The tumor immune microenvironment of chronic lymphocytic leukemia

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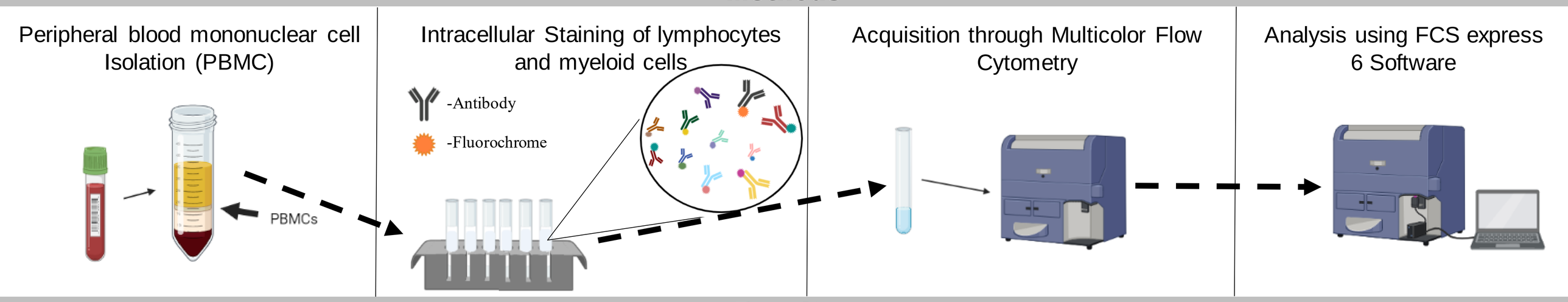
Background

Chronic lymphocytic leukemia (CLL) is a B cell malignancy characterized by the overexpression of $CD5^+$ B-cells.[1] CLL cells have been shown to have high levels of proliferation that is facilitated through interactions with the surrounding microenvironment. The immune microenvironment is populated by several cell types of the lymphoid and myeloid lineage. Including regulatory and memory T cells[2], monocytes, tumor associated macrophages (TAMs), and myeloid derived suppressor cells (MDSCs).[3] Currently, there are therapeutic agents such as BTK inhibitors (e.g.Ibrutinib) that target CLL cells by inhibiting the Bruton's tyrosine kinase, which is involved in the growth and proliferation of leukemic cells.[4] One major setback from the use of these therapies is the immune suppression that results. Some patients that undergo treatment are prone to serious fungal infections and other immune dysfunctions.[5] This evidence opens the door for the possibility that these therapies could interfere with non-neoplastic immune cells as well as CLL cells. There is a gap in knowledge that exists about the current state of immune cells that interact with CLL cells. It is important to analyze the microenvironment of CLL in order to correctly identify how to repair immune dysfunction that is a common feature of patients with CLL. We hypothesize that the tumor immune microenvironment of CLL includes dysfunctional memory T cells, but also lymphoid and myeloid cells with pro-tumor function.

