The role of PANE1 as a model for minor histocompatibility restricted antigen in hematopoietic stem cell transplantation for leukemic patients

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Abstract

Background:
In hematopoietic stem cell transplantation (HSCT), minor histocompatibility antigens (MiHA) can be used as tools for immunotherapy especially if they are restricted to hematopoietic cells like PANE1 MiHA.

Purpose of study:
We aimed at studying PANE1 as MiHA model in human leucocyte antigen (HLA) matched related Egyptian HSCT patients regarding its frequency and the effect of mismatching in PANE1 on HSCT outcomes.

Methods:
Ninety-six patient/donor pairs were studied for the prevalence of disparities in PANE1 MiHA and its effect on graft versus host disease (GvHD) and graft versus leukemia (GvL). A sequence- specific primer (SSP) approach was used to determine the immunogenic ($PANE^R$) and non-immunogenic (PANES) alleles of PANE1 gene. Student t-test, Mann-Whitney U test, ANOVA F-test, and Kruskal-Wallis H tests were used to determine the significance of the difference for quantitative data. Relations of qualitative data were determined using Chi-square test. Survival analysis was done using Kaplan-Meier method to determine overall survival (OS).

Results:
High prevalence $PANE^R$ allele of PANE1 was observed in both patients and donors (192/192,100% of 96 patient/donor pairs). Mismatches in PANE1 gene were observed in 9 patient /donor pairs. Nine cases showed relapse post-transplantation and there was no association between PANE1 mismatch and relapse (p=0.8).

Conclusion:
No association between mismatches in PANE1 gene and any transplant - related outcome was observed. Which might decrease its use as a target for immunotherapy in Egyptian leukemia patients.

Key words: Stem cell transplantation; Leukemia; Minor Histocompatibility antigens; PANE1

Introduction:

Hematopoietic stem cell transplantation (HSCT) is a curative procedure for different malignant and non-malignant conditions, with much of the efficiency of HSCT in malignant diseases is restricted by the immunologic reactions of donor cells towards the malignant host cells making GvL a beneficial outcome following HSCT **[1].**

The first suggestion of a graft versus leukemia (GvL) outcome was in 1956 when Barnes et al. used a mouse transplantation model, where rejected leukemia cells seemed to be removed by the incoming bone marrow when irradiation was postponed .This made the conception that the donor marrow cells may allow leukemia eradication **[2].**

Following human leucocyte antigen (HLA) matched allogenic HSCT, certain polymorphic peptides displayed on patient cells by shared HLA are distinguished as “non-self” by donor T-cells. These polymorphic peptides known to be minor histocompatibility antigens (MiHA) are encoded by the male-specific Y-chromosome (H-Y antigens) or other

chromosomes (autosomal MiHA) and are due to genetic disparities between patient and donor **[3].** MiHAs are small peptides which are present in association with class I or class II major histocompatibility antigens (MHC) molecules on the cell surface**[4].** These peptides are normally about 9-12 amino acids in length **[5].** About a third of the characterized MiHAs arise from the Y chromosome **[6].**

Up to date, over 60 MiHAs have been discovered **[7]**. Genes on autosomal chromosomes encode 54 of these MiHAs. The MHC/minor H peptide complexes can serve as transplantation barriers in allogeneic-HLA-matched HSCT and in solid organ transplantation **[8].**

Minor histocompatibility antigens (MiHAs) are causal agents of graft rejection and GvHD in HLA-identical sibling transplants. They may be effective in GvL, if these antigens expression is on hematopoietic cells **[9].**

The potential for MiHAs to be targets for a GvL response after allogeneic stem-cell transplantation was shown in mouse models. In these mice, the adoptive transfer of CD8+ CTLs that were specific for a single recipient MiHA eradicated leukemia **[10].** CD8+MiHA-specific T-cell clones lyse primary acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) cells, inhibit the growth of leukemic colonies, and prevent the engraftment of AML **[11].**

All MiHAs may be possibly utilized in immunotherapy owing to their restricted distribution. The other type of MiHAs' tissue distribution is their presence on neoplastic epithelial cells, for example, HA-1 and Acetyl –CoA carboxylase (ACC-1/ACC-2), though in normal circumstances they show restriction to hematopoietic cells only and are not existing on epithelial cells **[12].**

