PR1 Peptide Vaccine Induces Specific Immunity With Clinical Responses In Myeloid Malignancies

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Abstract

PR1, an HLA-A2-restricted peptide derived from both proteinase 3 and neutrophil elastase, is recognized on myeloid leukemia cells by cytotoxic T lymphocytes (CTL) that preferentially kill leukemia and contribute to cytogenetic remission. To evaluate safety, immunogenicity and clinical activity of PR1 vaccination, a phase I/II trial was conducted. Sixty-six HLA-A2+ patients with acute myeloid leukemia (AML: 42), chronic myeloid leukemia (CML: 13) or myelodysplastic syndrome (MDS: 11) received three to six PR1 peptide vaccinations, administered subcutaneously every 3 weeks at dose levels of 0.25, 0.5 or 1.0 mg. Patients were randomized to the 3 dose levels after establishing the safety of the highest dose level. Primary endpoints were safety and immune response, assessed by doubling of PR1/HLA-A2 tetramer-specific CTL, and the secondary endpoint was clinical response. Immune responses were noted in 35 of 66 (53%) patients. Of the 53 evaluable patients with active disease, 12 (24%) had objective clinical responses (complete: 8, partial: 1 and hematological improvement: 3). PR1-specific immune response was seen in 9 of 25 clinical responders vs. 3 of 28 clinical non-responders (p=0.03). In conclusion, PR1 peptide vaccine induces specific immunity that correlates with clinical responses, including molecular remission, in AML, CML and MDS patients.

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Conflict of Interest : None

Keywords

Vaccine; Immune Response; PR1; Myeloid Leukemia; CTL

Introduction

PR1 is a 9 amino acid-long peptide derived from the myeloid proteins proteinase 3 (P3) and neutrophil elastase (NE) and it is recognized on the surface of myeloid leukemia cells by cytotoxic T lymphocytes (CTL) when it is bound to HLA-A2 [1-3]. PR1-specific CTL (PR1-CTL) mediate specific lysis of CML and AML and preferentially suppress CML progenitor cells. PR1-CTL, normally present at low precursor frequency in the blood of healthy donors (less than 1/200,000 CD8+ cells), are more numerous in CML and AML patients (0.1% to 3%) and they contribute to cytogenetic and molecular remission of CML in patients treated with interferon-α or allogeneic stem cell transplant [4]. PR1-CTL may also contribute to molecular remission of CML in patients treated with imatinib and interferon. However, a high number of CML cells that overexpress P3 and PR1 can also induce apoptosis of PR1-CTL that express high affinity T cell receptors (TCRs), leading to a loss of effective immunity and the outgrowth of CML [5]. Collectively, these results suggest that disease burden may be a critical factor for effective anti-leukemia immunity.

Peptide vaccine studies with WT1, an HLA-A2-restricted nonomer peptide derived from the Wilms tumor antigen that is similarly targeted by CTL that specifically lyse myeloid leukemia, or a combination of WT1 and PR1, have shown that leukemia-specific immunity can be induced [6-10]. Likewise, vaccination with peptides derived from the BCR-ABL fusion region within the 9;22 translocation in CML can also induce specific T cell immunity, supporting the feasibility of this approach [11-13]. Importantly, objective clinical responses have been observed in these trials, although some responding patients received treatment with imatinib and other active agents that confound a more complete interpretation of the clinical activity of peptide vaccination.

We studied 66 patients with CML, MDS, and AML that had refractory, relapsed, or smoldering disease or were beyond first remission to determine whether 3 to 6 doses of PR1 peptide vaccine was well tolerated and whether vaccination with a single peptide epitope induces high avidity PR1-CTL. Here we report the results showing that PR1 vaccination was minimally toxic and increased circulating PR1-CTL, which was associated with clinical responses in patients with low leukemia burdens.

Materials and Methods

Patient Eligibility

Sixty-six patients were enrolled in the study. Eligible patients were 18 years of age or older, were HLA-A2 positive (+), had AML with relapsed/refractory disease or ≥ 2nd CR, CML refractory to first line treatment or > 2nd chronic phase, MDS with refractory anemia with excess blasts (RAEB) or refractory anemia with excess blasts in transformation (RAEB-t) by FAB classification [14]. Patients were excluded if they received chemotherapy or

immunosuppressive treatment within one month of study entry; or if they had detectable anti-proteinase 3 antibodies (cANCA) or history of Wegener's granulomatosis or other autoimmune vasculitis. Antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis is associated with ANCA specific for myeloperoxidase or proteinase 3. Therefore patients with a history of Wegener's granulomatosis or autoimmune vasculitis were excluded to prevent potential exacerbation by anti-PR1 antibodies or T lymphocytes. The study was conducted under an IND by the Food and Drug Administration. The protocol was reviewed and approved by the University of Texas-MD Anderson Cancer Center Institutional Review Board and all patients provided written informed consent to participate (ClinicalTrials.gov Identifier: NCT00004918).

Study Design and Treatment

PR1 peptide (VLQELNVTV) was provided by the Cancer Therapy Evaluation Program (CTEP/NCI). The peptide solution was added to an equal volume of the incomplete Freund's adjuvant Montanide ISA51 (CTEP/NCI) and injected as an emulsion into the deep subcutaneous tissue, alternating between anterior thigh and upper arm. All patients received GM-CSF at a dose of 75 µg subcutaneously at the same site [15]. The first 9 patients were treated in phase I, with 3 patients enrolled at each successive dose of PR1 vaccine (0.25 mg, 0.50 mg, or 1.0 mg). In phase II, 57 patients were randomized among the three PR1 doses. Fifty-four patients (9 in phase I and 45 in phase II) received a total of 3 injections at 3-week, intervals (weeks 0, 3 and 6). Because of the lack of significant toxicity in the first 54 patients, the number of vaccinations was extended from 3 to 6 (given at weeks, 0, 3, 6, 9, 12 and 15) in the last 12 patients to explore whether more doses increased PR1-specific immunity beyond 9 weeks.

Endpoints

The primary endpoints were dose limiting toxicity (DLT), defined as an autoimmune vasculitis, and induction of specific immune response (IR) to PR1 vaccine, defined as a 2-fold increase in the peak percentage of peripheral blood PR1/HLA-A2 tetramer-positive CD8+ T cells compared to baseline [3, 16]. Primary endpoints were assessed three weeks after each vaccination. Toxicity other than autoimmune vasculitis was graded by NCI Common Terminology Criteria (version 2.0). Patients were monitored for potential autoimmune vasculitis with chest X-ray and cANCA titers every 3 weeks until 3 weeks after the last vaccine.

