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Perspective

A clinical road map for single-cell omics

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SUMMARY

In a matter of years, single-cell omics has matured from a pioneering technique employed by just a handful of specialized laboratories to become a ubiquitous feature of biological research and a key driver of scientific discovery. The widespread adoption and development of single-cell omic assays has sparked mounting enthusiasm that these technologies are poised to also enhance the precision of diagnosis, the monitoring of disease progression, and the personalization of therapeutic strategies. Despite initial forays into clinical settings, however, single-cell technologies are not yet routinely used to inform medical or surgical decision-making. Here, we identify and categorize key experimental, computational, and conceptual barriers that currently hinder the clinical deployment of single-cell omics. We focus on the potential for single-cell transcriptomics to guide clinical decision-making through the development of combinatorial biomarkers that simultaneously quantify multiple cell-type-specific pathophysiological processes. We articulate a framework to identify patient subpopulations that stand to benefit from such biomarkers, and we outline the experimental and computational requirements to derive reproducible and actionable clinical readouts from single-cell omics.

INTRODUCTION

The parcellation of human pathology into taxonomies of diseases and their subtypes has been a central aim of medicine for millennia. These taxonomies initially grew out of attempts to organize clinical observations and were gradually refined as new technologies for physiological, biochemical, and radiological measurement were developed and then implemented in clinical practice.[1] Over the past century, the taxonomy of human disease has been refined based on increasingly precise molecular measurements.[2] The application of genomics, transcriptomics, proteomics, metabolomics, and lipidomics to human tissues has produced detailed molecular portraits of many diseases. Refinements to these assays that allowed them to achieve single-cell resolution, and the advent of single-cell omics as a ubiquitous feature of biomedical research, are further deepening our ability to measure human pathophysiology by capturing the full spectrum of molecular and cellular dysregulation that occurs in disease (see Table 1 for glossary of terms).

Since the sequencing of the first single-cell transcriptome in 2009,[3] single-cell omics has scaled to entire organisms[4–10] and has become a ubiquitous feature of biomedical research. The ability to measure the cellular and molecular alterations induced by disease and biological perturbations has enabled profound discoveries across many fields of science. In neuroscience, single-cell omics has identified subpopulations of neurons that control torpor, regulate thirst, or can restore walking after paralysis.[11–13] In cancer biology, single-cell omics has revealed that the transcriptional programs of malignant cells in glioblastoma converge on four cardinal cellular states, each associated with distinct genetic drivers.[14] In regeneration, single-cell omics has uncovered recovery-organizing cells that orchestrate the regrowth of injured limbs, organs, and damaged nervous systems.[15–19]

The impact of single-cell omics on scientific discovery stands in marked contrast with its impact on clinical practice, which has to date been minimal. Why have the impressive increases in the resolution of molecular and cellular measurements attendant on the maturation of single-cell omics not translated into improved diagnosis, prognosis, or management of human disease? We argue that this limited clinical impact is due to multifaceted experimental, computational, and conceptual barriers that remain to be overcome for single-cell omics to be translated into clinical and surgical environments. We focus our discussion on single-cell transcriptomics as the most technically, conceptually, and commercially mature single-cell omic assay and therefore the most primed for clinical translation. However, the experimental, computational, and conceptual barriers that we outline extend to other single-cell omic approaches, such as

Table. 1. Glossary of terms

Term	Definition
Single-cell omics	a family of technologies that measure thousands of analytes (for instance, transcripts, proteins, or metabolites) simultaneously within individual cells, allowing the detection of disease-associated molecular changes at the resolution of individual cells or cell types
Bulk omics	in contrast to single-cell omics, technologies that measure the average abundance of analytes across all the cells in a biological sample, which can mask molecular alterations within specific cell types or conflate changes in cell-type abundance with cell-type-intrinsic alterations
Single-cell transcriptomics	a single-cell omic assay that involves measuring the expression of thousands of genes within individual cells by RNA sequencing
Single-nucleus transcriptomics	a variation of single-cell transcriptomics that sequences RNA from isolated nuclei instead of whole cells, enabling analysis of frozen or difficult-to-dissociate tissues
Molecular biomarkers	biomolecules present in human tissues or biofluids that reflect physiological or pathophysiological processes and can be used to diagnose disease, stage disease severity, prognosticate disease outcomes, or guide treatment
Coordinated multicellular biomarkers	diagnostic or prognostic tools that integrate measurements made within multiple cell types simultaneously
Library preparation	in sequencing assays, the process of converting the DNA or RNA present in a biological sample into a format compatible with the chosen sequencing technology
Gene signature	a coordinated group of genes implicated in the same physiological or pathophysiological process, whose expression is often summarized with a single statistic (e.g., the mean expression of all genes in the signature)
Multimodal single-cell omics	the simultaneous measurement of multiple molecular features—such gene expression and surface protein abundance—within the same cell
Standard operating procedures (SOPs)	reproducible protocols for experimental and computational workflows, often in the form of step-by-step instructions, which codify routine laboratory operations
Clustering	a form of unsupervised machine learning in which a set of objects is grouped in such a way that objects in the same group are more similar to one another than to those in other groups; a fundamental operation in the analysis of single-cell omics data, which is used to identify cells of the same type or state
Differential expression	a form of statistical analysis that enables the identification of genes whose expression demonstrates significant differences between groups of cells; a fundamental operation in the analysis of single-cell transcriptomics data, which enables the identification of cell types and disease-associated molecular programs
Pseudotime analysis	a computational method that orders cells along a trajectory in order to assign each cell a position along a continuous biological process, such as cell differentiation or disease progression
Hierarchical data	a structure in which data are nested—e.g., cells within the same cell type or originating from the same patient—and which may require statistical methods that account for non-independence between observations
Count splitting	a computational approach in which a matrix of gene expression values derived from RNA sequencing is partitioned into two gene expression matrices based on an underlying distributional assumption, enabling cell-type identification (via clustering) to be performed on a different subset of the data from that used to identify marker genes of each cell type (via differential expression)
Double dipping	a statistical error where the same dataset is used for both hypothesis generation and testing, leading to overfitting and spurious findings
Pseudobulk analysis	an approach that aggregates data from individual cells, often of the same inferred cell type, to produce a single gene expression profile for each biological replicate, and which has been repeatedly demonstrated to reduce false discoveries
Differential abundance	A form of statistical analysis that seeks to identify cell types or states whose relative abundances change significantly between two conditions (for instance, health versus disease)
Validation cohort	a group of patients independent from those used to identify a biomarker, in which the diagnostic or prognostic performance of that biomarker can be evaluated
Phenotype prediction	in the context of this perspective, the use of statistical or machine learning methods to infer clinical features such as disease status or treatment response based on single-cell omics data

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Table. ***Continued***

Term	Definition
Cell quality control, doublet removal, ambient RNA removal, data integration, and cell-type annotation	a series of steps that have become established as routine steps in the preprocessing of single-cell transcriptomics data, before any biological inferences are drawn; specifically: ● cell quality control: removal of low-quality single-cell transcriptomes ● doublet removal: removal of single-cell transcriptomes that actually arise from mixtures of two cells sequenced together ● ambient RNA removal: removal of artifactual gene expression associated with cell lysis ● data integration: analysis of single-cell omics data acquired at different times, in different batches, or in different laboratories in a manner that removes or mitigates technical differences associated with the time, batch, or laboratory of origin ● cell-type annotation: computational assignment of cell types (e.g., T cell, fibroblast) to each single-cell transcriptome
Clinically actionable readout	a measurement that alters the standard of care; for instance, identifying a new biological pathway active in the innate immune response to bacteria from clinical data is not itself a clinically actionable readout, but identifying bacterial resistance to a given antibiotic in a patient with pneumonia is clinically actionable
Multiple instance learning	a machine learning paradigm in which a model receives a set of data points (for instance, individual cells) that all share the same label (for instance, disease versus control), rather than being labeled individually, and is tasked with making a single prediction for all data points
Attention	an architectural component of many modern deep neural networks, which allows these models to determine the relative importance of each item in a set (for instance, an individual cell), relative to the other items in that set, when making a prediction
Benchmarking	the process of comparing different computational or experimental workflows on the basis of a common set of objective metrics
Single-cell assay for transposase-accessible chromatin (ATAC-seq)	a single-cell omics method that enables measurement of chromatin accessibility across the genome within individual cells
Spatial omics	techniques that measure many analytes at once within cells in their native tissue context, maintaining the spatial relationships between cells that occur within a healthy or diseased tissue

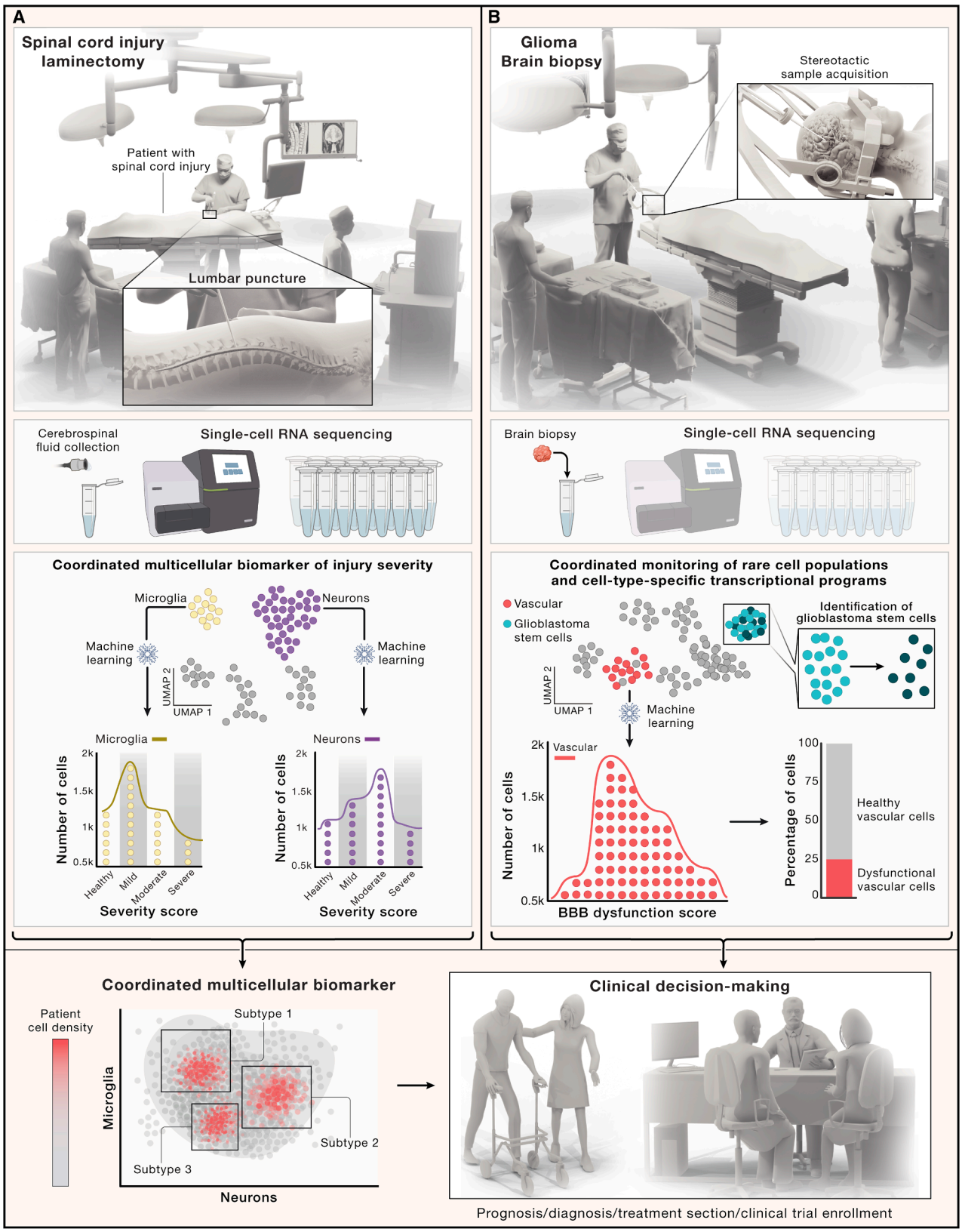
single-cell measurements of chromatin accessibility or protein abundance.

