Background: Hemophagocytic lymphohistiocytosis (HLH) is a life-threatening condition characterized by prolonged fever, hepatosplenomengaly, cytopenia, and hemophagocytosis. Primary HLH is considered fatal unless treated by hematopoietic stem cell transplantation (HSCT).

Method: Herein we report six Iranian patients as a case series with primary HLH and their outcome from a single tertiary-care center between 2000-2012.

Results: Griscelli syndrome type 2 (G52) was diagnosed in 3 patients based on clinical, laboratory, and microscopic features of partial albinism and finding of RAB27A mutations in 2 patients tested. Two patients were on the HLH-2004 continuation therapy and waiting for HSCT, but no matched donor yet; The first case is in 2nd remission after 1.5 years; he had history of relapse in maintenance phase of HLH-2004 protocol which more immunosuppressive treatment was advised. The second case is in remission after 1 year. Two other patients died, while waiting for a suitable donor: case 3 had history of recurrent HLH and died after 6 months; and the case 4 had history of progression to T-cell ALL after 2 years and died due to relapse of ALL, sepsis and DIC despite chemotherapy after 3 years. Two patients underwent HSCT; patient 5 had a HLA-identical sibling donor and patient 6 had a cord blood-, unrelated, one antigen mismatched donor. Both patients were treated according to the HLH-2004 treatment protocol and none had active disease at the time of HSCT. Patient 5 who were diagnosed with G52 responded well and remained in remission through 24 months of follow-up, while another case died because of graft versus host disease and infection on day 136 after HSCT.

Conclusion: HSCT seems to be the only curative treatment for primary HLH which drastically improve survival of the patients. Making a definite diagnosis confirmed by gene mutation studies is helpful to provide genetic counseling and prenatal diagnosis and, more important, dictate the need for HSCT later in the patient’s course.

Key words: Hematopoietic stem cell transplantation; Hemophagocytic lymphohistiocytosis; Familial HLH; Griscelli syndrome type 2

Platform Independent Multiplex qPCR Research Assay for Chimerism Analysis

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Aim: Following Hematopoietic stem cell transplantation (HSCT), the accurate monitoring of donor-derived cells prospectively is critical for understanding sustained engraftment or whether therapeutic intervention is needed, in turn predicting the success or failure of HSCT. We designed a highly sensitive, quantitative PCR (qPCR) research assay specifically for engraftment monitoring following HSCT in a research setting. It can accurately detect recipient cells up to 0.01% while also reducing workflow and analysis burdens as compared to routine STR testing. To enable broader access to the AlleleSEQR qPCR Chimerism Assay RUO across more clinical research laboratories, we have assessed performance of the multiplexed screening assay across five different qPCR platforms from four manufacturers.

Method: A panel of 34 qPCR research assays to bi-allelic indels across the genome enables informative marker identification and quantification to occur within a few hours and without the need for capillary electrophoresis. To decrease our current sample volume requirements, increase throughput and enable multiple donor screening scenarios across multiple transplantation centers, we evaluated a multiplexed assay approach for marker identification utilizing five different qPCR platforms. The alleles are scored in relation to amplification of an endogenous control assay and 6-8 samples can be evaluated simultaneously across all platforms.

Results: Multiplexing the assays to 2-4 assays/well yielded identical results when compared to single well 34 assays. In addition, using five different qPCR platforms i.e. Applied Biosystems 7500 and Viia7™ Real-Time PCR System, QIAGEN Rotor-Gene Q, Focus 3M™ Integrated Cycler and Roche LightCycler™ 480 System, the described multiplexed qPCR approach yielded results with 100% concordance in identifying informative markers on six unique Coriell DNA across platforms.

Conclusions: The results from this multiplexed qPCR research system for screening of informative markers were 100% concordant to results generated when using the assays individually. Performance of the multiplex qPCR assays were independent of the platform providing supportive results for possible future use in transplant centers.

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Maintenance Hypomethylating Therapy Post Allogeneic Stem Cell Transplantation Provides Favorable Progression Free Survival in High Risk Acute Myelogenous Leukemia or Non Responsive Myelodysplastic Syndrome

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Introduction: Patients with acute myelogenous leukemia (AML) or non responsive myelodysplastic syndrome (MDS) who fail to achieve a complete remission, have unfavorable cytogenetics or are refractory to therapy have a poor prognosis. Allogeneic HSCT is frequently considered a salvage option for these patients although remissions are short-lived. New strategies are needed for maintaining remission in this extremely high risk patient group. We hypothesized hypomethylating agents post allogeneic HSCT would enable patients to remain in complete remission.

Methods: Eleven patients were treated. Patient characteristics: median age, 48 years (range, 18-70 years), PBSC (n=9) or bone marrow (n=2); Diagnoses AML= 10, Non- responsive MDS= 1, CIBMTR disease risk category: High [n=8], Intermediate [n=1], Low [n=2]; 36% of the patients had primary induction failure, 54% had complex molecular abnormalities with 18% of the patients having deletion 11q 23 molecular abnormalities. Donors were unrelated (36%) and related (64%). All but 1 donor-recipient pairs were fully matched. Preparative regimen- busulfan based (16mg/kg or equivalent) (82%), TBI ≥ 12 Gy (9%) based or reduced-intensity haplo-identical regimen (9%). The median number of prior chemotherapy regimens was 2.5 (range, 2-6), Azacitidine (n=10) or decitabine (n=1) was selected at physician discretion as a planned prophylactic regimen post allogeneic HSCT engraftment. Initial starting doses were based on hematological conditions at the time of therapy initiation.

