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**ABSTRACT BOOK**

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## Non-Hodgkin & Hodgkin lymphoma - Biology

### PB2040

#### PROLIFERATING KI67 EXPRESSING B-CELLS ASSOCIATE WITH CD4+PD1+ T-CELLS IN MARGINAL ZONE LYMPHOMA

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**Background:** Specific microbial antigens have been implicated in the development and maintenance of several types of marginal zone lymphoma suggesting that an abnormal immune response is essential for driving B-cell proliferation. Reasoning that T-cell stimulation, especially from CD4+ T-cells, might make important contributions to promoting B-cell proliferation we carried out a detailed analysis of infiltrating T-cells in 8 cases of nodal marginal zone lymphoma.

**Aims:** The aim of the study was to undertake the first detailed study of T-cell subsets in marginal zone lymphoma in order to determine the relationship of individual subsets to proliferating lymphoma B-cells.

**Methods:** We carried out multiplex immunohistochemistry and validated the results to show that, for the combinations of antibodies employed, there was firstly no reduction in intensity after several rounds of staining and destaining and secondly that there was no significant carry over from one round to the next. The stained slides were scanned and, utilising a custom macro written for ImageJ software, we enumerated CD4+ T-cell subsets (TH1- CD4+TBET+, Treg - CD4+FoxP3+, follicular helper (Tfh) - CD4+PD1+ and follicular regulatory (Tfr) - CD4+FoxP3+PD1+).

**Results:** In all cases CD4+ T-cells constituted a major portion of infiltrating T-cells, mean 39.8% (range 13.5 to 70.3%). There were, however, large differences in the CD4+ T-cell subset composition; Tfh cells varied from 2.5 to 36% of all CD4+ T-cells whilst Tregs accounted for 2.7 to 24.7%. We also compared architecture of T-cell infiltration across cases and found that T-cells were not homogeneously distributed and that CD4+PD1+ cell clusters could show some association or no association with CD4+FoxP3+ clusters and, in one case, repulsion from CD4+FoxP3+ clusters. In order to quantitate the associations we carried out Pearson correlations. For comparison normal tonsil showed a Pearson correlation of -0.4 i.e. no overlap, between CD4+PD1+ and CD4+FoxP3+ cells whereas there were varying degrees of association (range 0.3 to 0.8) for the lymphoma samples. By contrast proliferating Ki67+ lymphoma B-cells associated with CD4+PD1+ cell clusters (Pearson 0.1 to 0.6) whatever the relation to CD4+PD1+ cells to CD4+FoxP3+ cells. To confirm this result we used an alternative method to analyse clustering (the Morisita index). This produced similar results with normal tonsil having a Morisita index of 0.2 for CD4+PD1+ and CD4+FoxP3+ whereas lymphoma samples showed higher degrees of association (range 0.3 to 0.9). The Morisita index also confirmed association between proliferating B-cells and CD4+PD1+ cells; normal tonsil 0.8 and lymphoma (0.4 to 0.8).

**Summary/Conclusions:** Collectively our data suggests an unsuspected association between CD4+PD1+ T-cells and proliferating lymphoma B-cells in marginal zone lymphoma.

### PB2041

#### CLINICAL IMPLICATIONS OF MYD88 L265P MUTATION IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA

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**Background:** It is now well known fact that recurrent mutation in the MYD88 gene (L265P MYD88) is identified in about 90% of Waldenström macroglobulinemia, in approximately one-quarter of patients (pts) with diffuse large B-cell lymphoma (DLBCL) and in a minority of cases of other lymphoproliferative disorders. Evidence is beginning to accumulate that L265P MYD88 mutation in pts with DLBCL has strong association with clinical phenotypes and outcomes.

**Aims:** To evaluate the clinical implications of MYD88 L265P mutation in pts with DLBCL.

**Methods:** We analyzed the data of 75 pts (median age 50 years, range 18-77, 33/42 female/male ratio, 39 extranodal and 36 nodal disease) with de novo DLBCL diagnosed between 1999 to 2015 at National Research Center for Hematology. Pts were classified as germinal center B-cell-like (GCB) or non-GCB DLBCL using the Hans algorithm. For molecular analysis DNA was extracted from 56 cryopreserved and 19 formalin-fixed paraffin-embedded tumor tissue. The mutational status of a sample was determined by real-time PCR (RT-PCR).