Centromere protein M (CENPM), also known as proliferation associated nuclear element1 (PANE1) is a protein that is encoded by the CENPM gene in humans **[13].** The PANE1 gene is encoded by a new HLA-A*0301-restricted MiHA that is expressed mainly in B-lymphoid cells. Sequencing of PANE1 alleles in MiHA-positive and MiHA-negative cells shows that differential T-cell recognition of donor and recipient cells owes to a nonsynonymous single nucleotide polymorphism (SNP) within the variant exon that substitutes an arginine codon with a translation termination codon **[14].**

The PANE1 transcript through which the MiHA is encoded shows expression at high levels in resting CD19+ B cells and B-chronic lymphocytic leukemia (CLL) cells, and at lower levels in activated B cells. Therefore indicating that PANE1 may be a promising therapeutic target in B-CLL **[14].** New studies are now done based on using certain proteins such as PANE 1 gene to be used to identify GVL without GVHD after allogeneic donor lymphocyte infusion (DLI) through a proteomic signature**[15].**

There are very few studies concerning MiHA role in modifying HSCT outcome **[16],** and no study was done on Egyptian transplant patients. Given that the distribution of MiHA is various among different populations and ethnic groups, studying PANE1 as MiHA model in HLA matched related Egyptian HSCT patients would pave the way to the effect of MiHA on transplant outcomes and their distribution in Egyptian patients/donors pairs.

The aim of this study was to identify the prevalence of PANE1 alleles among Egyptian patient/donors pairs undergoing HSCT especially for hematologic malignancies to uncover the effect of mismatching in minor histocompatibility antigens that are expressed on hematopoietic cells on transplantation outcome mainly the useful graft versus leukemia and thus the use of PANE1 as a target for immunotherapy among mismatched pairs.

Patients and Methods:

This prospective study was conducted on 96 patients who received peripheral blood stem cell transplantation at Nasser Institute, Ministry of Health (MOH), Cairo, Egypt in the period between 2010 to 2019. The study was performed according to Helsinki declaration for studies performed on human. It was approved by the Institutional Review Board of Nasser Institute and a written informed consent was obtained from patients and their donors.

Patients included 64 males and 32 females with an age range of (3-60), median 31 years. Patient's characteristics are summarized in table (1).

All patients received stem cells from HLA matched siblings, and in all patients; the stem cell source was granulocyte-colony stimulating factor (G-CSF)-mobilized peripheral blood. Gender mismatch as a female donor to a male patient in 28 (29%) of cases. HLA typing of patients and donors were extracted from patient's files.

Time to neutrophil engraftment: defined as the 1st of 3 consecutive days with neutrophil count $\geq$ 0.5 x 109/L. Similarly, time to platelet engraftment: defined as the first of 3 consecutive days with a count of $\geq$ 20 x 109/L without any platelet transfusion. All HSCT showed successful engraftment.

Overall survival (OS) was defined as the time from stem cell infusion to death from any cause.

Genotyping of PANE1:

Genomic deoxyribonucleic acid (DNA) was isolated from peripheral blood leukocytes using the spin column extraction method (Qiagen, Germany). MiHA (PANE1) genotyping for both immunogenic ($PANE^R$) and non-immunogenic (PANES) alleles for both 96 patients and their HLA matched sibling donors, was determined by using polymerase chain reaction – sequence - specific primer (PCR-SSP) [17] shortly 1 unit Amplitaq® DNA polymerase, 1x Type I buffer and 2ul of 10 nM deoxyribonucleotide triphosphate (dNTPS) (Thermo Fisher Scientific, MA USA) and 1X My Taq red master mix (Bioline Reagents Limited, London, UK). Alleles assignments were determined according to visualized bands after electrophoresis migration on 2% agarose gel.

Statistical Analysis:

Data were analyzed by SPSS version 21 (IBM Inc., USA). Quantitative data were summarized as mean ± standard deviations (SD) if it is normally distributed

and as median (range) if it is not. Qualitative data were described as frequencies and percentages.

