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Reviewing the possible cure of drug-resistant hematologic malignancies by innovative cell-mediated immunotherapy using intentionally mismatched pre-activated donor lymphocytes

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ABSTRACT

Preliminary data summarized in the present article provides support to a new treatment strategy based on cell-mediated immunotherapy of patients with multi-drug-resistant hematologic malignancies, based on a 2-step cell-mediated immunotherapy approach. First, attempting to induce a stage of minimal residual disease or minimizing tumor burden by conventional modalities, which can usually be accomplished at an early stage of the disease. Next, apply innovative cell-mediated immunotherapy by mismatched pre-activated killer cells following mild immunosuppressive conditioning. Out of a total of 33 patients with different drug-resistant hematologic malignancies, 23 accomplished complete remission and 6 observed for more than 5 years with no further treatment are probably cured. Using pre-activated mismatched killer cells was based on short-term circulation of multi-potent cancer killer cells including a mixture of T, NK & NKT cells, could result in most effective cytoreduction of all drug-resistant malignant cells, possibly even accomplishing cure, with no need for allogeneic stem cell transplantation, thus avoiding the hazardous risks of acute and chronic graft-vs-host disease. Our working hypothesis supported by pre-clinical and preliminary clinical investigations confirms the feasibility to cure patients with different hematologic malignancies considered otherwise incurable using mismatched pre-activated killer cells following mild immunosuppressive conditioning. Our suggestion that our innovative cell-mediated immunotherapy could eliminate fully resistant malignant cells in patients with a broad range of otherwise incurable hematologic malignancies needs to be confirmed by prospective randomized clinical trials.

Keywords: Hematological malignancies; leukemia; lymphoma; multiple myeloma; cell-mediated immunotherapy; mismatched donor lymphocytes; IL-2 activated killer cells

Abbreviations:

IMAK, Immunotherapy by intentionally Mismatched Activated Killer cells; MRD, minimal residual disease; NK cells, natural killer cells; NKT cells, natural killer T cells; MDR, multi-drug-resistant cancer cells; SCT, allogeneic stem cell transplantation; DLI, donor lymphocyte infusion; GVHD, graft-vs-host disease; GvL, graft-versus-leukemia effects; RIC, reduced intensity conditioning; NST, non-myeloablative stem cell transplantation; ADCC, antibody-dependent cell-mediated cytotoxicity.

Introduction

Immunotherapy represents a most promising approach for control of hematologic malignancies otherwise resistant to available anti-cancer modalities. One of the most effective immunotherapies approaches available for treatment of patients with advanced and resistant hematologic malignancies may be accomplished by allogeneic stem cell transplantation (SCT) using fully matched related or haploidentical donor or fully matched unrelated donor following myeloablative or even non-myeloablative stem cell transplantation (NST) or reduced intensity conditioning (RIC).[1,2] The role of graft versus leukemia (GvL) as one of the most effective immunotherapy procedures for treatment of drug-resistant hematologic malignancies was supported by comparing the superior disease-free survival of patients undergoing similar myeloablative conditioning prior to syngeneic stem cells transplantation with no risk of graft-vs-host disease (GVHD).[3] Also, higher relapse rates and improved disease-free survival were always observed among patients undergoing successful allogeneic SCT with no evidence of acute or chronic GVHD in comparison with patients with evidence of GVHD. Recognized that much of the therapeutic effects accomplished by SCT are induced primarily by (GvL) mediated by alloreactive donor lymphocytes, we could demonstrate that the hazardous myeloablative SCT could be replaced by much safer and better tolerated non-myeloablative stem cell transplantation (NST) or reduced intensity conditioning (RIC), confirming that the main role of engraftment of donor stem cells was induction of transplantation tolerance or unresponsiveness to donor's alloantigens, thus allowing durable engraftment of donor's alloreactive lymphocytes.[1,2] Unfortunately, GvL effects induced following both NST or RIC were still accompanied by risky unavoidable acute and chronic graft-vs-host disease (GVHD) that could not be completely prevented despite the use of optimal immunosuppression anti-GVHD prophylaxis. Moreover, post grafting immunosuppressive treatment mandatory for prevention or treatment of GVHD also impaired the intensity and clinical efficacy of the anticipated GvL effects for treatment of resistant leukemia and higher incidence of leukemia relapse was observed when higher doses of cyclosporine A was used after allogeneic SCT.[4] On the other hand, T cell depletion, the only effective procedure for consistent prevention of GVHD and the need for post grafting anti-GVHD prophylaxis is associated with both increased incidence of relapse and allograft rejection, confirming the important role of alloreactive T cells for elimination of residual malignant cells on the one hand, and residual patient's hematopoietic stem cells on the other.[5] Accordingly, the use of T cell depletion for prevention of GVHD had to be supported by more intensive immunosuppression conditioning.[6,7] We have pioneered the use of donor lymphocyte infusion (DLI) to maximize the GvL effects after allogeneic SCT and documented that GvL effects could be maximized by over activation of donor lymphocytes with interleukin 2 (IL-2).[8,9] More recently we have also documented that post-grafting DLI could be used for prevention of relapse in patients with high-risk disease.[10] Much more disturbing was the fact that recurrent disease could occur despite development of severe acute and/or chronic GVHD and sometimes even after successful DLI, suggesting that the therapeutic potential of durable engraftment and maximally activated HLA compatible donor lymphocytes activated by GVHD may not be sufficient for complete elimination of all drug-resistant malignant cells and the much more resistant cancer stem cells even following successful SCT.

