.15 μM in 4/4 MM and 18/20 AML cases, respectively. ITF2357 induced hyperacetylation of histone H4 in both resistant and sensitive lines, suggesting that histone hyperacetylation is not sufficient for apoptosis. Through RT-PCR and western blot analysis, we also examined the expression of several molecules involved in the control of apoptosis and found that ITF2357 upregulates p21 RNA and protein and down-modulates bcl-2 and bcl-xL gene expression. We also set up a co-culture system at least in part mimicking the bone marrow microenvironment, in which either the IL6-dependent MM cell line CMA-03 or freshly isolated AML samples were grown *in vitro* on human multipotent mesenchymal stromal cells (MSCs). In this system ITF2357 was still strongly cytotoxic for leukemic cells but not for MSCs, with an IC50 of 0.3-0.4 μM. Interestingly, ITF2357 inhibited by 80-95% the production of IL-6, VEGF and IFN-γ by MSCs, but had no effect on IL-8 secretion. Altogether these data demonstrate that ITF2357 has potent anti-neoplastic activity through direct induction of apoptosis and inhibition of IL-6 and VEGF production by bone marrow stromal cells.

DIFFERENT *IN VITRO* ALEMTUZUMAB-INDUCED APOPTOSIS ON T AND B LYMPHOCYTES OF B-CLL PATIENTS

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Alemtuzumab, a monoclonal anti-CD52 antibody, has shown high efficacy against lymphoproliferative disorders such as B-cell chronic lymphocytic leukemia (B-CLL). However, its use results in a profound immunosuppression with decrease of T lymphocytes leading to increased susceptibility to infections. The aim of our study was to assess the *in vitro* cytotoxic effect of alemtuzumab on normal T and neoplastic B lymphocytes obtained from B-CLL patients. Peripheral blood mononuclear cells (PBMC) from 12 B-CLL patients (5 at diagnosis and 7 previously treated) were collected and treated *in vitro* with alemtuzumab (10 μg/mL) and autologous serum in the culture as complement source. Spontaneous and alemtuzumab-induced apoptosis were quantified in T (CD3+) and B (CD20+) lymphocytes after 24 hours using an annexin-V flow cytometry based asssay. Alemtuzumab was able to induce apoptosis on both B and T lymphocytes of CLL patients. However, the cytotoxic activity on neoplastic B cells was comparable in all cases analyzed, irrespective of stage or previous treatments. Spontaneous apoptosis of B CLL cells was also comparable in all studied samples. On the contrary, the activity of alemtuzumab on normal T lymphocytes varied according to stage of disease and previous treatment. In early stages (0-I) alemtuzumab induced apoptosis in 19 % of T cells vs 60% of advanced stages (II-IV) (p=0,009). Differences were also relevant when patients were split by treatment: in fact, alemtuzumab induced apoptosis in 15% of T cells of untreated patients vs 52% of T cells of previously treated patients (p=0,005). Moreover, spontaneous apoptosis of T cells was more pronounced in treated vs. untreated patients (15% vs 2%, p=0.03) while the difference was not statistically significant in early vs advanced stage (p=0.06). Our *in vitro* data confirm that alemtuzumab is active against B-CLL cells in all stage of disease. However, in advanced stages and in previously treated patients, T cell compartment seems to be more fragile and susceptible to both spontaneous and alemtuzumab-induced apoptosis. This observation supports the hypothesis that using Alemtuzumab earlier in the treatment of B-CLL might result in less immunosuppression.

FRONTLINE HIGH DOSE SEQUENTIAL CHEMOTHERAPY WITH RITUXIMAB (R-HDS) AND AUTOLOGOUS STEM CELL TRANSPLANTATION INDUCES HIGH RATES OF COMPLETE RESPONSE AND PROLONGS SURVIVAL IN MANTLE CELL LYMPHOMA