Student t-test, Mann-Whitney U test, ANOVA F-test, and Kruskal-Wallis H tests were used to determine the significance of difference for quantitative data. Relations of qualitative data were determined using Chi-square test. Survival analysis was done using Kaplan-Meier method to determine OS. Log- rank (Mantel-Cox) test was used to examine the difference between survivals of different groups. P- value ≤ 0.05 was considered significant.

Results:

Out of the 96 patient/donor pairs, only 9 showed mismatches in Pane1 gene where all patients and donors had at least one PANE1^{R} allele with 3 pairs showing one PANE1 stop codon allele in the donor only while homozygous PANE^{R} allele in patient and 6 pairs showing one PANE1stop codon allele in patient while donor is homozygous for PANE^{R}, only four cases out of the nine showed gender mismatch (A female donor to a male recipient). The rest 87 pairs were homozygous for PANE1^{R} with 24 cases showing gender mismatch (A female donor to a male recipient). Thus, the immunogenic PANE1 phenotype was 100% present among Egyptian patient/donor pairs with no subjects homozygous for the non-immunogenic PANE1 phenotype.

Effect of PANE1 mismatch on GvHD:

Only 13 patients showed acute GvHD (grade II-IV) with 10 patients showing gastrointestinal tract (GIT) GvHD, six with liver GvHD, five with skin GvHD. While five patients had both liver and GIT GvHD, three patients showed skin and GIT GvHD and only one patient showed skin and liver GvHD.

Acute GvHD grade 0-I was found in 83 (86%) of patients while acute GvHD grade II-IV was found in only 13 (14%) of patients.

All nine PANE1 mismatched cases showed no signs of acute GvHD however there was no association between mismatch in PANE1 towards GvHD direction and acute GvHD (p=0.2).

Chronic GvHD was observed in 7 (8%) patients and mismatched PANE1 cases were not associated with occurrence of chronic GvHD (p=0.99).

Effect of PANE1 mismatch on survival:

Median survival of patients was 60 months (53-68); Median OS of mismatched PANE1 cases was 65 (44-85) months while median OS of matched PANE1 was 58 (50-66) months. There was no association between PANE1 mismatch on either overall survival of patients (p=0.75) (Figure 1) or disease-free survival (DFS) (p=0.9) (Figure 2).

Effect of PANE1 mismatch on relapse:

Nine cases showed relapse post-transplantation and there was no association between PANE1 mismatch and relapse (p=0.8).

Table (1): Characteristics of (96) patients undergoing HSCT from HLA-matched sibling donor and the respective PANE1 genotypes of patients and donors

Total number= 96	
Gender (n %)	
Female	32 (33.3%)
Male	64 (66.7%)
Age (years)	
Mean ± SD	31.1±12.9
Diagnosis	
AML	59 (62%)
ALL	16 (16.7%)
CML	14 (14.3%)
MDS	4 (4%)
MPAL	3 (3%)
Conditioning regimen:	
BU/CY	45 (47%)
FLU/Mel	6 (6%)
FLU/BU	9 (9%)
FLU/BU/post Cy	18 (19%)
TBI/CY	18 (19%)
Acute GVHD:	
Grade 0-1	83 (86%)
Grade 2-4	13 (14%)
Chronic GVHD:	
No	89 (92%)
Yes	7 (8%)
PANE1 status* (Patient/Donor):	
RR/RR	87 (90%)
RS/RR	6 (6.6%)
RR/RS	3 (3.4%)

AML: *Acute myeloid leukemia;* ***ALL:*** *Acute lymphoblastic leukemia;* ***CML:*** *Chronic myeloid leukemia;* ***MDS:*** *Myelodysplastic syndromes;* ***MPAL:*** *Mixed phenotype acute leukemia;* ***BU:*** *Busulfan;* ***CY:*** *Cyclophosphamide;* ***FLU:*** *Fludarabine;* ***Mel:*** *Melphalan;* ***TBI:*** *Total body irradiation;* ***GVHD:*** *Graft-versus-host disease;* ***PANE1:*** *Proliferation associated nuclear element 1*

