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Review article

Applications of single-cell RNA sequencing in drug discovery and development

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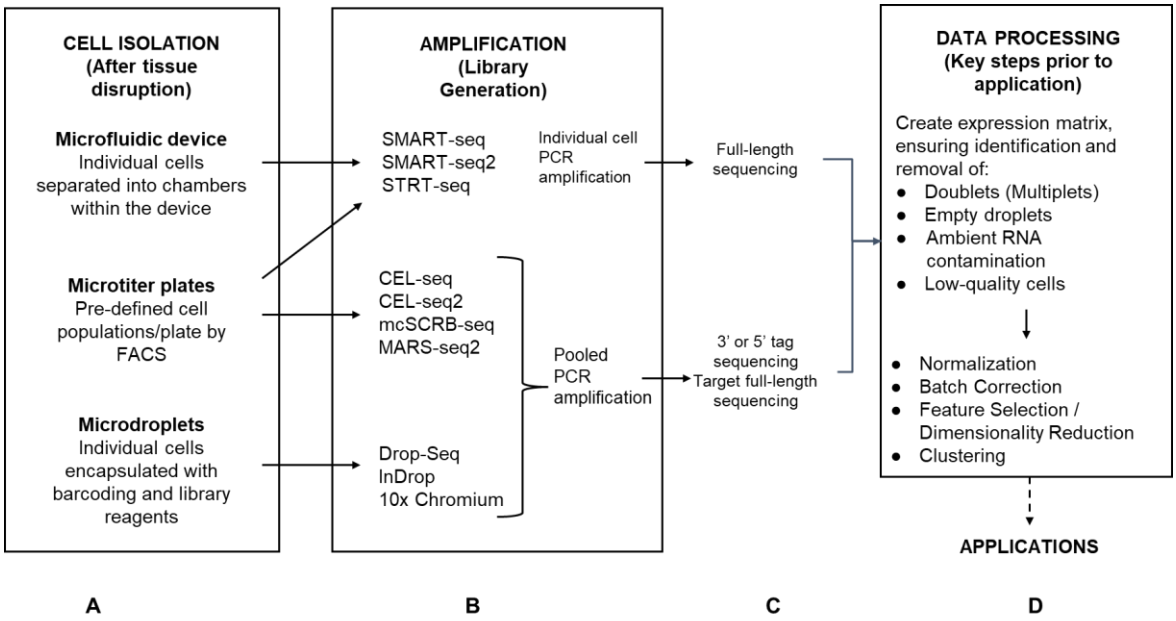
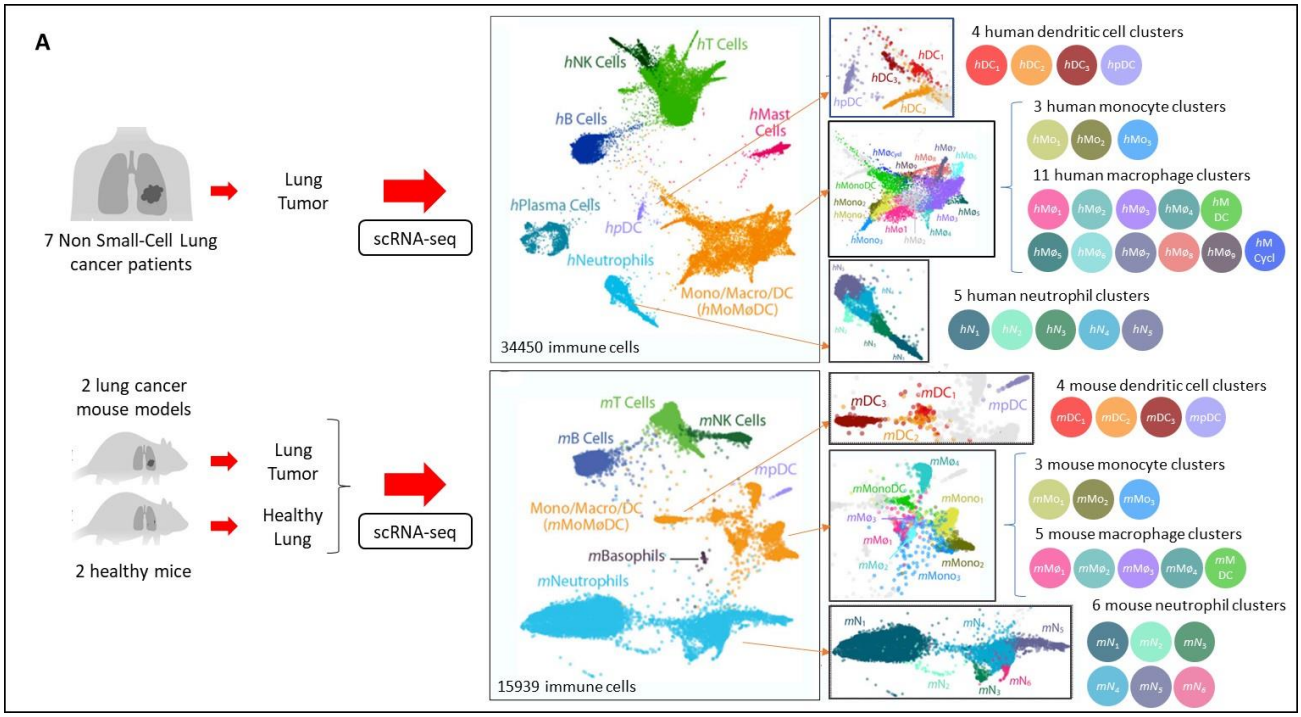


