# Large granular lymphocyte (LGL) subsets in smoldering multiple myeloma (SMM): Immunophenotypic profiles that predict progression to multiple myeloma (MM)

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## Background

Smoldering multiple myeloma (SMM) is an asymptomatic precursor condition to multiple myeloma (MM). The rate of progression of SMM to MM is approximately 10% per year for the first five years after diagnosis[1]. Two independent studies from the Mayo Clinic and the Spanish PETHEMA group have suggested risk-stratification schemes to classify SMM patients as low, intermediate, or high-risk for progression[2,3]. However, these models are often discordant with each other[4]. This necessitates a need for better biomarkers to predict the progression of SMM patients to MM[4].

The immune system is known to play an important role in the progression of precursor disease to MM[2,3]. Large granular lymphocytes (LGL) are a distinct subset of lymphocytes that comprise of effector lymphocytes such as natural killer cells (NK-cells), natural killer T-cells (NK-T-cells) and cytotoxic T-cells[5].These cells normally exert effector functions against tumor cells during carcinogenesis.

We hypothesized that changes in certain LGL subsets in SMM patients could predict progression to MM. These subsets could thus potentially serve as biomarkers of progression to MM.

## Materials and Methods

Flow cytometric analysis was used to quantify LGL subsets in the peripheral blood (PB) of SMM patients (N=85) enrolled in the NCI Natural History of SMM/MGUS prospective clinical trial (NCT 01109407). All patients received regular 3-6 month clinical follow-ups. We conducted an analysis of LGL subsets using samples collected at diagnosis or follow-up on SMM patients who progressed to MM within the subsequent 12-month period (N=8) versus SMM patients who did not progress in that time period (N=71).

Flow cytometric analysis of LGL subsets was performed using the following phenotypic markers: CD16, CD56, CD57, CD3, CD4, CD8, CD2, CD5, CD7, CD19, CD94, TCRab, and TCRgd. B-cells, T-cells, NK-cells, and NK-T-cells were defined as CD19(+), CD3(+), CD3(-)CD16(+)56(+), and CD3(+)CD16(+)56(+) lymphocytes, respectively.

Jonckheere-Terpstra trend test, Mann-Whitney test, Fisher's exact test and chi-squared tests were used for statistical analyses.

## Objectives

1. To quantify LGL subsets in the peripheral blood of SMM patients
2. To identify differences in LGL subsets in SMM patients who did not progress (SMM non-progressors) vs those who progressed (SMM progressors) to MM within 12 months

## Results

A

**Table 1.** Distribution of B-, T-, NK-, and NK-T-cell populations in the PB of all SMM patients, stratified based on Mayo and Spanish PETHEMA risk-stratification models of SMM progression to MM

| | N | B-cells (%) *median (IQR)* | T-cells (%) *median (IQR)* | NK-cells (%) *median (IQR)* | NK-T-cells (%) *median (IQR)* |
|---|---|---|---|---|---|
| All SMM patients | 85 | 6.80 (4.34-9.40) | 76.72 (69.02-81.86) | 12.70 (8.96-17.56) | 7.90 (5.45-14.67) |
| **Mayo risk-stratification** | | | | | |
| Low | 40 | 7.95 (5.06-10.50) | 74.55 (68.03-79.81) | 13.16 (9.52-19.30) | 8.43 (5.41-14.60) |
| Intermediate | 45 | 6.34 (3.90-8.55) | 79.32 (71.59-82.25) | 11.39 (8.82-16.77) | 7.74 (5.84-14.67) |
| High | 0 | | | | |
| *p-value* | | - | .065 | - | - |
| **Spanish risk-stratification** | | | | | |
| Low | 11 | 9.22 (7.44-12.64) | 68.79 (68.27-73.92) | 15.63 (9.37-19.84) | 6.36 (4.30-11.59) |
| Intermediate | 26 | 7.73 (5.10 – 10.01) | 73.98 (69.33-81.25) | 12.61 (9.75-17.35) | 8.27 (6.68-11.00) |
| High | 48 | 5.94 (3.61-8.37) | 79.09 (73.63-82.54) | 12.78 (8.42-17.43) | 8.13 (5.30-17.15) |
| *p-value* | | 0.0031 | 0.013 | - | - |

