Immunotherapy of multi-drug resistant hematologic malignancies with IL-2-activated intentionally mismatched lymphocytes

Shimon Slavin (slavinMD@gmail.com)
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Research Article

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Abstract

Donor lymphocyte infusion using IL-2 activated donor lymphocytes following allogeneic stem cell transplantation (SCT) can sometimes eliminate multi-drug resistant (MDR) cancer cells and result in a cure. We hypothesized that more effective and faster immunotherapy could be accomplished by non-engrafting Intentionally Mismatched IL-2 Activated Killer cells (IMAK) with no prior SCT, avoiding the risks of GVHD due to consistent spontaneous rejection of killer cells after induction of cancer cytotoxicity. IMAK was applied for the compassionate treatment of 33 patients with MDR hematological malignancies following mild immunosuppressive conditioning. IMAK infusion was followed by low-dose subcutaneous IL-2 injections for ≤ 5 days for *in vivo* activation of donors' and patients' lymphocytes. Cumulative experience confirmed major anti-cancer responses following treatment with IMAK including in patients relapsing after myeloablative SCT. Treatment of relatively low tumor burden resulted in cure (> 5 to > 28 years unmaintained disease-free survival). Treatment was reasonably well-tolerated or manageable with no grade IV toxicity. Circulating donor lymphocytes were undetected beyond day + 6, confirming that early spontaneous rejection prevented the risk of GVHD-like toxicity. Immunotherapy potentially resulting in cure could be accomplished in patients with resistant hematologic malignancies when treated with relatively low tumor burden by transient circulation of IMAK. Since MRD can be accomplished in most patients with hematologic malignancies following successful first-line or high-dose chemotherapy, eradication of MRD in otherwise incurable patients could possibly be accomplished by IMAK. Prospective randomized clinical trials are indicated to confirm our working hypothesis.

Key Points

Immunotherapy represents a promising future method for the treatment of multi-drug resistant hematologic malignancies but in most cases cure remains an unmet need.

We present a new cell-mediated immunotherapy protocol using non-engrafting mismatched activated killer cells that can eliminate multi-drug resistant cancer.

Introduction

Although most patients with hematological malignancies respond initially to conventional anti-cancer modalities, recurrent disease represents the major cause of failure due to recurrent resistant disease. We have pioneered the use of donor lymphocytes infusion (DLI) for treatment of relapse following myeloablative allogeneic stem cell transplantation (SCT) as early as 1986, confirming the feasibility to eliminate chemo-radiotherapy-resistant leukemia in a proportion of multi-drug resistant patients using alloreactive donor lymphocytes (1–3). The effective role of DLI for induction of graft-versus-leukemia (GvL) effects was confirmed by many transplant centers worldwide (4–7). Subsequently, graded increments of DLI, while watching for early signs of acute graft-vs-host disease (GVHD), was applied successfully for the prevention of relapse by pre-emptive DLI in patients with high-risk disease (8). Unfortunately, whereas DLI treatment following allogeneic SCT for treatment of acute and chronic

hematologic malignancies could sometimes eliminate resistant malignant cells, durable circulation of donor lymphocytes following induction of transplantation tolerance by engraftment of donor's stem cells was always risky due to unpredicted toxicity, morbidity and mortality due to unavoidable acute and chronic GVHD. Furthermore, even maximal GvL effects induced by risky acute and chronic GVHD following MHC-compatible allogeneic SCT was not always sufficient for eliminating all resistant malignant cells (1–7).