Results: Ten patients received azacitidine subcutaneously daily for seven days for a median of 5 cycles (range, 1-10)
cycles) and one patient received decitabine 20mg/m² intravenously for 5 days for 3 cycles. Median initial Azacitidine dose of 50 mg/m² (range, 25-75 mg/m²) were given at a median of 40 days (range, 38-101 days) post HSCT engraftment. Azacitidine doses were increased based on counts to a maximum dose of 75mg/m². With a median follow-up of 24 months (range, 1.7-49 months) 6 patients are alive in complete remission. Twenty-four month progression free survival is 51% with overall survival of 76%.

Conclusion: Given the dismal results of progression free survival and overall survival following allogeneic HSCT in high risk AML or non responsive MDS, hypomethylating agents given post transplantation provide a valuable approach to prolonging remission.

Leukocytosis Post Engraftment Is Associated with Increased Mortality Among Adult Allogeneic Hematopoietic Cell Transplant (HCT) Recipients

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Engraftment among allogeneic HCT recipients is associated with endothelial damage, cytokine release and elevated white count. The impact of persistent leukocytosis after engraftment on survival and transplant related complications such as graft versus host disease is not well established.

In this study, we retrospectively reviewed the charts of 74 consecutive patients who underwent allogeneic HCT in a single center between 2009 and 2011. Neutrophil engraftment was defined as an absolute neutrophil count of 0.5x10³/µl for 3 consecutive days. Patients’ blood counts were assessed for 60 days post engraftment (three times per week). Persistent Leukocytosis (group A) was defined as a white blood cell count of >10x10³/µl for more than four different blood draws. Patients who had leukocytosis for three or less blood draws were called group B. Conditioning regimens included myeloablative MA (Fludarabine/Busulfan or cytoxan/total body irradiation) and reduced intensity RIC (fludarabine/busulfan or fludarabine/cytoxan/low dose TBI). Graft versus host disease prophylaxis included methotrexate/tacrolimus for myeloablative and tacrolimus/Myco- phenolate for reduced intensity conditioning.

The median age was 51 years (range: 18-74 years). Graft source included matched related (n=24), Unrelated donor (n=48) and cord blood (n=2). 36 patients received MA conditioning and 37 had RIC conditioning. Median follow up for the cohort was 556 days. Median time to neutrophil engraftment was 13 days. 22 patients had persistent leukocytosis (group A) during the first 60 days post engraftment. The two groups did not differ in terms of age, gender, diagnosis (acute leukemia versus other), disease risk and conditioning regimen (table 1). Group A had a higher proportion of related donor source (50% versus 31%; P = .05). One year overall survival was significantly worse in group A (40.6% versus 74.7%; P = .009). Grade II-IV acute graft versus host disease was higher among group A patients (68% versus 48%; P = .18) although not statistically significant. The cause of death among patients with persistent leukocytosis included acute GVHD (n=6), relapse (n=5), infection (n=2) and thrombosis (n=1).

In conclusion, Persistent leukocytosis post engraftment in allo-HCT recipients is associated with worse overall survival and may be an independent risk factor for acute GVHD. Despite a larger proportion of related donors among the leukocytosis group, our data showed a trend towards higher incidence of acute GVHD. The exact mechanism leading to prolonged leukocytosis is not clear and further studies to confirm the association between leukocytosis and clinical outcomes are warranted.

Incidence of Invasive Fungal Disease After Unmanipulated Haploidentical Stem Cell Transplantation Was Significantly Higher Than That After HLA-Matched Sibling Transplantation

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The aim of this study was to determine the incidence, clinical features and outcome of invasive fungal disease (IFD) after either an unmanipulated haploidentical hematopoietic stem cell transplantation (HSCT) or a human leukocyte antigen (HLA)-matched sibling HSCT. This was a head-to-head comparative study performed at a single center. Patients were admitted between 2007 and 2010, and IFD was evaluated according to the revised EORTC/MSG criteria, with only proven and probable cases included. Of the 1,042 consecutive patients enrolled, 390 received the HLA-matched HSCT and 652 received an unmanipulated haploidentical HSCT. A total of 61 (5.8%) patients had IFD, which was broken down into 15 proven cases and 46 probable cases. The median time of diagnosis was 35 days (range 6-405) after transplantation. The most common involved site was the lung (52/61), and Aspergillus was the most common (18/29) pathogen. The incidence of IFD after an unmanipulated haploidentical HSCT was significantly higher than the HLA-matched transplant (7.1% versus 3.3%, respectively, P = .007), which was caused by the more early IFD (before day 40). IFD occurred later in patients receiving an HLA-matched transplant compared to patients receiving the unmanipulated haploidentical HSCT (141.5 versus 23 days, respectively, P = .04). The response to therapy and the mortality rate corresponding to IFD were similar between the two types of transplantation. In conclusion, patients received an unmanipulated haploidentical HSCT had higher risk of IFD than those patients received an HLA-matched HSCT, but the prognosis of IFD was not associated with the HLA type.