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REVIEW ARTICLE

High-throughput sequencing unveils tumor-immune interactions: From genomic alterations to clinical translation

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Abstract

High-throughput sequencing (HTS) has revolutionized tumor immunology by enabling precise dissection of tumor-immune interactions, directly informing the development of precision immunotherapies. This review highlights key advances in HTS technologies—including whole genome sequencing (WGS), RNA sequencing, assay for transposase-accessible chromatin using sequencing, and single-cell immunogenomics (scTCR-seq/scBCR-seq)—and their clinical translation in personalized cancer vaccines, engineered T-cell therapies, and combination regimens. We discuss how these tools decode tumor-specific mutations, immune evasion mechanisms, and therapeutic targets, while addressing challenges in data standardization, sample processing, and computational integration. Emerging breakthroughs such as spatial multiomics, real-time monitoring, and artificial intelligence-driven discovery are transforming the field by enabling dynamic, personalized treatment strategies. Finally, we outline future directions to overcome current barriers and expand equitable access to HTS-driven precision immunotherapies.

Keywords: High-throughput sequencing; Tumor immunology; Neoantigen vaccines; Spatial multiomics; Artificial intelligence-driven discovery

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1. Introduction

The advent of high-throughput sequencing (HTS) has fundamentally transformed our ability to decipher the complex interplay between tumors and the immune system, providing unprecedented insights that are directly shaping the development of precision immunotherapies.[1,2] By enabling comprehensive molecular profiling across genomic, transcriptomic, and epigenomic dimensions, HTS technologies have emerged as indispensable tools for uncovering the mechanisms of immune evasion and identifying actionable targets for therapeutic intervention.[3,4] Whole-genome sequencing (WGS) enables systematic identification of tumor-specific mutations—including single-nucleotide variants, insertions/deletions, and structural variations—that generate neoantigens presented on major histocompatibility complex (MHC)

molecules to activate cytotoxic T cells, positioning these mutation-derived targets as central to cancer vaccines and T cell-based therapies.[5-7] Complementing this genomic perspective, RNA sequencing (RNA-seq) provides dynamic snapshots of gene expression patterns within the tumor microenvironment, revealing critical insights into immune cell infiltration dynamics, T cell exhaustion states, and cytokine signaling pathways that collectively determine treatment responses.[8-10] Meanwhile, assay for transposase-accessible chromatin using sequencing (ATAC-seq) has opened new avenues for understanding how epigenetic regulation of chromatin accessibility influences immune gene expression, particularly in the context of T cell dysfunction.[11-13] The recent development of single-cell immunogenomics approaches, including single-cell T-cell and B-cell receptor sequencing (scTCR-seq and scBCR-seq, respectively), has further revolutionized the field by enabling high-resolution mapping of adaptive immune responses at the level of individual T and B cell receptors (BCRs), thereby revealing clonal expansion patterns, antigen specificity, and functional states of tumor-infiltrating lymphocytes (TIL) with remarkable precision.[14-16]

Advances across genomic, transcriptomic, and epigenomic profiling platforms have fundamentally transformed cancer immunotherapy, leading to three particularly promising therapeutic applications that are currently redefining clinical investigation. First, neoantigen-based vaccines, designed through the integration of WGS, RNA-seq, and ATAC-seq data to predict MHC-binding immunogenic mutations while considering expression levels, have demonstrated exceptional clinical potential in early-phase trials by consistently eliciting strong T cell-mediated antitumor responses and achieving prolonged tumor regression.[17,18] Second, scTCR-seq serves as a pivotal technology for engineering tumor-specific T-cell therapies by enabling the discovery of high-affinity, functionally persistent T-cell receptors (TCR) while simultaneously allowing, through integration with single-cell transcriptomic data, the selection of less exhausted T-cell subsets—a critical advancement that directly mitigates a principal limitation of current adoptive cell therapies.[19,20] Third, the integration of multiomics-derived molecular characterization enables the rational design of next-generation combination therapies that strategically target complementary resistance mechanisms through orthogonal molecular pathways, as demonstrated by synergistic preclinical and early-phase clinical investigations.[21,22]

Despite these remarkable advances, the translation of sequencing-driven technological breakthroughs into routine clinical practice faces substantial challenges, including the inherent heterogeneity of tumor-immune interactions that hinders comprehensive data integration, technical constraints in single-cell sequencing regarding capture efficiency and scalability, and the growing computational demands for processing high-dimensional biological data. The development of spatial multiomics platforms enabling high-resolution mapping of immune cell spatial architecture, coupled with real-time liquid biopsy monitoring for dynamic treatment response assessment, along with advanced artificial intelligence (AI) frameworks for multimodal data integration and predictive modeling, collectively represent transformative solutions that are progressively addressing these barriers. The synergistic integration of these technological advancements is expected to fundamentally transform the clinical implementation and therapeutic outcomes of sequencing-guided immunotherapeutic interventions.

The current review systematically evaluates the transformative impact of HTS technologies on tumor-immune interaction studies and their pivotal role in advancing precision immunotherapy. We provide a critical assessment of the analytical capabilities and technical constraints inherent to leading sequencing platforms, while highlighting their clinically validated applications and remaining translational gaps. Through an integrative synthesis of multi-dimensional data across diverse cancer types, we identify key challenges in data harmonization, computational scalability, and biomarker standardization that currently impede clinical implementation. Our analysis establishes a roadmap for future research directions, emphasizing the development of robust analytical frameworks, rigorous clinical validation paradigms, and innovative computational strategies to fully realize the potential of sequencing-driven precision immunotherapy.

2. Core sequencing technologies for elucidating tumor-immune crosstalk

To systematically contextualize the technical profiles and translational value of these core sequencing platforms, their defining attributes are synthesized and presented in Table 1. This comparative overview delineates the principal applications, key advantages, and prevailing limitations of WGS, RNA-seq, ATAC-seq, and single-cell immunogenomics in dissecting tumor–immune crosstalk. By summarizing the distinctive and complementary roles of each technology, this table serves as a practical reference to guide the selection and integration of HTS approaches in both research and clinical settings.

Table 1. Comparison of major high-throughput sequencing platforms in tumor immunology

Technology	Key applications in tumor immunology	Major advantages	Current limitations
Whole genome sequencing (WGS)	• Comprehensive discovery of somatic mutations (SNVs, Indels, SVs, CNVs) • Neoantigen prediction and prioritization • Calculation of tumor mutational burden	• Unbiased detection of mutations across the entire genome, including non-coding regions • Foundation for personalized vaccine and TCR-T therapy design	• High computational and storage demands • "Signal-to-noise" problem; majority of mutations are passengers • Cannot confirm protein expression or antigen presentation
RNA-seq	• Profiling immune cell composition and gene expression in the TME • Characterizing T-cell exhaustion and functional states • Filtering and prioritizing neoantigens by expression level	• Reveals active transcriptional programs and immune pathways • Bulk RNA-seq is cost-effective for population-level analysis • scRNA-seq resolves cellular heterogeneity	• Bulk RNA-seq obscures cellular heterogeneity (averaging effect) • scRNA-seq is costly and sensitive to sample quality/technical artifacts (e.g., dropouts) • Complex bioinformatics analysis
ATAC-seq	• Mapping chromatin accessibility landscapes in immune and stromal cells • Identifying epigenetic drivers of T-cell dysfunction • Understanding CAF-mediated immune exclusion	• Reveals regulatory mechanisms invisible to genomics/transcriptomics • Explains gene silencing despite an intact DNA sequence • Can be performed at single-cell resolution (scATAC-seq)	• Chromatin accessibility is dynamic and context-dependent • Functional interpretation of distal open regions can be complex • Requires intact nuclei, which can be challenging with clinical specimens
Single-cell immunogenomics (scTCR-seq/ scBCR-seq)	• Tracking clonal expansion and diversity of T/B cells • Identifying tumor-reactive TCRs/BCRs and their functional states • Informing the design of engineered T-cell therapies (e.g., TCR-T)	• Unprecedented resolution to link clonality with cell state • Enables isolation of potent, less exhausted T-cell subsets for therapy • Elucidates the role of B cells and TLS in antitumor immunity	• Stringent sample requirements (high-quality, viable single-cell suspensions) • Suboptimal cell capture efficiency, potential loss of rare clones • Exceptionally complex data integration and analysis • High cost limits routine clinical application

Abbreviations: ATAC-seq: Assay for transposase-accessible chromatin using sequencing; BCR: B cell receptor; CAF: Cancer-associated fibroblasts; CNVs: Copy number variations; Indels: Insertions/Deletions; RNA-seq: RNA sequencing; scBCR-seq: Single-cell B-cell receptor sequencing; scTCR-seq: Single-cell T-cell receptor sequencing; SNVs: Single nucleotide variants; SVs: Structural variants; TCR: T cell receptor; TCR-T: T-cell receptor-engineered T-cell therapy; TLS: Tertiary lymphoid structures; TME: Tumor microenvironment.