We have used a spontaneous murine B cell leukemia (BCL1) (9) in an attempt to develop new approaches for improving the anti-cancer effects inducible by naïve donor lymphocytes. First, we documented that even the GvL effects induced by syngeneic donor lymphocytes could be amplified by treatment with interleukin 2 (IL-2) (10,11). Similarly, the opportunity to document similar enhancement of GvL effects by IL-2-activated DLI following super-myeloablative conditioning and allogeneic SCT from a fully matched sibling was already confirmed in late 1986 when a patient with fully resistant relapsed ALL following super-myeloablative conditioning was rescued with IL-2 activated DLI after failing to respond to treatment with naïve donor lymphocytes (2,3). This patient is alive and well today, more than 35 years later, with no further treatment, luckily with no acute or chronic GVHD. Next, we have investigated the therapeutic effects of haploidentical or fully mismatched donor lymphocytes activated with IL-2 in pre-clinical animal models in an attempt to maximize their anticipated capacity to eradicate leukemia. Using both BALB/c mice inoculated with lethal doses of BCL1, and SJL/J mice inoculated with lethal doses of acute murine myeloid leukemia, we have confirmed that infusion of IL-2-activated MHC mismatched spleen cells resulted in complete eradication of leukemia 12,13). Interestingly, elimination of a lethal challenge of BCL1 inoculated in (BALB/c X C57BL/6)F1 mice following infusion of recipients with IL-2 activated C57BL/6 lymphocytes resulted in cure, in sharp contrast to similar treatment using IL-2 activated BALB/c spleen cells, although both IL-2 activated BALB/c or C57BL/6 spleen cells could result in equal GVHD against F1 recipients (14). These observations indicated that effective GvL effects were induced by activation of alloreactivity against mismatched target cells and not by non-specific alloreactivity induced by GVHD per se. Taken together, it became obvious that much more effective and much faster GvL effects could be induced by IL-2 pre-activated intentionally mismatched killer cells consisting of a mixture of both T and NK cells. We hypothesized that due to more effective and much faster induction of GvL effects by mismatched and IL-2 activated killer cells, elimination of malignant cells could be accomplished within a few days by non-engrafting donor lymphocytes, thus avoiding the need for prior SCT in order to ensure consistent rejection of mismatched donor lymphocytes after induction of cytotoxicity against leukemia for prevention of GVHD. Accordingly, it seemed reasonable to assume that clinical application of intentionally mismatched activated killer cells (IMAK) at the stage of minimal residual disease (MRD) or against a low tumor burden could similarly result in complete elimination of all multi-drug resistant malignant cells, including cancer stem cells. In November 1994 we had the first opportunity to confirm our working hypothesis in a patient with fully resistant AML after failure of conventional chemotherapy and myeloablative autologous SCT (15). This patient is currently alive and well with no further treatment for more than 28 years. Since then, we have applied our new IMAK immunotherapy program for the compassionate treatment of patients considered fully resistant to all

available conventional modalities. The present investigations represent the cumulative experience of compassionate treatment of 33 patients similarly treated with IMAK.

Patients And Methods

Patients

A total of 33 consenting patients, 21 males and 12 females, aged 3–63 (median 30) with different hematopoietic malignancies detailed in Table 1 were treated with IL-2 activated lymphocytes obtained from haploidentical or fully mismatched unrelated consenting donors on a compassionate basis between 1994 and 2016 due to multi-drug resistant relapse. Treatment protocol was approved by the Ethical Committee of the Hadassah Medical Center & University Hospital in Jerusalem, Israel, and each patient signed an approved informed consent according to the principles expressed in the declaration of Helsinki.

Table 1
Diagnosis of patients with advanced multi-drug resistant hematologic malignancies treated with IMAK

Multiple myeloma	**2**
Non-Hodgkin lymphoma	9
AML	8
ALL	14
Total	**33**

Preparation Of Activated Donor Lymphocytes

Peripheral blood lymphocytes were obtained by aphaeresis using a COBE spectra cell separator from haploidentical related donors (n = 20) or unrelated volunteers (n = 13). Cells were transferred into 250 ml tubes and loaded over ficoll gradient at room temperature and spun for 20 minutes at 2,000 rpm using a Sorval centrifuge. Cells were cultured in a 1 L Lifecell bag (Baxter, USA) at a concentration of 2x10^6 cells/ml in RPMI medium supplemented with 10% heat-inactivated human AB serum, glutamine 1%, and antibiotics (Gentamicin 0.1%). Recombinant human IL-2 (Proleukin, The Netherlands) was added at 6,000 IU/ml. Cells were placed in a 5% CO2 in air incubator at 37^0C, at a maximum volume of 1,500 ml in 3 L Lifecell bags for 4 days. Activated lymphocytes were harvested following 4 days of culturing. Upon termination of incubation, cells were transferred to a 600 ml transfer pack by plasma transfer set. Cells were spun at 1,200 rpm for 15 minutes, washed and resuspended in saline in a 150 ml transfusion bag ready for infusion.

IMAK enriched for NK cells was used in 3 patients with AML. CD56 positive NK cells were selected using Miltenyi's immunomagnetic bead system (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany). Positively selected NK cells consisted of 30–71% $CD56^+$ (median 39%) cells and 2–21% $CD3^+$ (median 3%) T cells.

IMAK infusion was combined with infusion of Rituximab 100mg in an attempt to target Fc receptor-positive NK cells against malignant B cells in 7 patients with ALL and 5 patients with NHL.

