''focus on...'' session: lymphoma biology

127 DETECTION OF *MYC* MUTATIONS IN MOLECULARLY CLASSIFIED AGGRESSIVE LYMPHOMAS

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Introduction: Mutations of the *MYC* gene found in 50-60% of Burkitt-lymphoma (BL) probably play a role in its oncogenic activation. They affect the *MYC* promoter region (*MYC* PR) and the´transactivation-domaiń (TAD) containing *MYC* boxes I and II.

Methods: 267 mature aggressive B-cell lymphomas (Hummel et al. NEJM 2006) were investigated for somatic mutation in *MYC*-PR and TAD. RESULTS were correlated to molecular subgroups defined by gene expression profiling (GE) and morphologic, molecular cytogenetic and clinical features.

Results: 91/267 cases (34%) displayed *MYC* PR mutations. The mutation frequency (MF) was significantly higher in molecular BL (mBL) identified by GE than in non-molecular BL (non-mBL) (0,63% vs. 0,18%, p<0,0001). Interestingly 27 non-mBL lacking *MYC* translocations carried *MYC* PR mutations indicating aberrant somatic hypermutation. *MYC* TAD mutations were present in 62/267 cases (23%) including 36/64 mBL. TAD mutations were strongly associated with *MYC-IGH fusions* (p<0,0001) but also occured in 8 MYC-negative cases. In 17 cases, TAD mutations involved known phosphorylation sites at *MYC* box I (Pro 57, Thr58, Pro59, Pro60, Ser64) and in 13 cases *MYC* box II including a novel mutational hotspot at Phe138. *MYC* MF did not correlate with *IGH* MF, genetic complexity and gene expression of *MYC*or *MYC* target genes. However, mBL with *MYC* PR mutations had an unfavourable prognosis in comparison with unmutated mBL.

Conclusion: Heterogenous patterns of *MYC* mutations are found among mature aggressive B-cell lymphomas. Though predominatly *MYC*+ lymphomas are affected, *MYC* mutations also occur in MYC negative cases indicating aberrant SHM. *MYC* PM and TAD mutations in *MYC* negative lymphomas might represent an alternative mechanism of altered MYC function leading to lymphoma development.

128 *CMYC* TRANSLOCATIONS MAY BE FOUND IN FOLLICULAR LYMPHOMA IN THE ABSENCE OF TRANSFORMATION OR PROGRESSIVE DISEASE

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Deregulation of c*MYC* through immunoglobulin gene translocations is a defining feature of Burkitt Lymphoma (BL) and is found in aggressive forms of Diffuse Large B-cell Lymphoma (DLBCL) and in rapidly progressive transformed Follicular Lymphoma (FL). In these clinical settings rearrangement of c*MYC* is closely correlated with clinically aggressive disease. The aim of this study was to investigate the temporal relationship between the detection of a c*MYC*translocation in FL and the onset of clinically aggressive transformed disease. Using interphase FISH a *cMYC* rearrangement was detected in 18/68 biopsies from an unselected series of transformed FL. In some cases this was present in a minority of cells in the tumour. Pre-transformation biopsies from 12 of the 18 cases showing a c*MYC* rearrangement were available for study. In this group *MYC* rearrangements were found in 5 pre-transformation biopsies, 4 of these at presentation. These biopsies were taken between 7-105 months prior to the diagnosis of transformation. In a second series of 100 FL biopsies taken at relapse, but with no evidence of morphological transformation, a c*MYC* rearrangement was found in 9 tumours, sometimes in a minority of cells. In 4/9 of these cases a c*MYC*rearrangement was also found in the presentation specimen. After a minimum of 12 months follow up, none of these patients have developed transformed FL. Tumours containing a c*MYC* rearrangement were not morphologically or phenotypically distinctive and did not show an elevated Ki67 fraction. This study shows that stable tumour clones of FL containing a c*MYC* rearrangement may persist for a protracted period without the development of transformation or disease progression. The wide spectrum of clinical effects associated with c*MYC*rearrangement may reflect variation in translocation partners or association with other abnormalities, such as inactivation of P53, that are known to modify the oncogenic potential of c*MYC*.

129 THE PROGNOSTIC VALUE OF THE TUMOR/MICROENVIRONMENT SIGNATURE COMPARED TO EARLY PET SCAN IN ADVANCED-STAGE, ABVD-TREATED HODGKIN LYMPHOMA PATIENTS

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Background: Early interim PET scan is the only prognostic tool able to predict treatment outcome in advanced-stage HL (AsHL) We evaluated the prognostic role of Tissue Micro Arrays (TMAs) in 138 patients enrolled in the already published Italian-Danish trial on early PET in AsHL (Gallamini, JCO 2007).

Patients and methods: TMAs were assembled at the hemopathology unit of Bologna. Eight parameters according to Sanchez-Aguilera (Blood 2006) were assessed by immunohistochemistry: STAT-1, PCNA, SAP, TOP2A both in neoplastic cells (HRSC), and microenvironment cells (MC), RRM2, CDC2, MAD2 in HRSC, ALDH1A1 in MC. Moreover, CD 20, EBER(1/2), BCL-2 and p53 were evaluated in HRSC. All patients were treated with standard ABVD chemotherapy ± Rx therapy. Interim PET after 2 ABVD was evaluated accordingto the criteria of JCO study.

