IS KIR HAPLOTYPE MATCHING IMPORTANT?
Gendzekhadze, K.1, Oki, A.1, Shen, Y., Palmer, J.2, Nademanee, A.3, Nakamura, R.3, Forman, S.2, Senitzer, D.4, City of Hope, Duarte, CA; 2 City of Hope, Duarte, CA; 3 Hospital & Research Center, Riyadh, Saudi Arabia

KIR haplotypes and their Centromeric (Cen) and Telomeric (Tel) motifs have been shown to affect HCT outcome for unrelated donor transplantation in AML patients. Little is known regarding KIR haplotype matching. HLA and KIR segregate independently. Only 25% of sibling donors were expected to be KIR fully matched with the patient. Our pilot study included 108 AML patients transplanted in complete remission at City of Hope (2005-2009).

Table. Patient Demographic and Clinical Characteristics

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<th>N or median</th>
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<tbody>
<tr>
<td>Patient Female/Male ratio</td>
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<tr>
<td>Donor Type Bone Marrow/Stem Cell</td>
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<tr>
<td>1st/2nd/3rd remission</td>
</tr>
<tr>
<td>Survival / Death</td>
</tr>
<tr>
<td>Disease Progression / No</td>
</tr>
<tr>
<td>Relapse / No</td>
</tr>
<tr>
<td>Death without relapse</td>
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<tr>
<td>aGVHD / No</td>
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<td>cGVHD / No</td>
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The patient and HLA-fully matched sibling donor were typed for KIR genes and 3DL1 alleles (Ssp-Multiple). We sequenced 3DL1/3DS1 alleles and Ig domains for 3DL2 and 3DL3 genes. A KIR-fully matched pair is defined patient and donor share the same KIR genotype, have identical 3DL1/S1 alleles, and the same SNP for 3DL2 and 3DL3.

24 (22%) patients had KIR fully matched donors. Interestingly, if the patient and sibling had identical Bx KIR genotypes, then their 3DL1 alleles or 3DL2/3 SNP were also identical, with only few exceptions (8/28), which is not true for AA patient/donor combination (14/18 AA pairs). The data appears to confirm that KIR B haplotypes are less diverse in terms of KIR alleles than KIR A haplotypes. Therefore, if the patient and sibling are identical based on KIR Bx genotypes, most probably they are KIR fully matched (70% probability).

Patients with fully matched KIR donors have similar relapse and aGVHD rates however, disease free survival is slightly higher (RR = 0.553; 95% CI = 0.23-1.3; P = NS). Patients with KIR Bx donor genotype have a higher incidence of aGVHD (RR = 1.96; 95% CI = 1.01-3.73; p = 0.04), due to Tel-Bx motif (RR = 2.26; 95% CI = 1.2-3.5; p = 0.009). Higher donor B content is associated with increased aGVHD (RR = 1.94; 95% CI = 1.12-3.34; p = 0.018). Non of those parameters had any impact on rates of relapse, or disease-free survival.

In conclusion, KIR haplotype matching does not appear to affect related HCT outcome. However, KIR B haploype was associated with aGVHD. These results suggest that inclusion of ALL patients may reveal greater impact of the KIR genes on HCT.

1 National Marrow Donor Program, Minneapolis, MN; 2 Fred Hutchinson Cancer Research Center, Seattle, WA

GENETIC ANCESTRY: AN ALTERNATIVE TO SELF-IDENTIFIED RACE AND ETHNICITY FOR THE EVALUATION OF UNRELATED TRANSPLANTATION OUTCOMES

Madbouly, A.1, Gragert, L.1, Maldoki, M.1, Petersdorf, E.2, Maters, M.1
1 National Marrow Donor Program, Minneapolis, MN; 2 Fred Hutchinson Cancer Research Center, Seattle, WA

We are investigating genetic ancestry in transplant pairs as a means to understand differences in outcomes across ethnic groups and also as a potentially informative new parameter of matching.