B

**Table 2.** Baseline clinical characteristics of SMM Non-progressors (N=71) vs SMM Progressors (N=8)

| | SMM Non-progressors (N=71) | SMM Progressors (N=8) | p-value |
|---|---|---|---|
| Median age, years (range) | 64 (33-79) | 60 (50-73) | .44 |
| Sex M/F, n | 40/31 | 7/1 | .13 |
| Race White/Black, n | 62/9 | 8/0 | .59 |
| BMPC, median % (range) | 12.5 (10-45) | 35 (15-80) | <.001 |
| Serum M protein, median g/dL (range) | 1.20 (0-3.4) | 2.15 (0-2.9) | .21 |
| Skewed Free Light Chain Ratio (<0.125 or >8.0), n/N (%) | 33/71 (46) | 8/8 (100) | .006 |
| Isotype IgG/IgA/IgD/biclonal/free-κ/free-λ, n | 48/15/2/4/2 | 4/3/-/1/- | - |
| >=95% aberrant PCs, n/N (%) | 50/71 (70) | 8/8 (100) | .10 |
| Immunoparesis, n/N (%) | 49/71 (69) | 7/8 (88) | .43 |
| Mayo risk-stratification, n/N (%) | | | |
| Low | 36/71 (51) | 1/8 (12) | |
| Intermediate | 34/71 (48) | 7/8 (88) | |
| High | 1/71 (1) | 0/8 (0) | |
| PETHEMA risk-stratification, n/N (%) | | | |
| Low | 11/71 (15) | 0/8 (0) | |
| Intermediate | 21/71 (30) | 1/8 (12) | |
| High | 39/71 (55) | 7/8 (88) | |
| Mayo vs Spanish inter-model concordance, n/N (%) | 17/71 (24) | 0/8 (0) | .19 |

**Table 3.** Distribution of LGL subsets in the PB of SMM Non-progressors (N=71) vs SMM Progressors (N=8)

| | SMM Non-progressors (N=71) *median (IQR)* | SMM Progressors (N=8) *median (IQR)* | p-value |
|---|---|---|---|
| **Total Lymphocytes (%)** | 1.64 (1.25-1.91) | 1.80 (1.55-1.94) | - |
| Total (ALC) | 7.01 (4.95-9.07) | 4.04 (2.44-5.65) | .003 |
| CD57(-) CD56(+) CD57(+) | 17.61 (12.17-24.14) | 19.62 (17.88-32.24) | .23 |
| **NK-cells (%)** | | | |
| Total | 13.57 (9.46-19.15) | 13.42 (7.54-16.36) | - |
| CD57(-) CD16(+) | 6.11 (4.56-8.72) | 3.70 (2.45-5.26) | .035 |
| CD57(+) CD8(-) | 17.61 (12.17-24.14) | 7.94 (4.91-9.19) | .13 |

C

**Figure 1.** Subsets of total lymphocytes in SMM Non-progressors vs SMM Progressors

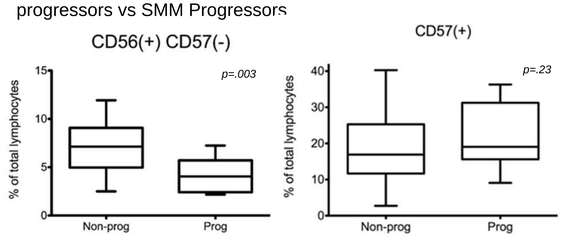



**Figure 2.** Subsets of NK-cells in SMM Non-progressors vs SMM Progressors

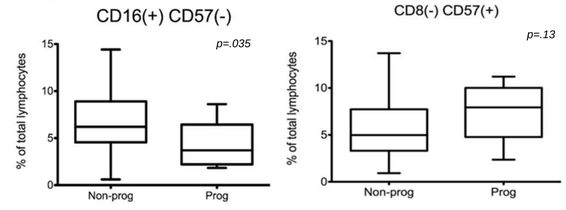
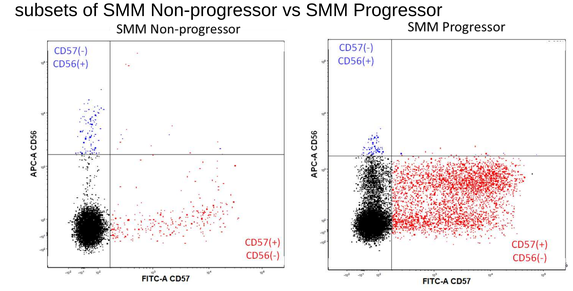



**Figure 3.** Representative flow cytometry charts of CD56 and CD57 subsets of SMM Non-progressor vs SMM Progressor




## Results / Conclusions

### Results

**Panel A**

SMM patients risk-stratified per the Mayo criteria show differences in T-cells, while SMM patients risk-stratified per the Spanish criteria show differences in both B-cells and T-cells. No differences were seen in NK-cells or NK-T-cells per either criteria.

**Panels B/C**

SMM patients who progress to MM possessed distinct LGL profiles 6-12 months before progression. Their LGL profile was characterized by a decrease in CD57(-) lymphocyte subsets and trending increases in CD57(+) lymphocyte subsets. These differences were also seen in CD57(-) and CD57(+) NK-cell subsets.

### Conclusions

Current clinical risk scores for SMM have limitations. There is a need for biological markers that can reliably predict progression from SMM to MM. Such markers would be clinically useful as they would allow more tailored clinical monitoring of SMM patients and guide clinicians regarding initiation of early treatment to delay or prevent progression. Based on our LGL findings in SMM patients, we suggest CD57 subsets as potential markers of progression from SMM to MM.

## References / Acknowledgements

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