Bcr-abl peptides for chronic myeloid leukemia

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In these recent years, treatment of CML has been notably improved by imatinib mesylate a potent tyrosine kinase inhibitor that blocks the kinase activity of p210, thus inhibiting the proliferation of Ph-positive progenitors.[1] In chronic phase patients treated with Imatinib mesylate, the kinetic of response, particularly cytogenetic response, is most of the time rapid, with a major and even complete cytogenetic remission (CCR) observed within 6-12 months of therapy.[2,3] However, molecular remissions are rare[4] and up-front resistance to Imatinib as well as loss of response during treatment is of increased concern.[5,6] For all these reasons, despite the fact that Imatinib represents the current most effective *de-bulking* therapy for chronic phase CML and the actual best *conventional* treatment for CML patients, the eradication of residual disease and possibly the cure without bone marrow transplantation still appears a difficult goal for a tyrosine kinase inhibitor approach alone in these patients.[7]

An alternative attempt to target CML is an active specific immunotherapy (like a vaccine)[8] and a CML-specific vaccine approach could be considered as part of treatment's strategy in CML with the intent to eliminate and/or control residual cells by inducing a leukemia-specific immune surveillance. As a matter of fact, the chimeric p210 fusion protein resulting from the bcr-abl fusion gene produced by the t(9;22)(q34;q11) translocation, in virtue of the unique sequence of amino acid contained in the junctional regions, can be considered a CML specific antigen potentially immunogenic. In particular the breakpoint in the bcr gene occurs either between bcr exon 2 (b2) and 3 (b3) or alternatively between bcr exon 3 (b3) and 4 (b4) this resulting in two alternatively chimeric p210 bcr-abl proteins, comprising either the b2-a2 or b3-a2 junction.[9,10] As such, the two different amino acid sequences at the point of the junction represent unique tumor-specific determinants.

The possible immunogenicity of p210 fur-

nished the rationale for a peptide vaccine strategy in this disease. Firstly, peptides derived from amino acid sequences crossing the b3a2 breakpoint in p210, were shown able to bind to purified HLA class I and class II molecules with a binding affinity similar to that of naturally processed peptides and to elicit *in vitro* a specific T cell response both in normal donors[11-12] and in CML patients.[13] In particular 4 peptides (8-11 amino acid in length) binding to the HLA class I molecules A3, A11 and B8 and one peptide (25 amino acids long) binding to the HLA class II molecule DR11, have been identified. Subsequently, the relevance of P210 peptides as tumor associated antigens has been further confirmed by observing peptide-specific HLA restricted cytotoxic T cells (CTL) and CD4+ cells able to mediate killing of b3a2-CML cells and proliferation in the presence of b3a2 containing cell lysates, respectively.[14] The latter findings were the indirect proof of a *natural* CML cell processing of the fusion protein, presentation of junctional peptides on the cell surface within the groove of HLA molecules and recognition by T cells. Nevertheless, the final proof of an endogenous presentation of breakpoint peptides onto class I molecules by CML cells, came from the elution from purified HLA A3-positive b3a2-CML cells, of *KQSSKALQR*, one of the b3a2 peptides previously identified.[15] Furthermore, the finding of HLA class II-restricted antigen presentation of endogenous bcr-abl fusion protein by CML-derived dendritic cells to CD4+ T lymphocytes suggests that CML cells can naturally process and present breakpoint-peptides also in the context of HLA class II molecules.[16] All together, these findings furnished powerful scientific support for pursuing a breakpoint-peptide vaccine strategy in CML.

The first b3a2-breakpoint peptides phase I dose escalation vaccine trial included 12 patients with chronic phase CML with b3a2 breakpoint but no HLA restriction.[17] The multivalent peptide vaccine contained all 5 peptides previously described 12 associat-

ed with the immunological adjuvant QS-21.[18] All but one enrolled patient had large tumor burden, however, the vaccine could induce a peptide-specific delayed hypersensitivity (DTH) and a peptide-specific T cell proliferation in 2/6 and 3/6 patients treated at the two highest dose levels of vaccine, respectively.

More recently, a similar vaccine strategy was started at the Hematology Department of University of Siena, and in the attempt to improve vaccine immunogenicity and anti-tumor activity, in b3a2-CML patients with at least one proper HLA molecules and in stable major cytogenetic response (MCR) we added to the same 5 peptides and QS-21, low doses of GM-CSF as co-immunoadjuvant.[19] We conducted a multicenter study enrolling so far 25 b3a2-CML patients in which a stable minimal residual disease, during conventional treatment with Imatinib or Interferon-alpha (IFN-α), was documented before starting vaccinations. All patients continued their conventional therapy through out the study while receiving at least 6 vaccinations at two weeks interval with the CML-derived vaccine (5 breakpoint peptides + QS-21 + GM-CSF). The results observed in the first 16 patients (10 while on Imatinib and 6 while on IFN-α) have been recently published.[20] Briefly, 9/10 Imatinib patients started CMLVAX100 with a median of 10 months stable cytogenetic disease and a median 10% Ph+ metaphases. All patients showed a progressive improvement after 3-6 vaccinations with 5/9 becoming CCR. Remarkably, 3/5 CCR patients also reached an undetectable level of b3a2 transcript by real-time quantitative (Taqman) RT-PCR (BCR-ABL/B2-microglobulin ratio <0.00001). Six patients were vaccinated while on IFN-α with a median of 17 months stable residual disease (13% median Ph+ cells). All but one of the patients improved, and 2/6 achieved CCR. Immunological activity of CML-VAX100 consisted of *in vitro* peptide-specific CD4+ cell proliferation (13/14 evaluated patients) and IFN-γ production (5/5 evaluated patients). At present, 18 patients have been vaccinated while on Imatinib and 7 while on IFN-α. In conclusion, If these preliminary results will be confirmed in a larger cohort of patients, the addition of CMLVAX100 in b3a2-CML patients showing persistent disease during conventional therapy could be considered as part of treatment's strategy with the intent to control and possibly eradicate minimal residual disease. Furthermore, studies evaluating a peptide vaccine strategy also for patients with b2a2-CML are currently ongoing.

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