Conditioning Of Patients Before Cell Infusion

Conditioning was applied by a single intravenous cyclophosphamide 1,000 mg/m^2 on day − 1 with forced hydration 3L/m^2 before infusion of IMAK on day 0. Only 4 patients were pre-treated with heavier immunosuppressive conditioning as previously described (16).

Starting with the conditioning, all patients were treated with prophylactic anti-inflammatory indomethacin 25mg tablets x2/day and ranitidine (Zantac) tablets 150-300mg/day to minimize adverse reactions COX 2-induced fever and inflammatory reactions that could result from infusion of IL-2-activated killer cells followed by low-dose IL-2 administration following cell infusion. Just before cell infusion patients received promethazine 12.5 mg against any potential hypersensitivity reaction.

Cell Therapy Based On The Use Of Imak

A total of 1.5–18.0 (median 3.0) x10^7 cells/kg were slowly injected intravenously on day 0. A total of 7 patients with CD20-positive ALL out of a total of 14, and 5 patients out of 9 with CD20-positive non-Hodgkin lymphoma were treated with IMAK targeted against the malignant cells using anti-CD20 monoclonal antibody (Rituximab) together with cell infusion.

Treatment Of Patients After Cell Infusion

Starting on day 0, subcutaneous injections of low doses IL-2 (< 6x10^6 IU/m^2) individually adjusted to prevent adverse reactions for up to 5 consecutive days to continue activation of killer cells until anticipated rejection.

Engraftment And Gvhd Following Treatment With Imak

All patients were closely monitored for toxicity and clinical and laboratory signs of acute and later on for possible chronic GVHD. Chimerism in female recipients of male cells was checked by cytogenetic analysis and later using male-specific amelogenin gene by PCR as previously described [17]. Donor-specific VNTR-PCR was used in female- to-female, male-to-male, or female-to-male cases for detection of circulating donor cells to confirm complete rejection of mismatched IMAK.

Long-term Observations Of Patients

All patients were observed for signs of toxicity, acute and chronic GVHD and possible recurrent disease as long as they were under our observation.

Data Sharing Statement

Individual participant data will not be shared but deidentified individual participant data will be available in the filed medical records available at the Hadassah University Hospital in Jerusalem.

Results

Procedure-related toxicity and risk of GVHD

As shown in Table 2, conditioning before cell infusion was accomplished with acceptable toxicity with no grade 4 among the first cohort of 33 heavily pre-treated patients with different resistant hematologic malignancies. Conditioning with cyclophosphamide alone was much simpler and much better tolerated among 29 patients in comparison with a previous cohort of 4 patients pre-treated with heavier immunosuppressive conditioning (16). Also, the only suspected 2 cases of possible grade I GVHD occurred among the patients pre-treated with heavier immunosuppressive conditioning. Administration of IL-2 following cell infusion was accompanied by local erythema at the site of injection in 22 patients, and malaise or mild fever responded to anti-inflammatory agents. Skin erythema compatible with grade I GVHD was observed in 2 patients. A total of 16 patients, 8 with grade II and 2 with grade III developed significant fever and/or chills requiring additional treatment. Other adverse reactions are shown in Table 2 with grade III observed in 14 patients but none with grade IV. As shown in Table 2, patients conditioned with mild cyclophosphamide conditioning had less severe adverse reactions as compared with patients in a previous cohort (16) pre-treated with more immunosuppressive conditioning.

All procedures were carried out in the outpatient setting and no patient required admission to the hospital.

Due to subjective intolerance to IL-2, the daily dose was reduced to 0.5x10^6 IU and IL-2 administration was reduced from 5 to 3 days in 8 patients. Importantly, apart from the adverse reactions shown in Table 2, no patient developed any sign of clinically significant acute GVHD and no patient developed any sign of chronic GVHD, due to consistent rejection of donor lymphocytes, since circulating donor lymphocytes were never detected beyond day + 6.

Table 2
Adverse reactions of all 33 patients treated with IMAK following mild or moderate immunosuppressive conditioning

	Grade 0	Grade I	Grade II	Grade III	Grade IV
Hemoglobin	23	4	6	0	0
Leucocytes	19	6	6	2*	0
Platelets	14	7	9	3*	0
ALT	22	4	5	2*	0
AST	24	4	3	2*	0
GGTP	27	1	2	3*	0
ALK phosphatase	28	3	2	0	0
Erythema at IL-2 injection site	11	20	2†	0	0
Fever and/or shaking chills	17	6	8	2*	0
Skin erythema; grade I GVHD?	31	0	2*	0	0

* Adverse reactions among patients conditioned with more aggressive lymphoablation consisting (16).

