Targeting Interleukin-2 to the Bone Marrow Stroma for Therapy of Acute Myeloid Leukemia Relapsing after Allogeneic Hematopoietic Stem Cell Transplantation

Christoph Schliemann1, Katrin L. Gutbrodt2, Andrea Kerkhoff1, Michele Pohlen1, Stefanie Wiebe1, Gerda Silling1, Linus Angenendt1, Torsten Kessler1, Rolf M. Mesters1, Leonardo Giovannoni3, Michael Schäfers4, Bianca Altvater5, Claudia Rossig6, Inga Grünewald6, Eva Wardelmann6, Gabriele Köhler6,7, Dario Neri2, Matthias Stelljes1, and Wolfgang E. Berdel1

Abstract

The antibody-based delivery of IL2 to extracellular targets expressed in the easily accessible tumor-associated vasculature has shown potent antileukemic activity in xenografts and immunocompetent murine models of acute myelogenous leukemia (AML), especially in combination with cytotoxic agents. Here, we report our experience with 4 patients with relapsed AML after allogeneic hematopoietic stem cell transplantation (allo-HSCT), who were treated with the immunoconjugate F16-IL2, in combination with low-dose cytotoxic agents. One patient with disseminated extramedullary AML lesions achieved a complete metabolic response identified by PET/CT, which lasted 3 months. Two of 3 patients with bone marrow relapse achieved a blast reduction with transient molecular negativity. One of the 2 patients enjoyed a complete remission before AML relapse occurred 2 months after the first infusion of F16-IL2. In line with a site-directed delivery of the cytokine, F16-IL2 led to an extensive infiltration of immune effector cells in the bone marrow. Grade 1 fever was the only nonhematologic side effect in 2 patients. Grade 3 cytokine-release syndrome developed in the other 2 patients but was manageable in both cases with glucocorticoids. The concept of specifically targeting IL2 to the leukemia-associated stroma deserves further evaluation in clinical trials, especially in patients who relapse after allo-HSCT. Cancer Immunol Res; 3(5): 547–56. ©2015 AACR.

Introduction

Therapeutic approaches interfering with tumor angiogenesis have become fully incorporated into clinical practice nowadays (1). In addition to antiangiogenesis, a second, conceptually different strategy, ‘vascular targeting,’ takes advantage of tumor vessels for therapeutic purposes and aims at the selective pharmacodelivery of therapeutic payloads (e.g., drugs, cytokines, radionuclides) to the tumor site by their conjugation to carrier antibodies, which specifically home to tumor-associated vasculature (2–5). The approach benefits from the intrinsic accessibility of vascular targets and bypasses many of the hurdles associated with the targeting of cancer cell markers, such as physical and kinetic barriers of bulky tumors or antigen heterogeneity among tumor cells. Furthermore, whereas cancer cell surface proteins are in many cases shared by normal cells, vascular and stromal targets are available, allowing a clear-cut discrimination between healthy and neoplastic tissues (2, 3). Although it was initially assumed that vascular-targeting antibodies would mainly be relevant for solid tumors, we recently discovered that well-characterized vascular targets are abundantly expressed in hematologic malignancies, such as Hodgkin and non-Hodgkin lymphomas, allowing the antibody-mediated deposition of bioactive payloads at the lymphoma site (6, 7).

In 2000, we reported that acute myelogenous leukemia (AML) is associated with extensive neovessel formation in the bone marrow (8). In an attempt to evaluate a vascular-targeting strategy in AML, we have recently shown that the alternatively spliced extr-domains A (EDA-Fn) and B (EDB-Fn) of fibronectin and the extra-domain A1 of tenascin-C (TnC-A1) are abundantly expressed in AML bone marrow and extramedullary AML lesions (5). These markers are almost exclusively found in the tumor-associated vasculature and stroma while being virtually absent in normal organs and have been shown to be suited for site-specific pharmacodelivery approaches (2, 3, 9, 10). In immunocompromised and immunocompetent mouse models of myelosarcoma-type AML, the antibody-mediated delivery of the proinflammatory cytokine IL2 to the leukemia-associated vasculature led to...
substantial leukemia growth retardation in a process mediated by CD8+ T cells and natural killer (NK) cells, whereas equivalent doses of nontargeted IL2 were ineffective (5). Most importantly, the combination of the immunocytokine with cetarabine promoted complete leukemia eradications.