**Results:** MYD88 L265P mutation was detected in 14 (18.7%) out of 75 pts. Half of pts with MYD88-positive MYD88 L265P mutation DLBCL are older than 60

years compared with only 24.6% of those pts with MYD88 unmutated DLBCL. In terms of sex ratio the data show a double preponderance of males at MYD88 L265P positive DLBCL (9:5) as compared with MYD88 wild type DLBCL with the same number of males and females (33:28). There was equal distribution of pts across high-intermediate and high risk groups of the international prognostic index both in MYD88 mutated and MYD88 unmutated pts (71.7% vs 63.9%). The majority of pts in both groups had elevated serum lactate dehydrogenase levels, 12/14 (85.7%) and 47/61 (77%) respectively. Tumors with high Ki-67>80% expression were found in 13 (92.8%) of pts MYD88 mutation versus 43 (70.5%) pts with MYD88 wild type DLBCL. All 14 (100%) pts with MYD88 L265P DLBCL were non-GCB DLBCL as compared to 42/61 (72%) pts with MYD88 unmutated DLBCL. Eleven (78.6%) out of 14 pts with MYD88 mutation had extranodal lesions (p<0.05). Five (45.4%) of 11 cases MYD88 L265P extranodal DLBCL were present in immune-privileged site DLBCL (central nervous system, testis) versus 4 (14.2%) out of 28 pts with MYD88 wild type extranodal DLBCL (p<0.05). The table summarizes baseline characteristics pts.

Table 1.

Patients Characteristics n=75 (%)		
	MYD88 mutation	MYD88 wild type
Number of patients	14 (18.7)	61 (81.3)
Sex (male:female)	9:5	33:28
Age at diagnosis, years		
Median	58	49
Range	18-70	20-77
Over 60 years old	7 (50)	16 (26.2)
Serum LDG		
Level > normal	12 (85.7)	47 (77)
Ki67 > 80%	13 (92.8)	43 (70.5)
International Prognostic Index		
Low	-	7 (11.5)
Low-intermediate	4 (28.6)	15 (24.6)
High-intermediate	3 (21.4)	20 (32.8)
High	7 (50)	19 (31.1)
Lymph node involvement	3 (21.4)	33 (54.1)
Extranodal sites:	11 (78.6)	28 (45.9)
Bone	2	8
Skin	2	4
Gastrointestinal tract	1	10
Adrenal	1	-
Breast	-	2
Immune-privileged site		
Testis	-	2
Central nervous system	3	2
Bone marrow	3 (21.4)	14 (22.9)

LDG: lactate dehydrogenase

**Summary/Conclusions:** It is apparent from the present study that MYD88 L265P mutation was significantly associated with extranodal DLBCL (78.6%) and prevalent in immune-privileged site DLBCL. Detection of MYD88 L265P mutation by RT-PCR could improve diagnosis non-GCB DLBCL as a complement to immunohistochemistry.

### PB2042

#### QUINACRINE SYNERGIZES WITH DOXORUBICIN IN INHIBITING THE SURVIVAL OF DLBCL CELL BY SUPPRESSING NF-KB AND ALTERING CELL-CYCLE PROGRESSION

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**Background:** Diffuse large B-cell lymphoma (DLBCL) is an aggressive B-cell non-Hodgkin lymphoma that affects patients of all ages with a wide range of clinical presentations. Although DLBCL is curable even in advanced stages, up to one-third of patients will not achieve cure with initial therapy. To test drug combinations that could improve the efficacy of chemotherapeutics used currently. We combined doxorubicin (dox) with quinacrine (qc), which inhibits the FACT complex that is required for NF- $\kappa$ B transcriptional activity.

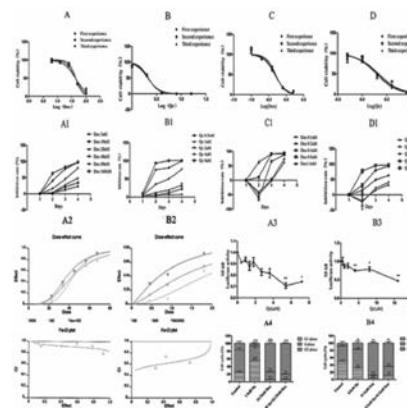


Figure 1.