BCR-ABL DERIVED PEPTIDE VACCINES FOR CHRONIC MYELOID LEUKAEMIA

Bocchia M, Ippoliti M, Defina M, Gozzetti A, Chitarrelli L, Lauria F.

Ematology and Transplant Unit, Dept. of Clinical Medicine and Immunological Sciences
University of Siena

INTRODUCTION
Chronic Myeloid Leukemia (CML) is a myeloproliferative clonal stem cell disorder characterized by the presence of a cytogenetic hallmark, the Philadelphia (Ph) chromosome, and accounts for 15% of adult leukemias (1). The disease progresses from a chronic phase through an accelerated phase to a blast phase and its natural course accounts for a median 4 years survival (1). The Ph chromosome is derived by a reciprocal translocation termed t(9;22) in which the c-abl oncogene has moved from chromosome 9 into the breakpoint cluster region (bcr), within the bcr gene on chromosome 22, resulting in a chimeric bcr-abl fusion gene that encodes a 210 KD protein (p210) with constitutive tyrosine kinase activity (2). Two major alternative chimeric p210 can result from this fusion gene: p210-b2a2 where the junction occurs between bcr exon 2 (b2) and abl exon 2 (a2) and p210-b3a2 where the junction occurs between bcr exon 3 (b3) and abl exon 2 (a2) (3) (Fig.1). About 40% of CML patients harbor the p210-b2a2 and about 60% of them show the p210-b3a2.

At diagnosis CML is often clinically asymptomatic, with patients showing a various degree leukocytosis with myeloid precursors at the differential and often normal/slightly altered hemoglobin and platelets values. Two third of the cases present also splenomegaly, often as well asymptomatic. The natural course of the disease usually includes 3 to 5 years a quite indolent phase (chronic phase, CP) that inexorably exerts into a highly chemoresistant and fatal acute leukemia (blastic crisis, CB) through a various length accelerating phase (AP).

The identification of the Ph chromosome as well as the underneath genetic BCR-ABL fusion, allowed researcher to fine check the response to any given treatment in CML. In fact, different “level” of response can be observed ranging from a “complete hematologic response” (HR: normalization of blood cells count, normal differential and disappearance of all signs and symptoms of disease), to a “complete cytogenetic response” (CCyR: disappearance of Ph+ metaphases in the bone marrow as measured by standard cytogenetic analysis) up to what’s now the level of response closest to a “cure” that is a “complete molecular response” (CMoIR: absence of BCR-ABL fusion transcript in peripheral blood and in bone marrow even after the most sensitive “nested” RT-PCR analysis (Reverse transcriptase-polymerase chain reaction) (Table 1).

Table 1. Definition of Response to treatment of CML

<table>
<thead>
<tr>
<th>Response</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematologic</td>
<td>WBC counts &lt;10^9/L, with normal differential; PLT count &lt;450x10^9/L; disappearance of all signs and symptoms of disease</td>
</tr>
<tr>
<td>Cytogenetic</td>
<td>Ph positive metaphases = 0%</td>
</tr>
<tr>
<td></td>
<td>Ph positive metaphases 1%-35%</td>
</tr>
<tr>
<td></td>
<td>Ph positive metaphases 35%-95%</td>
</tr>
<tr>
<td></td>
<td>Ph positive metaphases 100%</td>
</tr>
<tr>
<td>Molecular</td>
<td>No detectable BCR-ABL transcript by Q-PCR</td>
</tr>
<tr>
<td></td>
<td>BCR-ABL/ABL ratio &lt;0.1%</td>
</tr>
<tr>
<td></td>
<td>or &gt; 3 log reduction from baseline</td>
</tr>
</tbody>
</table>

CML CONVENTIONAL THERAPY
The treatment of Ph+ CML over the last 25 years has included oral chemotherapy (Hydroxyurea, Busulphan), intensive chemotherapy followed by bone marrow transplantation (BMT), Interferon-alpha (IFN-α) with or without cytarabine and more recently the highly innovative tyrosin kinase inhibitor imatinib mesylate (GLIVEC , Novartis Pharma) (4). The use of Hydroxyurea permitted a relative good control of clinical symptoms with nearly all patients achieving a HR but this agent was unable to modify the natural course of the disease (5). With the introduction of IFN-α about 10-20% of patients achieved CCyR and a statistically significant longer overall survival over Hydroxyurea was observed in several randomized trials (6,7). So far BMT is thought to be the solely “curative” treatment for CML but since the advent of imatinib it is not usually used as a front-line therapy, due to limited donor availability and a relatively high toxicity of the procedure (8).

