Iranian Journal of Blood & Cancer

Journal Home Page: www.ijbc.ir

Original article

The long-term outcome and efficacy of PR1/BCR-ABL multipeptides vaccination in chronic myeloid leukemia: results of a 7-year longitudinal investigation

Seyed H. Ghaffari [1], Ebrahim Osfouri [1], Mohammad Ahmadvand [1], Davood Bashash [2], Parisa Ghaffari [1], Ahmadreza Niavarani [3], Elham Hossaini [1], Marjan Yaghmaie [1], Roghieh Koohi [1], Andisheh Ghashghaie [1], Atieh Pourbagheri-Sigaroodi [4], Seyed A. Mousavi [1], Kamran Alimoghaddam [1], Ardeshir Ghavamzadeh [5,6]

[1]Hematology, Oncology and Stem Cell Transplantation Research Center, Tehran University of Medical Sciences, Tehran, Iran
[2]Department of Hematology and Blood Banking, School of Allied Medical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[3]Digestive Oncology Research Center, Digestive Disease Research Institute, Tehran University of Medical Sciences, Tehran, Iran
[4]Department of Biotechnology, faculty of Advanced Sciences and Technology, Pharmaceutical sciences branch, Islamic Azad University (IAUPS), Tehran, Iran
[5]Hematologic Malignancies Research Center, Tehran University of Medical Sciences, Tehran, Iran
[6]Cell Therapy and Hematopoietic Stem Cell Transplantation Research Center, Tehran, Iran

ARTICLE INFO

Article History:
Received: 14/09/2022
Accepted: 22/12/2022

Keywords:
Multi-peptide vaccination
BCR-ABL
PR1 peptide
Chronic myeloid leukemia

*Corresponding authors:
Seyed H. Ghaffari, Ph.D
Associate professor, Hematology, Oncology and Stem Cell Transplantation Research Center, Shariati Hospital, Tehran 14114, Iran
Email: shghaffari@tums.ac.ir

Abstract

Background: Although Imatinib has revolutionized the treatment of chronic myeloid leukemia (CML), not all patients reach complete remission and a considerable proportion of the patients develop resistance to Imatinib.
Material and Methods: In an attempt to increase the tail on the survival curve, we conducted a Phase I/II study of PR1/BCR-ABL multipeptides vaccination trial in CML patients with at least 15 months of Imatinib treatment and 5 months of persistent molecular residual disease.
Results: One month after the completion of the vaccinations, 4 patients nearly developed a 1-log fall in their BCR-ABL transcript level, with 4 patients achieving a major molecular response (MMR). Nine patients were followed for more than a period of 7 years. The vaccinations were associated with a MMR in five patients and a complete molecular response (CMR) in one patient. The removal of Imatinib in two patients who achieved MMR after the vaccinations led to a resurgence of the leukemia population and relapse.
Conclusion: Our study suggests that a combination of immunotherapy with Imatinib targeted therapy keeps the leukemia population under control, improving the long-lasting clinical and molecular response of CML patients, for at least 7 years.

Please cite this article as: Ghaffari SH, Osfouri E, Ahmadvand M, Bashash D, Ghaffari P, Niavarani A, Hossaini E, Yaghmaie M, Koohi R, Ghashghaie A, Pourbagheri-Sigaroodi A, Mousavi SA, Alimoghaddam K, Ghavamzadeh A. The long-term outcome and efficacy of PR1/BCR-ABL multipeptides vaccination in chronic myeloid leukemia: results of a 7-year longitudinal investigation. Iranian Journal of Blood and Cancer. 2022; 14(4): 84-94.

1. Introduction

Disclosing molecular alterations associated with the malignant state have accomplished at an astonishing pace and ushered in a new field of targeted therapy [1, 2]. Chronic myeloid leukemia (CML), which is characterized by the presence of a chimeric BCR-ABL fusion gene, represents as one of the first human cancers in which molecular targeted therapy using selective ABL tyrosine kinase inhibitors (TKI) has drawn broad attention [3, 4]. In spite of the fascinating effect of Imatinib (as the standard first-line TKI in CML treatment), several mechanisms

have been reported to be responsible for drug resistance including ABL mutations, BCR-ABL gene amplification or over-expression, and decreased intracellular drug concentrations caused by drug influx (OCT-1) or efflux (Pgp/ABCB1) proteins [5-9]. Notwithstanding the fact that mutations occurring in the ABL kinase domain is the main mechanism of resistance, studies suggest that Imatinib, even in the absence of resistance mutations, fails to completely eliminate residual leukemia stem cells, inevitably resulting in relapse after the cessation of treatment. Taking advantages of these facts, alternative approaches such as vaccine-based therapy would particularly be beneficial for eradicating Philadelphia positive (Ph+) cancer stem cells, possibly providing a long-term leukemia-free survival and permanent cure.

Although vaccines are cable of provoking an immune response, translating this observation into clinical gain has proved to be exceptionally challenging [10]. Vaccine therapy using a single agent for immunotherapy has disclosed a long history of generally unimpressive and disappointing results. Recently, an improved conception of cancer pathogenesis has led to the rise in the development of novel combined-modality strategies, including targeted therapy and cancer immunotherapies [11-13]. Targeted approaches act by blocking essential biochemical pathways that are critical for tumor cell growth and survival, whereas immunotherapy attempts to stimulate a host response that leads to a long-lived destruction of the tumor [14]. Furthermore, some targeted therapies might sensitize tumor cells to immune-mediated destruction by increasing the expression of death receptors and diminishing the expression of pro-survival signals [15, 16]. In the present study, we report the results of a Phase I/II clinical trial evaluating the combination of immunotherapy with ongoing-targeted therapy in CML patients in an attempt to increase the tail on the survival curve by the means of generally increasing the number of long-term survivors.

2. Patients and Methods

2.1. Patients

In the year 2009, from the enrollment of a total 20 CML patients into this study, we selected only 10 for our vaccination trial. The protocol was approved by the official ethics committee and central institutional review board of Tehran University of Medical Sciences. The patients were thoroughly educated to all the procedures and processes throughout this study and a written informed consent was obtained from each patient. The criteria for selection include all patients to be over the age of 18, to have the e14a2 breakpoint, and remain in the first chronic phase of the disease. Moreover, they must have received Imatinib treatment for at least 15 months prior to entering the trial as well as being with at last 5 months of persistence molecular residual disease. All the patients had measurable active disease in the peripheral blood (PB) and bone marrow (BM) (any level of BCR-ABL transcript), and altogether, were treated previously and concurrently with Imatinib. The participants continued to receive Imatinib treatment as the same dose prescribed at the time of their enrollment in the trial (at least 400 mg/day). This trial is registered at Clinicaltrials.gov NCT00455221.

