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Cord blood–derived cytokine-induced killer cells combined with blinatumomab as a therapeutic strategy for CD19$^+$ tumors

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Abstract

Background: Cytokine-induced killer cells (CIKs) are an advanced therapeutic medicinal product (ATMP) that has shown therapeutic activity in clinical trials but needs optimization. We developed a novel strategy using CIKs from banked cryopreserved cord blood units (CBUs) combined with bispecific antibody (BsAb) blinatumomab to treat CD19$^+$ malignancies. *Methods:* CB-CIKs were expanded *in vitro* and fully characterized in comparison with peripheral blood (PB)–derived CIKs. *Results:* CB-CIKs, like PB-CIKs, were mostly CD3$^+$ T cells with mean 45% CD3$^+$CD56$^+$ and expressing mostly TCR(T cell receptor)$\alpha\beta$ with a TH1 phenotype. CB-CIK cultures had, however, a larger proportion of CD4$^+$ cells, mostly CD56$^-$, as well as a greater proportion of naïve CCR7$^+$CD45RA$^+$ and a lower percentage of effector memory cells, compared with PB-CIKs. CB-CIKs were very similar to PB-CIKs in their expression of a large panel of co-stimulatory and inhibitory/exhaustion markers, except for higher CD28 expression among CD8$^+$ cells. Like PB-CIKs, CB-CIKs were highly cytotoxic *in vitro* against natural killer (NK) cell targets and efficiently lysed CD19$^+$ tumor cells in the presence of blinatumomab, with 30–60% lysis of target cells at very low effector:target ratios. Finally, both CB-CIKs and PB-CIKs, combined with blinatumomab, showed significant therapeutic activity in an aggressive PDX Ph$^+$ CD19$^+$ acute lymphoblastic leukemia model in NOD-SCID mice, without sign of toxicity or graft-versus-host disease. The improved expansion protocol was finally validated in good manufacturing practice conditions, showing reproducible expansion of CIKs from cryopreserved cord blood units with a median of 28.8×10^6 CIK/kg. *Discussion:* We conclude that CB-CIKs, combined with bispecific T-cell–engaging antibodies, offer a novel, effective treatment strategy for leukemia.

Key Words: *adoptive therapy, bispecific antibody, cord blood, cytokine-induced killer, T lymphocytes*

Introduction

Cytokine-induced killer cells (CIKs) are activated T lymphocytes expanded for about 3 weeks with interleukin-2 *in vitro* after stimulation with interferon-ɣ (IFN-ɣ) and anti-CD3 antibody. Large numbers of CIKs for adoptive therapy are easily expanded in good manufacturing practice (GMP) conditions from peripheral blood (PB) sources, in general HLA (human leukocyte antigen)-matched mononuclear cells collected by apheresis for donor lymphocyte infusions (DLI), in the context of hematopoietic stem cell transplantation (HSCT) or PB from patients in an autologous setting (1–3)<?show -aptara_TEMP_aptara-?>. CIKs show natural killer (NK)–like cytotoxic properties against tumor cells, but do not induce significant graft-versus-host disease (GvHD), both in animal models and in humans (3–8). Importantly, allogeneic HLA-matched, haplo-identical or unmatched CIKs have been used in several clinical trials to treat patients with cancer and have shown therapeutic activity with limited toxicity (3,5,6,8–10).

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Nonetheless the wider use of CIKs as a therapeutic agent in cancer treatment still needs to overcome a number of hurdles, in particular: (i) CIKs alone have shown so far therapeutic activity mostly in a minimal residual disease (MRD) or low–tumor burden context (6,11); (ii) rapid access to sufficient starting material may be difficult from either patients (due to concurrent chemotherapy or their possible immunosuppressed state) or from normal donors outside a HSCT setting, especially if some HLA matching is required; (iii) at least 3–4 weeks are required for large-scale production and quality controls of the drug and (iv) strategies to increase the therapeutic efficacy of CIKs feasible in the clinic are still required.