BIOMARKERS: THE ENTRY POINT INTO CLINICAL MEDICINE

Historically, advances in measurement technologies have generally had their most immediate impact on clinical care by advancing the diagnosis of human disease. These diagnostic advances have often taken the form of new molecular biomarkers.[20] The development of conventional "bulk" omic techniques that measure the average abundance of analytes over all of the cells within a biological sample led to numerous discoveries in preclinical models that have now been directly integrated into patient care through the clinical implementation of new biomarkers. For instance, sequencing technologies are now routinely used in the clinic to identify relevant cancer mutations, direct targeted therapy, or diagnose genetic diseases.[21–26]

The increased resolution afforded by single-cell transcriptomics has sparked enthusiasm that this technology could further enhance the precision of diagnoses, monitoring of disease progression, and accuracy of personalized medicine.[27,28] Because bulk transcriptomic profiling of biological tissues conflates changes in cell-type proportions with cell-type-intrinsic alterations in gene expression, these measurements can overlook important aspects of disease physiology or even lead to false discoveries.[29,30] A canonical example is in neurodegenerative diseases, where the apparent downregulation of genes associated with synaptic function actually reflects loss of neurons and the attendant increase in glial cell fraction.[30] Bulk transcriptomic profiling can also overlook cell-type-specific transcriptional changes, particularly in cell types that are comparatively rare in the tissue of interest; for instance, microglia differentially express hundreds of genes in response to neuroinflammatory stimuli, which cannot be detected by bulk transcriptomic analysis of whole-brain tissue.[30] By decoupling these axes of biological variability, single-cell transcriptomics has the potential to expose clinically meaningful alterations within specific cellular subpopulations that may provide more precise and accurate biomarkers of disease. Studies of the central nervous system, for instance, have decoupled shifts in cell-type proportions from cell-type-specific differential expression by revealing neuronal subtype-specific responses to spinal cord injury (SCI)[31] or by identifying subpopulations of neurons that are uniquely resilient or susceptible to injury or disease.[32,33]

The clinical impact of biomarkers derived from single-cell transcriptomics could be more pervasive than is currently appreciated (Figure 1). Consider, for example, the potential clinical application of single-cell transcriptomics in the context of traumatic SCI. This is a disease for which the clinical diagnosis is rarely difficult and for which effective pharmacotherapies do not exist. Nonetheless, cell-type-specific biomarkers could guide the clinical implementation of promising therapeutic



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approaches that are emerging in preclinical models. In SCI, the initial traumatic insult to the spinal cord is not necessarily the primary determinant of neurological outcome. This insult also initiates a complex and progressive cascade of biochemical processes, collectively termed the "secondary injury,[34]" involving all the major cell types and subtypes of the central nervous system.[35–38] Historically, therapeutic approaches to SCI have generally sought to target aspects of the secondary injury response within individual cell types—for instance, by inhibiting immune responses or promoting neuronal sprouting.[39,40] However, despite decades of research, these approaches have failed to translate into effective therapies.[41–43] More recently, coordinated targeting of multiple cell-type-specific responses, including astrocyte, neuronal, vascular, and immune cell-specific programs, has produced more promising therapies that can reverse paralysis in preclinical models.[44,45] Clinical translation of these therapies requires that these multicellular responses are similarly modulated and targetable in human patients. Yet, if the responses of each cell type to each individual component of these multipronged strategies cannot be measured, it could remain permanently unclear which aspects of these treatments have succeeded or failed. It is reasonable to expect, then, that translating multipronged therapies that target multiple cell-type-specific responses in tandem may require multicellular biomarkers to determine the profile of patients who stand to benefit from these therapies.

The example of SCI illustrates the potential clinical impact of biomarkers that monitor coordinated but cell-type-specific molecular programs, even in diseases for which there is little diagnostic uncertainty. The potential benefit of biomarkers based on single-cell transcriptomics could be considerably larger in diseases for which diagnosis is challenging, or when clinically meaningful subtypes exist; where accurate prognostication requires the integration of multiple cell-type-specific responses; or where more precise diagnosis or prognosis would alter the nature or timing of therapy—points to which we return below. Conversely, many clinical contexts are unlikely to benefit from the deployment of single-cell omics, either because diagnosis or management is relatively unambiguous or because clinically actionable information can be obtained without measuring many analytes across multiple cell types, and we outline a framework to prioritize settings that justify the additional cost and complexity of single-cell omic analysis.

BARRIERS TO CLINICAL TRANSLATION

At present, however, this potential benefit is entirely theoretical. Why has single-cell transcriptomics failed to impact clinical practice thus far, and what factors have prevented its integration into clinical decision-making? Here, we distinguish three categories of barriers that currently hinder single-cell technologies from realizing their transformative potential in clinical practice: experimental barriers, computational barriers, and conceptual barriers (Figure 2).

Experimental barriers

Arguably the most significant barrier to the identification and translation of biomarkers based on single-cell transcriptomics is the relatively high cost and limited scalability of existing single-cell assays. Clinical discoveries that emerged from bulk omics were enabled by studies that profiled large cohorts of patients, often comprising hundreds of individuals. In contrast, only a handful of studies have profiled cohorts of comparable size with single-cell transcriptomics.[46–49] Even cohorts comprising dozens of patients have been uncommon.

Human diseases are inherently heterogeneous. Cellular and molecular manifestations of disease vary according to the age, sex, genetic background, exposure history, disease severity, time since onset, and medication history of each individual patient. Consequently, it is unlikely that cohorts comprising only a handful of patients will support the discovery of robust and generalizable biomarkers. Instead, assembling large patient cohorts that capture the full spectrum of disease manifestations will be necessary to identify disease-associated molecular programs that transcend patient-specific biological heterogeneity and inevitable technical variability.

On the other hand, assembling large patient cohorts alone cannot overcome arbitrary variability introduced at the level of the study design. For example, atlases that span multiple primary cancer types in a single study are instrumental for biological discovery, but they will not support the identification of reliable biomarkers within specific patient populations, such as a group of patients undergoing an identical course of therapy for a single type of cancer. Studies that seek to identify robust molecular and cellular biomarkers will require a more thoughtful approach to cohort ascertainment in order to mitigate biological variability that is irrelevant to the clinical deployment of these biomarkers.

Until recently, efforts to profile large cohorts of patients with single-cell transcriptomics have been hindered by the cost of these measurements and the time-intensive nature of library preparation, combined with the difficulty of procuring fresh biological tissues. Advances in the scalability of single-cell technologies (for instance, probe-based multiplexing or well-based combinatorial indexing) are poised to increase the number of samples that can be profiled in a single experiment.[5,50,51] Moreover, technological advances in the recovery of RNA transcripts from formalin-fixed or frozen tissues may open new opportunities to leverage existing hospital tissue banks for biomarker discovery.[52] Combining these advances with judicious patient ascertainment could accelerate the pace at which reliable biomarkers linked to cell-type-specific molecular alterations are discovered and then translated into clinical practice.

Figure 1. Transformative possibilities for actionable clinical biomarkers derived from single-cell omics

Illustrative examples of clinical applications for biomarkers derived from single-cell omics.

(A) A coordinated multicellular biomarker to stratify patients with spinal cord injury (SCI) based on continuous cell-level distributions of neuronal and microglial gene expression programs.

(B) A cellular biomarker for glioma based on the presence of rare cell populations and concomitant dysregulation of the blood-brain barrier (BBB).

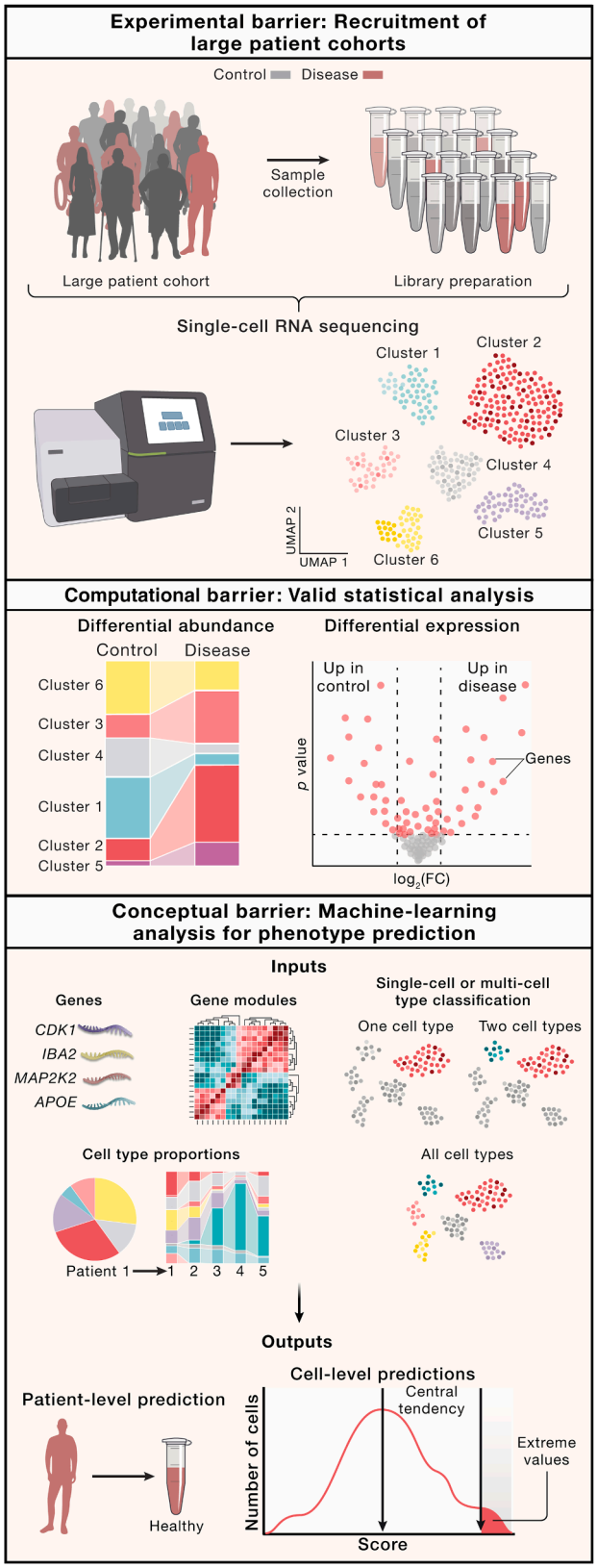


Figure 2. Experimental, computational, and conceptual barriers to the implementation of coordinated multicellular biomarkers based on single-cell transcriptomics

