



## The 65th ASH Annual Meeting Abstracts

## POSTER ABSTRACTS

## 641. CHRONIC LYMPHOCYTIC LEUKEMIAS: BASIC AND TRANSLATIONAL

**NK Cell Dysfunction in CLL Is Mediated through SHP-1 Signaling and Is Associated with Poor Prognosis**

Hila Shaim, MD<sup>1</sup>, Hind Rafei, MD<sup>1</sup>, Mecit Kaplan<sup>1</sup>, Rafet Basar, MD<sup>1</sup>, May Daher, MD<sup>1</sup>, Mayra Shanley, PhD<sup>1</sup>, Pinaki Banerjee, PhD<sup>1</sup>, Bijender Kumar, PhD<sup>1</sup>, Ana K. Nunez Cortes, MD<sup>1</sup>, Sanjida Islam<sup>1</sup>, Xin Ru Jiang<sup>1</sup>, Lucila Nassif Kerbauy, MD PhD<sup>1</sup>, Paul Lin, MD PhD<sup>1</sup>, Elif Gokdemir<sup>1</sup>, Nadima Uprety<sup>1</sup>, Sunil Acharya, PhD<sup>1</sup>, Mayela Carolina Mendt, PhD<sup>1</sup>, Luciana Melo Garcia, MD MSc<sup>1</sup>, Alexander Biederstädt, MD<sup>1</sup>, Bin Liu, PhD<sup>1</sup>, Enli Liu, MD<sup>1</sup>, Sonny Ang<sup>1</sup>, Jan A. Burger, MD PhD<sup>2</sup>, William G. Wierda, MD PhD<sup>2</sup>, Alessandra Ferrajoli, MD<sup>3</sup>, Elizabeth J. Shpall, MD<sup>1</sup>, Katayoun Rezvani, MD PhD<sup>1</sup>

<sup>1</sup> Department of Stem Cell Transplantation and Cellular Therapy, The University of Texas MD Anderson Cancer Center, Houston, TX

<sup>2</sup> Department of Leukemia, MD Anderson Cancer Center, Houston, TX

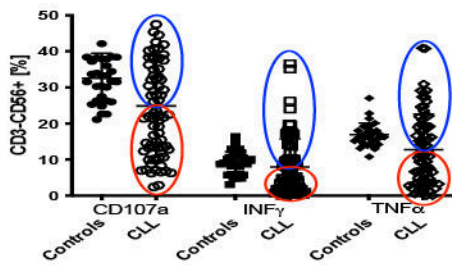
<sup>3</sup> Department of Leukemia, The University of Texas MD Anderson Cancer Center, Houston, TX

Chronic lymphocytic leukemia (CLL) is a heterogeneous disease, presenting with a highly variable clinical course, outcome and response to therapy. This variability has been linked to a complex tumor microenvironment and genetic/epigenetic modifications that lead to immunosuppression. Indeed, the clinical hallmark of this disease is the susceptibility to infections and secondary malignancies. While T cell dysfunction in CLL has been well described, conflicting information exists on NK cell changes in CLL and their role in CLL immunosurveillance. Here, we analyzed samples from 75 untreated (or completed treatment >2 years ago) CLL patients and 30 healthy controls. We discovered a substantial variation in NK cell function compared with healthy controls, which we further classified into hyperfunctional, normofunctional and dysfunctional based on their ability to degranulate, and produce IFN- $\gamma$  in response to K562 targets ( **Figure 1**). NK cells from 'dysfunctional' CLL samples also displayed impaired cytotoxicity against K562 targets, weaker synapse formation and Syk/Zap70 signaling in response to antibody-dependent cellular cytotoxicity (ADCC) compared to those from 'functional' samples. The NK functional status was strongly associated with multiple prognostic markers, including cytogenetics, IgVH mutation status, ZAP-70 expression and disease stage, with patients with dysfunctional NK cells having significantly higher rates of secondary malignancies and viral infections ( **Table 1**). Using multispectral flow cytometry we discovered high phenotypic heterogeneity with unique NK cell clusters present in the dysfunctional samples. Those clusters express a pattern of activation-induced exhaustion, with upregulation of multiple checkpoint molecules such as PD-1, TIGIT, LAG-3, KLRG-1, TIM-3 but not evidence of terminal differentiation (CD57 negative). Those clusters also showed concomitant expression of activating molecules such as NKG2D and DNAM-1, suggesting reversibility of their dysfunctional status. We subsequently found significantly higher ( $p < 0.0001$ ) constitutive phosphorylation of SHP-1 protein, a phosphatase downstream of multiple inhibitory NK cell receptors, in NK cells from dysfunctional samples. Treatment with a SHP-1 inhibitor NSC-87877 (EMD Millipore) or use of siRNA targeting SHP-1 (ThermoFisher scientific), resulted in reversal of NK dysfunction with full restoration of their functional capacity. Taken together, our data suggest that NK cells functional status plays an important role in CLL disease progression and secondary complications. The novel mechanism of NK cell immune dysfunction in CLL described here has the potential to improve outcomes with novel immunotherapies such as chimeric antigen receptors, checkpoint inhibitors and bi-specific antibody therapy that have shown disappointing results in CLL to date when compared with other B cell malignancies.