FIG. S1 | Core scRNA-seq methods for library generation and data processing.

Figure shows commonly used methods for SC isolation (A), library generation (B), types of data obtainable from the libraries (C) and data processing (D) prior to application. Amplification methods described are: SMART-seq[1], SMART-seq2[2], STRT-seq[3], CEL-seq[4], CEL-seq2[5], mcSCRB-seq[6], MARS-seq2[7], Drop-Seq[8], InDrop[9], 10x Chromium[10]. Widely used data processing include: a) Scrublet[11], DoubletFinder[12] and DoubletDecon[13] for removing multiplets; b) EmptyDrops[14] and DropletQC[15] for removal of empty droplets; c) DecontX[16] and SoupX[17] for removal of ambient RNA contamination; d) Cellity[18] for removal of low-quality cells; and e) SCRAN[19], SCTransform[20], and SCnorm[21] for normalization. Tran *et al*.(2020)[22] provide a review of batch correction methods. Feature Selection/Dimensionality Reduction methods are uniform manifold approximation and projection (UMAP)[23], t-Distributed Stochastic Neighbor Embedding (t-SNE)[24] and the application of a generalization of PCA to exponential family likelihoods for feature selection (reviewed by Townes *et al* [25]). For Clustering see benchmarking review in Duò, Robinson and Soneson[26].