Imatinib was approved in 2001 by the Food and Drug Administration for the treatment of CML in chronic phase and has been the most exciting breakthrough in the treatment of hematologic malignancies in the last 7 years (9). Imatinib, originally designated signal transduction inhibitor 571 (STI571), arose from a time-consuming process of random screening of large numbers of compounds. Imatinib is a 2-phenyl-amino-pyrimidine and it emerged as one of the most potent substances inhibiting the ABL protein, the key protein up-regulated by the oncogenic BCR-ABL fusion in CML cells and thus the main actor in leukemic transformation (10). Imatinib also inhibits other kinases,
predominantly those related to platelet-derived growth factor receptors and c-Kit (10).

Fig.2: Imatinib mesylate (formerly STI571) chemical structure

Early studies have shown that imatinib can induce HRs in excess of 90% (9) and estimated CCyR in more than 80% at a median follow-up of 19 months. Imatinib has soon shown superiority over IFN-α in combination with cytarabine in a randomized trial with newly diagnosed CML (11). Recent data indicate that the median time to achieving a CCyR after the start of imatinib therapy is about 5.5 months and that more than 90% of patients achieving a CCyR are able to maintain it with imatinib for at least two years (12). Very recently imatinib has finally showed statistically significant superiority in term of 5 years overall survival over IFN-α (13).

Overall, since the inception of imatinib mesylate therapy many trials have yielded excellent cytogenetic results with most patients achieving a CCyR. Thus, it has been suggested that quantitative RT-PCR is crucial in determining an accurate response to treatment, monitoring minimal residual disease, and detecting relapse (14). RT-PCR is a highly sensitive assay, which has the ability to detect one leukemia cell in the background of 10⁷–10⁸ normal cells (15). It is undoubtedly that the introduction of Imatinib mesylate in the scenario of treatment options for CML has dramatically modified the quality of life and life expectancy of CML patients. Yet, a CMoRI is achieved only in about 15-20% of patients and even in those the withdrawing of the drug leads to a loss of the response suggesting that imatinib mesylate usually cannot eliminate the malignant primitive progenitors which cause the disease (16). Thus, additional treatments to synergize with imatinib with the intent to possibly cure minimal residual disease are under investigation.

AN IMMUNOTHERAPEUTIC APPROACH TO CONTROL MINIMAL RESIDUAL DISEASE

There are consolidated evidences that in CML patients the immune system plays an important role in eliminating minimal residual disease and ultimately “curing” this disease as witnessed by results following BMT, donor lymphocyte infusion and to some extents also IFN-α as an immunomodulant agent (17-19). Thus an alternative specific immunotherapeutic approach to be added to imatinib could both increase the number of patients reaching a CMoRI and possibly overcome the intrinsic resistance to imatinib of Ph+ stem cells. On this regard the p210 protein is unique to the CML clone and in virtue of the unique sequence of amino acids contained in the junctional region represents a tumor specific determinant which can be used as a target for an immunological attack against the tumor cell (20-21). In fact both b3a2 and b2a2 p210 junction sequences contain a series of amino acid that doesn’t belong either to normal bcr or abl protein and in addition a new amino acid is created at the exact fusion point (a lysine, K, for b3a2-p210 and a glutamatic acid, E, for b2a2-p210) (Fig.1). Based on this assumption, 5 peptides, all derived from amino acid sequences crossing the b3a2 breakpoint in p210, were identified by us within 36 possible candidates and shown first to bind to certain HLA class I and class II molecules (20). In these initial studies no fusion peptides belonging to the b2a2-p210 sequences were identified as potential HLA molecules binders instead.

Subsequently, b3a2-p210 derived peptides were also proved to elicit in vitro a specific T cell response both in normal donors (21) and in CML patients (22). The relevance of p210 peptides as immunogenic tumor associated antigens has been further confirmed by several crucial observations:

1) peptide-specific HLA restricted cytotoxic T cells (CTL) and CD4+ cells were able to mediate killing of b3a2-CML cells and proliferation in the presence of b3a2 containing cell lysates, respectively (23);

2) b3a2-CML-derived dendritic cells showed HLA class II restricted antigen presentation of endogenous bcr-abl fusion protein by to CD4+ T lymphocytes (24);

3) KQSSKALQR, one of the peptides previously identified, has been directly eluted from HLA A3-positive CML cells (25).

