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Review article

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Emerging clinical applications of single-cell RNA sequencing in oncology

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Abstract

Single-cell RNA sequencing (scRNA-seq) has revolutionized our understanding of complex tissues both in health and in disease. Over the past decade, scRNA-seq has been applied to tumour samples obtained from patients with cancer in hundreds of studies, thereby advancing the view that each tumour is a complex ecosystem and uncovering the diverse states of both cancer cells and the tumour microenvironment. Such studies have primarily investigated and provided insights into the basic biology of cancer, although considerable research interest exists in leveraging these findings towards clinical applications. In this Review, we summarize the available data from scRNA-seq studies investigating samples from patients with cancer with a particular focus on findings that are of potential clinical relevance. We highlight four main research objectives of scRNA-seq studies and describe some of the most relevant findings towards such goals. We also describe the limitations of scRNA-seq, as well as future approaches in this field that are anticipated to further advance clinical applicability.

Sections

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Key points

- Single-cell RNA sequencing (scRNA-seq) technologies facilitate a comprehensive understanding of the tumour ecosystem, particularly the intratumour heterogeneity of cancer cells and those of the tumour microenvironment.
- With the maturation of the field, scRNA-seq is now increasingly being applied to address clinically important questions in a way that might ultimately inform routine patient management.
- Potential clinical applications of scRNA-seq might be broadly described by four objectives: the refinement of tumour subtyping, the characterization of treatment-induced changes, the identification of expression programmes predictive of treatment response and the discovery of novel therapeutic targets.
- Barriers to a more streamlined embedding of scRNA-seq into clinical research include difficulties in sample acquisition and the computational challenges inherent in integrating varied datasets.

Introduction

Cancer research has traditionally considered the tumour as a single entity, primarily ascribing biological features — genetics, histology and classification — to the entire tumour. However, this approach fails to reflect that each tumour is a complex ecosystem with diverse interacting components that together give rise to its phenotypes and clinical characteristics. Thus, a more realistic paradigm of cancer is developing that recognizes the tumour's various cellular components, their specific functions, their interactions and the emergent clinical phenotype. Such a renewed 'systems biology' understanding of cancer is ushering in a revolution in cancer research and will hopefully also promote the development of various new therapeutic strategies. One driver of this revolution has been the development and instrumental success of immunotherapies, which has highlighted the important role of the immune microenvironment as well as the interactions of immune cells with other tumour components. A second driver of interest in this area is technological — single-cell RNA sequencing (scRNA-seq) has risen over the past decade from a novel technology into a standard molecular biology tool and its widespread adoption has transformed cancer research.

In this Review, we highlight the most pertinent and clinically relevant findings gleaned from scRNA-seq studies of patient-derived tumour material. We found that potential clinical applications can be broadly categorized into one of four research objectives: the refinement of tumour subtyping, the characterization of treatment-induced changes, the identification of expression programmes predictive of treatment response and resistance, and the discovery of novel therapeutic targets (Table 1 and Fig. 1). We first focus on progress made towards these four objectives, and then discuss the prospects for incorporation of scRNA-seq technologies and insights into clinical practice, as well as the current limitations that might constrain further progress.

scRNA-seq methodologies and initial discoveries

The various single-cell sequencing methodologies have been described in detail elsewhere[1,2]. Briefly, typical scRNA-seq protocols encompass five main steps: (1) tissue dissociation, (2) encapsulation of individual cells in well plates or in drops of microfluid devices (most commonly by the 10x Genomics platforms), (3) generation of barcoded cDNA from poly-adenylated mRNAs, (4) sequencing and (5) quantification of expression level per gene. Conceptually, scRNA-seq can be viewed as providing similar data to that of bulk RNA sequencing (RNA-seq), albeit at a single-cell level of resolution. Practically, however, scRNA-seq data are affected by higher levels of noise, which requires complex analysis, as well as batch effects and the computational challenges associated with the large volumes of data generated (typically 10–100 MB per cell, reaching terabytes of data for atlas-level studies). Accordingly, numerous methods have been developed to visualize, normalize and analyse scRNA-seq datasets and have led to diverse discoveries across molecular biology.

Over the past decade, scRNA-seq studies dealt primarily with the fundamentals of cellular states that underlie intratumour heterogeneity (ITH). Recurrent ITH expression programmes related to cell cycle and stress responses in cancer cells have been described in numerous studies[3–6]. Malignant programmes enriched in mesenchymal genes that were traditionally associated with epithelial-to-mesenchymal transition (EMT) were also uncovered and, therefore, thought to reflect the presence of partial or hybrid EMT cell states[7]. In a more context-dependent fashion, cancer cells in various tumour types were shown to express programmes that recapitulate the developmental and/or physiological processes of the tissue of origin, albeit usually in a partial and sometimes distorted fashion, as seen in lung and colorectal cancer (CRC)[8,9]. Many other scRNA-seq studies examined the tumour microenvironment (TME)[5,10], resulting in important observations on immune and stromal cell diversity as well as possible modulation of immune cell activity by certain cancer cell populations.

Taken together, these early findings substantially improved our understanding of the tumour ecosystem, although their applicability to the clinical space remained limited. With the maturation of the field, particularly owing to a series of technological developments enabling easier analysis of archival samples and hence of larger cohorts (hundreds of samples, or potentially even more), efforts are now underway to correlate key scRNA-seq findings with clinical outcomes in a way that will ultimately inform patient management.

Defining clinically relevant objectives of scRNA-seq studies

Briefly, consensus tumour subtyping, which has thus far largely relied on distinct tumour profiles based on bulk-sequencing data, can be further refined using single-cell analysis, which also accounts for the prevalence and distribution of previously unknown subpopulations. A more granular stratification of tumours at diagnosis might aid in determining prognosis and in tailoring treatments; as one example, two new subtypes of CRC, iCMS2 and iCMS3, underpinned by dichotomous malignant epithelial populations, were shown to confer differential sensitivity to standard-of-care chemotherapy[11].

The second objective, characterizing treatment-induced changes, has been investigated using scRNA-seq in the context of every major nonsurgical treatment modality, including chemotherapy, immunotherapy, targeted therapy and radiotherapy. Studies in this area typically compare treatment-naive versus treated samples, either from different patients or those obtained longitudinally from the same patient. A common goal of these analyses is to pinpoint the post-treatment changes associated with either a favourable

Table 1 | Summary of single-cell RNA sequencing cancer studies with clinically relevant findings

Tumour type	Findings	Ref.
Refined tumour subtyping		
HNSCC	Confirmed three of four previously described subtypes, while also finding that the TCGA mesenchymal subtype is derived from nonmalignant cells	20
HGSOC	Two previously described intrinsic subtypes are driven by the abundance of immune and mesenchymal cells	21
Glioblastoma	Commonly accepted subtypes are determined by the abundance of different malignant cellular states rather than by distinct cell types. These states also correlate with the presence of specific genetic aberrations	22
HPV$^+$ HNSCC	HPV$^+$ tumours with limited expression of HPV-related genes tend to have an inferior prognosis	6
NSCLC	Specific subtypes correlate with metastatic dissemination and an inferior prognosis	23
Prostate cancer	A specific malignant subtype correlates with the presence of a specific gene fusion event	24
CRC	Two intrinsic epithelial subtypes demonstrated to have differential sensitivity to chemotherapy	11
Chemotherapy-induced changes		
PDAC	A neural-like progenitor programme is enriched in residual tumour material and patient-derived organoids after cytotoxic therapy and is associated with inferior clinical outcomes	25
Breast cancer	Post-treatment samples harbour resistant malignant cell populations with upregulated immune-checkpoint expression, including PD-L1 and CD80	26
PDAC	Post-neoadjuvant treatment samples have substantially elevated levels of CAFs, among them the protumorigenic inflammatory CAF (iCAF) subtype	33
PDAC	Post-treatment samples have reduced expression of inhibitory checkpoint molecules, as well as fewer inhibitory ligand–receptor interactions	34
NPC	Cisplatin plus gemcitabine activates an innate-like B cell-dominant antitumour immune response that is positively associated with disease-free and overall survival	35
Predictive signatures for chemotherapy		
HGSOC	TCGA samples with a 'stress-high' gene expression score associated with significantly shorter PFS on standard-of-care platinum-based therapy	14
Effects of targeted therapies		
Breast cancer	Combination of endocrine therapy plus the CDK4/6 inhibitor ribociclib leads to rapid loss of oestrogen signalling and activation of the JNK signalling pathway. Tumours that maintain oestrogen signalling have increased levels of CDK4/6 activation and ERK signalling through ERBB4	66
IDH-mutant glioma	The IDH inhibitor vorasidenib induces lineage differentiation to a more differentiated astrocytic-like state	68
Myelodysplastic syndromes	Patients develop resistance to venetoclax, which targets BCL-2-mediated anti-apoptotic signalling, by shifting to compensatory TNF-driven prosurvival NF-κB signalling	67
Predictive signatures for targeted therapies		
NSCLC	Responders to tyrosine kinase inhibitors have increased T cell and decreased macrophage infiltration, whereas those with progressive disease have reduced T cell infiltration and an increase in IDO1-expressing macrophages	70
CML	Resistance to imatinib linked with dysfunctional NK cells, whereas responders have hyperfunctional adaptive-like NK cells	72
NSCLC	Accumulation of lipid-associated macrophages in patients with resistance to osimertinib	71
Effects of immunotherapies		
TNBC	T cell infiltration increased in patients with a pathological complete response following neoadjuvant pembrolizumab±radiotherapy	15
CRC	An extensive reduction in proinflammatory immune and stromal cell numbers in samples from patients with mismatch repair-deficient CRC with a response to ICIs	12
CRC	Antitumour T cells can be found in the vicinity of myeloid and malignant cells expressing interferon-stimulated genes	53
Basal or squamous cell carcinoma	Clonal replacement of tumour-specific T cells following PD-1 blockade	42
HNSCC	Tissue-resident memory and circulating T cells mediate early responses to neoadjuvant anti-PD-1, with or without anti-CTLA4 antibodies	44
NSCLC	Expanded T cell clones post-therapy often have tumour-binding properties, which are associated with better clinical outcomes	45
NSCLC	Described transcriptional programmes of neoantigen-specific tumour-infiltrating lymphocytes in samples from patients who received anti-PD-1 antibodies	46
Bladder cancer	CD4$^+$ T cells have enhanced cytotoxicity following treatment with anti-PD-1 or anti-PD-L1 antibodies	40
Breast cancer	Described immune cell subsets associated with response to anti-PD-L1 antibodies	41