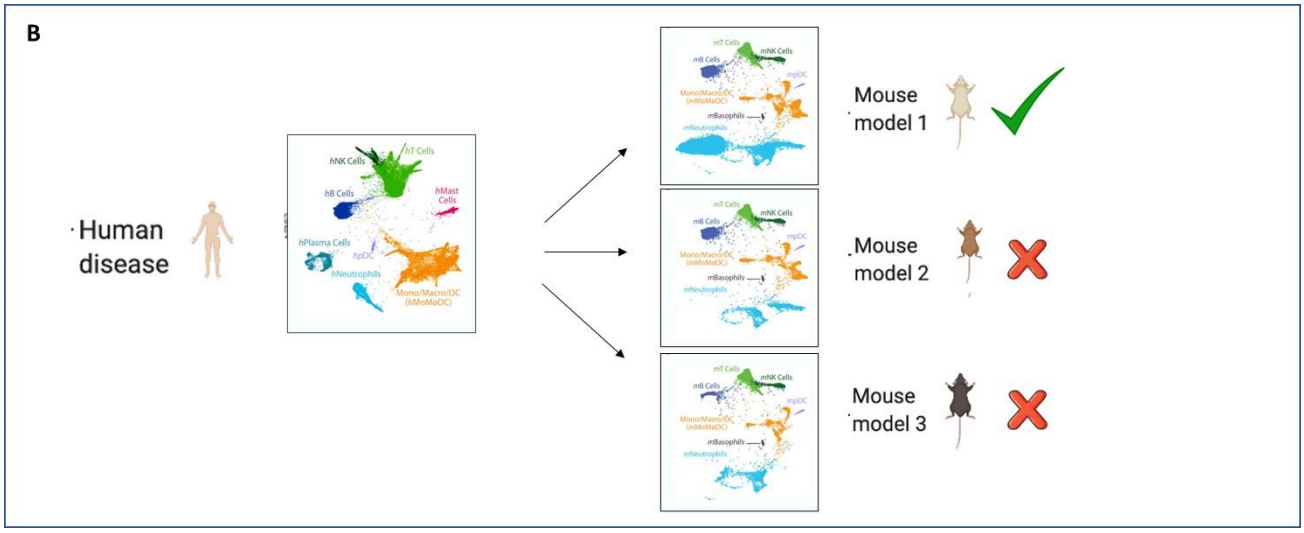


FIG. S2| Comparison of pre-clinical models to human disease

(A) Systematic comparison of tumor-infiltrating myeloid cell states in human and murine lung tumors reveals conserved myeloid populations across species. Five out of six neutrophil clusters in mice have similar expression to the 5 neutrophil clusters found in human. Main differences were found between macrophages; with 6 additional cluster in human, probably reflecting differences between the state of patient cancers or because of other reasons. In the Figure a same color was used for corresponding clusters between human and mouse.

(B) SC methodologies can be used to assess and compare tissue components between several animal models and human tissue collected from patients. Systematically benchmarking human versus different mouse models at the single cell level would thus open the possibility of better selecting the right model in preclinical studies (mouse model 1 in the here schematized example).