Here, we report the use of the immunocytokine F16-IL2, which mediates the selective delivery of human IL2 to TnC-A1, in combination with low-dose cetarabine (LDAC) in heavily pretreated AML patients with relapsed or refractory disease after allogeneic hematopoietic stem cell transplantation (allo-HSCT).

Case reports

Patient 1 was a woman diagnosed with complex karyotype AML French-American-British classification (FAB) M1 in February 2007 at 45 years of age. Her detailed medical history has been described previously (5). Briefly, in May 2013, the patient ultimately presented with rapidly progressing, disseminated myelosarcoma (chloroma) nodules after multiple lines of intensive therapy, including two unrelated donor allo-HSCTs, while the bone marrow was free of leukemia. [18F]fluoro-2-deoxy-D-glucose PET/CT revealed hypermetabolic manifestations in the left deep cervical area, in the mediastinum, in the hilum of the liver (leading to cholestasis), in the mesentery, in the hilum of the right kidney, in the sternum, in the transverse process of the 9th thoracic vertebra, and subcutaneously in the left gluteal area.

A 50-year-old woman (patient 2) had a diagnosis of NPM1-mutated, normal karyotype AML FAB M1 in August 2010. Induction therapy with two cycles of high-dose cetarabine and mitoxantrone (HAM) resulted in a first complete remission (CR) and was followed by consolidation with 6-thioguanine, cetarabine, and daunorubicin (TAD) and maintenance therapy according to the Acute Myeloid Leukemia Cooperative Group 2008 protocol. In June 2011, the patient’s first AML relapse occurred, and she underwent matched related donor allo-HSCT, leading to a second CR. Ten months later, the second AML relapse occurred, and another allo-HSCT from a matched unrelated donor was performed after chemotherapy with amsacrine, fludarabine, and high-dose cetarabine (FLAMSA) and conditioning with melphalan, busulfan, fludarabine, and antithymocyte globulin (ATG). The second allo-HSCT resulted in another CR, but a third AML relapse was diagnosed 9 months later.

Patient 3 was a 53-year-old man diagnosed with AML secondary to chronic myelomonocytic leukemia (CMML). CMML was first diagnosed in August 2011 and was treated with azacitidine until August 2012, when the disease transformed to AML. Although he was refractory to HAM induction therapy, he received allo-HSCT from a matched unrelated donor in December 2012 after conditioning with melphalan, fludarabine, ATG, and 8-Cy total body irradiation (TBI) and achieved a CR that lasted 6 months. However, AML relapse was diagnosed in July 2013.

In January 2007, NPM1-mutated AML FAB M5 with normal karyotype was diagnosed in patient 4 at the age of 39 years. After induction therapy with two courses of daunorubicin and cetarabine (DA), he achieved a CR, followed by consolidation with three cycles of high-dose cetarabine. In May 2011, the first AML relapse occurred (now FLT3-ITD positive), and the patient underwent allo-HSCT with a matched unrelated donor after chemotherapy with HAM and conditioning with cyclophosphamide, ATG, and 12-Cy TBI in July 2011, leading to a second CR of 2 years. In September 2013, NPM1-positive, FLT3-ITD-negative AML relapse was diagnosed with the karyotype 46,XY,t(2;3)(q13;p21)[5]/46,XY[13]. At that time, the patient was reluctant to receive intensive chemotherapy followed by a second allo-HSCT.

Patients and Methods

Patients and treatment

From June 2013 to December 2013, 4 AML patients who relapsed after multiple chemotherapies and one (n = 2) or two (n = 2) allo-HSCCs were treated in a compassionate-use setting with F16-IL2 and LDAC at the University Hospital Muenster, Germany. The joint ethical board of the University of Muenster and the regional Physician’s Chamber of Westfalen-Lippe was consulted for each individual case. Written informed consent from each patient was obtained in accordance with the Declaration of Helsinki. F16-IL2, consisting of the human anti–TnC-A1 antibody F16 fused to human IL2, was provided by Philogen. F16-IL2 was administered weekly as a 3-hour intravenous infusion. Patients received 30 Mio IU IL2 equivalents on the first infusion and 50 Mio IU in the following infusions when the first was well tolerated. LDAC was administered s.c. on days 1 to 10 at 5 mg twice daily. With the exception of patient 1, who required low-dose systemic steroids for chronic skin graft-versus-host disease (GVHD), none of the other 3 patients received immunosuppressive medication when post–allo-HSCT AML relapse was diagnosed and treatment with F16-IL2 and LDAC was initiated. A phase Ib study with F16-IL2 in combination with chemotherapy in patients with solid tumors is ongoing and has shown that doses up to 70 million IU can be safely administered (NCT01134250 and NCT01131364).