Disease status was assessed at study entry (baseline) and 3 weeks after the last vaccination (post-vaccine). Clinical response was defined as one of the three outcomes: complete remission (CR), partial remission (PR) or hematologic improvement (HI), according to International Working Group's criteria [17]. CR was defined as < 5% bone marrow or peripheral blood myeloblasts, PR required at least 50% reduction in measurable disease parameters and HI was defined as an improvement in blood counts, or less than 50% reduction in measurable disease parameters. In addition to morphological assessment of bone marrow and conventional karyotype analysis, fluorescent in-situ hybridization (FISH) and real-time polymerase chain reaction (RT-PCR) assays were used to detect known cytogenetic abnormalities and molecular abnormalities.

Statistical Methods

The primary outcomes were DLT and IR, both defined over an eighteen-week period from initiation of treatment. After 3 patients were treated at the highest dose level of 1.0 mg in phase I, all subsequent patients were randomized among the 3 dose levels. IR and DLT were monitored within a dose level using the method of Thall and Sung [18]. Within each dose level, the goals were to achieve at least 20% probability of IR while maintaining at most a 20% probability of DLT, with any dose level terminated if either (1) none of the first 12 patients had an IR or (2) [# patients with DLT]/ [# patients scored] was greater than or equal to 3/4, 4/8, 5/12, or 6/16. Comparisons between continuous variables were done using the Wilcoxon-Mann-Whitney test [19]. Associations between categorical variables were assessed via Fisher's exact test or its generalizations [19]. The Cox proportional hazards regression model was used to assess the ability of individual patient covariates and immune response to predict event-free survival (EFS) time, with goodness-of-fit assessed by the Grambsch-Therneau test and martingale residual plots [20, 21]. All computations were carried out in S-Plus. [22]

Cell phenotype

Peripheral blood mononuclear cells (PBMC) were prepared by ficoll separation and stained with the following antibody cocktail (Caltag, Burlingame CA): CD8 FITC, CD4/CD14/CD16/CD19 TC, PR1/HLA-A2 or pp65/HLA-A2 tetramer PE [23] and aqua live/dead stain (Invitrogen, Carlsbad, CA). Events were acquired on a FACScan flow cytometer (Becton Dickinson, San Jose, CA). After gating on live, CD4/CD14/CD16/CD19- negative cells, a CD8+TC- gate was used to identify CTL. PR1-CTL were then identified from this gating schema.

Detailed phenotyping of total CD8 lymphocytes and PR1-CTL at the time of immune response was performed in patients with sufficient sample material. Cells were stained with antibodies as above for tetramer and with antibodies to CD45RA, CCR7, and a dead cell exclusion by aqua live/dead (Invitrogen, Carlsbad, CA). Cells were then acquired on a Cyan flow cytometer (Dako, Ft. Collins, CO). Cell compartments were defined as: Naïve (N=CD45RA+CCR7+), central memory (CM=CD45-CCR7+), effector memory (EM=CD45RA-CCR7-), and terminally differentiated (TD=CD45RA+CCR7-). Statistics were analyzed by paired t test (two-tailed).

TCR Avidity Measurement

For comparison of overall PR1-specific TCR avidity, median fluorescence intensity (MFI) of PR1 PR1/HLA-A2 tetramer staining was measured on 10^6 PBMCs incubated at 4°C with tetramer. After washing, cells were incubated with FITC-labeled CD8 (Caltag Laboratories Inc., Burlingame, California, USA) for 30 minutes on ice. The minimum concentration of tetramer necessary to show a distinct population of tetramer+CD8+ cells was determined in tetramer titration experiments and the same tetramer reagent was used to compare MFI. Propidium iodide (PI) (Becton Dickinson Immunocytometry Systems) staining (1μg/ml) was performed to exclude dead cells, according to the manufacturer's instructions. Surface expression of TCR was determined with FITC-labeled TCR-αβ (PharMingen, San Diego, California, USA), and a "dump" channel was used to eliminate monocytes and B

lymphocytes with nonspecific binding to the HLA-A2 heavy chain by staining with PerCP-labeled CD14 and CD19 (both Becton Dickinson Immunocytometry Systems). Cells were washed and fixed in 2% paraformaldehyde and analyzed on a FACScan (Becton Dickinson Immunocytometry Systems), and data were analyzed using CELLQuest (Becton Dickinson Immunocytometry Systems) software.

Results

Patient characteristics

Sixty-six patients were enrolled between January 2000 and November 2005 and their clinical characteristics are summarized in Table 1. Characteristics of the randomized groups were well balanced (Table 1). Eleven (17%) patients did not receive all of the intended vaccine doses due to disease progression (211 total vaccine doses were administered to 66 patients out of 234 (90%) planned doses), but all patients were assessed for toxicity after the first 3 weeks.

Vaccine safety

No grade 3 or 4 toxicity was observed. Grade 1 (30 patients) and grade 2 (4 patients) toxicity was observed, primarily as injection site reactions. In one patient a grade-2 skin reaction required a 2-week delay of the second scheduled dose. There was no association between grade I or grade II toxicity and vaccine dose level (p=0.21 and 0.48, respectively). Of 16 patients with prior allogeneic stem cell transplant (SCT), there was no worsening of GVHD after vaccination. None of the patients developed cANCA (anti-proteinase 3 antibodies) [24], including one patient (UPN #8) that had pANCA (anti-myeloperoxidase antibody) with no clinical evidence of an autoimmune disease prior to study entry.

PR1-specific T Cell Immune Responses

IR was defined as a   2-fold increase in the percentage of PR1-CTL in peripheral blood (Figure 1a), at any time during the study period. IR occurred in 35 (53%) patients and was not associated with PR1 dose. There was no significant difference in IR between the 3 dose levels (p=0.48). IR rate was not different between patients that received 3 doses versus those that received 6 doses (54% versus 50%, respectively). Furthermore, IR occurred by week 3 in 31 (89%) patients, at week 6 in 3 (9%) patients, and at week 9 in only 1 patient, as shown in Figure 1b-e. Of the 31 patients with no IR, the percentage of PR1-CTL varied   2-fold during the study period. In the 12 patients that received 6 doses of PR1 peptide vacine, no IR was observed after the third dose of the vaccine (Figure 1d, e).

