# QUALITY AND QUANTITY OF NK CELL LIGAND EXPRESSION ON TUMOR CELLS IMPACT OVERALL SURVIVAL OF CHILDHOOD ACUTE B LYMPHOBLASTIC LEUKEMIA

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## Introduction

Consistent with the "missing self" hypothesis, loss of expression of class I human histocompatibility leukocyte antigen (HLA-I) renders targets more susceptible to NK cell-mediated killing due to the loss of inhibitory signals via self-HLA-recognizing receptors. NK cell activity is regulated by the balance of interactions between several families of activating (aRec) and inhibitory (iRec) NK cell receptors and their cognate ligands on target cells. KIR molecules are a family of receptors expressed on the surface of NK cell that play an important role in tumor immunesurveillance. Importantly quality and quantity of their ligands on the tumor cells can effectively modulate NK cell response.

Four major inhibitory KIRs, KIR2DL2/3, KIR2DL1, KIR3DL1, and KIR3DL2 recognize HLA-C allotypes with asparagine at position 80 (C1), HLA-C allotypes with lysine at position 80 (C2), HLA-A and HLA-B allotypes with Bw4 motifs at positions 77-83, and HLA-A3/11, respectively. And two major activating KIRs, KIR2DS2 and KIR2DS1 recognize HLA-C allotypes C1 and HLA-C C2, respectively.

## Objective

Evaluate the role of tumor KIR-ligand/NK-cell interactions on childhood B-acute lymphoblastic leukemia (B-ALL) three-year overall survival (3yOS).

## Patients and methods

A total of thirty-five patients with B-ALL (23 males and 12 females; aged 10m-14y) were enrolled into the study, and fifty-three sex and age matched unrelated healthy individuals (32 males and 21 females; aged 13m-14y) were enrolled as controls (Table 1). All patients and controls gave written informed consent prior to enrolment.

HLA and KIR typing of patient and control was performed using the commercial typing kit from Luminex. Expression level of HLA-I and HLA-C ligands by flow cytometry were evaluated at diagnosis of patients using FACSCanto-II flow cytometer and to evaluate increase/decrease of HLA ligands, mean fluorescence intensity (MFI) of ligands on tumor cells were normalized with the expression on residual healthy lymphocytes (Figure 1).

| | | B-ALL | Control |
|---|---|---|---|
| Age | Years | Med. 5.57 (Std. Dev. 3.8)<br>Min. 0 - Max. 14 | Med. 5.53 (Std. Dev. 3.1)<br>Min. 1 - Max. 14 |
| Gender | Male | 23 (65.7%) | 32 (60.4%) |
| | Female | 12 (34.3%) | 21 (39.6%) |
| Total | | 35 | 53 |

Table 1. Crosstab with age and gender frequency, and percentages of each group.

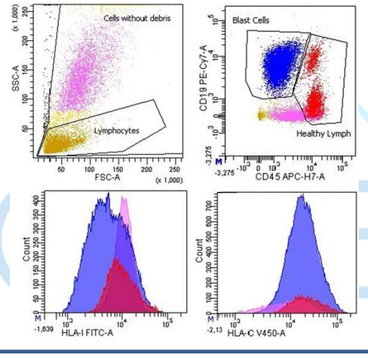



Fig. 1. Representative flow cytometry dot plot of Bone Marrow sample.

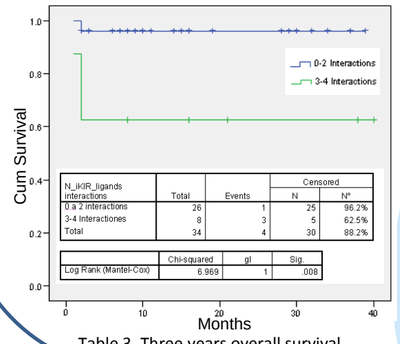
## Results

No differences in the genetic frequency of either iRec/aRec KIRs or HLA-C1/C2, HLA-A*03/A*11 and HLA-Bw4 ligands were found between controls and B-ALLs (Table 2). Although, neither SEHOP-PETHEMA-2013 risk-stratification nor day-14 minimal-residual-disease impacted 3yOS, both higher number of iKIR/ligands interactions (>3 iKIR/ligand interactions) (62.5% vs. 96.2% 3yOS, p<0.01) and decreased membrane expression of total HLA-I (77.0% vs. 95.5% 3yOS, p=0.07) and HLA-C (75.0% vs. 100% 3yOS, p=0.019) on tumor cells negatively impacted patient 3yOS. Cox regression analysis confirms that the number of iKIR/ligand interactions (0R=10.5; p=0.042) is an independent prognostic factors in childhood B-ALL (Table 3).