Genetic ancestry of transplanted patient/donor combinations was analyzed using two types of ancestry informative polymorphisms (AIMs): HLA and SNPs. The dataset included 491 individuals (76 African-American (AFA), 48 Asian/Pacific Islanders (API), 243 Caucasian (CAU), 104 Hispanic (HIS) and 20 Native American (NAM) genotyped for HLA-A, B, C, DRB1 and DQB1 and 30 autosomal SNPs from a published (Nassir 2009) reference AIMs panel. 23 of the chosen SNPs had >40% allele frequency difference between AFA and CAU, 23 >40% difference between AFA and API, and 9 >40% difference between CAU and API. Out of the last 9 SNPs, only one had a difference >40% between CAU and South Asian (SAS) and three between East Asian (EAS) and SAS. Assignment of admixture proportions was determined using STRUCTURE v2.3.2. Preliminary results recaptured ancestry of self-identified AFA, EAS and CAU individuals, however distinguishing SAS from CAU individuals proved challenging due to sample size disparities and the lack of sufficiently differentiating SNPs. When the pilot cohort was augmented with EAS and SAS individuals from the published reference cohort (Nassir 2009) to balance sample proportions, SAS individuals remained as a separate cluster distinct from EAS and CAU.

We additionally used Bayesian inference to assign racial categories to the pilot cohort using HLA AIMs. The algorithm used reference haplotype frequencies and knowledge of population sizes within the Be The Match® Registry as priors to calculate the overall likelihood of a race assignment. Classifying race using HLA frequencies gave >80% recovery of self-identified race for AFA and API, with lower recovery for CAU, HIS, and NAM. HLA ancestry classification is used as a supplement to ancestry classification using SNP AIMs.

THE IMPACT OF SINGLE CLASS I HLA-ANTIGEN MISMATCH ON OUTCOME OF CHILDREN UNDERGOING HEMATOPOIETIC CELL TRANSPLANTATION FROM RELATED DONORS

Ayan, M., Al-Seraisyh, A., Siddiqui, K., Al-Jefri, A., Al-Abnami, A., Khatri, A., Shabeen, H., Markiz, S., El-Solh, H. King Faisal Specialist Hospital & Research Center, Riyadh, Saudi Arabia

Several studies have shown that single class I HLA-antigen mismatch adversely affect the outcome of hematopoietic cell transplantation (HCT) from unrelated donors. Few data are however available on the significance of such mismatch in related donor HCT, particularly in children. This study was designed to retrospectively compare the outcomes of children who underwent HCT from HLA class I single antigen mismatched related donors and children who underwent HCT from fully matched related donors.

Patients and Methods: 38 patients (pts), age =14 years, underwent HCT from HLA class I single antigen mismatched related donors from January 1993 to December 2010 at King Faisal Specialist Hospital & Research Center. Out of this group, 30 pts (the study group: MIS) were matched with 90 pts that underwent HCT from fully matched related donors (the control group: MRD) with respect to age, gender, donor gender, indication for HCT, donor relationship and source of stem cells.

Results: There were 18 males in MIS and 55 in MRD (P = 1.00). Median age at HCT was 2.1 years in MIS and 4.5 years in MRD (P = 0.4). The locus of mismatch was A in 22 pts and B in 8 pts. Median follow up time was 5.7 years (95% CI: 2.5-8.8) in MIS and 4.6 years (95% CI: 2.9-6.4) in MRD. Incidence of Acute graft vs. host disease (aGVHD) was 63% (19 pts) in MIS and 35% (32 pts) in MRD (P = 0.01), and the incidence of severe (grade III-IV) aGVHD was 33.3% (10 pts) and 9% (8 pts) in the 2 groups (P = 0.007). One hundred pts were evaluable for chronic graft vs.host disease (cGVHD), 23 in MIS and 77 in MRD. Incidence of cGVHD was 26.1% (6 pts) in MIS and 6.5% (5 pts) in MRD (P = 0.017). Among MIS, 13 % (3 pts) experienced extensive GvHD in contrast to 3.9% (3 pts) in MRD (P = 0.029). Incidence of Graft Failure (Primary/secondary) was not different in the 2 groups; 40% (12 pts) in MIS compared to 27.8% (25 pts) in MRD (P = 0.26). No significant difference was found between the overall survival (OS) of the pts from MIS (55%) and MRD (38%) (P = 0.072). However, pts in MRD had a significantly better Event Free Survival (EFS) of 32% compared to 21% in MIS (P = 0.027); events were defined as primary/secondary graft failure, relapse of primary disease, or death.

Conclusion: Our analysis demonstrates that in related donor HCT, a single antigen, HLA-class I mismatch is associated with a significantly higher risk of acute and chronic GVHD, and with a lower survival when compared with HCT from fully matched related donors.
Further work is currently ongoing to study the effect of sub-continen-
tal ancestry on HSCT outcomes for fully HLA matched patient/donor pairs. This work has the potential to demonstrate clinical
relevance of ancestry beyond the categories where it is typically self-identified.

