Simultaneous genotype inference and cell-to-clone mapping in the tissue of Follicular Lymphoma

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A major challenge in developing efficient immunotherapy is intratumour heterogeneity. During the course of tumour development, its replicating cells acquire different mutations in their genome, resulting in a clonal structure, in which the tumour is populated with *clones* – groups of cells with similar *genotypes*. Since different mutations in the genotype can impact the clone's *phenotype*, the clones can react differently to therapy; therefore, extensive efforts are made to gain insight into the genotype-phenotype relation in tumour. Thanks to the development of single-cell transcriptomics, we can measure the phenotypes on the level of individual cells using single-cell RNA sequencing (scRNA-seq). However, to study it on the clonal level, we need to infer clonal genotypes present in the tissue, map the sequenced cells to their clone of origin and only then analyse their combined gene expression. The genotype inference and cell-to-clone mapping was done in previous approaches separately as a two step procedure, in which genotypes were typically inferred from additional bulk data and only then the mapping was performed. This is potentially inefficient, as the information on the clonal structure from the scRNA was not used in calling the genotypes, and requires an additional sequencing experiment.

A new approach combining the inference of clonal genotypes and the assignment of cells to clones in one model would both facilitate and improve the analysis of tumour heterogeneity. Moreover, by getting rid of the previously needed whole exome sequencing (WES) experiment, it would enable the study of the heterogeneity of small tumour samples, on which it cannot be performed, due to insufficient genetic material. However, genotype inference from only scRNA data is troublesome, as it is both sparse and noisy, with some mutations not being expressed and/or observed in their carrier cells. To make that inference reliable, we need an additional information source that can tell us, which groups of cells should come from the same evolutional clone; then, we can pool their scRNA information together, to obtain better genotype inference and clonal mapping quality.

In the case of Follicular Lymphoma (FL) we do have such an information source. FL is a cancer of the B-cells of the immune system, which as part of their function in the human adaptive immune system express a highly mutable B-cell receptor (BCR) protein. Therefore, BCR mutations can serve as markers in the tumour evolution history and we should regard cells with identical or very similar BCR sequences as belonging to the same evolutional clone. This approach was successfully used in our previous research, greatly improving mapping quality to genotypes inferred from WES, that were also corrected with the scRNA information in our model.

Here, we aim to develop a method for simultaneous genotype inference and cell-to-clone mapping in FL tissue, which will only require scRNA and BCR sequencing. Motivated by our previous findings, we anticipate it will provide an easier and more effective method of studying the clonal structure of FL, enabling the study of smaller tumour samples, and furthering our understanding of the tumour evolutionary mechanisms.