Single cell characterization of longitudinal biopsies from breast cancer patients treated with the aromatase inhibitor letrozole and the CDK4/6 inhibitor ribociclib

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Xavier Tekpli[1,*,#], Salim Ghannoum[1], Chloé Beate Steen[1], Marie Fongaard[1], Marius Bornstein[1], Barbro Holm[2], Tatjana Bosnjak[3], Laurens Cornelus Reitsma[4], Stephanie Beate Geisler[5], Kamilla Fjermeros[5], Manouchehr Seyedzadeh[6], Unn-Cathrin Buvarp[5], Marie Loeng[5], Aino Katri Vuoriluoto[5], Torben Lüders[8], Diether Lambrechts[9], Marianne Lyngra[10], Vessela Kristensen[1,7], Jürgen Geisler[5,7,*]

1) Department of Medical Genetics, Oslo University Hospital, Oslo, Norway 2) Novartis, Medical Affairs Oncology, Kista, Sweden 3) Novartis, Medical Affairs Oncology, Oslo, Norway 4) Department of Breast and Endocrine Surgery, Akershus University Hospital, Oslo, Norway 5) Department of Oncology, Akershus University Hospital, Oslo, Norway 6) Department of Radiology, Akershus University Hospital, Oslo, Norway 7) University of Oslo, Institute of Clinical Medicine, Faculty of Medicine, Oslo, Norway; 8) Department of Clinical Molecular Biology (EPIGEN), Akershus University Hospital, Oslo, Norway 9) Laboratory for Translational Genetics, Department of Human Genetics, KU Leuven, Leuven, Belgium 10) Department of Pathology, Akershus University Hospital, Oslo, Norway; * Jointly supervised the work, shared corresponding authors; # conflict of interest: the presenter received research honorary from Novartis by participating in science advisory board. The NEOLETRIB trial is a research collaboration between OUH, AHUS and Novartis Norway AS.

THE NEOLETRIB TRIAL

Multicenter | Single-arm | Open-label
Neoadjuvant | Phase II trial | NCT05163106

Neoadjuvant combination therapy for 6 months with:
- Ribociclib (600 mg daily) [21 days on / 7 days off]
- Letrozole (2.5 mg daily)

Biopsies were collected:
- *Pre-treatment*
- *On-treatment* (21 days post treatment)
- *End-of-treatment*

Locally advanced breast cancer patients luminal-A / luminal-B

From biopsy collection to single cell RNA sequencing using single-cell v2 5' chemistry (10x Genomics)

Introduction

Methods

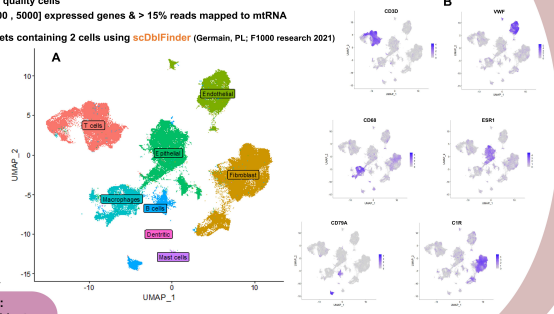
- Loading raw gene expression matrices to Seurat (v4.0.2) (Hao, Y; Cell 2021)
- Filtering out empty droplets using emptyDrops (Lun, ATL; Genome Biol 2019)
- Filtering out low quality cells
  < 500 UMIs & [300 , 5000] expressed genes & > 15% reads mapped to mtRNA
- Filtering out droplets containing 2 cells using scDblFinder (Germain, PL; F1000 research 2021)

Normalizing gene expression of the remaining good cells

Constructing principal components using variable genes

Cluster cells based on significant principal components

Identifying 8 main cell types:
T cells, B cells, epithelial cells, fibroblasts, endothelial cells, macrophages, mast cells and dendritic cells

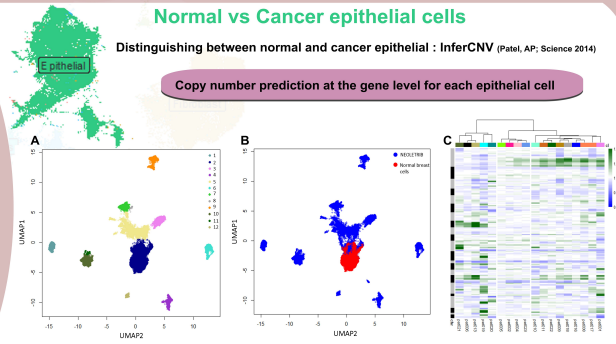


(A) UMAP of 24177 cells obtained from 45 biopsies of the NEOLETRIB trial. UMAP is colour-coded for annotated cell types.
(B) UMAP with marker gene expression related to the cell types identified.

Normal vs Cancer epithelial cells

Distinguishing between normal and cancer epithelial : InferCNV (Patel, AP; Science 2014)

Copy number prediction at the gene level for each epithelial cell



(A) Clustering of 48901 epithelial cells of the NEOLETRIB trial clustered with 10865 normal epithelial cells from GSE164898 (Bhat-Nakhshatri, P, Cell Reports Medicine 2021) . Cells are clustered according to their copy number profile as inferred by InferCNV. Twelve clusters with distinct copy number are identified. (B) Colour coding of the cells inferred copy number according to provenance of the cells either from the NEOLETRIB trial (blue) or GSE164898 (red). (C) Visualization of the copy number profile chromosome wise (y-axis) for the different patient analyzed by single cell RNA sequencing.

