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Is there any role left for p210-derived peptide vaccines in chronic myeloid leukemia?

Despite the fact that the idea of *educating* the immune system against tumor-specific antigens by using an active immunotherapy such as a vaccine has been pursued by many researchers, consistent clinical data on the effectiveness of anticancer vaccines have not yet been produced. Lack of tumor specific targets, low immunogenicity of the tumor-associated antigens, inappropriate vaccine formulation and large tumor burdens of the vaccinated patients are some of the most frequent reasons accounting for the current disappointing results with anticancer vaccines.[1]

In chronic myeloid leukemia (CML), 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 acids contained in the junctional regions, which is CML-specific, furnished the rationale for a peptide vaccine strategy in this disease.[2] In fact, peptides derived from amino acid sequences crossing the b3a2 breakpoint in p210, were shown to be 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[3,4] and in CML patients.[5] In particular 4 peptides (8-11 amino acids 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. 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.[6] 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.

Recently, the elution from HLA A3-positive CML cells of *KQSSKALQR*, one of the previously identified peptides, has finally proven endogenous presentation of breakpoint peptides onto class I molecules by CML cells.[7] In addition, 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.[8]

Both these findings retrospectively furnished powerful scientific support for pursuing a breakpoint-peptide vaccine strategy in CML.

A short time ago, Scheinberg *et al.*[9] completed the first b3a2-breakpoint peptides phase I dose escalation vaccine trial in 12 patients with CML and b3a2 breakpoint. The multivalent peptide vaccine contained all 5 peptides previously described[4] associated with the immunologic adjuvant QS-21.[10] The patients' characteristics included hematologic remission, interferon-α (IFN-α) therapy and no HLA restriction.

The peptide vaccine appeared safe with 60% of patients experiencing only minimal discomfort at the site of injection. All but one of the patients enrolled had large tumor burden, however, the vaccine induced 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. It is noteworthy that the only patient vaccinated in cytogenetic remission had a transient disappearance of positivity for the b3a2 mRNA by reverse-transcription-polymerase chain reaction (RT-PCR).

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 a HLA DR11 b3a2-CML patient in stable major cytogenetic response (MCR) we added to the peptide vaccine the same QS-21 adjuvant and low doses of granulocyte-monocyte colony-stimulating factor (GM-CSF) as co-immunoadjuvant.[11] The patient had obtained MCR (4/40) after 1 year of treatment with interferon (IFN)-α at 9MU/day plus cytarabine for 14 days/month, and did not improve any further despite continuing IFN-α treatment at 3 MU/day for another year. Two months before starting vaccinations, IFN-α was reduced to 3 MU/3 times a week, which she continued during vaccinations and thereafter. The vaccine consisted of a mixture of 100 μg/each 5 b3a2-derived peptides (4 binding to HLA class I A3, A11 or B8; and one binding to HLA class II DR11) plus 100 μg of QS-21. The day before peptide-QS-21 vaccination and for 4 consecutive days, GM-CSF (50 $\mu g/m^2$/day) was subcu-

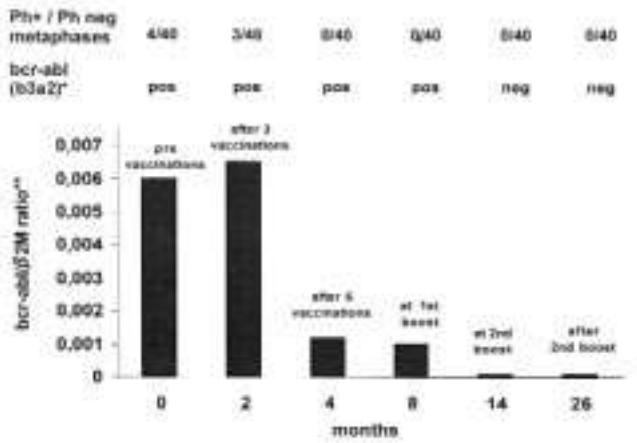

Figure 1. Cytogenetic and molecular responses during vaccinations with CMLVAX100. *Standard qualitative RT-PCR analysis for bcr-abl b3a2 subtype. **Quantification of bcr-abl b3a2 subtype by real-time *quantitative* RT-PCR analysis.[12]

taneously injected in proximity of the vaccination site. Two further boosts of vaccine and adjuvants after 4 and 10 months were planned. No major side-effects occurred after vaccinations, except mild erythema following the injections of GM-CSF. A peptide-specific immune response was documented by a strong DTH reaction which appeared after 3 vaccinations and is still detectable at the present time, and by proliferation (after 3 and 6 vaccinations and after each boost) of freshly purified $CD4^+$ T-cells in the presence of all 5 CML peptides or DR11-binding peptide alone but not in the presence of control peptides.