Top, recruitment of large patient cohorts as a central experimental barrier. Bottom left, valid statistical analysis as a central computational barrier. Bottom right, design of machine learning models for patient-level phenotype prediction from single-cell profiles as a central conceptual barrier.

Despite these advances, technical challenges remain. For instance, investigators wishing to profile transcriptomes at single-cell resolution must choose between sequencing RNA from whole, intact cells (single-cell transcriptomics per se) or sequencing only nuclear RNA from isolated nuclei (single-nucleus transcriptomics). The latter has the advantage that it enables profiling of tissues that are difficult to dissociate mechanically or enzymatically, and it mitigates transcriptional dysregulation associated with the dissociation procedure itself. Yet, it is becoming clear that single-cell and single-nucleus transcriptomics both fail to recover certain cell types or gene signatures, raising the possibility that relevant biomarkers may be overlooked by either methodology.[53,54] Moreover, it is possible that accurate diagnosis or prognosis of certain diseases may require information that is not captured in the transcriptome but that may manifest exclusively in the epigenome, proteome, or metabolome. Despite advances in multimodal single-cell profiling,[55] the cost and complexity of these technologies generally restrict investigators to the selection of a single modality for analysis, particularly when studying larger cohorts of patients.

An additional experimental challenge for the clinical translation of single-cell transcriptomics relates to the regulatory environment under which these biomarkers will be approved for clinical deployment. Current standards require that the results obtained from any given clinical sample can be independently reproduced by other laboratories provided with the same sample.[56] Achieving this level of reproducibility will require major investments of time and resources to standardize the preparation of tissues, the single-cell profiling of these tissues, and computational analyses of the resulting sequencing data. This standardization will need to account for the heterogeneity of hospital environments and will require the training of skilled personnel across hospitals and jurisdictions to perform and interpret identical assays according to standard operating procedures (SOPs). The cost and complexity of this standardization will likely foster new industrial partnerships that will be necessary to achieve the marriage of biomedical expertise with the biotechnological foundation required to develop and implement single-cell assays.

Computational barriers

Beyond experimental barriers, there are also important computational barriers that will need to be overcome in order to realize the potential clinical impact of single-cell transcriptomics. These challenges can be divided broadly into barriers to the discovery of candidate biomarkers and barriers to their clinical implementation.

In the early stages of single-cell transcriptomics, both experimental methodologies and computational pipelines advanced rapidly and in tandem. During this formative period, computational biologists drew heavily on methods originally developed

in the context of bulk transcriptomics.[57–59] Later developments identified purportedly distinct statistical properties of single-cell transcriptomics data, and methods tailored to these properties were introduced.[60–63] Many of these assumptions and computational approaches became entrenched in standard workflows.[64] Over time, it has become evident that many of these workflows rely on flawed methodological or statistical assumptions.[65–68]

The widespread use of inappropriate statistical methodologies to interrogate transcriptomic data presents a major barrier to the discovery of robust and generalizable biomarkers, because of the tendency for these methodologies to produce false discoveries. For instance, clustering a single-cell dataset to identify cell types and then using the same data to identify genes differentially expressed between cell types represents a form of statistical "double dipping" that produces false discoveries, even in the absence of any underlying structure.[69,70] The same fundamental issue recurs in other widespread computational paradigms, such as pseudotime analysis. The emergence of false discoveries is further compounded by the ubiquitous use of statistical methods that inappropriately treat individual cells as the experimental unit of observation.[68,71] In reality, single-cell data have a hierarchical structure, such that cells from the same patient cannot be treated as statistically independent. Failing to account for biological variation between samples conflates variation within and between replicates and can produce thousands of false discoveries in the absence of meaningful differences.[68] A third challenge arises from the desire to identify cell types or states that vary in abundance as a function of disease. Because single-cell transcriptomics measures the relative proportions of cell types and states rather than their absolute abundances, an increase in the abundance of one cell type can produce the false impression that the abundances of all other cell types have decreased.[72]

Efforts to clinically validate and translate biomarkers identified by statistical methods that are prone to producing false discoveries are unlikely to be successful. Fortunately, alternatives are available. Count splitting separates latent variable estimation from statistical inference to enable valid differential expression after clustering or pseudotime analysis.[69] Generalized linear mixed models or "pseudobulk" differential expression approaches account for variation between biological replicates and thereby avoid false discoveries.[68,71,73,74] Numerous methods enable valid differential abundance analysis by accounting for the compositional nature of single-cell datasets.[72,75] However, adoption of these methods has been variable and incomplete.[68] Replacing inappropriate methodologies with statistically valid methods in standard analysis workflows is an important first step in making generalizable discoveries from single-cell data that can translate into clinical advances.

The application of single-cell transcriptomics to human tissues in clinical and surgical environments also introduces new computational challenges. The promise of biomarkers based on single-cell transcriptomics is to integrate multiple coordinated and cell-type-specific alterations into a single diagnostic or prognostic readout. Statistical or machine learning models provide a natural framework to realize this promise (albeit by no means the only one). In this framework, a model trained on single-cell transcriptomics data from one cohort of patients is then tasked with predicting phenotypes in new cohorts: for instance, to diagnose disease, stratify disease severity, or monitor therapy response.[76] This scenario, however, presents formidable computational challenges that have been addressed to only a limited extent. Even though a handful of models have been developed to predict phenotypes in unseen patients, they have taken contrasting methodological approaches; and because these efforts are still in their early stages, none of these methods has yet been tested in an independent validation cohort.[76–79] Consequently, there are numerous open questions about how the challenge of phenotype prediction from single-cell transcriptomics data might be most productively addressed by machine learning. Initial attempts have already exposed the central importance of accounting for both technical batch effects and biological heterogeneity in a principled manner. Other open questions related to the optimal representation of the input data and nature of the prediction task are more conceptual in nature, and they are therefore discussed further below.

Beyond phenotype prediction, the need to establish reproducible SOPs for the computational analysis of single-cell transcriptomics data will also introduce new computational challenges. At present, standard computational workflows for data analysis require a considerable degree of subjective manual intervention at essentially every stage after read alignment, including cell quality control, doublet removal, ambient RNA removal, data integration, and cell-type annotation.[80] The values of specific parameters that are selected at each of these steps can markedly alter the final dataset. The degree of subjectivity that is inherent to the analysis of single-cell datasets is at odds with the regulatory requirement that different laboratories produce identical clinical results for any given sample. Consequently, clinical deployment of single-cell transcriptomics will require the development of computational protocols that standardize and automate these steps for each specific disease context. This standardization is a daunting challenge that has not yet been successfully addressed. In order for single-cell omics to meet regulatory standards for clinical use, we argue that the development of reproducible, automated analysis workflows must be elevated to a central priority within the computational research community. Meeting this requirement may require a realignment of academic incentives, which currently favor the introduction of novel methods over the less glamorous work required to enable translation into clinical settings.

Conceptual barriers

Beyond the concrete experimental and computational barriers related to the implementation of single-cell transcriptomics in clinical settings, there are also broader unresolved conceptual issues that relate to realizing the clinical potential of these technologies. For example, which patient populations stand to benefit from biomarkers based on single-cell transcriptomics? And, for these patients, how can the coordinated and cell-type-specific molecular alterations associated with each disease be integrated to produce clinically actionable readouts?

For many diseases, clinical care will not directly benefit from single-cell transcriptomics or single-cell omics more broadly. Diagnosis, for instance, is unlikely to be advanced by

Box 1. Potential clinical applications of single-cell omics

Single-cell omics is now routinely being applied to profile human tissues, including in prospective clinical trials that seek to reveal new pathobiological mechanisms of human disease or nominate putative biomarkers of disease progression and responses to therapy.[99] Such studies, although exciting, fall short of implying a requirement for single-cell omics itself to be deployed in the clinic. Instead, biomarkers identified through such discovery efforts may be more straightforwardly measured with existing technologies that are well-established in diagnostic settings (for instance, immunochemical assays or flow cytometry).

We argue that there is a rationale for clinical deployment of single-cell omics only when clinical decision-making is contingent on the simultaneous measurement of many analytes across multiple cell types. For instance, clinical translation of therapies that target multiple cell-type-specific aspects of the response to SCI may require molecular biomarkers that can measure the corresponding cell-type-specific responses in order to identify patients who stand to benefit from these therapies. In this section, we highlight additional clinical scenarios that could benefit from such coordinated multicellular biomarkers.

In neurosurgical oncology, tumors such as meningiomas and chordomas are noted for the complexity of their evolution and response to therapies.[100,101] Most are indolent and can be managed conservatively or with limited surgical resection. A subset, however, follow a far more aggressive course characterized by repeated recurrence and progressive neurological impairment. Recent single-cell studies have begun to identify transcriptional programs and immune or stromal subpopulations—such as reactive leptomeningeal cells and immunosuppressive macrophages—that are enriched in tumors with early or frequent recurrence.[101–103] These cellular and molecular features have not historically been appreciated by histopathology and therefore have not informed prognostication or management, but their monitoring by single-cell omics could stratify patients to more intensive surveillance regimens or inform the enrollment of high-risk patients in trials of novel adjuvant therapies.