**I87**

**SIBLING CORD AND BONE MARROW INFUSION TO CURE BETA THALAS-
SAEMIA MAJOR**

Raj, R., Munirathnam, D., Khandelwal, V., Sri, K., Kumar, M., Laksmanan, V. Apollo Specialty Hospital, Chennai, Tamilnadu, India

There are 10,000 new births of beta thalassemia major in India
each year. The burden of long term transfusion and chelation in
such children is huge and the majority of such patients in our country
face early death in their teenage due to cardiac or liver haemosidero-
sis. Private cord blood banks have been operational in India since
2002. We present a series of 8 children with thalassemia major who
have been cured of their disease by their sibling bone marrow.
All 8 children were transfusion dependent thalassemia major with age
range 2 years to 9 years. Three children were Lucarelli class I and
the other five were class III. Six children received thiotepa, clophos-
phamide and fludarabine conditioning and two had busulphan and cy-
clophosphamide. The cord was thawed and infused first followed
by bone marrow harvested from the sibling. The sibling donors
were between 7 months to 3 years of age. Bone marrow was used
in addition to cord for two reasons – cord nucleated cell count alone
was inadequate in all 8 cases and data was not yet available in our
country regarding post thaw nucleated count from private cord
banks. Total nucleated cell count from the cryopreserved cord ranged
from 0.5 x 10^6 / kg to 1 x 10^7 / kg in these eight children and
the CD 34 ranged from 0.18 x 10^4 / kg to 1.8 x 10^5 / kg. Bone
marrow harvest yielded 1 to 7 x 10^7 / kg CD 34 count after harvesting
less than 5 ml/kg marrow of the recipient body weight. There was on
average 38% cell loss after thawing from different private cord blood
banks in India. All patients engrafted between days 12 to day 17 with
persistent donor chimerism between 90 to 100% after more than
a year follow up. There was no graft versus host disease or cytomeg-
aloavirus reactivation in any of these children.

We conclude that the use of cord and bone marrow helps cure
thalassaemia with the benefit of durable engraftment without graft
versus host disease. Cord blood banking should be recommended
by all physicians managing children with thalassaemia. It is an ideal
source of stem cells for transplantation and should be used even if the
cell dose seems suboptimal and balanced with addition of cells from
bone marrow.

**IMMUNE RECONSTRUCTION**

**I88**

**TIM-3 IS AN INDOUCIBLE HUMAN NATURAL KILLER (NK) CELL RECEPTOR
THAT ENHANCES INTERFERON GAMMA PRODUCTION IN RESPONSE TO
GALECTIN-9 (GAL-9)**

Gleason, M.K.1, Lewick, T.R.1, McCullar, V.1, Felices, M.1, O’Brien, M.S.2, Cooley, S.A.1, Verneesi, M.R.1, Citluki, F.1, Holman, C.1, Panoskaltsis-Mortari, A.1, Niki, T.1, Hirahama, M.1, Blazar, B.R.1, Miller, J.S.2 1 University of Minnesota Masonic Cancer Center, Minneapolis, MN; 2 University of Minnesota, Minneapolis, MN; 3 Kagawa University, Kagawa, Japan

Natural killer cells are an attractive option for immunotherapy, as
the adoptive transfer of alloreactive NK cells has demonstrated anti-
leukemia effects in transplant and non-transplant settings. Natural
killer cell function is regulated by the integration of signals received
from activating and inhibitory receptors. Here we show that a novel
immune receptor, Tim-3, is expressed on resting human NK cells
and is upregulated upon activation. The NK92 NK cell line engi-
neered to overexpress Tim-3 showed a marked increase in IFN-γ
production in the presence of soluble rhGal-9 or Raji tumor cells en-
gineered to express Gal-9. The Tim-3+ population of low dose IL-
12/IL-18-activated primary NK cells significantly increased IFN-γ
production in response to soluble rhGal-9. Gal-9 presented by cell
lines, and primary acute myelogenous leukemia (AML) targets that
dedogenously express Gal-9. This effect is highly specific as Tim-3
crosslinking induced extracellular signal-regulated kinase activation,
degradation of the NF-κB inhibitor IκBα, and Tim-3 antibody
blockade significantly decreased IFN-γ production. Exposure to
Gal-9 expressing target cells had little effect on CD107a expression.
Reconstituted NK cells obtained from patients after hematopoietic
cell transplant had diminished expression of Tim-3 compared to
paired donors and correlated with a decrease in IFN-γ production,
a known defect seen early after transplant. Upon low dose IL-12/
IL-18 activation, Tim-3 expression was rescued in post-transplant
developing NK cells, which correlated with an increase in IFN-γ
production. Modeling human NK cell development in vitro, NK
cells derived from umbilical cord blood (UCB) CD14+ progenitor
cells cultured on the murine stromal cell line ELO8.1D displayed
lower levels of Tim-3 expression and IFN-γ production in the presen-
tce of rhGal-9 and low dose IL-12/IL-18 cytokine priming. Tim-3
overexpressing UCB-derived NK cells activated with low dose
IL-12/IL-18, however, significantly increased IFN-γ production in
response to rhGal-9. Application of the Tim-3 blocking antibody
abrogated this effect, supporting the premise that Tim-3 is develop-
mentially regulated in mice. In conclusion, we show that Tim-3 func-
tions as an activating co-receptor in human NK cells to enhance
IFN-γ production. Understanding Tim-3 mechanisms can lead to
strategies to enhance NK cell function after transplantation.

