Targeting apoptotic resistance in chronic lymphocytic leukemia (CLL)

Author: Nils Lilienthal

Chronic lymphocytic leukemia (CLL), as the most frequent leukemia in the Western world is still considered incurable by conventional therapeutic means. Although being a heterogeneous disease, biologically and clinically, a common underlying molecular feature of CLL, namely an abnormally low rate of spontaneous and drug-induced tumor cell death, mediates a very limited vulnerability to available chemotherapeutic drugs. As this significantly hampers our current treatment in CLL, strategies to target its apoptotic resistance are of urgent need and particular interest.

The tumor suppressor death-associated protein kinase 1 (DAPK1) is thought to play a key role in conferring such apoptotic resistance in CLL. DAPK1 activity is impaired via mutational or epigenetic gene silencing in ≈98% of CLL across all of its molecular and clinical subsets. Another common cause of abnormally robust tumor cell survival in CLL is thought to be the over-expression of the oncogene T-cell leukemia 1 (TCL1), which acts as a co-activator of the pro-survival kinase AKT. This postulates that CLL's biology is with respect to apoptosis resistance crucially dependent on a deficiency of the DAPK1 tumor suppressor along with activation of the TCL1/AKT oncogene axis. Consequently, experimental strategies to harness these molecular relays towards reconstituted cell death sensitivity will be of great therapeutic potential. This thesis reports on encouraging data about novel strategies to target these nodal apoptotic resistance-mediating pathways.

In detail, experimental evidence that TCL1 increases drug resistance was provided. To neutralize TCL1´s AKT kinase coactivating capacity, 20aa peptides mimicking the interaction domain of TCL1 with AKT were designed. These peptides were capable of (1) reducing AKT-kinase activity in *in vitro* kinase activity assays, (2) interfering in the oncogenic AKT-TCL1 interaction, and (3) inducing cell death in primary CLL and both TCL1(+) and TCL1(-) cell lines.

In a proof-of-principle and feasibility approach to target DAPK1 silencing in CLL by CLL-specific reconstitution of pro-apoptotic DAPK1 activity, the absence of DAPK1 protein in the majority of CLL patient samples was demonstrated, a panel of constitutively active DAPK1 mutants (i.e. DK1KD) was generated, and their ability to induce cell death in various B-cell lines after over-expression was shown. To achieve CLL-specific delivery, the most promising DAPK mutant DK1KD was fused to various ligands targeting the CLL cell surface, including scFv SGIII targeting CD22. The resulting fusion protein DK1KD-SGIII was: (1) highly purified via FPLC-based IMAC & CEC; (2) identity-confirmed by mass spectrometry; (3)

capable of target-cell specific, robust binding, and internalization into CD22 antigen-positive cells including CLL patient samples. It further (4) exhibited kinase activity in *in vitro* kinase activity assay and (5) induced apoptosis in various B-cell lines and primary CLL in a dose-dependent manner irrespective of resistance to the conventional chemotherapeutic agent fludarabine.

Overall, our findings demonstrate that targeting the TCL1/AKT interaction as well as reconstitution of pro-apoptotic DAPK in CLL cells can overcome apoptotic resistance and show great therapeutic potential for the treatment of CLL.