Histochemical analyses

For analysis of TnC-A1 expression, cryosections of pretherapeutic bone marrow biopsies were stained with biotinated F16 as described (5). Antibody detection was performed with streptavidin Alexa Fluor 488 (Invitrogen). For analysis of in vivo targeting, F16-IL2 bound to its target in situ was detected in posttherapeutic bone marrow specimens using the labeled streptavidin–biotin (LSAB) method. Sections of formalin-fixed paraffin-embedded bone marrow biopsies were prepared, followed by antigen retrieval in citrate buffer (pH 6, 30 minutes, 99°C). Sections were incubated with a rabbit anti-human IL2 antibody (1:125; Abcam) overnight at 4°C. Visualization was performed using Dako REAL Detection System/AP following the manufacturer’s instructions. Samples were analyzed on a Nikon Eclipse 50i microscope with the NIS-Elements 4.13 acquisition software (Nikon).

Flow cytometry

Lymphocyte phenotypes were determined using fluorescence-conjugated mAbs against CD3, CD4, CD8, CD16, CD19, CD56, and TCR γδ (all from BD Pharmingen). For each sample, 20,000 cells were analyzed with FACS Canto and FACS Diva Software. Intracellular FoxP3 expression was analyzed with the anti-human FoxP3 staining Kit according to the manufacturer’s recommendations (eBioscience). The percentage of CD25+FoxP3+ cells was determined after gating on CD3+/CD4+ cells.

Bone marrow chimerism

Chimerism analyses were performed as previously described using a semiquantitative PCR approach based on the amplification of short tandem repeat markers (11). Briefly, genomic DNA for multiplex PCR of microsatellite markers was extracted directly from mononuclear cells. Nine tetra-nucleotide microsatellite regions were coamplified with dye-labeled primers.
using the AmpFLSTR Profiler PCR amplification Kit (Applied Biosystems).

Results

Target expression in pretherapeutic bone marrow

TnC-A1 was expressed with a vascular and stromal pattern of staining in the bone marrow of all 3 patients with bone marrow AML involvement (Fig. 1A), providing the molecular basis for an F16-based targeting approach. In patient 1, only paraffin-embedded chloroma material was available, which could not be analyzed for TnC-A1 expression, because the F16 antibody does not work in paraffin-embedded tissues (12). However, we have previously observed that extramedullary AML lesions abundantly express TnC-A1 (5).

In vivo targeting of F16-IL2

Posttherapy bone marrow biopsies were available for patients 2 and 4 to analyze the in vivo–targeting capabilities of F16-IL2. Using an anti-IL2 antibody to detect F16-IL2 bound to its target in situ, IL2 signals decorating the extracellular matrix were detectable in the bone marrow 6 days after the last infusion in patient 2, but not in pretherapeutic bone marrow (Fig. 1B), suggesting that F16-IL2 has efficiently targeted TnC-A1 in vivo and persisted for several days. On day 42, however, 3 weeks after the last infusion of F16-IL2, stromal IL2 signals were not observed any more (Fig. 1B, inset). In patient 4, no significant F16-IL2 signals could be observed 6 days after infusion.