| | Id_KIR | | HLA-A*03/A*11 | | HLA-Bw4 | | HLA-C | | |
|---|---|---|---|---|---|---|---|---|---|
| | AA | Bx | No A*03/*11 | A*03/*11 | No Bw4 | Bw4 | C1/C1 | C2/C2 | C1/C2 |
| B-ALL | 14 (40%) | 21 (60%) | 24 (68.6%) | 9 (25.7%) | 17 (48.6%) | 15 (42.9%) | 8 (22.9%) | 8 (22.9%) | 16 (45.7%) |
| Control | 16 (30.2%) | 37 (69.8%) | 39 (73.6%) | 14 (26.4%) | 17 (32.1%) | 36 (67.9%) | 14 (26.4%) | 14 (26.4%) | 20 (37.7%) |

Table 2. Frequency and percentages of KIR and HLA.

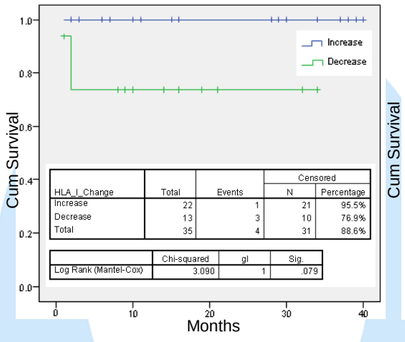
iKIR/ligands interactions and 3yOS




| N_iKIR_ligands interactions | Total | Events | Censored N | Censored N° |
|---|---|---|---|---|
| 0 a 2 interactions | 26 | 1 | 25 | 96.2% |
| 3-4 Interactiones | 8 | 3 | 5 | 62.5% |
| Total | 34 | 4 | 30 | 88.2% |

| | Chi-squared | gl | Sig. |
|---|---|---|---|
| Log Rank (Mantel-Cox) | 6.969 | 1 | .008 |

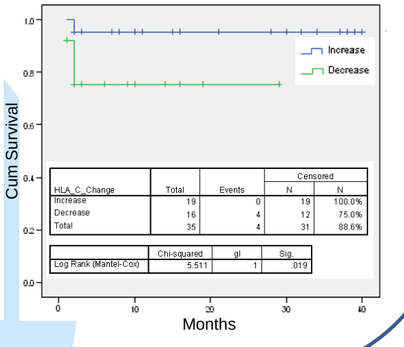
Increase/decreased membrane expression of total HLA-I on tumor cells and 3yOS




| HLA_I_Change | Total | Events | Censored N | Censored Percentage |
|---|---|---|---|---|
| Increase | 22 | 1 | 21 | 95.5% |
| Decrease | 13 | 3 | 10 | 76.9% |
| Total | 35 | 4 | 31 | 88.6% |

| | Chi-squared | gl | Sig. |
|---|---|---|---|
| Log Rank (Mantel-Cox) | 3.090 | 1 | .079 |

Increase/decreased membrane expression of total HLA-C on tumor cells and 3yOS




| HLA_C_Change | Total | Events | Censored N | Censored N |
|---|---|---|---|---|
| Increase | 19 | 0 | 19 | 100.0% |
| Decrease | 16 | 4 | 12 | 75.0% |
| Total | 35 | 4 | 31 | 88.6% |

| | Chi-squared | gl | Sig. |
|---|---|---|---|
| Log Rank (Mantel-Cox) | 5.511 | 1 | .019 |

Table 3. Three years overall survival.

## Conclusion

Although these results should be confirmed in larger series, both the number of iKIR/ligands interactions and the expression level of KIR-ligand on tumor cells could modulate NK cell inmunoserveillance of B-ALL, which apparently have an impact on patient survival.

## References

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Acknowledgements: To Dr. Simon Brackenridge, University of Oxford, who kindly provided DT-9 antibody. This work was supported by Asociación Pablo Ugarte.