*PANE^{R} is represented as R and PANE1 stop codon is represented as S

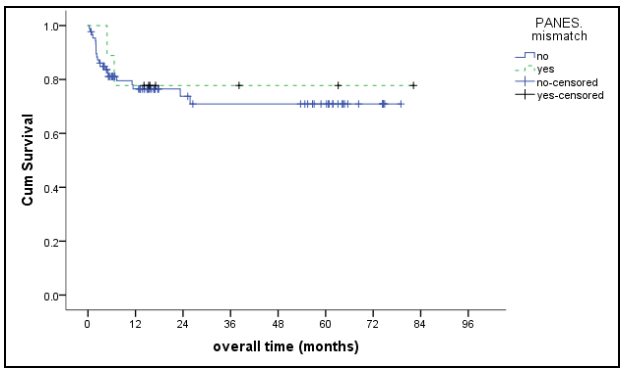

Figure (1): Effect of mismatches in PANE1 gene on OS of 96 patients undergoing matched related stem cell transplantation

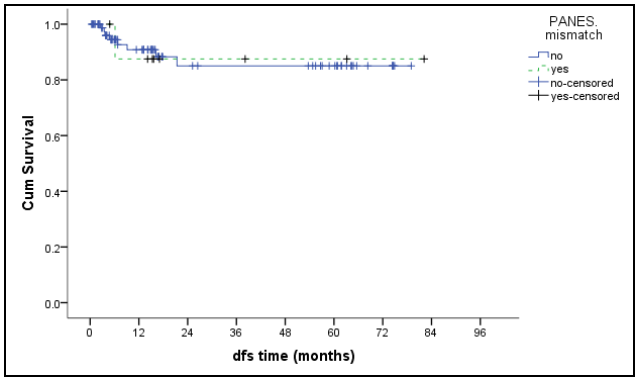

Figure (2): Effect of mismatches in PANE1 gene on disease - free survival of 96 patients undergoing matched related stem cell transplantation

Discussion:

Minor histocompatibility antigens (MiHAs) belong to genetic factors which may vary between the donor and the recipient despite matching in HLA loci and thus they may influence allo-HSCT results [17].

Even though the human MHC system has been described in details over the years, the repertoire and characteristics of MiHAs have continued to be largely undiscovered [18]. Several human MiHAs, as PANE1, show expression by hematopoietic cells only and are being considered as possible targets for a GvL outcome. The ultimate goal of known hematopoiesis-specific MiHA immunotherapy is to enhance the GvL effect while minimizing unfavorable GvHD. So, MiHA encoded by hematopoiesis-specific genes such as PANE1 is a good candidate for immunotherapy for some of hematological malignancies, depending on their preferential expression in hematopoietic cells [19].

Very few studies tried to emphasis the role of PANE1 in stem cell transplantation outcome either from HLA matched related and unrelated donors [12,20,21].

The probability of the interaction of MiHA with respective HLA restricted molecule differs depending on the population frequency of both MiHA alleles and the HLA molecule restricting the immunogenic peptide [21]. Thus, population-based assessment of MiHA is advised to explore the allele frequency of MiHA to assess their further use in immunotherapy on a population-based evidence.

The prevalence of the immunogenic $PANE^R$ allele was 100% present among Egyptian patient/donor pairs in our study. Other studies observed similar high prevalence by a range of 90-100% of $PANE^R$ allele with the exception of only the Mulatto population which showed 77.3% prevalence [22], however a Korean group also reported lower frequency of $PANE^R$ allele of 51.2% in a cohort of 329 healthy unrelated subjects[23].

In our current study the PANE1 gene has been explored in 96 HLA matched related patients/donors pairs where the most frequent genotype was the homozygous (RR) about 87% followed by the heterozygous (RS) about 9% while the non-immunogenic homozygous (SS) was not found at all in our study which led to a relatively low probability of their immunogenic disparity in donor/recipient pairs. The same results were found in studies made by Markiewicz et al, as the distribution of PANE1 gene was not uniform and the most frequent were the RR and RS genotype [19]. Then again Dzierzak-Mietla et al found there was a uniform distribution of homozygous RR and heterozygous RS genotypes but still a low frequency of the non-immunogenic homozygous genotype SS [12]. A study made on a Korean group revealed relatively higher frequency of the non-immunogenic homozygous genotype (SS) 25%, yet it was performed on healthy unrelated subjects [23].

PANE1 gene mismatch did not influence the presence, duration or clinical stage of acute GvHD or chronic GvHD. The same results were found in different studies [12,20,21].

