Abstract

Despite several recent advancements in the treatment of B-cell lymphoproliferative disorders, still a considerable number of lymphoma cases either cannot be cured (as for indolent cases) or become incurable when relapsing (as for aggressive lymphomas). This issue reflects the need for better and more effective therapies that should target in a tailored manner pathogenic mechanisms specifically acting in each form of the disease. The process of mechanism-based targeting implies several steps (Figure 1): first, identify genes that are involved in lymphomagenesis; second study the function of the protein through *in vitro* and *in vivo* approaches leading to the selection of the most promising targets; third design new specific drugs and investigate their effectiveness and overall toxicity in preclinical testing and fourth identify biomarkers predicting for response in early-phase clinical trials. Mouse models have a central role in drug development as demonstrated by their usage at multiple stages of the process.

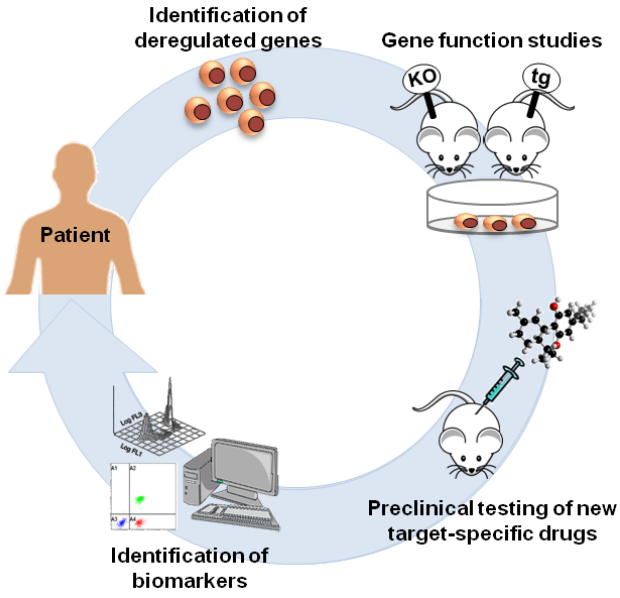

FIGURE 1. Preclinical steps of targeted drug design, including the usage of mouse models.

Aim of this thesis was to understand the molecular mechanisms leading to mature B-lymphoid malignancies and in particular to chronic lymphocytic leukemia (CLL). CLL originates from the accumulation of mature $CD5^+$ B lymphocytes in the peripheral blood, bone marrow, lymph nodes and spleen. It is well established that the reservoir of kinetically resting, circulating leukemic cells is sustained by proliferative compartments in the lymphoid organs. CLL cells are dependent on the tumor microenvironment for their growth and survival and are still responsive to external signals, including stimulation through the B-cell receptor (BCR). New insights on CLL and on B-cell malignancies have been gained in this study by using both genetically-engineered and xenograft mouse models.

We have established a new CLL xenograft model by injecting immunodeficient *Rag2*$^{-/-}\gamma_c^{-/-}$ mice (subcutaneously or intravenously) with the CLL cell line MEC1. The disease arising in the recipient mice has 100% engraftment efficiency, displays systemic involvement and resembles the aggressive form of human CLL. Moreover responsiveness to systemic treatment with fludarabine, alone or in combination with cyclophosphamide, indicates that this model is a reliable preclinical tool for testing new therapeutic agents.

We then focused on the role of Hematopoietic cell-specific Lyn substrate 1 (HS1) and Single immunoglobulin domain-containing IL1R-related protein (SIGIRR/TIR8), respectively, in the pathogenesis of CLL. The function of these molecules in malignant B lymphocytes has been investigated *in vitro* and *in vivo*, through genetic ablation of the gene of interest in the *Eμ-TCL1* transgenic mouse, an established animal model of CLL. HS1 is a hematopoietic-specific intracellular protein and its phosphorylation status has a prognostic value in CLL. We have demonstrated that HS1 is important for the proper organization of the cellular cytoskeleton and for spontaneous migration, adhesion and homotypic aggregation of the leukemic cells. HS1-deficient MEC1 cells preferentially infiltrate the bone marrow in the *Rag2*$^{-/-}\gamma_c^{-/-}$ xenograft model. *In vivo* migration studies with primary CLL cells revealed that HS1 hyper-phosphorylation status associates with the preferential localization of the leukemic cells in the bone marrow. Moreover the absence of HS1 leads to an early onset of the disease and a reduced survival in the *Eμ-TCL1* mouse model. Taken together this evidence suggests that HS1 hyper-phosphorylation might induce functional inactivation of the protein and might increase the leukemogenic potential of the B-lymphocytes.

Finally we showed that the lack of the negative regulator of TLR/IL1R signalling, TIR8, accelerates disease progression in *Eμ-TCL-1* transgenic mice and favours transformation of the disease into a prolymphocytoid-like phenotype, supporting the role of BCR costimulatory signals in the natural history of the disease. B-cell prolymphocytic leukemia is a rare aggressive disease that in some cases originates from transformation of CLL. It is characterized by the accumulation of large B cells (prolymphocytes) that may lose the expression of CD5. Histopathological evaluation of lymphoid tissues of TIR8-deficient Eμ-TCL1 transgenic mice revealed clear areas of “prolymphocytoid” transformation. These results indicate that chronic inflammation, as the one occurring after unabated TLR signalling, may favour CLL progression to this aggressive form.

Overall our results highlight the importance of dissecting the molecular mechanism of cancer and specifically of lymphomagenesis, in order to select mechanism-based targeting strategies. In this process mouse models have a double beneficial function: genetically-engineered mouse models, which can mimic the progression of specific types of B-lymphoid malignancies, can contribute to the prioritization of the therapeutic targets, while xenograft model can add new insights on human disease and facilitate preclinical testing.