Clinical course

The most impressive response was observed in patient 1 with disseminated extramedullary AML. Shortly after the first infusion of F16-IL2, the patient experienced pain in the cervical and the right upper abdominal chloroma lesions, suggesting a site-specific targeting of the cytokine. The palpable tumors became softer and started to shrink on the following day, and swelling problems due to the cervical mass resolved within 48 hours, while head mobility improved. All these signs of activity occurred before the beginning of additional radiotherapy, which was initiated on day 4 to ensure that the two most critical chloroma lesions (cervical mass and liver hilum) were efficiently treated (23.4 Gy). On day 14 (i.e., after two infusions of F16-IL2), a complete metabolic response of all irradiated and nonirradiated AML lesions could be observed in PET/CT, accompanied by a partial morphologic response (Fig. 2A). Although the first three infusions of F16-IL2 were well tolerated, with transient fevers of 2 to 3 days after infusion, pain at sites of chloroma, and slight signs of skin and liver GVHD worsening, the patient had to be hospitalized 3 days after the fourth administration of the fusion protein (day 25) due to prolonged high fevers escalating to 40.5°C, respiratory distress, and increasing skin GVHD (stage 3, erythroderma). A CT scan revealed pulmonary infiltrates in the middle lobe and bilateral pleural effusions. Because clinical and laboratory findings were compatible with cytokine release associated with a macrophage activation syndrome, with elevation of ferritin levels (15,575 μg/L) on day 28, hypofibrinogenemia (81 mg/dl, 263 mg/dl before F16-IL2), and elevated IL6 levels (32 pg/ml, 6-fold increase from baseline), high-dose corticosteroids were administered in addition to broad-spectrum antimicrobials. The patient’s fever resolved within 48 hours, her other clinical symptoms improved within the following days, and ferritin levels decreased to 2806 μg/L within 2 weeks. She was discharged on day 39. The patient did not receive the planned second cycle of therapy but continued treatment with a reduced dose of 30 Mio IU of F16-IL2 in longer intervals of 3 weeks on an outpatient basis. She resumed normal activity with a fair quality of life (Karnofsky index of 80%–90%), no clinical signs of chloroma disease, and preexisting skin GVHD that was manageable with

Figure 1.

Expression of TnC-A1 in the bone marrow (BM) and in vivo targeting of F16-IL2. A, pretherapeutic stromal expression of TnC-A1 (green) could be detected in all patients with bone marrow involvement of AML using biotinylated F16(SIP) antibodies (>200). B, the presence of F16-IL2 in posttherapeutic bone marrow was highlighted using an antibody specific to human IL2 (>400). In patient 2, extracellular IL2 signals could be detected in BM aspirates (day 42, inset) after the last infusion, suggesting successful targeting and on-target persistence of the immunocytokine for several days in vivo. Stromal IL2 signals were not detectable in pretherapeutic bone marrow that was not exposed to F16-IL2 treatment and used as control. C, schematic representation of the dimeric scFv–huIL2 fusion protein F16-IL2 in diabody format.
local and oral methylprednisolone. However, in September 2013, almost 4 months after the first infusion of F16-IL2, a follow-up PET/CT revealed disseminated recurrence of chloromas. Treatment was intensified to weekly applications of 50 Mio IU F16-IL2 in combination with LDAC; however, the patient did not respond a second time. Because she expressed her distinct wish for further therapy, she was offered intensive chemotherapy with sequential high-dose cytarabine and idarubicin. Unfortunately, the patient died due to infectious complications in the phase of neutropenia in November 2013 on day 176.

The other patients displayed “classical” medullary AML. Patient 2 showed a 50% to 60% bone marrow infiltration before treatment. After two infusions of the immunocytokine, bone marrow blasts as determined by cytologic analyses were reduced to 6% on day 14, and were further reduced to <5% on day 28, after four administrations of F16-IL2. Microscopic evaluation revealed therapy-induced extensive infiltration of activated lymphocytes, some with blast-like morphology in the bone marrow at the same time. The reduction of myeloid blasts was paralleled by a disappearance of mutated NPM1 in PCR analyses and by an increase of bone marrow donor cell chimerism from 63% to 98% (Fig. 3A). No significant leukemia infiltration was detected in flow-cytometric analyses (0.81% and 0.98% CD117+/L blasts on days 14 and 28, respectively; 23.85% at baseline). Each infusion of the immunocytokine led to fever of up to 39.0°C, which typically lasted 2 days, but no other symptoms of cytokine release developed. Because there was no evidence of residual leukemia at the molecular level, therapy with F16-IL2 was paused for 2 weeks to allow regeneration of normal hematopoiesis. By day 42, however, neutrophopil and thrombocyte counts did not regenerate and AML progressed rapidly again. Treatment was resumed with higher doses of F16-IL2 [50 Mio international units (IU) on day 1, 70 Mio IE on days 8 and 15] in combination with LDAC, which, however, did not lead to a second response. The patient expressed a clear wish for further therapy, and she received a third allo-HSCT 3 months after treatment initiation with F16-IL2. This resulted in a short CR with incomplete platelet recovery (CRp) of approximately 2 months, until AML relapse ultimately occurred in February 2014.

