



Single cell transcriptomics approach reveals ATRT intratumoral heterogeneity and putative cells of origin

M. Andrianteranagna^{1,2,3,4}, M. Lobon-Iglesias^{1,4}, Z.-Y. Han^{1,4}
N. Servant^{2,3} & F. Bourdeaut^{1,4}

¹ PSL Research University, Institut Curie, INSERM U830, RTOP

² INSERM, U900, Bioinformatics Platform, Analysis group

³ MINES ParisTech, PSL Research University, CBIO-Center for Computational Biology

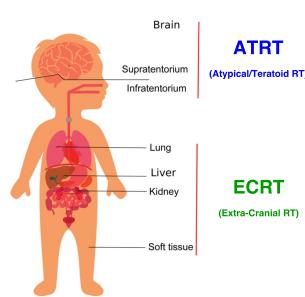
⁴ PSL Research University, Institut Curie, Translational Research Department

June 27, 2023

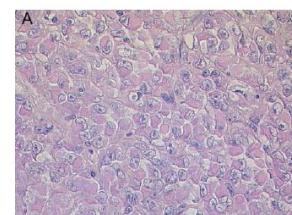
Rhabdoid tumors (RT)

rare but highly malignant pediatric tumors (90 % of cases occur before 3 years of age)

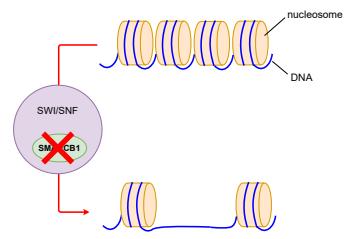
can grow anywhere in the body



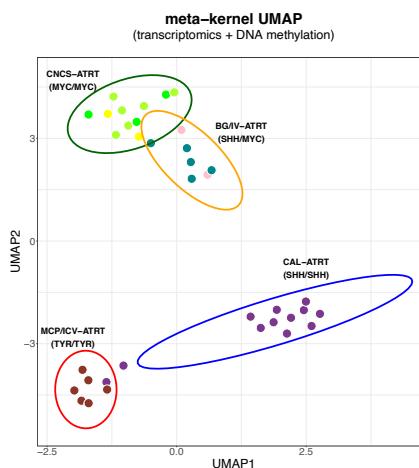
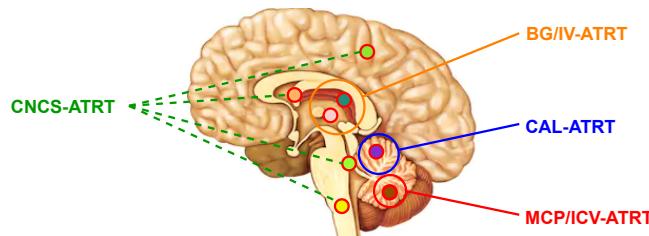
rhabdoid feature