More recently, newer more effective and safer strategies were introduced in an attempt to control resistant malignant cells while avoiding the use of SCT using more sophisticated approaches for activation and targeting patient's immune system cells against the malignant cells first using monoclonal antibodies such as anti-CD20, anti-CD19 and anti-CD38 for treatment of in patients with B cells malignancies and multiple myeloma, respectively, aiming for induction of antibody-dependent cell-mediated cytotoxicity (ADCC) for improving the cytotoxic effects of circulating patient's lymphocytes. More recently, the efficacy of cell mediated immunotherapy was further improved by more advanced technologies using bispecific antibodies and most recently using CAR-T cells by engineering T cells to target malignant B cells in patients with ALL and non-Hodgkin lymphoma and multiple myeloma cells.[11-13] Although complete remission can be accomplished in a significant proportion of patients with B cell positive acute leukemia, non-Hodgkin lymphoma and multiple myeloma as documented in many studies that are still ongoing, yet it seems that unmaintained disease-free survival and cure could possibly be accomplished only in a small fraction of patients with multi-drug-resistant hematologic malignancies using available conventional immunotherapy procedures. Taken together, the limited cytolytic capacity of patient's own T cells and NK cells maximally activated by currently available immunotherapy procedures prompted us to pioneer the use of much more potent universal killer cells that could possibly eliminate every multi-drug-resistant (MDR) malignant cell, using non-engrafting intentionally Mismatched pre-Activated Killer cells (IMAK).

Materials and methods

Compassionate treatment using IMAK was applied in 33 consenting patients with different hematologic malignancies, all with MDR but in good performance status. Conditioning was based on the use of cyclophosphamide 1,000mg/m^2 alone, or in combination with a-interferon.[14] Haploidentical related donors (n=20) or mismatched unrelated donor lymphocytes (n=13) obtained by apheresis were activated for 4 days using interleukin 2 (IL-2) at 6,000 IU/ml in RPMI medium supplemented with 10% heat-inactivated human AB serum, glutamine 1%, and antibiotics (Gentamicin 0.1%) in a 5% CO2 in air incubator at 37°C. A total of 7 out of 14 patients with ALL and 5 out of 9 with NHL, all with documented CD20- positive malignant B cells were treated with monoclonal anti-CD20 monoclonal antibodies (Rituximab) in an attempt to target killer cells against residual malignant cells as antibody-dependent cell-mediated cytotoxicity (ADCC).

Freshly harvested killer cells were infused one day following conditioning with no immediate adverse effects. Starting with intravenous treatment of patients with mismatched killer cells, subcutaneous injections of low dose IL-2 was applied for no more than 5 days for continuous in vivo activation of killer cells before their anticipated rejection.[14] It seems reasonable that in parallel with activation of donor lymphocytes, patient's own lymphocytes too could be activated to facilitate the mandatory rejection of mismatched killer cells for prevention of the risk of GVHD-like toxicity as previously confirmed.[14]

Residual circulating XY cells in female recipients was checked by amelogenin based PCR as previously described.[15]

Disease-free patients in complete remission for >5 years with no further treatment were considered as cured.