Patient 3 presented with highly proliferative AML relapse in peripheral blood (4,900 leukemic blasts/L) and bone marrow infiltration of 70%. Treatment with F16-IL2 (30 Mio IU) and LDAC led to a blast reduction in peripheral blood (1,080/L) within 1 week (Fig. 3B). However, the patient had to be hospitalized after the first dose of F16-IL2 due to fevers (39.7°C), chills, nausea, and a reduced general condition. He was treated with broad-spectrum antibacterials, but it was again suspected that the fevers were more likely due to noninfectious hyperinflammation/cytokine release. Indeed, signs of capillary leakage developed within the next few days with generalized edema, weight gain (8 kg), and mild dyspnea. Laboratory findings showed elevated levels of IL6 (139 pg/mL, 10-fold increase from baseline), hyperferritinemia (11,600 μg/L), and a transient increase in creatinine (2.1 mg/dL). Fevers resolved by day 9 after 4 days of glucocorticoids. Creatinine normalized within 4 days, the patient’s weight tolerated at this time. Five days later, however, the patient had a severe accident at home and died due to intracerebral hemorrhage in the hospital shortly after.

Patient 4 presented with at least 10% leukemic blasts (focally more) in the bone marrow and 2% in peripheral blood.
Pretherapeutic bone marrow donor cell chimerism decreased to 83%. Peripheral blasts disappeared within the first few days of treatment with F16-IL2 and LDAC, and the first bone marrow evaluation on day 14 revealed a reduction of blasts to approximately 2% (CD33+/CD117− cells on flow cytometry, 12.3% at baseline in Fig. 3C). Microscopic quantification showed ≤5% myeloid leukemic blasts and an extensive infiltration with activated lymphocytes. In molecular and conventional cytogenetic analyses, the markers NPM1, t(2;3) and t(5;6), were undetectable, and bone marrow donor cell chimerism increased to 96% to 97%. Thrombocyte counts increased to 157,000/µL, and neutrophil counts improved to 2,214/µL after the first cycle (Fig. 3C), thereby meeting the criteria for a CR. However, after two cycles (2 months after the first infusion of F16-IL2), the patient experienced a bone marrow relapse with 15% blasts and reappearance of the molecular and cytogenetic abnormalities. The patient received two infusions of donor-derived lymphocytes without response and underwent a second matched unrelated alternative donor allo-H SCT in February 2014, but eventually died from infectious complications in the recovery time following transplantation.

Toxicities
All toxicities are summarized in Table 1. The most frequent adverse events were fever, chills, and fatigue, which occurred in all patients. IL2-related infusion-associated hypotension was not observed. Tumor pain as observed in the patient with chloroma is an uncommon effect of recombinant IL2 that could be related to F16-mediated targeting of the cytokine. In patients 2 and 4, fevers typically occurred either toward the end of the infusion or shortly after, regularly resolved by day 2 or 3, and were easily manageable at home with acetaminophen. In these patients, no other relevant nonhematologic toxicities occurred (apart from mild skin GVHD in patient 2).

However, the other 2 patients became significantly ill after the first infusion of 30 Mio IU F16-IL2 (patient 3) or after the fourth infusion of 50 Mio IU F16-IL2 (patient 1), respectively. Both patients developed symptoms of the cytokine-release syndrome described above, with laboratory features compatible with macrophage activation syndrome, leading to hospitalization in both cases. The syndrome could be effectively treated with glucocorticoids. No anticytokine antibodies, mechanical ventilation, or vasopressor support were required. Nonhematologic grade 4 events were not observed.