The role of mismatch in PANE1 gene on patients' survival in our study is very restricted. The same results were observed in different studies [[12,20,21]].

There was no association between PANE1 mismatch and relapse in our study. The same results were found in different studies [12,20,21].

In our cohort, PANE1 had little effect on transplant outcome, together with high prevalence of $PANE^R$ allele in both patients and donors, which may decrease its use as a target for immunotherapy in Egyptian leukemia patients.

Conclusion:

No association between mismatches in PANE1 gene and any transplant - related outcome was observed. Which might decrease its use as a target for immunotherapy in Egyptian leukemia patients.

Minor histocompatibility antigen (MiHA) phenotype frequencies is variable among populations and this exerts extra difficulty on the possible effect of MiHA use in immunotherapy and every population should address the phenotype of various hematopoietic expressed MiHA to identify future candidate genes with high immunogenic disparity rate between patient/donors pairs.

Further studies should be done to elaborate more on the use of other restricted MiHA especially that their distribution among populations is variable especially those used for future use in immunotherapy.

List of abbreviations:

ACC	Acetyl –CoA carboxylase
ALL	Acute lymphoblastic leukemia
AML	Acute myeloid leukemia
BU	Busulfan
CENPM	Centromere protein M
CLL	Chronic lymphocytic leukemia
CML	Chronic myeloid leukemia
CY	Cyclophosphamide
DFS	Disease free survival
DNA	Deoxyribonucleic acid
dNTPS	deoxyribonucleotide triphosphate
FLU	Fludarabine
G-CSF	Granulocyte-colony stimulating factor
GIT	Gastrointestinal tract

GvHD	Graft versus host disease
GvL	Graft versus leukemia
HLA	Human leucocyte antigen
HSCT	Hematopoietic stem cell transplantation
MDS	Myelodysplastic syndromes
Mel	Melphalan
MHC	Major histocompatibility antigens
MiHA	Minor histocompatibility antigens
MOH	Ministry of Health
MPAL	Mixed phenotype acute leukemia
OS	Overall survival
PANE 1	Proliferation associated nuclear element 1
PCR	Polymerase chain reaction
SNP	Single nucleotide polymorphism
SSP	Sequence- specific primer
TBI	Total body irradiation

Conflict of interests:

The authors declare no conflict of interest.

References:

[1] Gyurkocza B, Rezvani A, Storb RF. Allogeneic hematopoietic cell transplantation: the state of the art. Expert Rev. Hematol. 2010;3:285–299.

[2] Barnes DWH, Corp MJ, Loutit JF, et al. Treatment of Murine Leukaemia with X Rays and Homologous Bone Marrow. Br. Med. J. 1956;2:626–627.

[3] Zilberberg J, Feinman R, Korngold R. Strategies for the Identification of T Cell–Recognized Tumor Antigens in Hematological Malignancies for Improved Graft-versus-Tumor Responses after Allogeneic Blood and Marrow Transplantation. Biol. Blood Marrow Transplant. J. Am. Soc. Blood Marrow Transplant. 2015;21:1000–1007.

[4] Roy DC, Perreault C. Major vs minor histocompatibility antigens. Blood. 2017;129:664–666.

[5] Spierings E, Wieles B, Goulmy E. Minor histocompatibility antigens--big in tumour therapy. Trends Immunol. 2004;25:56–60.

[6] Hirayama M, Komada EA and Y. Major and Minor Histocompatibility Antigens to Non-Inherited Maternal Antigens (NIMA). Histocompatibility [Internet]. 2012 [cited 2018 Oct 5]; Available from: https://www.intechopen.com/books/histocompatibility/major-and-minor-histocompatibility-antigens-to-non-inherited-maternal-antigens.

[7] Romaniuk, Dmitrii S., Anna M. Postovskaya, Alexandra A. Khmelevskaya, Dmitry B. Malko, and Grigory A. Efimov. "Rapid Multiplex Genotyping of 20 HLA-A*02:01 Restricted Minor Histocompatibility Antigens." Frontiers in Immunology 2019 10:1226. doi: 10.3389

[8] Spierings E. Molecular Typing Methods for Minor Histocompatibility Antigens. Bone Marrow Stem Cell Transplant. [Internet]. Humana Press, New York, NY; 2014 [cited 2018 Apr 11]. p. 115–138. Available from: https://link.springer.com/protocol/10.1007/978-1-4614-9437-9_8.

