Cytotherapy, 2012; 14: 851–859

informa
healthcare

A phase I/II clinical trial of autologous cytokine-induced killer cells as adjuvant immunotherapy for acute and chronic myeloid leukemia in clinical remission

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Abstract

Background aims. Cytokine-induced killer (CIK) cells have shown remarkable cytotoxicity against various tumors *in vitro* and in animal studies. We report on the clinical outcome of autologous CIK cells for patients with acute (AML) and chronic (CML) myeloid leukemia in remission. *Methods*. Eleven of the 13 recruited AML patients undergoing autologous peripheral blood stem cell transplant (autoPBSCT) were given autologous CIK cell infusion upon engraftment post-transplant and followed-up for disease relapse. Eleven CML patients on Imatinib with residual disease detectable by polymerase chain reaction (PCR) were given infusion and monitored by quantitation of the bcr-abl transcript. *Results*. Despite the presence of interferon (IFN)-γ-secreting T cells against various AML- and CML-associated peptides at sporadic time-points and demonstration of *in vitro* cytotoxicity of CIK cells against autologous and allogeneic AML targets, there was no survival benefit in AML patients post-autoPBSCT given CIK cells compared with historical controls. For CML patients, all continued to have a detectable bcr-abl transcript fluctuating within a range comparable to their pre-treatment baseline, although two had a transient but non-sustainable disappearance of bcr-abl transcript. There were no adverse reactions except for fever within the first day of infusion. *Conclusions.* Our small series, while confirming safety, failed to demonstrate a clinical benefit of autologous CIK cells given in its current form for AML and CML. Further manipulation of CIK cells to improve anti-leukemic potency and specificity, together with the preparation of patients to create a more conducive milieu for *in vivo* expansion and persistence of infused CIK cells, should be explored.

Key Words: *acute myeloid leukemia, cellular immunotherapy, chronic myeloid leukemia, cytokine-induce killer cells*

Introduction

Cytokine-induced killer (CIK) cells are a polyclonal T-cell population expanded *in vitro* under cytokine stimulation and exhibit non-major histocompatibility complex (MHC)-restricted recognition and killing of target cells. Over the past two decades, the cytotoxicity of CIK cells against a variety of hematologic malignancies, including lymphoma (1,2), acute myeloid leukemia (AML) (3), chronic myeloid leukemia (CML) (4,5), chronic lymphocytic leukemia (6) and myeloma targets (7), has been demonstrated by *in vitro* and mice studies.

We have shown previously that CIK cells are able to kill autologous AML cells to a variable degree (3). CIK cells have also been shown to suppress the growth of CML-positive colonies in cultures and eradicate CML in Severe Combined Immunodeficiency (SCID) mice engrafted with autologous CML cells (4,5). With the availability of a good manufacturing practice (GMP) facility, we have embarked on a phase I/II clinical trial to study the feasibility, safety and efficacy of autologous CIK cells in patients with AML and CML, where we have previously demonstrated feasibility of expansion. We report on the clinical outcome of autologous CIK cells in these two groups of patients. As for any immunotherapeutic modalities where efficacy is expected to be maximal when tumor load is low, we included patients with AML who were in remission and undergoing autologous peripheral blood stem cell transplant

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(Received 1 March 2012; accepted 14 May 2012)

ISSN 1465-3249 print/ISSN 1477-2566 online
DOI: 10.3109/14653249.2012.694419

(autoPBSCT), and CML patients with a residual bcr-abl transcript detectable at a molecular level.

Methods

Patients

Two groups of patients were studied prospectively. The AML group was designed to study a total of 20 patients with AML in remission undergoing autoPBSCT as consolidative therapy. The CML group was targeted to study 10 patients with CML on Imatinib with residual disease detectable by polymerase chain reaction (PCR) only. Exclusion criteria included renal impairment with Creatinine (Cr) $>$ 200 mmol/μL, liver impairment with transaminase $> 5 \times$ upper limit and life expectancy $<$ 3 months. The studies were approved by an institutional review board and patients signed informed consent. The studies were registered with www.clinicaltrials.gov as NCT00394381 and NCT00815321, respectively.