Single-cell omics could also advance cancer diagnostics at earlier stages—for instance, through the molecular characterization of circulating tumor cells (CTCs). These rare cells, which detach from primary tumors and enter the bloodstream or lymphatic system, can be isolated from whole blood and profiled by single-cell omics. Genomic or trancriptomic profiles of isolated CTCs could enable the evaluation of multiple clinically relevant parameters simultaneously, for instance, by inferring cell proliferation rates, predicting cell type and tissue of origin, or identifying the recurrence of treatment-resistant subclones. These readouts in turn could stratify high-risk patients to more intensive surveillance regimens, direct medical imaging to locate the primary tumor, enable diagnosis and staging of surgically inaccessible tumors, or guide therapeutic selection.

The broader potential for single-cell omics to guide personalized therapeutic selection based on patient-specific molecular alterations has already been recognized.[104] For instance, single-cell transcriptomics has informed personalized therapy for patients with refractory drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms (DiHS/DRESS) and idiopathic multicentric Castleman's disease.[27,105] In both cases, pathway enrichment analysis of single-cell transcriptomics data identified dysregulated signaling pathways that could be pharmacologically targeted with existing agents. The availability of large-scale resources cataloging transcriptional responses to pharmacological and genetic perturbations raises the possibility of matching patient-specific patterns of transcriptional dysregulation with compounds or knockouts that can elicit or reverse similar transcriptomic signatures.[106–108] In the setting of oncology, this approach could even be extended to individual subclones to enable the personalization of combination therapies. However, many unresolved questions remain about the regulatory frameworks that would be required for such computational approaches to advance from compassionate use to broader clinical adoption.

multicellular biomarkers when disease status can be unambiguously determined by simple laboratory or clinical investigations. Single-cell omics is not necessary to determine whether, for instance, a patient is hypertensive or presents with iron deficiency anemia. Similarly, for diseases with unambiguous indications for surgical management, such as appendicitis or hydrocephalus, single-cell omics is unlikely to have a meaningful impact at least on the initial stages of clinical decision-making.

Which diseases, then, do stand to benefit from single-cell biomarkers? The diseases that stand to benefit the most are those whose pathophysiology involves coordinated molecular alterations across multiple cell types and for which assessing those multicellular alterations can meaningfully improve diagnosis, management, or prognosis.[81] Improvements in diagnosis are most relevant when they deliver actionable information that rapidly alters management and changes the patient's prognosis. Improvements in management are most relevant when clinicians must consider a large number of possible therapeutic regimens or when the timing at which an intervention should be delivered is unclear.

Technical considerations also play a role. If clinically actionable information can be obtained without measuring many genes across multiple cell types, then clinical benefit may be more straightforwardly realized by simpler approaches that sort individual cellular subpopulations and quantify the abundance of individual transcripts or surface proteins—as is the standard of care, for instance, in many hematological malignancies.[82] Moreover, if the tissues within which a disease manifests are not clinically accessible, and other accessible tissues like blood, urine, or cerebrospinal fluid (CSF) cannot capture the relevant cellular and molecular alterations, then the potential benefit of biomarkers based on single-cell transcriptomics will be considerably diminished.

A number of diseases meet all of the above criteria and may therefore benefit from biomarkers derived from single-cell transcriptomics (Box 1). Most solid tumors, for instance, will be biopsied as part of standard practice, meaning that the diseased tissue is clinically accessible. Tumors are complex ecosystems that often comprise multiple malignant subclones as well as a microenvironment of stromal and immune cells, and each of these cellular compartments may influence disease progression.[83] Consequently, there is a clear rationale to monitor multicellular alterations within tumors at the scale of the whole transcriptome. Decades of research have shown that histologically uniform tumors can be differentiated based on their molecular profiles, and some of these molecular subgroups have distinct prognoses and characteristic responses to specific therapeutic regimens.[84–86] A single-cell biomarker for glioma, for instance, could discern the glial cell state of origin, inform prognosis by identifying the presence of treatment-resistant subclones or

glioblastoma stem cells, and guide clinicians through clinical considerations such as conservative versus aggressive surgical resection, the role of chemotherapy and radiotherapy in the management plan, and whether these interventions are likely to alter the patient's prognosis. In a similar vein, diagnosis and treatment of autoimmune disease could be guided by the identification of specific inflammatory cell subpopulations in peripheral blood, particularly when each subpopulation responds differently to targeted immunotherapies.

Once a disease has been prioritized as a rational target for biomarker development, and single-cell transcriptomics data have been collected from a large and carefully ascertained patient cohort, the question next arises as to how to translate multifaceted single-cell data obtained from any given patient into a clinically meaningful readout. Existing computational paradigms designed to produce diagnostic or prognostic readouts from bulk omics data are unlikely to translate directly into the setting of single-cell transcriptomics because of profound differences in the structure of the data. Instead, efforts to deploy coordinated multicellular biomarkers must carefully consider the conceptual framework by which data from tens of thousands of genes measured across thousands of cells, potentially grouped into dozens of cell types, will be translated into readouts that clinicians have historically appreciated as scalar values. From a machine learning perspective, we might ask: what are the inputs to our model, and what is it trained to predict as output?

Clinically meaningful readouts derived from single-cell biomarkers may take more forms that have been described in the literature to date. Among the handful of computational frameworks that have been developed thus far, all have assumed the need to predict a single patient-level output: for instance, a binary diagnostic classification of disease versus control.[76–79] However, it is equally possible that in some scenarios, more actionable biomarkers may be derived from predictions made at the level of individual cells. This might be the case when, for instance, the presence or proportion of a small number of cells with a particular molecular signature is of critical diagnostic or prognostic importance. The former case could be exemplified by the presence of a rare group of drug-resistant cancer cells that will ultimately lead to recurrence. In the latter case, the proportion of astrocytes within a glioma that express a particular molecular signature might increase the resolution of tumor staging and therefore the accuracy of prognostication.

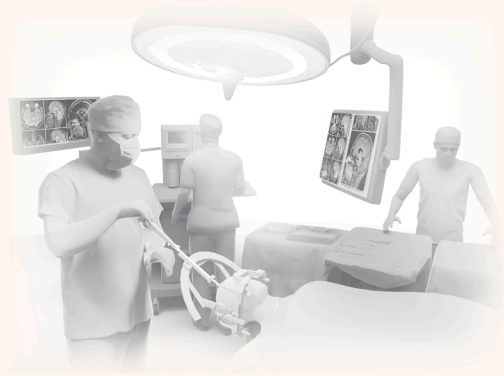
Machine learning models that are designed to predict phenotypes for individual cells instead of producing patient-level outputs will generate thousands of predictions per patient. In order for these predictions to inform clinical decision-making, they will need to be summarized into interpretable patient-level readouts. How might cell-level predictions be aggregated to the patient level? Cells from a single individual will demonstrate molecular responses to disease that are heterogeneous in both their nature and intensity. The distribution of these responses could be summarized in multiple ways: for instance, by a measure of central tendency reflecting the average molecular response to disease, or by the presence of extreme values, as in the case of rare drug-resistant cancer cells.

Regardless of whether a machine learning model produces patient- or cell-level predictions, the next question will be whether the biomarker must incorporate information from every cell type present in the tissue or only from the cell types that are most relevant to the disease in question. A related consideration is whether more accurate predictions can be achieved by training separate models for each cell type in turn or whether it is preferable to design a single model that can integrate information across multiple cell types. Integration of information across cell types in a single model can be achieved either through the representation of the input data or through the architecture of the model itself. For instance, representing a sample as a vector of cell-type or cell-state frequencies allows models to learn from multiple cell types in tandem, whereas neural network architectures based on attentive multiple instance learning can automatically prioritize cells and cell types most relevant to disease when making patient-level predictions.[78]

The introduction of new computational methods to draw clinically actionable inferences from hierarchical single-cell data, along with careful benchmarking of these methods supported by statistical comparisons rather than point estimates of model performance, will guide the field toward the identification of optimal analytical approaches. However, the questions enumerated above will not have unique answers. Instead, the pathobiology of specific diseases and the clinical contexts in which a given biomarker is to be deployed will guide the design and implementation of biomarkers based on single-cell technologies. Close collaboration between computational scientists and clinicians will be required to realize the full clinical potential of these technologies.

BUILDING ON EXISTING FOUNDATIONS

In the sections above, we have focused on the major barriers to the clinical translation of biomarkers based on single-cell transcriptomics. It is equally important to appreciate the impressive progress that has already been made and the existing computational and experimental technologies that are poised to enable biomarker discovery. It is only by virtue of these advances that the field is now in a position to begin contemplating the clinical deployment of single-cell technologies. Experimental approaches now unambiguously achieve the scale and throughput required to profile large, carefully designed patient cohorts in prospective clinical trials, as evidenced by the handful of studies that have collected and analyzed single-cell datasets spanning hundreds of patients.[46–49] Computational advances have scaled in parallel to enable the analysis of these large datasets using standard workflows, although many aspects of these workflows are still the subjects of debate.[87] We argue that despite ongoing efforts to refine important steps in single-cell data analysis workflows, such as normalization, clustering, or data integration, these tools are now sufficiently mature that their deployment in clinical and surgical environments will not compromise the clinical promise of single-cell transcriptomics. Each of the above steps has been extensively benchmarked, and these benchmarks have generally affirmed a high degree of similarity in performance between top-ranking methods.[88–91] Conversely, for



Clinic

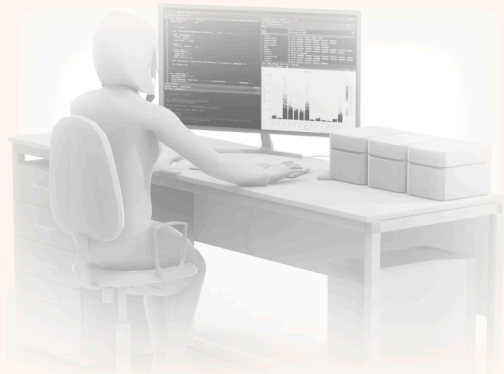
Experimental barrier: Biological and technical heterogeneity in single-cell profiles of human disease	
Solutions	• Profiling of large patient cohorts • Careful patient ascertainment to mitigate axes of biological variability irrelevant to clinical deployment • Technological innovations to facilitate sample procurement (e.g., profiling fixed tissues)

Conceptual barrier: Only a subset of patient populations stand to potentially benefit from single-cell omics	
Solutions	• Exclude clinical settings where diagnosis, prognosis, or management are already straightforward • Exclude clinical settings where information can be obtained without measuring many analytes across multiple cell types • Exclude clinical settings where the disease tissue is not clinically accessible and accessible tissues do not capture cellular and molecular alterations • Include clinical settings where pathophysiology involves coordinated molecular alterations across multiple cell types and for which assessing those multicellular alterations can meaningfully improve diagnosis, management, or prognosis