All these findings furnished powerful scientific support for a b3a2-breakpoint peptides vaccine approach.

CMLVAX100 VACCINE FOR MINIMAL RESIDUAL DISEASE IN CML

Based on these pre-clinical experimental data in the Department of Hematology of Siena we developed a vaccine (CMLVAX100) containing 5 peptides of different length all encompassing the b3a2-p210 breakpoint (Table 2). Four peptides are 8-11 aa long and are able to bind to HLA class I molecules, while the 5th peptide is 25 aa long and is able to bind to HLA class II molecules. The peptides included in CMLVAX100 and the relative HLA binding molecules are listed in Table 2.

With the intent to increase the immunogenicity of oncogenic peptides otherwise poorly immunogenic, we added to the 5 p210 derived sequences two immunological adjuvants: QS-21 and GM-CSF. QS-21 is an immune adjuvant designed to boost immune responses. It’s a carbohydrate extracted from the bark of the South American tree Quillaja saponaria Molina (26). The monosaccharide composition, molecular

<table>
<thead>
<tr>
<th>Peptide sequence</th>
<th>Peptide name</th>
<th>HLA binding molecules</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>class I</td>
</tr>
<tr>
<td></td>
<td></td>
<td>class I1</td>
</tr>
<tr>
<td>ATGFK/QSSSK</td>
<td>CML-A11</td>
<td>A11</td>
</tr>
<tr>
<td>KV/QSSKALQR</td>
<td>CML-A3</td>
<td>A3</td>
</tr>
<tr>
<td>FSSATGFK/QSSSK</td>
<td>CML-A3/A11</td>
<td>A3 and A11</td>
</tr>
<tr>
<td>GY/SS/SSKAL</td>
<td>CML-B8</td>
<td>B8</td>
</tr>
<tr>
<td>FSVHATGFK/QSSKAL</td>
<td>CML-B32-25</td>
<td>DR11, DR1, DR4, DR5 and DR15</td>
</tr>
</tbody>
</table>

The K in bold represents the new amino acid that is created at b2a2-p210 fusion point.
weight, adjuvant effect and toxicity for a series of saponins has been described. QS-21 has been selected due to its adjuvanticity and lack of toxicity (26). With regards to GM-CSF (granulocyte-macrophages colony stimulating factor), it has been shown, in vitro, to stimulate the growth of antigen presenting cells (APCs) such as dendritic cells and macrophages and to increase the immunogenicity of tumors in animal models (27). In particular the use of GM-CSF as adjuvant for peptide vaccine is based on the evidence that subcutaneous injection of this biologic modifier increases the percentage of MHC class II positive cells (potential APCs) in the injected area (27). In murine animal models, injections of GM-CSF subcutaneously for 5 days were shown to produce a recruitment of subcutaneous APCs that peaked within 24 to 48 hours after first injection and declined at 72 hours despite continuing the injections.

CMLVAX100 vaccine was given in a pivotal multicenter phase II study to b3a2-CML patients presenting a stable minimal cytogenetic residual disease during imatinib treatment. Each vaccination consisted of a subcutaneous injection in the upper arm of a mixture containing 100 μg each peptide together with 100 μg of QS-21. In order to recruit APCs and possibly augment the probability of peptide to be presented to effectors T cells, on the day before and the day of vaccination, 50 μg/m² of GM-CSF were injected s.c. at the same injection site. The “immunization” phase consisted of six vaccinations, each at two weeks interval, with additional boosts of vaccine planned every 4 to 6 months after last vaccination. During the study protocol, patients were evaluated for peptide-specific T cell response as well for disease reduction induced by vaccinations.