†Dose reduction of IL-2 injections due to fever and malaise and reduction of treatment to 3 days instead of 5 days subcutaneous IL-2 injections was indicated in 8 patients.

Anti-cancer Effects Induced By Imak

Details and outcomes following cell therapy with IMAK are shown in Table 3 and summarized in Fig. 1. Interestingly, the anti-cancer effects of IMAK seemed to be equally effective against all hematologic malignant cells investigated including lymphoid, myeloid and malignant plasma cells.

Table 3
All 33 poor prognosis patients with multi-drug resistant relapse treated with IMAK for the past 28 years

	Type of cell therapy	Number of patients treated	Number of patients in CR, PFS or cure	Number of patients with PR or VGPR	Number of patients with PD
Relapsed resistant ALL	IL-2 activated mismatched PBL	7	3	1	3
Relapsed resistant ALL	IL-2 activated mismatched PBL + anti-CD20 monoclonal antibody	7	4	2	1
Relapsed resistant AML*	IL-2 activated mismatched PBL	5	3	1	1
Relapsed resistant AML	IL-2 activated mismatched NK cells	3	3		
Relapsed non-Hodgkin's lymphoma	IL-2 activated mismatched PBL	4	4		
Relapsed non-Hodgkin's lymphoma	IL-2 activated mismatched PBL + anti-CD20 monoclonal antibody	5	3	1	1
Advanced relapsed multiple myeloma (one after HSCT)	IL-2 activated mismatched PBL	2	2		
Total number of patients treated with IMAK		33	22	5	6

AML, acute myeloid leukemia; ALL, acute lymphocytic leukemia; PBL, peripheral blood lymphocytes; CR, complete remission; PFS, progression-free

survival; PR, partial remission; VGPR, very good partial response; PD, progressive disease; HSCT, hematopoietic stem cell transplantation.

* Including the first patient with fully resistant AML that failed conventional chemotherapy and relapsed following myeloablative autologous hematopoietic stem cell transplantation (HSCT) that was successfully treated with IMAK using IL-2 activated maternal (haploidentical mismatched) lymphocytes. Currently, more than 28 years later, she is alive and well with no further treatment. This patient is the first one ever treated with IMAK that confirmed the feasibility of cure of fully resistant leukemia using IL-2 activated non-engrafting intentionally mismatched donor lymphocytes with no need for prior allogeneic stem cell transplantation and no risk of acute or chronic GVHD.

Out of the first cohort of 33 patients treated with IMAK for recurrent multi-drug resistant disease a total of 22 were with confirmed complete response, 5 with partial response or very good partial response, and only 6 with progressive disease. The first patient treated with IMAK at the age of 12 years is currently

more than 28 years out, a physician in perfect clinical condition with no further treatment since then happily married with 2 children. Following the diagnosis of acute promyelocytic leukemia, complete remission could not be accomplished in the Philippines and residual disease was confirmed following chemotherapy in a major medical center in Los Angeles. Accordingly, the patient was referred to the Hadassah Medical Center in Jerusalem but residual AML was confirmed even following myeloablative hematopoietic stem cell transplantation. Therefore, it was suggested to try an alternative cell-mediated immunotherapy using intentionally mismatched lymphocytes as supported by the pre-clinical investigations detailed above, first using unsuccessfully DLI infusions using naïve maternal lymphocytes. Next, complete eradication of all detectable AML was only accomplished following treatment with IMAK, using maternal lymphocytes pre-activated with IL-2 for 4 days prior to cell infusion and with subcutaneous injections of IL-2 for 3 days following cell infusion. Accordingly, a similar therapeutic strategy was applied as compassionate treatment in the next cohort of 32 patients considered incurable by any available conventional procedure. Out of the 22 responders that can be seen in Table 3, at least 6 observed for > 5 to > 28 years with no further treatment should be considered cured. All 6 patients with progressive disease were treated with IMAK at the time they presented with progressive disease.

Successful induction of complete remission, progression-free survival or possibly even cure in patients with different types of multi-drug resistant hematologic malignancies following treatment with intentionally mismatched activated killer cells (IMAK).

