''Focus on...'' session: plasma cell disorders and Waldenström macroglobulinemia

114 CYTOGENETIC ABNORMALITIES IN A COHORT OF 175 PATIENTS WITH WALDENSTRÖM MACROGLOBULINEMIA BEFORE TREATMENT: CLINICAL AND BIOLOGICAL CORRELATIONS

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The genetic bases of WM are poorly understood. Because of the difficulty in obtaining tumor metaphases, few recurrent chromosomal abnormalities have been reported.

We studied a cohort of 175 patients, enrolled in a prospective randomized trial from the French Cooperative Group on CLL and WM, and compared the efficacy of fludarabine to that of chlorambucil, using karyotype (CC) and fluorescence *in situ* hybridization (FISH).

CC was performed on bone marrow or peripheral blood, and FISH was carried out using 8 probes CEP4, CEP12, 13q14, 11q22 (ATM), 17p13 (TP53), IGH, BCL2 Abbott, 6q21 Q-Biogene. The gender ratio M:F was 2.12, the median age at inclusion was 67 y [58-73]. The mean percentage of lymphoplasmacytic cells was 50% [4-93]. Out of 142/175 (81%) successful CC, 67 (38%) showed clonal abnormalities. Out of 67 abnormal CC, 20 (30%) were complex, with at least 3 chromosomal changes, and 23 (34%) showed translocations. Using FISH, we observed 44/136 (32.4%) del6q, 15/108 (13.9%) tri18, 18/144 (12.5%) del13q14, 11/137 (8%) tri4, 11/138 (8%) del*TP53*, 10/135 (7.4%) del*ATM*, 5/139 (3.6%) tri12, and 3/122 (2.5%) *IGH* rearrangements.

Chromosomal abnormalities were compared to adverse characteristics described by Morel et al (Blood 2009,113,4163-70): age >65y, hemoglobin (Hb) <11.5g/dL, platelet count <100x10^9/L, b2microglobulin (b2M) >3mg/L, and IgM >7g/dL. Patients with a 6q deletion had significantly more frequently b2M >3mg/L (29/44 [66%] vs. 43/92 [47%], p=.04), Hb <10g/dl (28/44 [64%] vs. 40/92 [44%], p=.04), and IgM >7g/dl (4/44 [9%] vs. 1/92 [1%], p=.03). Moreover, they had more frequently albumin <3.5g/dl (24/44 [55%] vs 14/92 [15%], p<.001). Similarly, patients with trisomy 4 had significantly more frequently b2M >3 mg/l (10/11 [91%] vs. 61/126 [48%], p=.009). Of note, all patients with trisomy 4 had an IgM >2 g/dl (11/11 [100%] vs. 83/126 [66%], p=.01). Finally, there were significantly more patients with age >65y (9/10 [90%] vs. 68/125 [54%], p=.04) and with albumin <3.5g/dl (6/10 [60%] vs. 33/125 [26%], p=.03), when *ATM* deletion was observed. Even if cytogenetic abnormalities did not influence the response rate (RR) and overall survival (OS) (median follow up: 39.7 months), the del*TP53* was associated with a shorter time until treatment failure (TTF) (7.8 vs.25.7 m, p=.001) as assessed in a multivariate analysis.

Cytogenetic abnormalities in WM differ from those commonly reported in other B-cell neoplasms and confirm the originality of this disease. The 6q deletion is the most frequently reported cytogenetic abnormality in WM. Even if this abnormality is correlated with biological adverse parameters, it did not influence the RR, progression free survival, TTF and OS. The *TP53* deletion could be detected early in the course of the natural disease, and is associated with shorter TTF. This indicates that treatment could be adapted for these patients, as it is for chronic lymphocytic leukemia.