2.1. WGS: Decoding neoantigen landscapes

WGS employs distinct workflows for reference and individual genomes (Figure 1). For reference genome sequencing, the genome is initially fragmented into large pieces, cloned, and then further subdivided into smaller fragments to generate thousands of sequence reads per clone for assembly. In contrast, individual genome sequencing begins with direct fragmentation into small fragments, producing millions of sequence reads that are aligned to the reference genome for assembly. WGS also serves as the foundational technology for identifying the full spectrum of somatic mutations in tumor cells, encompassing single-nucleotide variants (SNVs), insertions/deletions (Indels), copy number variations, and structural variants (SVs).[23-25] Among these genetic alterations, non-synonymous mutations represent a pivotal category as they induce amino acid substitutions in protein-coding regions, thereby generating neoepitopes—novel tumor-specific peptide antigens—that are processed through the proteasomal degradation pathway and subsequently presented on the cell surface by MHC class I molecules, where their recognition by cytotoxic T lymphocytes through T-cell receptors (TCRs) initiates adaptive anti-tumor immune responses mediated by tumor-specific $CD8^+$ T cell activation.[26-28]

Key technical considerations encompass sequencing depth and coverage parameters, wherein sufficient depth ensures robust detection of low-abundance somatic mutations while comprehensive genomic coverage facilitates the identification of both coding and non-coding variants that may modulate antigen presentation machinery.[29,30] Bioinformatics pipelines (e.g., GATK Best Practices workflow, Mutect2) systematically integrate variant calling with curated databases (e.g., COSMIC, dbSNP) to prioritize putative driver mutations exhibiting functional relevance to immune recognition processes.[31-34] The personalized design of neoantigen vaccines and TCR-engineered T-cell therapies (TCR-T) is fundamentally dependent on a complete catalog of a patient's tumor mutations. Without WGS, therapeutic strategies would be limited to a pre-defined set of genes or regions, potentially missing highly immunogenic neoantigens derived from non-coding regions, structural rearrangements, or novel driver mutations. The clinical translation of WGS data hinges on its ability to predict and prioritize neoantigens capable of eliciting robust antitumor immunity, which is

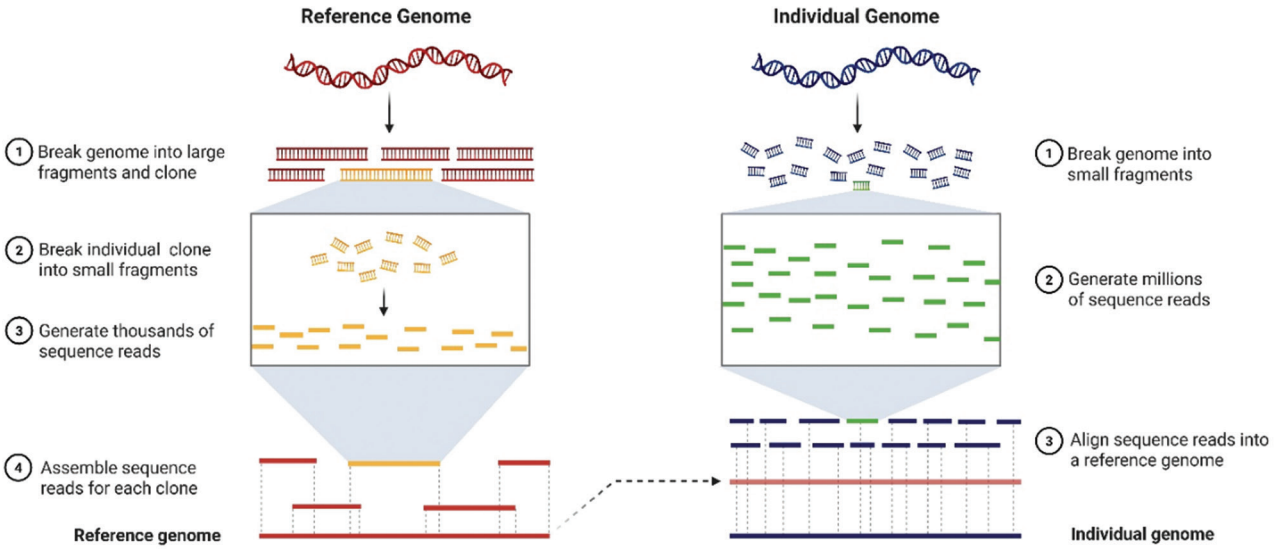

Figure 1. Schematic of whole-genome sequencing workflows for reference and individual genomes. Image created by the author.

determined by tumor mutational burden and neoantigen prediction accuracy.[35,36] Tumor mutational burden serves as a surrogate for neoantigen load, with elevated levels demonstrating positive correlations to immune checkpoint inhibitor (ICI) response rates, particularly in melanoma cohorts.[37,38] However, tumor mutational burden alone is insufficient, as mutation context—including MHC-binding affinity and tumor microenvironment (TME) factors such as programmed death-ligand 1 (PD-L1) expression—further modulates ICI efficacy.[39,40] To refine neoantigen prediction, WGS data are integrated with transcriptomic (RNA-seq) and proteomic profiles: RNA-seq filtering removes mutations in transcriptionally silent regions, while computational algorithms predict mutant peptide binding to patient-specific HLA alleles based on affinity thresholds.[41-43] Advanced tools such as PRIME and pVACtools further incorporate MHC-binding characteristics, TCR contact residues, and epitope stability to hierarchically rank neoantigens by immunogenic potential.[44-46]

The integration of WGS-driven neoantigen discovery into immunotherapy has demonstrated transformative potential across diverse clinical applications. Personalized neoantigen vaccines, exemplified by NeoVax, have proven feasible for synthesizing patient-specific vaccines targeting multiple tumor neoantigens; clinical studies in melanoma patients have confirmed durable $CD4^+$ and $CD8^+$ T-cell responses, with extended recurrence-free survival and absence of severe treatment-related adverse events.[47-50] Notably, vaccine-induced T cells demonstrated the capacity to infiltrate metastatic lesions, establishing systemic antitumor immunity. Engineered T-cell therapies targeting neoantigens have also exhibited promising clinical outcomes, including partial responses in a subset of metastatic colorectal cancer patients treated with KRAS G12D neoantigen-specific TCR-T in early-phase trials, underscoring their therapeutic potential despite challenges in overcoming TME suppression and ensuring consistent neoantigen expression.[51,52] Combination strategies have further augmented treatment efficacy, as demonstrated by clinical trials evaluating WGS-informed neoantigen vaccines in conjunction with ICIs (e.g., pembrolizumab), which have shown improved progression-free survival (PFS) in non-small cell lung cancer patients relative to ICI monotherapy.[53-55]

Despite its power, WGS faces significant practical challenges. The immense volume of data requires substantial computational resources for storage and analysis. A major analytical hurdle is the "signal-to-noise" problem; the vast majority of somatic mutations are passenger mutations with no immunogenic potential, making the computational prediction of the few functionally relevant neoantigens complex and prone to false positives. Furthermore, WGS has a fundamental functional blind spot: it identifies genomic alterations but cannot confirm whether the resulting mutant peptide is actually processed and presented on the cell surface by the MHC.

2.2. RNA-seq: Quantifying immune cell functional states

RNA-seq has transformed the study of tumor immunology by enabling precise measurement of gene expression patterns in immune cells and their microenvironment,

which not only provides critical insights into the mechanisms of immune activation and evasion but also facilitates the development of novel therapeutic strategies (Figure 2).