[9] Dickinson AM, Norden J, Li S, et al. Graft-versus-Leukemia Effect Following Hematopoietic Stem Cell Transplantation for Leukemia. Front. Immunol. [Internet]. 2017 [cited 2018 Mar 17];8. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5461268/.

[10] Fontaine P, Roy-Proulx G, Knafo L, et al. Adoptive transfer of minor histocompatibility antigen-specific T lymphocytes eradicates leukemia cells without causing graft-versus-host disease. Nat. Med. 2001;7:789–794.

[11] Bonnet D, Warren EH, Greenberg PD, et al. CD8 (+) minor histocompatibility antigen-specific cytotoxic T lymphocyte clones eliminate human acute myeloid leukemia stem cells. Proc. Natl. Acad. Sci. U. S. A. 1999;96:8639–8644.

[12] Dzierzak-Mietla M, Markiewicz M, Siekiera U, et al. Occurrence and Impact of Minor Histocompatibility Antigens' Disparities on Outcomes of Hematopoietic Stem Cell Transplantation from HLA-Matched Sibling Donors [Internet]. Bone Marrow Res. 2012 [cited 2018 Mar 23]. Available from: https://www.hindawi.com/journals/bmr/2012/257086/.

[13] Foltz DR, Jansen LET, Black BE, et al. The human CENP-A centromeric nucleosome-associated complex. Nat. Cell Biol. 2006;8:458.

[14] Brickner AG, Evans AM, Mito JK, et al. The PANE1 gene encodes a novel human minor histocompatibility antigen that is selectively expressed in B-lymphoid cells and B-CLL. Blood. 2006; 107:3779–3786.

[15] Liu, Xiaowen, Zongliang Yue, Yimou Cao, Lauren Taylor, Qing Zhang, Sung W. Choi, Samir Hanash, et al. "Graft-Versus-Host Disease–Free Antitumoral Signature After Allogeneic Donor Lymphocyte Injection Identified by Proteomics and Systems Biology." JCO Precision Oncology 3 2019; 3: doi: 10.1200.

[16] Spierings E. Minor histocompatibility antigens: past, present, and future. Tissue Antigens. 2014;84:374–360.

[17] Spierings E, Drabbels J, Hendriks M, et al. A Uniform Genomic Minor Histocompatibility Antigen Typing Methodology and Database Designed to Facilitate Clinical Applications. PLOS ONE. 2006;1:e42.

[18] Barrett AJ, Melenhorst JJ. Minor histocompatibility antigen discovery: turning up the HEATR. Blood. 2010;115:4630–4631.

[19] Akatsuka Y, Morishima Y, Kuzushima K, et al. Minor histocompatibility antigens as targets for immunotherapy using allogeneic immune reactions. Cancer Sci. 2007;98:1139–1146.

[20] Spierings E, Kim Y-H, Hendriks M, et al. Multicenter analyses demonstrate significant clinical effects of minor histocompatibility antigens on GvHD and GvL after HLA-matched related and unrelated hematopoietic stem cell

transplantation. Biol. Blood Marrow Transplant. 2013;19:1244–1253.

[21] Markiewicz M, Siekiera U, Karolczyk A, et al. Immunogenic disparities of 11 minor histocompatibility antigens (mHAs) in HLA-matched unrelated allogeneic hematopoietic SCT. Bone Marrow Transplant. 2009;43:293–300.

[22] Linscheid C, Petroff MG. Minor Histocompatibility Antigens and the Maternal Immune Response to the Fetus During Pregnancy. Am. J. Reprod. Immunol. N. Y. N 1989. 2013;69:304–314.

[23] Spierings E, Hendriks M, Absi L, et al. Phenotype Frequencies of Autosomal Minor Histocompatibility Antigens Display Significant Differences among Populations. PLOS Genet. 2007;3:e103.

[24] Jung H, Ki CS, Kim JW, et al. Frequencies of 10 autosomal minor histocompatibility antigens in Korean population and estimated disparities in unrelated hematopoietic stem cell transplantation. Tissue Antigens. 2012;79:42–49.