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 experiences suggests that Tipifarnib, a farnesyl transerase 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 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 KIR2DL2, KIR2DS5, KIR2DL2 and KIR2DS5 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:

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 Mibiteny 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

Correlation between clinical and molecular data of the patients

KIR2DL4, 3DL2, 3DL3, 2DP1 and 3DP1 were presented in all patients, whereas 2DS5 (7/55; 13%), 2DS5 (16/55; 29%), and 2DL5 (9/55, 16%) were less frequent (Figure 2). These results corroborate the Allele Frequency in Worldwide Populations Net Database showing similar distribution in the normal American population (Fig 2).

Correlation between clinical and molecular data of the patients

Conflict of Interest Disclosure:

Results (continued):

Correlation between KIR2DS2/2DL5/2DS5/2DS1 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 KIR2DS1, 2DS5, and 2DS1 genes [p<0.05] (Fig. 3A, B & C). In addition, survival was different between the four haplotypes groups Cenn/TaL, Cenn/TaB, CenB/TaL, and CenB/TaB [p<0.06; Fig. 3D] but a subset analysis of CenA/TaL and CenB/TaB haplotypes showed a significantly different survival between two groups [p<0.01; Fig 3E]. There was no correlation between gene expression data and survival.

Conclusions:
We report on the methodology and its use to define a reportable range of KIR2DS2, KIR2DS5, KIR2DL2 and KIR2DS5 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.

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

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.