Table 1 (continued) | Summary of single-cell RNA sequencing cancer studies with clinically relevant findings

Tumour type	Findings	Ref.
Predictive signatures for immunotherapies		
ESCC	A progenitor/precursor exhausted CD8$^+$ T cell population is associated with favourable outcomes following neoadjuvant anti-PD-1 antibodies	58
ccRCC	A population of CD8$^+$ tissue-resident T cells is positively associated with a favourable response to anti-PD-1 antibodies either as monotherapy or in combination with an anti-CTLA4 antibody	13
ccRCC	A transcriptomic signature reflecting macrophage–CD8$^+$ T cell interactions was shown to be predictive of response to ICIs in real-world data	59
Breast, melanoma, colon and rectal cancers	TCR clonotypes can be leveraged to identify tumour-specific immunogenic antigens	47
Novel therapeutic strategies		
Glioma	NK-receptor CD161 is expressed on clonally expanded tumour-infiltrating T cells and might be a target for immunotherapy	49
H3K27M glioma	PDGFRA drives the development of an oligodendrocyte precursor-like state, and might be a potential target for differentiation therapy	16
Neuroblastoma	MYCN and loss of TFAP2b identified as a possibly targetable barrier to differentiation in a subset of neuroblastoma with mesenchymal features	74
IDH-mutant glioma	In responding patients, the IDH inhibitor vorasidenib induces lineage differentiation to a more differentiated astrocytic-like state	68
HNSCC	An epithelial senescence-associated cellular state confers differential sensitivity to EGFR inhibitors in patients with head and neck cancer	76
PDAC	The basal cellular state potentially confers sensitivity to MAPK inhibitors and might be inducible with TGFβ	77
HGSOC	JAK/STAT inhibitors show activity against specific immune-related HGSOC cell states	21
Prostate cancer	KIT inhibitors explored for potential targeting of adenocarcinoma-to-neuroendocrine transition in prostate cancer	75
Melanoma	CDK4/6 inhibitors reverse an immune resistance-associated cellular state	17
ccRCC	A subpopulation of C1Q$^+$TREM2$^+$APOE$^+$ TAMs is associated with postsurgical disease recurrence, and is proposed as a candidate therapeutic target	78
Pan-cancer	The terminally exhausted T cell state is associated with inferior survival outcomes and reduced responsiveness to immunotherapy. Two distinct developmental trajectories leading to T cell exhaustion were discovered, which could be targeted to promote responsiveness to immunotherapy	80
PDAC	Combined inhibition of IL-1β signalling and COX2 proposed as a strategy for targeting IL-1β$^+$ TAMs driving epithelial-to-mesenchymal transition and a PGE2-driven response in PDAC cells	79
Melanoma	Selective depletion of CD39$^+$TIM3$^+$CD8$^+$ T cells from tumour infiltrates sensitizes ex vivo tumours to anti-PD-1 antibodies	48
Leptomeningeal metastasis	Iron chelation therapy for leptomeningeal metastases in patients with lung or breast cancers starves cancer cells that adapted to the nutrient-depleted cerebrospinal fluid	83

CAFs, cancer-associated fibroblasts; ccRCC, clear-cell renal cell carcinoma; CML, chronic myelogenous leukaemia; CRC, colorectal cancer; ESCC, oesophgeal squamous cell carcinoma; HGSOC, high-grade serous ovarian carcinoma; HNSCC, head and neck squamous cell carcinoma; HPV, human papilloma virus; ICIs, immune-checkpoint inhibitors; NK, natural killer; NPC, nasopharyngeal carcinoma; NSCLC, non-small-cell lung cancer; OS, overall survival; PDAC, pancreatic ductal adenocarcinoma; PFS, progression-free survival; TAMs, tumour-associated macrophages; TCGA, The Cancer Genome Atlas; TCR, T cell receptor; TNBC, triple-negative breast cancer.

or unfavourable response to treatment, such as the upregulation of markers of T cell exhaustion, which are associated with an inferior response to immune-checkpoint inhibitors (ICIs) in patients with CRC[12].

Occasionally, the post-treatment changes detected in such studies might be leveraged to promote the third research objective: identifying pretreatment predictive signatures. Here, a common approach involves examining whether an ITH programme, once identified at the single-cell level and potentially associated with a specific response pattern, can be identified and quantified in treatment-naive samples (even from bulk RNA-seq data) and then correlated with clinical outcomes. Through this basic approach, several putative predictive signatures have been proposed both for chemotherapy and ICIs across a diverse range of cancer types, including high-grade serous ovarian carcinoma (HGSOC), breast cancer and renal cell carcinoma[13–15].

Finally, data from various studies have led to the proposal of novel therapeutic targets based on scRNA-seq findings. These are typically targets with a role in the differentiation and propagation of aggressive malignant cell states[16]. Notably, in some scenarios, the proposed target might be druggable with one or more compounds already in widespread use for a different tumour type, for example, a resistance programme identified in patients with melanoma can be repressed using CDK4/6 inhibitors in mouse xenograft models harbouring this programme[17].

Refinement of tumour subtyping

Substantial progress has been made by integrating genetics (specific gene sequence alterations) and epigenetics (DNA methylation markers) with bulk-level transcriptomic (tissue-level RNA-seq) data for tumour subtype classification. This molecular framework

provides an improved understanding of intertumour heterogeneity, thereby potentially influencing diagnosis, prognosis and treatment strategies. Methodologically, bulk RNA expression data are used to perform unsupervised clustering of tumour samples, which is then followed by differential expression analysis to identify the dominant genes that distinguish each cluster. In some tumour types, such molecular classifications are more clinically relevant than histology-based classification[18,19]. For example, an immune-rich subtype of melanoma was first described based on unsupervised analysis of bulk RNA-seq data, and was associated with improved survival outcomes[19].

Multiple scRNA-seq studies have now stratified tumours based on single-cell transcriptomic data and compared their results against stratification by bulk RNA-seq. Although scRNA-seq often reveals the same or similar subtypes as those derived using bulk RNA-seq, important differences have emerged from multiple studies. First, bulk subtypes probably reflect the abundance of shared cell types and cellular states rather than the presence of unique cells. Second, more granular classifications are likely to be provided by scRNA-seq.

Bulk subtypes often reflect the abundance of specific cell types

A study mapping single head and neck squamous cell carcinoma (HNSCC) cells to bulk The Cancer Genome Atlas (TCGA) subtypes revealed alignment with only three of the four consensus subtypes (basal, classical and atypical), whereas cancer-associated fibroblasts (CAFs) mapped to the fourth subtype (mesenchymal)[20]. All HNSCCs contain some fibroblasts, and those with a high fraction of fibroblasts were classified as mesenchymal. Notably, on follow-up analysis, the malignant cells of tumours originally classified as mesenchymal by bulk RNA-seq were more consistent with the basal subtype, highlighting the differences between tumour classification by single-cell versus bulk-sequencing data. Similarly, the mesenchymal and immunoreactive subtypes of HGSOC were found not to reflect the gene expression patterns of malignant cells, but were instead derived from an abundance of fibroblasts and macrophages, respectively[21].