Since only 7 out of 14 patients with ALL and 5 out of 9 with NHL were treated with IMAK targeted against malignant B cells with Rituximab, no information is available if targeting IMAK against B cells with anti-CD20 monoclonal antibodies was contributary. Likewise, since only 3 out of 8 patients with AML were treated with T cell-depleted NK cells-enriched IMAK and none of the 8 developed significant GVHD, no conclusions can be drawn if depletion of T cells is essential to prevent GVHD or if the only effective killer cells are NK cells.

Discussion

The main purpose of the present investigations was to provide proof of principle of feasibility, safety, and efficacy of a simple and inexpensive protocol for more effective cell-mediated immunotherapy of patients fully resistant to all available anti-cancer modalities. Based on successful cure with no severe side effects of the first patient treated with IMAK currently 28 years out (15), 32 additional incurable patients with different hematologic malignancies were recruited to assess the feasibility and safety of cell therapy with IMAK against multi-drug resistant disease. We have previously confirmed that DLI, especially DLI induced with IL-2 activated donor lymphocytes, can sometimes eliminate resistant malignant cells escaping following maximally tolerated myeloablative conditioning following allogeneic SCT [1–3]. There was a need to develop more effective treatment program for attempting elimination of malignant cells of hematopoietic origin resistant to all other available procedures, including allogeneic SCT and GvL induced by IL-2 activated DLI, since control of relapse was neither consistent even in patients with severe GVHD nor in patients with overt relapse. In contrast, our experience in our pre-clinical animal models [9,

12, 14] confirmed that immunotherapy based on transient circulation of mismatched activated lymphocytes against malignant target cells by readily available killer cells can be fast-acting and most effective, thus accomplished within a few days, before consistent rejection of anti-cancer effector cells occurs for prevention of GVHD. These observations supported by successful treatment of our first patient treated with IMAK with no clinically overt GVHD confirmed the expectation that elimination of malignant cells could be accomplished before rejection of mismatched killer cells, a mandatory pre-requisite for prevention of GVHD. As such, it seemed reasonable to anticipate that cure could be anticipated only when IMAK will be applied against MRD or against a relatively low tumor burden. Alternatively, the procedure would have to be repeated using different donor cells. Indeed, as shown in Table 3 and Fig. 1, most patients treated with IMAK using either haploidentical related donor cells or mismatched lymphocytes obtained from consenting unrelated donors showed evidence of response, and at least 6 of 22 patients observed for more than 5 years may even be considered cured.

Although our cumulative experience confirmed the feasibility, safety and efficacy of immunotherapy induced by IMAK, many questions remain to be answered. First, the role of IMAK needs to be confirmed in a prospective randomized clinical trial in different disease categories, both for treatment of patients with overt relapse and for treatment of patients at risk with minimal residual disease. Fortunately, induction of complete remission can be accomplished by conventional 1st line chemotherapy even in patients with high-risk hematologic malignancies at an early stage of the disease, or if indicated following high-dose chemotherapy and supportive autologous SCT, as per the routine procedure in patients with multiple myeloma. Therefore, once the safety and efficacy of IMAK can be confirmed, eradication of MRD resulting in cure could be easily accomplished at an early stage in patients with high risk disease before relapse occurs, when another opportunity of MRD may be difficult of impossible to accomplish.

Other important parameters that need to be investigated include whether treatment outcome could be improved by targeting the mismatched killer cells with relevant monoclonal antibodies. Also, as shown in Table 3, targeting T cell-depleted IMAK against 3 patients with AML was also successful, suggesting that IL-2 activated NK cells may be the dominant anti-cancer effector cells. Using T cell depletion or NK cells enriched killer cells will certainly minimize any risks of GVHD but it has to be determined if IMAK containing both IL-2 activated T and NK cells is more effective than IMAK based on the use of activated NK cells alone. Accordingly, we need to investigate if treatment with T cell-depleted or NK-enriched killer cells is worth the extra-complicated procedure and higher cost as compared with using an unmanipulated mixture of killer cells.

Another potentially important question is whether the anti-cancer effects inducible by IMAK could be improved by synergistic control of negative regulators such as regulatory T cells, checkpoint inhibitors, myeloid-derived suppressor cells and even mesenchymal stromal cells. Also, in the case of CD20-positive lymphoid malignancies, the anti-cancer effects induced by IMAK will have to be compared to the anti-cancer effects induced by CAR-T cells.