PR1-CTL were present in 56 (85%) patients prior to vaccination (0.07% to 3.6%, mean of 0.37%), which was independent of disease type (p = 0.4), disease status (p = 0.2), and prior transplant history (p = 0.6). Importantly, the percentage of pre-vaccination PR1-CTL was not associated with IR after vaccination (p = 0.4). Similarly, IR was not associated with disease type, prior transplant, or abnormal cytogenetics prior to vaccination. However, IR after vaccination was associated with < 5% bone marrow blasts (p = 0.01) and younger age (51 vs. 60 years, p = 0.03). This suggests that patients with higher leukemia burden may not derive much benefit from active vaccination strategies alone, as was shown in a previous

study of combined PR1 and WT1 peptide vaccination [7]. The association of IR with younger age may reflect retained thymic function, since thymic output declines with age [25].

To investigate the possibility that IR merely reflected an increase of generalized immune responsiveness rather than an antigen specific response to vaccination, we measured T cell immunity to CMV using pp65/HLA-A2 tetramers in 31 IR patients that were seropositive without CMV reactivation. The change in the percentage of pp65-CTL from week 0 to week 9 did not correlate with the increase in PR1-CTL ($R^2 = 0.02$), supporting the conclusion that an increase of PR1-CTL was an antigen-specific response to vaccination.

Together, these data show that PR1 vaccine induces an antigen-specific immune response that occurs early after vaccination, which is independent of the percentage of pre-existing PR1-CTL and which may not be further increased by administering more than 3 vaccine doses.

To determine whether PR1 vaccine induced memory responses, we performed detailed immunophenotyping and compared changes in the percentage of PR1-CTL with naïve (N), central memory (CM), effector memory (EM), and terminally differentiated (TD) phenotype to the same compartments of overall CD8 T cells (Figure 2a). Compared to total CD8 T cells, as shown in Figure 2b, we found that PR1-CTL were significantly enriched in the CM cell compartment (p = 0.008) although they were depleted of EM cells (p = 0.007). Because CM cells contain a subset of cells with self-renewal capacity [23, 26], this suggests the possibility that vaccine-induced enrichment of the CM PR1-CTL compartment may increase the potential for long-term immunity, which may benefit patients by preventing relapse.

The PR1-CTL phenotype analysis was performed on the samples at the point of highest IR to provide a sufficient number of cells for phenotype analysis. Had the phenotyping been performed earlier during the PR1 immune response, it is likely that the phenotype of the PR1-CTL would have been skewed toward an EM phenotype. Since the phenotyping was performed later during the immune response, the point of highest IR, it is therefore not surprising that the phenotype of the PR1-CTL was of CM. Importantly, the detection of PR1-CTL with a CM phenotype highlights the ability of the PR1 peptide vaccine to elicit long-lasting immune responses that are expected after conventional peptide vaccines.

Increase in TCR avidity of PR1-CTL after vaccination

Since TCR avidity correlates with more specific lysis of leukemia, we sought to determine whether PR1 vaccine altered TCR avidity of PR1-CTL. We compared the PR1-CTL avidity of 18 patients before and after PR1 vaccination. We studied patients with IR (n = 10) or without IR (n = 8), and with clinical response (n = 8) or without clinical response (n = 10), who had a sufficient number of lymphocytes available for this assay.

Representative tetramer MFI data from 4 patient groups are shown in Figure 3. As shown in Figure 3a, among the IR patients, post-vaccine PR1-CTL avidity was higher in patients who achieved a clinical response compared to patients without clinical response (MFI = 326 vs. 238, p < 0.01). PR1-CTL avidity of 3 patients with clinical response but without IR was comparable to the TCR avidity in patients with IR and without a clinical response (p = 0.7),

but was higher than the no-IR patients without a clinical response, although it did not reach statistical significance (p=0.2). Finally, among the clinical responders, patients who achieved an IR demonstrated significantly higher PR1-CTL avidity than those who did not achieve an IR (P=0.02).

As shown in Figure 3b, the overall avidity of PR1-CTL increased in all 18 patients studied. However, the increase in avidity was greater in the clinical responders compared to non-clinical responders (p < 0.01), regardless of IR status. Interestingly, the increase in avidity of the clinical responders in the no-IR group was higher compared to the non-clinical responders in the IR group (p < 0.01). These results suggest that increases in TCR avidity of PR1-CTL, even in the absence of increases in the percentage of PR1-CTL, may be critical for clinical benefit. Furthermore, in one patient who remains in remission 16 years following vaccination with PR1 peptide vaccine, high TCR avidity (mean MFI= 343) was maintained 4 years after vaccination, which was the time of the last TCR avidity measurement.

Together, these results show that PR1-specific TCR avidity increased after vaccination in some patients with IR and clinical responses, and they suggest that TCR avidity did not increase in patients with no IR and no clinical response.

Clinical Responses

Although this study was designed to evaluate toxicity and vaccine-induced IR rate, clinical responses were studied in 53 patients with measurable disease at study entry. Clinical responses occurred in 12 (23%) patients in all 3 disease-types (8 in AML, 2 in MDS and 2 in CML patients) predominantly in patients with a low amount of leukemia. *Low disease burden* was considered as < 10% BM blasts (morphologically identified as 5-10% BM blasts, or 5% blasts with either cytogenetic (metaphase analysis) or molecular (PCR or FISH analysis) evidence of disease), and *high disease* burden was 10% BM blasts. As summarized in Table 2, 11 (33%) of 33 patients with low disease burden (< 10% BM blasts) had a clinical response, versus only 1 of 20 patients with high disease burden (> 10% BM blasts) (p=0.01). Six (50%) responses occurred in patients with cytogenetic or molecular abnormalities documented 1 to 4 months after the last anti-leukemia therapy.

Clinical responses were also associated with IR. Nine (36%) of 25 patients with IR had a clinical response, compared to 3 (10%) of 28 patients that did not have IR (p=0.04). These responses were independent of the dose of PR1 peptide vaccine, although there was a trend in favor of lower dose (p = 0.05) or the number of vaccinations that was administered (3 versus 6). Because of the heterogeneity of the patients, including the type and status of the disease, it was not possible to adequately compare clinical responses within groups.

With a median follow up in living patients of 120.7 months (range: 4 to 188), 26 patients (39%) are still alive, and 14 (21%) are alive and in remission. Out of the 26 patients still alive, 17 (65%) had an IR to PR1 vaccine. Out of 14 patients still alive and in remission, 10 (71%) had an IR to the vaccine.

Survival

With a median follow up of 120 months, 10-year EFS was 20% and 10-year OS was 38% (Figure 4a and b). We also evaluated the outcome based on the IR to PR1 vaccine. As shown in Supplementary Figures 1a and b, both EFS (p=0.03) and OS (p=0.05) were significantly longer in immune responders vs. non-responders. We further evaluated the outcome based on clinical response to the vaccine. Supplementary Figures 2a and b show that both EFS (0.001) and OS (<0.001) were significantly longer in clinical responders vs. non-responders.