Immunologic effects of F16-IL2
Circulating lymphocytes in peripheral blood significantly increased to levels 2 to 4 times as high as baseline levels by day 11 in all patients. Therapy-induced changes of peripheral lymphocyte subpopulations in patients 2 and 4 are shown in Fig. 4A. CD8+ lymphocytes peaked early after treatment initiation between days 8 and 11 with a 5.7-fold increase to 4,640/µL in patient 2 and a 2-fold increase to 747/µL in patient 4. In contrast, peripheral NK cells showed a more or less steady rise with repeated administrations of F16-IL2 to values as high as 3,567/µL and 1,303/µL, respectively—a 17.7-fold and 16.5-fold increase from baseline. At peak levels, NK cells accounted for more than 50% of circulating lymphocytes. There were no significant changes in CD19+ B cells. Interestingly, activated lymphocytes and lymphocytes with large granular morphology could be readily detected in peripheral blood (Fig. 4B).

The most prominent changes were observed in the bone marrow. F16-IL2 led to a massive accumulation of lymphocytes in the bone marrow (Fig. 4C and D). Whereas lymphocytes
accounted for 15% to 20% of cells in pretherapeutic bone marrow aspirates, lymphocytic infiltration of the bone marrow was approximately 80% in patient 2 and 65% in patient 4 on day 28, coinciding with the marked reduction of leukemic blasts to molecular negativity in both patients. This phenomenon was not solely explained by the reduction of myeloid cells during therapy, but by an absolute expansion of lymphocytes. Indeed, a 10-fold increase of absolute CD8⁺ lymphocyte numbers, a 38-fold increase of NK cells, and a 24-fold increase of γδ T cells from baseline have been observed on day 28 in patient 4 (Fig. 4D). Comprising 45% to 50% of lymphocytes, NK cells represented the predominant lymphocyte fraction in the bone marrow in both patients, with maximum absolute numbers of 2,798/µL and 4,933/µL. In microscopy of bone marrow aspirates, clusters of lymphocytes tightly attached to leukemic blasts suggestive of immunological synapse formation have been frequently observed (Fig. 4E).

**Discussion**

The increased angiogenic activity in AML bone marrow has provided the scientific rationale for the clinical evaluation of...
antiangiogenic agents in AML (8, 13–15). However, therapeutic outcomes of angiogenesis inhibitors in AML have been mostly disappointing so far (16, 17), stimulating the search for alternative strategies that take therapeutic advantage of angiogenic neo-vessels. Here, we report first-in-human experiences with the targeted delivery of IL2 to the leukemia-associated vasculature and stroma using the immunocytokine F16-IL2 in heavily pretreated AML patients. We have chosen the situation after allo-HSCT to allow for a graft-versus-leukemia (GVL) response of a transplanted cellular immune system to the immunocytokine, and because we had previously treated 2 patients with late-stage refractory AML without previous allo-HSCT and had not observed significant antileukemic activity (data not shown).

The therapeutic principle of allo-HSCT is based on an active donor immune system that fights tumor cells of the host. We hypothesized that accumulating high doses of IL2 in the bone marrow as the site of origin of the disease would promote GVL activity in post-allo-HSCT AML patients. Indeed, immunocytokine treatment resulted in a dramatic expansion of cytotoxic effectors, which was paralleled by a striking reduction of leukemic blasts or extramedullary AML lesions. It was unexpected that only two infusions of the immunocytokine in combination with LDAC would result in a (transient) complete molecular clearance of the leukemic clone in 2 of 3 patients and in a complete metabolic response of extramedullary AML lesions as early as day 14 after treatment initiation. The antileukemic effects observed with this combination therapy are highly encouraging, given that all patients were heavily pretreated with multiple lines of intensive therapy, with 2 of the 4 patients having received even two allo-HSCTs. The observation that even patients who were previously refractory to high-dose cytarabine (3 g/m²) responded supports a substantial contribution of the targeted cytokine to the therapeutic effect. In fact, the cumulative dose of cytarabine (100 mg per cycle) was 3 to 4 times lower than the doses that are usually used in LDAC regimens (10 mg/m² or 20 mg twice daily for 10 days; ref. 18). Furthermore, LDAC alone has not been shown to induce remissions in patients with complex karyotype AML (18).