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Mantle cell lymphoma (MCL) is characterized by its aggressiveness and refractoriness to standard therapy. Recently we showed that the dismal outcome of MCL might be improved in 28 young patients (<61 years) by an up-front rituximab supplemented high-dose sequential chemotherapy (R-HDS) supported by stem cell autograft (A.M. Gianni *et al.*, Blood, 2003). Following this encouraging experience we treated other 26 patients aged<61 years with standard R-HDS and 19 aged >60 years with an age-adapted R-HDS. The present study reports the outcome of 54 young patients (group 1), including the first 28 cases and that of 19 elderly patients (group 2). At enrolment, the median age of group 1 was 50 years, range 33-60 years, while in group 2 it was 65 years, range 61-70 years. The majority of both groups had Ann Arbor stage >II (96% in young and 100 % in old patients) and bone marrow infiltration (70 and 89% , respectively), while in elderly patients prevail B symptoms (53% vs. 19%) and more than 1 extranodal site (58% vs. 28%). One third of cases had more than two adverse prognostic features according to age-adjusted IPI (aaIPI). After a debulking phase consisting of two or three cycles of either doxorubicin- or cisplatin-containing chemotherapy, group 1 received standard R-HDS including: HD-cyclophosphamide 7 gr/sqm and HD-Ara-C (2 g/sqm every 12 hours for 6 days). Following the HDS chemotherapy regimen a myeloablative transplantation program with HD-melphalan (180 mg/sqm) and/or HD-mitoxantrone plus melphalan (60 and 180 mg/sqm, respectively) with autologous PBSC transplantation was planned. Rituximab (375 mg /sqm) was given for a total of 6 dos-

es, twice after HD-CTX and HD-Ara-C , as in vivo purging before CD34+ cells harvest and twice after the final myeloablative step. In elderly patients the dosage and schedule of R-HDS was age-adapted: HD-cyclophosphamide (3-4 gr/sqm) and HD-Ara-C (1-1.5 g/sqm every 12 hours for 3-5 days), followed by HD-melphalan (120 mg/sqm) and HD-mitoxantrone plus melphalan (40 and 120 mg/sqm, respectively). Thirty five patients (65%) in group 1 and nine (47%) in group 2 completed the planned program and a median number of 7.6 and 6.8×10^6 cells CD34+/kg were transplanted. After autologous transplantation, the CR rate was 88% in young and 95% in elderly patients. Two patients (4%) in group 1 achieved a PR and 3 (6%) showed no response or progressive disease, while in group 2 only one patient (5%) had a progressive disease. One young patient (2%) died during treatment, while none of elderly patients died of acute toxicity. Eventually, 13 remitters of group 1 relapsed (25%), one developed secondary MDS and five died of late toxicity including a case of lung carcinoma. Among elderly patients six relapsed (33%) and only one died tardily because of cardiac disease. With a median follow-up of 48 months (range 8-101) in group 1 and 25 months (range 9-68) in group 2, the 5-year estimated overall survival (OS), event-free survival (EFS) and disease-free survival (DFS) are 77%, 60% and 71%, in group 1 and 55%, 53% and not yet achieved in group 2. The Cox multivariate analysis failed to identify within potential prognostic markers factors predictive for OS and EFS.. In conclusion, these data obtained on a large population of patients followed for a relatively long period of time suggest that rituximab-supplemented HDS chemotherapy is an effective regimen for the induction of complete remissions in patients with newly diagnosed MCL. The manageable toxicity of the program in elderly patients, proved that an age-adjusted R-HDS regimen can be safely applied to this age subgroup still producing long-term remissions. Larger prospective randomized trials are needed to define its role compared with novel, competitive strategies.