In other scenarios, tumours largely share the same malignant cellular states and bulk subtypes reflect the abundance of these shared states. For example, in glioblastoma, the abundance of each of the four main malignant states (astrocyte-like, oligodendrocyte progenitor-like, neural progenitor-like and mesenchymal-like) is consistent with a specific bulk subtype previously defined by TCGA, with the TCGA proneural (TCGA-PN) subtype reflecting co-occurrence of the oligodendrocyte progenitor-like and neural progenitor-like states, and the TCGA mesenchymal (TCGA-MES) subtype corresponding to a combination of the mesenchymal-like malignant state and immune cells[22].

Thus, bulk RNA subtypes might primarily reflect one of three main features: the presence of unique cellular phenotypes that differ between patients (for example, basal, classical and atypical subtypes in HNSCC), the abundance of shared TME cell types (such as fibroblasts in mesenchymal subtypes or immune cells in immune subtypes in various cancer types) or the abundance of shared malignant states (such as glioblastoma subtypes). scRNA-seq allows us to better distinguish these effects and, therefore, better understand the previous classifications, as well as potentially define new subtypes based on a combination of unique cellular signatures and/or cellular frequencies.

scRNA-seq enables more granular classifications

Several studies have demonstrated the ability to uncover novel cancer subtypes on the basis of the higher resolution afforded by scRNA-seq, including subtypes of potential clinical relevance. One example comes from human papilloma virus (HPV)-positive HNSCC, in which scRNA-seq analysis revealed that the expression of HPV-related genes is repressed in a subset of cells and suggests that HPV$^+$ tumours could be further stratified by the degree of such repression. Tumours with extensive HPV repression, and accordingly with lower levels of expression of HPV-related genes, were deemed 'HPV low' and associated with a worse prognosis in this study[6].

In an analysis of 44 lung adenocarcinoma samples[23], including samples from both primary tumours and metastases from various anatomical locations, two dominant subtypes were identified. One subtype was specifically associated with metastatic dissemination and correlated with a poor prognosis when assessed in large bulk RNA expression datasets. This subtype was associated with malignant cells with upregulation of genes associated with detoxification and immune responses, as well as with dysfunctional T cells and protumorigenic macrophages. Testing for this subtype in high-risk populations could help identify and treat patients who are likely to have the worst prognosis at earlier disease stages.

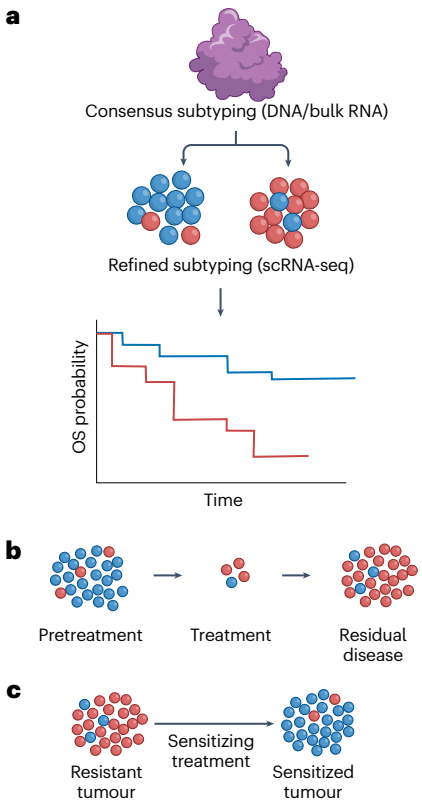

Fig. 1 | Common clinically relevant objectives of single-cell RNA sequencing cancer studies. a, The refinement of consensus tumour subtyping facilitates more accurate prognostic stratification, which might inform treatment decisions at diagnosis. **b**, Analysis of treatment-induced changes, highlighting emergent intratumour heterogeneity programmes driving resistance. **c**, Analysis of the mechanisms underlying treatment resistance processes, suggesting possible tumour-sensitizing treatments. OS, overall survival; scRNA-seq, single-cell RNA sequencing.

In another example, the ERG-positive prostate cancer subtype was identified using scRNA-seq. When compared with the established TCGA prostate cancer subtypes, no direct correlation with any known subtype was found. Notably, this transcriptional subtype had a robust association with the gene expression pattern of the *TMPRSS2–ERG* fusion, thus highlighting the capability of scRNA-seq to connect genotypic classifications with phenotypic stratification at the mRNA level[24]. Such phenomena were also described in glioblastoma, in which malignant cellular states correlate with the presence of specific genetic alterations, such as high-level amplifications of *EGFR*, *PDGFRA*, and *CDK4* with the astrocyte-like, oligodendrocyte-like and neural progenitor-like states, respectively[22].

Response to chemotherapy

Chemotherapy remains the standard-of-care approach for patients with many cancers, although efficacy is often undermined by both innate and acquired resistance. By comparing baseline tumour samples obtained before treatment with on-treatment and/or post-treatment samples, scRNA-seq can be used to identify post-treatment phenomena that might warrant targeting with additional therapies; these include reprogramming of cancer cell states, alongside various cell-type enrichments and transcriptional alterations within the TME.

Shifts in cancer cell states

Cancers that might be treated with neoadjuvant therapy before surgery, including locally advanced breast cancer and borderline resectable pancreatic ductal adenocarcinoma (PDAC), are especially suitable for analysing chemotherapy-induced shifts in cancer cell states. For example, scRNA-seq analysis of resected PDAC specimens from patients who received neoadjuvant chemotherapy (mostly folinic acid, fluorouracil, irinotecan and oxaliplatin (FOLFIRINOX)) with or without radiotherapy showed enrichment of a newly identified malignant neural-like progenitor programme, as well as a neuroendocrine-like programme, at the expense of the hallmark PDAC classical and basal gene expression programmes[25]. The authors then leveraged TCGA bulk RNA-seq data to demonstrate a correlation between the neural-like progenitor programme and an inferior prognosis, an effect driven by shortened time to disease progression. In another example, post-neoadjuvant therapy samples from patients with breast cancer were shown to harbour resistant cancer cell subpopulations that upregulate established immunomodulatory genes, including *CD247* (encoding PD-L1) and *CD80*. The authors suggested, therefore, that a combination chemo–immunotherapy regimen might overcome resistance in this setting[26].

Repeat longitudinal samples can be obtained more easily from patients with haematological malignancies than from those with solid tumours, and serial longitudinal sampling has the potential to provide multiple insights into treatment-induced changes[27–29]. For example, in acute myeloid leukaemia (AML), a subpopulation of dormant miR-126^{high} leukaemia stem cells was shown to persist and correlate with treatment failure following induction chemotherapy[27]. In a related study, samples from patients with treatment-refractory AML were found to harbour a population of proliferating stem/progenitor-like cells with evidence of reprogramming towards a quiescent stem-like expression programme[28]. Similar efforts have been directed towards paediatric leukaemia[30–32]. In one analysis of samples obtained from paediatric patients with AML, combining scRNA-seq and the assay for transposase-accessible chromatin with sequencing (ATAC-seq), samples from patients with relapsed tumours showed evidence of having undergone distinct shifts in cellular hierarchies, including an enrichment of primitive cells and a transition away from the myeloid lineage[30].

Chemotherapy-induced changes in the TME

scRNA-seq also provides a useful method of exploring how the TME is reshaped by chemotherapy. In one study, investigators collected primary PDAC samples from 21 patients, of whom 7 were treatment naive, while 14 received various neoadjuvant regimens, including FOLFIRINOX, gemcitabine plus nab-paclitaxel or preoperative chemoradiotherapy[33]. The authors demonstrated substantially increased levels of CAFs in treated samples, driven primarily by a threefold increase in the inflammatory CAF subpopulation, which is considered protumorigenic.

Another pertinent finding from the post-treatment PDAC space relates to the effect of chemotherapy on subsequent refractoriness to ICIs, which reflects a common clinical challenge. Comparing primary and metastatic samples from treatment-naive and previously treated patients (who received either FOLFIRINOX or gemcitabine plus nab-paclitaxel), investigators found reduced expression of *PDCD1*, *CTLA4*, *TIGIT* and the genes encoding other inhibitory immune-checkpoint molecules, among CD8$^+$ T cells present in post-treatment samples[34].

Finally, scRNA-seq analysis of samples from patients with nasopharyngeal carcinoma (NPC) demonstrates that cisplatin plus gemcitabine chemotherapy, which is often administered before definitive radiotherapy, activates an innate-like B cell-dominant antitumor immune response[35]. Notably, a positive association between innate-like B cell frequency and both overall and disease-free survival has been demonstrated in an analysis of 139 NPC samples obtained in a phase III trial.

Predictive markers of response to chemotherapy

Although the above examples highlight the utility of scRNA-seq in uncovering effects of chemotherapy that might drive responsiveness or disease progression on subsequent therapies, another avenue of investigation focuses on the identification of pretreatment gene expression programmes that might be predictive of upfront chemotherapy resistance and, therefore, warrant a change in the initial treatment strategy.