Taken together, the concept of fast and effective killing of malignant cells despite resistance to maximally tolerated doses of chemoradiotherapy and other available anti-cancer modalities by cell therapy alone, while avoiding much more complicated allogeneic SCT and the risks of GVHD, could possibly represent a new approach for more effective yet much simpler approach for immunotherapy of otherwise incurable hematologic malignancies. The available proof of principle should provide the basis and justification for future investigations and prospective clinical trials to investigate the safety and efficacy of cell-mediated immunotherapy based on the use of intentionally mismatched donor lymphocytes.

Remembering that no available anti-cancer modality can compete with the capacity of mismatched lymphocytes to reject any MHC mismatched target cells, including even kilograms of organ allografts in recipients sub-optimally immunosuppressed, our prediction based on multiple studies in pre-clinical animal models and our limited clinical experience is that IMAK application against a true stage of MRD could result in complete eradication of all resistant malignant cells. Future studies are indicated to prove or disprove our working hypothesis.

Declarations

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Authorship Contributions

Slavin has developed the concept of cell therapy using door lymphocytes first in pre-clinical animal models and then was personally in charge of clinical application of cellular therapy at the patients' bedside in conjunction with stem cell transplantation. Based on past experience, Slavin developed the working hypothesis that cell-mediated immunotherapy could be improved using intentionally mismatched IL-2 activated lymphocytes avoiding the need of stem cell transplantation and pioneered clinical application of the new modality for compassionate treatment of patients in need.

Conflict of Interest Disclosure

Slavin declares no conflict of interest.

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Figures

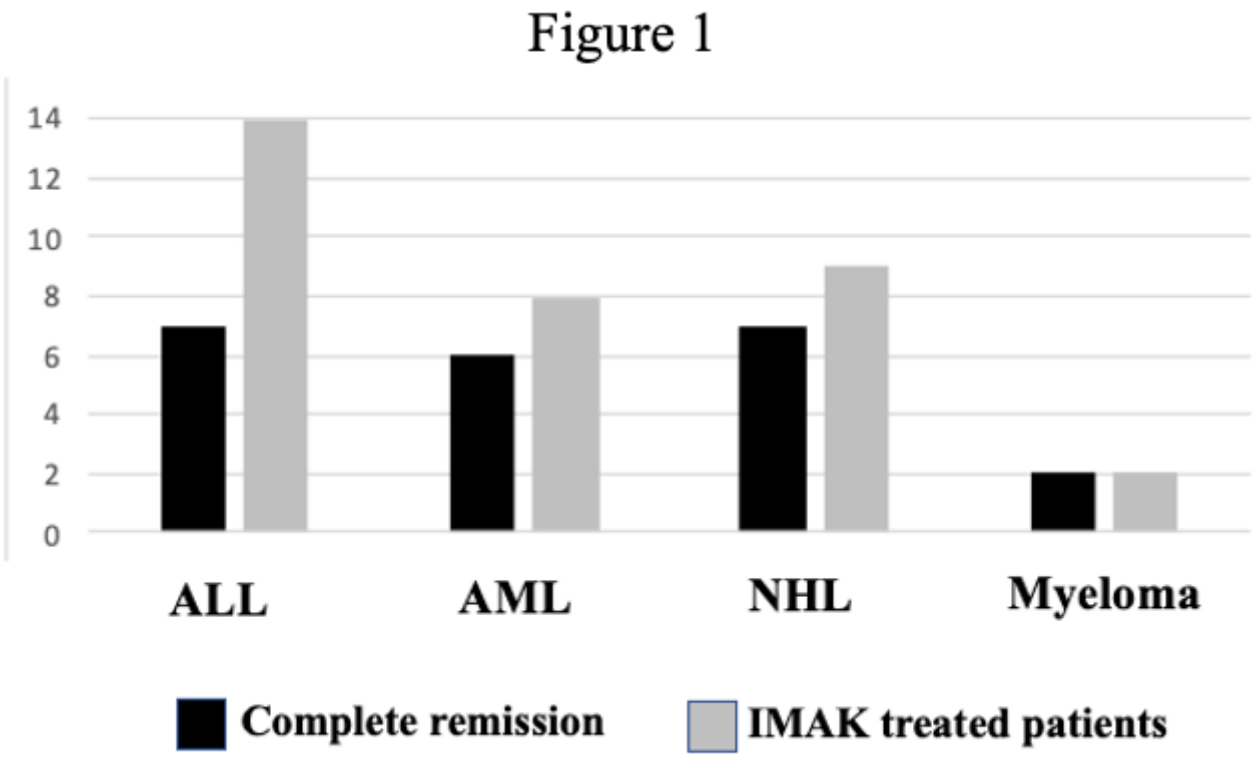

Figure 1

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