YTTRIUM-90-LABELED IBRITUMOMAB TIUXETAN (ZEVALIN) FOR PRIMARY CNS LYMPHOMA

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Background. Salvage treatment has not yet been established in primary CNS lymphoma (PCNSL). Here we report preliminary results of an ongoing study with a single treatment course of Y-90 anti-CD20 antibody ibritumomab tiuxetan in relapsed/resistant PCNSL. *Methods.* Eligibility criteria include histologically confirmed, recurrent PCNSL after at least one prior treatment, HIV negativity and adequate bone marrow and cardiac function. Primary endpoint is overall response, secondary endpoints are response duration, survival, and toxicity including late neurotoxicity. Treatment includes rituximab 250 mg/m^2 on day -7 and day 0, followed by Y-90-ibritumomab tiuxetan 15 MBq/kg IV. Response evaluation by contrast-enhanced magnetic resonance imaging (MRI) and fluorodeoxyglucose positron emission tomography (FDG-PET) is scheduled before, one month and two months after treatment as well as every three months thereafter in responders. In three patients single photon emission computed tomography (SPECT) target imaging with gamma-emitting 111-Indium-ibritumomab tiuxetan was performed repeatedly. *Results.* Four of seven patients enrolled thus far responded. In one patient complete response (CR) with minimal residual contrast enhancement (uncertain CR) lasting 18+ months was observed. In two additional patients CR was achieved lasting 0.5 and one month, respectively. One patient had partial response lasting 0.5 months. Relapse usually occurred distant of target lesions. Three patients had disease progression. Three patients developed CTC grade 2 leukopenia with grade 3 pneumonia in two patients (with fatal outcome in one patient). Four patients developed grade 3 thrombocytopenia lasting up to 6 weeks. SPECT indicated target accumulation in the tumor starting 48 hours and still ongoing 7 days after injection of 111-Indium-ibritumomab tiuxetan. *Conclusions.* This is the first report on activity of Y-90-ibritumomab tiuxetan in PCNSL and penetration of the antibody into the tumor, warranting further investigation. Hematotoxicity should be regarded.

COMBINING *ERADICATING THERAPY* AND RITUXIMAB FOR HIGH-GRADE GASTRIC MUCOSA-ASSOCIATED LYMPHOID TISSUE (MALT) LYMPHOMA IN LIMITED STAGE

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Background. Complete and durable clinical remission can be obtained in about 64% of patients with high-grade Helicobacter Pylori (HP) positive, gastric lymphoma in stage IE, following HP *eradicating therapy* (ET), without the need of additional chemo and/or radiotherapy. Aim of the present study was to verify whether combining ET with anti-CD20 therapy (Rituximab) may improve the complete remission rate in early-stage high grade HP+ gastric lymphoma. *Patients and methods.* the prospective trial was started in 2003; patients entered the study protocol if presenting with a previously untreated HP+, gastric high grade MALT lymphoma in stage IE. Patients were treated initially with ET, including omeprazole 20 mg twice/day x 2 weeks followed by 20 mg day x 2 further weeks, claritromicine 500 mg twice/day x 2 weeks, tinidazole 500 mg twice/day x 2 weeks. Patients were re-evaluated at six weeks since therapy start, by performing gastric endoscopic ultra sonography (EUS). Patients who did not obtain at least a 50% reduction of the volume or depth of lesions were then treated with anti-CD20 Rituximab at the standard schedule of 375 mg/m^2 once a week for four weeks. A second clinical re-assessment was then performed, including endoscopic, histological and EUS evaluation. If a complete remission was not achieved after this period an additional four-week Rituximab treatment was planned. Patients who reached more than 50% of reduction after six weeks were followed up with EUS. Patients not achieving complete remission (CR) were re-treated with Rituximab while patients with non-responsive disease were shifted to chemotherapy ± radiotherapy. *Results*. four consecutive patients with high grade HP+ and CD20+ gastric lymphoma, stage 1E1, were treated according to the protocol. One patient reached a less than 50% response following ET; he then underwent Rituximab and 6 weeks later a CR was documented and maintained up to date for 19 months. Two patients obtained a partial remission (more than 50 % of mass reduction): at the second evaluation one patient obtained CR after ET only; this patient is presently in continuous CR after 31 months of follow-up; the second patient did not reach CR and was treated with the four-week Rituximab program, which allowed CR achievement; also this patient is still in CR at 8 months since Rituximab. The last patients obtained a CR at the first re-assessmemnt following ET only; he is still in CR at 7 months. *Conclusions.* these preliminary results suggest that the combination of ET and Rituximab can allow to obtain a long-lasting remission in early stage high grade HP+, CD20+ gastric lymphoma; thus, invasive treatments, including chemotherapy, radiotherapy and ultimately surgery, have a minor role, most likely as salvage therapy, in the management of limited-stage aggressive HP+ gastric lymphoma.