Results

The successful use of IMAK was pioneered starting in 1992 with treatment of a 12-year-old girl with AML with residual disease following several courses of chemotherapy and myeloablative chemotherapy supported by autologous stem cell transplantation in 1992.[16] Although a stage of MRD was induced by prior autologous stem cell transplantation, residual disease was still documented. Luckily, MRD was successfully eliminated by IMAK with no clinically overt GVHD and patient remained disease-free with no further treatment until today, already more

than 30 years. Since then, a total of 33 patients with MDR of different hematologic malignancies were similarly treated on compassionate basis using IMAK,14 with ALL, 8 with AML, 9 with NHL and 2 with multiple myeloma.[17] Complete remission was confirmed in 22 patients out of 33 treated with IMAK (a total of 23 including the first patient treated with IMAK). Cure could be accomplished in at least 6 patients that remained in complete remission for >5 years with no further treatment (2 AML; 2 multiple myeloma, 1 ALL & 1 NHL). Unfortunately, other patients with documented complete remission were not observed for longer than 5 years at the time of the latest follow up so the proportion of longer disease-free survival, possibly even cure, could even be higher. Adverse reactions were observed but none with grade 4 toxicity and early rejection of all mismatched killer cells was confirmed as could be documented in female recipients of male killer cells by PCR.[15] Due to early rejection of mismatched donor derived killer cells no patient developed overt clinical signs of GVHD.

Prevention of the risks of GVHD were accomplished by non-engrafting killer cells that were consistently rejected by patient's mismatched immune system, since no XY positive cells could be detected in female recipients beyond 6 days following cell infusion.[14,17]

Discussion

In the past, it was considered that high dose myeloablative chemo-radiotherapy was the main therapeutic component of the bone marrow transplant procedure and that transplantation of genotypically or phenotypically matched stem cells, or sometimes even using stem cells derived from an identical tween, was only indicated for rescue of patients treated with myeloablative conditioning. Hence, much attention was given to try and eradicate all tumor cells by maximally tolerated combinations of chemotherapy and whole-body radiation. However, it was recognized that the incidence of relapse was high among recipients rescued with autologous or even syngeneic hematopoietic stem cells as compared with recipients of allogeneic stem cells. The documented correlation between one of the most serious complications of allogeneic SCT, GVHD, and more successful eradication of malignant cells as a result of alloreactive donor lymphocytes, despite the mandatory use of immunosuppressive agent post grafting in an attempt to control GVHD, suggested that much of the therapeutic effects of SCT were due to GvL effects induced by alloreactive donor lymphocytes.[3,18-21]

The role of immune-mediated GvL effects in the course of SCT was further supported by observations suggesting that relapse while patients were on immunosuppressive treatment with cyclosporine A (CSA) was occasionally reversed by discontinuing immunosuppression.[22] Likewise, it has been documented that the incidence of relapse is lower in patients treated with sub-optimal doses of CSA used as prophylaxis against GVHD.[4]

Starting in early 1987, we have confirmed for the first time that therapeutic GvL effects could be induced post SCT for successful treatment of relapse by donor lymphocyte infusion (DLI), even after failure of the most aggressive myeloablative conditioning.[8,9] The first patient that confirmed the therapeutic role of DLI even after failure of maximally tolerated dosed of chemo-radiotherapy, was a young patient with ALL in third fully resistant relapse considered incurable. The patient was treated with supralethal myeloablative chemo-radiotherapy conditioning and SCT was accomplished from a fully matched sister with successful early outcome, with 3-linear engraftment of female cells. Unfortunately, aggressive systemic and extra-medullary relapse, with one large leukemic lesion obstructing his airway treated with emergency tracheotomy. Surprisingly, this patient was cured following 5 fresh intravenous injections of 20ml donor's blood, followed by the 6th dose of IL-2 activated donor lymphocytes administered one or two weeks apart. Following DLI this patient went into complete remission, fully reconstituted with female cells, with disappearance of all visible extra-medullary lesions and tracheotomy was closed. This patient is

currently alive and well with no evidence of disease with no further treatment and with no evidence of chronic GVHD, disease free for >37 years. This patient and many other that were similarly treated with DLI for treatment of relapse following SCT in our center and as confirmed by other transplant centers, confirmed that relapse following SCT may be reversed by DLI.[22-26] Later on, we have confirmed that pre-emptive DLI may be used also for successful prevention of relapse following SCT in patients with high-risk disease.[10]