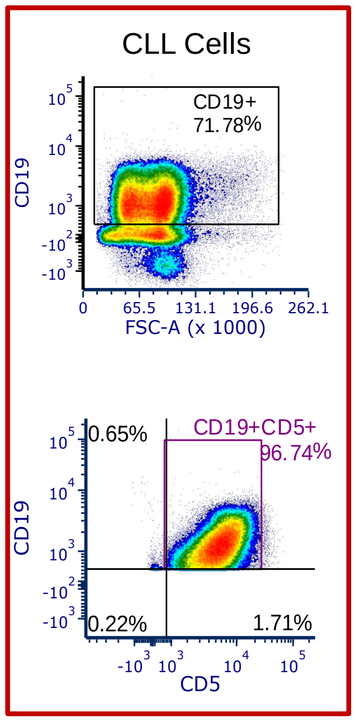
Methods



Results

Lymphoid Cells

Myeloid Cells



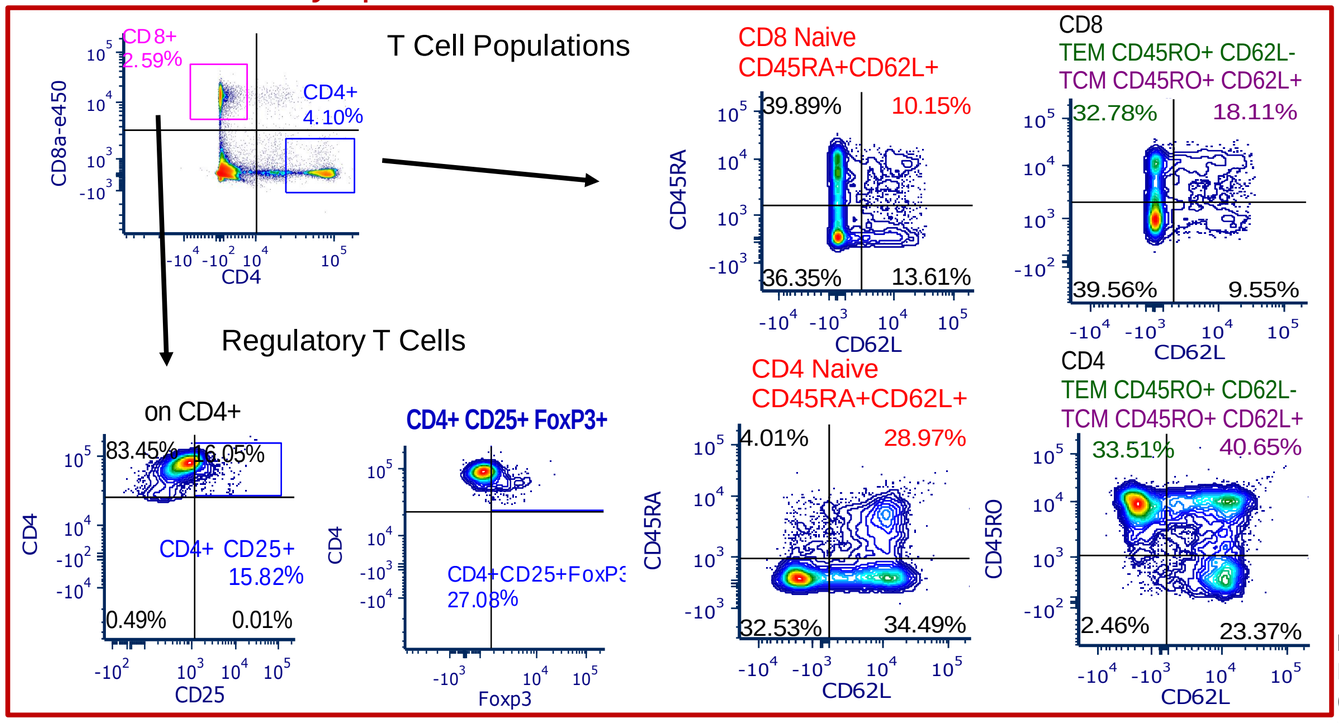


Figure 1 | Staining of human lymphocytes. CLL cells were identified based on their surface expression of CD5 and CD19 molecules. Human naïve, $CD4^+$ and $CD8^+$ effector (TEM) and central memory (TCM) T cells have been identified based on the differential surface expression of CD45RO, CD45RA, and CD62L. $CD4^+$ regulatory T lymphocytes expressing CD25 and Foxp3 were also analyzed. Representative PBCMs from a CLL patient are described. This experiment was executed on three different patient samples.