Bulk *RNA-seq analyzes* gene expression across entire cell populations, offering a comprehensive view of immune cell composition within tumors and enabling researchers to quantify mRNA levels of key immune markers to assess the abundance and activity of different immune cell subsets.[56,57] Computational tools such as ESTIMATE have been developed to calculate immune scores based on signature genes—such as CD8A (cytotoxic T cells), CD274/PD-L1 (antigen-presenting cells [APCs]), and FOXP3 (regulatory T cells)—which have demonstrated prognostic value for predicting patient responses to ICIs.[58-61] Clinical studies have demonstrated that high immune scores are strongly associated with improved PFS, defined as the time from treatment initiation to disease progression or death, in melanoma and non-small cell lung cancer.[62,63] Patients with high immune scores exhibit markedly prolonged PFS following anti-PD-1 therapy, whereas those with low scores show substantially shorter PFS durations.[64,65] Beyond quantifying immune cell populations, bulk RNA-seq facilitates the identification of molecular pathways linked to treatment resistance. RNA-seq is vital for characterizing the functional state of the TME. It can reveal the balance between cytotoxic effector functions and immunosuppressive networks, identifying upregulated exhaustion markers (e.g., LAG-3, TIM-3) or immunosuppressive cytokines (e.g., TGF-β, IL-10), thereby elucidating the mechanistic basis of treatment failures and informing the development of combination therapies targeting multiple resistance mechanisms.[66,67]

Single-cell RNA-seq (scRNA-seq) represents a major advancement by not only enabling the analysis of gene expression at the individual cell level but also revealing significant heterogeneity within immune cell populations through the identification of distinct subsets with unique functional properties.[68,69] One notable discovery is the characterization of T-cell exhaustion as a spectrum of states rather than a uniform phenotype, with scRNA-seq studies identifying both specific transcription factors like TOX that drive exhaustion programs and progenitor exhausted T cells marked by TCF-1 expression, which retain reinvigoration potential, thereby highlighting their therapeutic significance for enhancing immunotherapy efficacy through targeted strategies.[70-72] In the B-cell compartment, scRNA-seq has elucidated the critical role of tertiary lymphoid structures (TLS) in antitumor immunity by demonstrating their function as specialized microenvironments where B cells undergo affinity maturation and class switching to generate high-affinity antibodies, directly enhancing humoral immune responses and correlating with improved outcomes following ICI therapy.[73] Further, the presence of TLS, identified through distinct scRNA-seq transcriptional signatures, serves as a predictive biomarker for therapy response, highlighting their dual utility in both mechanistic elucidation and clinical translation.

RNA-seq acts as a crucial filter for neoantigen prioritization. A mutation identified by WGS is unlikely to be immunogenic if the gene is not expressed. By confirming the transcriptional activity of mutated genes,

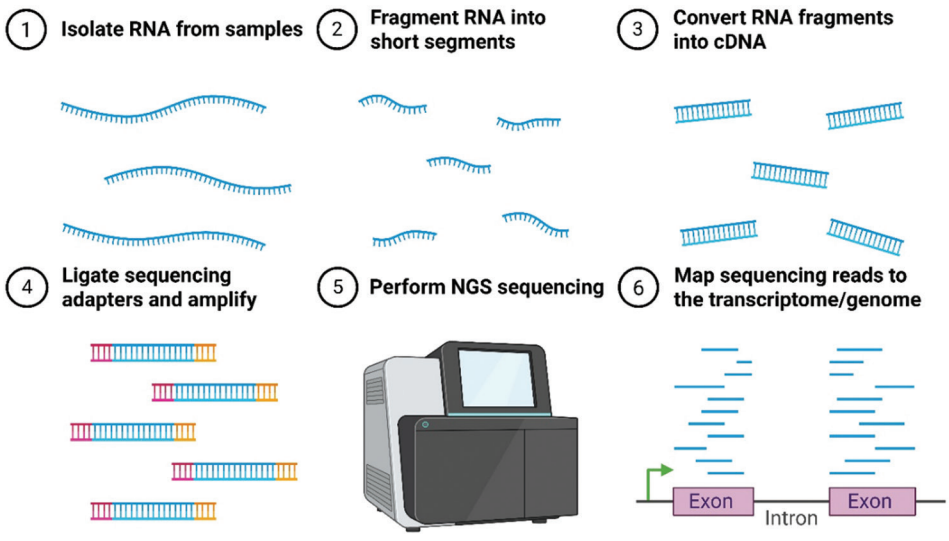

Figure 2. RNA Sequencing (RNA-seq) workflow from RNA isolation to transcriptome profiling. Image created by the author.
Abbreviation: NGS: Next-generation sequencing.

RNA-seq refines neoantigen candidate lists, significantly improving prediction specificity and the efficacy of vaccine design.[74] For adoptive cell therapies, scRNA-seq helps select optimal T-cell subsets with desirable functional properties, improving the efficacy of treatments such as chimeric antigen receptor T-cell (CAR-T cell) therapy.[75] By enabling systematic decoding of tumor-immune ecosystem interactions and facilitating single-cell resolution immune monitoring with longitudinal tracking, RNA-seq is not only advancing personalized cancer therapeutics through molecularly informed treatment design but also revolutionizing therapeutic optimization by detecting early resistance mechanisms and enabling adaptive therapeutic adjustments—a paradigm shift from conventional approaches to real-time molecularly guided strategies that hold transformative potential for oncologic outcomes.[76,77]

The primary limitation of bulk RNA-seq is the averaging effect, which obscures cellular heterogeneity and masks rare but critical cell subpopulations, such as progenitor exhausted T cells. While scRNA-seq resolves this by profiling individual cells, it introduces its own set of challenges.[68,69] scRNA-seq data are plagued by technical artifacts like dropouts (false-negative transcripts) and are highly sensitive to sample quality and cell viability. Furthermore, the high cost and complex bioinformatics analysis required for scRNA-seq currently limit its routine application in clinical workflows.

2.3. ATAC-seq: Decoding chromatin accessibility in the TME

Epigenomic profiling, particularly through ATAC-seq, has emerged as a powerful tool for deciphering the regulatory mechanisms governing immune responses in the TME by identifying chromatin accessibility landscapes and transcription factor binding patterns (Figure 3), thereby revealing key regulatory elements that drive immune cell infiltration, activation states, and functional polarization.[78] By integrating ATAC-seq data with multiomics datasets—including gene expression and DNA methylation profiles—researchers can construct comprehensive regulatory networks to elucidate the molecular basis of immunosuppression, identify potential therapeutic targets, and translate these insights into the development of epigenetic therapies aimed at reprogramming immune-suppressive microenvironments, thereby not only enhancing our understanding of TME biology but also advancing clinical translation.[79,80]

Recent advances in ATAC-seq studies have established chromatin accessibility as a central regulator of immune gene expression and T-cell functional dynamics. Epigenetic regulation, particularly through histone acetylation modifications, has been shown to modulate the transcriptional activity of effector genes critical for T-cell-mediated immune responses.[81,82] Genome-wide analyses consistently identify hypoacetylation of H3K27ac-marked enhancers near cytotoxicity-associated genes—including interferon gamma (IFN-γ) and granzyme B—as a hallmark of dysfunctional T cells, which not only leads to transcriptional repression of these key effector molecules but also, as supported by multiple independent studies, reduces chromatin accessibility at regulatory regions and contributes to impaired T-cell cytotoxicity against tumor cells.[83,84] Cross-sectional epigenomic comparisons further demonstrate that tumor-infiltrating T cells show reduced enhancer accessibility relative to T cells from healthy tissues or peripheral blood, underscoring the role of epigenetic remodeling in immune evasion.[85-87]

Beyond immune cell dysfunction, ATAC-seq has shed light on how stromal cells, particularly cancer-associated fibroblasts (CAFs), remodel the TME to create physical and functional barriers to immune infiltration.[88] Single-cell ATAC-seq (scATAC-seq) studies have identified fibroblast-specific open chromatin regions associated with genes involved in collagen deposition and extracellular matrix (ECM) remodeling, revealing that CAFs actively reorganize the TME to establish physical barriers against immune infiltration through distinct chromatin accessibility profiles featuring open regions proximal to key ECM-related genes, including those regulating collagen fibril assembly and proteolytic degradation.[89-91]

ATAC-seq provides insights that are invisible to genomic and transcriptomic assays. It explains why certain genes are silenced in the TME even when their DNA sequence is intact. This is crucial for understanding and overcoming T-cell exhaustion, a major barrier to durable immunotherapy responses. Transcription factors such as TEAD1 and AP-1 have been implicated as key regulators of CAF-specific chromatin states, highlighting their potential as therapeutic targets to disrupt ECM remodeling and enhance immune infiltration.[92,93] Targeting the epigenetic programs governing fibroblast-mediated immune exclusion holds potential to reprogram the TME, enhance T-cell infiltration, and improve ICI efficacy. Pharmacological inhibition of TEAD1 or AP-1 signaling pathways may reduce collagen deposition and ECM stiffness, creating a more permissive environment for T-cell penetration.[94,95] Furthermore, combining these epigenetic therapies with direct CAF-targeting approaches, such as fibroblast activation protein (FAP)-targeted antibodies, could synergistically enhance immune cell infiltration into tumors.[96]

