Technology	Example Methods	Advantages	Limitations	References
Genomic Sequencing	Whole exome sequencing (WES)	• Provides comprehensive coverage of coding regions and is a cost-effective alternative to whole-genome sequencing • Identifies copy number variants • Can determine mutational signature and total mutation burden • Ability to detect subclonal population with high depth of sequencing	• WES covers only a small fraction of the genome (does not detect variants in most non-coding regions) • Not validated for the detection of structural variations (SVs) • Cannot demonstrate functional relevance of abnormal findings	[1]
Genomic Sequencing	Whole genome sequencing	• Captures mutations in non-coding regions, including potentially important regulatory regions • Detects structural variants (e.g., translocations)	• Higher cost • Relatively low depth (compared to WES) limits ability to detect smaller subclonal populations • Cannot demonstrate functional relevance of abnormal findings	[2]
Transcriptomic Sequencing	Whole transcriptome sequencing	• Provides comprehensive understanding of phenotypes and identifies biomarkers across the broadest range of transcripts. • Captures known and novel gene fusions • Can infer the frequency of	• Dependent on RNA quality, which is variable to poor from FFPE rather than frozen tissue. • Expensive and high turnaround times in clinical settings. • Lack of single cell information limits the ability to define cellular composition and determine relevant pathways/gene signaling within	[3–5]

Technology	Example Methods	Advantages	Limitations	References
		immune cell types can aid in determining immune archetypes	immune cell types • Cannot demonstrate functional relevance of abnormal findings	
Single cell RNA sequencing	Whole transcriptome sequencing on a single cell basis (scRNA-seq)	• Comprehensive assessment of cellular composition and phenotypic states • Computational methods can also be applied to infer repertoire sequences from scRNA-seq not specifically enriched for V(D)J sequences • Methods for multiplexing allow for high sample throughput • Can be paired with chromatin accessibility sequencing (ATAC-seq) • Methods available for lineage tracing (ex: MAESTER)	• High cost • Technically challenging, which limits feasibility, and requires immediate dissociation of fresh tissue • Sequencing depth • Requires familiarity with bioinformatics for data analysis and large amount of data generated, leading to slow adoption for clinical use • Lack of spatial information • Cannot demonstrate functional relevance of abnormal findings	6–10
Immune repertoire sequencing	Whole transcriptome sequencing with paired sequencing of T and B cell receptors	• Can be used with single cell or bulk RNA sequencing • Paired receptor information (variable heavy and light chains or TCR α and β chains) can be obtained • Identification of antigen-specific receptors in some cases	• Antigen specificity not available for most receptor sequences • Cannot demonstrate functional relevance of abnormal findings	11–13

Technology	Example Methods	Advantages	Limitations	References
Proteomic Profiling	Flow cytometry Mass cytometry (CyTOF)	• Inexpensive • Validated laboratory and clinical tests • Quantifies expression of multiple parameters/analytes on per-cell basis	• Requires immediate dissociation of fresh tissue • Requires large quantity of tissue / cells (CyTOF) • Parameters limited by detection technology • Validated and conjugated detection antibodies needed • Cannot demonstrate functional relevance of abnormal findings unless combined with extra labor-intensive steps, e.g., intracellular cytokine staining	[14]
Proteomic Profiling	Secreted cytokine assessment (e.g., ELISA, Luminex)	• Inexpensive • Functional information • Multiplex capability (Luminex)	• Requires supernatant from fresh tissue/cell culture or immediate processing of fresh tissue • Limited to 1 parameter for traditional ELISA	
Proteomic Sequencing	Cellular Indexing of Transcriptomes and Epitopes by Sequencing (CITE-seq)	• Enables the simultaneous analysis of protein and RNA-level expression data by combining traditional scRNA-seq with staining of cells with DNA oligo-tagged antibodies	• High cost • Many techniques still limited in number of proteins analyzed • Cannot demonstrate functional relevance of abnormal findings	[15,16]
Spatial Imaging (traditional)	Immunohistochemistry/ Immunofluorescence	• Preserves spatial architecture and heterogeneity	• Limited multiplexing capabilities • Inefficient for analyzing immunotherapy response	[17]