bi-allelic inactivation of *SMARCB1*

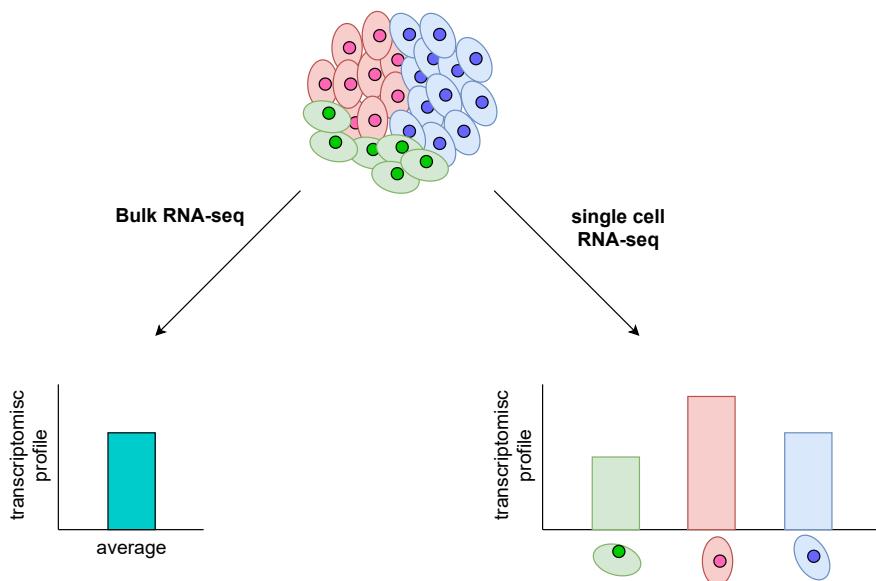


molecular intertumoral heterogeneity of ATRT (Johann et al. (2016); Torchia et al. (2016); Ho et al. (2019)) that are correlated with anatomic localization (Lobon-Iglesias et al. (submitted))



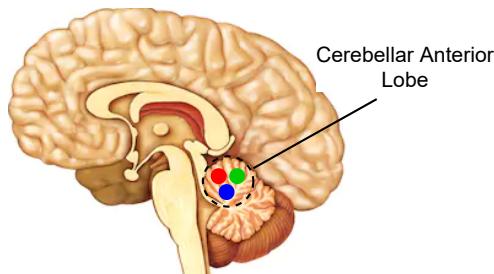
anatomic location
Basal Ganglia
Cerebellar Anterior Lobe
Cerebral Cortex
Cranial Nerves
Intraventricular
MCP/ICV
Septum
Spinal Cord

Single cell transcriptomics



- ▶ intratumoral heterogeneity
- ▶ putative cells of origin

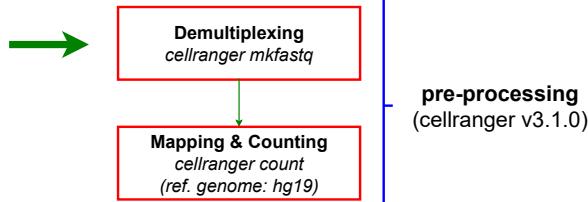
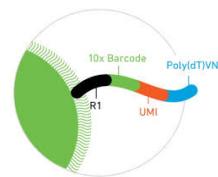
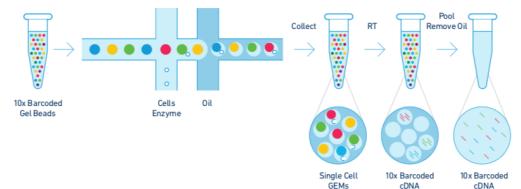
Biological sample and data pre-processing



FACS-sorted
(CD45-) tumor
cells

INI254, INI255, INI254
(all from the CAL region)

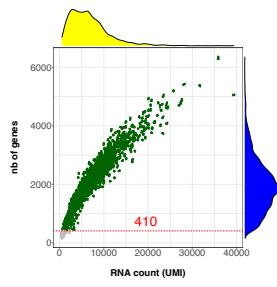
Droplet-based technology
(Illumina 10x genomics)



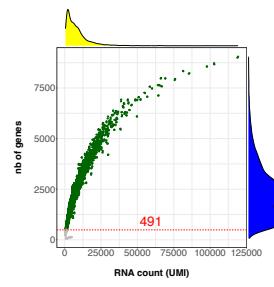
Cell filtering

Adaptive threshold based filtering using the number of detected genes and mitochondrial transcript content as criteria applied for each sample