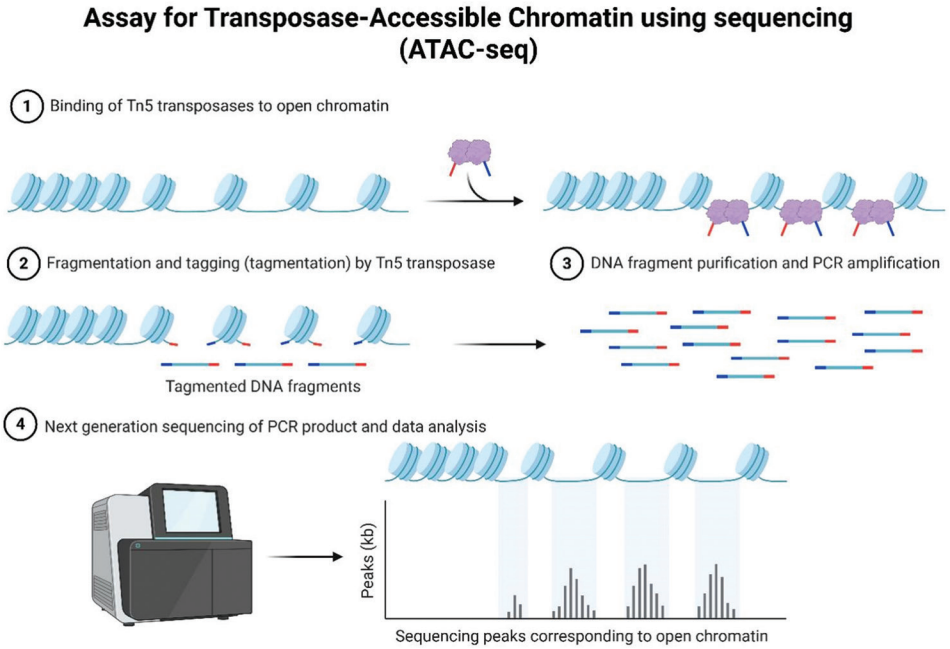

Figure 3. Comprehensive workflow of the assay for transposase-accessible chromatin using sequencing for profiling chromatin accessibility. Isolated nuclei are incubated with Tn5 transposase to bind open chromatin regions including promoters and enhancers, where DNA is simultaneously fragmented and sequencing adapters are inserted through tagmentation. Purified DNA fragments undergo polymerase chain reaction amplification using barcoded primers followed by sequencing library preparation. Paired-end sequencing reads align to reference genomes, with open chromatin regions identified as peaks for genome-wide regulatory element discovery. Image created by the author.

In the context of clinical translation, ATAC-seq is being integrated into biomarker discovery pipelines to identify patients most likely to benefit from epigenetic therapies. Combination regimens of histone deacetylase (HDAC) inhibitors with immune checkpoint blockers are being evaluated in patients with advanced solid tumors, with ATAC-seq employed to monitor dynamic changes in chromatin accessibility and immune gene expression during treatment, aiming to establish molecular criteria for patient stratification that ensure only tumors exhibiting specific epigenetic alterations receive combination therapy to maximize efficacy while minimizing adverse effects.[97,98] The ability of ATAC-seq to track temporal changes in chromatin accessibility further enables real-time monitoring of treatment response and resistance. Serial analyses of tumor biopsies or liquid biopsies (e.g., circulating tumor cells [CTCs]) are being employed to detect early signs of resistance, such as increased hypoacetylation of IFN-γ and granzyme B enhancers, which may guide therapeutic adjustments like the addition of HDAC inhibitors to restore T-cell function.[99,100]

A significant challenge is the dynamic nature of chromatin accessibility, which can change rapidly in response to environmental cues. This means a snapshot measurement may not fully represent the epigenetic state over time. In addition, interpreting the functional impact of open chromatin regions, particularly those distal to gene promoters, remains complex and often requires integration with complementary datasets like gene expression or transcription factor binding data. Sample quality is also paramount, as the assay requires intact nuclei, which can be difficult to obtain from clinical specimens.

2.4. Single-cell immunogenomics: Tracing adaptive immunity at single-cell resolution

Single-cell immunogenomics, encompassing scTCR-seq and scBCR-seq, has revolutionized the study of adaptive immune responses by enabling the precise tracking of T-cell and B-cell receptor (TCR/BCR) repertoires at unprecedented resolution, allowing researchers to map clonal expansions, identify antigen-specific receptors, and decipher the functional states of adaptive immune cells within the TME.[101] As illustrated in Figure 4, the single-cell sequencing workflow involves tissue dissociation to generate single-cell suspensions, followed by cellular composition analysis to evaluate heterogeneity. Target cells, such as antigen-specific immune populations, are isolated using fluorescence-activated cell sorting and subjected to single-cell sequencing, enabling simultaneous capture of TCR/BCR repertoires and transcriptomes. The resulting data is processed into expression profiles, revealing clonal

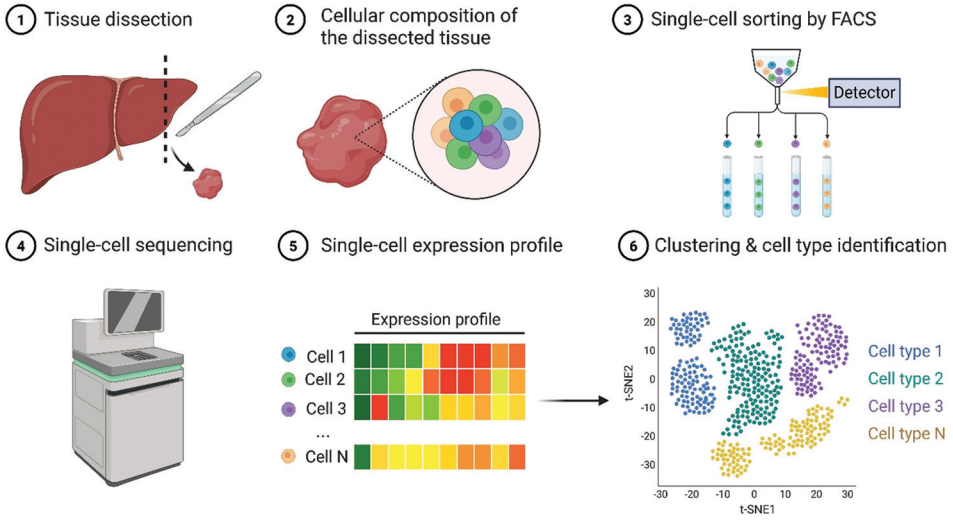

Figure 4. Workflow of single-cell sequencing and cell type identification. The diagram shows the key steps of single-cell sequencing: Tissue dissection, analysis of cellular composition, single-cell sorting by fluorescence-activated cell sorting, sequencing, expression profiling, and clustering for cell type identification. The output includes individual cell expression profiles (Cell 1 to Cell N) and their classification into distinct cell types (Cell type 1 to Cell type N). Image created by the author.

and functional signatures, and further analyzed through clustering to define distinct cell types and states. The final output includes annotated expression matrices linking individual cells (Cell 1.N) to their respective identities (Cell Type 1.N).

scTCR-seq represents a transformative approach in adaptive immune profiling by enabling precise identification and tracking of expanded TCR clones specific to tumor-associated antigens, distinguishing it from bulk TCR sequencing that primarily provides an aggregate view of T-cell populations and lacks the resolution to dissect clonal architecture, diversity, frequency, and functional states of tumor-reactive T cells at single-cell resolution.[102,103] TCR clonality has been established as a predictive biomarker for ICI efficacy, with expanded clones targeting driver mutations (e.g., *BRAF* V600E) in melanoma patients showing prolonged PFS following anti-PD-1 treatment, an association further supported by TIL profiling data where high clonality indices correlate with enhanced checkpoint blockade sensitivity.[104,105] Mechanistically, TCR clonality dynamics reflect bidirectional interactions between clonal expansion and the TME, wherein functional clones exhibit transcriptional upregulation of effector molecules while TME-induced exhaustion manifests as transcriptional suppression of cytotoxic genes and inhibitory receptor upregulation.[106]

scBCR-seq has similarly transformed our understanding of B-cell responses in the TME, particularly within TLS. By dissecting clonal diversity and affinity maturation of BCRs, this technology has elucidated B cells' dual roles in antitumor immunity: (i) generating high-affinity antibodies through somatic hypermutation (SHM) and class switching enhances antigen binding; (ii) mediating antibody-dependent cellular cytotoxicity, whereby tumor cells are lysed through the recruitment of natural killer cells and macrophages.[107-109] These mechanistic insights directly link B-cell activity to clinical outcomes, demonstrating that tumors enriched with TLS and high-affinity BCR clones exhibit improved responses to ICIs and enhanced efficacy of antibody-based therapies. Notably, high-affinity BCRs enable the design of bispecific antibodies that redirect T cells to tumors by bridging tumor antigens and CD3 on T cells, leveraging preclinical evidence of enhanced T-cell infiltration and tumor cytotoxicity in BCR-high tumor models.[110,111] Furthermore, integrating scBCR-seq with spatial transcriptomics reveals that TLS harbor structured germinal centers where follicular helper T cells (Tfh) guide affinity maturation, a spatial organization that is critical for sustaining long-term immune memory, as confirmed by imaging mass cytometry demonstrating phased B-cell differentiation in these niches, underscoring TLS preservation as a key consideration in cancer immunotherapy.[112,113]