2.2. HLA typing

The patients underwent HLA-typing to define their HLA A, B, and DR haplotypes. By using the salting-out method, high molecular weight DNA was extracted from 10 mL of the PB samples. HLA-A and -B were typed to two digits by the ELPHA Primer HLA-AB LowRes (Biotest AG, Dreieich, Germany) kit according to the manufacturer's instruction. Also, HLA-DR was typed by the ELPHA DRB1 (Biotest) and PCR products were separated on a 2% agarose gel.

2.3. Peptide synthesis

Each patient received a cocktail of nine peptides, eight peptides spanning the e14a2 junction and one PR1 peptide. Peptides sequences along with their corresponding HLAs were listed in **Table 1.** The following CML multipeptides were included 7 HLA class-I binding peptides plus one HLA class-II binding peptide. Moreover, we used PR1 which is a nine-amino-acid HLA-A*0201-restricted peptide (VLQELNVTV) derived from proteinase 3. All peptides were synthesized by the Genosphere Biotechnologies (Paris, France) with at least 97.5%. To prepare the vaccine, 100 mg of each peptide (900 g total peptide) were mixed in 0.5 mL phosphate-buffered saline (PBS) (pH 7.4). The vaccine was stored frozen at -80 °C.

2.4. Preparation of adjuvant plasmids

In this study, we used two DNA plasmids encoding the human GM-CSF (pORF-hGM-CSF) and IL-12 (pORF-hIL-12) as the immune adjuvants. The plasmids (InvivoGen, San Diego, CA) were transformed into DH5α competent cells and subsequently purified

Table 1: Sequences of peptides used for vaccination.

Peptide	*Sequence*	HLA binding
Peptide A	ATGFKQSSK	A11, A1, A26, A3, A68
Peptide B	ATGFKQSSKA	A2, A1, A26
Peptide C	GFKQSSKAL	A24, B15, B8
Peptide D	KALQRPVAS	B51
Peptide E	TGFKQSSKAL	A2, A24, A26, B44
Peptide F	KQSSKALQR	HLA-A3 and -A11
Peptide G	HSATGFKQSSK	HLA-A3/11
Peptide H	IVHSATGFKQSSKALQRPVASDFEP	DR1, DR4, and DR11
Peptide J (PR1)	VLQELNVTV	HLA-A*0201

using the EndoFree Plasmid Giga Kit (Qiagen, Santa Clarita, CA, USA). The plasmids' DNA was dissolved into the endotoxin-free buffer TE at a concentration of 100 µg/ml.

2.5. Trial design

The treatment schedule consisted of eight subcutaneous vaccinations administered every 3 weeks. In other words, each patient received eight vaccinations over a 21-week period, administered on weeks 0, 3, 6, 9, 12, 15, 18 and 21, accordingly (**Fig. 1**). Patients received 900 µg of peptides (100 µg each of the 9 peptides) per dose. All vaccinations were administered at multiple sites on the limbs, with vaccination sites rotated between arms. One day prior to the initial vaccination, patients received 50 µg of each IL-12 and GM-CSF plasmid in 1 ml of PBS at multiple subcutaneous sites at the same site as the vaccination in order to augment the antigen-presenting function. The usual Imatinib dose was continued in the discretion of the treating physician. BM aspirates were obtained from the patients for the examination of morphology, cytogenetics, and BCR-ABL level at two time points: one day before (pre-trial) and one month after the completion of vaccinations (post-trial). Similarly, the PB was examined at three different time points for the morphology and BCR-ABL level by qRT-PCR: one day before (pre-trial), four months after the first vaccination (mid-trial), and one month after the last vaccination (post-trial-1). Moreover, plasma samples were collected for immunological assessments prior to each vaccination. We investigated the long-term follow up of the patients' molecular responses (with respect to the BCR-ABL transcript level) and survival over a 7-year period.

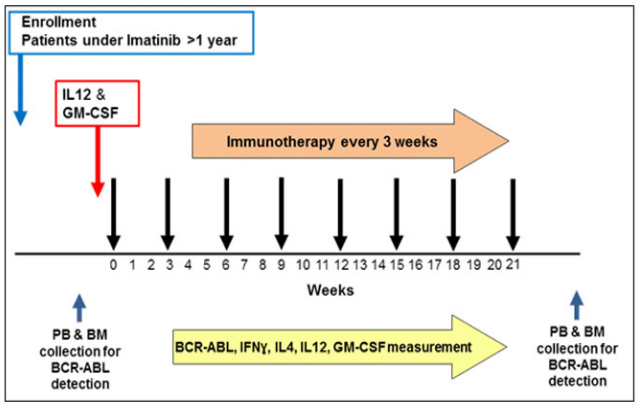

Figure 1. Protocol design for the multipeptide vaccination. The vaccine was given on weeks 0, 3, 6, 9, 12, 15, 18, and 21. DNA plasmids encoding the human GM-CSF and IL-12 were used as immune adjuvants in order to enhance the immune response to the vaccine. The BM aspirates were examined at two different time intervals, one day before the first vaccination (pre-trial) and one month after the last vaccination (post-trial-1). The PB samples were examined at three different times, one day before (pre-trial), four months after the first vaccination (mid-trial), and one month after the last vaccination (post-trial-1).

2.6. RNA isolation, cDNA synthesis and BCR-ABL mRNA quantification

10 ml of the PB and 5 ml of the BM samples were collected in EDTA. Mononuclear cells (MNC) were separated by using the density-gradient centrifugation on Ficoll-Hypaque. Total RNA was extracted by using TRIZOL (Gibco-BRL, Gaitherburg, MD) according to the manufacturer's instructions. The quantity of RNA samples were assessed spectrophotometrically by using Nanodrop ND-1000 (Nanodrop Technologies, Wilmington, Delaware, USA). Next, 1 µg of the extracted RNA was reverse transcribed into cDNA using the Prime ScriptTM RT reagent kit (Takara Bio) according to the manufacture's specifications. BCR-ABL qRT-PCR reactions were performed using the m-bcr FusionQuant® Kit (IPSOGEN, Luminy Biotech Entreprises, France) for the quantification of BCR-ABL transcripts in the BM or PB samples of CML patients in a Roche LightCycler Instrument (Roche Diagnostics GmbH, Mannheim, Germany). In a final volume of 20 µl, 2 µl of the cDNA (100 ng of RNA equivalent), 0.8 µl of the IPSOGEN Primers & Probe mix, 10 µl TaqMan Premix Ex Taq (Takara Bio Inc.) and 7.2 µl of nuclease-free water (Qiagen, Hilden, Germany) were mixed in a capillary tube to conduct real-time PCR. The thermal cycling conditions were as follows: 10 min

at 95 ˚C (initial activation), 40 cycles including 10 sec at 95 ˚C and 60 sec at 60 ˚C. Copies of BCR-ABL/100 copies of ABL were used as a reference for a baseline tumor burden. The BCR-ABL ratio was reported in accordance to the international scale (IS). BCR-ABL/ABL ratio of ≤ 0.1% is considered as the criteria for major molecular response (MMR) achievement, while a complete molecular response (CMR) was defined as becoming undetectable[17, 18]. Furthermore, we determined the molecular response by comparing the individual patients' BCR-ABL transcript levels to the level at the beginning of the vaccination trial (Pre-trial).