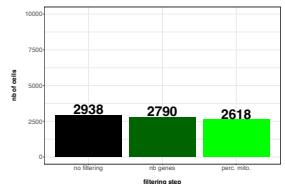
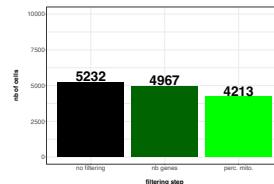
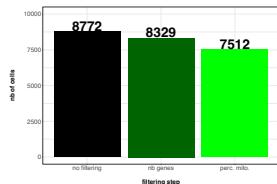
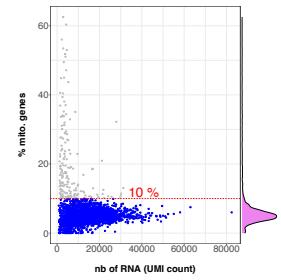
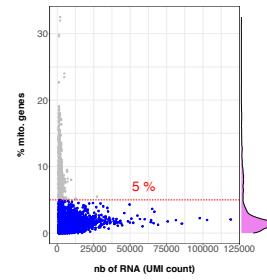
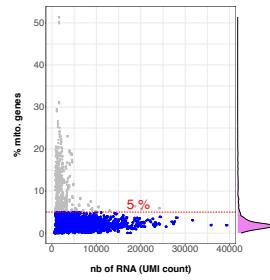
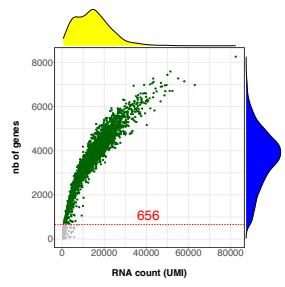
INI254



INI255

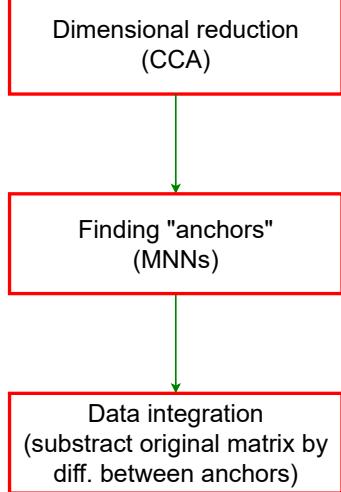


INI267

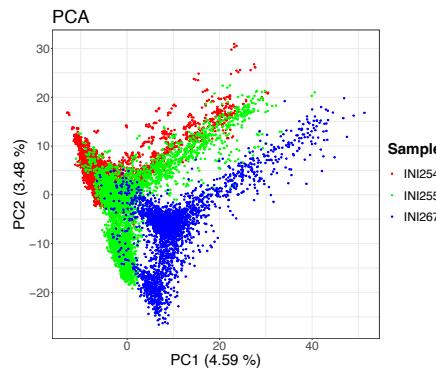


Data integration

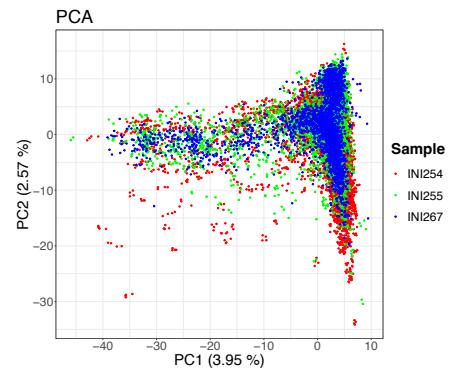
Seurat v3



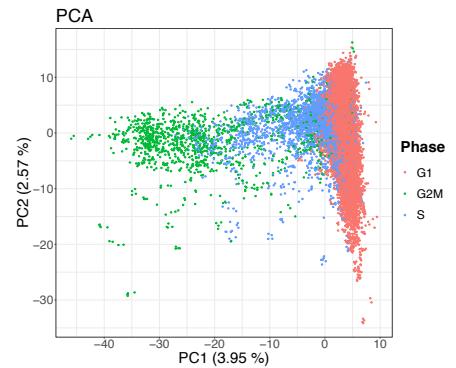
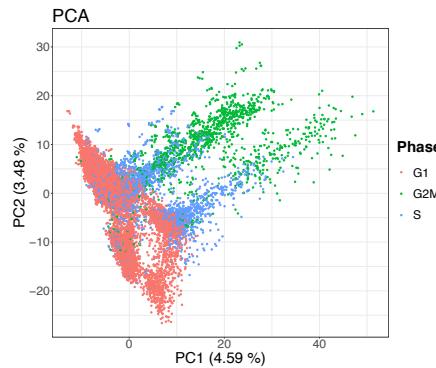
before integration



after integration



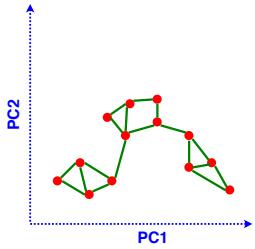
Stuart et al. (2019)