Discussion

In this phase I/II safety and immune efficacy study, the PR1 vaccine showed minimal toxicity, no adverse autoimmunity, and no vaccine-associated peripheral blood cytopenia. The accrual for this trial was completed in 2005. However, it took us several years to obtain and analyze all the post treatment samples for immune response and other correlative immunological studies. We elected to have a longer follow up to assess the durability of response and survival outcomes. Indeed, a study with PR1 and WT1 peptide vaccines was conducted by Rezvani et al in a small number of patients and they reported the results in 2008[7]. Their study was important because the investigators used a dose and route similar to our study (0.5 mg subcutaneously for a single dose). However, it was limited to 8 patients with myeloid malignancies and they characterized T-cell responses with limited follow up, at a median of approximately 8 months. In contrast, our study tested PR1 vaccination in more patients (66) with a mix of AM, MDS and CML. Moreover, because the clinical impact of immunotherapies is often delayed, we felt it would be important to report more mature clinical outcomes from this larger phase I/II study. Thus, we delayed publication to determine detailed immune results in a maximum number of patients and to correlate this with long term clinical outcome.

IR after vaccination occurred in 35 (53%) patients and was independent of PR1 peptide vaccine dose. Compared to overall CD8 T cells, PR1-specific CD8 T cells were significantly enriched in central memory cells following vaccination [26, 27]. In a subset of 18 patients studied in greater detail, higher TCR avidity of PR1-specific CD8 T cells was observed after vaccination in patients with IR and with clinical response compared to non-responders.

Significantly, a reduction of disease activity was observed in 12 (22%) of 53 patients vaccinated with active disease, including conversion to cytogenetic remission in 2 CML patients and molecular remission in 4 AML patients who had a low leukemia burden prior to vaccination. IR was confirmed in a subset of patients by measuring PR1-specific intracellular interferon-γ production and CD69 upregulation [4, 28]. Immune responses were likely to be specific for PR1 since immunity to the dominant CMV pp65 epitope in 31 CMV antigen seropositive patients was not correlated with immunity to PR1 following vaccination. Taken together, these results show that vaccination with a single PR1 peptide dose results in functionally active antigen-specific immunity against leukemia and is associated with molecular remission.

P3, the source of PR1 peptide, has been demonstrated in chronic phase CML progenitor cells, including leukemia stem cells (LSC) [1, 3, 29]. While aberrant expression of P3 and NE

in leukemia target cells increases their susceptibility to lysis by PR1-CTL, overexpression also leads to peripheral tolerance by inducing selective deletion of PR1-CTL with high avidity TCR, facilitating leukemia outgrowth [5]. However, high avidity PR1-CTL also show higher specific lysis of leukemia and are present in higher frequency in the bone marrow, the site of disease, compared to peripheral blood. In this study, we observed increased TCR avidity of PR1-CTL in patients with clinical responses, regardless of the quantitatively determined IR status, although these comparisons could not be performed on all patients. Nevertheless, this suggests the possibility that PR1 peptide vaccination may cause the selective outgrowth of clones expressing high affinity TCR, which could be evaluated in future trials. In addition, we found that vaccine-induced PR1-CTL were enriched in CM cells compared to the overall CD8+ population, which might be important for sustained immunity after vaccination [26, 27].

A separate phase I trial of PR1 peptide combined with WT1 peptide has been reported on 8 patients with AML, CML, and MDS by Rezvani, et al at NIH [7]. In that trial, PR1 was administered using the same schedule and adjuvant plus low-dose GM-CSF. Immune responses were observed against both peptides, and WT1 expression, a measure of disease activity, decreased in 3 of 6 evaluable patients. Although the differential effects of PR1-versus WT1-specific immunity could not be distinguished in this study, the results are similar to those reported here. Another trial with a polyvalent WT1 peptide vaccine was performed in patients with AML in CR but with residual molecular disease. WT1-specific T cell responses were seen in 7 of 8 evaluable patients, 5 patients remained in CR for up to 30 months after treatment and their WT1 transcript levels either decreased or remained stable[10]. There have been clinical trials using BCR-ABL peptides in patients with CML that demonstrated an immunologic response[11, 12, 30, 31]. At least two of these trials also showed some evidence of clinical response[12, 31]. However, subsequent studies showed that BCR-ABL peptides are either insufficiently processed or presented on the antigen presenting cell surface, or elicit a weak T cell response to induce a clinical response[13]. Taken together, the results of these trials suggest that peptide vaccination with certain immunogenic peptides for myeloid leukemia is safe, can induce specific immunity, and is associated with reduced disease activity.

The results of this trial also show that high avidity PR1-CTL can emerge after PR1 peptide vaccination and may contribute to molecular remission. Furthermore, since high avidity PR1-CTL are more frequent in the bone marrow compared to peripheral blood, it is possible that a higher percentage of these cells homed to the bone marrow [32]. However, since leukemia can shape immunity through deletion of peripheral PR1-CTL, it is possible that high leukemia burden results in the selective loss of high avidity PR1-CTL[5]. Our data are consistent with this hypothesis and suggest that vaccination strategies aimed at increasing the number of highly active high avidity T cells would not be effective in the setting of advanced disease. The conversion to molecular remission that was observed suggests there was a significant elimination of LSC. Because the clinical response to single agents targeting FLT3 or BCR-ABL is short-lived and not curative [33], our results support future studies to test whether peptide vaccines in combination with targeted therapies, such as tyrosine kinase inhibitors, or immune checkpoint inhibitors, such as ipilimumab or nivolumab, may be additive or synergistic in further reducing leukemia burden or eliminating remaining LSC[34].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

References

1. Molldrem J, Dermime S, Parker K, Jiang YZ, Mavroudis D, Hensel N, et al. Targeted T-cell therapy for human leukemia: cytotoxic T lymphocytes specific for a peptide derived from proteinase 3 preferentially lyse human myeloid leukemia cells. Blood. 1996 Oct 1; 88(7):2450–2457. [PubMed: 8839835]

2. Molldrem JJ. Vaccination for leukemia. Biol Blood Marrow Transplant. 2006 Jan; 12(1 Suppl 1):13–18.

3. Molldrem JJ, Clave E, Jiang YZ, Mavroudis D, Raptis A, Hensel N, et al. Cytotoxic T lymphocytes specific for a nonpolymorphic proteinase 3 peptide preferentially inhibit chronic myeloid leukemia colony-forming units. Blood. 1997 Oct 1; 90(7):2529–2534. [PubMed: 9326217]

4. Molldrem JJ, Lee PP, Wang C, Felio K, Kantarjian HM, Champlin RE, et al. Evidence that specific T lymphocytes may participate in the elimination of chronic myelogenous leukemia. Nature medicine. 2000 Sep; 6(9):1018–1023.