Elements of Part A and B are adapted with permission from ref 27, ELSEVIER

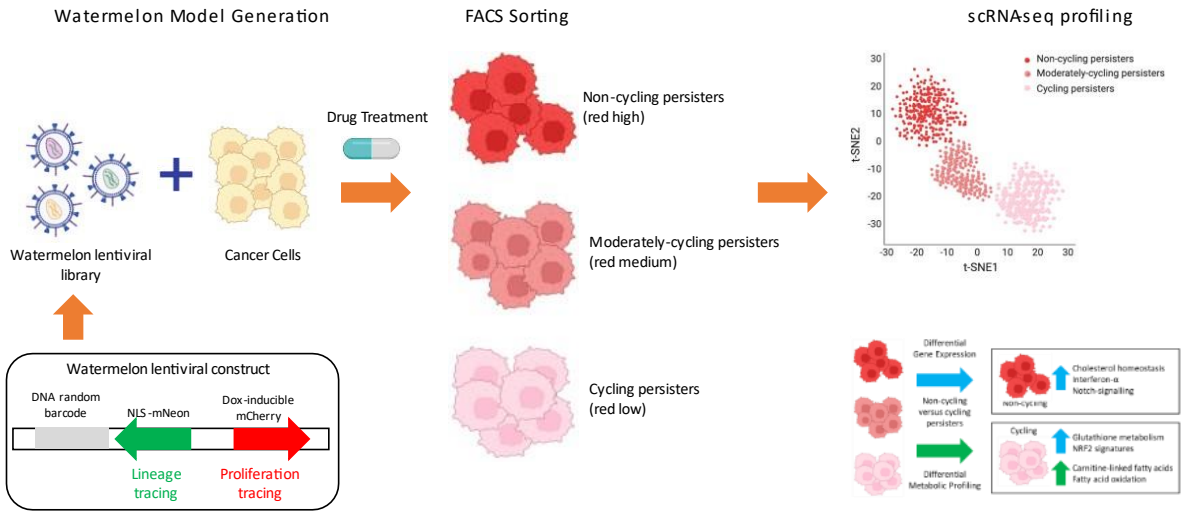


FIG. S3 | Drug Response Monitoring using Watermelon libraries

Relapse after cancer treatment is unfortunately common. Some cancer cells, called persisters, survive therapy and a subset can even keep on proliferating during treatment (cycling persisters). Watermelon, a high-complexity lentiviral barcode library, was developed to detect cycling persisters[28]. Green (mNeon) and red fluorescent (mCherry, inducible by doxycycline) reporters are used to simultaneously trace clonal lineages as well as the transcriptional and proliferative state of each cell in the population. The library is used to infect lung cancer cells which are then treated with a drug (the EDGF inhibitor osimertinib) for 14 days (+ doxycycline during the first 3 days). As red fluorescence is diluted with division events, cell-cycling persisters are less fluorescent than non-cycling ones and can thus be detected. After FACS sorting persister cells can be classified into three groups: non-cycling; cycling and an intermediate group between (low cycling). Single cell RNA sequencing of the cells and other approaches allows for further characterization of the molecular mechanisms associated with cycling persisters and why some of them can keep on cycling under treatment. Note that characterization of rare cell populations, like cycling persisters, can only be done by using single cell approaches.

Table S1: Computational tools typically used in drug discovery and development

PURPOSE	ALGORITHM/TOOL	DATA TYPE	URL TO ALGORITHM
Application | Data processing, quality control			
Ambient RNA removal	DecontX[16]	scRNA-seq	https://bioconductor.org/packages/release/bioc/html/celda.html
	SoupX[17]	scRNA-seq	https://github.com/constantAmateur/SoupX
Doublet detection	Scrublet[11]	scRNA-seq	https://github.com/swolock/scrublet
	DoubletFinder[12]	scRNA-seq	https://github.com/chris-mcginnis-ucsf/DoubletFinder
	DoubletDecon[13]	scRNA-seq	https://github.com/EDePasquale/DoubletDecon
Empty drops identification	DropletQC[29]	scRNA-seq	https://github.com/powellgenomicslab/DropletQC
	EmptyDrops[14]	scRNA-seq	https://bioconductor.org/packages/release/bioc/html/DropletUtils.html
Low Quality cells	Cellity[18]	scRNA-seq	https://github.com/Teichlab/cellity
Cell specific bias normalisation	SCRAN[19]	scRNA-seq	https://bioconductor.org/packages/release/bioc/html/scran.html
	SCTransform[20]	scRNA-seq	https://satijalab.org/seurat/articles/sctransform_vignette.html
	SCnorm[21]	scRNA-seq	https://www.bioconductor.org/packages/release/bioc/html/SCnorm.html
Batch correction	Harmony[30]	scRNA-seq	https://github.com/immunogenomics/harmony
	LIGER[31]	scRNA-seq	https://github.com/welch-lab/liger
	CCA in seurat	scRNA-seq	https://satijalab.org/seurat/
Application | Biological insights – cell type/genotype/phenotype classification			
Dimensional reduction	UMAP[23]	scMultiomics	https://cran.r-project.org/web/packages/umap/index.html
	t-SNE[24]	scMultiomics	https://github.com/klugerlab/FIt-SNE
Annotation	Seurat	CITE-seq scATAC-seq	Joint multimodal analysis https://satijalab.org/seurat/
	SingleR[32]	scRNA-seq	Cell type annotation http://www.bioconductor.org/packages/release/bioc/html/SingleR.html

