Functional Roles of PD-L1 and PD-L2 in the Pathogenesis of Chronic Lymphocytic Leukaemia – an *in vivo* Analysis

Doctoral thesis from Sebastian Reinartz

Abstract

Chronic lymphocytic leukaemia (CLL) is one of the most prevalent lymphoid malignancies in the western world, with a median onset of 72 years of age and a highly heterogeneous disease course. In recent years, the development of small-molecule inhibitors targeting essential effectors of the B cell receptor pathway such as ibrutinib or the anti-apoptosis pathway such as venetoclax has vastly improved the treatment of CLL patients. However, the emergence of therapeutic resistance often leaves patients with limited other treatment options, which underlines the demand for novel strategies to cure this disease. The restoration of endogenous anti-tumour immune responses by blocking the Pd-1/Pd-1-ligand immune checkpoint axis might represent a promising approach, as it has become a paradigm shift for the treatment of diverse cancer entities. However, relapsed/refractory CLL patients do not respond to immune checkpoint blockade, although PD-1 and PD-L1 are found to be frequently overexpressed. This highlights the urgent need to better understand the functional role of the PD-1/PD-L1 immune checkpoint axis in CLL to identify potential strategies to enhance the response to immune checkpoint blockade (ICB).

In this study, we generated two novel *Pd-l1*$^{-/-}$ and *Pd-l2*$^{-/-}$ mouse lines with pure C57BL/6J background and crossed them to the *Eμ-TCL1*^{tg} mouse model, which is frequently used to study CLL pathogenesis *in vivo*. By performing a comprehensive characterization of the disease course, we observed a functional role for Pd-l1, but not Pd-l2, in CLL development during the initiation and early progression phase. This was reflected in delayed disease onset with a significantly reduced CLL burden in peripheral blood and lymphoid tissues (spleen, bone marrow, and liver) of *Eμ-TCL1*$^{tg/wt}$*Pd-l1*$^{-/-}$ mice compared to *Eμ-TCL1*$^{tg/wt}$ controls until 8 months of age. However, the initial growth blockade could be overcome by *Pd-l1*$^{-/-}$ CLL cells during the acceleration phase, eventually leading to a similar disease progression between *Eμ-TCL1*$^{tg/wt}$*Pd-l1*$^{-/-}$ and *Eμ-TCL1*$^{tg/wt}$ mice with no survival benefit.

To identify the immunological alterations facilitating the early-stage immune escape of CLL cells upon Pd-l1 deficiency, we performed a longitudinal characterization of major immune cell populations, including untransformed B cells, CLL cells, Cd11b^{+} myeloid cells as well as Cd4^{+} and Cd8^{+} T cells. Interestingly, we could not detect significant CLL-independent differences in the overall abundance of these cell types between *Eμ-TCL1*$^{tg/wt}$*Pd-l1*$^{-/-}$ and *Eμ-TCL1*$^{tg/wt}$ mice except for the untransformed B cells, which were considerably less in lymphoid tissues of *Eμ-TCL1*$^{tg/wt}$*Pd-l1*$^{-/-}$ mice. Moreover, we did not observe compensatory upregulation of the remaining Pd-1 ligand in *Eμ-TCL1*$^{tg/wt}$*Pd-l1*$^{-/-}$ and *Eμ-TCL1*$^{tg/wt}$*Pd-l2*$^{-/-}$ mice, which was

consistent with previous publications. In contrast, we detected a significant increase of the Pd-1 receptor on the surface of both $Cd4^+$ and $Cd8^+$ T cell subsets upon systemic loss of Pd-l1 in both peripheral blood and lymphoid tissues. Accordingly, we observed lower surface levels of Cd69 in *Eμ-TCL1*$^{tg/wt}$*Pd-l1*$^{-/-}$ mice at 3 months of age, indicating reduced T cell activity.

To identify the mechanisms underlying the phenotype of *Eμ-TCL1*$^{tg/wt}$*Pd-l1*$^{-/-}$ mice, we performed a transcriptomic analysis of untransformed B cells, CLL cells, $Cd4^+$ and $Cd8^+$ T cells isolated from the spleen of *Eμ-TCL1*$^{tg/wt}$*Pd-l1*$^{-/-}$ and *Eμ-TCL1*$^{tg/wt}$ mice when the most significant difference in CLL burden between the two genotypes was reached at 8 months of age, which was considered a turning point for the establishment of compensatory immune escape mechanisms. The overall transcriptional changes were low upon global loss of Pd-l1. However, the gene signature of *Eμ-TCL1*$^{tg/wt}$*Pd-l1*$^{-/-}$ CLL cells suggested an acquisition of an aggravated phenotype, which could be the result of selective pressure triggered by enhanced anti-tumour immune responses. This was reflected by the differential expression of multiple cancer-associated genes and changes in cellular adhesion and mobilization processes. Together these adaptations might provide the CLL cells with a better fitness upon the loss of Pd-l1 expression.

Most interestingly, the transcriptional profiles of splenic $Cd4^+$ and $Cd8^+$ T cell subsets in *Eμ-TCL1*$^{tg/wt}$*Pd-l1*$^{-/-}$ mice suggested a highly responsive and activated phenotype, with $Cd8^+$ T cells simultaneously exhibited high levels of multiple exhaustion markers, indicating the acquisition of a dysfunctional phenotype. This might eventually propagate and facilitate the expansion of CLL cells in *Eμ-TCL1*$^{tg/wt}$*Pd-l1*$^{-/-}$ mice during advanced disease stages.

In parallel, we examined the role of Pd-1 ligand expression in the CLL tumour microenvironment (TME) by the adoptive transfer of *Eμ-TCL1*$^{tg/wt}$ CLL cells onto *Pd-l1*$^{-/-}$, *Pd-l2*$^{-/-}$ and WT recipients. Strikingly, we did not observe any effect for the lack of either Pd-1 ligand in the TME on CLL progression. However, there was a consistent appearance of T cell-mediated graft rejection for a considerable proportion of both *Pd-l1*$^{-/-}$ (40 %) and *Pd-l2*$^{-/-}$ (30 %) recipients, accounting for the potential of cytotoxic T cells to become activated in Pd-1 ligand deficient mice.

Taken together, the *Eμ-TCL1*$^{tg/wt}$*Pd-l1*$^{-/-}$ mouse line represents a suitable *in vivo* model to study compensatory immune escape mechanisms upon the loss of Pd-1 – Pd-l1 mediated inhibitory signalling. Findings from this thesis suggest the acquisition of an aggravated CLL cell phenotype as well as the induction of cytotoxic T cell exhaustion caused by the expression of compensatory immune checkpoint molecules such as Ctla4, Lag3 and Tim3. Further investigations of the underlying molecular mechanisms will help identify novel strategies to improve ICB therapy for CLL patients.