The insights from scTCR-seq and scBCR-seq directly advance precision immunotherapy development by enabling the precise identification of tumor-reactive TCRs and high-affinity BCRs, which inform the design of antigen-specific therapies with reduced off-target effects. For TCR-T therapy, scTCR-seq allows for the precise identification of TCRs that are reactive to tumor neoantigens. Crucially,

by pairing this with transcriptomic data, clinicians can isolate these tumor-reactive T cells from functional, less exhausted subsets, thereby generating more potent and persistent therapeutic products. Similarly, scBCR-seq has elucidated the critical role of B cells and TLS in anti-tumor immunity by tracking clonal expansion, SHM, and class switching, all of which are associated with improved responses to immunotherapy. TCR/BCR clonality itself has emerged as a dynamic biomarker for monitoring response to ICIs. Beyond cellular therapies, integrated scTCR-seq and scBCR-seq data enable personalized cancer vaccine design by pinpointing immunogenic tumor-specific mutations and predicting their capacity to elicit robust T/B-cell responses, which holds potential to improve vaccine efficacy through antigen prioritization tailored to individual tumor mutational landscapes.

The widespread clinical application of single-cell immunogenomics is currently constrained by stringent sample requirements and cost. The technology demands high-quality, viable single-cell suspensions, which are challenging to obtain from small core biopsies or necrotic tumors. Cell capture efficiency, while improving, is still suboptimal, leading to the potential loss of rare but biologically important clones. Finally, the data analysis is exceptionally complex, requiring sophisticated computational pipelines to integrate clonal tracking with transcriptional clustering, posing a significant bottleneck for non-specialist labs.

3. Integrating multimodal HTS for precision immunotherapy

The convergence of advanced sequencing technologies has enabled a paradigm shift in tumor immunology, providing an integrated framework to dissect the molecular and cellular mechanisms of tumor-immune interactions while enhancing understanding of the complex interplay between tumors and the immune system and driving the development of next-generation immunotherapies tailored to the unique genomic and immunological landscape of individual patients.

3.1. Neoantigen vaccines: Optimizing design through multimodal data integration

Multimodal HTS enables the development of personalized neoantigen vaccines by leveraging the immune system's capacity to target tumor-specific mutations. WGS identifies somatic mutations—including SNVs, Indels, and SVs—across the tumor genome, with non-synonymous changes generating neoantigens: tumor-specific peptides presented on MHC molecules that cytotoxic T cells recognize.[114,115] Comprehensive mutation cataloging via WGS informs neoantigen prediction, prioritizing mutations with the highest immunogenic potential.

Neoantigen vaccines, leveraging tumor-specific non-synonymous somatic mutations that generate immunogenic peptides with enhanced MHC binding affinity to bypass central tolerance, potently activate both $CD8^+$ cytotoxic and $CD4^+$ helper T cells (Figure 5). Neoantigen vaccine efficacy hinges on identifying mutations that are not only genomically present but also functionally translatable into immunogenic targets, necessitating multiomics integration with RNA-seq and ATAC-seq, validating functional relevance and predicting antigen accessibility, respectively, to bridge genomic variation and immune recognition. RNA-seq serves as a functional filter to confirm transcriptional activity of mutated genes, ensuring predicted neoantigens are translated into proteins and processed by APCs while eliminating transcriptionally silent mutations to refine prediction specificity for genuinely immunogenic mutations.[116-118] ATAC-seq maps chromatin accessibility at MHC loci to identify regulatory elements governing MHC expression and peptide loading, prioritizing mutations in open chromatin regions to ensure neoantigens are expressed and presented on the cell surface in a T-cell-accessible configuration, thereby linking transcriptional activity to antigen presentation machinery and complementing RNA-seq.[119-121]

3.2. Engineered T-cell therapies: Enhancing efficacy through single-cell insights

Engineered T-cell therapy has revolutionized cancer treatment through two pioneering approaches: TCR-engineered T cells, which precisely target tumor-specific antigens presented by HLA molecules, and CAR-T cells, which directly recognize cancer surface markers independent of HLA restrictions (Figure 6). Multimodal HTS is transforming TCR-T/CAR-T therapies by enabling precise redirection of T-cell specificity toward tumor antigens and enhancement of their effector functions.

scTCR-seq enables the identification of high-affinity TCRs targeting tumor-specific mutations by resolving TCR clonal diversity and antigen specificity at single-cell resolution, thereby facilitating the isolation of receptors with optimal neoantigen binding affinity for robust tumor targeting.[122-124] T-cell dysfunction remains a critical hurdle, often driven by inhibitory checkpoint activation and metabolic stress.[125,126] scRNA-seq enables the isolation of resilient T-cell subsets that maintain function within suppressive tumor microenvironments by resolving transcriptional differences between exhausted and durable antitumor populations.[127,128] Among these, progenitor-like exhausted T cells, which exhibit stemness features

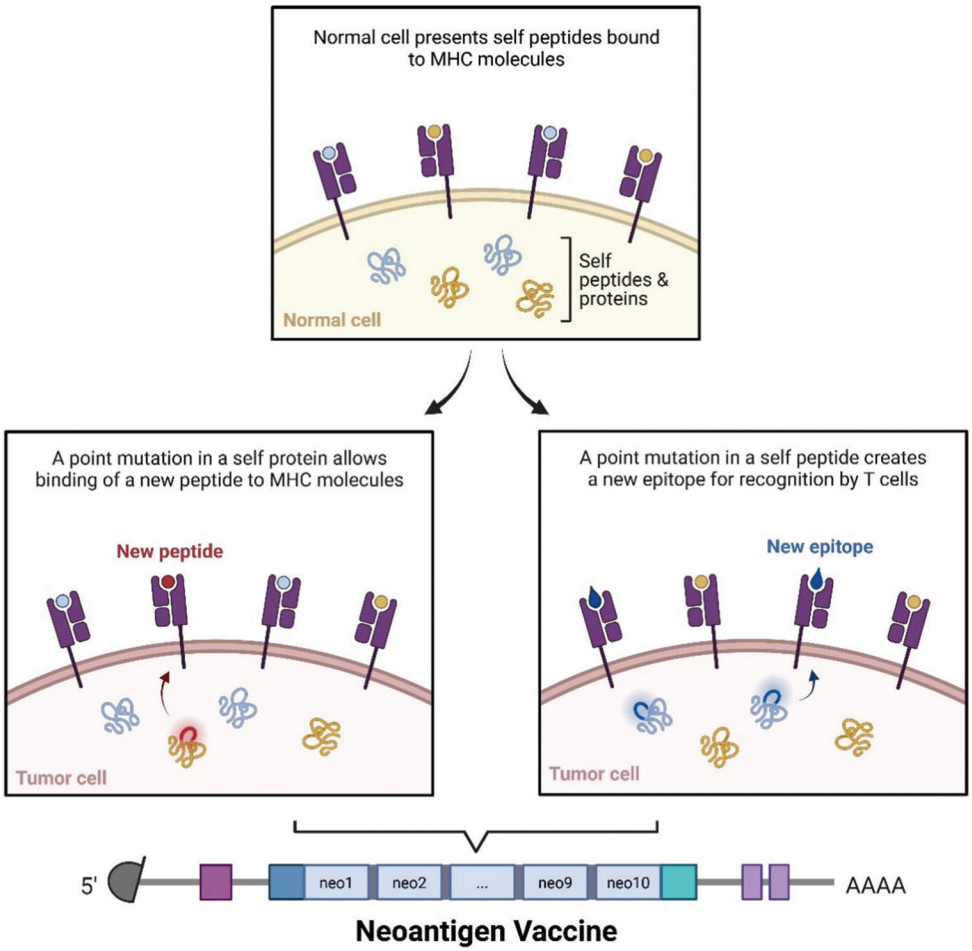

Figure 5. Mechanism of neoantigen vaccine action. This schematic illustrates the rationale for neoantigen-based cancer vaccines. In normal cells, self-peptides derived from endogenous proteins are stably presented on MHC molecules without eliciting immune responses. Tumor cells, however, harbor somatic mutations that either generate entirely novel peptides (left panel) or alter existing self-peptides to create immunogenic neoepitopes (right panel) capable of T-cell recognition. Neoantigen vaccines (bottom panel) are engineered with a defined set of tumor-specific mutated antigens (e.g., neo1-neo10), aiming to selectively activate T-cell responses against these uniquely expressed targets while sparing normal tissues. Image created by the author.

and long-term survival capacity, are now prioritized for enrichment strategies.[129,130]

The integrated use of scTCR-seq and scRNA-seq is pushing the boundaries of T-cell therapy development. In a representative clinical implementation, scTCR-seq enabled the precise identification of neoantigen-reactive TCRs, while concurrent scRNA-seq profiling allowed for the selection of functionally preserved T-cell subsets. These complementary technologies working in tandem produced clinically meaningful responses, underscoring multimodal HTS's potential to simultaneously overcome T-cell dysfunction and tumor immune evasion.