Platinum resistance in HGSOC has already been investigated with this purpose in mind. Investigators longitudinally analysed paired pre- and postchemotherapy samples obtained from 11 patients and described the fractional changes in cancer cell states brought about by treatment with platinum-based chemotherapy plus a taxane[14]. Gene expression signatures associated with cellular stress and proliferation were both enriched in cancer cells following treatment with chemotherapy, enabling the authors to compute a 35-gene 'stress score' deconvoluted from bulk TCGA RNA-seq data. On a Kaplan–Meier survival analysis, TCGA samples obtained from treatment-naive patients with HGSOC with a 'stress-high' score had a significantly shorter median progression-free survival on standard-of-care first-line platinum-based therapy compared with the 'stress-low' group (14.9 months versus 21.2 months; $P = 0.0037$), suggesting that this 'stress state' exists before administration of chemotherapy and becomes enriched during treatment.

Response to ICIs

ICIs have transformed the treatment of patients with cancer and are now first-line therapies or part of the first-line regimens for many

advanced-stage solid tumours including driver-negative non-small-cell lung cancer, melanoma, triple-negative breast cancer (TNBC) and others[36]. Despite their widespread use, most patients with advanced-stage disease do not respond to these agents[37]. Even among patients with an initial response, most will eventually have disease relapse[38,39]. A better understanding of the immunological processes underlying these treatments is therefore required for better utilization and to inform the development of more effective immunotherapies or combinations.

T cell analysis in the context of immunotherapy

Data from scRNA-seq studies have provided detailed insights into the diversity of T cell populations and their association with response to anti-PD-1 and anti-PD-L1 antibodies. Unlike bulk sequencing, which provides an average of gene expression across large populations of cells, scRNA-seq enables the identification of specific T cell subsets at high levels of resolution. For example, data from two studies investigating bladder cancer and TNBC[40,41] identified unique subpopulations of cytotoxic T cells that differ from the broader signatures of T cell cytotoxicity and that specifically correlated with treatment response. In bladder cancer, researchers found a $CD4^+$ cytotoxic T cell state that was predictive of clinical response to the anti-PD-L1 atezolizumab[40]. Similarly, in TNBC, a specific $CD8^+$ cytotoxicity cell state was correlated with a better response to atezolizumab[41]. Thus, the abundance of particular cytotoxic T cell states (as opposed to the overall expression of markers associated with cytotoxicity) might have important clinical consequences and can only be quantified accurately using single-cell analysis.

Another key observation is the expansion of certain T cell populations in post-treatment samples[34–36], sparking questions regarding the origins of the expanded T cells. Exhausted T cells that have been reactivated by an ICI might be tissue-resident memory T cells that were triggered to respond, or potentially newly recruited T cells that infiltrated the tumour[42,43]. An analysis of HNSCC tumour-infiltrating $CD8^+$ T cells that underwent clonal expansion during treatment with an anti-PD-1 antibody demonstrates the expression of genes associated with tissue-resident memory and cytotoxicity phenotypes, leading the investigators to hypothesize that the therapeutic response is dependent on the presence and activity of non-exhausted tissue-resident memory T cells[44].

Leveraging the robustness of single-cell technologies, investigators have sequenced the variable regions of T cell receptor (TCR) genes[45]. These studies demonstrate that a subset of post-treatment expanded T cell clones are able to recognize cancer neoantigens[45,46]. However, this expansion did not clearly correlate with treatment response, suggesting that recognition of cancer neoantigens might not be the only bottleneck for an effective ICI response. In another study, investigators demonstrated how identifying unique rearrangements in TCR genes, via TCR-seq, can be used to uncover immunogenic targets. By analysing expanded T cell clones post-therapy, this approach enabled the identification of novel tumour-reactive TCRs. These findings pave the way for developing novel therapies, such as personalized T cell-based immunotherapies or vaccines targeting these tumour-specific antigens[47].

Additional markers of T cell exhaustion have been identified in patients receiving ICIs, offering insights into novel therapeutic strategies. In melanoma, for example, $CD8^+$ T cells associated with better treatment responses were found to gain expression of exhaustion markers such as ENTPD1 and TIM3 following immune-checkpoint inhibition[48]. Combination therapies targeting these markers, such as inhibitors of the cell-surface ectonucleotidase enzyme ENTPD1, which catalyses the breakdown of extracellular ATP to adenosine, with anti-PD-1 antibodies, have shown improved tumour control and prolonged survival in mouse models[47].

In patients with glioma, clonally expanded tumour-infiltrating T cells have been found to express *KLRB1*, which encodes CD161, a natural killer (NK) receptor capable of acting as an immune checkpoint. Inhibiting CD161 has been shown to promote T cell-mediated cytotoxicity in patients with glioma[49]. This discovery has led to an ongoing clinical trial (NCT05565417) investigating the efficacy of anti-CD161 monoclonal antibodies in patients with advanced-stage solid tumours or lymphomas.

Immunotherapy-induced changes in the TME

Clinical responses to ICIs are primarily thought to be mediated by T cells[50–52], although an increasing recognition exists that the activity of these therapies also depends on various components of the TME. Accordingly, numerous scRNA-seq studies have examined ICI-induced changes in other TME components. In one study, investigators described an extensive reduction in the populations of proinflammatory immune and stromal cells in patients with CRC who respond to ICIs[43]. Based on scRNA-seq data from 62 patients with CRC, another research group found that antitumour T cells are located in the vicinity of myeloid and malignant cells expressing interferon-stimulated genes and are involved in antigen presentation[53]. This observation is supported by a large pan-cancer study showing an association between cytotoxic $CD8^+$ T cells and interferon-stimulated gene-expressing cells within the TME[7].

Predictive markers for immunotherapy response

Tumour mutational burden, microsatellite-instability status, and PD-L1 expression are all used clinically to predict a response to ICIs, although additional and more effective biomarkers are needed[54–56]. In melanoma, a subset of $CD8^+$ T cells characterized by high levels of TCF7 expression has been shown to predict a favourable clinical response to ICIs[48,57]. This $CD8^+$ T cell subset, commonly referred to as progenitor/precursor exhausted T cells owing to the co-expression of markers associated with memory and exhaustion, was found to be associated with favourable outcomes in patients with oesophageal cancer treated with anti-PD-1 antibodies in the neoadjuvant setting[58]. Similarly, a population of $CD8^+$ tissue-resident T cells has been positively associated with a favourable response to ICIs in an analysis of samples from six patients with clear-cell renal cell carcinoma (ccRCC)[13]. In another study involving real-world patients with ccRCC, the presence of a combined signature incorporating cytotoxic $CD8^+$ T cells with factors related to antigen presentation and interferon signalling correlates with favourable outcomes following treatment with ICIs[59].

Response to targeted therapies

Targeted therapies, including small-molecule inhibitors, monoclonal antibodies and endocrine therapies, have dramatically improved the outcomes of patients with certain cancer types[60–62]. Nevertheless, many patients either do not respond to these therapies or develop resistance and have disease relapse on or following treatment[63–65]. The mechanisms of resistance are diverse and include resistant subpopulations that expand in the presence of the selective therapeutic pressures created by the targeted therapy, acquisition of various mechanisms of resistance owing to on-treatment changes in tumour biology and drug tolerance induced by the TME.

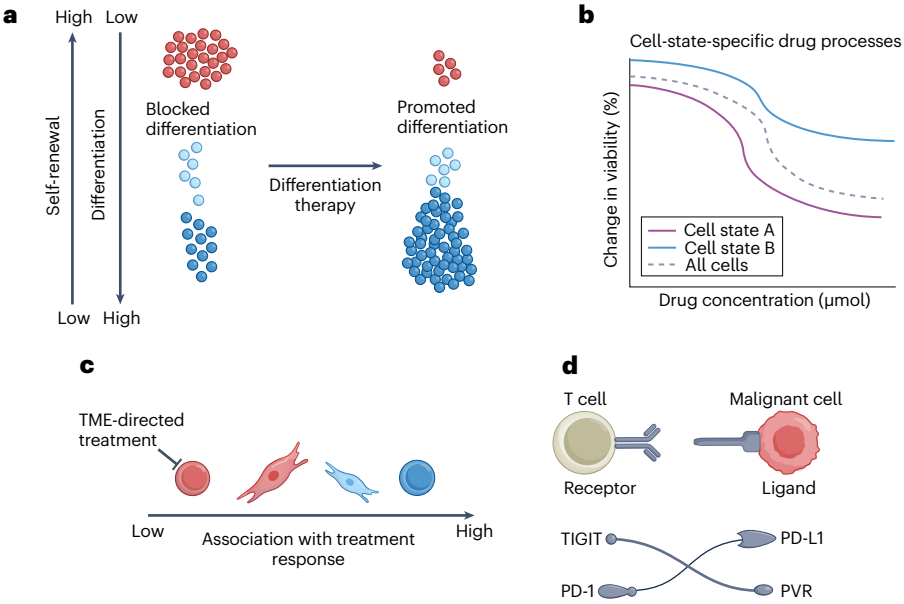

Fig. 2 | Discovery of novel therapeutic targets. **a**, Differentiation therapy. In this approach, barriers to differentiation are targeted to drive transitions from a proliferative, poorly differentiated cancer cell state back to a more differentiated one. **b**, Cell-state-specific drug responses. Drug screens in cell lines or organoid models can be used to guide differential targeting of co-existing cancer cell states. **c**, Tumour microenvironment (TME)-directed therapy. TME subpopulations associated with an inferior response to therapy (such as immunosuppressive cells) could be selectively targeted and depleted as a therapeutic strategy. **d**, Uncovering targetable immune interactions. Ligand–receptor interaction analyses highlight prominent immunomodulatory interactions that might be candidates for therapy.