2.7. Immunologic responses to the CML peptide vaccination

The immunologic responses elicited by the peptide vaccinations were assessed by the ELISA systems. A blood sample was collected before and after the vaccinations and the plasma was harvested and stored at -80 ˚C for subsequent analysis. Plasma concentrations of IL-4, IFN-γ, IL-12 and GM-CSF were evaluated according to the manufacturer's instructions (BioSource International, Inc., Camarillo, CA). A standard curve was generated by using the control cytokines diluted in PBS at different concentrations, beginning with IL-4 (1696 pg/ml), IFN-γ (30 IU/ml), IL-12 (320 pg/ml), and GM-CSF (320 pg/ml). Finally, the OD450 and OD490values were determined and the results were calculated by interpolation of the OD sample values with the relevant standard curve.

2.8. Cytogenetic studies

The BM metaphase cytogenetics was evaluated by standard techniques. Complete cytogenetic remission is defined as an absence of Philadelphia chromosome positive (Ph+) metaphases.

2.9. Long-term longitudinal follow-up of CML patients

The long-term follow up of the patients' molecular responses and survival over a 7-year period were investigated. Between the years of 2010-2016, starting one year after the post-trial-1, the PB samples were collected and analyzed every year for the BCR-ABL transcript copy number by using the qRT-PCR assay. The data was expressed as the BCR-ABL/100 ABL copy number, also as the fold change in the BCR-ABL transcript levels of the PB compared to the level at the beginning of the vaccination trial (pre-trial).

3. Results

3.1. Patient characteristics

The clinical details, cytogenetics, HLA class-I and class-II types for all the 10 entrants were summarized in **Table 2.** All the 10 participants, had both the e14a2 breakpoint and measurable active disease, and were previously as well as concomitantly treated with Imatinib (400 mg daily). Respectively, all the patients had been stable on the same dose of Imatinib for at least 5 months before the entry to the trial. Patients completed all series of the 8 vaccinations. In all of the patients, at least one HLA molecule was associated with peptide binding.

3.2. Vaccine safety and toxicity

The patients were closely followed during and after each vaccination for any signs of adverse events. The peptide vaccine was administered on an outpatient basis. The vaccine was well tolerated creating no problems in any of the patients, and importantly, no signs of toxicity or clinically serious side effects attributable to the vaccine treatment or the adjuvant injection were observed in any of the patients during the short- and long-term follow-ups. During the injections of over a period of 21 weeks, we only detected mild local skin reactions (similar to delayed type hypersensitivity) following the patients' second and subsequent vaccinations. As reported by the patients, nurses and physicians, an overall clinical improvement was observed in all of the cases during their routine periods of vaccination and subsequent follow-ups. Accordingly, we conclude that the use of DNA plasmids encoding the human GM-CSF and IL-12 and repeated injections of BCR-ABL peptides combined with PR1 was safe and did not show any signs of toxicity or adverse effects in our study.

3.3. Cytogenetic and hematologic responses following vaccinations

The cytogenetic responses to the multipeptides vaccine were listed in **Table 2.** As presented, all the patients' cytogenetic responses improved after the completion of 8 vaccinations, with 5 of the participants reaching a complete cytogenetic remission. In addition, these patients proved to be BCR-ABL negative by means of the FISH technique. The complete hematologic response rate after the vaccination was 100% in all of the CML patients.

Table 2. Details of patient characteristics.

Patients	*Age/Sex*	*Cytogenetics*	*Last cytogenetic*	*HLA-I*	*HLA-II*	*Peptide cocktail*
P1	32M	46, XY, t(9;22)	46, XY	A3/A24(9)-B35	DRB1*11-DRB1*3	A-J
P2	42M	46, XY, t(9;22;15)	46, XY	A*02-B*49,*51	DRB1*11-DRB1*13-DRB3*-	A-J
P3	33F	46, XX, t(9;22)	46, XX, t(9;22)	A*02-B*35,*51	DRB1*13-DRB*14-DRB3*	A-J
P4	25M	46, XY, t(9;22)	46, XY	A*02/A*74	DRB1*04-DRB1*14	A-J
P5	43M	46, XY, t(9;22)	46, XY, t(9;22)	A*03/A*24-B*35/B*BW6	DRB1*04/DRB4-DRB1*11/DRB3	A-J
P6	69M	46, XY, t(9;22)	46, XY, t(9;22)	A2/A33-B35/B58	DRB1*04-DRB1*13	A-J
P7	26M	46, XY, t(9;22)	46, XY	A02/A02-B44/B51	DRB1*04-DRB1*09	A-J
P8	41M	46, XY, t(9;22)	46, XY	A02/A68-B51/B51	DRB1*04-DRB1*16	A-J
P9	29M	46, XY, t(9;22)	46, XY, t(9;22)	A24,A33-B08,B14	DRB1*01-DRB1*03	A-J
P10	41M	46, XY, t(9;22)	46, XY, t(9;22)	A01,A02-B08,B52-Bw4,Bw6	DRB1*03-DRB1*15	A-J