3.3. Combination therapies: Synergizing epigenetic modulation and immune agonism

While neoantigen vaccines and engineered T-cell therapies represent groundbreaking advances, multimodal HTS is now enabling the identification of synergistic combination strategies to overcome ICI resistance. Recent advances highlight the potential of simultaneously targeting epigenetic reprogramming and immune co-stimulation to disrupt distinct yet mechanistically complementary pathways underlying T-cell dysfunction.[131,132] DNA methyltransferase inhibitors (DNMTis) have emerged as potent tools to reverse T-cell exhaustion and restore effector function.[133,134] By demethylating gene promoters associated with exhaustion markers, DNMTis reactivate transcriptional programs critical for cytokine production and proliferation, thereby restoring the ability of exhausted T cells to produce key effector molecules such as IFN-γ and granzyme B.[135-137] DNMTis significantly enhances ICI efficacy by reprogramming exhausted T cells, restoring their functional capacity within the TME.

In parallel, immune agonists such as anti-OX40 antibodies are being explored to enhance the formation

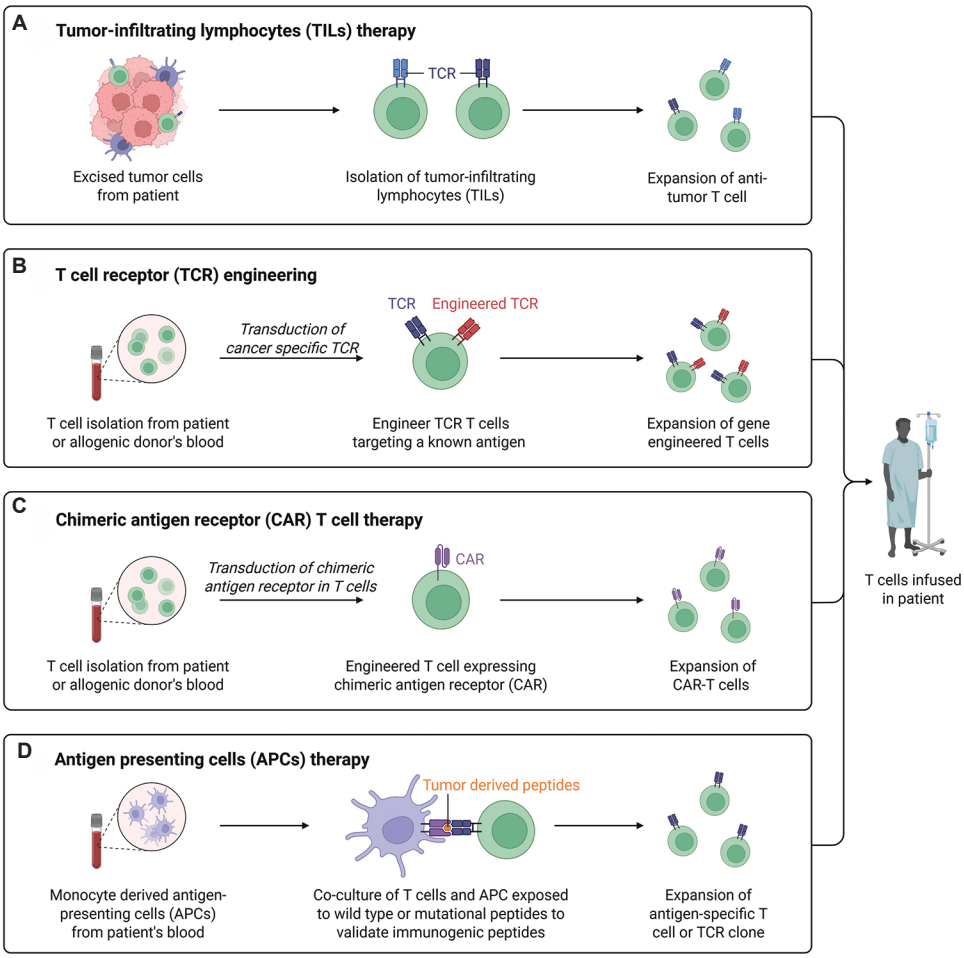

Figure 6. Strategies and workflows of key engineered T-cell cancer therapies: (A) tumor-infiltrating lymphocytes therapy, (B) T-cell receptor engineering, (C) chimeric antigen receptor T cell therapy, and (D) antigen-presenting cells therapy. Each section details the process from initial cell sourcing or modification to the expansion of therapeutic T cells for patient infusion. Image created by the author.

of TLS, which serve as critical hubs for orchestrating antitumor immunity.[138,139] By binding to the OX40 receptor on T cells, anti-OX40 antibodies promote T-cell survival, proliferation, and recruitment of B cells and other immune cells to form TLS, which facilitates dendritic cell activation, B-cell maturation, and T-cell priming to create a microenvironment conducive to sustained tumor attack.[140-142] The dual action of anti-OX40 antibodies not only strengthens local immune surveillance but also addresses the spatial organization of immune cells within the TME, a critical factor for effective antitumor responses.

The integration of DNMTis, anti-OX40 antibodies, and ICIs has demonstrated remarkable synergy in preclinical models by simultaneously targeting T-cell exhaustion, immune cell recruitment, and checkpoint blockade, thereby overcoming resistance mechanisms, enhancing T-cell infiltration and TLS formation, achieving durable tumor regression, and providing a roadmap for next-generation immunotherapies through multimodal HTS-guided identification of rational combination strategies. By leveraging HTS to uncover non-redundant targets and mechanisms, researchers can develop strategies that dismantle multifactorial ICI resistance and improve clinical outcomes for patients with cancer.

4. Current challenges

Despite its transformative potential, HTS-driven precision immunotherapy faces significant technical challenges that hinder its clinical translation. These bottlenecks primarily involve data standardization, sample acquisition and processing, and computational analysis capabilities.

4.1. Data standardization

A critical challenge in sequencing-based research lies in platform-specific discrepancies that compromise data reliability by causing substantial variations in

the detection of complex genomic alterations, where differing sensitivity and error rates between platforms pose significant risks of omitting or misidentifying clinically actionable mutations.[143,144] Platform-specific detection variations directly undermine the reliability of downstream applications like neoantigen prediction and vaccine development, where precision is paramount.[145-147] Cross-platform validation strategies are implemented to address these challenges, with key findings from WGS workflows routinely undergoing secondary confirmation via orthogonal technologies. Nevertheless, bioinformatics workflow disparities across institutions, particularly variations in variant calling parameters and analytical pipelines, introduce additional barriers to data harmonization.[148,149]

The establishment of universal standards is urgently required to resolve systemic challenges in genomic data interoperability, as demonstrated by key initiatives like the quality control framework by the U.S. Food and Drug Administration. These standardized frameworks not only enhance data comparability across platforms but also serve as essential bridges for translating genomic discoveries into clinical applications. By enforcing standardized protocols and quality metrics, the research community can systematically eliminate discrepancies in variant calling and analytical workflows, thereby ensuring that sequencing data maintains both reliability and clinical relevance in diverse environments.

4.2. Sample limitations and mitigation strategies

Single-cell sequencing imposes stringent sample requirements, with platforms like Drop-seq requiring minimal input material at the milligram scale—a demand that proves particularly challenging given the limited availability of clinical samples, especially when isolating rare tumor populations or metastatic lesions, where inherent variability in sample quality further compounds the problem by reducing viable cell numbers through factors such as degradation, necrosis, and stromal contamination.[150,151] Current cell capture efficiency remains suboptimal, a limitation that not only results in significant waste of precious clinical samples but also leaves a substantial proportion of input cells uncaptured, ultimately leading to incomplete representation of cellular heterogeneity.