Results: The mean age was 33.3 years (14-79), stage III-IVB in 98, IIB in 40; the mean follow-up 38.1 months (7.6-71.9). Histopathology review showed: Nodular Sclerosis (NS) type1: 75, NS type2: 22, mixed cellularity: 20, lymphocyte depletion: 3, classical, lymphocyte-rich: 18 cases. Interim PET scan was positive in 30 (21.7%) patients. Treatment failure was recorded in 32 (23.2%) patients, 24 for progression and 8 for relapse. In unvaried analysis the factors related to treatment outcome were bcl-2 on HRSC (cut off value 50%), STAT-1/SAP on MC and PET scan (log-rank 6.9, 7.9 and 93.9, respectively). The combined expression of STAT-1 and SAP was scored in three levels depending on the architectural pattern of expression: score 0 (expression of both markers with a diffuse/rosetting pattern); score 1 (discordant: a diffuse/rosetting combined with a scattered pattern); score 2: (both markers with a scattered pattern); the 3-y PFS were 87.4%, 69.9% and 61.9%, respectively. In multivariate analysis PET, bcl-2 and STAT-1/SAP remained significant (hazard ratio. 24.8, 4.6, 7.5 and 5.6, respectively; p<0.01).

Conclusion. The proposed model based on TMA study is able to predict treatment response in AsHL treated with ABVD, even if with a lower efficacy than PET. However, unlike PET, it can be applied upfront therapy.

130 HIGH NUMBERS OF TUMOR INFILTRATING PD-1-POSITIVE REGULATORY LYMPHOCYTES ARE ASSOCIATED WITH IMPROVED SURVIVAL IN FOLLICULAR LYMPHOMA

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Background: Tumor microenvironment influences the behavior of follicular lymphoma (FL), but the specific cell subsets involved are not completely known.

Patients and methods: To determine the impact of PD-1-positive inhibitory immunoregulatory lymphoid cells in the outcome of FL we examined samples from 100 patients (53M/47F; median age 54 years; 5-year overall survival (OS) 76%) at diagnosis by means of a recently generated monoclonal antibody. Cells were quantified using computerized image analysis.

Results: PD-1 expression was alternative to FOXP3 in lymphoid cells from both reactive tonsils and FL. At diagnosis, the median percentage of PD-1-positive cells was 14% (range, 0.1 to 74%). Patients with grade 3 FL, poor performance status and high serum LDH showed lower numbers of PD-1-positive cells. No differences were found in CR rate according to PD-1. After a median follow-up of 6.2 years, the data of progression-free survival (PFS) and OS are detailed in the table. PD-1 and FLIPI maintained prognostic value for OS in multivariate analysis. Patients with PD-1-positive cells ≤5% showed a higher risk of histological transformation. At that time, transformed diffuse-large-B-cell lymphomas had lower percentage of PD-1-positive cells than FL.

Conclusion: A high content of PD-1-positive cells predicted favorable outcome in patients with FL, while a marked reduction is observed in transformation.

	PD-1+ cells ≤5%(N=25)	PD-1+cells 6-33%(N=50)	PD-1+ cells >33% (N=25)
CR rate*	48	65	50
5-year PFS %**	20	46	48
5-year OS %***	50	77	95
5-year risk of transformation %**	27	10	0

*89 patients with assessable response; **p<0.05; ***p<0.01

131 THE IMPACT OF TUMOUR MICROENVIRONMENT IN DIAGNOSTIC FOLLICULAR LYMPHOMA SAMPLES USING TISSUE MICROARRAYS

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Background: Evidence of the importance of the immune microenvironment in FL is increasing but the contribution of subsets and their impact on outcome is conflicting. The goal of this study was to examine the immune microenvironment in diagnostic samples from a widely representative cohort of patients with FL and correlate this to transformation and overall survival (OS).

Materials and methods: TMAs were constructed of 1mm cores in triplicate from 137 patients with initial diagnostic biopsies of FL. Immunohistochemistry was performed on the TMAs using a panel of antibodies detecting T cell antigens, FOXP3, CD68 and follicular dendritic cells (FDC) (CD21, CD23). The immune infiltrates were scored for number and location. FDCs were scored on the presence of the FDC meshwork (disrupted versus non disrupted).

Results: The median age of the 137 patients at diagnosis was 56 years (range 27 -85); the grade at diagnosis was grade one in 60 patients, grade two in 51 patients and grade 3a in 17 patients, median stage was 4. Thirty nine of the patients transformed to DLBCL. Median OS was 5.9 years and the median time to transformation was 3 years. The presence of >5/hpf CD68+ macrophages and FOXP3 positive cells in an intrafollicular location was more often observed in patients with median OS of less than 8 years (p=0.004 and p=0.08 respectively). Disruption of CD23 positive FDC meshwork was more frequent in patients with OS of less than 8 years (p=<0.05). Number and location of CD4 and CD8 T cells in the diagnostic biopsies were not significantly associated with time to transformation or OS. The median overall survival in patients with >5 CD68+ macrophages/hpf was 5.05 years compared to 12.95 years for patients with <5 CD68+ macrophages/hpf (p=0.06).

Conclusion: The presence of increased numbers of macrophages, FOXP3+ cells in an intrafollicular location and disruption of FDC meshwork in diagnostic FL samples identify patients with worse outcome.