Investigation of Multiple Myeloma transformation into Secondary Plasma Cell Leukemia under severe viral infection

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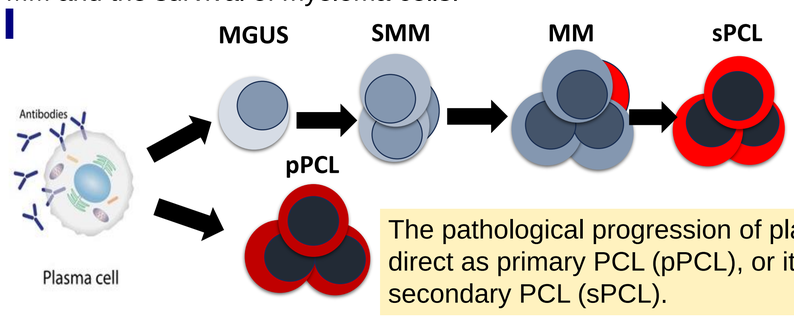
Background

Multiple myeloma (MM) is a plasma cell neoplasm that causes clonal plasma cell proliferation within the bone marrow. Patients with multiple myeloma are at **risk** for **severe infections**.

Plasma Cell Leukemia (PCL) is also a plasma cell neoplasm, but is rare and the most aggressive plasma cell dyscrasia. It is defined by the presence of (≥5%) aberrant plasma cells in the peripheral blood.

The **NF-κB** signaling pathway plays a major role in inflammatory and immune responses. Dysregulation of this pathway is one of the factors said to play a role in the pathogenesis of MM and the survival of myeloma cells.



The pathological progression of plasma cells to PCL can be direct as primary PCL (pPCL), or it can emerge from MM as secondary PCL (sPCL).

Aims

- Uncover the immunophenotypical changes of clonal plasma cells during viral infection in cases showing MM to PCL transformation.
- Determining the involvement and role of the NF-kB signaling pathway in the transformation process.
- Reveal the difference between pPCL and sPCL transformation.

Methods

Used Samples

Virus infected patients showed MM to sPCL transformation:

Patient samples	Viral infection	Genetic abnormality
Patient 1	COVID+	t(4;14), TP53del, 1q21 ampl, +13q
Patient 2	COVID+	t(11;14)
Patient 3	COVID+	t(11;14)
Patient 4	COVID+	t(4;14), 1q21 ampl
Patient 5	HEV+	t(14;16), TP53del+mut, 1q21ampl

Control samples:

I. **N = 9** newly diagnosed **MM patients** (non-infected) with 7/9 cases t(11;14), cases 2/9 t(4;14).

II. **N = 17 non-infected PCL samples** in total, 9 of which are pPCL samples; The remaining 6 are sPCL samples:

Mutation distripution frequency	t(11;14)	t(4;14)	17p deletion	1q12Amp	3 IgH	1p amp	del 1 q12	t(14;16)
Across 6/17 sPCL samples	2	1	4	0	1	1	0	0
Across 9/17 pPCL samples	4	0	1	3	2	4	1	1

Flow cytometric panels

BD FACS LyricTM 10-color flow cytometer – 50000 events measured.

	Violet 405nm, 40mW			Blue 488nm, 40mW				Red 640nm, 40mW
Fluorescent channels	FL1	FL2	FL3	FL4	FL5	FL6	FL7	FL8
Filters	448/45	528/45	606/36	527/32	586/42	700/54	783/56	660/10
Panel 1	Syto40	**CD138** BV510	**CD45** BV605	**CD81** FITC	**CD56** PE	**CD19** PERCP-Cy5.5	**CD117** PC7	**CD38** APC
Panel 2	Syto40	**CD138** BV510	**CD45** BV605	**CD69** FITC	**CD38** PE	**CD44** PERCP-Cy5.5		**CD86** APC
Panel 3	Syto40	**CD138** BV510	**CD45** BV605	**CD38** FITC	**CD184** PE			**CD49d** APC

Clinical data

The **CRP levels** (<10mg/L is normal,>100mg/L severe)**, Hemoglobin, Free kappa-lambda and WBC** was collected & compared **before** and **after the infection** for the 5 infected sPCL patient samples (shown above in the sample table).

Software tools and Statistical analysis:
Kaluza 2.1.1 software, to analysis Flow cytometric data.
Sigma Plot 12.5. for statistical analysis (Paired t-test, Wilcoxon signed-rank test, T-test, Mann-Whitney test).