	SingleCellNet[33]	scRNA-seq	Cell type annotation https://github.com/pcahan1/singleCellNet
	scmap[34]	scRNA-seq	Cell type annotation https://bioconductor.org/packages/release/bioc/html/scmap.html
	scPred[35]	scRNA-seq	Cell type annotation https://github.com/powellgenomicslab/scPred
	CellAssign[36]	scRNA-seq	Cell type annotation https://github.com/irrationone/cellassign
	Garnett[37]	scRNA-seq	Cell type annotation https://github.com/cole-trapnell-lab/garnett
	Cell-ID[38]	scRNA-seq	Cell type annotation https://bioconductor.org/packages/release/bioc/html/CelliD.html
Genotype/Copy Number Variation (CNV)	HMMcopy[39]	scDNA-seq	CNV detection https://bioconductor.org/packages/release/bioc/html/HMMcopy.html
	CopyNumber[40]	scDNA-seq	CNV detection https://bioconductor.org/packages/release/bioc/html/copynumber.html
	SCOPE[41]	scDNA-seq	CNV detection https://github.com/rujinwang/SCOPE
	CHISEL[42]	scDNA-seq	CNV detection https://github.com/raphael-group/chisel
	InferCNV[43]	scRNA-seq	CNV detection http://www.bioconductor.org/packages/release/bioc/html/infercnv.html
	CopyKat[44]	scRNA-seq	CNV detection https://github.com/navinlabcode/copykat
Genotype/Single Nucleotide Variation (SNV)	MonoVar[45]	scDNA-seq	SNV detection https://bitbucket.org/hamimzafar/monovar/src/master/
	SCcaller[46]	scDNA-seq	SNV detection https://github.com/biosinodx/SCcaller/
	SCAN-SNV[47]	scDNA-seq	SNV detection https://github.com/parklab/scan-snv

	SCIΦ[48]	scDNA-seq	SNV detection https://github.com/cbg-ethz/SCIPhI
	VarTrix[49]	scRNA-seq	SNV detection https://github.com/10XGenomics/vartrix
	SCmut[50]	scRNA-seq	SNV detection https://github.com/nghiavtr/SCmut
Genotype/eQTL	SCeQTL[51]	scRNA-seq	eQTL Analysis https://github.com/XuegongLab/SCeQTL
Reconstruction of TCR/BCR sequences	TraCer[52]	scRNA-seq	https://github.com/Teichlab/tracer
	BraCer[53]	scRNA-seq	https://github.com/Teichlab/bracer
	BALDR[54]	scRNA-seq	https://github.com/BosingerLab/BALDR
	VDJPuzzle[55]	scRNA-seq	https://github.com/simone-rizzetto/VDJPuzzle
	TRUST4[56]	scRNA-seq	https://github.com/liulab-dfci/TRUST4
TCR/BCR integrative analysis	scRepertoire[57]	scTCR/BCR-seq	http://www.bioconductor.org/packages/release/bioc/html/scRepertoire.html
	CellaRepertorium	scTCR/BCR-seq	http://www.bioconductor.org/packages/release/bioc/html/CellaRepertorium.html
Application | Biological insights – cell states and signalling			
Trajectory analysis	Monocle suite	scRNA-seq	https://cole-trapnell-lab.github.io/monocle3/
	PAGA[58]	scRNA-seq	https://github.com/theislab/paga
	Slingshot[59]	scRNA-seq	https://www.bioconductor.org/packages/release/bioc/html/slingshot.html
	STEMNET[60]	scRNA-seq	https://bioinformaticshome.com/tools/rna-seq/descriptions/STEMNET.html
	Scorpius[61]	scRNA-seq	https://github.com/rcannood/SCORPIUS
RNA velocity	Velocyto	scRNA-seq	https://github.com/velocyto-team
	CellRank[62]	scRNA-seq	https://github.com/theislab/cellrank
	scVelo[63]	scRNA-seq	https://github.com/theislab/scvelo
Pathway analysis	PAGODA2[64]	scRNA-seq	Identification of Cell Relationships