3.4. BCR-ABL transcript levels following vaccinations

Following immunotherapy, the qRT-PCR of PB and BM samples from the post-trial-1 (one month after the completion of the vaccinations) was performed. As presented in **Table 3**, quantitative detection of BCR-ABL from PB samples of the patients revealed that 4 patients (P4, P8, P9 and P10) have achieved MMR, 4 patients (P1, P2, P5 and P6) had significant reduction and only 2 patients (P3 and P7) remained with stable level of measurable disease. Moreover, as compared to the levels at the beginning of the vaccination program, 4 of the patients (P2, P4, P8 and P10) have almost achieved a one log reduction, 4 patients (P1, P5, P6 and P9) had slight reductions in their transcript levels and 2 patients (P3 and P7) remained unchanged **(Table 3).** Overall, the results presented in **Fig. 2A** outlined that there was a relative correlation between BCR-ABL transcript levels of PB and BM samples. Interestingly, a similar trend in BCR-ABL transcript levels was observed in BM samples as compare to the PB samples during time of the post-trial-1 **(Fig. 2B)**. After vaccinations, the patients were further followed for more than 7 years with respect to the long-term molecular responses and survival. The overall survival was 100%. Although clinically well, one of the participants (P2) did not participate in the follow up study. Based on the aforementioned definition of a molecular response (BCR-ABL/ABL ≤ 0.1%), after 7 years, one patient (P3) experienced a CMR (undetectable), while five of the patients (P1, P5, P7, P8 and P10) developed a major molecular response (**Table 4**). As represented in **Fig. 3,** when compared to the levels measured at the beginning of the vaccination trial, one patient (P1) almost had a one log reduction in the BCR-ABL transcript levels and 4 patients (P3, P5, P8 and P10) had >2 log reduction in BCR-ABL transcript levels. Two early responding patients (P4 and P9) who achieved a MMR after vaccinations, ceased Imatinib treatment for 16 months (during 2013-2014) and 24 months (during 2013-2015), respectively. Noteworthy, both of these patients experienced an increase in the BCR-ABL trends **(Fig. 4)**. During this time, they were hospitalized for a re-emergence of their CML disease and thus, underwent treatment with an increased dose of Imatinib.

Taken together, our results showed that the small changes in the tumor burden observed during the immunotherapy were somewhat different from the results attained from a long-term follow up. Although two of our patients (P3 and P7) developed no change in their PCR trends following the vaccinations, both of them reached the level of CMR and MMR at some point during the 7-year follow-up. This possibly confirms that the overtime trends in the values of BCR-ABL may be a better reflection of the true clinical effect.

3.5. Immunologic responses

The 10 participants were assessed for the plasma levels of IFN-γ, IL-4, IL-12 and GM-CSF in response to the vaccination (Data not shown). Although the responses were heterogeneous, there was an obvious trend regarding the increase in the frequency of IFN-γ after completion of the 8 vaccinations. Attributable to the ineffective sample storing, extended periods of sample freezing, and the usage of non-fresh samples for detection of plasma concentration of the cytokines could be the reasons for appropriate data processing.

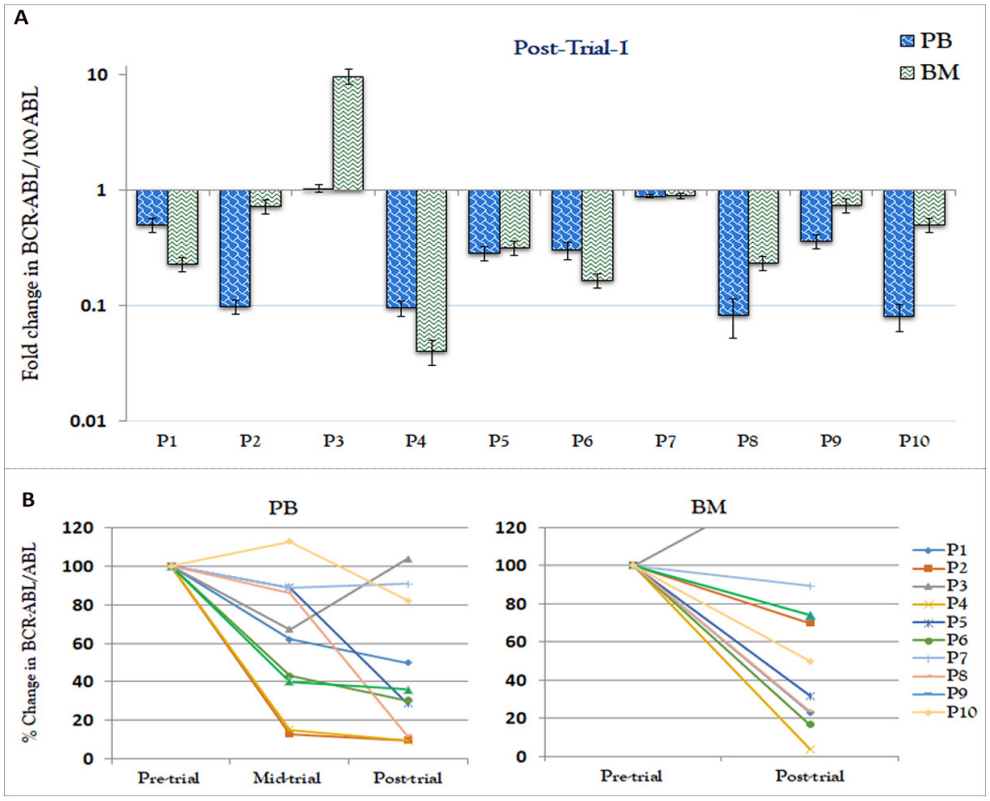

Figure 2. Molecular responses in the PB and BM of all CML patients. A) The qRT-PCR of PB and BM samples from the post-trial-1 (one month after the completion of the vaccinations) outlined that there was a relative correlation between BCR-ABL transcript levels of PB and BM samples. B) The BCR-ABL transcript levels in the PB and BM samples during the vaccination is expressed as a percent reduction compared to the level at the beginning of the vaccination. The PB samples were examined at three time intervals, one day before (pre-trial) and four months following the first vaccination (mid-trial), and one month after the last vaccination (post-trial-1). The BM aspirates were examined at two time intervals, one day before the first vaccination (pre-trial) and one month after the last vaccination (post-trial-1).