Since it became obvious that the main component of therapeutic effects of SCT are mediated by engraftment of alloreactive donor lymphocytes, the next step was to change the paradigm of SCT being a method to allow development of successful replacement of myeloablative SCT based on the need to try and eliminate MDR malignant cells using much more aggressive conditioning, focusing on the need to use the transplant procedure as a means to induce transplantation tolerance to allow durable engraftment of alloreactive donor lymphocytes, realizing that cytolysis of MDR cancer cells occurs mostly following SCT. Accordingly, myeloablative SCT could be replaced with lymphoablative non-myeloablative stem cell transplantation (NST) or reduced intensity conditioning (RIC) the goal in mind to engraft donor's hematopoietic cells and a means to allow durable circulation of donor's immune system cells.[1,2] Due to significant reduction of procedure-related toxicity and mortality the transplant procedure could be accomplished for older patients with no upper age limit and also for very sick patients that would not be considered candidates for conventional SCT. NST and RIC made it possible to apply SCT for treatment of young children with genetic disorders treatable by SCT while avoiding procedure-related short and long-t6rm complications of growth and development including multiple endocrinopathies. Unfortunately, acute and chronic GVHD remained unavoidable complications following durable engraftment of alloreactive donor lymphocytes due to induction of transplantation tolerance following NST or RIC conditioning despite the mandatory use of of optimal post-transplant immunosuppressive treatment in an attempt to prevent or modify GVHD. Accordingly, the full potential benefits of GvL effects were suppressed by the mandatory post-transplant prophylactic immunosuppressive medications in an attempt to control GVHD.

As we were disappointed by lack of sufficiently potent GvL effects inducuble by MHC compatible donor lymphocytes for treatment of all patients with MDR disease, our attempt was to develop superior cell-mediated immunotherapy procedure based on the use of intentionally mismatched universal killer cells. Following proof of principle in pre-clinical animal models[27,28] this was successfully pioneered in one patient treated by maternal killer cells using IMAK at a stage of MRD following failure of myeloablative autologous SCT.[17] The feasibility and safety of IMAK was confirmed in a cohort of 40 patients with very advanced MDR metastatic solid tumors.[14] Subsequently, the use of IMAK was applied for compassionate treatment of a cohort of 33 patients with MDR hematologic malignancies as reviewed here, with the first patient treated with IMAK still alive and well, already disease-free for 32 years.

The development of IMAK was based on the following four principles. First, mismatched T cells can kill by a mechanism of rejection any cancer cell. Second, mismatched NK cells can kill any cancer cell that does not express identical MHC antigens, according to the "missing self" theory.[29] Third, IL-2 activation of both mismatched T cells and NK cells, possibly also NKT cells, acting together simultaneously are likely to represent the most potent cancer killer cells. may represent the ideal universal killer cells. Fourth, early rejection of non-engrafting mismatched killer cells would prevent development of hazardous GVHD-like toxicity. Additionally, in case mismatched activated killer T cells would cause GVHD-like effects against normal recipient cells before being rejected, safer IMAK procedure could be accomplished by depletion of T cells before or after IL-2 activation, or by positive selection of IL-2 activated NK cells. We have previously documented in pre-clinical

animal models[27,28] and in several patients treated successfully with T cell depleted IL-2 activated CD56-positive NK cells that no GVHD was observed even among patients that were treated with activated NK and NKT cells following allogeneic SCT followed by durable engraftment of donor lymphocytes (unpublished observations).

Encouraged by the cure of the first patient that was successfully treated with IMAK, additional investigations were carried out to confirm feasibility, safety and potential efficacy of IMAK in a total of 40 consenting patients with very advanced metastatic solid tumors with heavy tumor burden.[14] This pilot clinical trial confirmed that the conditioning was reasonable safe and that administration of fully mismatched killer cells could be safely accomplished with no clinically overt acute or chronic GVHD.[14] Mild immunosuppressive conditioning administered prior to treatment with IMAK was intended to maintain the cytotoxic activity of infused donor killer cells, control regulatory T cells and facilitate homeostatic proliferation, and also to activate patient's immune system to facilitate early rejection of mismatched door lymphocytes. IMAK treatment was reasonably well tolerated with different WHO grade 1 to 3 adverse effects but no grade 4 toxicity was observed. Only 2 patients developed mild, self-limited skin rash possibly compatible with acute grade 1 GVHD.[14]