Leukapheresis

Leukapheresis to collect peripheral blood mononuclear cells (PBMNC) was done either in steady state or as part of a stem cell-harvesting process to collect $CD34^+$ cells upon recovery of counts after granulocyte–colony-stimulating factor (G-CSF) ± mobilization chemotherapy. A target of two to three times the blood volume was processed using a COBE Spectra continuous flow system (CaridianBCT, Lakewood, CO, USA), when done for the purpose of $CD34^+$ cell collection, or 1.5 times the blood volume using a MCS^+ Haemonetics (Haemonetics Corp, Braintree, MA, USA) discontinuous flow system, with the sole purpose of collecting PBMNC for CIK expansion.

Expansion of CIK cells

Autologous CIK cells were expanded from either fresh or frozen PBMNC as described previously (8). Briefly, starting cells were seeded in LIFECELL culture bags (Baxter, Deerfield, IL, USA), with 1000 U/mL interferon (IFN)-γ (Imukin; Boehringer, Ingelheim, Wien, Austria) added, followed by 50 ng/mL OKT-3 (Orthoclone; Janssen-Cilag, Schaffhausen, Switzerland) and 300 U/mL IL-2 (Proleukin; Novartis, Basel, Switzerland) 24 h later, and maintained with the addition of IL-2 and medium consisting of 10% fetal calf serum (FCS)/RPMI every week. Previous experience with CIK expansion has shown that proliferation plateaus after 28 days, therefore CIK cells are considered matured and are harvested by 28 ± 1 days (8). Matured CIK cells were pooled, washed and frozen in aliquots to allow sterility tests to be carried out. The target dose was 5×10^9 per infusion. Additional leukapheresis sessions for further cultures were offered to the patients if the target dose was not achieved.

Cytotoxicity assay

The cytotoxicity of CIK cells was measured by chromium release assay as described previously (3). Targets consisting of autologous or allogeneic AML blasts frozen at diagnosis were thawed and cultured overnight in granulocyte–macrophage colony-stimulating factor and 10% FCS/RPMI. These were then labeled with 100–200 μCi ^{51}Cr (PerkinElmer, Waltham, MA, USA) in 200 μL medium for 3–4 h in a 5% CO_2 incubator at 37°C and washed. Frozen CIK cells were thawed and plated with ^{51}Cr-labeled targets at 20 000 targets/well at effector-to-target (E:T) ratios of 40:1, 10:1, 2.5:1 and 1:1 in 200 μL final volume for 4 h, and the radioactivity of the supernatant was measured with a gamma counter.

CIK cell infusion

CIK cell infusion was given at 3-weekly intervals. For AML patients undergoing autoPBSCT, the first infusion was scheduled between days 11 and 14 of transplantation upon engraftment, when patients have recovered from cytopenic and toxic complications related to autoPBSCT. For CML patients this was done at steady state without interruption of Imatinib. Infusions were done as an outpatient procedure, except for the first dose for AML patients who were still recovering from autoPBSCT. Premedication included intravenous diphenhydramine 25 mg, per-oral (p.o). Celecoxib 200 mg and p.o. Paracetamol 1000 mg given 0.5–1 h before the infusion. Frozen CIK cells were brought to the bedside, thawed rapidly and infused over 10–20 min. Patients were monitored for vital signs for 3 h after infusion.

Clinical monitoring

For monitoring of adverse reactions, patients were asked for symptoms that developed within the first 24–48 h after infusion. AML patients were followed-up with routine laboratory tests, including blood counts and biochemistry, as indicated clinically. A marrow study was done only when there was clinical suspicion of relapse. A database-derived, historical cohort of AML patients who have undergone autoPBSCT was used as a comparison for blood counts and survival data. CML patients underwent quantitation of the bcr-abl transcript with the following schedule: during leukapheresis, at the first infusion, at the fourth infusion and every 3 months thereafter for 1 year. At each infusion and 3 monthly for 1 year, an additional