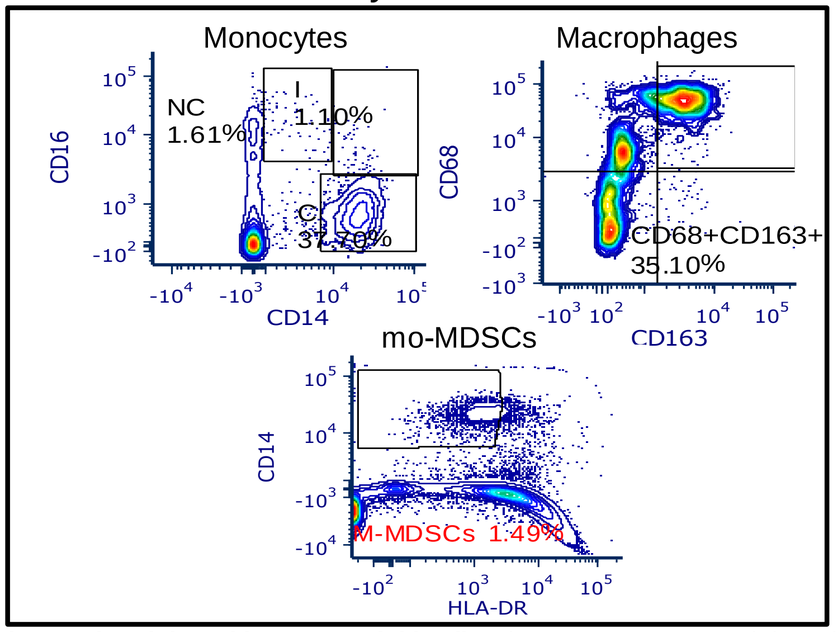


Figure 2 | Staining of human myeloid cells. Live PBMCs were gated through LIVE/DEAD Aqua Cell Stain (not shown). After the exclusion of neutrophils (through CD66b molecule, not shown) and then the exclusion of lymphoid cells through lineage cocktail including CD56, CD3, CD19, CD20 (not shown), monocytes were visualized as $CD14^+$ $CD16^{++}$ non-classical (NC), $CD14^{++}$ $CD16^+$ intermediate (I) and $CD14^{++}$ $CD16^-$ classical subsets (C). $CD14^+$ HLA-DR-/low mo-MDSCs were identified. Macrophages expressing CD68 and CD163 were also identified. Representative PBCMs from a CLL patient are described. This staining was executed on three different patient samples.

Conclusions

We successfully identified and characterized through multi-color flow cytometry a number of cell types of the lymphoid and myeloid lineage. As hypothesized, some of these cell types have pro-tumor phenotype/function and include immunosuppressive regulatory CD4 cells (T_{REG}), tumor associated macrophages (TAM) and monocytic-MDSCs. In addition, naïve, effector and central memory $CD4^+$ and $CD8^+$ T cells were identified. The characterization of the cellular tumor immune microenvironment will pave the way toward future studies aimed at repairing the function of some anti-tumor immune cells, but also at depleting immune cells with immunosuppressive function.

References

[1] Kipps, T. *et al.* "Chronic lymphocytic leukaemia." *Nat Rev Dis Primers* Vol. 3, 16096 (2017).

[2] Hacken, T. *et al.* "Microenvironment Interactions and B-cell Receptor Signaling in Chronic Lymphocytic Leukemia: Implications for Disease Pathogenesis and Treatment." *Biochimica Et Biophysica Acta (BBA) - Molecular Cell Research* Vol. 1863.3 (2016).

[3] Banerjee, P. *et al.* "Trabectedin Reveals a Strategy of Immunomodulation in Chronic Lymphocytic Leukemia." *Cancer immunology research* Vol. 7,12 (2019): 2036-2051.

[4] Nitin, J. *et al.* "Ibrutinib and Venetoclax for First-Line Treatment of CLL." *The New England journal of medicine* Vol. 380,22 (2019): 2095-2103.

[5] Varughese, T *et al.* "Serious Infections in Patients Receiving Ibrutinib for Treatment of Lymphoid Cancer." *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America* Vol. 67,5 (2018): 687-692.

Acknowledgements

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