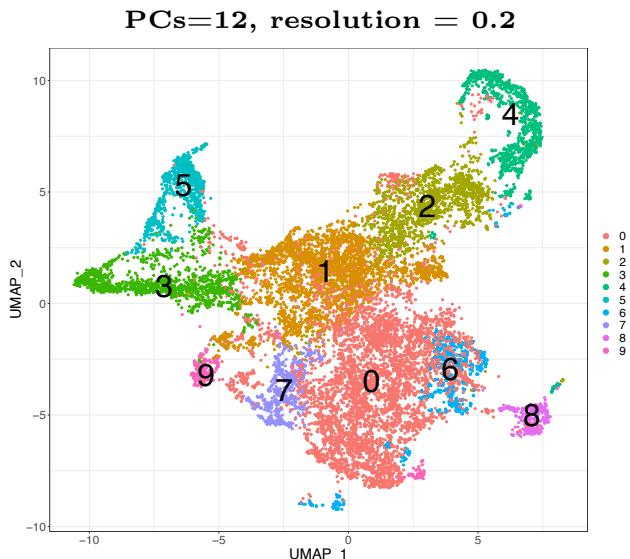
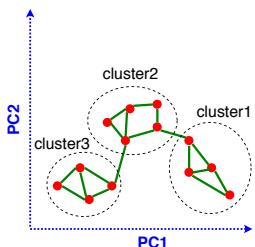
Cell clustering

Seurat v3

step 1: build
KNN/SNN graph (cells
as nodes)



step 2: cluster cells by
modularity
optimization (Louvain)