Targeted therapy-induced changes in cancer cells and the TME

Breast cancer cells are able to adapt to treatment by shifting towards a reliance on alternative signalling pathways to support tumour growth: in response to endocrine therapy alone, cells compensate for the loss of growth signals via increased ERBB4 signalling while maintaining oestrogen dependence; whereas combination with a CDK4/6 inhibitor might enable a subset of cells to completely bypass oestrogen signalling by upregulating the JNK signalling pathway[66]. These pathways are typically upregulated earlier in the disease trajectory in nonresponders and later in the remaining malignant cells of patients with an initial response. Similarly, patients with myelodysplastic syndromes are able to develop resistance to venetoclax, which targets BCL-2-mediated anti-apoptotic signalling, by shifting to compensatory TNF-driven prosurvival NF-κB signalling[67]. Conversely, an scRNA-seq analysis of samples from three patients with *IDH*-mutant oligodendrogliomas who responded to IDH inhibitors showed a shift in cellular states, characterized by an increase in differentiated astrocytic-like cells, a reduction in stem-like cells and decreased cellular proliferation[68]. This example suggests that IDH inhibitors function as a form of differentiation therapy in this setting and highlight a general strategy that might be effective in other contexts[68,69].

Predictive marker for response to targeted therapies

Immune modulation has been shown to have a role in resistance to targeted therapies across multiple cancer types[70–72]. Among patients with metastatic non-small-cell lung cancer, those who respond to tyrosine kinase inhibitors often have increased T cells and decreased macrophages in tumour samples, whereas those with progressive disease often have fewer T cell levels and an increase in *IDO1*-expressing macrophages[70]. Similarly, resistance to imatinib in patients with chronic myelogenous leukaemia has been linked with the presence of dysfunctional NK cells, whereas long-term responders have hyperfunctional adaptive-like NK cells[72]. Finally, the presence of lipid-associated macrophages in cerebrospinal fluid has been shown to contribute to acquired resistance to the EGFR inhibitor osimertinib, as well as the development of leptomeningeal metastases[71].

Discovery of novel therapeutic targets

scRNA-seq of patient-derived tumour samples has led to the identification of cancer cells harbouring recurrent expression states, reflecting subpopulations of cancer cells that can be common to many patients with a given cancer type, although the abundance of these subpopulations varies substantially across tumours[6,11,16,20–22,73]. For example, a 'partial EMT' programme was shown to be recurrently expressed in a majority of HNSCC samples[6]. Furthermore, these states are often highly plastic, such that cancer cells are likely to transition between states and often possess tumour-initiating capacity in vivo. These insights have led to the development of novel therapeutic strategies targeting either specific malignant cell states, specific TME subpopulations or the drivers regulating the transitions between such states (Fig. 2).

Inducing differentiation and state transitions

scRNA-seq of samples obtained from paediatric patients with histone mutant (H3K27M) gliomas demonstrated that such gliomas consist primarily of proliferative oligodendrocyte precursor (OPC)-like cells driven by PDGFRA signalling, with a minority of cells in more differentiated glial states, suggesting that differentiation beyond this point might be suppressed[16]. These data also suggest that OPC differentiation might be obstructed by inactivation of polycomb repressive complex 2 (PRC2), leading to compensatory upregulation of the PRC1 subunit BMI1. Taken together, these findings suggest that PDGFRA provides a likely target, either alone or in combination with BMI1 inhibition, and that differentiation therapy to drive transitions from an OPC-like state to a more differentiated state might be a viable therapeutic approach[16]. Similarly, another group of investigators proposed differentiation therapy for a subset of poorly differentiated neuroblastomas with mesenchymal features, identifying oncogenic MYCN and loss of TFAP2B as barriers to differentiation[74].

In addition to differentiation therapies, interventions designed to induce or repress other types of state transitions might also have clinical utility. For example, adeno-to-neuroendocrine transitions are an established mechanism of resistance to androgen-deprivation therapy in men with prostate cancer. Data from another scRNA-seq study suggest KIT inhibitors as a method of inhibiting such transitions[75]. Similarly, CDK4/6 inhibitors have been proposed to reverse the development of an immune resistance-associated cancer cell state in melanoma, supported by promising data from mouse models[17].

Targeting state-specific vulnerabilities

Several studies have used cell lines or organoid models to demonstrate differential drug sensitivity of co-existing cancer cell states originally identified in human tumour material. These studies provide proof-of-concept for differential targeting of specific cellular states as a therapeutic paradigm. For example, in vitro HNSCC cells in an epithelial senescence-associated state, defined by a 38-gene signature, were found to respond preferentially to EGFR inhibitors[76]. Consistent with this observation, the estimated abundance of epithelial senescence-associated cells in tumour samples was correlated with improved patient survival after treatment with EGFR inhibitors.

Another study involving patients with PDAC demonstrated that the basal cell state is less sensitive to standard-of-care chemotherapies, underscoring the need to develop novel approaches specifically for this subpopulation. To this end, organoid models were screened for drugs capable of targeting state-specific vulnerabilities. While the PDAC cells characterized by a classical-like RNA signature were more sensitive to chemotherapy and drugs targeting DNA damage repair, those with a basal-like signature were more sensitive to MAPK inhibitors. Investigators also found that exposing organoid models to TGFβ could induce the basal-like state, whereas TGFβ withdrawal resulted in reversion back to the classical state, which resensitized the cancer cells to chemotherapy and highlights the role of plasticity in drug responsiveness[77].

Targeting immune cell states and interactions

Clinically relevant cellular states have been identified within the immune TME, particularly in macrophages and T cells. For example, the presence of specific C1Q$^+$TREM2$^+$APOE$^+$ tumour-associated macrophages has been correlated with post-surgery disease recurrence in patients with ccRCC[78]. Additionally, IL-1β$^+$ tumour-associated macrophages have been associated with EMT, with induction of a PGE2-driven response, and poor clinical outcomes observed in patients with PDAC[79]. This association suggests that agents designed to inhibit IL-1β signalling could be effectively combined with COX2 inhibition. A pan-cancer analysis of tumour-infiltrating T cells revealed a spectrum of exhaustion states, in which terminal exhaustion, marked by high levels of *ENTPD1* expression, is associated with poor survival outcomes and responsiveness to ICIs[80]. This study uncovered two distinct developmental trajectories leading to T cell exhaustion — one through effector memory cells and another through tissue-resident memory cells — both of which are regulated by key transcription factors such as TOX and TCF7 (ref. 80). These findings support the idea that targeting specific exhaustion pathways could improve the outcomes of patients receiving ICIs.

Beyond defining cellular states, scRNA-seq can facilitate the investigation of receptor–ligand interactions (Fig. 2d). In this context, the TIGIT–PVR interaction has been identified as a clinically relevant immune checkpoint in several studies. TIGIT levels are often high in treatment-naive PDAC[25] and serve as a major mechanism of inhibitory immune regulation of CD8$^+$ T cell activity[34]. Interactions between TIGIT and PVR decrease in samples from patients who received chemotherapy, suggesting an important role in the TME and the promotion of resistance to established ICIs. Clinically, and unlike other immune checkpoints, a notable correlation exists between intratumoral CD8$^+$ T cell TIGIT expression and that of peripheral CD8$^+$ T cells, making TIGIT a potentially accessible predictive biomarker[81]. In line with these observations, several anti-TIGIT antibodies are currently being tested in patients with advanced-stage solid tumours in various clinical trials[82].

Targeting niche-specific cellular adaptation

Similar therapeutic strategies to those targeting immune cells are being directed specifically at the metastatic niche. For example, cancer cells that had metastasized to the leptomeninges in lung cancer and breast cancer were profiled using scRNA-seq and found to upregulate the iron-binding protein LCN2 and its receptor, SLC22A17. This adaptation enables cancer cells to survive and proliferate in the nutrient-depleted cerebrospinal fluid by scavenging iron from macrophages[83]. In this model, administration of the iron chelator deferoxamine inhibits LCN2/SLC22A17-mediated iron uptake, thereby starving the cancer cells. Accordingly, intrathecal deferoxamine is now being tested as a therapeutic strategy in a phase I trial involving patients with advanced-stage solid tumours with leptomeningeal metastases (NCT05184816)[84]. While the therapeutic possibilities raised by most scRNA-seq studies have not yet been translated into effective clinical interventions, this is one example of a few approaches that are being tested in clinical trials.