Western blot

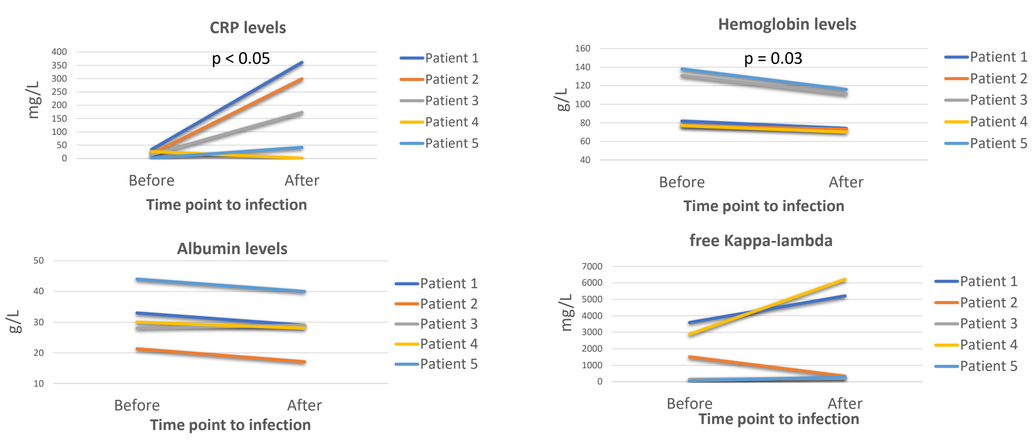
Western blot techniques were used on 14 samples, including the samples of the 5 infected patients exhibited the MM-to-sPCL transformation, to measure for the NF-kB signaling pathway activation.

Tested proteins:
- RelA
- RelB
- TNFR2
- MMP9

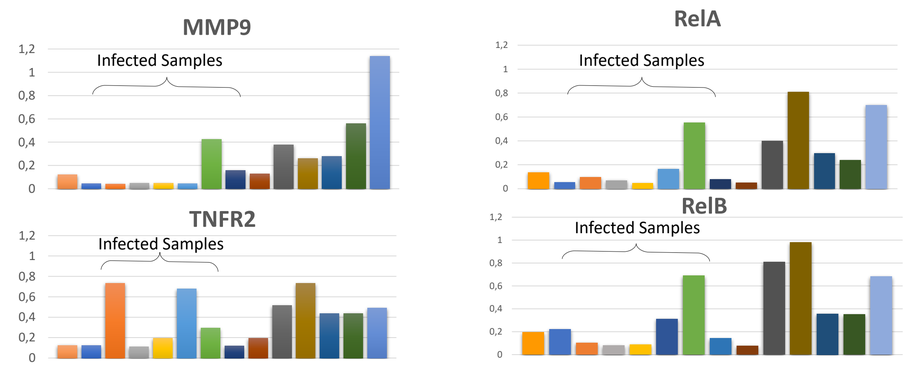
Key components of the NF-kB signaling pathway

Results

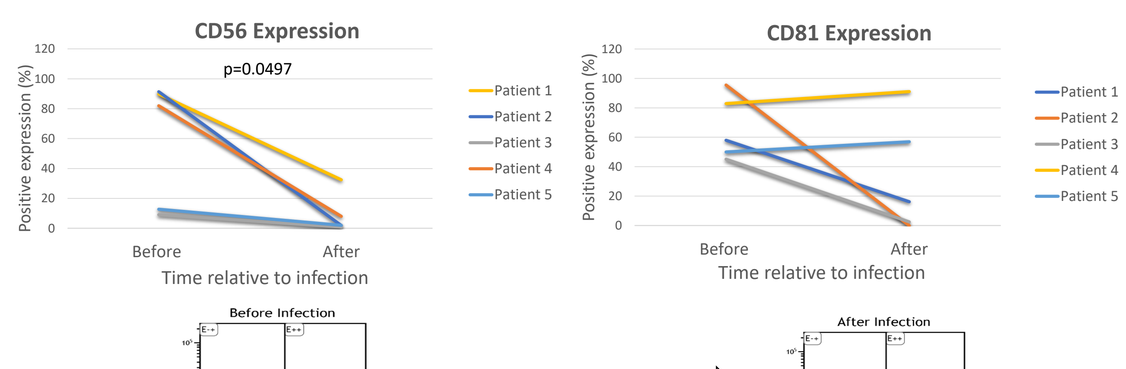
Clinical changes before and after infection for n = 5 transformed patient samples