Aims and goals of the single cell experiments of the NEOLETRIB trial:

- Assess the evolution of the tumor cell profile during neoadjuvant therapy with CDK4/6 inhibitor ribociclib and aromatase inhibitor in HR+/HER2- BC (luminal-A/luminal-B).
- Identify the cell sensitive and resistant to the combinatory treatment.
- Assess the effect of the aromatase and ribociclib on the tumor microenvironment.

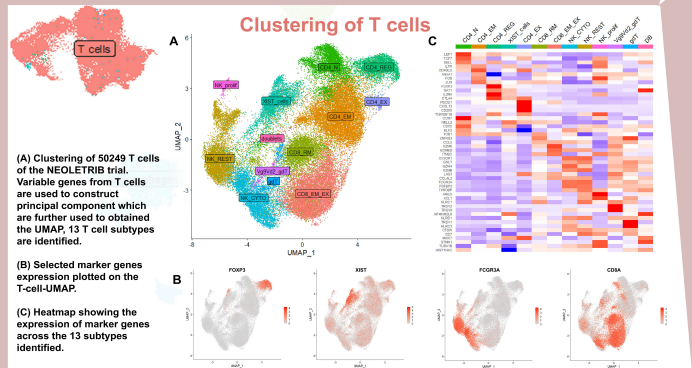
Identifying specific and specialized subtypes:

To identify specific and specialized cell subtype, we clustered 3 of the cell types independently and annotated the clusters obtained using validated marker genes.

Conclusions and Perspectives

- We successfully performed single cell RNA-seq on serial biopsies of the NEOLETRIB trial.
- We identifed 8 main cell types in biopsies which we further divided in specialized subtypes.
- Our methodological setup provides a framework to identify the cells sensitive or resistant to therapy and characterize the changes induced by drugs in the tumor microenvironment.

Clustering of T cells

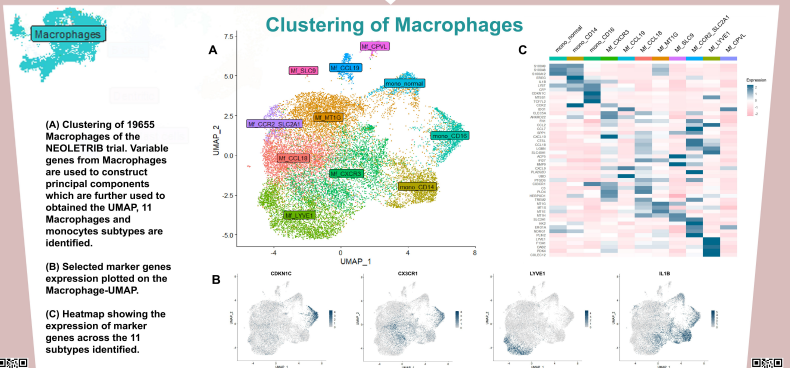


(A) Clustering of 50249 T cells of the NEOLETRIB trial. Variable genes from T cells are used to construct principal component which are further used to obtained the UMAP, 13 T cell subtypes are identified.

(B) Selected marker genes expression plotted on the T-cell-UMAP.

(C) Heatmap showing the expression of marker genes across the 13 subtypes identified.

Clustering of Macrophages

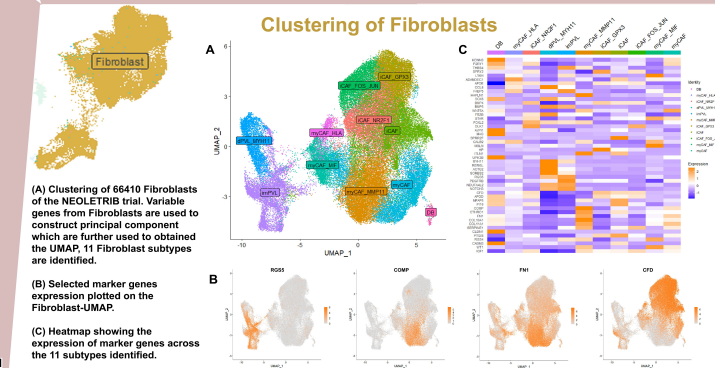


(A) Clustering of 19655 Macrophages of the NEOLETRIB trial. Variable genes from Macrophages are used to construct principal components which are further used to obtained the UMAP, 11 Macrophages and monocytes subtypes are identified.

(B) Selected marker genes expression plotted on the Macrophage-UMAP.

(C) Heatmap showing the expression of marker genes across the 11 subtypes identified.

Clustering of Fibroblasts



(A) Clustering of 66410 Fibroblasts of the NEOLETRIB trial. Variable genes from Fibroblasts are used to construct principal component which are further used to obtained the UMAP, 11 Fibroblast subtypes are identified.

(B) Selected marker genes expression plotted on the Fibroblast-UMAP.

(C) Heatmap showing the expression of marker genes across the 11 subtypes identified.

Please contact: juergen.geisler@medisin.uio.no and/or xavier.tekpli@medisin.uio.no for permission to reprint, distribute or further information