Leukemia-specific T cells in chronic lymphocitic leukemia and the development of a leukemia specific vaccination approach

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B-cell chronic lymphocytic leukemia (B-CLL) accounts for approximately 30% of all leukemias and is characterized by a highly variable prognosis. This makes it necessary to define individually-tailored treatments on the basis of the age and of the risk of each patient. Especially in the early stage and for low-risk patients, an immunotherapy approach seems ideally suited for the treatment of this disease, considering that B-CLL patients may spontaneously mount a leukemia specific T cell response[1] and that it is recognized that a vaccine therapy is more likely to be successful when the tumour burden is low[2].

My research project aimed at investigating and providing the background for the development of a leukemia-specific vaccination approach. Previous studies performed in our research group[3,4] compared *ex vivo* different types of tumor cell antigen preparations (leukemic cell DC hybrids, tumor cell lysate, tumor cell apoptotic bodies and total tumor RNA) and observed that DC pulsed with apoptotic bodies represented the best approach for the induction of leukemia cell-specific T cell responses. Moreover, in a parallel study, we showed the feasibility of producing a sufficient number of DC as a single batch and subsequently cryopreserve them in aliquots ready for clinical application. This study demonstrated that cryopreserved, tumour cell-loaded DC retain their functional characteristics after thawing with a high recovery of viable cells. These observations provided the background for the a phase I/II trial recently started at our Institution, in which B-CLL patients are vaccinated with dendritic cells loaded with autologous apoptotic leukemic cells. As one of the secondary endpoints of this study, I am also evaluating CLL-specific immunological cellular responses by *in-vitro* tests such as proliferation, cytotoxicity and Enzyme-linked immunospot (ELISPOT) assays. Results from this trial are awaited in the near future.

In the search for new leukemia-specific vaccination approaches, we then focused on telomerase, a specialized RNA-dependent, DNA polymerase, which adds TTAGGG repeats onto the 3' ends of chromosomes. In normal cells telomerase activity compensates only to a limited extent for the loss of telomeric repeats which occurs at each cell division and ultimately drives the cell to an irreversible growth arrest known as "replicative senescence". It is well known that more than 85% of human tumors have high telomerase activity. Tumour cells thus mantain their telomeres long and this partially accounts for the acquisition of unlimited proliferative capacity by clinically significant cancers. Telomerase activity was shown to correlate with the expression of hTERT, the catalytic subunit of the complex, which levels are increased at a low-moderate degree in 75% of CLL cases.

hTERT as tumour-associated antigen (TAA) has been targeted in a number of peptide vaccination trials by our (pancreatic carcinoma) and other research groups. The ability of cytotoxic T lymphocytes (CTLs) to lyse hTERT-expressing human cancer cell lines and cells derived from primary tumours has been shown by a number of investigators[5-8], but studies investigating the feasibility of targeting this TAA in CLL are lacking and no clinical trials have been performed so far in this disease setting.

We thus performed this study 1) to evaluate the presence of spontaneously occurring telomerase-specific T cells in B-CLL patients with telomerase-positive leukemic cells and 2) to investigate whether these T cells could be expanded *in vitro* by means of dendritic cells loaded with an hTERT peptide. For this purpose, we performed PCR-based hTERT expression assay on the cDNA samples synthetised from the the purified B cells of 25 B-CLL patients. We then selected 7 telomerase-positive patients, 3 telomerase-negative patients and 3 healthy controls for monocyte-derived dendritic cells (DC) generation and hTERT-specific CTL expansion. Monocyte-derived DC were pulsed either with the relevant hTERT peptide or the Ras control peptide and then used to pulse effector T cells to be used in proliferation and cytotoxicity assays. In proliferation assay, the peptide-loaded DC were added to autologous T cells and incubated at 37°C for 5 days. The proliferation rate of fresh T cells pulsed either with the hTERT peptide or the control peptide was then assessed by [^{3}H] thymidine incorporation as quantitated by a beta scintillation counter. In cytotoxicity assay the pre-pulsed effector T cells were co-incubated with the target tumour B-cells at 37°C for 4 hours and the percentage of lysis of tumour cells achieved by the cytotoxic T cells was assessed at flow-cytometry by propidium iodide release.We were then able to provide evidence[9] that telomerase-positive leukemic cells induce spontaneously occurring telomerase-specific T cells that can be expanded when pulsed *in vitro* by means of DC loaded with the hTERT peptide. This hTERT-specific autologous T cells expansion can be achieved only in patients with hTERT overexpression, as shown by the fact that, at proliferation assay, dendritic cells pulsed with the hTERT peptide could expand autologous specific T cells in most patients with hTERT overexpression, while this could not be achieved neither in telomerase-negative patients nor in healthy controls. Moreover, in 6 out of 7 hTERT-positive patients, DC pulsed with hTERT peptide could generate cytotoxic T lymphocytes able to lyse leukemic cells upon two rounds of restimulation, while in the same patients this did not occur with DC pulsed with the control peptide. The difference between the lytic

capacity of T cells pulsed with the relevant peptide and the T cells pulsed with the control peptide was statistically significant. On the other hand, in 3 telomerase-negative patients, telomerase-specific CTLs against the autologous leukemic B cells could not be expanded. The difference in lytic capacity of leukemic cells between telomerase-positive and telomerase-negative patients was found to be statistically significant as well. Moreover, our data show that the proliferative T cell response was both HLA I and HLA II-dependent, while the cytotoxic activity was HLA I-dependent.

On the basis of these encouraging results, we now believe that telomerase might be a valid target structure for vaccine development in CLL and are currently planning to start a clinical trial using telomerase-pulsed DC.

In summary, considering that an immunotherapeutic approach could be a valuable treatment option for low-risk patients with early-stage B-CLL, we believe that the possibility to develop a telomerase-targeted vaccine to be used in this setting deserves further investigation.

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