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BCR-ABL-SPECIFIC T-CELL THERAPY IN Ph+ ALL PATIENTS ON TYROSINE-KINASE INHIBITORS

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Key points:

BCR-ABL-specific CTLs may be obtained by stimulation with peptides derived from BCR-ABL junctional region and alternative splicing.

T cell therapy with BCR-ABL-specific CTLs from healthy donors or patients mediate molecular or hematologic CR in patients with Ph+ ALL.

ABSTRACT

While the emergence of bone marrow-resident p^{190} BCR-ABL-specific T lymphocytes has been correlated with hematologic and cytogenetic remissions in patients with Philadelphia chromosome positive, acute lymphoblastic leukemia (Ph+ ALL) undergoing maintenance tyrosine-kinase inhibitor treatment, little is known about the possibility of culturing these cells ex vivo and employing them in T-cell therapy strategies. We investigated the feasibility of expanding/priming p^{190} BCR-ABL-specific T cells in vitro by stimulation with dendritic cells pulsed with p^{190} BCR-ABL peptides derived from the BCR-ABL junctional region and alternative splicing, and of adoptively administering them to patients with relapsed disease. We report on the feasibility of producing clinical grade BCR-ABL-specific cytotoxic T lymphocytes (CTLs) from Ph+ ALL patients and healthy donors, endowed with anti-leukemia activity. We treated 3 patients with Ph+ ALL with autologous or allogeneic p^{190} BCR-ABL-specific CTLs. No post-infusion toxicity was observed, except for a grade II skin graft-versus-host disease in the patient treated for hematologic relapse. All patients obtained a molecular or hematologic complete remission after T-cell therapy, upon emergence of p^{190} BCR-ABL-specific T cells in the bone marrow. Our results show that p^{190} BCR-ABL-specific CTLs are capable of controlling treatment-refractory Ph+ ALL in vivo, and support the development of adoptive immunotherapeutic approaches with BCR-ABL CTLs in Ph+ ALL.

Patrizia Comoli*, Sabrina Basso*, Mario Luppi* and Leonardo Potenza* contributed equally to this study.

The online version of the article contains a data supplement.

Introduction

Philadelphia chromosome positive acute lymphoblastic leukemia (Ph+ ALL) was formerly burdened by uniformly poor prognosis.¹ Widespread application of allogeneic hematopoietic stem cell transplantation (alloHSCT) and advent of targeted BCR-ABL-specific tyrosine-kinase inhibitors (TKI) have significantly improved complete response rates and disease-free survival.^{1,2} Despite these therapeutic advances, some unresolved issues remain, including the high prevalence in older patients,³ often ineligible for alloHSCT, and the extremely poor prognosis of relapsed Ph+ ALL, particularly following alloHSCT.^{1,4}

Prolonged hematologic and cytogenetic remissions have been observed with imatinib mesylate (IM) alone, even in the presence of persisting levels of minimal residual disease (MRD).⁵⁻⁷ Our group was able to demonstrate that attainment of such clinical responses directly correlated with the emergence of BCR-ABL-specific T cells in the bone marrow and, to a lesser extent, in the peripheral blood of non-allografted Ph+ ALL patients, undergoing post-remission maintenance treatment with either IM or other second generation TKIs.^{8,9} These observations extended previous evidence of functional leukemia-specific cellular immune responses developing in patients receiving IM, and possibly acting in synergy with IM to reach disease control,^{10,11} and represent the basis for a combined TKI and T-cell therapy approach to Ph+ ALL in elderly patients, or in patients relapsing after alloHSCT.

We report on the feasibility of inducing durable MRD clearance and leukemia control, without additional toxicity, by transfer of donor-derived or autologous cytotoxic T-lymphocytes (CTLs) specific for the BCR-ABL fusion product in patients receiving TKI treatment for leukemia relapse after alloHSCT, or for molecular relapse in patients ineligible for alloHSCT. In addition, we describe the immunological parameters correlated with clinical response.