Laboratory

Experimental barrier: High cost of single-cell profiling	
Solutions	• Technological innovations to decrease cost or technical variation (e.g., combinatorial indexing, sample multiplexing) • Judicious selection of omics modality based on pathophysiological hypothesis • Public-private partnerships

Experimental barrier: Regulatory accreditation requires reproducibility of assay readouts between laboratories	
Solution	• Development of standard operating procedures for single-cell profiling in specific clinical contexts



Computational analysis

Computational barrier: Inappropriate statistical methodologies lead to false discoveries of disease-associated genes and cell types	
Solution	• Valid computational methods that account for compositional data, model within-sample variation, and avoid statistical "double dipping"

Computational barrier: Computational methods to predict patient-level phenotypes have not yet been evaluated in independent cohorts	
Solutions	• Validation of computational readouts in independent, prospectively collected cohorts • Accounting for batch effects and technical heterogeneity in method design

Computational barrier: Regulatory accreditation requires reproducibility of assay readouts between laboratories	
Solution	• Reproducible and automated computational workflows that eliminate subjective decisions and accelerate data analysis

Actionable clinical readout

Conceptual barrier: Translation of thousands of single-cell profiles into simple and actionable clinical readouts	

Solutions	• Development of new statistical or machine-learning frameworks to interpret multifaceted single-cell data, tailored to specific clinical scenarios
	• Judicious design of novel analytical methods, accounting for
	  - Input (e.g., all cells, cells of specific subtypes, cell type or state frequencies)
	  - Model output (e.g., patient- or cell-level predictions, binary classification or continuous stratification, summarization to patient-level readouts)
	• Statistically rigorous benchmarking of computational methods to derive clinical readouts from hierarchical single-cell data

(legend on next page)

other steps in the analysis workflow, such as differential expression and differential abundance analysis, benchmarks have identified clear differences in performance between methods and in some cases elucidated the mechanisms underlying these differences.[68,71,74,75] Whereas further optimization of existing computational infrastructure is certainly valuable, this optimization may yield only incremental advances from a clinical perspective. Instead, we believe that overcoming the experimental, computational, and conceptual barriers outlined above must be the most urgent priority if the clinical potential of single-cell transcriptomics is to be realized.

BEYOND THE TRANSCRIPTOME

To this point, we have focused on single-cell transcriptomics, on the basis that the experimental and computational workflows for this modality are the most technically and commercially mature of any single-cell omic assay and are therefore the best positioned for clinical translation. Although other single-cell omic assays are now routinely being deployed in preclinical contexts or in basic studies of disease pathobiology in human tissues,[92] additional obstacles must be overcome in order to realize their implementation in clinical settings. In single-cell assay for transposase-accessible chromatin sequencing (ATAC-seq), for instance, fundamental aspects of data analysis, such as whether chromatin accessibility should be considered a qualitative or quantitative measurement[93–95] or whether regions of accessible chromatin should be identified *de novo* from a given dataset or defined on the basis of an external reference, remain debated. Such debate does not preclude the translation of coordinated multicellular biomarkers based on chromatin accessibility profiles, but it will hinder their clinical validation insofar as the risk of false positives or false negatives in biomarker discovery is increased.

On the other hand, there are also fundamental similarities in the nature and clinical utility of single-cell profiles obtained with single-cell transcriptomics and techniques such as single-cell chromatin accessibility, T or B cell receptor sequencing, proteomics (either by mass spectrometry or with antibody-based sequencing workflows), or more nascent techniques such as lipidomics and metabolomics, as well as multi-omic combinations of these approaches that monitor multiple modalities in individual cells. Development and translation of coordinated multicellular biomarkers based on any of these technologies will also require the profiling of large and carefully ascertained patient cohorts, the implementation of experimental and computational SOPs that can satisfy regulatory requirements, the use of valid statistical analysis methods, and the design of computational frameworks that can translate hierarchical data from thousands of individual cells into reproducible patient-level readouts.

As other single-cell omic assays mature, the question will increasingly arise as to which is best suited for a particular clinical context. Although we have focused on single-cell transcriptomics for technical reasons, it is unlikely that any particular assay will be optimal in all contexts. Instead, the selection of a specific assay to enable the development of coordinated multicellular biomarkers should reflect the biochemical and pathophysiological mechanisms underlying biomarker development. For instance, when durable responses to anticancer therapies are limited by the persistence of a small number of resistant cells characterized by a specific genomic alteration, sequencing of genomic DNA may provide a more sound basis for prognostication than transcriptomic assays.

The advent of spatial omic assays presents new and more conceptually divergent opportunities with respect to clinical translation.[96] Such assays are most likely to be integrated into the practice of pathology, wherein morphological and molecular alterations within individual cells have long been appreciated within their cytoarchitectural context. AI-guided workflows for digital pathology based on conventional histological stains have been approved by regulatory bodies,[97,98] but the incorporation of spatial omics data to derive clinically actionable readouts is more nascent. Consequently, it remains to be seen to what extent and in which contexts the incorporation of spatial coordinates of gene expression and cells can specifically augment clinical decision-making, as compared to single-cell assays that profile dissociated cells.

A ROAD MAP TO THE CLINIC

The maturation of single-cell technologies now opens the possibility to move beyond descriptive analysis and toward the integration of single-cell omics into medical and surgical practice (Figure 3). Past experience suggests that the most immediate mechanism by which these technologies will inform clinical decision-making will be through the introduction of new biomarkers that achieve the vision of precision medicine by improving diagnosis, facilitating treatment selection, monitoring therapeutic efficacy, and prognosticating disease progression. Realizing this potential will require profiling large and carefully ascertained patient cohorts and analyzing the resulting data with valid statistical methods, in order to discover robust biomarkers. Once biomarkers have been identified, experimental and computational SOPs will have to be developed in order to empower laboratories in any hospital environment to translate single-cell omics data into reproducible clinical readouts. Surmounting this challenge will require new computational infrastructure that automates subjective and manual aspects of single-cell data analysis. Because not all diseases or clinical decisions stand to benefit from single-cell biomarkers, this level of effort is best invested in diseases where single-cell omics can have the greatest impact on patient care. Condensing multifaceted single-cell datasets into actionable clinical readouts will require computational protocols that are tailored to the clinical context and careful consideration of methodological points that have received

Figure 3. A road map toward the integration of single-cell omics into medical and surgical practice
Summary of the key experimental, computational, and conceptual barriers for the clinical deployment of single-cell omics, and the solutions proposed in this manuscript.

relatively little attention to date, particularly with respect to the use of machine learning to assign patient-level phenotypes. Overcoming all of these barriers will require close collaboration between clinicians, computational scientists, and the biotechnological industry in order for single-cell omics to impact routine clinical practice.

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AUTHOR CONTRIBUTIONS

M.A.S., G.C., J.B., and J.W.S. wrote and edited the manuscript.

DECLARATION OF INTERESTS

M.A.S. is a member of the Rutgers Cancer Institute of New Jersey (RCINJ).

