**Supplementary material to the manuscript by Karolova et al.: PD-1, PD-L1 and PD-L2 expression in Mantle cell lymphoma and healthy population**

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**Table S1. Detailed information about patients with new diagnosis of MCL and CLL**

**a**. MCL patients

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Samples** | **DG** | **REL** | **CS** | **Date of last control / death** |  |
| MCL1 | 06/2013 | / | IVA | x 28/04/2014 |  |
| MCL2 | 01/2014 | / | IVBS | 06/06/2019 |  |
| MCL3 | 03/2013 | / | IVSA | x 03/05/2018 |  |
| MCL4 | 13/01/2014 | / | IVA | 10/01/2019 |  |
| MCL5 | 12/11/2013 | 03/2015 | IVBS | x 05/08/2015 |  |
| MCL6 | unknown | IV | 04/04/2019 |   |
| MCL7 | 04/2013 | 17/12/2013 | IVBS | x 18/02/2014 |   |
| MCL8 | 04/2013 | / | IVA | 11/04/2019 |   |
| MCL9 | 11/12/2013 | / | IVB | 18/01/2019. |   |
| MCL10 | unknown | x 30/10/2013 |   |
| MCL11 | 06/2013 | / | IVA | 04/04/2019. |   |
| MCL12 | 03/2013 | / | IVBS | 18/06/2019 |   |
| MCL13 | 22/05/2013 | 23/04/2015 | IVA | x 11/06/2016 |   |
| MCL14 | 01/2014 | 01/2015 | IVA | 20/06/2019 |   |
| MCL15 | 12/02/2014 | / | IVA | 06/06/2019 |   |
| MCL16 | unknown | x 04/09/2016 |   |
| MCL17 | 04/2013 | 23/02/2017 | IVA | 18/04/2019 |  |
| MCL18 | 06/2013 | 16/09/2014 | IVAS | x 06/12/2015 |  |
| MCL19 | 07/2013 | / | IVAS | 19/02/2019 |  |
| MCL20 | 03/2014 | 05/2016 | IVBS | x 28/09/2017 |  |
| MCL21 | 02/2014 | / | IVB | x 02/01/2019 |  |
| MCL22 | 05/2015 | / | IVAS | 14/03/2019 |  |
| MCL23 | 10/2015 | / | IVASB | 20/06/2019 |  |
| MCL24 | 02/2015 | / | IVA | 20/09/2018 |  |
| MCL25 | 02/2015 | / | IVA | 14/05/2019 |  |
| MCL26 | 04/2015 | / | IVBS | 18/06/2019 |  |
| MCL27 | 09/2014 | 20/06/2018 | IVA | x 03/02/2019 |  |
| MCL28 | 09/02/2015 | / | IVAB | 06/09/2018 |  |
| MCL29 | 01/2016 | / | IVB | 09/04/2019 |  |
| MCL30 | 06/2015 | / | unknown | 29/01/2018 |  |
| MCL31 | unknown | x 29/01/2015 |  |
|  |  |  |  |  |  |
| 1. CLL patients

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Samples** | **DG** | **REL/PROG** | **CS** | **Date of last control / death** |
| CLL1 | 15/09/2011 | 11/03/2015 | Rai I | 25/07/2017 |
| CLL2 | 09/1999 | / | Rai 0 | 24/05/2019 |
| CLL3 | 06/2005 | 05/2015 | Rai 0 | 26/04/2019 |
| CLL4 | 01/2003 | 18/01/2016 | Rai 0 | 02/05/2019 |
| CLL5 | 23/05/2012 | 30/03/2015 | Rai II | 07/06/2019 |
| CLL6 | 12/2006 | 12/11/2013 | Rai I | x 28/04/2015 |
| CLL7 | 08/2008 | 09/2013 | Rai 0 | 04/06/2019 |
| CLL8 | 03/2007 | 10/2010 | Rai I | x 27/06/2018 |
| CLL9 | unknown |
| CLL10 | unknown | 10/06/2019 |
| CLL11 | unknown | 12/02/2019 |
| CLL12 | 01/2013 | / | Rai I | 31/05/2019 |
| CLL13 | 04/2003 | 01/2015 | Rai I | x 07/03/2019 |
| CLL14 | 02/02/2015 | / | / | 28/02/2019 |
| CLL15 | unknown | 28/06/2019 |
| CLL16 | unknown | x 12/02/2019 |
| CLL17 | 11/ 2014 | / | IIIa | 17/05/2019 |
| CLL18 | 2013 | 09/2015 | unknown | 11/04/2016 |
| CLL19 | unknown | 02/01/2019 |
| CLL20 | 09/2015 | 11/2016 | IVB | x 12/08/2018 |
| CLL21 | unknown | 28/11/2018 |
| CLL22 | unknown | x 04/02/2016 |
| CLL23 | unknown | x 30/08/2016 |
| CLL24 | unknown | 15/04/2019 |
| CLL25 | 08/2013 | 11/2013 | Rai II | x 26/07/2016 |
| CLL26 | 2008 | 12/2013 | Rai 0 | 28/06/2019 |

 |  |
|  |
|  |

CLL = chronic lymphocytic leukaemia, CS = clinical stage, DG = date of the diagnosis, MCL = Mantle cell lymphoma, PROG = date of the progression, REL = date of the relapse, x = death.