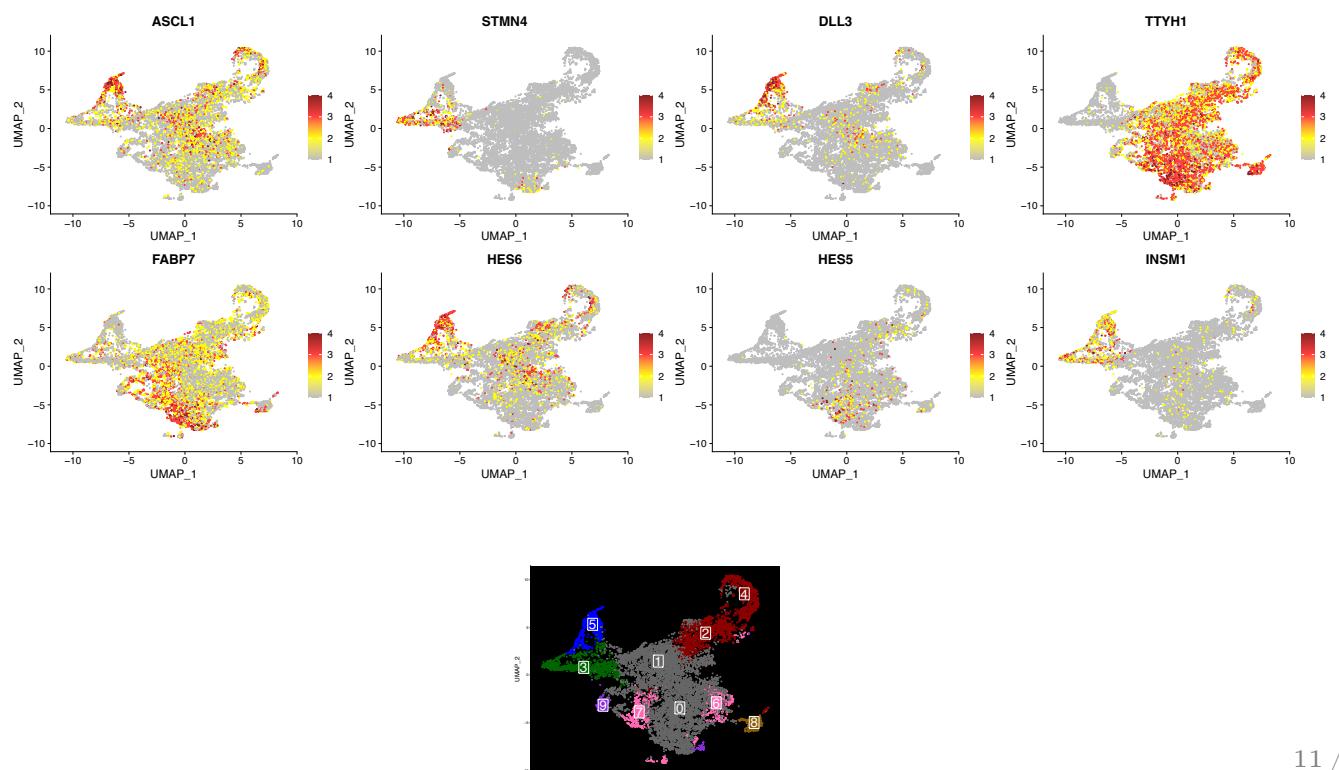


Stuart et al. (2019)

10 / 34

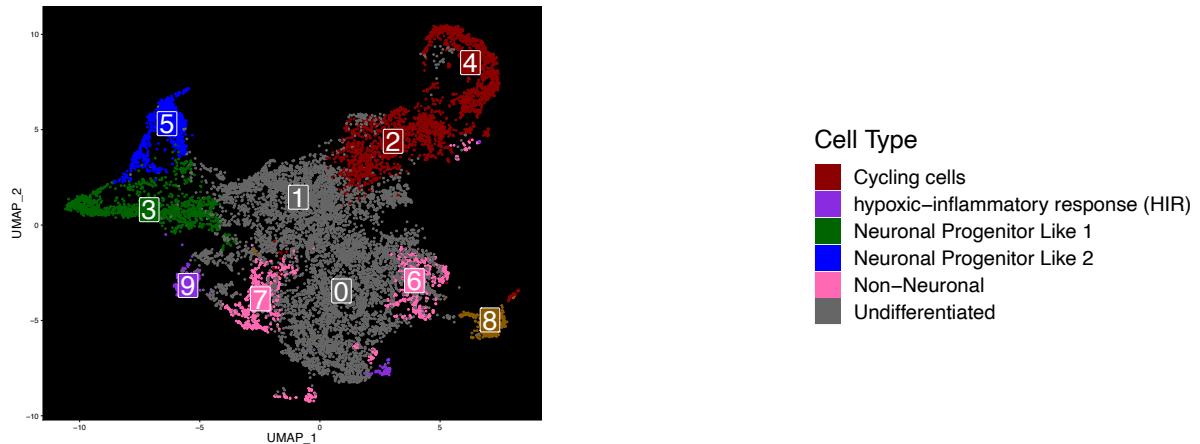
Intratumoral heterogeneity of CAL-ATRT (ATRT-SHH)

Previously identified ATRT-SHH gene signatures using bulk RNA-seq data (compared to other ATRT, Johann et al. (2016); Torchia et al. (2016)) show heterogenous expression at single cell level



Cell type annotation

- ▶ one versus all or pairwise differential analyses between clusters
- ▶ database and literature based cell annotation (Zeisel et al. (2018), Saunders et al. (2018))



- ▶ “undifferentiated” cluster 0 and 1 seem to be the core tumoral cells
- ▶ other methods such as correlation with published datasets and gene regulatory based network analysis were applied to confirm these annotations

Putative cells of origin

- ▶ “undifferentiated” cluster 0 and 1 the core tumoral cells and the neuronal progenitor cells the cells of origin?
- ▶ Trajectory inference analysis with cluster 0, 1, 3 and 5 to validate this hypothesis