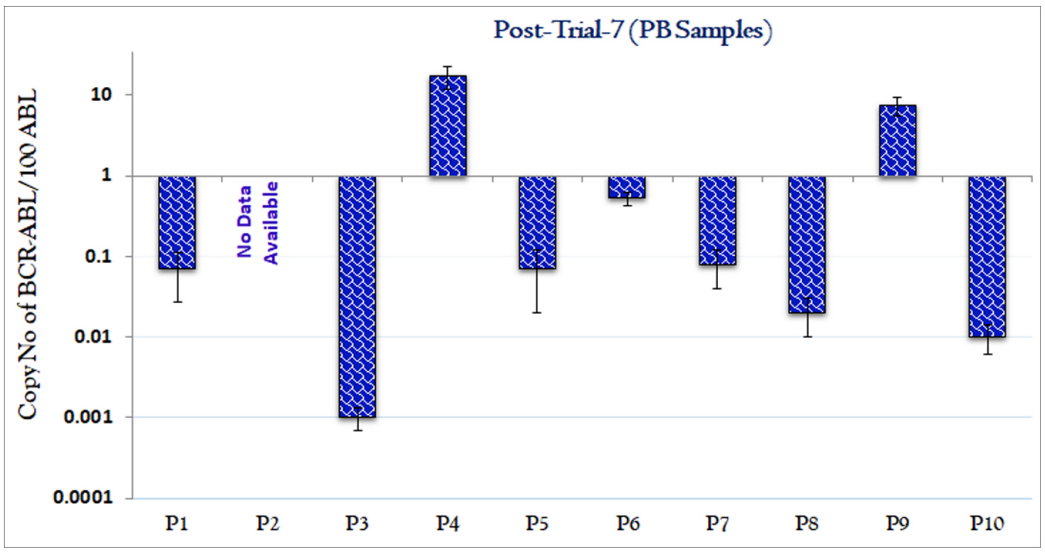

Figure 3. The molecular responses in the PB of CML patients at 7-years post vaccination. BCR-ABL transcript levels in PB at 7-years post vaccination expressed as a log reduction compared to the level at the beginning of vaccination.

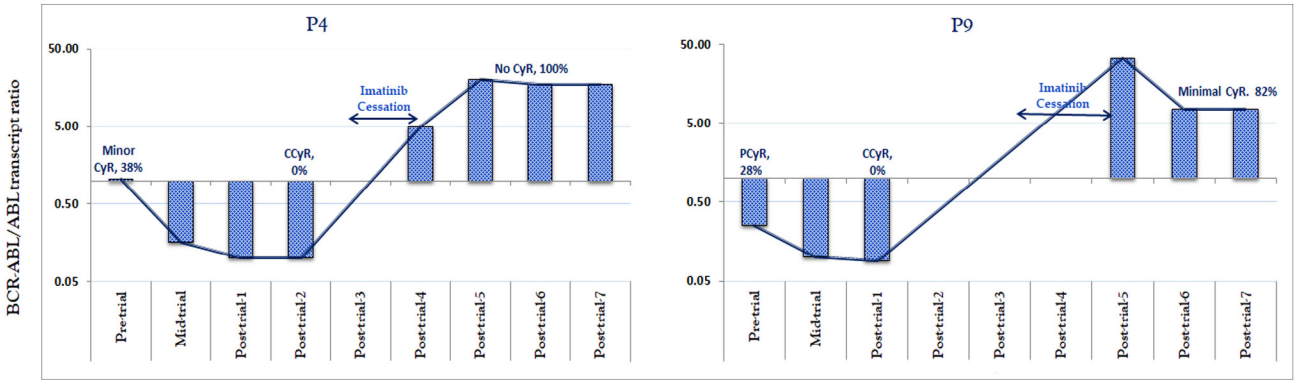

Figure 4. The molecular responses of 2 CML patients who ceased Imatinib treatment during follow-up. Two of the early responding patients (P4 and P9) who achieved a major molecular remission (MMR) after completing the last vaccination, ceased Imatinib treatment (P4; 16 months, during 2013-2014) and (P9; 24 months, during 2013-2015). Both patients experienced an increase in the BCR-ABL trends. Arrow indicates that during that time patient ceased Imatinib treatment.

Table 3. The BCR-ABL/100 ABL transcript ratios in the PB and BM for all 10 patients during a 1-year follow-up.

	PB			BM		
Patients	*Pre-trial*	*Post-trial-1*	*Fold changes*	*Pre-trial*	*Post-trial-1*	*Fold changes*
P1	0.66	0.33	-2.00	0.26	0.06	-4.33
P2	40.03	3.90	-10.26	75.41	54.73	-1.38
P3	0.55	0.57	1.04	0.44	4.29	9.75
P4	1.05	0.10	-10.50	4.01	0.16	-25.06
P5	36.93	10.61	-3.48	79.63	25.25	-3.15
P6	0.58	0.18	-3.29	1.26	0.21	-6.00
P7	0.26	0.23	-1.13	0.28	0.25	-1.12
P8	1.20	0.10	-12.00	0.17	0.04	-4.25
P9	0.25	0.09	-2.78	0.71	0.53	-1.34
P10	0.60	0.05	-12.41	0.20	0.10	-2.00

Table 4. The BCR-ABL/100 ABL transcript ratios in the PB for all 10 patients during a 7-year follow-up.

	PB									
Patients	*Pre-trial*	*Mid-trial*	*Post-trial-1*	*Post-trial-2*	*Post-trial-3*	*Post-trial-4*	*Post-trial-5*	*Post-trial-6*	*Post-trial-7*	*Fold change (7-year)*
P1	0.66	0.41	0.33	0.18	0.23	0.22	0.16	0.22	0.07	-9.43
P2	40.03	5.19	3.90	5.20					NA	-
P3	0.55	0.37	0.57	0.69					UD	-550.00
P4	1.05	0.16	0.10	0.10	←	4.00	19.80	17.30	17.10	16.29
P5	36.93	32.77	10.61	14.90	5.90	2.60	0.60	0.40	0.07	-527.52
P6	0.58	0.25	0.18						0.53	-1.09
P7	0.26	0.23	0.23						0.08	-3.25
P8	1.20	0.80	0.10	0.20	0.17	0.09	0.04		0.02	-60.00
P9	0.25	0.10	0.09		←		33.0	7.40	7.40	29.60
P10	0.60	0.67	0.05	0.02	0.05	0.01	0.01	0.01	0.01	-59.56

Pre-trial, 2010/5; Post-trial-1, 2011; Post-trial-2, 2012; Post-trial-3, 2013; Post-trial-4, 2014, Post-trial-5, 2015; Post-trial-6, 2016; Post-trial-7, 2017.
UD: undetectable. NA: no data available. Numbers in green: patients in CMR (undetectable) or MMR (BCR-ABL/ABL ≤ 0.1%). Arrow indicates that during that time, the patient stopped taking imatinib.

4. Discussion

Recently, the advancements in the progress and comprehension of the molecular processes involved in CML pathogenesis has given rise to an array of groundbreaking treatment possibilities, such as targeted therapy and immunotherapy [19, 20]. Whereas TKIs such as Imatinib results in the inhibition of the ABL tyrosine kinase pathways [21], immunotherapy stimulates a host response that effectuates a long-lived destruction of leukemic cells. Imatinib also conveys to modulate immune responses, possibly by the induction of the innate immune response that promote the patient's immune system to function alongside of Imatinib to sustain remission [22, 23] . In an attempt to increase the tail on the survival curve, we conducted a prospective, longitudinal study of a Phase I/II clinical trial to test the safety and immunogenicity of multipeptide vaccines in CML patients with previous incomplete response to Imatinib treatment.

