Assessment of Tumor Purity in CD19$^+$ Sorted Cells from Waldenström Macroglobulinemia Patients and a Comparison of Data Integration Methods Using Neoplastic and Healthy Cell Annotation

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Recent advancements in single-cell RNA sequencing (scRNA-Seq) technologies allow the analysis of thousands of cells, providing valuable insights into the cellular heterogeneity of tumors and the biological characteristics of different cell subpopulations. Normal cells infiltrating the tumor tissue perturb the tumor signal in molecular studies. In this study we use computational approaches in order to assess tumor purity on sc-RNA-seq of CD19$^+$ sorted cells from bone marrow aspirates of seven patients with Waldenström macroglobulinemia (WM). We then evaluate various integration methods in terms of limiting technical variability (batch effects) while preserving true biological heterogeneity of malignant and non-malignant cell populations between samples. Integrating data across different patients is crucial to uncovering shared cell states underlying disease pathogenesis.

We analyzed a total of 40,087 single CD19$^+$ cells: 35,131 from WM patients and 4,956 from two healthy donors (HDs), with an average of 4,454.11 (range 1,956–7,731) cells for each sample. To distinguish the malignant from the non-malignant cell populations, we calculated and visualized the IGKC-fraction (IGKC/(IGKC+IGLC2)) of every cell, and calculated the kappa-to-lambda ratio in each cluster for each patient[1]. Malignant clusters display a consistently even lambda and kappa light chain expression among every cell in the cluster, whereas B cells in healthy clusters express lambda and kappa light chain genes in random different proportions.

This was further validated by exploring somatic large-scale chromosomal copy number alterations (CNVs) inferred by inferCNV[2,3]. We examined the inferred CNV regions in each patient looking for chromosomal regions that are associated with WM (chromosomes 6q and 11q or affecting the following genes[4]: *MYD88, CXCR4, BCL2, TP53, PAX5, ATM*, immunoglobulin heavy and light chain. Our findings further support that the vast majority of patient cells are malignant and that there is heterogeneity within the clonal cells of the patients. We observed higher CNVscores[5,6] in malignant clusters and lower medians of the CNVscores in healthy clusters. We performed an unpaired Wilcox test between the inferCNV scores of HD's healthy cells, patient malignant cells and patient healthy cells, under the Bonferroni correction. Statistically significant differences were found between the aforementioned groups.

We have compared seven single-cell data integration tools[7]: BBKNN[8], Scanorama[9], scGen[10], scVI[11], Harmony[12], Seurat.v3[13] (CCA, RPCA). For the evaluation of data integration performance the median LISI score is used to assess batch (sample), individual cell types and

healthy/neoplastic cells mixing. Seurat.v3-CCA, Harmony, scVI tend to remove batch variation whereas scGen prioritizes biological variance conservation by using cell-type information. While Harmony kept each isolated cell label together, it overlapped these populations. We noticed that the above integration methods are prone to overcorrection. Scanorama is less vulnerable to overcorrection because it finds matches between all pairs of datasets. We chose to merge our datasets using Scanorama, to create a scRNA-seq 'panorama' and to proceed with clustering and trajectory analyses using Harmony's embedding[14]. We stress the importance of benchmarking tools when analyzing scRNA-Seq data for WM, to ensure accurate and reproducible results.

Citations:

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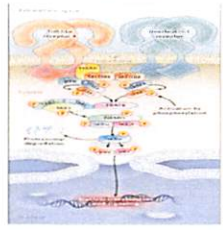
Clinical Records

Patients

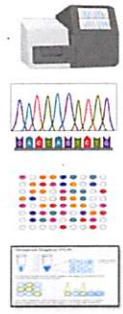
[N=385]

- ✓ BM [N=356]
- ✓ PB [N=22]
- ✓ FFPE [N=7]

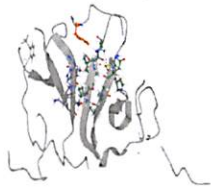
MYD88 mutation
[N=385]



- Sanger sequencing [N=233]
- NGS [N=246]
- AS-PCR [N=322]
- dd-PCR [N=214]



CXCR4 mutation
[N=362]



- Sanger sequencing [N=273]
- NGS [N=246]
- AS-PCR [N=318]