5. Molldrem JJ, Lee PP, Kant S, Wieder E, Jiang W, Lu S, et al. Chronic myelogenous leukemia shapes host immunity by selective deletion of high-avidity leukemia-specific T cells. The Journal of clinical investigation. 2003 Mar; 111(5):639–647. [PubMed: 12618518]

6. Keilholz U, Letsch A, Busse A, Asemissen AM, Bauer S, Blau IW, et al. A clinical and immunologic phase 2 trial of Wilms tumor gene product 1 (WT1) peptide vaccination in patients with AML and MDS. Blood. 2009 Jun 25; 113(26):6541–6548. [PubMed: 19389880]

7. Rezvani K, Yong AS, Mielke S, Savani BN, Musse L, Superata J, et al. Leukemia-associated antigen-specific T-cell responses following combined PR1 and WT1 peptide vaccination in patients with myeloid malignancies. Blood. 2008 Jan 1; 111(1):236–242. [PubMed: 17875804]

8. Scheibenbogen C, Letsch A, Thiel E, Schmittel A, Mailaender V, Baerwolf S, et al. CD8 T-cell responses to Wilms tumor gene product WT1 and proteinase 3 in patients with acute myeloid leukemia. Blood. 2002 Sep 15; 100(6):2132–2137. [PubMed: 12200377]

9. Oka Y, Tsuboi A, Taguchi T, Osaki T, Kyo T, Nakajima H, et al. Induction of WT1 (Wilms' tumor gene)-specific cytotoxic T lymphocytes by WT1 peptide vaccine and the resultant cancer regression. Proceedings of the National Academy of Sciences of the United States of America. 2004 Sep 21; 101(38):13885–13890. [PubMed: 15365188]

10. Maslak PG, Dao T, Krug LM, Chanel S, Korontsvit T, Zakhaleva V, et al. Vaccination with synthetic analog peptides derived from WT1 oncoprotein induces T-cell responses in patients with complete remission from acute myeloid leukemia. Blood. 2010 Jul 15; 116(2):171–179. [PubMed: 20400682]

11. Cathcart K, Pinilla-Ibarz J, Korontsvit T, Schwartz J, Zakhaleva V, Papadopoulos EB, et al. A multivalent bcr-abl fusion peptide vaccination trial in patients with chronic myeloid leukemia. Blood. 2004 Feb 1; 103(3):1037–1042. [PubMed: 14504104]

12. Bocchia M, Gentili S, Abruzzese E, Fanelli A, Iuliano F, Tabilio A, et al. 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. Lancet. 2005 Feb 19-25; 365(9460):657–662. [PubMed: 15721470]

13. Maslak PG, Dao T, Gomez M, Chanel S, Packin J, Korontsvit T, et al. A pilot vaccination trial of synthetic analog peptides derived from the BCR-ABL breakpoints in CML patients with minimal disease. Leukemia. 2008 Aug; 22(8):1613–1616. [PubMed: 18256684]

14. Vallespi T, Torrabadella M, Julia A, Irriguible D, Jaen A, Acebedo G, et al. Myelodysplastic syndromes: a study of 101 cases according to the FAB classification. British journal of haematology. 1985 Sep; 61(1):83–92. [PubMed: 3863667]

15. Cortes J, Kantarjian H, O'Brien S, Kurzrock R, Keating M, Talpaz M. GM-CSF can improve the cytogenetic response obtained with interferon-alpha therapy in patients with chronic myelogenous leukemia. Leukemia. 1998 Jun; 12(6):860–864. [PubMed: 9639411]

16. Falkenburg JHF, Goselink HM, Van der Harst D, Van Luxemburg-Heijs SAP, Kooy-Winkelaar YMC, Faber LM, et al. Growth inhibition of clonogenic leukemic precursor cells by minor histocompatibility antigen-specific cytotoxic T lymphocytes. Journal of Experimental Medicine. 1991; 174(1):27–33. [PubMed: 2056279]

17. Cheson BD, Bennett JM, Kantarjian H, Pinto A, Schiffer CA, Nimer SD, et al. Report of an international working group to standardize response criteria for myelodysplastic syndromes. Blood. 2000 Dec 1; 96(12):3671–3674. [PubMed: 11090046]

18. Thall PF, Sung HG. Some extensions and applications of a Bayesian strategy for monitoring multiple outcomes in clinical trials. Statistics in medicine. 1998 Jul 30; 17(14):1563–1580. [PubMed: 9699230]

19. Snedecor, GWC., Cochran, WG. Iowa State University Press; Ames, Iowa, U.S.A: 1980.

20. Cox DR. Regression Models and Life Tables (with Discussion). J R Stat soc B. 1972; 34:187–220.

21. Therneau, TMG., Grambsch, PM. Modelling Survival Data:Extending the Cox Model. Springer; New York, USA: 2000.

22. Venables, WNR., Ripley, BD. Modern applied statistics with S-PLUS. Springer; New York, USA: 1999.

23. Kanodia S, Wieder E, Lu S, Talpaz M, Alatrash G, Clise-Dwyer K, et al. PR1-specific T cells are associated with unmaintained cytogenetic remission of chronic myelogenous leukemia after interferon withdrawal. PLoS One. 5(7):e11770.

24. Savage CO, Harper L, Holland M. New findings in pathogenesis of antineutrophil cytoplasm antibody-associated vasculitis. Current opinion in rheumatology. 2002 Jan; 14(1):15–22. [PubMed: 11790991]

25. Douek DC, McFarland RD, Keiser PH, Gage EA, Massey JM, Haynes BF, et al. Changes in thymic function with age and during the treatment of HIV infection. Nature. 1998 Dec 17; 396(6712): 690–695. [PubMed: 9872319]

26. Lanzavecchia A, Sallusto F. Dynamics of T lymphocyte responses: intermediates, effectors, and memory cells. Science (New York, NY). 2000 Oct 6; 290(5489):92–97.

27. Lanzavecchia A, Sallusto F. Understanding the generation and function of memory T cell subsets. Current opinion in immunology. 2005 Jun; 17(3):326–332. [PubMed: 15886125]

28. Komanduri KV, Viswanathan MN, Wieder ED, Schmidt DK, Bredt BM, Jacobson MA, et al. Restoration of cytomegalovirus-specific CD4+ T-lymphocyte responses after ganciclovir and highly active antiretroviral therapy in individuals infected with HIV-1. Nature medicine. 1998 Aug; 4(8):953–956.