The targeted delivery of IL2 to the bone marrow led to a massive expansion of immune effector cells, with CD8⁺ T cells and NK cells representing the most upregulated lymphocyte subsets. These observations are encouraging, in light of the fact that they reproduce preclinical findings, which revealed a dominant role of
CD8\(^+\) and NK cells for the therapeutic activity of IL2-based immunocytokines in AML-bearing mice (5).

In line with the broad immunomodulatory properties of IL2, Tregs also increased during therapy. It is entirely conceivable that Tregs might have negatively influenced the therapeutic GVL effect. Recent studies, however, which have used ultralow doses of unconjugated IL2 to preferentially induce Tregs in an attempt to ameliorate GVHD, did not reveal a diminished antileukemia activity or an enhanced risk of leukemia relapse after allo-HSCT (19, 20). Indeed, in the context of GVHD, Tregs were shown to allow for robust GVL activity in several leukemia models (21–23). Interestingly, there is evidence that Tregs do not impair the immune response against tumors residing within the bone marrow, whereas they readily suppress antitumor immunity if the same tumor is located outside the bone marrow (24, 25). This phenomenon has been explained by an IL2-mediated neutralization of the suppressive activity of Tregs by the bone marrow stroma (in part through conversion of Tregs into IL17-producing T cells), leading to a conditional loss of Treg function in the bone marrow and providing a favorable environment for effector cells to mediate antitumor immunity (24). Thus, it is not entirely obvious that alternative immunomodulatory cytokines that do not significantly act on Tregs (e.g., IL15) would represent better effector functions for bone marrow–targeted immunocytokines, especially in patients with relevant GVHD. In fact, it is possible that the peripheral expansion of Tregs may have prevented even more severe manifestations of GVHD in patient 1 while still allowing for significant antileukemic activity of cytotoxic effectors in extramedullary sites. Combination strategies, however, which keep Treg levels low (e.g., anti–CTLA-4 antibodies; ref. 26), might be even more efficient, yet at the expense of a potentially higher toxicity.

The fact that we observed aggravation rather than amelioration of GVHD symptoms contrasts with the notion of using IL2 to stimulate Tregs and suppress GVHD (19, 20). Weekly IL2 equivalents that were administered in our patients were approximately 10 to 100 times higher than the doses used for GVHD. The difference of the dose of IL2 available in the bone marrow is likely to be even higher, when taking into account the targeting aspect. Indeed, although CD8\(^+\) T cells and \(\gamma\delta\) T cells did not change and NK cells only doubled under treatment with low-dose IL2 (19), we have observed an up to 38-fold expansion of NK cells, a 10-fold expansion of CD8\(^+\) T cells, and a 24-fold increase of \(\gamma\delta\) T cells in the bone marrow. Thus, the local dose of IL2 equivalents is likely to determine the net outcome of IL2-induced stimulation of proinflammatory and suppressive lymphocytes.

Although F16-IL2 was well tolerated in 2 patients, the other 2 patients had substantial toxic side effects that either occurred immediately after the first infusion or accumulated after several infusions of F16-IL2. The cytokine-release syndrome that developed in both patients was manageable with high-dose glucocorticoids and did not reach the severity reported for patients receiving chimeric antigen receptor–modified T cells (27). Both patients tolerated reexposure to lower doses of F16-IL2.