Study design and Methods

Patient 1 was a 61-year-old man in second molecular recurrence after matched unrelated donor (MUD) alloHSCT and unmanipulated donor lymphocyte infusions (DLI). Patient 2 was a 30-year-old man diagnosed with Ph+ ALL with hyperleukocytosis and CNS involvement, in third hematologic relapse (BM blast 66%, F317L mutation) after MUD-HSCT, DLI, and rescue therapy with Nilotinib. Patient 3 was a 62-year-old woman diagnosed with Ph+ ALL with CNS involvement, showing persistent molecular disease (last MRD before T-cell therapy 0.1% BCR-

ABL/ABL) after induction, maintenance chemotherapy, and prolonged TKI treatment. She was not eligible for alloHSCT due to comorbidities. Details on patients' clinical histories are reported in supplemental data.

Methods for ^{190}P BCR-ABL-specific CTL preparation and testing are detailed in supplemental data. BCR-ABL peptide pool employed in the stimulation procedure has been previously reported.⁸ BCR-ABL-specific treatment was administered on a compassionate basis according to Bioethical Committee approval.

MRD values were measured sequentially on bone marrow mononuclear cell (BM MC) samples at baseline, and after each CTL infusion, by means of a previously described reverse transcriptase-polymerase chain reaction quantification of BCR-ABL transcripts.¹² Immunological responses were evaluated sequentially, by flow cytometry (Document S1).^{8,13}

Results and Discussion

BCR-ABL-specific CTLs were expanded from peripheral blood MCs collected from the patient (case 3) or from HSC donors (cases 1 and 2) (Figure 1A). CTL lines were polyclonal (supplemental Figure S1) and included both CD3+CD8+ and CD3+CD4+ T cells (Figure 1B). Each CTL line produced IFN γ in response to at least 1 BCR-ABL peptide pool (Figure 1C), and recognized autologous targets pulsed with BCR-ABL peptides and/or patient leukemia blasts (Figures 1D, 1E). Cytotoxic activity was likely mediated by both CD8+ and CD4+ T cells, since we observed lysis in both 5h- and -12h assays, the latter being HLA-class II-restricted (supplemental Figure S2). Regarding specific activity against BCR-ABL peptides, we observed a broad response to the different peptide pools; of note, all CTLs recognized mix3 peptides, that included epitopes derived from products of BCR-ABL alternative splicing, confirming data on the suitability of these proteins as leukemia-specific antigens capable of eliciting an effective tumor-specific CTL response in vitro and also in vivo.¹⁴ As previously reported,^{15,16} by stimulating with dendritic cells pulsed with BCR-ABL peptides that included long peptides along with 9-mer epitopes, and employing homeostatic cytokines in the culture process,¹⁷ we were able to prime leukemia-specific responses also in healthy donors.

Cells were administered in a dose-escalating manner on a monthly schedule. The three patients received a mean of 10 monthly infusions (range 6-13). No immediate infusion-related adverse events were observed, and no grade 2-4 toxicities, including development of cytokine release syndrome, attributable to the T-cell infusions were recorded during follow-up. Patient 2, treated for

hematologic relapse after alloHSCT, showed grade II skin graft-versus-host disease after administration of dose-level 2, successfully treated with topic steroids.

Molecular or hematologic CR (MCR, HCR) was obtained in all patients, associated with emergence of p^{190} BCR-ABL-specific T-cells (Figure 2). Patient 1, who had detectable BCR-ABL transcripts in the BM prior to donor CTL treatment, achieved MCR after 4 weeks from the first CTL dose. He was maintained in CMR with monthly T-cell infusions, associated with IM treatment, for 12 months. After T-cell therapy discontinuation, he persists in CMR at 57-month follow-up (Figure 2A). Patient 3, who had persistent molecular disease and was ineligible for an alloHSCT, was treated with autologous p^{190} BCR-ABL-specific CTLs. She achieved MCR at month +6 from cell therapy initiation (Figure 2C). In the patient with hematologic relapse (Nr.2), we obtained HCR after 6 monthly p^{190} BCR-ABL-specific CTL infusions at lower doses ($0.1-0.5 \times 10^6$ T cell/Kg), in combination with Ponatinib treatment. Of note, in this last patient, the sole administration of p^{190} BCR-ABL-specific CTLs, before the introduction of Ponatinib, reduced BM blasts from 66% to 25%. Leukemia-specific T-cell responses were undetectable in the BM prior to CTL administration in all 3 patients. Progressive emergence of BM-resident, polyfunctional (cases 1, 3) or IFN γ secreting (case 2) p^{190} BCR-ABL-specific CD4+ and CD8+ T cells was associated with clearance of residual disease (Figure 2). Parallel epitope spreading to WT-1-antigen was observed in the patients BM (supplemental Figure S3).