29. Yong AS, Keyvanfar K, Eniafe R, Savani BN, Rezvani K, Sloand EM, et al. Hematopoietic stem cells and progenitors of chronic myeloid leukemia express leukemia-associated antigens: implications for the graft-versus-leukemia effect and peptide vaccine-based immunotherapy. Leukemia. 2008 Sep; 22(9):1721–1727. [PubMed: 18548092]

30. Pinilla-Ibarz J, Cathcart K, Korontsvit T, Soignet S, Bocchia M, Caggiano J, et al. Vaccination of patients with chronic myelogenous leukemia with bcr-abl oncogene breakpoint fusion peptides generates specific immune responses. Blood. 2000 Mar 1; 95(5):1781–1787. [PubMed: 10688838]

31. Rojas JM, Knight K, Wang L, Clark RE. Clinical evaluation of BCR-ABL peptide immunisation in chronic myeloid leukaemia: results of the EPIC study. Leukemia. 2007 Nov; 21(11):2287–2295. [PubMed: 17637811]

32. Melenhorst JJ, Scheinberg P, Chattopadhyay PK, Gostick E, Ladell K, Roederer M, et al. High avidity myeloid leukemia-associated antigen-specific CD8+ T cells preferentially reside in the bone marrow. Blood. 2009 Mar 5; 113(10):2238–2244. [PubMed: 18997173]

33. Carter BZ, Mak DH, Cortes J, Andreeff M. The elusive chronic myeloid leukemia stem cell: does it matter and how do we eliminate it? Semin Hematol. Oct; 47(4):362–370.

34. Sharma P, Allison JP. Immune checkpoint targeting in cancer therapy: toward combination strategies with curative potential. Cell. 2015 Apr 9; 161(2):205–214. [PubMed: 25860605]
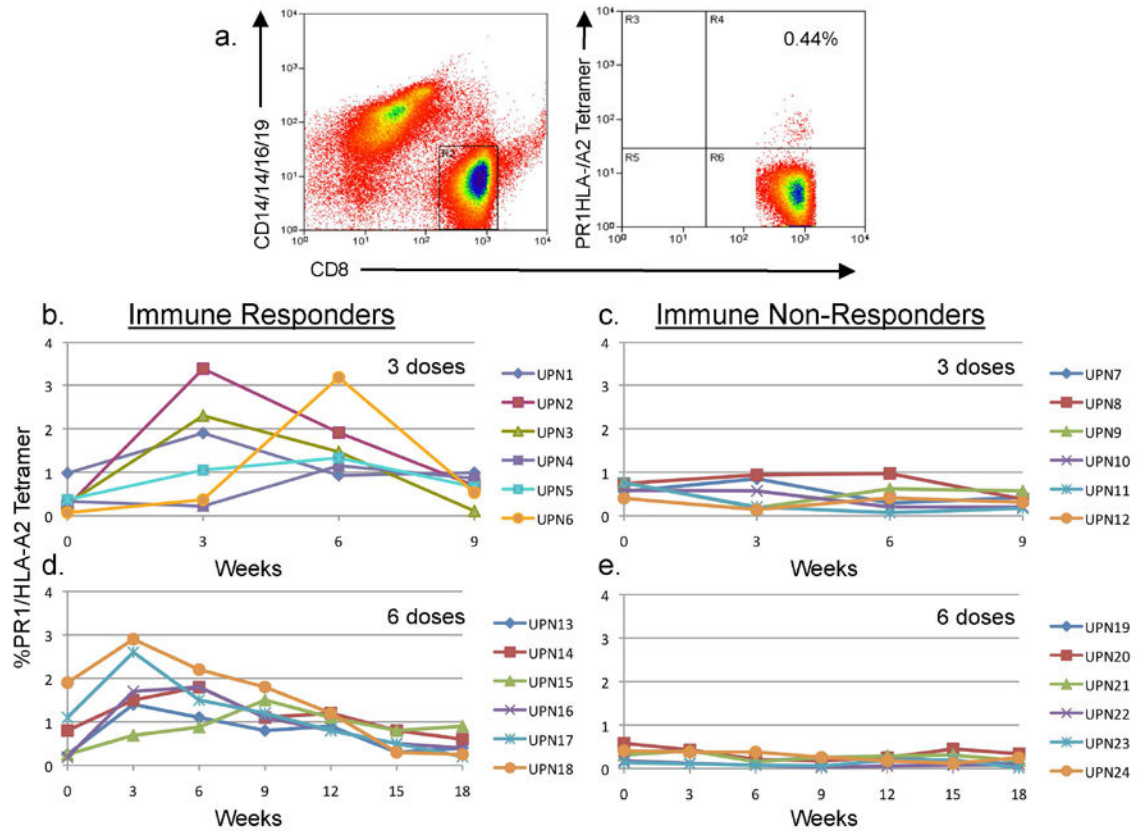
Figure 1. PR1 –specific CTLs were detected in patient peripheral blood following PR1-peptide vaccination