115 MICRORNA-9* REGULATES HISTONE ACETYLATION IN WALDENSTRÖM'S MACROGLOBULINEMIA

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Introduction: Primary Waldenström's Macroglobulinemia (WM) cells are characterized by a specific microRNA (miRNA) signature compared to the related normal cellular counterpart; among deregulated miRNAs, we found that primary WM cells present with a reduced expression of miRNA-9* that has been predicted to target histone acetylation regulatory genes. Importantly, it has been shown that epigenetic regulation of gene-expression, such as histone-acetylation, is commonly deregulated in tumors. We therefore looked at miRNAs as possible regulators of histone-acetylation in WM.

Material and methods: miRNA- and gene-expression-profiling have been performed on primary CD19+ cells isolated from bone marrow of patients with WM, and compared to their normal cellular counterparts. Validation was performed by using stem-loop-qRT-PCR on matched samples. Functional assays were performed on WM and IgM secreting lymphoma cells, transfected with either scramble- or precursor-miRNA-9*-probe: modulation of signaling cascades and apoptosis have been evaluated by either immunofluorescence or western-blot. DNA synthesis, cell survival, apoptosis, cell cycle progression were assessed by thymidine uptake, MTT, PI staining, Apo2.7 and flow cytometry analysis, respectively. HDAC activity was measured by a colorimetric HDAC activity assay kit.

Results: miRNA profiling performed on primary WM cells showed decreased expression of miRNA-9*, as compared to normal cells ($P< 0.01$). Predicted targets for miRNA-9* included histone-deacetylases (HDAC4; HDAC5) and histone-acetyltransferases (Myst3), as shown by using TargetScan, PicTar, and miRanda algorithms. We first described that primary WM cells present with an unbalanced expression of HDACs and HATs at gene level; together with a decreased acetylated-histone-H3 and -H4, at protein level, and an increased HDAC activity. We next found that miRNA-9* regulated histone-acetylation and HDAC activity in WM cells, based on its ability to target HDACs and HATs; leading to induction of toxicity in precursor-miRNA-9*-transfected cells, as shown by reduced proliferation rate, cell cycle arrest, induction of apoptosis. This was supported by PARP-, caspase-8-, caspase-9-cleavage. In addition, miRNA-9* induced autophagy in WM cells by modulating Rab7 and LC3B. Similar data were confirmed in other IgM secreting lymphoma cells.

Conclusion: These findings indicate that histone-modifying genes and HDAC activity are de-regulated in WM cells, partially driven by a reduced expression of miRNA -9* in the tumor clone, providing the basis for miRNA-based-targeted therapies in this disease.

116 FAMILIAL DISEASE STATUS IS ASSOCIATED WITH AN INFERIOR TREATMENT OUTCOME IN PATIENTS WITH WALDENSTRÖM'S MACROGLOBULINEMIA.

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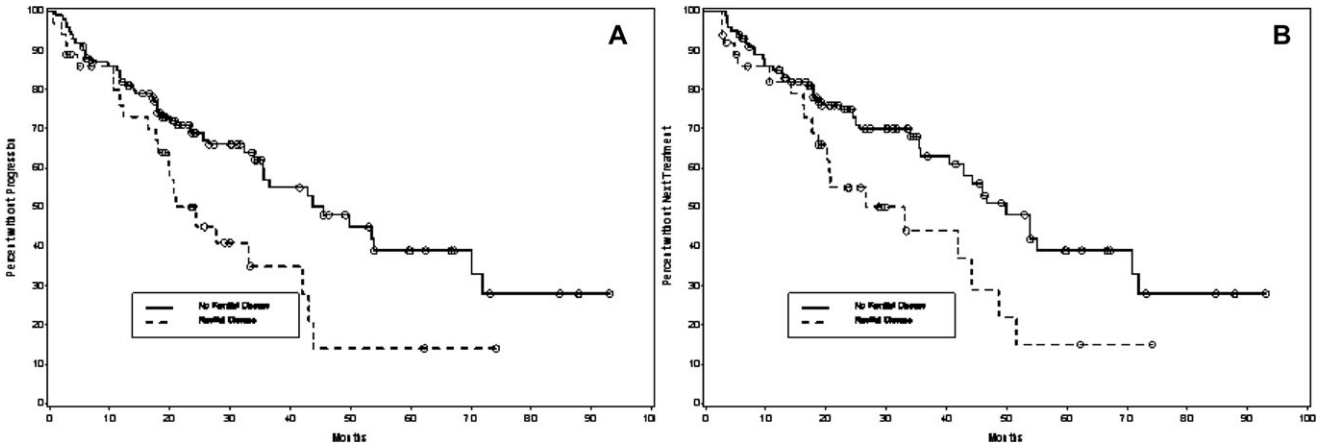
Background: Previous studies by us and others support a familial predisposition in certain patients with Waldenstrom's Macroglobulinemia (WM). The incidence, as well as clinical implications for familial WM disease need to be clarified.