In principle, it is conceivable that the observed therapeutic effects could have also partially been achieved with the administration of free, unconjugated IL2. However, several lines of evidence strongly support the contribution of the targeting aspect to the therapeutic efficacy. First, the most pronounced changes in effector cell populations have been observed in the bone marrow as compared with peripheral blood (e.g., up to 38-fold vs. 17-fold increase in NK cell numbers in bone marrow vs. peripheral blood, respectively), which we would not expect from a nontargeted application of IL2. The fact that we still have seen considerable immunologic effects in the peripheral blood (and systemic toxicities) could be attributed to the "fluid" nature of the bone marrow, which is—in contrast with solid tumors—part of the immune system and dynamically connected to the peripheral blood, allowing immune effector cells to traffic between bone marrow and the periphery and to aggravate GVHD effects. Indeed, a comparable IL2-based immunocytokine only led to a modest increase in peripheral effector cell counts in patients, in which solid tumors have been targeted (28). A dose-finding phase I study, which is currently in preparation, will show whether lower starting doses of IL2 equivalents may lead to a more prominent discrimination between bone marrow and peripheral blood immune effects. Second, the delivery of IL2 to the bone marrow was demonstrated using immunohistochemical analyses of postinfusion bone marrow biopsies. Stromal structures decorated with IL2 were detectable 6 days after the last administration of F16-IL2, indicating that the immunocytokine exhibited on-target persistence times of several days in vivo. In patients with solid tumors, comparable on-target persistence times have been described for vascular-targeting antibodies (29–33). The lack of posttherapeutic IL2 bone marrow signals in patient 4 might be explained by interindividual differences in targeting performance in vivo. Interestingly, preliminary data suggest that angiogenesis-related matrix proteins may persist even after normalization of microvessel density in patients achieving a CR (5), potentially making TnC-A1 an attractive target for targeted cytokines also in a postremission setting. Third, patient 1 developed pain at extramedullary AML sites after each of the first two infusions of the immunocytokine, a side effect that is not commonly observed with nontargeted IL2. Tumor pain upon infusion has also been observed in patients with solid tumors receiving L19-IL2, an immunocytokine that targets EDB-Fn (28, 34). Last, preclinical experiments using different syngeneic and xenograft models of AML have clearly shown that complete leukemia eradications could only be achieved with vascular-targeted IL2 in combination with cytarabine, whereas equimolar amounts of IL2 fused to an antibody of irrelevant specificity were ineffective in inhibiting leukemia progression (5).

In summary, a combination of F16-IL2 and LDAC can provide clinically meaningful benefit to patients in the desperate situation of AML relapse after allo-HSCT. We believe that F16-IL2 combined with either LDAC or with additional immunologic maneuvers to increase on-target activity of the stimulated effector cells at the bone marrow site deserves further evaluation in prospective clinical trials, with a dedicated part on dose finding, as it appears that patients with an alloengenic immune system may tolerate lower doses of F16-IL2 as compared with patients with solid tumors.

Disclosure of Potential Conflicts of Interest
D. Neri is a board member, has ownership interest (including patents), and is a consultant/advisory board member for Philogen. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions

Published OnlineFirst February 11, 2015; DOI: 10.1158/2326-6066.CIR-14-0179
Analysis and interpretation of data (e.g., statistical analysis, biostatistics): C. Schliemann, S. Wiebe, R.M. Mesters, W.E. Berdel

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): C. Schliemann, M. Pohlen, S. Wiebe, C. Silling, R.M. Mesters, B. Alt,var, I. Grünewald, G. Köhler, M. Stelljes, W.E. Berdel

Data analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): C. Schliemann, T. Kessler, B. Alt, var, C. Rossig, E. Wardelmann, W.E. Berdel

Writing, review, and/or revision of the manuscript: C. Schliemann, M. Pohlen, S. Wiebe, T. Kessler, R.M. Mesters, L. Giovannoni, M. Schaliers, C. Rossig, D. Neri, M. Stelljes, W.E. Berdel

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): C. Schliemann, C. Rossig, L. Giovannoni, I. Grünewald, E. Wardelmann, D. Neri, W.E. Berdel

Study supervision: C. Schliemann, R.M. Mesters, W.E. Berdel

Other (in involved in patient care and treatment): A. Kerkhoff

Other (patient care and treatment, immunohistochemical analyses of Tn-C-A1 expression, and edited and approved the article): L. Angenendt

Other (morphologic and immunohistochemical analysis): E. Wardelmann

Acknowledgments

C. Schliemann, M. Schaliers, and W.E. Berdel are supported by Deutsche Forschungsgemeinschaft, DFG EXC 1003.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received September 23, 2014; revised January 5, 2015; accepted January 30, 2015; published OnlineFirst February 11, 2015.

References


Targeting Interleukin-2 to the Bone Marrow Stroma for Therapy of Acute Myeloid Leukemia Relapsing after Allogeneic Hematopoietic Stem Cell Transplantation

Christoph Schliemann, Katrin L. Gutbrodt, Andrea Kerkhoff, et al.


Updated version
Access the most recent version of this article at: doi:10.1158/2326-6066.CIR-14-0179

Cited articles
This article cites 34 articles, 16 of which you can access for free at:
http://cancerimmunolres.aacrjournals.org/content/3/5/547.full#ref-list-1

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.