(a) Gating schema for PR1-CTLs. CTLs, defined by the expression of CD8 and lack of expression of CD4, 14, 16, and 19, were analyzed for binding to PR1-HLA-A2 tetramer. PR1-CTLs were detected in peripheral blood from immune responders following vaccination with 3 (b) and 6 (d) doses of PR1-peptide vaccine, in contrast to non-responders (c) and (e) where <1% of CD8+ CTLs were PR1-specific.
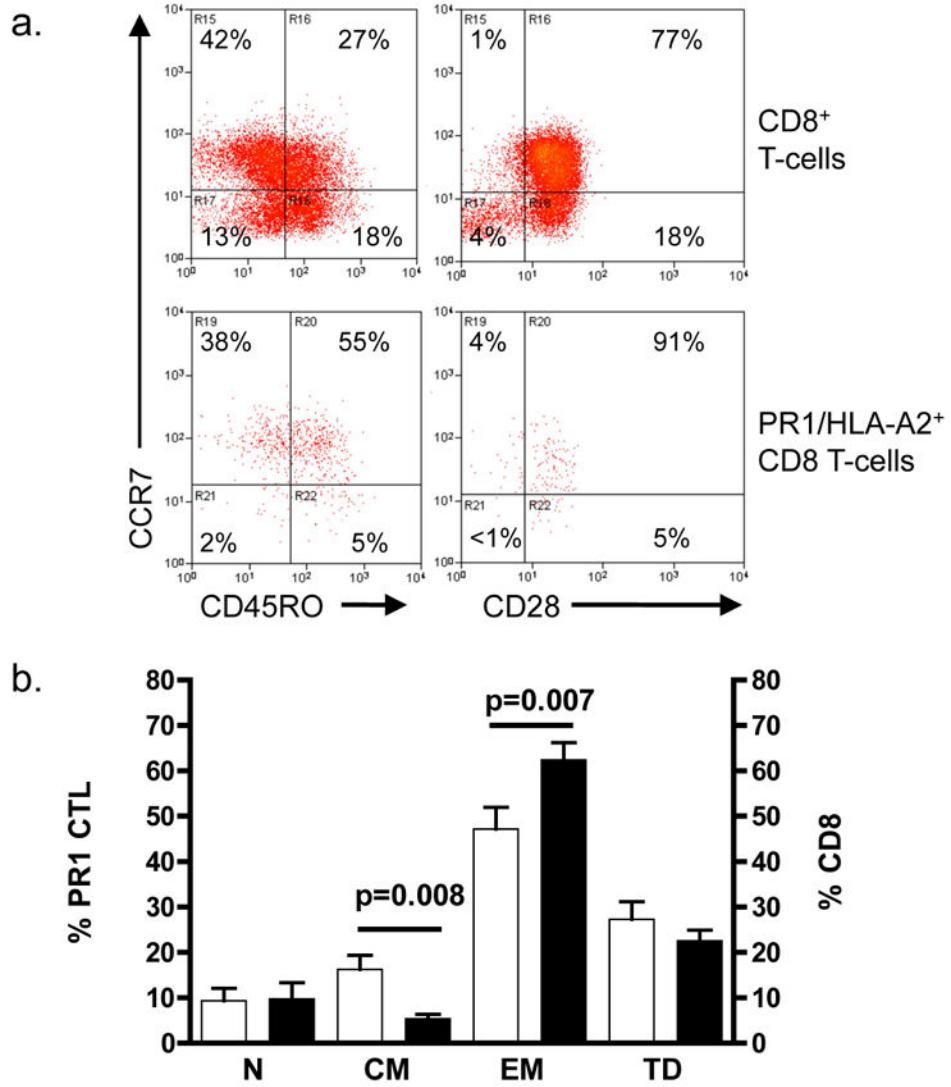
Figure 2. PR1-CTLs are enriched in central memory cells following PR1-peptide vaccination
CD8+ T-cells and PR1-specific CTLs were stained for CCR7, CD45RO and CD28 to
determine cell T- cell phenotype. (A) Representative flow cytometry staining of one patient
sample showing percentages of central memory (CD45RO+ CCR7+)/(CD28+ CCR7+) and
effector memory (CD45RO+ CCR7-)/ (CD28+ CCR7-) in CD8+ T-cell and PR1-specific
CTL compartments. (B) CD8+ T-cells and PR1-CTLs demonstrating CM phenotype were
significantly increased within PR1 CTL (*p*=0.008) while cells with an EM phenotype were
significantly decreased (*p*=0.007) compared to the overall CD8+ cell population. Open bars
are PR1-CTL; black bars are the remaining CD8+ T cells. N, naive; CM, memory; EM,
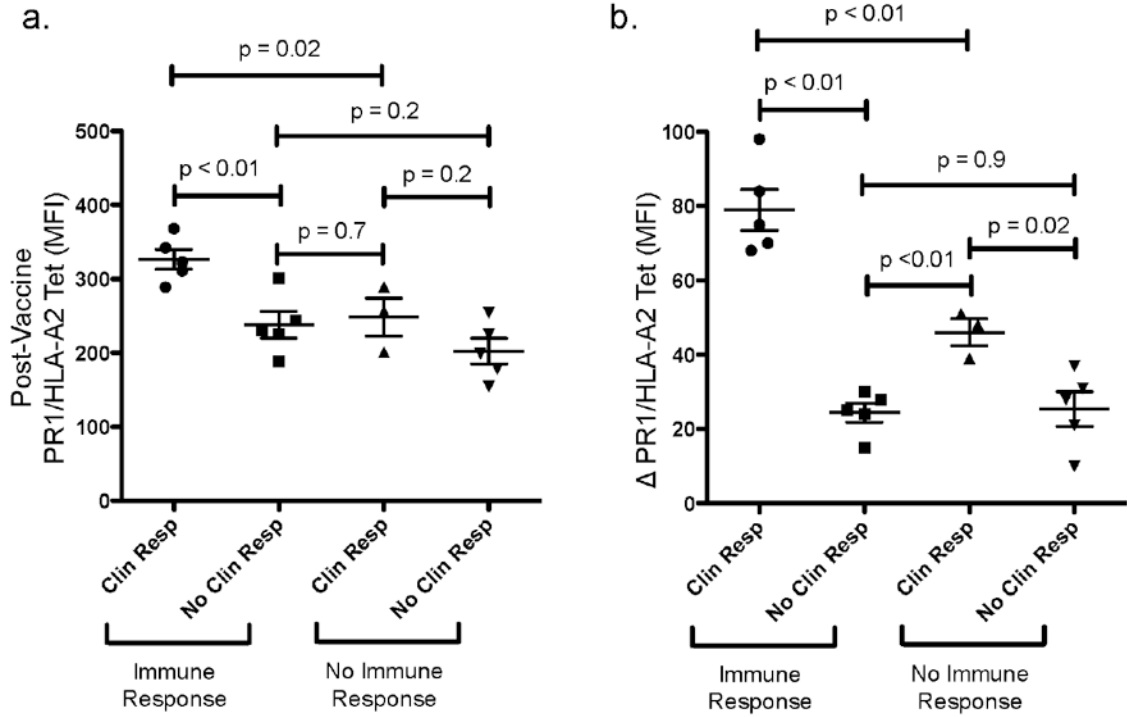effector memory; TD, terminally differentiated.