Despite concerns on deleterious effects of TKIs on immune effectors, experimental and clinical evidence obtained in patients on long-term imatinib treatment suggests that the immune system is functional, and may be harnessed towards antitumor surveillance.¹⁸ After underscoring the role of p^{190} BCR-ABL-specific T cells, emerging during TKI treatment, in controlling Ph+ ALL,⁸ we show the feasibility of expanding/priming these T cells from patients and HSCT donors, and demonstrate their excellent safety profile and in vivo antileukemic activity, in combination with TKI therapy.

In the last 5 years, impressive results have been obtained in the control of relapsed/refractory ALL by administration of T lymphocytes genetically modified to express chimeric antigen receptors (CARs) targeting B cell-associated antigens.¹⁹⁻²² The increase in CAR-T cell clinical efficacy, however, has been paralleled by the potential to induce severe adverse events, such as cytokine release syndrome, and on-target off-tumor toxicities.^{19,21,23} In this regard, BCR-ABL-specific CTLs may represent a valuable immunotherapeutic option for patients not amenable to or experiencing severe adverse events after CAR-T cell infusion, or for patients with persistent levels of MRD during TKI maintenance treatment after HSCT.

Clinical trials using immune checkpoint inhibitors (ICIs) have shown enhancement of naturally-occurring T-cell immunity against cancers, with promising therapeutic results.²⁴ In perspective, combination treatments with ICIs or the bispecific T-cell antibody blinatumomab²⁵ could allow to prevent T-cell anergy and exhaustion after ^{p190}BCR-ABL-specific CTL infusions, thus improving persistence of antileukemia CTLs and restoring a durable immune surveillance against Ph+ ALL. At the same time, leukemia-specific T cells mediating tumor lysis may expose neoantigens, further potentiating the activity of ICIs.

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Authorship

Contribution: PC, SB, GR, PBa, ML, LP conceived and designed the study, analyzed results, and wrote the manuscript; SB, IG, AG, GQ, LR, IL, DV, AP, produced and controlled CTL lines, processed samples and executed experiments; FF, MM, PBr, AC, AP, EC, provided clinical care, collected patient data and commented on the manuscript; RM, AC, LI, TT, FN, RF, MZ, supervised the study and critically revised the manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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FIGURE LEGENDS

Figure 1. Characteristics of the p^{190} BCR-ABL-specific T cells employed in the three patients.

(A) T-cell expansion of p^{190} BCR-ABL-specific T-cell lines achieved over a 40 day period based on cell counting using trypan blue exclusion (white square: patient 1; grey circle: patient 2; black triangle: patient 3). (B) Phenotype of p^{190} BCR-ABL-specific T-cell lines, reported as % positive cells (white square: patient 1; grey circle: patient 2; black triangle: patient 3). (C) Response, measured as IFN γ production in a ELISPOT assay, to the different peptide pools employed in the activation/expansion process. Mix1int: 9- and 10-mer peptides spanning the internal p190 breakpoint region; Mix1est: 9- and 10-mer peptides spanning the external p190 breakpoint region; Mix3: 9-mer peptides derived from the alternative BCR-ABL splice variants. (D) Cytotoxic activity of T-cell lines, measured as % specific lysis at a effector to target ratio of 5:1, against autologous PHA blasts pulsed with p^{190} BCR-ABL peptides (BCR-ABL-PHA targets, cytotoxicity measured at 5 hours and 12 hours, calculated after subtraction of background, consisting of cytotoxicity against autologous PHA blasts pulsed with irrelevant peptides), P815 cell line, non-pulsed PHA blasts from patients 1 and 2 (allo-PHA), non-pulsed autologous PHA blasts (auto-PHA) (black column: patient 1; grey column: patient 2; white column: patient 3). (E) Cytotoxicity profile of p^{190} BCR-ABL-specific CTLs obtained from patient 2. The figure reports the percentage of specific lysis against patient ALL blasts (solid line and triangle), autologous PHA blasts pulsed with p^{190} BCR-ABL peptides (dashed line and solid circle), P815 cell line (dotted line and solid squares), non-pulsed PHA blasts of the patient (dashed-dotted line and empty circles). The mean percentage of lysis of duplicate wells for 5 different effector-to-target (E:T) ratios is shown.