To address this limitation, alternative sample sources such as CTCs and pleural effusion specimens are being explored, which present distinct challenges: CTCs typically exhibit low viability due to their fragile nature and the harsh conditions of the bloodstream, while their enrichment is frequently complicated by contamination from normal cells, particularly leukocytes that share similar physical properties, further exacerbating isolation difficulties.[152-155] The intrinsic fragility of CTCs significantly compounds isolation challenges, necessitating specialized equipment and protocols to safeguard cellular integrity during enrichment. Concurrently, pleural effusion specimens, despite their relative abundance, exhibit substantial heterogeneity stemming from the coexistence of non-tumor cells, inflammatory mediators, and ECM components. This compositional complexity disrupts downstream analytical workflows by introducing confounding biological variables. The presence of these heterogeneous elements mandates additional purification steps, inevitably leading to diminished cell yield and heightened technical variability, which collectively compromise the reliability of single-cell analysis.

4.3. Computational and data integration complexities

The integration of multiomics data, particularly from WGS and RNA-seq, demands robust computational infrastructure and sophisticated algorithms, presenting a series of nuanced challenges that constitute significant bottlenecks in the translational pipeline. While specialized tools have been developed, the scale and heterogeneity of the data often push current computational methods to their limits. The sheer magnitude of data generated by HTS platforms is the most immediate challenge. A single WGS run can produce over 100 gigabytes of raw data, while large-scale scRNA-seq studies can easily reach terabyte-scale dimensions.[156,157] This necessitates not only scalable, high-performance computing storage architectures but also parallelized processing pipelines to ensure computational efficiency. The management, transfer, and long-term archiving of these datasets incur substantial financial and operational costs for research institutions and clinical laboratories, creating a barrier to entry for wider adoption. The alignment of WGS reads and subsequent variant calling is particularly computationally intensive, with accuracy highly dependent on sequencing depth, tumor purity, and algorithmic choice.[31,34] For RNA-seq, analytical challenges include accurate transcript quantification, normalization against technical variations, and deconvolution of cellular mixtures—all of which become increasingly difficult in complex TMEs.[58]

Data standardization poses even greater challenges, including harmonizing genomic coordinates across different sequencing platforms, normalizing expression levels across varying experimental conditions, and correcting batch effects in multicenter studies. Although specialized tools such as DeepVariant for variant calling and Seurat for single-cell data integration have optimized

specific workflows, algorithmic performance declines markedly in highly heterogeneous tumor samples with substantial stromal contamination, where dense stromal-tumor intermixing disrupts feature extraction and elevates false-negative rates in variant calls or cell-type clustering.[158-161]

Stringent data privacy regulations significantly complicate clinical data sharing, necessitating the development of storage solutions that ensure security while maintaining accessibility.[162,163] To address this challenge, distributed analytical pipelines that integrate encryption protocols and federated learning frameworks are being developed to enable direct analysis of encrypted datasets, thereby simultaneously satisfying regulatory requirements and preserving statistical power. Current priorities include advancing algorithmic robustness for complex biological contexts and establishing scalable frameworks for multiomics data processing.[164-166]

5. Emerging breakthroughs

Despite technical and clinical hurdles, HTS-powered precision immunotherapy is achieving transformative advances across multiple domains, establishing clinically actionable strategies that reshape cancer treatment by enabling more precise, dynamic, and personalized approaches through decoding tumor-immune interactions with unprecedented resolution and integrating real-time data streams to guide treatment decisions.

5.1. Spatial multiomics: Decoding the TME

Spatial transcriptomics has emerged as a transformative technology for deciphering the spatial organization of immune cells within the TME, enabling clinicians and scientists to map cellular interactions with sub-micrometer precision.[167,168] High-resolution platforms such as Visium HD now identify "immune-suppressive hotspots"—regions densely populated with PD-L1$^+$ tumor-associated macrophages (TAMs)—within the TME.[169-171] In breast cancer, regions densely populated with PD-L1$^+$ TAMs correlate with reduced T-cell infiltration and poor response to ICI therapy, providing critical insights for radiation therapy target delineation to disrupt physical and molecular barriers impeding immune cell infiltration.[172]

The rapid clinical translation of spatial transcriptomics is establishing a new paradigm where spatial biology directly informs precision radiotherapy design. By superimposing spatial transcriptomics data onto patient-specific anatomical imaging, PD-L1$^+$ TAM-enriched immunosuppressive regions can be systematically identified and targeted with precision radiotherapy dose sculpting—simultaneously disrupting dense stromal compartments that form physical barriers and inhibiting the PD-L1/PD-1 axis to restore immune cell infiltration.

Furthermore, spatial transcriptomics is expanding its applications through integration with complementary omics technologies, enabling a multiscale dissection of the TME.[173] Spatial proteomics advanced by mass cytometry facilitates concurrent mapping of protein expression and gene activity within the same tissue sections, revealing functional protein networks that underpin immune cell behavior.[174-176] Spatial epigenomics, particularly through chromatin accessibility profiling, identifies regulatory elements governing immune evasion-related gene expression, bridging the gap between genetic alterations and phenotypic manifestations.[177,178] By triangulating these multiomics datasets, the approach systematically pinpoints biomarkers and therapeutic targets, ultimately constructing a systems-level framework that deciphers the complex interplay between tumor cells and the immune microenvironment.

5.2. Real-time monitoring: Liquid biopsies for dynamic tumor tracking

Liquid biopsies have transformed cancer monitoring by enabling real-time, minimally invasive tracking of tumor dynamics through circulating tumor DNA (ctDNA) and CTCs analysis.[100,179] Unlike conventional imaging or tissue biopsies, liquid biopsies provide a dynamic, system-wide view of tumor evolution, offering critical advantages in assessing treatment response and detecting resistance.

The serial analysis of ctDNA has emerged as a powerful tool for early disease progression detection, outperforming conventional imaging modalities. Tumor-derived DNA fragments circulating in blood provide a direct window into molecular residual disease, enabling the identification of treatment response shifts before radiographic changes become visible.[180,181] Rising ctDNA levels during immunotherapy signal emerging resistance, prompting timely therapeutic adjustments from monotherapy to combination regimens that have been shown to improve clinical outcomes.[182,183] This approach also addresses a critical limitation of imaging by differentiating pseudoprogression from true disease progression, thereby preventing unnecessary treatment changes.

CTCs, as intact living cells, provide valuable functional information about key aspects of tumor biology, including metastatic potential and drug resistance.[184,185] Advanced microfluidic platforms such as CellSearch and Parsortix enable high-sensitivity isolation of viable CTCs, facilitating downstream molecular analyses such as single-cell RNA-seq.[186-188] This approach identifies transcriptional patterns linked to treatment resistance and metastasis, revealing

tumor heterogeneity and adaptation mechanisms beyond genomic analysis.

The integration of ctDNA and CTC analyses provides a comprehensive view of tumor dynamics by combining ctDNA's strength in detecting genomic alterations with CTCs' ability to reveal phenotypic characteristics, including metastatic potential and therapeutic vulnerabilities, ultimately enabling more informed treatment decisions and improved patient outcomes.

5.3. AI-driven discoveries: From biomarker identification to personalized immunotherapy strategies

AI is revolutionizing precision immunotherapy by enabling unprecedented advances in the analysis of complex biological data and accelerating the translation of discoveries into clinical applications.[189,190] Deep learning models, particularly Transformer architectures, have redefined the landscape of neoantigen immunogenicity prediction by systematically integrating multidimensional datasets including tumor mutation profiles, MHC binding affinities, and TCR repertoires.[191,192] These models identify the most immunogenic neoantigens for personalized cancer vaccine development, with AI-driven platforms demonstrating superior performance in preclinical models to predict neoantigens that elicit robust T-cell responses, thereby advancing next-generation immunotherapies with enhanced precision and efficacy. Recent advances in AI-driven image analysis, such as those applied in medical imaging for cervical cancer detection[193] underscore the potential of integrating visual and molecular data for improved diagnostic and therapeutic strategies.

Beyond biomarker discovery, generative AI approaches such as diffusion models are engineering novel antigen epitopes that evade immune evasion mechanisms by synthesizing synthetic peptides mimicking tumor-specific mutations while incorporating immunostimulatory motifs.[194-196] This innovation significantly improves cytotoxic T cell recognition and expands therapeutic possibilities for vaccine development. Concurrently, AI optimizes combination therapies through the integration of multiomics data with electronic health records (EHRs) and clinical trial outcomes, where advanced machine learning algorithms predict optimal drug combinations including synergistic pairs of ICIs and targeted therapies that achieve superior therapeutic responses compared to conventional regimens.[197,198]

AI systems fuse real-time multiomics data with EHRs and clinical guidelines to deliver personalized treatment recommendations, analyzing individual patients' tumor mutational burden, immune cell infiltration patterns, and genetic biomarkers to dynamically optimize therapy selection while incorporating emerging scientific evidence.[199,200] These AI-driven personalized treatment recommendations are translating into improved treatment outcomes, reduced healthcare costs, and elevated care quality for cancer patients worldwide, marking a paradigm shift toward data-driven, personalized medicine that redefines standards of care in oncology.