Patients and Methods: We sought to delineate the relative incidence of familial and sporadic WM in a large population of WM patients, as well as treatment outcomes resulting from the presence of familial disease. As part of these efforts, we examined the impact of familial disease status on a well defined WM patient population whose treatment outcome was previously reported, and who were rituximab-naïve, and received a rituximab containing regimen. WM patients with familial disease were defined as those individuals who had a first or second degree relative with a B-cell disorder.

Results: Among 924 consecutive patients seen at our center, 254 (27.4%) reported having a first or second degree relative with a B-cell disorder, including 45 (4.9%) patients with > 1 case of WM in their family. One-hundred-thirty-five patients were rituximab-naïve, and received a rituximab containing regimen with either cyclophosphamide, fludarabine, IMID, or bortezomib. Of these patients, 36 (26.7%) and 99 (73.3%) had familial and sporadic WM disease, respectively. No differences in age, baseline IgM, hemoglobin, platelet count, serum B_2M, WM IPSS score, or treatment regimen received was observed among these patients. Overall (74.8% vs. 55.6%; p=0.032) and CR/VGPR (23.2% vs. 16.7%; p<0.0001) responses, time to progression (45.5 vs. 21 months; p=0.015; Fig. 1 A), as well as time to next therapy (50.0 vs. 33.0 months; p=0.024; Fig. 1B) were greater among sporadic versus familial WM patients.

Conclusions: Familial predisposition is common in WM, and associated with an inferior treatment outcome. Prospective studies examining the impact of familial disease status on treatment outcome in WM are warranted.



117 RESPONSES TO SINGLE-AGENT CARFILZOMIB (CFZ) ARE NOT AFFECTED BY CYTOGENETICS IN PATIENTS (PTS) WITH RELAPSED AND REFRACTORY MULTIPLE MYELOMA (R/R MM)

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Introduction: The impact of cytogenetic abnormalities on therapeutic response in pts with MM is well-established. Bortezomib (BTZ) overcomes the adverse impact of several common unfavorable cytogenetic features, as do lenalidomide (LEN) /dexamethasone, and pomalidomide, albeit to a lesser extent. CFZ is a novel, highly selective epoxyketone proteasome inhibitor with durable single-agent activity. This analysis evaluated the influence of cytogenetics in a large phase 2b study (PX-171-003-A1) of single-agent CFZ in pts with R/R MM.

Methods: 229/266 pts enrolled were response evaluable and had available metaphase cytogenetic data (200 pts [75%]) and/or fluorescence in situ hybridization (FISH) data (205 pts [77%]). Unfavorable cytogenetic abnormalities were defined per mSMART criteria: del 17p, t(4:14), and t(14:16) by FISH; hypodiploidy and chr 13 deletions by metaphase cytogenetics. All pts received CFZ at 20 mg/m2 IV on days 1, 2, 8, 9, 15, and 16 of a 28-day cycle (C) in C1 followed by 27 mg/m2 in C2–12. The overall response rate (ORR) by International Myeloma Working Group criteria and the clinical benefit response rate (CBR) were assessed by investigators and confirmed by Independent Review Committee.