			https://github.com/kharchenkolab/pagoda2
	PLAGE[65]	scRNA-seq	http://dulci.biostat.duke.edu/pathways DEAD
	GSVA[66]	scRNA-seq	https://bioconductor.org/packages/release/bioc/html/GSVA.html
	Vision[67]	scRNA-seq	https://github.com/YosefLab/VISION
Cell-to-cell communication	CellPhoneDB[68]	scRNA-seq	https://github.com/Teichlab/cellphonedb
	ICELLNET[69]	scRNA-seq	https://github.com/soumelis-lab/ICELLNET
	NicheNet[70]	scRNA-seq	https://github.com/saeyslab/nichenetr
	CellChat[71]	scRNA-seq	https://github.com/sqjin/CellChat
Gene regulatory network inference	SCENIC[72]	scRNA-seq	https://scenic.aertslab.org/
	PIDC[73]	scRNA-seq	https://github.com/Tchanders/NetworkInference.jl
Application | Biological insights – functional assignment/multi-modal & bulk tissue type composition			
Joint multi-modal analysis	WNN in Seurat[74]	scRNA-seq/scATAC-seq	https://satijalab.org/seurat/index.html
	WNN in Seurat[75]	CITE-seq	
	TotalVI[76]	scMultiomics	https://scvi-tools.org/
	CiteFuse[77]	CITE-seq	https://github.com/SydneyBioX/CiteFuse/
	MOFA+[78]	scMultiomics	https://github.com/bioFAM/MOFA2
	LIGER[79]	scMultiomics	https://github.com/welch-lab/liger
	iNMF	scMultiomics	
Bulk transcriptomics deconvolution	MuSiC[80]	scRNA-seq/Bulk RNA-seq	https://github.com/xuranw/MuSiC
	scMappR[81]	scRNA-seq/Bulk RNA-seq	https://cran.r-project.org/web/packages/scMappR/index.html
	RNASieve[82]	scRNA-seq/Bulk RNA-seq	https://github.com/songlab-cal/rna-sieve
	CIBERSORTx[83]	scRNA-seq/Bulk RNA-seq	https://cibersortx.stanford.edu/
	EcoTyper[84]	scRNA-seq/Bulk RNA-seq/Spatial transcriptomics	https://ecotyper.stanford.edu/
	bMIND[85]	scRNA-seq/Bulk RNA-seq	https://github.com/randel/MIND
Spatial transcriptomics (ST) deconvolution	RCTD[86]	Spatial transcriptmics/scRNA-seq	https://github.com/dmcable/spacexr

	SPOTlight[87]		https://github.com/MarcElosua/SPOTlight
	Stereoscope[88]	Spatial transcriptmics/scRNA-seq	https://docs.scvi-tools.org/en/stable/user_guide/models/stereoscope.html
	Cell2location[89]	Spatial transcriptmics/scRNA-seq	https://github.com/BayraktarLab/cell2location
	SpatialDWLS[90]	Spatial transcriptmics/scRNA-seq	https://github.com/rdong08/spatialDWLS_dataset
	CARD[91]	Spatial transcriptmics/scRNA-seq	https://github.com/YingMa0107/CARD
ST neighborhood analysis	Giotto[92]	Spatial transcriptmics/scRNA-seq	https://github.com/RubD/Giotto
	Squidpy[93]	Spatial transcriptmics/scRNA-seq	https://github.com/scverse/squidpy
	CellTrek[94]	Spatial transcriptmics/scRNA-seq	https://github.com/navinlabcode/CellTrek
Application | Biological insights – functional assignment/target and biomarker discovery			
Perturbation analysis	MIMOSCA[95]	scCRISPR screening	https://github.com/asncd/MIMOSCA
	MUSIC[96]	scCRISPR screening	https://github.com/bm2-lab/MUSIC
	scMAGeCK[97]	scCRISPR screening	http://www.bioconductor.org/packages/release/bioc/html/scMAGeCK.html
	Mixscape[98]	scCRISPR screening	https://satijalab.org/seurat/articles/mixscape_vignette.html
Cell type prioritization	Augur[99]	scRNA-seq/scATAC-seq	https://github.com/neurorestore/Augur
	MELD[100]	scRNA-seq	https://github.com/KrishnaswamyLab/MELD
	Scissor[101]	scRNA-seq/Bulk RNA-seq	https://github.com/sunduanchen/Scissor

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