**2.**

**Table S2. Flow cytometry panels of antibodies used for the analysis**

**a*.*** 6-color tube for characterization of B-cells

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Antigen** | **Fluorochrome** | **Clone** | **Isotype** | **Target** | **Type** | **Manufacturer** |
| CD45 | Krome Orange | J33 | mouse  | human | monoclonal | Beckman Coulter |
| CD274 | FITC | MIH1 | mouse | human | monoclonal | BD Biosciences |
| CD279 | PE | eBioJ105 | mouse | human, monkey | monoclonal | eBioscience |
| CD5 | PerCP-Cy5.5 | L17F12 | mouse | human | monoclonal | Sony |
| CD19 | PE Cy7 | J3-119 | mouse | human | monoclonal | Beckman Coulter |
| CD273 | APC | MIH18 | mouse | human | monoclonal | Biolegend |

**b**. 7-color tube for characterization of T-cells

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Antigen** | **Fluorochrome** | **Clone** | **Isotype** | **Target** | **Type** | **Manufacturer** |
| CD4 | Pacific Blue | RPA-T4 | mouse | human | monoclonal | Sony |
| CD45 | Krome Orange | J33 | mouse | human | monoclonal | Beckman Coulter |
| CD8 | FITC | B9.11 | mouse | human | monoclonal | Beckman Coulter |
| CD279 | PE | eBioJ105 | mouse | human, monkey | monoclonal | eBioscience |
| CD197 | PerCP-Cy5.5 | 150503 | mouse | human | monoclonal | BD Biosciences |
| CD45RA | APC | HI100 | mouse | human | monoclonal | BD Biosciences |
| CD3 | APC-Alexa Fluor 750 | UCHT1 | mouse | human | monoclonal | Beckman Coulter |

**3.**

**Statistical analysis – details**

 In case of expression profile of PD-1/PD-L1/2 molecules on B and T cells of healthy population, we used mean fluorescent intensity to evaluate the expression level of these molecules and employed scatterplots with calculated regression lines to demonstrate linear relationship between the age and expression of those molecules in our data set. The strength of linear relationship between the variables was evaluated by Pearson's correlation coefficients (Pearson r). To assess the statistical significance of the relationship we report bootstrap estimate of P-value and bootstrap 95 % confidence intervals for the Pearson's correlation coefficient (5,000 bootstrap replications being employed). The individual significance level of 5 % was selected arbitrarily before the analysis.

 In expression analysis of PD-1, PD-L1 and PD-L2 molecules on the surface of B cells and T cells from peripheral blood of patients with newly diagnosed MCL and CLL we show particular distributions in the form of deciles and boxplots. To assess the statistical significance of differences in distributions in population of all patients and of all healthy samples the non-parametric Mann-Whitney statistical hypothesis test was carried out. As we simultaneously present results of 36 Mann-Whitney tests about identical distributions in both populations, the Bonferroni correction of 5% simultaneous significance level (selected arbitrarily before the analysis) was utilized, resulting in individual significance level of 0.1389 %.

 We also performed detailed descriptive statistics of expression profile of PD-1, PD-L1 and PD-L2 molecules and present particular distributions in CLL, MCL and CTRL data sets. The level of expression was evaluated by mean fluorescent intensity and percentage representation (see Supplemental Table 3).

 As for the analysis of specific subpopulations of T lymphocytes in MCL and expression of PD-1 molecule on the surface of these subpopulations we compared level of expression of PD-1 molecule (evaluated by MFI or percentage analysis) in 20 aged matched healthy controls and compared them with 31 samples of patients with MCL and 26 samples of patients with CLL. We simultaneously present results of 66 Mann-Whitney tests about identical distributions in both populations. The Bonferroni correction of 5 % simultaneous significance level (selected arbitrarily before the analysis) was utilized, resulting in individual significance level of 0.0758 %.

**4.**

**Descriptive statistics**

 Detailed descriptive statistics of expression profile of PD-1/PD-L1/2 molecules and complex descriptive statistics of PD-1 surface expression on T cell subpopulations available at: **Table S3 (**[**https://lymphoma-lab.lf1.cuni.cz/supplemental-data-file-karolova-et-al**](https://lymphoma-lab.lf1.cuni.cz/supplemental-data-file-karolova-et-al)**).**

**5.**

*Fig. S1.* Whisker-plots of PD-1/PD-L1/2 expression on B and T cells of newly diagnosed MCL and CLL in comparison with healthy controls (CTRL)



**Fig. S1**: Whisker-plots of PD-1 and PD-L1/L2 surface expression on B and T cells of newly diagnosed MCL and CLL. The figures on the right side show the expression level illustrated by MFI, on the left side by the percentage of positive cells (%). MFI = mean fluorescence intensity, CTRL = healthy controls, MCL= mantle cell lymphoma, CLL = chronic lymphocytic leukaemia.