

Peer Review File

Article information: <https://dx.doi.org/10.21037/ccco-22-121>

Reviewer comments

A vital constituent of innate immunity, neutrophils were previously considered functionally rigid with a fixed, defined roles in host pathogen response, in part due to their fleeting lifespan. In the manuscript “Single Cell Sequencing of Neutrophils demonstrates phenotypic heterogeneity and functional plasticity in health, disease, and cancer”, authors reviewed work done in neutrophil single cell RNA sequencing in mice and humans in health and a range of disease states including COVID-19 infection, and solid cancers to provide a template for understanding neutrophil biology in context.

Couple questions are required to be answered before it will be accepted.

Comment 1: In the background, please make a brief introduction about single cell sequencing.

Reply 1: Text added in more detail to introduce single cell RNA sequencing in section 1.2 rationale and knowledge gap

Changes in the text: Page 4 lines 110-116

Comment 2: In the introduction, it was proposed to add related reference (DOI: 10.21037/atm.2020.04.23) about the application of single cell sequencing in diseases.

Reply 2: Reference added (see reference 37) in section 1.2 rationale and knowledge gap

Changes in the text: Page 4 lines 115-117

Comment 3: How about the roles of neutrophils in injured brain?

Reply 3: Paragraph added to summarise roles of neutrophils in ischaemic brain injury and the two single cell transcriptomic studies that have been done in mice (none exist to date in the literature in human ischaemic brain injury).

Changes in the text: Page 7 Lines 212-224, page 8 lines 248-250

Comment 4: What is the prospective of single cell sequencing of neutrophils in the future? Please state in the conclusion.

Reply 4: Paragraph added to conclusion to summarise the role for single cell sequencing of neutrophils in the future (development of biomarkers to stratify treatment, greater understanding of cell-cell interactions, spatial transcriptomics)

Changes in the text: Page 13 Lines 496-507

Comment 5: What were your good ideas for the applications of neutrophils after single cell sequencing in the treatment of diseases, such as cancer?

Reply 5: Paragraph added to conclusion to summarise ideas for neutrophil sequencing in treatment of cancer (improving existing therapies, novel therapies targeting neutrophils, use of neutrophil phenotype as a biomarker to stratify treatment)

Changes in the text: Page 13 Lines 496-507