Killer Immunoglobulin-like Receptors (KIR) in Low-Risk Myelodysplastic Syndrome: Genotyping and Gene Expression Evaluation

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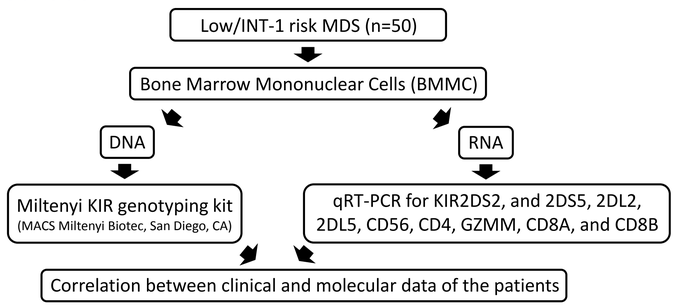
Background:

Myelodysplastic syndromes (MDS) are a group of myeloid malignancies arise from hematopoietic stem and/or progenitor cell. MDS is characterized by ineffective hematopoiesis, cytopenias in peripheral blood, and a higher risk of transformation into secondary acute myeloid leukemia. Natural killer (NK) cells mediate a key role in the immune surveillance, an important mechanism of cancer control, and they appear activated with high expression of perforin and granzyme in lower risk MDS (Chamuleau et al. Haematologica [2009]). Their activity is modulated by killer immunoglobulin-like receptors (KIR). Prior experience suggests that Tipifarnib, a farnesyl transferase inhibitor, may suppress abnormal NK cell function and autoimmunity that contributes to MDS. It is hypothesized that Tipifarnib could be especially of benefit in lower risk MDS patients where autoimmunity is known to play a role and immunosuppression is of clinical benefit for in some cases. We hypothesized that KIR molecular profile could be correlated with response to Tipifarnib and molecular characteristics of patients with MDS. The primary objective of this study was to define KIR genotype and expression of KIR2DS2, KIR2DS5, KIR2DL2 and KIR2DL5 in the bone marrow of lower risk MDS patients, and to explore potential interactions between KIR expression, patient demographics, mutational status and patient outcomes.

Methods:



Results

Performance specifications (precision, limit of detection, linearity, and reportable range) were determined for all assays used in this study. KIR typing was performed by conventional PCR using commercial Miltenyi KIR genotyping kit for all known 15 human KIR genes, while gene expression was accessed by qRT-PCR for two activating (2DS2, and 2DS5), two inhibitory (2DL2, and 2DL5) KIR genes and other leukocyte markers (Fig. 1).

Results (continued):

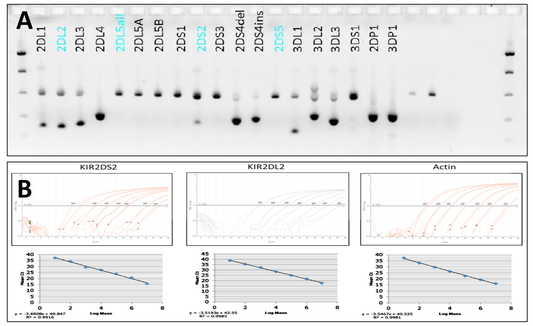


Figure 1. (A) representative DNA agarose gel showing internal control (~400 bp) and KIR bands (smaller bands). (B) A representative plot showing linearity and reportable range generated using plasmids with copy number 10 to 100,000 (log mass 1 to 6) for absolute quantification.

KIR2DL4, 3DL2, 3DL3, 2DP1 and 3DP1 were presented in all patients, whereas 2DS3 [7/55; 13%], 2DS5 [16/55; 29%], and 2DL5 [19/55; 35%] were less frequent (Figure 2). These results corroborate the Allele Frequency in Worldwide Populations Net Database http://www.allelefrequencies.net/default.asp) showing similar distribution in the normal American population (Fig. 2).