REFERENCES

1. United States Bureau of the Census (1909). Manual of the International List of Causes of Death (U.S. Government Printing Office).
2. National Research Council (US) Committee on A Framework for Developing a New Taxonomy of Disease (2011). Toward Precision Medicine (National Academies Press) https://doi.org/10.17226/13284.
3. Tang, F., Barbacioru, C., Wang, Y., Nordman, E., Lee, C., Xu, N., Wang, X., Bodeau, J., Tuch, B.B., Siddiqui, A., et al. (2009). mRNA-Seq whole-transcriptome analysis of a single cell. Nat. Methods *6*, 377–382. https://doi.org/10.1038/nmeth.1315.
4. Plass, M., Solana, J., Wolf, F.A., Ayoub, S., Misios, A., Glažar, P., Obermayer, B., Theis, F.J., Kocks, C., and Rajewsky, N. (2018). Cell type atlas and lineage tree of a whole complex animal by single-cell transcriptomics. Science *360*, eaaq1723. https://doi.org/10.1126/science.aaq1723.
5. Cao, J., Packer, J.S., Ramani, V., Cusanovich, D.A., Huynh, C., Daza, R., Qiu, X., Lee, C., Furlan, S.N., Steemers, F.J., et al. (2017). Comprehensive single-cell transcriptional profiling of a multicellular organism. Science *357*, 661–667. https://doi.org/10.1126/science.aam8940.
6. Han, X., Zhou, Z., Fei, L., Sun, H., Wang, R., Chen, Y., Chen, H., Wang, J., Tang, H., Ge, W., et al. (2020). Construction of a human cell landscape at single-cell level. Nature *581*, 303–309. https://doi.org/10.1038/s41586-020-2157-4.
7. Li, H., Janssens, J., De Waegeneer, M.D., Kolluru, S.S., Davie, K., Gardeux, V., Saelens, W., David, F.P.A., Brbić, M., Spanier, K., et al. (2022). Fly Cell Atlas: A single-nucleus transcriptomic atlas of the adult fruit fly. Science *375*, eabk2432. https://doi.org/10.1126/science.abk2432.
8. Tabula Muris Consortium, Overall coordination, Logistical coordination, Organ collection and processing, Library preparation and sequencing, Computational data analysis, Cell type annotation, Writing group, Supplemental text writing group, and Principal investigators (2018). Single-cell transcriptomics of 20 mouse organs creates a Tabula Muris. Nature *562*, 367–372. https://doi.org/10.1038/s41586-018-0590-4.
9. Han, L., Wei, X., Liu, C., Volpe, G., Zhuang, Z., Zou, X., Wang, Z., Pan, T., Yuan, Y., Zhang, X., et al. (2022). Cell transcriptomic atlas of the non-human primate Macaca fascicularis. Nature *604*, 723–731. https://doi.org/10.1038/s41586-022-04587-3.
10. Jones, R.C., Karkanias, J., Krasnow, M.A., Pisco, A.O., Quake, S.R., Salzman, J., Yosef, N., Bulthaup, B., Brown, P., et al.; Tabula Sapiens Consortium (2022). The Tabula Sapiens: A multiple-organ, single-cell transcriptomic atlas of humans. Science *376*, eabl4896. https://doi.org/10.1126/science.abl4896.
11. Hrvatin, S., Sun, S., Wilcox, O.F., Yao, H., Lavin-Peter, A.J., Cicconet, M., Assad, E.G., Palmer, M.E., Aronson, S., Banks, A.S., et al. (2020). Neurons that regulate mouse torpor. Nature *583*, 115–121. https://doi.org/10.1038/s41586-020-2387-5.
12. Kathe, C., Skinnider, M.A., Hutson, T.H., Regazzi, N., Gautier, M., Demesmaeker, R., Komi, S., Ceto, S., James, N.D., Cho, N., et al. (2022). The neurons that restore walking after paralysis. Nature *611*, 540–547. https://doi.org/10.1038/s41586-022-05385-7.
13. Pool, A.-H., Wang, T., Stafford, D.A., Chance, R.K., Lee, S., Ngai, J., and Oka, Y. (2020). The cellular basis of distinct thirst modalities. Nature *588*, 112–117. https://doi.org/10.1038/s41586-020-2821-8.
14. Neftel, C., Laffy, J., Filbin, M.G., Hara, T., Shore, M.E., Rahme, G.J., Richman, A.R., Silverbush, D., Shaw, M.L., Hebert, C.M., et al. (2019). An Integrative Model of Cellular States, Plasticity, and Genetics for Glioblastoma. Cell *178*, 835–849.e21. https://doi.org/10.1016/j.cell.2019.06.024.
15. Aztekin, C., Hiscock, T.W., Marioni, J.C., Gurdon, J.B., Simons, B.D., and Jullien, J. (2019). Identification of a regeneration-organizing cell in the Xenopus tail. Science *364*, 653–658. https://doi.org/10.1126/science.aav9996.
16. Gerber, T., Murawala, P., Knapp, D., Masselink, W., Schuez, M., Hermann, S., Gac-Santel, M., Nowoshilow, S., Kageyama, J., Khattak, S., et al. (2018). Single-cell analysis uncovers convergence of cell identities during axolotl limb regeneration. Science *362*, eaaq0681. https://doi.org/10.1126/science.aaq0681.
17. Qin, T., Zhang, G., Zheng, Y., Li, S., Yuan, Y., Li, Q., Hu, M., Si, H., Wei, G., Gao, X., et al. (2023). A population of stem cells with strong regenerative potential discovered in deer antlers. Science *379*, 840–847. https://doi.org/10.1126/science.add0488.
18. Li, Y., He, X., Kawaguchi, R., Zhang, Y., Wang, Q., Monavarfeshani, A., Yang, Z., Chen, B., Shi, Z., Meng, H., et al. (2020). Microglia-organized scar-free spinal cord repair in neonatal mice. Nature *587*, 613–618. https://doi.org/10.1038/s41586-020-2795-6.
19. Squair, J.W., Milano, M., de Coucy, A., Gautier, M., Skinnider, M.A., James, N.D., Cho, N., Lasne, A., Kathe, C., Hutson, T.H., et al. (2023). Recovery of walking after paralysis by regenerating characterized neurons to their natural target region. Science *381*, 1338–1345. https://doi.org/10.1126/science.adi6412.
20. Jain, K.K. (2017). The Handbook of Biomarkers (Humana Press), pp. 273–462. https://doi.org/10.1007/978-1-4939-7431-3_13.
21. Laskin, J., Jones, S., Aparicio, S., Chia, S., Ch'ng, C., Deyell, R., Eirew, P., Fok, A., Gelmon, K., Ho, C., et al. (2015). Lessons learned from the application of whole-genome analysis to the treatment of patients with advanced cancers. Cold Spring Harb. Mol. Case Stud. *1*, a000570. https://doi.org/10.1101/mcs.a000570.
22. Garcia, E.P., Minkovsky, A., Jia, Y., Ducar, M.D., Shivdasani, P., Gong, X., Ligon, A.H., Sholl, L.M., Kuo, F.C., MacConaill, L.E., et al. (2017). Validation of OncoPanel: A Targeted Next-Generation Sequencing Assay for the Detection of Somatic Variants in Cancer. Arch. Pathol. Lab. Med. *141*, 751–758. https://doi.org/10.5858/arpa.2016-0527-OA.
23. Cheng, D.T., Mitchell, T.N., Zehir, A., Shah, R.H., Benayed, R., Syed, A., Chandramohan, R., Liu, Z.Y., Won, H.H., Scott, S.N., et al. (2015). Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT): A Hybridization Capture-Based Next-Generation Sequencing Clinical Assay for Solid Tumor Molecular Oncology. J. Mol. Diagn. *17*, 251–264. https://doi.org/10.1016/j.jmoldx.2014.12.006.
24. Frampton, G.M., Fichtenholtz, A., Otto, G.A., Wang, K., Downing, S.R., He, J., Schnall-Levin, M., White, J., Sanford, E.M., An, P., et al. (2013). Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing. Nat. Biotechnol. *31*, 1023–1031. https://doi.org/10.1038/nbt.2696.

25. Rangachari, D., VanderLaan, P.A., Le, X., Folch, E., Kent, M.S., Gangadharan, S.P., Majid, A., Haspel, R.L., Joseph, L.J., Huberman, M.S., et al. (2015). Experience with targeted next generation sequencing for the care of lung cancer: Insights into promises and limitations of genomic oncology in day-to-day practice. Cancer Treat. Commun. *4*, 174–181. https://doi.org/10.1016/j.ctrc.2015.10.004.

26. Friedman, J.M., Jones, K.L., and Carey, J.C. (2020). Exome Sequencing and Clinical Diagnosis. JAMA *324*, 627–628. https://doi.org/10.1001/jama.2020.11126.

27. Kim, D., Kobayashi, T., Voisin, B., Jo, J.-H., Sakamoto, K., Jin, S.-P., Kelly, M., Pasieka, H.B., Naff, J.L., Meyerle, J.H., et al. (2020). Targeted therapy guided by single-cell transcriptomic analysis in drug-induced hypersensitivity syndrome: a case report. Nat. Med. *26*, 236–243. https://doi.org/10.1038/s41591-019-0733-7.

28. de Van de Sande, B.V., Lee, J.S., Mutasa-Gottgens, E., Naughton, B., Bacon, W., Manning, J., Wang, Y., Pollard, J., Mendez, M., Hill, J., et al. (2023). Applications of single-cell RNA sequencing in drug discovery and development. Nat. Rev. Drug Discov. *22*, 496–520. https://doi.org/10.1038/s41573-023-00688-4.

29. Zhuang, B., Mancarci, B.O., Toker, L., and Pavlidis, P. (2019). Meta-Analysis of Gene Expression in Mouse Models of Alzheimer's Disease. eNeuro *6*, ENEURO.0226-19.2019. https://doi.org/10.1523/ENEURO.0226-19.2019.

30. Srinivasan, K., Friedman, B.A., Larson, J.L., Lauffer, B.E., Goldstein, L.D., Appling, L.L., Borneo, J., Poon, C., Ho, T., Cai, F., et al. (2016). Untangling the brain's neuroinflammatory and neurodegenerative transcriptional responses. Nat. Commun. *7*, 11295. https://doi.org/10.1038/ncomms11295.

31. Skinnider, M.A., Gautier, M., Teo, A.Y.Y., Kathe, C., Hutson, T.H., Laskaratos, A., de Coucy, A., Regazzi, N., Aureli, V., James, N.D., et al. (2024). Single-cell and spatial atlases of spinal cord injury in the Tabulae Paralytica. Nature *631*, 150–163. https://doi.org/10.1038/s41586-024-07504-y.

32. Kamath, T., Abdulraouf, A., Burris, S.J., Langlieb, J., Gazestani, V., Nadaf, N.M., Balderrama, K., Vanderburg, C., and Macosko, E.Z. (2022). Single-cell genomic profiling of human dopamine neurons identifies a population that selectively degenerates in Parkinson's disease. Nat. Neurosci. *25*, 588–595. https://doi.org/10.1038/s41593-022-01061-1.

33. Tran, N.M., Shekhar, K., Whitney, I.E., Jacobi, A., Benhar, I., Hong, G., Yan, W., Adiconis, X., Arnold, M.E., Lee, J.M., et al. (2019). Single-Cell Profiles of Retinal Ganglion Cells Differing in Resilience to Injury Reveal Neuroprotective Genes. Neuron *104*, 1039–1055.e12. https://doi.org/10.1016/j.neuron.2019.11.006.

34. Amar, A.P., and Levy, M.L. (1999). Pathogenesis and pharmacological strategies for mitigating secondary damage in acute spinal cord injury. Neurosurgery *44*, 1027–1039. https://doi.org/10.1097/00006123-199905000-00052.

35. Popovich, P.G., Wei, P., and Stokes, B.T. (1997). Cellular inflammatory response after spinal cord injury in Sprague-Dawley and Lewis rats. J. Comp. Neurol. *377*, 443–464. https://doi.org/10.1002/(sici)1096-9861(19970120)377:3<443::aid-cne10>3.0.co;2-s.

36. Dusart, I., and Schwab, M.E. (1994). Secondary cell death and the inflammatory reaction after dorsal hemisection of the rat spinal cord. Eur. J. Neurosci. *6*, 712–724. https://doi.org/10.1111/j.1460-9568.1994.tb00983.x.

37. Bartholdi, D., and Schwab, M.E. (1997). Expression of pro-inflammatory cytokine and chemokine mRNA upon experimental spinal cord injury in mouse: An in situ hybridization study. Eur. J. Neurosci. *9*, 1422–1438. https://doi.org/10.1111/j.1460-9568.1997.tb01497.x.

38. Kerr, B.J., and Patterson, P.H. (2004). Potent pro-inflammatory actions of leukemia inhibitory factor in the spinal cord of the adult mouse. Exp. Neurol. *188*, 391–407. https://doi.org/10.1016/j.expneurol.2004.04.012.

39. Squair, J.W., Gautier, M., Sofroniew, M.V., Courtine, G., and Anderson, M.A. (2021). Engineering spinal cord repair. Curr. Opin. Biotechnol. *72*, 48–53. https://doi.org/10.1016/j.copbio.2021.10.006.

40. Anderson, M.A., Squair, J.W., Gautier, M., Hutson, T.H., Kathe, C., Barraud, Q., Bloch, J., and Courtine, G. (2022). Natural and targeted circuit reorganization after spinal cord injury. Nat. Neurosci. *25*, 1584–1596. https://doi.org/10.1038/s41593-022-01196-1.

41. Short, D.J., El Masry, W.S., and Jones, P.W. (2000). High dose methylprednisolone in the management of acute spinal cord injury – a systematic review from a clinical perspective. Spinal Cord *38*, 273–286. https://doi.org/10.1038/sj.sc.3100986.

42. Bracken, M.B., Collins, W.F., Freeman, D.F., Shepard, M.J., Wagner, F.W., Silten, R.M., Hellenbrand, K.G., Ransohoff, J., Hunt, W.E., and Perot, P.L. (1984). Efficacy of Methylprednisolone in Acute Spinal Cord Injury. JAMA *251*, 45–52. https://doi.org/10.1001/jama.1984.03340250025015.