HLA peptide vaccines are HLA restricted, therefore, are applicable to distinct patient populations expressing a particular HLA type. In the present study, we synthesized seven overlapping peptides with appropriate anchor motifs for binding to both classes of HLA molecules occurring in the Iranian population based on the information and screening numbers of the fusion peptides derived from junctional sequences (**Table 1**). The promising preliminary data acquired from a Phase I/II study evaluating the results of the PR1 vaccination encouraged us to use this peptide in combination with BCR-ABL-derived b3a2 fusion multipeptides [24, 25]. To the best of our knowledge, to date, no study has investigated the effect of CML vaccination using combined BCR-ABL-derived multipeptides with PR1 peptide, and, herein, we provide evidence which may support the findings of further studies integrating combination immunization strategies for leukemia patients. Overall, our results suggest that multipeptide antigen strategies with different HLA binding abilities may provide a more effective vaccine formulation to elicit an immune response in all populations.

A number of trials involving BCR-ABL peptides vaccinations have been reported with evidence of immunogenicity and clinical efficacy in CML patients, using different peptide antigens, peptide concentrations, vaccination schemes, HLA types and adjuvants (**Table 4**). In a Phase II clinical study, Jain et al. vaccinated 10 chronic-phase CML patients in CCyR undergoing Imatinib treatment with different peptides derived from BCR-ABL. Three out of 10 patients developed a transient 1-log reduction in the BCR-ABL transcripts [26]. In another study, Cathcart et al. vaccinated 14 chronic-phase CML patients regardless of their HLA type. Although after the vaccinations, 4 patients showed a decrease in the percentage of Ph chromosome and 3 patients became transiently PCR negative following allogeneic transplantation [27], the clinical responses could not be solely attributed to the vaccinations because of the other therapies that were concomitantly administered. The long-term follow up of peptide vaccines have rarely been investigated in longitudinal studies. **Table. 5** summarized BCR-ABL peptide vaccination trials for CML patients. In our study, changes in the tumor burden during the long-term follow-up of immunotherapy was intriguing, since 7 out of 9 (77.8%) of our patients elicited decreasing PCR trends following immunotherapy regimen. Further, 6 of these 7 responses appear to be long-lasting with these patients achieving MMR or CMR at some point during the 7-year follow-up. This sustained benefit, 7 years after the initial treatment, highlights the potential significance of immunotherapy in combination with targeted therapies for the achievement of long-lasting clinical and molecular responses.

The long-term outcome for patients with the concurrent use of Imatinib and immunotherapy resuming TKI treatment is unknown. In addition, the ability of the vaccinations to eradicate or hold the disease in the absence of Imatinib therapy is also remains to be an open to debate. Best insight in our clinical trial comes from the cessation of TKI in two patients (P4 & P9) who achieved a MMR after the completion of the final vaccination. We found that the removal of Imatinib led to resurgence in the leukemia population, as it was evident by the cytogenetic analysis along with an increase in the measured amount of BCR-ABL transcripts in PB samples. Kim et al. have reported similar clinical observations when the Imatinib treatment ceased at the twelfth month of their study [28]. It seems that the anti-leukemia T cell responses, even at low levels, prevent the relapse of leukemia, helping to maintain remission while under therapy with Imatinib; however, it worth to mention that T cell response is never strong enough

to overcome the rapidly growing leukemia population [28]. In conclusion, our study demonstrates that CML vaccination with BCR-ABL/PR1 multipeptides along with GM-CSF/IL-12 adjuvants was safe with no significant signs of toxicity and adverse effects. Moreover, the long-term follow-up indicates that the response of combined Imatinib targeted therapy with multipeptide immunotherapy keeps the leukemia population under control, improving the long-lasting clinical and molecular response of CML patients (**Fig. 5**).

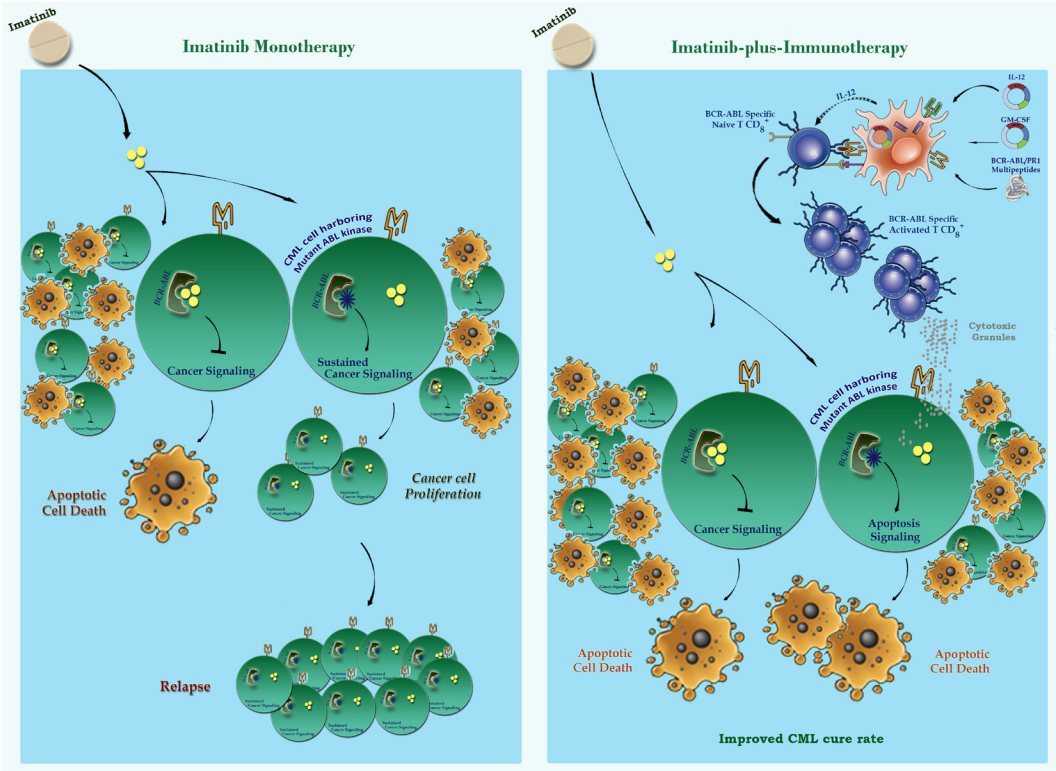

Figure 5. **Schematic representation proposed for CML targeted therapy in combination with BCR-ABL/PR1 vaccination.** As represented, combination of immunotherapy with Imatinib targeted therapy keeps the leukemia population under control, improving the long-lasting molecular response of CML patients.