Our successful preliminary study that confirmed the feasibility and safety of IMAK in 40 patients with most advanced metastatic solid tumors with heavy tumor burden with only 4 with hematologic malignancies encouraged us to consider the use of IMAK for additional patients with different hematologic malignancies considered otherwise incurable. Accordingly, the next cohort of 33 patients with drug-resistant hematologic malignancies (14 with ALL, 9 with non-Hodgkin lymphoma, 8 with AML and 2 with multiple myeloma) were treated with IMAK on compassionate basis.[17] The purpose of the immunosuppressive but non-myeloablative conditioning applied prior to infusion of killer cells was to combine several goals: (1) reduction of the number of host regulatory T cells; (2) optimize homeostatic proliferation of donor lymphocytes by establishing a "niche" for newly infused donor-derived killer cells; (3) eliminate unresponsiveness host's T cells and facilitate de novo proliferation of newly derived host T cells. Low dose IL-2 administration subcutaneously starting with cell infusion was accomplished as suggested by the preliminary investigations for continuous activation of circulating donor lymphocytes on the one hand and possibly also for activation of patient's own T cells in order to facilitate early rejection of mismatched killer cells. Selective targeting of IMAK was accomplished in about half of the patients with CD20-positive malignant B cells, including ALL and NHL, but the number of patients treated with anti-CD20 monoclonal antibodies is too small for driving any meaningful conclusions about the role of targeting IMAK for treatment of patients with malignant B cells.

Overall, the protocol was reasonably well tolerated with no >grade 3 toxicity and no GVHD-like toxicity. Complete remission was accomplished in 22 patients (23 if the first one is included) and 6 observed for more than 5 years with no additional treatment could be considered cured.[17] Other patients were not yet observed for >5 years at the time of reporting so theoretically, the cure rate may even be higher.

In conclusion, based on our successfully preliminary experience, the safety and clinical efficacy of IMAK should be confirmed in prospective future clinical trials in patients with different hematologic malignancies considered otherwise incurable by conventional modalities. In parallel, additional refinement of the optimal immunosuppressive conditioning is indicated in an attempt to optimize activation and homeostatic proliferation of both donor's and recipient's lymphocytes on the one hand, while reducing the risk of GVHD-like toxicity that could result from delayed rejection of mismatched donor lymphocytes on the other. In case of GVHD while trying to optimize the best parameters for

controlling the timing of circulation of non-engrafting donor lymphocytes, GVHD could be easily prevented by selective T cell depletion or positive selection of CD56-positive NK cells. Future confirmation of our preliminary results that could be further improved by maximizing cytokine-induced activation of donor killer cells combined with selective targeting donor's killer cells against patient's malignant cells by relevant monoclonal antibodies may represent an optimal immunotherapy procedure that should be considered for treatment of all patients with high-risk hematologic malignancies, preferably at an early stage of the disease following successful response to conventional chemotherapy. IMAK application against low tumor burden in patients with good performance status will certainly increase the option for cure of patients considered otherwise incurable. Since most patients with all types of hematologic malignancies respond initially to conventional chemotherapy, clinical application of IMAK at the stage of low tumor burden, ideally at the stage of MRD, could represent the optimal if not the only timing to accomplish cure.

In conclusion, cell-mediated immunotherapy based on the use of cytokine pre-activated intentionally mismatched killer cells including a mixture of T cells, NK cells and NKT cells could provide a most effective approach for immunotherapy of multi-drug-resistant hematologic malignancies. When applied against a low residual tumor load even one course of treatment could result in elimination of all drug-resistant malignant cells in patients considered otherwise incurable. Future prospective clinical trials should be recommended to confirm the safety and clinical efficacy of immunotherapy based on cytokine-induced activation of non-engrafting short circulation of mismatched killer cells. Whereas one treatment cycle may be sufficient for successful treatment of minimal residual disease, repeated treatment cycles may be indicated for treatment of patients with heavier tumor burden. Targeting pre-activated mismatched universal killer cells for selective anti-cancer immunotherapy using monoclonal antibodies against over-expressed cell-surface antigens may further optimize anti-cancer cytotoxicity and minimize the potential risks of GVHD for treatment of patients with otherwise incurable hematologic malignancies and possibly also for treatment of patients with drug-resistant metastatic solid tumors.[30,31]

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