6. Future directions

Precision immunotherapy hinges on harnessing HTS to decode tumor-immune interactions, yet widespread implementation remains constrained by technical and systemic barriers. Addressing these challenges demands concerted efforts across three pillars: workflow standardization and economic scalability, technological convergence, and global infrastructure development.

Current HTS adoption is impeded by assay variability and cost inefficiencies. Automated single-cell isolation platforms (e.g., microfluidic encapsulation systems) are reducing manual intervention in sample preparation, while AI-integrated liquid handlers are standardizing nucleic acid extraction and library construction across sequencing platforms.[201,202] Cloud-based federated learning frameworks enable secure multi-institutional collaboration for variant interpretation, with quantum-enhanced algorithms poised to optimize multiomics data integration. These advances collectively drive down per-sample processing costs, facilitating HTS deployment in mid-tier clinical laboratories.

Technological synergy is expanding HTS applications in immunotherapy development. Single-cell CRISPR screening (scCRISPR) combines microfluidic cell manipulation with genome editing to map immune evasion pathways at single-nucleotide resolution.[203,204] Organoid-on-a-chip models integrate 3D culture with CRISPR perturbation to simulate patient-specific tumor-immune dynamics, enabling high-content drug screening.[205,206] Wearable biosensors employing nanopore arrays facilitate continuous ctDNA monitoring in interstitial fluid, providing real-time insights into treatment response and resistance evolution.[207,208]

Global equitable access requires coordinated infrastructure investment. International consortia are standardizing immunotherapy-focused multiomics datasets (e.g., WGS, TCR-seq, spatial transcriptomics) through unified annotation protocols. Public-private partnerships are deploying HTS capacity in underserved regions through tiered pricing models, while open-source analysis suites (e.g., AI-driven variant callers) mitigate software costs. Mobile sequencing units equipped with

edge-computing capabilities support point-of-care molecular diagnostics in resource-limited settings.

7. Discussion

The integration of HTS technologies has fundamentally reshaped our understanding of tumor-immune interactions, laying the foundation for precision immunotherapies tailored to individual patients. WGS has been pivotal in identifying tumor-specific mutations and quantifying neoantigen loads, while RNA-seq provides critical insights into immune cell composition and functional states within the TME. ATAC-seq further elucidates epigenetic regulation of immune-related genes, revealing mechanisms of T-cell dysfunction and immune exclusion. Single-cell immunogenomics (scTCR-seq/scBCR-seq) adds another layer of resolution by mapping clonal T-cell and B-cell repertoires, enabling the identification of tumor-reactive lymphocytes and exhaustion signatures.

Despite these advances, significant hurdles remain. Data standardization is a pressing issue, as discrepancies between sequencing platforms (e.g., Illumina vs. Nanopore) and bioinformatics workflows can lead to inconsistent mutation calls, directly impacting neoantigen prediction and vaccine design. Sample acquisition and processing also pose challenges: single-cell sequencing requires high-quality input material, yet tumor biopsies often yield limited cells, and cell capture efficiency remains suboptimal. Computationally, integrating multiomics data demands substantial resources, and algorithms struggle with heterogeneous tumors (e.g., high normal cell contamination). In addition, translating preclinical discoveries into clinical workflows is complicated by regulatory requirements and the need for scalable, cost-effective solutions.

Emerging technologies are addressing these gaps. Spatial multiomics—such as Visium HD and organoid-

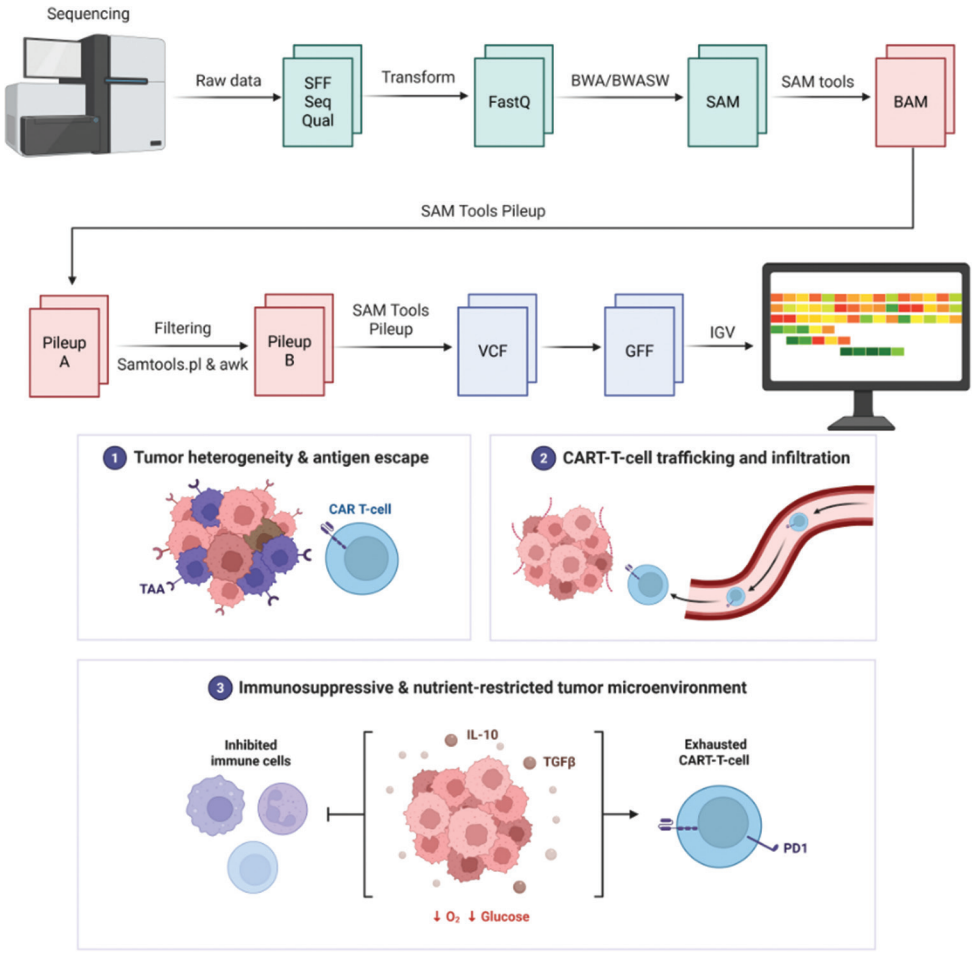

Figure 7. Integrative multiomics framework for precision immunotherapy. Image created by the author.
Abbreviations: CART-T cell: Chimeric antigen receptor T cell; IL-10: Interleukin 10; PD1: Programmed cell death protein 1; TAA: Tumor-associated antigen; TGFβ: Transforming growth factor beta.

immune co-cultures—provides spatially resolved maps of immune cell distribution and functional states, revealing "immune-suppressive hotspots" that correlate with treatment resistance. Real-time monitoring via liquid biopsies (ctDNA/CTCs) enables dynamic tracking of tumor evolution during therapy, allowing timely adjustments to treatment regimens. AI-driven discovery is transforming data interpretation: deep learning models predict neoantigen immunogenicity with higher accuracy than traditional algorithms, while generative AI designs novel antigen epitopes to evade immune evasion. AI also integrates multiomics data with EHRs to recommend personalized therapies, though validation in diverse patient cohorts is still needed.

8. Conclusion

HTS technologies have unlocked unprecedented opportunities to decode tumor-immune interactions and develop precision immunotherapies. WGS, RNA-seq, ATAC-seq, and scTCR-seq have collectively advanced our ability to identify neoantigens, characterize immune evasion mechanisms, and optimize therapeutic strategies (Figure 7). However, challenges persist in data standardization, sample processing, and computational integration, requiring continued innovation in experimental and analytical workflows. Emerging breakthroughs—spatial multiomics, real-time monitoring, and AI-driven discovery—are overcoming these barriers by enabling dynamic, personalized approaches to cancer treatment. Looking ahead, efforts must focus on standardizing protocols, reducing costs, and fostering global collaboration to ensure equitable access to HTS-driven precision immunotherapies. By addressing these challenges, we can accelerate the translation of these technologies into clinical practice, ultimately improving outcomes for patients with cancer.

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Conflict of interest

Ling Yin is the Guest Editor of this special issue, but was not in any way involved in the editorial and peer-review process conducted for this paper, directly or indirectly. The author declared that she has no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

Author contributions

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Availability of data

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