Table 5. BCR-ABL peptide vaccination trials for CML patients.

BCR-ABL Peptide	*No of patients*	*HLA binding*	*Adjuvant*	*Patients' status before trial inclusion*	*Response assessment after vaccination*	*Ref.*
5 peptides e14a2 2 peptides e13a2	10	Any	Montanide GM-CSF	In chronic phase, on imatinib for at least 12 months and had been on a stable imatinib dose for ≥ 6 months.	Three of 10 patients achieved 1-log reduction in BCR-ABL transcript levels and 3 other patients achieved a MMR.	[26]
6 peptides e14a2	14	Regardless of HLA	QS-21	Partial or complete hematologic remission induced by IFN-α, hydroxyurea, BMt, or imatinib.	Six patients with CCyR (3 with transient PCR improvement); 8 patients without CCyR (4 with cytogenetic improvement).	[27]
5 peptides e14a2	12	Any	QS-21	In partial or complete remission induced by IFN-α or hydroxyurea.	One CMR, one transient cytogenetic improvement.	[29]
6 peptides e14a2	16	4 class-I 1 class-II	QS-21	Chronic-phase, measurable and stable residual disease for at least 6 months with imatinib or IFN-α.	Five patients reached complete cytogenetic remission with 3 of those having undetectable levels of BCR-ABL transcripts.	[30]
3 peptides e14a2	19	Any	PADRE GM-CSF	In chronic phase, at least 6 months stable with imatinib, and in a complete hematological response.	13 of 14 patients in MCyR (1 log reduction in BCR-ABL transcript).	[31]
5 peptides b3a2 or b2a2	13	Any	Montanide GM-CSF	Either in major or CCyR, on a stable imatinib treatment, and to have molecular or cytogenetic measurable disease.	Two of 3 patients without CCyR achieved CCyR; inconsistent PCR results.	[32]
CML b2a2	1	Several HLA–DR molecules	GM-CSF	In CCyR but with a progressive rise in the BCR-ABL transcript.	In CMR with an undetectable level of BCR-ABL both in PB and in BM.	[33]

Acknowledgements

Authors would like to express their gratitude to Hematology, Oncology and Stem Cell Transplantation Research Center, Tehran University of Medical Sciences (Tehran, Iran) for supporting this study.

Funding

This study was funded by Hematology, Oncology and Stem Cell Transplantation Research Center, Tehran University of Medical Sciences, Tehran, Iran. (Grant number: 1125).

Conflict of Interest

Seyed H. Ghaffari has received research grants from. The authors declare that they have no conflict of interest.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the Regional Ethics Committee of Tehran University of Medical Sciences and with the 1964 Helsinki declaration.

Informed consent

Informed consent was obtained from all individual participants included in the study.

References

1. Y. Zhou, M.J. You, K.H. Young, P. Lin, G. Lu, L.J. Medeiros, C.E. Bueso-Ramos, Advances in the molecular pathobiology of B-lymphoblastic leukemia, Human pathology, 43 (2012) 1347-1362.
2. A. Holleman, M.H. Cheok, M.L. den Boer, W. Yang, A.J. Veerman, K.M. Kazemier, D. Pei, C. Cheng, C.-H. Pui, M.V. Relling, Gene-expression patterns in drug-resistant acute lymphoblastic leukemia cells and response to treatment, New England Journal of Medicine, 351 (2004) 533-542.
3. T. Hughes, M. Deininger, A. Hochhaus, S. Branford, J. Radich, J. Kaeda, M. Baccarani, J. Cortes, N.C. Cross, B.J. Druker, Monitoring CML patients responding to treatment with tyrosine kinase inhibitors: review and recommendations for harmonizing current methodology for detecting BCR-ABL transcripts and kinase domain mutations and for expressing results, Blood, 108 (2006) 28-37.
4. S. Abbasian, A. Ghotaslou, A. Ghasemi, F. Nadali, Analysis of expression Of SIRT1 gene in patients with chronic myeloid leukemia resistant to imatinib mesylate, Iranian Journal of Blood and Cancer, 7 (2015) 184-190.
5. J.F. Apperley, Part I: mechanisms of resistance to imatinib in chronic myeloid leukaemia, The Lancet. Oncology, 8 (2007) 1018-1029.
6. T. Illmer, M. Schaich, U. Platzbecker, J. Freiberg-Richter, U. Oelschlägel, M. Von Bonin, S. Pursche, T. Bergemann, G. Ehninger, E. Schleyer, P-glycoprotein-mediated drug efflux is a resistance mechanism of chronic myelogenous leukemia cells to treatment with imatinib mesylate, Leukemia, 18 (2004) 401.
7. S. Soverini, S. Colarossi, A. Gnani, G. Rosti, F. Castagnetti, A. Poerio, I. Iacobucci, M. Amabile, E. Abruzzese, E. Orlandi, Contribution of ABL kinase domain mutations to imatinib resistance in different subsets of Philadelphia-positive patients: by the GIMEMA Working Party on Chronic Myeloid Leukemia, Clinical Cancer Research, 12 (2006) 7374-7379.
8. N.P. Shah, J.M. Nicoll, B. Nagar, M.E. Gorre, R.L. Paquette, J. Kuriyan, C.L. Sawyers, Multiple BCR-ABL kinase domain mutations confer polyclonal resistance to the tyrosine kinase inhibitor imatinib (STI571) in chronic phase and blast crisis chronic myeloid leukemia, Cancer cell, 2 (2002) 117-125.
9. M. Copland, A. Hamilton, L.J. Elrick, J.W. Baird, E.K. Allan, N. Jordanides, M. Barow, J.C. Mountford, T.L. Holyoake, Dasatinib (BMS-354825) targets an earlier progenitor population than imatinib in primary CML but does not eliminate the quiescent fraction, Blood, 107 (2006) 4532-4539.
10. J. Ozao-Choy, D.J. Lee, M.B. Faries, Melanoma vaccines: mixed past, promising future, The Surgical clinics of North America, 94 (2014) 1017-1030, viii.
11. M. Vanneman, G. Dranoff, Combining immunotherapy and targeted therapies in cancer treatment, Nature reviews cancer, 12 (2012) 237.
12. I. Mellman, G. Coukos, G. Dranoff, Cancer immunotherapy comes of age, Nature, 480 (2011) 480.
13. P.E. Hughes, S. Caenepeel, L.C. Wu, Targeted therapy and checkpoint immunotherapy combinations for the treatment of cancer, Trends in immunology, 37 (2016) 462-476.
14. A.D. Fesnak, C.H. June, B.L. Levine, Engineered T cells: the promise and challenges of cancer immunotherapy, Nature Reviews Cancer, 16 (2016) 566.
15. H. Wang, F. Cheng, A. Cuenca, P. Horna, Z. Zheng, K. Bhalla, E.M. Sotomayor, Imatinib mesylate (STI-571) enhances antigen-presenting cell function and overcomes tumor-induced CD4+ T-cell tolerance, Blood, 105 (2005) 1135-1143.
16. J. Chen, A. Schmitt, K. Giannopoulos, B. Chen, M. Rojewski, H. Döhner, D. Bunjes, M. Schmitt, Imatinib impairs the proliferation and function of CD4+ CD25+ regulatory T cells in a dose-dependent manner, International journal of oncology, 31 (2007) 1133-1139.
17. T.P. Hughes, J. Kaeda, S. Branford, Z. Rudzki, A. Hochhaus, M.L. Hensley, I. Gathmann, A.E. Bolton, I.C. van Hoomissen, J.M. Goldman, J.P. Radich, S.T.I.S.G. International Randomised Study of Interferon versus, Frequency of major molecular responses to imatinib or interferon alfa plus cytarabine in newly diagnosed chronic myeloid leukemia, The New England journal of medicine, 349 (2003) 1423-1432.
18. B.D. Smith, Y.L. Kasamon, J. Kowalski, C. Gocke, K. Murphy, C.B. Miller, E. Garrett-Mayer, H.L. Tsai, L. Qin, C. Chia, B. Biedrzycki, T.C. Harding, G.H. Tu, R. Jones, K. Hege, H.I. Levitsky, K562/GM-CSF immunotherapy reduces tumor burden in chronic myeloid leukemia patients with residual disease on imatinib mesylate, Clinical cancer research : an official journal of the American Association for Cancer Research, 16 (2010) 338-347.
19. T. O'Hare, A.S. Corbin, B.J. Druker, Targeted CML therapy: controlling drug resistance, seeking cure, Current opinion in genetics & development, 16 (2006) 92-99.
20. M. Vakili, S.A. Rasoulinejad, Unusual ophthalmic manifestation in chronic myeloid leukemia: A case report, Iranian Journal of Blood and Cancer, 14 (2022) 58-60.