43. Hurlbert, R.J. (2000). Methylprednisolone for acute spinal cord injury: an inappropriate standard of care. J. Neurosurg. *93*, 1–7. https://doi.org/10.3171/spi.2000.93.1.0001.

44. Anderson, M.A., Burda, J.E., Ren, Y., Ao, Y., O'Shea, T.M., Kawaguchi, R., Coppola, G., Khakh, B.S., Deming, T.J., and Sofroniew, M.V. (2016). Astrocyte scar formation aids central nervous system axon regeneration. Nature *532*, 195–200. https://doi.org/10.1038/nature17623.

45. Anderson, M.A., O'Shea, T.M., Burda, J.E., Ao, Y., Barlatey, S.L., Bernstein, A.M., Kim, J.H., James, N.D., Rogers, A., Kato, B., et al. (2018). Required growth facilitators propel axon regeneration across complete spinal cord injury. Nature *561*, 396–400. https://doi.org/10.1038/s41586-018-0467-6.

46. Nathan, A., Beynor, J.I., Baglaenko, Y., Suliman, S., Ishigaki, K., Asgari, S., Huang, C.-C., Luo, Y., Zhang, Z., Lopez, K., et al. (2021). Multimodally profiling memory T cells from a tuberculosis cohort identifies cell state associations with demographics, environment and disease. Nat. Immunol. *22*, 781–793. https://doi.org/10.1038/s41590-021-00933-1.

47. Mathys, H., Boix, C.A., Akay, L.A., Xia, Z., Davila-Velderrain, J., Ng, A.P., Jiang, X., Abdelhady, G., Galani, K., Mantero, J., et al. (2024). Single-cell multiregion dissection of Alzheimer's disease. Nature *632*, 858–868. https://doi.org/10.1038/s41586-024-07606-7.

48. Green, G.S., Fujita, M., Yang, H.-S., Taga, M., Cain, A., McCabe, C., Comandante-Lou, N., White, C.C., Schmidtner, A.K., Zeng, L., et al. (2024). Cellular communities reveal trajectories of brain ageing and Alzheimer's disease. Nature *633*, 634–645. https://doi.org/10.1038/s41586-024-07871-6.

49. Perez, R.K., Gordon, M.G., Subramaniam, M., Kim, M.C., Hartoularos, G.C., Targ, S., Sun, Y., Ogorodnikov, A., Bueno, R., Lu, A., et al. (2022). Single-cell RNA-seq reveals cell type–specific molecular and genetic associations to lupus. Science *376*, eabf1970. https://doi.org/10.1126/science.abf1970.

50. Cao, J., O'Day, D.R., Pliner, H.A., Kingsley, P.D., Deng, M., Daza, R.M., Zager, M.A., Aldinger, K.A., Blecher-Gonen, R., Zhang, F., et al. (2020). A human cell atlas of fetal gene expression. Science *370*, eaba7721. https://doi.org/10.1126/science.aba7721.

51. Stoeckius, M., Zheng, S., Houck-Loomis, B., Hao, S., Yeung, B.Z., Mauck, W.M., Smibert, P., and Satija, R. (2018). Cell Hashing with barcoded antibodies enables multiplexing and doublet detection for single cell genomics. Genome Biol. *19*, 224. https://doi.org/10.1186/s13059-018-1603-1.

52. Slyper, M., Porter, C.B.M., Ashenberg, O., Waldman, J., Drokhlyansky, E., Wakiro, I., Smillie, C., Smith-Rosario, G., Wu, J., Dionne, D., et al. (2020). A single-cell and single-nucleus RNA-Seq toolbox for fresh and frozen human tumors. Nat. Med. *26*, 792–802. https://doi.org/10.1038/s41591-020-0844-1.

53. Thrupp, N., Sala Frigerio, C.S., Wolfs, L., Skene, N.G., Fattorelli, N., Poovathingal, S., Fourne, Y., Matthews, P.M., Theys, T., Mancuso, R., et al. (2020). Single-Nucleus RNA-Seq Is Not Suitable for Detection of Microglial Activation Genes in Humans. Cell Rep. *32*, 108189. https://doi.org/10.1016/j.celrep.2020.108189.

54. Denisenko, E., Guo, B.B., Jones, M., Hou, R., de Kock, L., Lassmann, T., Poppe, D., Clément, O., Simmons, R.K., Lister, R., et al. (2020).

Systematic assessment of tissue dissociation and storage biases in single-cell and single-nucleus RNA-seq workflows. Genome Biol. *21*, 130. https://doi.org/10.1186/s13059-020-02048-6.

55. Baysoy, A., Bai, Z., Satija, R., and Fan, R. (2023). The technological landscape and applications of single-cell multi-omics. Nat. Rev. Mol. Cell Biol. *24*, 695–713. https://doi.org/10.1038/s41580-023-00615-w.
56. Swanson, B.N. (2002). Delivery of High-Quality Biomarker Assays. Dis. Markers *18*, 47–56. https://doi.org/10.1155/2002/212987.
57. Xue, Z., Huang, K., Cai, C., Cai, L., Jiang, C.Y., Feng, Y., Liu, Z., Zeng, Q., Cheng, L., Sun, Y.E., et al. (2013). Genetic programs in human and mouse early embryos revealed by single-cell RNA sequencing. Nature *500*, 593–597. https://doi.org/10.1038/nature12364.
58. Shalek, A.K., Satija, R., Adiconis, X., Gertner, R.S., Gaublomme, J.T., Raychowdhury, R., Schwartz, S., Yosef, N., Malboeuf, C., Lu, D., et al. (2013). Single-cell transcriptomics reveals bimodality in expression and splicing in immune cells. Nature *498*, 236–240. https://doi.org/10.1038/nature12172.
59. Shalek, A.K., Satija, R., Shuga, J., Trombetta, J.J., Gennert, D., Lu, D., Chen, P., Gertner, R.S., Gaublomme, J.T., Yosef, N., et al. (2014). Single-cell RNA-seq reveals dynamic paracrine control of cellular variation. Nature *510*, 363–369. https://doi.org/10.1038/nature13437.
60. Finak, G., McDavid, A., Yajima, M., Deng, J., Gersuk, V., Shalek, A.K., Slichter, C.K., Miller, H.W., McElrath, M.J., Prlic, M., et al. (2015). MAST: a flexible statistical framework for assessing transcriptional changes and characterizing heterogeneity in single-cell RNA sequencing data. Genome Biol. *16*, 278. https://doi.org/10.1186/s13059-015-0844-5.
61. van Dijk, D., Sharma, R., Nainys, J., Yim, K., Kathail, P., Carr, A.J., Burdziak, C., Moon, K.R., Chaffer, C.L., Pattabiraman, D., et al. (2018). Recovering Gene Interactions from Single-Cell Data Using Data Diffusion. Cell *174*, 716–729.e27. https://doi.org/10.1016/j.cell.2018.05.061.
62. Kharchenko, P.V., Silberstein, L., and Scadden, D.T. (2014). Bayesian approach to single-cell differential expression analysis. Nat. Methods *11*, 740–742. https://doi.org/10.1038/nmeth.2967.
63. Bacher, R., Chu, L.-F., Leng, N., Gasch, A.P., Thomson, J.A., Stewart, R. M., Newton, M., and Kendziorski, C. (2017). SCnorm: robust normalization of single-cell RNA-seq data. Nat. Methods *14*, 584–586. https://doi.org/10.1038/nmeth.4263.
64. Luecken, M.D., and Theis, F.J. (2019). Current best practices in single-cell RNA-seq analysis: a tutorial. Mol. Syst. Biol. *15*, e8746. https://doi.org/10.15252/msb.20188746.
65. Svensson, V. (2020). Droplet scRNA-seq is not zero-inflated. Nat. Biotechnol. *38*, 147–150. https://doi.org/10.1038/s41587-019-0379-5.
66. Andrews, T.S., and Hemberg, M. (2018). False signals induced by single-cell imputation. F1000Res *7*, 1740. https://doi.org/10.12688/f1000research.16613.2.
67. Lun, A. (2018). Overcoming systematic errors caused by log-transformation of normalized single-cell RNA sequencing data. Preprint at bioRxiv. https://doi.org/10.1101/404962.
68. Squair, J.W., Gautier, M., Kathe, C., Anderson, M.A., James, N.D., Hutson, T.H., Hudelle, R., Qaiser, T., Matson, K.J.E., Barraud, Q., et al. (2021). Confronting false discoveries in single-cell differential expression. Nat. Commun. *12*, 5692. https://doi.org/10.1038/s41467-021-25960-2.
69. Neufeld, A., Gao, L.L., Popp, J., Battle, A., and Witten, D. (2023). Inference after latent variable estimation for single-cell RNA sequencing data. Biostatistics *25*, 270–287. https://doi.org/10.1093/biostatistics/kxac047.
70. Zhang, J.M., Kamath, G.M., and Tse, D.N. (2019). Valid Post-clustering Differential Analysis for Single-Cell RNA-Seq. Cell Syst. *9*, 383–392.e6. https://doi.org/10.1016/j.cels.2019.07.012.
71. Zimmerman, K.D., Espeland, M.A., and Langefeld, C.D. (2021). A practical solution to pseudoreplication bias in single-cell studies. Nat. Commun. *12*, 738. https://doi.org/10.1038/s41467-021-21038-1.
72. Büttner, M., Ostner, J., Müller, C.L., Theis, F.J., and Schubert, B. (2021). scCODA is a Bayesian model for compositional single-cell data analysis. Nat. Commun. *12*, 6876. https://doi.org/10.1038/s41467-021-27150-6.
73. Lee, H., and Han, B. (2024). Pseudobulk with proper offsets has the same statistical properties as generalized linear mixed models in single-cell case-control studies. Bioinformatics *40*, btae498. https://doi.org/10.1093/bioinformatics/btae498.
74. Crowell, H.L., Soneson, C., Germain, P.-L., Calini, D., Collin, L., Raposo, C., Malhotra, D., and Robinson, M.D. (2020). muscat detects subpopulation-specific state transitions from multi-sample multi-condition single-cell transcriptomics data. Nat. Commun. *11*, 6077. https://doi.org/10.1038/s41467-020-19894-4.
75. Simmons, S. (2022). Cell Type Composition Analysis: Comparison of statistical methods. Preprint at bioRxiv. https://doi.org/10.1101/2022.02.04.479123.
76. He, B., Thomson, M., Subramaniam, M., Perez, R., Ye, C.J., and Zou, J. (2022). CloudPred: Predicting Patient Phenotypes From Single-cell RNA-seq. Pac. Symp. Biocomput. *27*, 337–348.
77. Mao, Y., Lin, Y.-Y., Wong, N.K.Y., Volik, S., Sar, F., Collins, C., and Ester, M. (2024). Phenotype prediction from single-cell RNA-seq data using attention-based neural networks. Bioinformatics *40*, btae067. https://doi.org/10.1093/bioinformatics/btae067.
78. Litinetskaya, A., Shulman, M., Hediyeh-Zadeh, S., Moinfar, A.A., Curion, F., Szałata, A., Omidi, A., Lotfollahi, M., and Theis, F.J. (2024). Multimodal weakly supervised learning to identify disease-specific changes in single-cell atlases. Preprint at bioRxiv. https://doi.org/10.1101/2024.07.29.605625.
79. Xiong, G., Bekiranov, S., and Zhang, A. (2023). ProtoCell4P: an explainable prototype-based neural network for patient classification using single-cell RNA-seq. Bioinformatics *39*, btad493. https://doi.org/10.1093/bioinformatics/btad493.
80. Heumos, L., Schaar, A.C., Lance, C., Litinetskaya, A., Drost, F., Zappia, L., Lücken, M.D., Strobl, D.C., Henao, J., Curion, F., et al. (2023). Best practices for single-cell analysis across modalities. Nat. Rev. Genet. *24*, 550–572. https://doi.org/10.1038/s41576-023-00586-w.
81. Poste, G. (2011). Bring on the biomarkers. Nature *469*, 156–157. https://doi.org/10.1038/469156a.
82. Bain, B.J. (2017). Rodak's Hematology: Clinical Principles and Applications. By E.M. Keohane, L.J. Smith and J.M. Walenga (Eds), Elsevier, St Louis, 2016. Br. J. Haematol. *177*, 662. https://doi.org/10.1111/bjh.14687.
83. Rozenblatt-Rosen, O., Regev, A., Oberdoerffer, P., Nawy, T., Hupalowska, A., Rood, J.E., Ashenberg, O., Cerami, E., Coffey, R.J., Demir, E., et al. (2020). The Human Tumor Atlas Network: Charting Tumor Transitions across Space and Time at Single-Cell Resolution. Cell *181*, 236–249. https://doi.org/10.1016/j.cell.2020.03.053.
84. Guinney, J., Dienstmann, R., Wang, X., de Reyniès, A., Schlicker, A., Soneson, C., Marisa, L., Roepman, P., Nyamundanda, G., Angelino, P., et al. (2015). The consensus molecular subtypes of colorectal cancer. Nat. Med. *21*, 1350–1356. https://doi.org/10.1038/nm.3967.
85. Hoadley, K.A., Yau, C., Wolf, D.M., Cherniack, A.D., Tamborero, D., Ng, S., Leiserson, M.D.M., Niu, B., McLellan, M.D., Uzunangelov, V., et al. (2014). Multiplatform Analysis of 12 Cancer Types Reveals Molecular Classification within and across Tissues of Origin. Cell *158*, 929–944. https://doi.org/10.1016/j.cell.2014.06.049.
86. Alizadeh, A.A., Eisen, M.B., Davis, R.E., Ma, C., Lossos, I.S., Rosenwald, A., Boldrick, J.C., Sabet, H., Tran, T., Yu, X., et al. (2000). Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature *403*, 503–511. https://doi.org/10.1038/35000501.
87. Luecken, M.D., Gigante, S., Burkhardt, D.B., Cannoodt, R., Strobl, D.C., Markov, N.S., Zappia, L., Palla, G., Lewis, W., Dimitrov, D., et al. (2024). Defining and benchmarking open problems in single-cell analysis. Preprint at Res. Sq. https://doi.org/10.21203/rs.3.rs-4181617/v1.