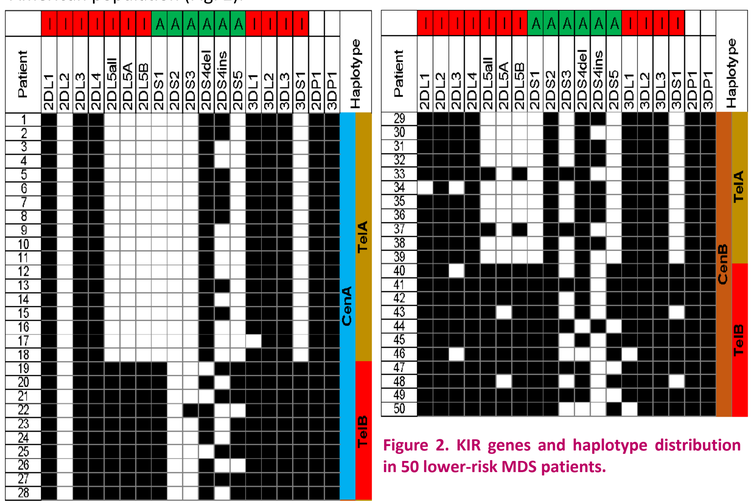


Figure 2. KIR genes and haplotype distribution in 50 lower-risk MDS patients.

Results (continued):

Correlation between KIR2DS2/2DL2/2DS5/2DL5 gene expression (copy number) and MDS subtype, BM cellularity, WBC, RBC, Hg, and platelets was not observed. Survival analysis showed poor survival for the patients carrying KIR2DL5, 2DS1, and 3DS1 genes [p<0.05] (Fig. 3A, B & C). In addition, survival was different between the four haplotypes groups CenA/TelA, CenA/TelB, CenB/TelA, and CenB/TelB (p=0.06; Fig. 3D) but a subset analysis of CenA-TelA and CenB-TelB haplotypes showed a significantly different survival between two groups (p= 0.01; Fig 3E). There was no correlation between gene expression data and survival.

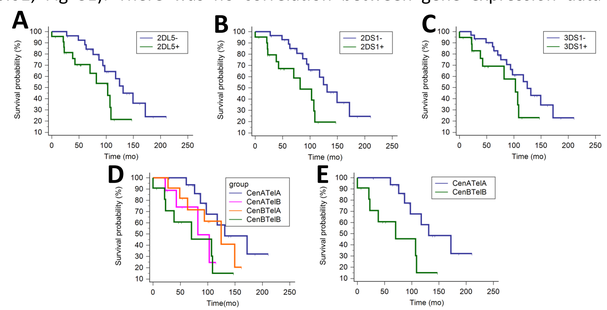


Figure 3. Kaplan-Meier overall survival analysis of KIR2DL5 (A), KIR2DS1 (B), KIR3DS1 (C), four haplotype groups CenA/TelA, CenA/TelB, CenB/TelA, and CenB/TelB (D), and two haplotype groups CenA/TelA and CenB/TelB (E).

Conclusions:

We report on the methodology and its use to define a reportable range of KIR2DS2, KIR2DS5, KIR2DL2 and KIR2DL5 RNA expression and their ratios by RT-PCR in 50 lower risk MDS patients. Our findings suggest that KIR genes might be associated with some clinical features in patients, mainly, survival. KIR expression will be correlated with clinical response to Tipifarnib as part of an ongoing clinical trial.

Conflict of Interest Disclosure:

Coutinho: Kura Oncology: Consultancy. **Ali**: Onconova Therapeutics: Consultancy; Kura Oncology: Consultancy. **Gualberto**: Kura Oncology: Employment, Equity Ownership, Other: Chief Medical Officer, Patents & Royalties. **Scholz**: Kura Oncology: Employment, Equity Ownership, Patents & Royalties. **Bracken**: Kura Oncology: Employment. **Jurcic**: Forma Therapeutics: Research Funding; Celgene: Research Funding; Alexion Pharmaceuticals: Consultancy; Genentech: Research Funding; Incyte: Consultancy; Actinium Pharmaceuticals, Inc.: Membership on an entity's Board of Directors or advisory committees, Research Funding; Astellas Pharma, Inc: Research Funding; Daiichi-Sankyo: Research Funding; Kura Oncology: Research Funding; Merck: Consultancy; Novartis: Membership on an entity's Board of Directors or advisory committees; Seattle Genetics: Consultancy, Research Funding; Syros Pharmaceuticals: Research Funding; Amgen: Consultancy. **Raza**: Novartis: Speakers Bureau; Genoptix: Speakers Bureau; Celgene Inc.: Research Funding; Kura Oncology: Research Funding; Janssen R&D: Research Funding; Syros Pharmaceuticals: Research Funding; Onconova Therapeutics: Research Funding, Speakers Bureau.

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