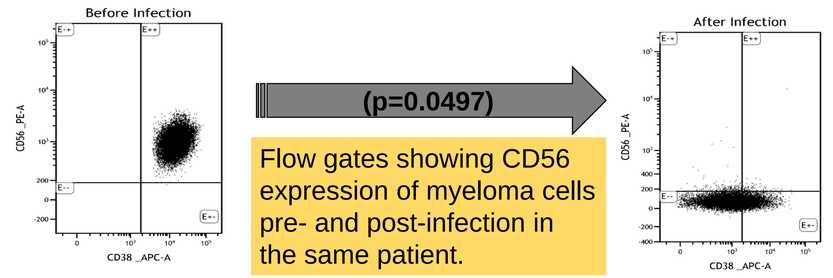


Western blot results showing the expression of TNFRII, Rel A/B and MMP9, for n = 14 patient samples (including the 5 sPCL/infected samples)



Flow cytometry I.: showing the change in immunophenotypic expression before and after infection for (n = 5) transformed patient samples





(p=0.0497)

Flow gates showing CD56 expression of myeloma cells pre- and post-infection in the same patient.

II.: Immunophentypic difference between Newly diagnosed MM and Infected samples

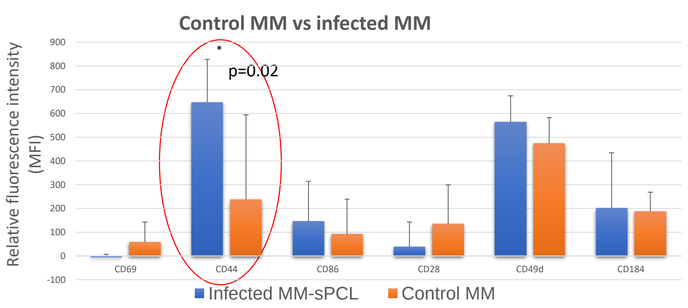
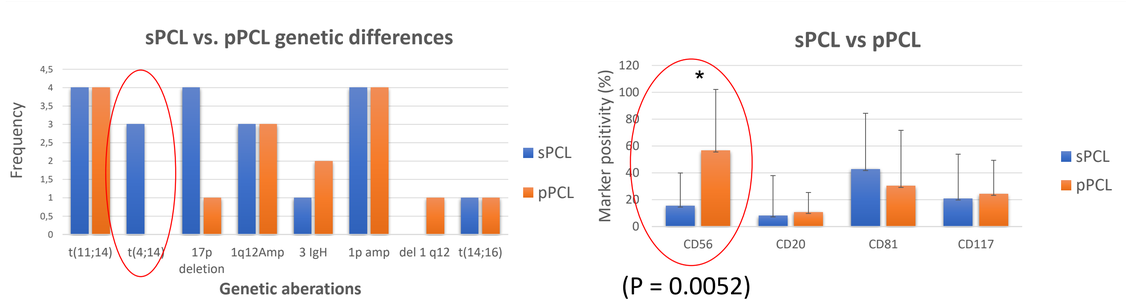


Figure showing CD marker expression of myeloma cells between infected patient group (n = 5) exhibiting the transformation to sPCL and non-infected newly diagnosed MM group (n = 9) serving as control.

III.: Differences between sPCL and pPCL



Comparison of genetic abnormality between sPCL and pPCL

Comparison of immunophenotypic changes between sPCL and pPCL

Findings

- The **CD56 marker** expression declined following the viral infection in our five infected patient samples. We measured this as the main immunophenotypic change during the transformation. The absence of this marker might have facilitated the dissemination of plasma cells into the circulation.
- The five-case post-infected group exhibited higher levels of **CD44 marker** expression when we compared them to the newly diagnosed MM control group (9 cases). CD44 is a marker for cancer stem cells and plays a role in the development of metastasis.
- Overall, the level of the **CD56 marker** in sPCL cases (6 + 5 infected cases) is much lower when compared to pPCL cases (9 cases). Also, the t(4;14) mutation is four times more common in sPCL case samples. This suggests that the transformation of sPCL is indeed different from that of pPCL.
- Lastly, our discovery suggests that a severe viral infection (e.g., COVID) could be a new potential risk factor for high-risk MM-to-sPCL transformation.