Figure 2. Clinical and immunological responses to p^{190} BCR-ABL-specific CTL infusion in three patients with molecular or hematological relapse of Ph+ ALL.

Longitudinal data tracking MRD kinetics (left y-axis) and frequency of IFN γ , IL-2 and TNF α producing, p^{190} BCR-ABL-specific, CD8+ and CD4+ T cells in the BM of patients, measured by flow cytometry and reported as % positive cells (right y-axis) are summarized in a single time-course graph for each patient. For each cytokine-producing T cell subset, memory profiles are depicted over the related time points, defined as following: CD62L- CD45RA- (effector memory, EM), CD62L+ CD45RA- (central memory, CM).

On each patient's graph, data on % donor chimerism, TKI treatment, and cell therapy (unmanipulated donor lymphocyte infusion, DLI; p^{190} BCR-ABL-specific CTLs) timing and dose are also reported.

Figure 1.

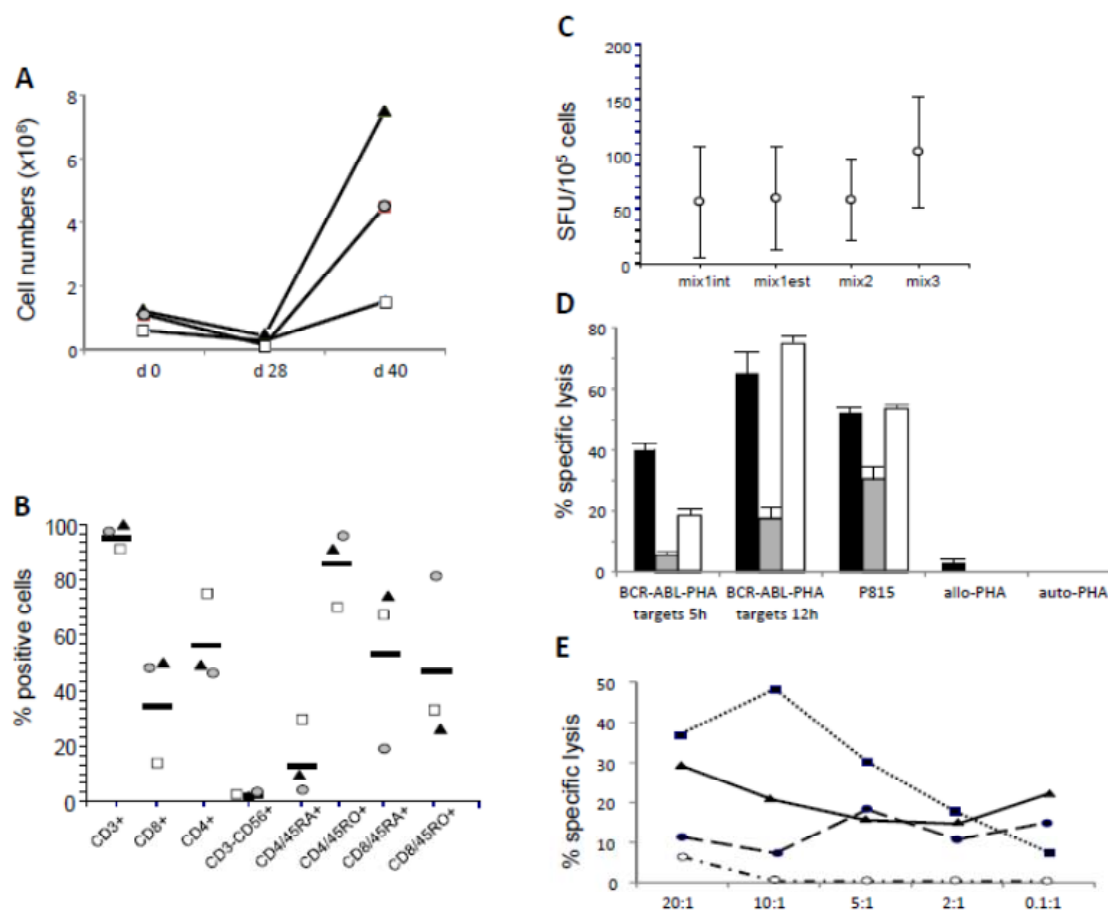
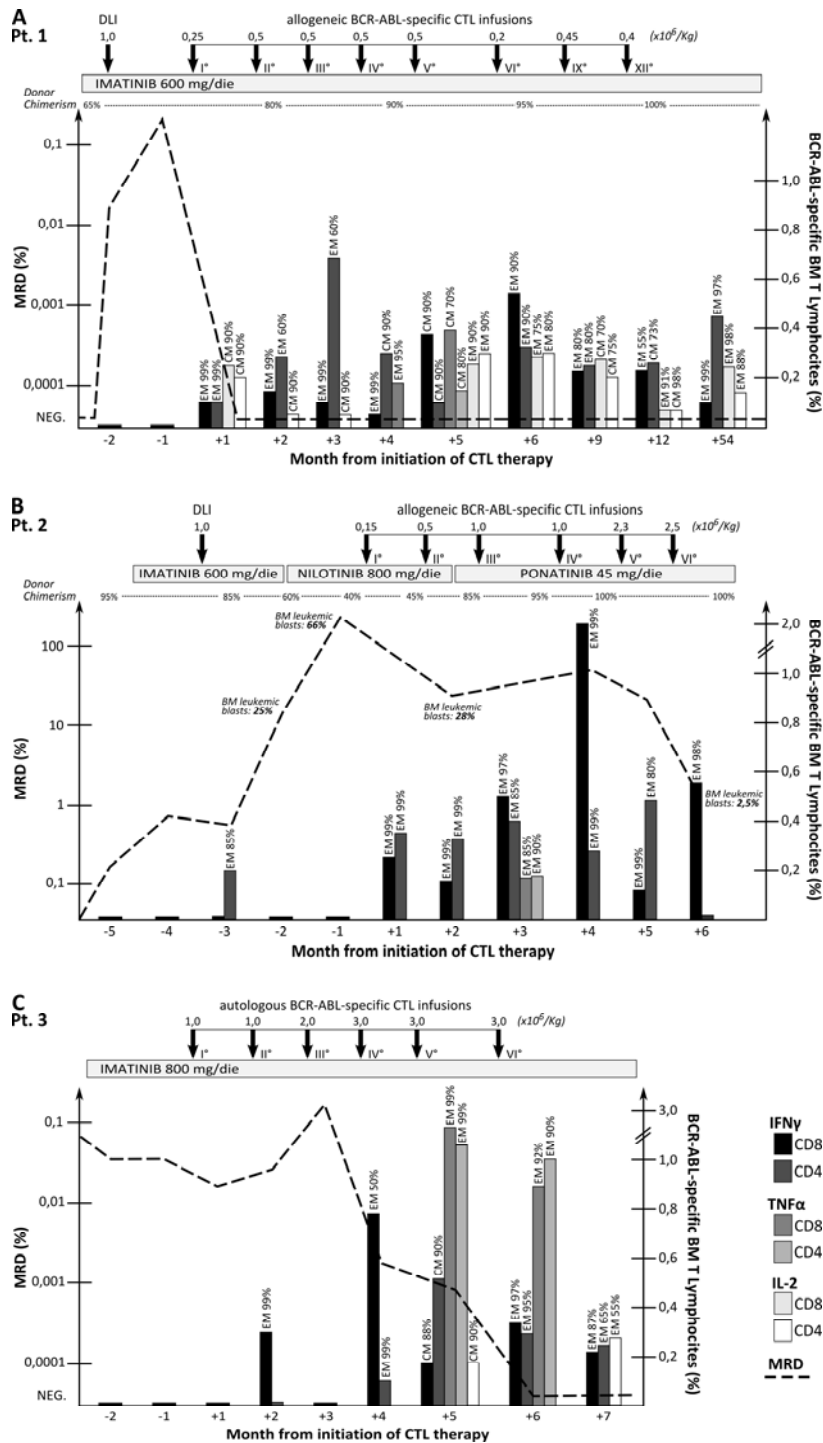


Figure 2.





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BCR-ABL-Specific T-cell therapy in Ph+ ALL patients on tyrosine-kinase inhibitors

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