Challenges in leveraging scRNA-seq for clinically relevant discoveries

Although scRNA-seq offers insights into cancer biology at a previously unprecedented level of resolution, several challenges hinder the ability of this technique to yield clinically relevant discoveries. These challenges span all of the major steps of scRNA-seq cancer studies, from sample acquisition to data generation, computational analysis and follow-up experiments (Fig. 3).

Sample acquisition

Ideally, most investigators seek to profile specific types of samples that address important clinical questions, such as metastatic versus matched primary tumours, on-treatment versus matched pretreatment samples, or samples obtained from exceptional responders. However, such clinical samples are often very difficult to obtain, especially as fresh samples (which must be processed within a few hours of resection) are required for many scRNA-seq protocols.

scRNA-seq studies have thus far largely focused on primary tumour samples from treatment-naive patients, and obtaining and profiling samples that would enable well-designed analyses of treatment effects or mechanisms of metastatic dissemination remains challenging. As the vast majority of scRNA-sequenced samples come from surgeries, patients with more disseminated disease, who are not eligible for surgery, are often underrepresented in scRNA-seq studies. Therefore, the insights from these studies are largely limited to patients with better prognoses. An additional group of underrepresented patients are those for whom the standard of care does not include surgery, for example, patients with locally advanced cervical cancer treated with definitive chemoradiation. Moreover, the complexity and costs of scRNA-seq (a typical experiment costs >1,000 USD per sample) have restricted most studies to small sample sizes. Hence, even for questions that

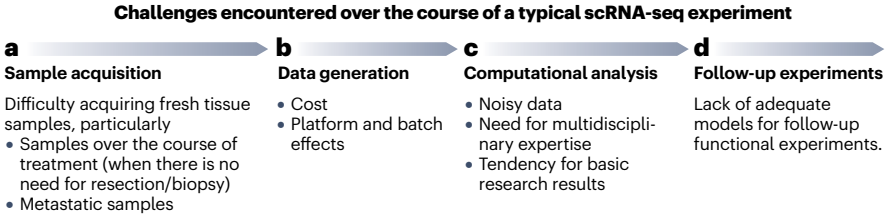

Fig. 3 | Challenges encountered over the course of a typical scRNA-seq experiment. The challenges faced during single-cell RNA sequencing (scRNA-seq) experiments can be divided into four main categories. **a**, Sample acquisition. Fresh tissue samples, including on-treatment samples from patients with metastatic disease, and particularly from exceptional responders, are notoriously difficult to obtain. This difficulty precludes making important experimental comparisons that could provide clinically meaningful results. **b**, Data generation. Differences in sample handling and sequencing protocols can induce 'batch effects' — technical artefacts that might be mistaken for genuine biological differences, thus complicating the interpretation of results. **c**, Computational analysis. Proper analysis of scRNA-seq data requires a multidisciplinary skill set. Additionally, the applicability of the results can be limited, given that RNA expression does not always directly correspond to the expression or functional proteins. **d**, Follow-up experiments. Functional validation of findings from scRNA-seq is often limited by a lack of suitable models capable of accurately replicating the complexity of the TME. This complicates efforts to translate scRNA-seq insights into clinically actionable outcomes.

might be addressable by investigating primary untreated samples, the use of small cohorts has reduced the statistical power of many studies, making significant conclusions difficult (Fig. 3a).

Data generation

The process of tissue acquisition and preparation for scRNA-seq is complex, requiring several delicate steps. Even minor variations in these procedures — including the exact protocol that is used, the time from sample extraction (such as surgery) to dissociation, and even the laboratory and person performing each of the steps — can all substantially affect the resulting data and generate 'batch effects'. As a result, integrating scRNA-seq data from multiple sources becomes extremely difficult and any observed patterns might not reflect true biological phenomena, but could instead stem from technical artefacts. Various data integration approaches have been developed in an attempt to mitigate these issues, although these also have limitations. While designed to remove technical artefacts, these methods might unintentionally also remove important biological signals (Fig. 3b).

Computational analysis

The analysis and interpretation of tumour scRNA-seq data comes with several additional challenges. First, performing the analysis requires expertise in multiple fields, including computational analysis, statistics, biology and medicine. Successful research is therefore challenging and often requires collaboration among experts from different fields. Second, the resulting insights primarily reflect levels of RNA expression rather than the activity of the related proteins, which might reduce the functional relevance and therefore applicability of the results. Third, although scRNA-seq enables the dissection of tumour biology at an unprecedented level of detail, such characterization usually falls into the scope of basic research as it promotes our understanding of the tumour ecosystem but might not lead to clinically relevant observations. Utilizing such information to develop better therapeutic strategies currently remains challenging (Fig. 3c).

Follow-up experiments

The results from scRNA-seq studies typically require follow-up functional experiments involving model systems to validate the conclusions and assess whether the findings might be translated into clinical settings. Although traditional model systems recapitulate many aspects of tumour biology, they often fail to recapitulate specific cellular states and/or cell–cell interactions, highlighting the difficulties in recapitulating the complex patterns and relationships observed in tumour samples obtained from patients. Moreover, even when distinct cellular states are indeed recapitulated in model systems, they tend to be subtle and dynamic, and are therefore often difficult to isolate (such as by flow cytometry) for subsequent experiments and drug screens (Fig. 3d).

Future directions

Spatial transcriptomics and proteomics

scRNA-seq provides a wealth of information on the transcriptional state of each individual cell, yet the exact location of cells is lost as tissues are dissociated and cells (or nuclei) are isolated. Multiple technologies, collectively termed spatial transcriptomics (as well as spatial proteomics), enable the profiling of individual cells while retaining spatial information[85]. This added layer of spatial information reveals potential interactions and the organizational structure of cell types and states within tumours, which might have various important consequences. For example, the proximity of immune cells to malignant cells and the aggregation of immune cells into tertiary lymphoid structures might predict responsiveness to ICIs[15,53]. Another emerging advantage of spatial transcriptomics is the ability to profile (almost) all cells in the tissue, thus ameliorating the biased cell capture that often occurs with scRNA-seq. Given these and other advantages, spatial transcriptomics and proteomics methods have seen rapid improvements and adoption and are projected to soon become widely used, possibly replacing scRNA-seq as the preferred single-cell tumour profiling technology. Although these techniques might not yet have immediate clinical applications, they provide a deeper understanding of the tumour ecosystem that will hopefully lead to clinically relevant discoveries (Supplementary Information).

Increased cohort sizes

Several developments are helping to overcome the challenges associated with small patient cohorts from whom samples are collected, a limitation that has restricted the clinical potential of scRNA-seq studies. First, atlases such as The Curated Cancer Cell Atlas (3CA) compile and analyse scRNA-seq data from multiple studies, helping to standardize analyses and overcome the limitations of individual datasets. Second, technological advances have led to the ability to sequence single nuclei in formalin-fixed, paraffin-embedded tissues.

Formalin-fixed, paraffin-embedded samples are more readily available and include extensive archives of valuable material, thus opening new opportunities for larger-scale studies and those involving rare sample types (such as matched primary and metastatic tumour samples)[86]. Third, multiple ongoing studies are profiling large cohorts of patients (>100 samples). The results of such studies will be published in the next few years for many common cancer types, potentially replacing the TCGA bulk datasets with single-cell atlases that would serve as the foundational resources for a diverse range of future studies (Supplementary Information).

Embedding insights from scRNA-seq insights into clinical contexts

Looking forward, scRNA-seq results could improve clinical cancer care in several possible ways. One example is provided by cancer screening, in which single-cell profiling of nonmalignant and premalignant tissues could shed light on the earliest stages of oncogenesis. By identifying molecular signatures associated with malignant transformation, scRNA-seq could support the development of more sensitive early detection strategies, particularly for high-risk patient populations. These assays could identify early changes in cellular behaviour that probably reflect or precede the development of cancer, helping clinicians make better-informed decisions on preventive interventions and/or closer monitoring.

Another promising application is in clinical trials, in which scRNA-seq of tumour material can provide valuable information. Clinical trials incorporating scRNA-seq can offer greater insight into why treatments fail or succeed, allowing for targeted adjustments of the treatment approach. scRNA-seq could improve patient outcomes by enabling the identification of mechanisms of resistance and uncovering new therapeutic targets or new biomarkers of response. Tumour profiling has thus far mostly focused on treatment-naive samples, therefore applying single-cell sequencing throughout the course of treatment could offer valuable insights into how tumours evolve in response to therapy. Patients can be stratified into responders and non-responders based on their expression profiles, which could potentially guide the development of more personalized therapeutic strategies (Supplementary Information).