Figure 3. PR1-peptide vaccine increases TCR avidity of PR1-CTLs

(a) PR1-HLA tetramer staining of 18 representative patient samples demonstrates increased PR1-CTL avidity following PR1-peptide vaccination. PR1-CTL avidity was significantly higher in patients with an IR and CR in comparison with patients with IR who failed to achieve a CR (P<0.01). In patients with a CR and IR, PR1-CTLs showed higher TCR avidity for PR1-HLA tetramer in comparison to patients with CR and no-IR (p=0.02). (b) Change in PR1/HLA-A2 tetramer staining of PR1-CTLs in patients according to IR and CR status. Patients with IR and CR had the greatest changes in PR1-CTL TCR avidity following vaccination, compared to PR1-CTL TCR avidity prior to vaccine administration. TCR, T-cell receptor; CTLs, cytotoxic T lymphocytes; IR, immunologic response; CR, clinical response.

a: Event-Free Survival (n=66)

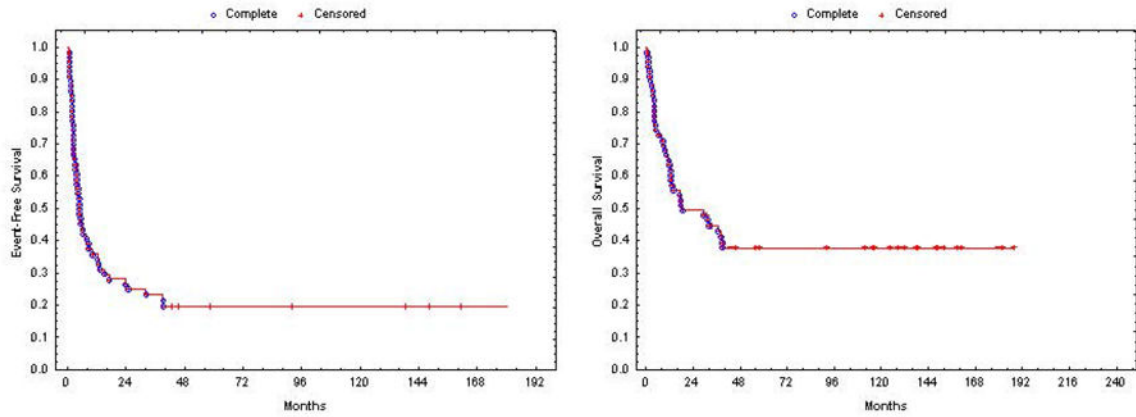## b: Overall Survival (n=66)

Figure 4. Event-free and Overall Survival of all 66 patients treated with PR1 Peptide Vaccine
a) EFS of 66 patients who received PR1 peptide vaccine; b) OS of 66 patients who received
PR1 peptide vaccine

Table 1

Patient Characteristics (N=66)

	Dose level 1 (0.25 mg) N=21	Dose level 2 (0.5 mg) N=22	Dose level 3 (1.0 mg) N=23	p value
Gender				
Female	8	6	12	0.2
Male	13	16	11	
Median Age	52	57	53	0.4
Diagnosis				
AML	14	16	12	0.6
MDS	3	2	6	
CML	4	4	5	
Disease Status				
MD	20	17	16	0.09
CR	1	5	7	
Prior SCT	8	6	6	0.6
Allogeneic	6	6	4	
Autologous	2	0	2	
Prior DLI	2	3	2	1.0
Vaccinations #				
3	17	19	18	0.8
6	4	3	5	
BM Blast 10%	8	5	7	0.5

MD = Measurable disease by bone marrow blasts > 5%, karyotype abnormality, or molecular defect detected by specific PCR or FISH

CR = Complete remission

SCT = HLA-matched allogeneic stem cell transplantation

DLI = Donor lymphocyte infusion from SCT donor

*
One patient received 1 vaccination, and four received 2 vaccinations

**
One patient each received 1, 2, 3 or 5 vaccinations

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Table 2

Clinical Response Characteristics

Pt #	Sex/ Age (yrs)	PR1 Dose Level	Disease	# Of Prior Regimens	Time From Last Treatment (weeks)	Immune Response	Disease Status *Before*	Disease Status *After*	Clinical Response	Response Duration (months)
4	M/26	2	AML-M3	7	12	+	CRp PCR: [t(5;17)] + Blasts: 1%	CR PCR: [t(5;17)] – Blasts: 1%	MR	75 +
7	F/48	3	AML-M2	2	24	+	CRp Leukemia cutis Diploid karyotype Blasts: 1%	CR Leukemia cutis resolved Diploid karyotype Blasts: 0 %	CR	60 +
15	M/31	1	AML-M4	3	12	+	CRp FISH: 2.5% PCR: [inv(16)]+ Blasts 2%	CR FISH: 0% PCR: [inv(16)] – Blasts: 2%	MR	50 +
16	F/28	1	AML-M2	4	16	+	CRp Diploid karyotype Blasts: 4%	CR Diploid karyotype Blasts: 3%	CR	23
36	F/28	1	AML-M1	3	12	–	CRp Complex karyotype Blasts: 0%	CR Diploid karyotype Blasts:1%	CR	26 +
45	M/30	1	AML-M4	3	4	+	CR PCR: [inv(16)]+ Blasts: 1%	CR PCR: [inv(16)] – Blasts: 1%	MR	24
54	F/74	3	MDS (RA)	2	52	–	Neutropenia and thrombocytopenia Blasts: 1%	Cytopenia resolved Blasts: 2%	HR	21 +
61	M/72	3	MDS (RA)	3	8	–	Neutropenia Blasts: 0 %	Neutropenia resolved Blasts: 0 %	HR	4
18	M/60	1	MDS (RAEB)	1	4	+	Pancytopenia Blasts: 17%	Pancytopenia persisted Blasts: 4%	PR	5
43	M/66	1	MDS (RAEB)	2	4	+	Thrombocytopenia Blasts: 7%	Thrombocytopenia resolved Blasts: 5%	HR	5
12	M/50	1	CML-CP	1	4	+	Major CR [bcr-abl/abl]: 0.084 Cyto:10% Ph$^+$ Blasts: 1%	MR [bcr-abl/abl]: 0.00 Cyto: 0% Ph$^+$ Blasts: 1%	MR	45
63	F/63	1	CML-CP	1	12	+	Major CR [bcr-abl/abl]: 12.22 Cyto: 25% Ph$^+$ Blasts: 1%	CCR [bcr-abl/abl]: 1.54 Cyto: 0% Ph$^+$ Blasts: 1%	CCR	9

Abbreviations:

+ Ongoing response at last clinical evaluation

AML = acute myeloid leukemia

MDS = myelodysplastic syndrome

CML–CP = chronic myelogenous leukemia, chronic phase

RA = refractory anemia

RAEB = refractory anemia with excess blasts

PCR = polymerase chain reaction

inv(16) = pericentric inversion of chromosome 16 assessed by PCR

bcr-abl/abl = ratio of bcr/abl transcripts to abl transcripts determined by real-time PCR

FISH = Fluorescence in-situ hybridization detection of inv(16)

Ph$^+$ = Number of Philadelphia chromosomes identified by standard metaphase chromosome analysis of 20 cells

MR = molecular response (sustained reduction of leukemia-specific PCR product by ≥1-log or is no longer detectable in two consecutive bone marrow biopsies)

CR = complete remission

Major CR = major cytogenetic response

CCR = complete cytogenetic response

CRp = complete remission without platelet recovery

PR = partial remission

HR = hematologic response

BM = bone marrow