To overcome these limitations, we and others have started more than 10 years ago to develop cord blood (CB)–derived CIKs as therapeutic agents (5,12–14). CB is a suitable source because large numbers of HLA-typed and quality-controlled cord blood units (CBUs) are cryopreserved and conserved in banks. Whereas these units are normally prepared for HSCT purposes, many have a relatively low content of total nucleated cells (TNCs) and/or $CD34^+$ cells and are, therefore, not adequate for this purpose. Several groups, including ours, have shown previously that CIKs can be expanded from freshly isolated or banked CBUs (5,12–14). CB-CIK cultures contain mostly $CD3^+$ T cells expressing CD56 on 40–80% of cells and show NK-like cytotoxic activity, some therapeutic activity and limited toxicity, like their PB counterparts (5,12,14). The use of frozen CBUs would resolve several of the disadvantages of CIKs as drugs, mentioned above.

In parallel, we and others have also developed the idea that bispecific antibodies (BsAbs) could be used to redirect CIK effector cells toward tumor targets, rendering these cells more effective and specific *in vitro* (15–17) and *in vivo* in animal models (18–20).

In this report, we performed the full pre-clinical validation of CB-CIKs, expanded from cryopreserved CBUs using an improved GMP-compliant method, to be combined with BsAb blinatumomab (CD3 x CD19). We demonstrated the efficacy and feasibility of this approach in an aggressive patient-derived (PDX) model of Ph^+ acute lymphoblastic leukemia (ALL).

Methods

Sources of cells

Cryopreserved CBUs were obtained from the Cord Blood Bank of the Careggi Hospital in Florence, Italy. Fresh CBUs were obtained from the Obstetrics and Gynecology Unit of the Hospital Papa Giovanni XXIII, Bergamo, Italy. Peripheral blood mononuclear cells (PBMCs) were collected from healthy donors using apheresis in the context of HSCT procedures and cryopreserved. Biological material was collected after informed consent and used in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008.

CIK expansion

Fresh CBUs were subject to Ficoll-Hypaque gradient centrifugation and mononuclear cells were frozen for further use. Cryopreserved CBUs or lymphocytoapheretic material were thawed, washed and put in culture directly. CIK expansion was performed according to written standard operating procedures (SOPs) in X-VIVO 15 medium, containing gentamycin, glutamine and holotransferrin (Lonza) and using standard T75 or T175 culture flasks (21). This same method has been used to expand CIKs from PB, to treat 73 patients in a previous clinical protocol (Eudract 2008-003185-26), as approved by Italian regulatory authorities (6). In the case of CBUs, 1% off-the-clot human serum (HS) from male AB donors (Akron Biotechnology or Euroclone SpA) was added to the culture medium, unless otherwise specified. Briefly, cells were plated in the medium described at 3×10^6/mL and 1000 U/mL IFN-γ (Imukin, Boehringer Ingelheim) were added. After 24 h of culture, 50 ng/mL anti-CD3 antibody (OKT3, Janssen-Cilag) and 500 U/mL recombinant human interleukin-2 (rhIL-2; Proleukin, Novartis) were added. All these are clinical-grade reagents for infusion. Thereafter cells were counted every 3–4 days and diluted to $0.5–1.5 \times 10^6$/mL with fresh medium containing 500 U/mL rhIL-2. From day 10 onward, an aliquot of cells was collected and the percentage of $CD3^+CD56^+$ cells was measured using flow cytometry (see below). Expansion lasted for a total of 17–25 days to reach adequate number of cells and the specified $\geq$30% $CD3^+CD56^+$ cells in the final product.

Immunophenotyping and viability

The immunophenotype of CIKs was performed according to detailed written operating procedures by staining with the following sets of antibodies, all from BD Biosciences, unless otherwise indicated: CD3-FITC(fluorescein isothiocyanate), CD56-, CD4-APC(allophycocyanin), CD8-PcP(phycoerythrin)Cy5.5; extended immunophenotype: CD4 PerCP(Peridinin-chlorophyll-protein)-Cy5.5, -APC or -PE, CD8-APC-Cy7 or -PerCP-Cy5.5, NKG2D-PE, CD27-PE, CD28-PE, CD137-PE, CD154-