Technology	Example Methods	Advantages	Limitations	References
		• Validated laboratory and clinical tests • Robust catalog of available antibodies • Easy and standard tissue/ cell processing • Low cost	• Cannot demonstrate functional relevance of abnormal findings	
Spatial Imaging (High dimensional)	Mass cytometry imaging (Imaging Mass Cytometry [IMC] or Multiplex Ion Beam Imaging [MIBI])	• Preserves spatial architecture and heterogeneity • >40 parameters (antibodies conjugated with stable isotopes) • High sensitivity, resolution, and throughput • Uses archival specimens	• Tissue is ablated during imagining • Difficult analysis • Cost, specialized platforms are needed • Suggests but cannot demonstrate functional relevance of abnormal findings	[18,19]
Spatial Imaging	Cyclic immunofluorescence (t-CyCIF)	• Preserves spatial architecture and heterogeneity • ~60 parameters (fluorescently labeled barcodes or secondary antibodies) • High sensitivity and resolution • Uses archival specimens	• Time, hours to 1 day per cycle per tissue section • Cost, specialized platforms are needed • Difficult analysis • Suggests but cannot demonstrate functional relevance of abnormal findings	[18]

Technology	Example Methods	Advantages	Limitations	References
Spatial Transcriptomics	Conventional methods (seqFISH, MERFISH, RNAscope, SABER)	• Serial imagining, or branched amplification • Good spatial resolution	• Lack of tools that can be easily used for multiplexing • Detects 3-4 targets • Error-prone, time consuming, laborious, and costly to scale up • Repeated processing can affect tissue integrity • Suggests but cannot demonstrate functional relevance of abnormal findings	[20]
Spatial Transcriptomics	Advanced methods (Visium, GeoMx, CosMx)	• Increased multiplexing capabilities • Can profile up to 10,000 genes • Cellular and subcellular 3D resolution • Compatible with FFPE and fresh frozen tissues	• Reduced spatial resolution and detection of low abundance targets compared to conventional methods • Optical crowding can limit the molecules that can be detected efficiently and accurately • Low mRNA detection efficiency • Cost, specialized platforms often are needed • Not high-throughput and difficult analysis • Suggests but cannot demonstrate functional relevance of abnormal findings	[20]
Proteomic and transcriptomic imaging	Multi Omic Single-scan Assay with Integrated Combinatorial Analysis (MOSAICA) and CosMx	• Visualization of 1,000 RNA and 100 proteins on one slide	• Reduced spatial resolution and detection of low abundance targets compared to conventional methods	[20]

Technology	Example Methods	Advantages	Limitations	References
	Spatial Molecular Imager	• Cellular and subcellular 3D resolution • Compatible with FFPE and fresh frozen tissues	• Optical crowding can limit the molecules that can be detected efficiently and accurately • Low mRNA detection efficiency • Cost, specialized platforms often are needed • Not high-throughput and difficult analysis • Suggests but cannot demonstrate functional relevance of abnormal findings	
Microbiome imaging	Fluorescent in situ hybridization (FISH)	• Single bacterial imaging technology that provides spatial information	• Not high throughput, tedious, must have known bacterial probes to test • Cannot demonstrate functional relevance of findings	21–24
Microbiome quantification	16S rRNA sequencing, Metagenomics	• Provides a quick look at relative abundances of microbes	• No species level specificity (16S rRNA sequencing) • difficult to analyze (Metagenomics) • Cannot demonstrate functional relevance of findings	25,26
Metabolomics	Mass Spectrometry-based methods	• Small samples • High amount of information • High sensitivity • Definition of metabolic fingerprints before and after therapy	• Data analysis requires high dimensional computational resources • Cannot demonstrate functional relevance of abnormal findings	27,28