**I89**

**IMMUNE RECONSTITUTION IN CHILDREN RECEIVING CORD BLOOD
TRANSPLANTATION (CBT) WITHOUT THE USE OF LYMPHOCYTOTOXIC
ANTIBODIES**

Martinez, C.A.1, Chan, S.K.2, Shearer, W.T.1, Leung, K.S.1, Kennedy-
Nasser, A.A.2, Bollard, C.M.1, Liu, H.1, Wu, M.-P.1, Brenner, M.K.1,2,
Hessel, H.E.1, Hanson, C.F.2, Kerence, R.A.2 1 Baylor College of Medicine,
Texas Children’s Hospital, Houston, TX; 2 Baylor College of Medicine,
Texas Children’s Hospital, Houston, TX, 3 Baylor College of Medicine,
Houston, TX

Immune reconstitution following CBT can be prolonged and
heighten the risk for infection. In this prospective study we substituted
fludarabine for a lymphocytotoxic antibody such as ATG or Campath
with the intent of accelerating immune reconstitution and reducing
the incidence of viral infections. Fifteen children (median age 12
months, range, 1 mo – 8 yr) with; Primary Immune Deficiency (n
= 5), HLH (n = 1), alpha mannosidosis (n = 1), Fanconi Anemia (n
= 1), Dyskeratosis Congenita (n = 1), SAA (n = 1) and acute leukemia
(n = 5) were treated. Four patients were HLA fully matched and 11
were one HLA antigen mismatched. The median total nucleated
cell dose was 10.2x10^6 / kg (range, 5.1 x 10^6 – 23.5 x 10^6). Ten patients
received myeloablative conditioning consisting of busulfan, cyclo-
phosphamide and fludarabine for their non-malignant conditions
while 5 patients with acute leukemia received cyclophosphamide,
fludarabine and total body irradiation. Patients with chromosomal
breakage/instability syndromes received reduced intensity condition-
ing without serotherapy. The median time to neutrophil and platelet
engraftment was 25d (range, 15d-35d) and 48d (range, 30d-60d), re-
spectively. All patients achieved 100% donor chimerism by day 42.
Median absolute number of CD3+ cells was 394/ul at day 42 (range,
79-918) and 638/ul at day 100 (range, 187-1632). Median absolute
number of CD14+ and CD8+ cells was 330/ul (range, 56-826) and
65/ul x 10^3 (range, 13-86) by day 42, respectively. The median absolute
B cell number was 1098/ul (range, 36-1202) by 6 months. In vitro lym-
phocyte proliferative responses to phytohemagglutinin were
$\leq 75,000$ cpm $^{3}$H-thymidine incorporation in 8/15 patients by day
42 and in all the patients by day 60. Stimulation index in response to
specific antigens (candida/tetanus) was $\geq 2$ by 6 months post UCBT.
One patient had EBV reactivation which resolved spontaneously
and one patient had CMV reactivation responsive to treatment with
donor derived CTL infusion. No patient developed viral disease
and aGVHD grade II-IV was seen in three patients. No cGVHD has
occurred. The 1-year overall survival is 72% (95% CI (41-89%)) with
a median follow up of 385 days (4d-798d). Hence omitting lymphocy-
totoxic antibodies from conditioning regimens for CBT may be asso-
ciated with earlier phenotypic and functional engraftment and
immune-reconstitution in the absence of increased risk for significant
GVHD, leading to a high overall survival of pediatric patients after
cord blood transplantation.