21. M.W. Deininger, J.M. Goldman, J.V. Melo, The molecular biology of chronic myeloid leukemia, Blood, 96 (2000) 3343-3356.
22 P.S. Kim, P.P. Lee, D. Levy, Dynamics and potential impact of the immune response to chronic myelogenous leukemia, PLoS computational biology, 4 (2008) e1000095.
23. V.P. Balachandran, M.J. Cavnar, S. Zeng, Z.M. Bamboat, L.M. Ocuin, H. Obaid, E.C. Sorenson, R. Popow, C. Ariyan, F. Rossi, P. Besmer, T. Guo, C.R. Antonescu, T. Taguchi, J. Yuan, J.D. Wolchok, J.P. Allison, R.P. DeMatteo, Imatinib potentiates antitumor T cell responses in gastrointestinal stromal tumor through the inhibition of Ido, Nat Med, 17 (2011) 1094-1100.
24. Q. Ma, C. Wang, D. Jones, K.E. Quintanilla, D. Li, Y. Wang, E.D. Wieder, K. Clise-Dwyer, G. Alatrash, Y. Mj, Adoptive transfer of PR1 cytotoxic T lymphocytes associated with reduced leukemia burden in a mouse acute myeloid leukemia xenograft model, Cytotherapy, 12 (2010) 1056-1062.
25. K. Rezvani, A.S. Yong, S. Mielke, B.N. Savani, L. Musse, J. Superata, B. Jafarpour, C. Boss, A.J. Barrett, Leukemia-associated antigen-specific T-cell responses following combined PR1 and WT1 peptide vaccination in patients with myeloid malignancies, Blood, 111 (2008) 236-242.
26. N. Jain, J.M. Reuben, H. Kantarjian, C. Li, H. Gao, B.N. Lee, E.N. Cohen, T. Ebarb, D.A. Scheinberg, J. Cortes, Synthetic tumor-specific breakpoint peptide vaccine in patients with chronic myeloid leukemia and minimal residual disease, Cancer, 115 (2009) 3924-3934.
27. K. Cathcart, J. Pinilla-Ibarz, T. Korontsvit, J. Schwartz, V. Zakhaleva, E.B. Papadopoulos, D.A. Scheinberg, A multivalent bcr-abl fusion peptide vaccination trial in patients with chronic myeloid leukemia, Blood, 103 (2004) 1037-1042.
28. P.S. Kim, P.P. Lee, D. Levy, Dynamics and potential impact of the immune response to chronic myelogenous leukemia, PLoS computational biology, 4 (2008) e1000095.
29. J. Pinilla-Ibarz, K. Cathcart, T. Korontsvit, S. Soignet, M. Bocchia, J. Caggiano, L. Lai, J. Jimenez, J. Kolitz, D. Scheinberg, Vaccination of patients with chronic myelogenous leukemia with bcr-abl oncogene breakpoint fusion peptides generates specific immune responses, Blood, 95 (2000) 1781-1787.
30. M. Bocchia, S. Gentili, E. Abruzzese, A. Fanelli, F. Iuliano, A. Tabilio, M. Amabile, F. Forconi, A. Gozzetti, D. Raspadori, Effect of a p210 multipeptide vaccine associated with imatinib or interferon in patients with chronic myeloid leukaemia and persistent residual disease: a multicentre observational trial, The Lancet, 365 (2005) 657-662.
31. J. Rojas, K. Knight, L. Wang, R. Clark, Clinical evaluation of BCR-ABL peptide immunisation in chronic myeloid leukaemia: results of the EPIC study, Leukemia, 21 (2007) 2287-2295.
32. P.G. Maslak, T. Dao, M. Gomez, S. Chanel, J. Packin, T. Korontsvit, V. Zakhaleva, J. Pinilla-Ibarz, E. Berman, D.A. Scheinberg, A pilot vaccination trial of synthetic analog peptides derived from the BCR-ABL breakpoints in CML patients with minimal disease, Leukemia, 22 (2008) 1613-1616.
33. M. Bocchia, M. Defina, L. Aprile, M. Ippoliti, R. Crupi, M. Rondoni, A. Gozzetti, F. Lauria, Complete molecular response in CML after p210 BCR-ABL1-derived peptide vaccination, Nat Rev Clin Oncol, 7 (2010) 600-603.