Conclusions

scRNA-seq has provided unprecedented insights into the transcriptional landscapes of individual cells within tumours, uncovering the diversity of malignant and nonmalignant populations and their functional states. In this Review we have outlined the progress made towards four clinically relevant objectives: refining tumour subtyping, characterizing treatment-induced changes, identifying predictive biomarkers and uncovering novel therapeutic targets. The identification of transcriptional patterns associated with therapeutic resistance or response illustrates the potential of scRNA-seq to refine patient stratification and support treatment planning. Additionally, the ability of this technique to reveal treatment-induced changes and novel therapeutic vulnerabilities emphasizes its utility in guiding the development of novel combination therapies and advancing precision medicine approaches.

However, translating these insights into clinical practice remains challenging. The technical and logistical complexities of scRNA-seq, including the need for fresh or, more recently, high-quality archival samples, specialized expertise for data generation and analysis, and the costs associated with large-scale studies, limit its accessibility and scalability. Furthermore, batch effects and variability between studies hinder the integration of datasets, while the need for functional validation of findings has slowed the path towards clinical application. Despite these challenges, the findings presented in this Review underscore the growing importance of scRNA-seq in bridging basic research and clinical applications, paving the way for improved diagnostic frameworks, therapeutic strategies and patient outcomes.

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References

1. Kashima, Y. et al. Single-cell sequencing techniques from individual to multiomics analyses. *Exp. Mol. Med.* **52**, 1419–1427 (2020).
2. Heumos, L. et al. Best practices for single-cell analysis across modalities. *Nat. Rev. Genet.* **24**, 550–572 (2023).
3. Simeonov, K. P. et al. Single-cell lineage tracing of metastatic cancer reveals selection of hybrid EMT states. *Cancer Cell* **39**, 1150–1162.e9 (2021).
4. Baron, M. et al. The stress-like cancer cell state is a consistent component of tumorigenesis. *Cell Syst.* **11**, 536–546.e7 (2020).
5. Barkley, D. et al. Cancer cell states recur across tumor types and form specific interactions with the tumor microenvironment. *Nat. Genet.* **54**, 1192–1201 (2022).
6. Puram, S. V. et al. Cellular states are coupled to genomic and viral heterogeneity in HPV-related oropharyngeal carcinoma. *Nat. Genet.* **55**, 640–650 (2023).
7. Gavish, A. et al. Hallmarks of transcriptional intratumour heterogeneity across a thousand tumours. *Nature* **618**, 598–606 (2023).
8. Bischoff, P. et al. Single-cell RNA sequencing reveals distinct tumor microenvironmental patterns in lung adenocarcinoma. *Oncogene* **40**, 6748–6758 (2021).
9. Becker, W. R. et al. Single-cell analyses define a continuum of cell state and composition changes in the malignant transformation of polyps to colorectal cancer. *Nat. Genet.* **54**, 985–995 (2022).
10. Taki, M. et al. Tumor immune microenvironment during epithelial–mesenchymal transition. *Clin. Cancer Res.* **27**, 4669–4679 (2021).
11. Joanito, I. et al. Single-cell and bulk transcriptome sequencing identifies two epithelial tumor cell states and refines the consensus molecular classification of colorectal cancer. *Nat. Genet.* **54**, 963–975 (2022).
12. Li, J. et al. Remodeling of the immune and stromal cell compartment by PD-1 blockade in mismatch repair-deficient colorectal cancer. *Cancer Cell* **41**, 1152–1169.e7 (2023).
13. Krishna, C. et al. Single-cell sequencing links multiregional immune landscapes and tissue-resident T cells in ccRCC to tumor topology and therapy efficacy. *Cancer Cell* **39**, 662–677.e6 (2021).
14. Zhang, K. et al. Longitudinal single-cell RNA-seq analysis reveals stress-promoted chemoresistance in metastatic ovarian cancer. *Sci. Adv.* **8**, eabm1831 (2022).
15. Shiao, S. L. et al. Single-cell and spatial profiling identify three response trajectories to pembrolizumab and radiation therapy in triple negative breast cancer. *Cancer Cell* **42**, 70–84.e8 (2024).
16. Filbin, M. G. et al. Developmental and oncogenic programs in H3K27M gliomas dissected by single-cell RNA-seq. *Science* **360**, 331–335 (2018).
17. Jerby-Arnon, L. et al. A cancer cell program promotes T cell exclusion and resistance to checkpoint blockade. *Cell* **175**, 984–997.e24 (2018).
18. Cancer Genome Atlas Research Network. Comprehensive molecular characterization of clear cell renal cell carcinoma. *Nature* **499**, 43–49 (2013).
19. Cancer Genome Atlas Network. Genomic classification of cutaneous melanoma. *Cell* **161**, 1681–1696 (2015).
20. Puram, S. V. et al. Single-cell transcriptomic analysis of primary and metastatic tumor ecosystems in head and neck cancer. *Cell* **171**, 1611–1624.e24 (2017).
21. Izar, B. et al. A single-cell landscape of high-grade serous ovarian cancer. *Nat. Med.* **26**, 1271–1279 (2020).
22. Neftel, C. et al. An integrative model of cellular states, plasticity, and genetics for glioblastoma. *Cell* **178**, 835–849.e21 (2019).
23. Kim, N. et al. Single-cell RNA sequencing demonstrates the molecular and cellular reprogramming of metastatic lung adenocarcinoma. *Nat. Commun.* **11**, 2285 (2020).
24. Song, H. et al. Single-cell analysis of human primary prostate cancer reveals the heterogeneity of tumor-associated epithelial cell states. *Nat. Commun.* **13**, 141 (2022).
25. Hwang, W. L. et al. Single-nucleus and spatial transcriptome profiling of pancreatic cancer identifies multicellular dynamics associated with neoadjuvant treatment. *Nat. Genet.* **54**, 1178–1191 (2022).
26. Shahbandi, A. et al. Breast cancer cells survive chemotherapy by activating targetable immune-modulatory programs characterized by PD-L1 or CD80. *Nat. Cancer* **3**, 1513–1533 (2022).
27. Naldini, M. M. et al. Longitudinal single-cell profiling of chemotherapy response in acute myeloid leukemia. *Nat. Commun.* **14**, 1285 (2023).
28. Li, K. et al. Single-cell analysis reveals the chemotherapy-induced cellular reprogramming and novel therapeutic targets in relapsed/refractory acute myeloid leukemia. *Leukemia* **37**, 308–325 (2023).
29. Stetson, L. C. et al. Single-cell RNA sequencing of AML initiating cells reveals RNA-based evolution during disease progression. *Leukemia* **35**, 2799–2812 (2021).