Technology	Example Methods	Advantages	Limitations	References
Metabolomics Isotope-labeled probes and PET:	• PD1, PDL1, CTLA4, LAG3 conjugated with ^{89}Zr or ^{64}Cu • ^{18}F-FDG PET/CT • FET • ^{18}F-Gln • ^{18}F-Glu • FLT • ^{18}F-choline • ^{11}C-acetate • ^{18}F-MISO • ^{68}Ga-DOTATOC • ^{68}Ga-PSMA	• More sensitive than IHC determining patient basal expression. • Monitor response predictive changes in metabolic activities before and after ICB treatment. • Some FDA approved • Non-invasive, i.e., does not require tissue biopsy • For ^{18}F-FDG PET: widely used clinical test to assess treatment response, provides functional readout of tumor metabolism	• Their large molecular size implies a long time for biodistribution and optimal image background control. • Still in development	[29–31]
Metabolomics Energy metabolism	• Microplate analyzers • Clark-type electrode chambers	• Small samples for microplate analyzers • Simultaneous measurement of different substrates • Friendly data analysis software • Allows tissue pieces	• Cellular structure is disrupted • Specific analysis software required • Different readouts require addition of detectors sensitive to other analytes. • High quantity of sample required for Clark-type electrode chambers	[32]

Technology	Example Methods	Advantages	Limitations	References
Nanotechnology	Can be integrated with current diagnostic methods. For example, two common methods are MRI imaging and biomarker assay based on human bio-fluid samples.	• Improve the sensitivity of current diagnostic methods • Non-invasive and longitudinal assessment • Track immune resistance early on treatment • Enable real-time monitoring of adoptive cell therapy	• Often requires injection of imaging agent or substrates, therefore clinical trials are required to establish safety • Biomarkers being assessed in a single assay is limited • cGMP manufacturing and clinical trials require large investments, which can lead to high cost for the patients.	33–36
Artificial Intelligence	Integrated analysis of medical imaging, histological analysis, genomics/ epigenomics, and clinical outcomes	• Automates analysis from multiple different sources • Has been successful at predicting responders and non-responders to immunotherapy	• Field is underdeveloped • Need of robust data set to train and iterate machine learning algorithms • Prospective randomized clinical trials are often small; larger data sets derived from retrospective analyses • Patient confidentiality concerns	37
Human *ex vivo* tumor models	Organotypic slice culture	• Biologic surrogate • Tumor 3-D architecture and all stromal and immune components maintained in similar spatial and stoichiometric relationship to patient's tumor • Provides a platform in which to test functional relevance of a therapeutic target	• Slice-to-slice variation, difficult to normalize readouts unless enough biologic slice replicates • Variable yield dependent on tumor type, viability, and preoperative chemo- or radiotherapy administered to the patient • Short viability (1-2 weeks depending on tumor type and starting viability) • Cannot be propagated • Large quantities of fresh tumor are	38–40

Technology	Example Methods	Advantages	Limitations	References
			needed from surgical resection specimen rather than core needle biopsy	
Human *ex vivo* tumor models	Organoid culture (short-term)	• Heterogeneous, maintain all cellular components • Small amount of tissue is needed • Provides a platform in which to test functional relevance of a therapeutic target	• Cell-cell spatial relationships and tissue architecture are lost • Short viability (~5 days), propagation leads to reduced immune and stromal cell composition • Fresh tumor digests are preferred	[41]
Human *in vivo* tumor models	Humanized allogeneic PDX mouse models	• Developed through various sources (PBMCs, $CD34^+$ hematopoietic stem cells, surgical transplant of fetal liver and thymus fragments) • Cellular diversity • Ease of development and cost varies between models • Provides a platform in which to test functional relevance of a therapeutic target	• High cost • Time required to generate model • Cells are typically naive and lack tumor antigen specificity • HLA-mismatched • Allogeneic response may be misinterpreted as anti-tumor response • Models develop GVHD	[42,43]
Human *in vivo* tumor models	Humanized autologous PDX mouse models	• HLA-dependent, autologous response • Patient-specific response can be evaluated • Provides a platform in which	• Tissue is often limiting • TIL expansion may alter TIL maturation/exhaustion phenotype • Current models lack full immune cell reconstitution	[44–47]

Technology	Example Methods	Advantages	Limitations	References
		to test functional relevance of a therapeutic target	• Time and cost for development limits use as a clinical surrogate	

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