Results of pilot phase II study with CMLVAX100

Immunological and clinical response of the first 10 patients included in our pilot study have been recently published (28). All patients were receiving standard dose of imatinib for a median of 15.5 months and they showed a median duration of unchanged residual disease of 10 months. Imatinib was not discontinued during or after vaccination treatment. Before starting the vaccine protocol, 9/10 patients still showed some degree of cytogenetic disease and 1/10 had molecular residual disease only. After 6 vaccinations the majority of them improved their residual cytogenetic disease with 5/10 reaching a complete cytogenetic response and 3/5 gaining even a complete molecular response. The clinical response was associated also to a peptide-specific immune response induced by the vaccine. After 6 vaccinations, we could measure a peptide-specific CD4+ T cell proliferation in vitro in all patients and a positive Delayed Type Hypersensitivity (DTH) skin reactions in 8/10. Based on our findings the predominant immunological effect produced by the vaccine appeared to be mediated by the 25 amino acids long peptide which contains several epitopes for different HLA class II molecules, some of which were newly identified along the study. Much less evident was the immunological effect mediated by the short peptides included in the vaccine as only few peptide-specific cytotoxic T cell lymphocytes were documented. In this pilot study, the peptide-specific immune response as well as the antitumor effect was supposed to be maintained by boosts of vaccine performed every 4-6 months. Along the study, it has been documented that a shorter interval between boosts (maximum 3-4 months) was necessary to maintained an adequate immune and tumor response. In addition the evidence that CMLVAX100 induced mainly a peptide-specific T cell proliferation suggested that the immunological adjuvant GM-CSF had a key role in the response while the role of QS-21 (known to favor mainly a cytotoxic response) was probably irrelevant.

With regards to vaccine related toxicity and compliance, CMLVAX100 was well tolerated by all patients. None of them experienced any toxicity other that local mild pain, redness and itching at the site of vaccination. No systemic adverse events and no severe adverse events have been recorded.

At the present time a total of 21 b3a2-CML patients have been vaccinated for a total of more than 200 vaccinations. We confirmed the previous clinical promising results also in this larger series of patients and the results of the total 21 patients are summarized in Table 3.

Table 3. Disease response after vaccinations with CMLVAX100 plus QS-21 and GM-CSF: results of the phase II clinical trial.

<table>
<thead>
<tr>
<th>Response after immunization (6 CMLVAX100)</th>
<th>Response after boosts</th>
</tr>
</thead>
<tbody>
<tr>
<td>13/21 improved (6/21 in CMR)</td>
<td>5/8 stable disease (3/8 in CMR)</td>
</tr>
<tr>
<td>5/21 stable disease</td>
<td>3/21 no response</td>
</tr>
</tbody>
</table>

Beside experiencing an improvement in their residual disease, b3a2-CML vaccinated patients showed an evident and effective immune response against the peptides contained in CMLVAX100. In particular, after immunization, 15/21 imatinib-patients developed a p210 peptide-specific in vivo Delayed Type Hypersensitivity skin reaction (DTH) and all of them demonstrated p210 peptide-specific unprimed CD4+T cell proliferation (29). These results demonstrated, for the first time in vivo, that imatinib treatment does not impair the immune system of CML patients contrasting with some earlier in vitro and in vivo in mice experimental evidences indicating imatinib as an immunosuppressive agent (30-31). As a matter of fact our immunological data confirmed the lack of infectious episode observed in the clinical management of imatinib treated CML patients.

Future directions

The Department of Hematology and Transplants of Siena is currently coordinating a multicenter study on behalf of the GIMEMA association, the Italian Study Group for Adult Hematologic Diseases. The impact of this active, CML specific, immune approach on the minimal residual disease during imatinib treatment will be evaluated in a larger scale. Meanwhile, studies have been carried out to investigated if the alternative b2a2 breakpoint of p210 for peptides suitable for a vaccine approach. A 25 amino acid long fusion spanning peptide, named b2a2-25, showed binding properties to several HLA class II molecules and it was able to mediate a peptide specific T cell response in vitro. The
use in b2a2-CML patients of b2a2-25 peptide have been recently approved by the Italian Institute of Health and a clinical phase II multicenter study, coordinated by Siena is planned for the near future. However the only way to definitely measure the impact of such immune approach in controlling and possibly eradicating minimal residual disease will be to carry out a phase III randomized study comparing imatinib alone vs imatinib plus peptide vaccine (b3a2 or b2a2). The preliminary results achieved in CML with a peptide vaccine approach encouraged us to extend a similar approach to other hematological diseases. In particular we are currently investigating immunogenic peptides derived from WT-1 a Wilms’ Tumor derived gene over-expressed in acute myeloid leukemias and in myelodisplasia that may represent a tumor specific target for a vaccine approach in these diseases.

References