30. Lambo, S. et al. A longitudinal single-cell atlas of treatment response in pediatric AML. *Cancer Cell* **41**, 2117–2135.e12 (2023).
31. Turati, V. A. et al. Chemotherapy induces canalization of cell state in childhood B-cell precursor acute lymphoblastic leukemia. *Nat. Cancer* **2**, 835–852 (2021).
32. Mumme, H. et al. Single-cell analysis reveals altered tumor microenvironments of relapse- and remission-associated pediatric acute myeloid leukemia. *Nat. Commun.* **14**, 6209 (2023).
33. Cui Zhou, D. et al. Spatially restricted drivers and transitional cell populations cooperate with the microenvironment in untreated and chemo-resistant pancreatic cancer. *Nat. Genet.* **54**, 1390–1405 (2022).
34. Werba, G. et al. Single-cell RNA sequencing reveals the effects of chemotherapy on human pancreatic adenocarcinoma and its tumor microenvironment. *Nat. Commun.* **14**, 797 (2023).
35. Lv, J. et al. The tumor immune microenvironment of nasopharyngeal carcinoma after gemcitabine plus cisplatin treatment. *Nat. Med.* **29**, 1424–1436 (2023).
36. Hamdan, F. & Cerullo, V. Cancer immunotherapies: a hope for the uncurable? *Front. Mol. Med.* **3**, 1140977 (2023).
37. Kwa, M. J. & Adams, S. Checkpoint inhibitors in triple-negative breast cancer (TNBC): where to go from here. *Cancer* **124**, 2086–2103 (2018).
38. Sharma, P., Hu-Lieskovan, S., Wargo, J. A. & Ribas, A. Primary, adaptive, and acquired resistance to cancer immunotherapy. *Cell* **168**, 707–723 (2017).
39. Menzies, A. M. et al. Pathological response and survival with neoadjuvant therapy in melanoma: a pooled analysis from the International Neoadjuvant Melanoma Consortium (INMC). *Nat. Med.* **27**, 301–309 (2021).
40. Oh, D. Y. et al. Intratumoral $CD4^+$ T cells mediate anti-tumor cytotoxicity in human bladder cancer. *Cell* **181**, 1612–1625.e13 (2020).
41. Zhang, Y. et al. Single-cell analyses reveal key immune cell subsets associated with response to PD-L1 blockade in triple-negative breast cancer. *Cancer Cell* **39**, 1578–1593.e8 (2021).
42. Yost, K. E. et al. Clonal replacement of tumor-specific T cells following PD-1 blockade. *Nat. Med.* **25**, 1251–1259 (2019).
43. Liu, X. et al. Immune checkpoint HLA-E:CD94-NKG2A mediates evasion of circulating tumor cells from NK cell surveillance. *Cancer Cell* **41**, 272–287.e9 (2023).
44. Luoma, A. M. et al. Tissue-resident memory and circulating T cells are early responders to pre-surgical cancer immunotherapy. *Cell* **185**, 2918–2935.e29 (2022).
45. Pai, J. A. et al. Lineage tracing reveals clonal progenitors and long-term persistence of tumor-specific T cells during immune checkpoint blockade. *Cancer Cell* **41**, 776–790.e7 (2023).
46. Caushi, J. X. et al. Transcriptional programs of neoantigen-specific TIL in anti-PD-1-treated lung cancers. *Nature* **596**, 126–132 (2021).
47. Lowery, F. J. et al. Molecular signatures of antitumor neoantigen-reactive T cells from metastatic human cancers. *Science* **375**, 877–884 (2022).
48. Sade-Feldman, M. et al. Defining T cell states associated with response to checkpoint immunotherapy in melanoma. *Cell* **175**, 998–1013.e20 (2018).
49. Mathewson, N. D. et al. Inhibitory CD161 receptor identified in glioma-infiltrating T cells by single-cell analysis. *Cell* **184**, 1281–1298.e26 (2021).
50. Kansy, B. A. et al. PD-1 status in $CD8^+$ T cells associates with survival and anti-PD-1 therapeutic outcomes in head and neck cancer. *Cancer Res.* **77**, 6353–6364 (2017).
51. van der Leun, A. M., Thommen, D. S. & Schumacher, T. N. $CD8^+$ T cell states in human cancer: insights from single-cell analysis. *Nat. Rev. Cancer* **20**, 218–232 (2020).
52. Pardoll, D. M. The blockade of immune checkpoints in cancer immunotherapy. *Nat. Rev. Cancer* **12**, 252–264 (2012).
53. Pelka, K. et al. Spatially organized multicellular immune hubs in human colorectal cancer. *Cell* **184**, 4734–4752.e20 (2021).
54. Schmid, P. et al. Pembrolizumab for early triple-negative breast cancer. *N. Engl. J. Med.* **382**, 810–821 (2020).
55. Dushyanthen, S. et al. Relevance of tumor-infiltrating lymphocytes in breast cancer. *BMC Med.* **13**, 202 (2015).
56. Denkert, C. et al. Tumour-infiltrating lymphocytes and prognosis in different subtypes of breast cancer: a pooled analysis of 3771 patients treated with neoadjuvant therapy. *Lancet Oncol.* **19**, 40–50 (2018).
57. Miller, J. S. & Lanier, L. L. Natural killer cells in cancer immunotherapy. *Annu. Rev. Cancer Biol.* **3**, 77–103 (2019).
58. Liu, Z. et al. Progenitor-like exhausted $SPRY1^+CD8^+$ T cells potentiate responsiveness to neoadjuvant PD-1 blockade in esophageal squamous cell carcinoma. *Cancer Cell* **41**, 1852–1870.e9 (2023).
59. Kinget, L. et al. A spatial architecture-embedding HLA signature to predict clinical response to immunotherapy in renal cell carcinoma. *Nat. Med.* **30**, 1667–1679 (2024).
60. Iqbal, N. & Iqbal, N. Imatinib: a breakthrough of targeted therapy in cancer. *Chemother. Res. Pract.* **2014**, 357027 (2014).
61. Russo, A. et al. A decade of EGFR inhibition in EGFR-mutated non small cell lung cancer (NSCLC): old successes and future perspectives. *Oncotarget* **6**, 26814–26825 (2015).
62. Sawyers, C. L. Herceptin: a first assault on oncogenes that launched a revolution. *Cell* **179**, 8–12 (2019).
63. Chhouri, H., Alexandre, D. & Grumolato, L. Mechanisms of acquired resistance and tolerance to EGFR targeted therapy in non-small cell lung cancer. *Cancers* **15**, 504 (2023).
64. Nahta, R. & Esteva, F. J. Herceptin: mechanisms of action and resistance. *Cancer Lett.* **232**, 123–138 (2006).
65. Volpe, G., Panuzzo, C., Ulisciani, S. & Cilloni, D. Imatinib resistance in CML. *Cancer Lett.* **274**, 1–9 (2009).
66. Griffiths, J. I. et al. Serial single-cell genomics reveals convergent subclonal evolution of resistance as early-stage breast cancer patients progress on endocrine plus CDK4/6 therapy. *Nat. Cancer* **2**, 658–671 (2021).
67. Rodriguez-Sevilla, J. J. et al. Hematopoietic stem cells with granulo-monocytic differentiation state overcome venetoclax sensitivity in patients with myelodysplastic syndromes. *Nat. Commun.* **15**, 2428 (2024).
68. Spitzer, A. et al. Mutant IDH inhibitors induce lineage differentiation in IDH-mutant oligodendroglioma. *Cancer Cell* **42**, 904–914.e9 (2024).
69. Jin, W. et al. Cellular hierarchy insights reveal leukemic stem-like cells and early death risk in acute promyelocytic leukemia. *Nat. Commun.* **15**, 1423 (2024).
70. Maynard, A. et al. Therapy-induced evolution of human lung cancer revealed by single-cell RNA sequencing. *Cell* **182**, 1232–1251.e22 (2020).
71. Li, Y.-S. et al. Lipid-associated macrophages for osimertinib resistance and leptomeningeal metastases in NSCLC. *Cell Rep.* **43**, 114613 (2024).
72. Krishnan, V. et al. A single-cell atlas identifies pretreatment features of primary imatinib resistance in chronic myeloid leukemia. *Blood* **141**, 2738–2755 (2023).
73. Peng, J. et al. Single-cell RNA-seq highlights intra-tumoral heterogeneity and malignant progression in pancreatic ductal adenocarcinoma. *Cell Res.* **29**, 725–738 (2019).
74. Jansky, S. et al. Single-cell transcriptomic analyses provide insights into the developmental origins of neuroblastoma. *Nat. Genet.* **53**, 683–693 (2021).
75. Han, M. et al. FOXA2 drives lineage plasticity and KIT pathway activation in neuroendocrine prostate cancer. *Cancer Cell* **40**, 1306–1323.e8 (2022).
76. Kinker, G. S. et al. Pan-cancer single-cell RNA-seq identifies recurring programs of cellular heterogeneity. *Nat. Genet.* **52**, 1208–1218 (2020).
77. Raghavan, S. et al. Microenvironment drives cell state, plasticity, and drug response in pancreatic cancer. *Cell* **184**, 6119–6137.e26 (2021).
78. Obradovic, A. et al. Single-cell protein activity analysis identifies recurrence-associated renal tumor macrophages. *Cell* **184**, 2988–3005.e16 (2021).
79. Caronni, N. et al. $IL\text{-}1\beta^+$ macrophages fuel pathogenic inflammation in pancreatic cancer. *Nature* **623**, 415–422 (2023).
80. Zheng, L. et al. Pan-cancer single-cell landscape of tumor-infiltrating T cells. *Science* **374**, abe6474 (2021).
81. Steele, N. G. et al. Multimodal mapping of the tumor and peripheral blood immune landscape in human pancreatic cancer. *Nat. Cancer* **1**, 1097–1112 (2020).
82. Rousseau, A., Parisi, C. & Barlesi, F. Anti-TIGIT therapies for solid tumors: a systematic review. *ESMO Open.* **8**, 101184 (2023).
83. Chi, Y. et al. Cancer cells deploy lipocalin-2 to collect limiting iron in leptomeningeal metastasis. *Science* **369**, 276–282 (2020).
84. Wilcox, J. A., Li, M. J. & Boire, A. A. Leptomeningeal metastases: new opportunities in the modern era. *Neurotherapeutics* **19**, 1782–1798 (2022).
85. Rao, A., Barkley, D., França, G. S. & Yanai, I. Exploring tissue architecture using spatial transcriptomics. *Nature* **596**, 211–220 (2021).
86. Cords, L. et al. Cancer-associated fibroblast phenotypes are associated with patient outcome in non-small cell lung cancer. *Cancer Cell* **42**, 396–412.e5 (2024).

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E.B., N.F., R.T., N.G.D., A.G., R.H., D.K., D.S., S.T. and L.Z. researched data for the manuscript, E.B., N.F., R.T. and I.T. made a substantial contribution to discussions of content. All authors wrote the manuscript, and E.B., N.F., R.T. and I.T. reviewed and/or edited before submission.

Competing interests

I.T. is a co-founder and adviser of Cellyrix Therapeutics and an adviser of Immunitas Therapeutic. The other authors declare no competing interests.

Additional information

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