Results: Pts had relapsed disease after ≥2 regimens including BTZ and either thalidomide (THAL) or LEN, and were refractory to their last regimen. In this heavily pre-treated pt population, 99.6% of pts previously received BTZ, 74% received prior THAL, 94% prior LEN, and 74% prior stem cell transplantation.

The ORR (≥ PR) for pts with available cytogenetic/FISH data was 25% and the CBR was 36%. 71/229 pts (31%) had ≥1 abnormality. These were detected by metaphase analysis in 47 pts (66%), by FISH in 44 (62%), and by both in 20 (28%). The presence of del13, hypodiploidy, del17p13, t(4;14),or t(14;16) did not significantly impact responses – updated data will be presented. The ORR was 28% in pts with ≥1 abnormality; 24% in those with 0. The CBR was similarly unaffected. The median OS for pts with ≥1 abnormality was 19.2 mo; the median OS for pts with 0 was not reached. The median duration of response (DOR) for all pts was 7 mo (95% CI 6–9); for pts with ≥1 unfavorable abnormality 6 mo (95% CI 4–10); and for those with 0, 8 mo (95% CI 6–10).

Response Category, n (%)	Normal/Favorable (N=158)	Unfavorable (N=71)	Total (N=229)
CR	1 (0.6)	0 (0)	1 (0.4)
VGPR	10 (6.3)	3 (4.2)	13 (5.7)
PR	27 (17)	17 (24)	44 (19)
MR	21 (13)	3 (4.2)	24 (11)
SD	49 (31)	28 (39)	77 (34)
PD	41 (26)	18 (25)	59 (26)
ORR	38 (24)	20 (28)	58 (25)
CBR	59 (37)	23 (32)	82 (36)

Conclusions: CFZ demonstrated comparable, durable activity in pts with R/R MM in both the absence and the presence of cytogenetic abnormalities. This study suggests that responses to CFZ and DOR in heavily pretreated pts are not impacted by poor prognostic cytogenetic features. *The authors wish to acknowledge the support of the Multiple Myeloma Research Consortium (MMRC).*

118 IL-6 MEDIATED HEMATOPOIESIS DEVIATION FAVORS APRIL PRODUCTION DURING MULTIPLE MYELOMA INVASION OF BONE MARROW

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Background: A proliferation inducing ligand (APRIL/TNFSF13) is a TNF member implicated in plasma-cell survival.

Methods: In order to study the *in vivo* role of APRIL in multiple myeloma (MM) development, we used two new multiple myeloma models, one xenogeneic based on the backcrossing of APRIL$^{-/-}$ mice onto an immunodeficient background, and one syngeneic based on the selection of a variant (MOPC-315$_{BM}$) of the MOPC-315 plasmocytoma cell line able to invade mouse BM following intravenous injection.

Results: In the xenogeneic model, we are showing for the first time that lack of APRIL severely impairs the *in vivo*development of multiple myeloma (MM). We recently showed that the bone marrow (BM), the orthotropic site of MM development, is an organ constitutively rich in APRIL. Indeed, the most abundant hematopoietic precursors, i.e. myeloid precursors, constitutively secrete high levels of APRIL. In the syngeneic model, we further observed that MOPC-315$_{BM}$ selectively protects APRIL-producing myeloid precursors in an IL-6 dependent manner upon BM invasion, while all other hematopoietic precursors are downregulated. This process insures a stable level of APRIL expression in MOPC-315$_{BM}$-invaded BM.

Conclusion: Our present study shows how MM selectively deviates hematopoiesis to maintain a favorable cytokine milieu for a maximal BM invasion. Hematopoietic reconstitution is mandatory for patients' recovery to multiple myeloma treatments. The link demonstrated here between hematopoiesis and multiple myeloma development may explain why multiple myeloma is still an incurable disease.