Abstract

Aberrations of two co-operating genes, the *p53* and *ATM*, significantly deteriorate prognosis and treatment options for B-CLL patients. Monoclonal antibody rituximab (anti-CD20) is preferably used in combination regimens in B-CLL, often in those containing fludarabine. Very few *in vitro* data exist, however, showing an effect of such common treatment on B-CLL cells with aberrant *p53* and/or *ATM*. In this respect, the data are also missing for a potential application of rituximab with chlorambucil.

The aims were to assess the *in vitro* effect of the above mentioned combinations of drugs on B-CLL cells with various *p53/ATM* status. A metabolic WST-1 assay monitored the drugs effect on cell viability. An *in vitro* system lacking active human plasma was used, thus omitting the CDC pathway. After rituximab pre-treatment (10 $\mu g.ml^{-1}$, 72 h) the chemotherapeutics were applied in four concentrations for additional 48 h (F: 25 - 0,4 $\mu g.ml^{-1}$; CLB: 50 - 6,25 μM). Two-way analysis of variance (MANOVA) was used for determination of rituximab pre-treatment significance.

For the rituximab/fludarabine combination we tested 46 samples having a median 90 % of B-CLL lymphocytes, with the following characteristics: 14 were wild-type, 14 harbored *ATM* deletion (median 83 % of deleted cells) and 18 exhibited *p53* defects of various complexity. The sensitivity to fludarabine was determined for the concentration 1,56 $\mu g.ml^{-1}$, which provided significant differences among the samples. The sensitivity was assessed as follows: resistant - viability is over 60 %; medium - viability is between 60 % and 40 %; sensitive - viability is lower 40 %.

The *p53*-affected samples were mostly resistant (72 %) and none were sensitive. Among *ATM* deleted samples, on the contrary, 35,5 % were sensitive, what was more than in wild-type subgroup (14 %). When the viability of fludarabine-tretead and rituximab/fludarabine-treated samples was assessed in relation to fully untreated control, the positive sensitization effect of rituximab pre-treatment ($P = 0,05$) was noted as follows: within the *p53*-affected as well as *ATM*-deleted subgroups in 33 % (36 %) of samples and within the wild-type subgroup in 57 % of samples.

For the rituximab/chlorambucil testing, which was performed as a pilot study in the same manner in sixteen samples, the positive effect of antibody pre-treatment was also noted in some samples of all the three subgroups.

Our results indicate that the *p53/ATM* status is critical for the sensitivity of B-CLL cells to fludarabine. Regardless of the *p53* and *ATM* aberrations, some samples are available for the rituximab-mediated sensitization to this agent. Our pilot data also support a warranty of testing a combined regimen containing rituximab and chlorambucil.