The patient, who started the study with 4/40 Ph^+ metaphases steadily present for at least one year, after 6 vaccinations no longer showed any detectable Ph^+ metaphases (Figure 1). In addition, after the first vaccine boost, standard qualitative RT-PCR for bcr-abl became negative and remained negative thereafter. Minimal residual disease was also monitored by real-time *quantitative* RT-PCR analysis as the ratio between bcr-abl and the housekeeping gene β2-microglobulin:[12] a 60-fold progressive reduction of this ratio, still maintained 1 year after the second boost, was observed (Figure 1). It is likely that the measurable and durable anti-tumor effect obtained in the patient has been induced by the peptide vaccine. In fact, a *late* therapeutic effect of IFN-α in this patient seems very unlikely as the patient never reached a complete cytogenetic response (CCR) during treatment with IFN-α, MCR was stable for one additional year despite continuing IFN-α 3 MU/day and two months before starting vaccinations IFN-α was further reduced to 3 MU/3 times/week.

The recent and very exciting results observed after the introduction of a selective inhibitor of the Abl tyrosine kinase (STI571) in the therapy of CML have certainly revolutionized the scenario of CML treatments.[13] In fact, clinical results, although preliminary, suggest that almost all CML patients in chronic phase obtain hematologic complete response with many of them achieving CCR within 6-12 months. Therefore, on the basis of these very promising results, the role of conventional treatments, including allogeneic bone marrow transplantation (BMT) is currently a matter of debate. Nevertheless, the significant results achieved with STI571 should be regarded with caution since the follow-up is still very short and the possibility of developing resistance to this drug has also been reported.[14] On the other hand, there is consolidated evidence that in CML patients the immune system plays an important therapeutic role in eliminating minimal residual disease and ultimately *curing* this disease as witnessed by results following BMT, donor lymphocyte infusion and to some extent also IFN-α as a biological modifier. In this context, a vaccine approach should be reconsidered as part of the treatment strategy in CML. Besides peptide vaccines, the use of dendritic cells (DC) as powerful inducers of an *active* immune approach in CML is now under evaluation.[15-16] Interestingly, most CML-derived DC carry the t(9;22) translocation and therefore could *naturally* present p210-derived peptides. Ultimately, a vaccine strategy which combines Ph^+ DC and breakpoint peptides could be investigated. In the future, a DNA vaccine containing the nucleotide fusion sequence of this oncogenic protein could also be proposed for CML patients.

In conclusion, the eradication of minimal resid-

ual disease in CML is a crucial step for the cure of the patient and therefore the role of breakpoint peptide vaccines in eliminating and /or controlling residual cells by inducing a leukemia-specific immune surveillance needs to be assessed in a wider cohort of patients, possibly as part of a prospective randomized trial.

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A milestone in the study of the vascular system: Wilhelm Roux's doctoral thesis on the bifurcation of blood vessels

Jonathan Bard wrote that «*If the science of embryology has an hero, it is probably Wilhelm Roux because he, through the force of his thinking, writing and experimentation, changed the direction of embryology from interest in evolution and teleology to a concern with mechanisms, or in the language of those times, from final to efficient causes*».[1] Roux (1850-1924) inaugurated his program of mechanisms (*Entwicklungsmechanik*), the physiological approach to embryology.

He was one of the first to attempt a causal analysis of early development. With a hot needle, he killed one of the two cells of a frog embryo after the first cleavage and then watched the development of the surviving cell. A typical half embryo was seen to emerge just as if an older embryo had been sliced in two with a razor. Only very few embryos survived as far as the gastrula stage, a finding that he thought lent support to the idea of qualitative cell division. Conversely, Hans Dreisch (1867-1941) discovered that when he separated blastomers of sea-urchin eggs by shaking, they developed into half-sized embryos, some of which reached the larval stage. It seemed, after all that each cell retained its *totipotency* enabling it to develop into any part of the organism as the occasion demanded. In 1878, at the medical faculty of Jena, Roux discussed his doctoral thesis entitled *On the bifurcation of blood vessels. A morphological study*. As underlined by Kurz, Roux *realized that an enormous number of*