88. Duò, A., Robinson, M.D., and Soneson, C. (2018). A systematic performance evaluation of clustering methods for single-cell RNA-seq data. F1000Res *7*, 1141. https://doi.org/10.12688/f1000research.15666.3.

89. Tian, L., Dong, X., Freytag, S., Lê Cao, K.-A.L., Su, S., JalalAbadi, A., Amann-Zalcenstein, D., Weber, T.S., Seidi, A., Jabbari, J.S., et al. (2019). Benchmarking single cell RNA-sequencing analysis pipelines using mixture control experiments. Nat. Methods *16*, 479–487. https://doi.org/10.1038/s41592-019-0425-8.

90. Luecken, M.D., Büttner, M., Chaichoompu, K., Danese, A., Interlandi, M., Mueller, M.F., Strobl, D.C., Zappia, L., Dugas, M., Colomé-Tatché, M., et al. (2022). Benchmarking atlas-level data integration in single-cell genomics. Nat. Methods *19*, 41–50. https://doi.org/10.1038/s41592-021-01336-8.

91. Tran, H.T.N., Ang, K.S., Chevrier, M., Zhang, X., Lee, N.Y.S., Goh, M., and Chen, J. (2020). A benchmark of batch-effect correction methods for single-cell RNA sequencing data. Genome Biol. *21*, 12. https://doi.org/10.1186/s13059-019-1850-9.

92. Morabito, S., Miyoshi, E., Michael, N., Shahin, S., Martini, A.C., Head, E., Silva, J., Leavy, K., Perez-Rosendahl, M., and Swarup, V. (2021). Single-nucleus chromatin accessibility and transcriptomic characterization of Alzheimer's disease. Nat. Genet. *53*, 1143–1155. https://doi.org/10.1038/s41588-021-00894-z.

93. Martens, L.D., Fischer, D.S., Yépez, V.A., Theis, F.J., and Gagneur, J. (2024). Modeling fragment counts improves single-cell ATAC-seq analysis. Nat. Methods *21*, 28–31. https://doi.org/10.1038/s41592-023-02112-6.

94. Teo, A.Y.Y., Squair, J.W., Courtine, G., and Skinnider, M.A. (2024). Best practices for differential accessibility analysis in single-cell epigenomics. Nat. Commun. *15*, 8805. https://doi.org/10.1038/s41467-024-53089-5.

95. Miao, Z., and Kim, J. (2024). Uniform quantification of single-nucleus ATAC-seq data with Paired-Insertion Counting (PIC) and a model-based insertion rate estimator. Nat. Methods *21*, 32–36. https://doi.org/10.1038/s41592-023-02103-7.

96. Bressan, D., Battistoni, G., and Hannon, G.J. (2023). The dawn of spatial omics. Science *381*, eabq4964. https://doi.org/10.1126/science.abq4964.

97. Hondelink, L.M., Hüyük, M., Postmus, P.E., Smit, V.T.H.B.M., Blom, S., von der Thüsen, J.H., and Cohen, D. (2022). Development and validation of a supervised deep learning algorithm for automated whole-slide programmed death-ligand 1 tumour proportion score assessment in non-small cell lung cancer. Histopathology *80*, 635–647. https://doi.org/10.1111/his.14571.

98. da Silva, L.M., Pereira, E.M., Salles, P.G., Godrich, R., Ceballos, R., Kunz, J.D., Casson, A., Viret, J., Chandarlapaty, S., Ferreira, C.G., et al. (2021). Independent real-world application of a clinical-grade automated prostate cancer detection system. J. Pathol. *254*, 147–158. https://doi.org/10.1002/path.5662.

99. Sheridan, C. (2024). Can single-cell biology realize the promise of precision medicine? Nat. Biotechnol. *42*, 159–162. https://doi.org/10.1038/s41587-024-02138-x.

100. Bai, J., Shi, J., Li, C., Wang, S., Zhang, T., Hua, X., Zhu, B., Koka, H., Wu, H.-H., Song, L., et al. (2021). Whole genome sequencing of skull-base chordoma reveals genomic alterations associated with recurrence and chordoma-specific survival. Nat. Commun. *12*, 757. https://doi.org/10.1038/s41467-021-21026-5.

101. Nassiri, F., Liu, J., Patil, V., Mamatjan, Y., Wang, J.Z., Hugh-White, R., Macklin, A.M., Khan, S., Singh, O., Karimi, S., et al. (2021). A clinically applicable integrative molecular classification of meningiomas. Nature *597*, 119–125. https://doi.org/10.1038/s41586-021-03850-3.

102. Zhang, Q., Fei, L., Han, R., Huang, R., Wang, Y., Chen, H., Yao, B., Qiao, N., Wang, Z., Ma, Z., et al. (2022). Single-cell transcriptome reveals cellular hierarchies and guides p-EMT-targeted trial in skull base chordoma. Cell Discov. *8*, 94. https://doi.org/10.1038/s41421-022-00459-2.

103. Lucas, C.G., Mirchia, K., Seo, K., Najem, H., Chen, W.C., Zakimi, N., Foster, K., Eaton, C.D., Cady, M.A., Choudhury, A., et al. (2024). Spatial genomic, biochemical and cellular mechanisms underlying meningioma heterogeneity and evolution. Nat. Genet. *56*, 1121–1133. https://doi.org/10.1038/s41588-024-01747-1.

104. Keller, L., and Pantel, K. (2019). Unravelling tumour heterogeneity by single-cell profiling of circulating tumour cells. Nat. Rev. Cancer *19*, 553–567. https://doi.org/10.1038/s41568-019-0180-2.

105. Mumau, M.D., Gonzalez, M.V., Ma, C., Irvine, A.H., Sarmiento Bustamante, M.S., Shyamsundar, S., Chen, L.Y.C., Koslicki, D., and Fajgenbaum, D.C. (2025). Identifying and Targeting TNF Signaling in Idiopathic Multicentric Castleman's Disease. N. Engl. J. Med. *392*, 616–618. https://doi.org/10.1056/NEJMc2412494.

106. Subramanian, A., Narayan, R., Corsello, S.M., Peck, D.D., Natoli, T.E., Lu, X., Gould, J., Davis, J.F., Tubelli, A.A., Asiedu, J.K., et al. (2017). A next generation connectivity map: L1000 platform and the first 1,000,000 profiles. Cell *171*, 1437–1452.e17. https://doi.org/10.1016/j.cell.2017.10.049.

107. Barretina, J., Caponigro, G., Stransky, N., Venkatesan, K., Margolin, A.A., Kim, S., Wilson, C.J., Lehár, J., Kryukov, G.V., Sonkin, D., et al. (2012). The Cancer Cell Line Encyclopedia enables predictive modelling of anticancer drug sensitivity. Nature *483*, 603–607. https://doi.org/10.1038/nature11003.

108. Tsherniak, A., Vazquez, F., Montgomery, P.G., Weir, B.A., Kryukov, G., Cowley, G.S., Gill, S., Harrington, W.F., Pantel, S., Krill-Burger, J.M., et al. (2017). Defining a Cancer Dependency Map. Cell *170*, 564–576.e16. https://doi.org/10.1016/j.cell.2017.06.010.