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Figure 1



Degranulation (CD107a), IFN- $\gamma$  and TNF- $\alpha$  production in response to K562 targets cells by peripheral blood CLL NK cells and healthy control NK cells. Blue circle represents hyperfunctional/normofunctional NK cells and red circle represents dysfunctional NK cells. Degranulation, IFN- $\gamma$  and TNF- $\alpha$  production correlate with  $p < 0.0001$ .

Table 1

	Hyperfunctional NK (N=6)	Normofunctional NK (N=30)	Dysfunctional NK (N=39)
Age (mean $\pm$ SD)	63.4 $\pm$ 14.31	65.16 $\pm$ 10.00	64.56 $\pm$ 9.84
Sex			
M (%)	3 (50)	17 (56.7)	25 (64)
F (%)	3 (50)	13 (43.3)	14 (36)
CLL-IPI			
Low 0-1 (%)	6 (100)	13 (43.3)	9 (23)
Intermediate 2-3 (%)	0	9 (30)	6 (15.5)
High 4-6 (%)	0	6 (20)	11 (28.5)
Very high 7-10 (%)	0	0	10 (25.5)
ND	0	2 (6.7)	3 (7.5)
Rai Stage			
0 (%)	6 (100)	15 (50)	7 (18)
1 (%)	0	11 (36.7)	14 (36)
2 (%)	0	1 (3.3)	1 (2.5)
3 (%)	0	1 (3.3)	4 (10)
4 (%)	0	1 (3.3)	13 (33.5)
ND	0	1 (3.3)	0
ZAP70 status			
Positive	0	9 (30)	19 (49)
Negative	5 (83.3)	17 (56.7)	16 (41)
ND	1 (16.7)	4 (13.3)	4 (10)
IgVH status			
Mutated (%)	6 (100)	22 (73.3)	15 (38.5)
Unmutated (%)	0	7 (23.3)	21 (54)
ND (%)	0	1 (3.3)	3 (7.5)
Mutation status			
del(p17)/TP53 mutation (%)	0	1 (3.3)	13 (33.5)
del(q11)/ATM mutation (%)	0	1 (3.3)	3 (7.5)
T12 (%)	0	6 (20)	6 (15.5)
del(q13) (%)	4 (66.7)	18 (60)	12 (31)
Negative (%)	2 (33.3)	4 (13.3)	4 (10)
ND (%)	0	0	1 (2.5)
Secondary malignancies			
SCC, BCC, melanoma (%)	0	4 (13.3)	10 (25.5)
Richter transformation (%)	0	0	2 (5)
Other (%)	1 (16.7)	1 (3.3)	5 (12.5)
Infections			
Viral/fungal (%)	1 (16.7%)	1 (3.3)	8 (20.5)
Bacterial (%)	0	1 (3.3)	5 (12.5)
B2M (mean $\pm$ SD)	1.95 $\pm$ 0.58	2.81 $\pm$ 1.07	4.39 $\pm$ 4.22
ND= not determined			

Figure 1

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