

Harnessing the Potential of Multiomics Studies for Precision Medicine in Infectious Disease

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The field of infectious diseases currently takes a reactive approach and treats infections as they present in patients. Although certain populations are known to be at greater risk of developing infection (eg, immunocompromised), we lack a systems approach to define the true risk of future infection for a patient. Guided by impressive gains in “omics” technologies, future strategies to infectious diseases should take a precision approach to infection through identification of patients at intermediate and high-risk of infection and deploy targeted preventative measures (ie, prophylaxis). The advances of high-throughput immune profiling by multiomics approaches (ie, transcriptomics, epigenomics, metabolomics, proteomics) hold the promise to identify patients at increased risk of infection and enable risk-stratifying approaches to be applied in the clinic. Integration of patient-specific data using machine learning improves the effectiveness of prediction, providing the necessary technologies needed to propel the field of infectious diseases medicine into the era of personalized medicine.

Keywords. high-throughput technologies; infectious diseases; invasive fungal infections; systems immunology.

Infectious diseases (ID) physicians currently take a reactive approach in which we provide diagnostic and therapeutic advice on patients with established infection. For example, *Staphylococcus aureus* bacteremia is commonly encountered in inpatient settings. Although *S aureus* colonization and unsterile techniques contribute to incidents of infection, there is clearly a role for host factors in modulation of potential infection and disease severity. The most common conditions that portend increased risk for *S aureus* bacteremia are diabetes, intravenous drug use, presence of central lines in patients requiring dialysis, cancer chemotherapy, and corticosteroid use [1]. The underlying assumption is that bacteremia in this patient population cannot

be accurately predicted, and therapeutic approaches are purely reactive—treatment begins once the infection is established and subsequently diagnosed. Unfortunately, this approach permits widespread host damage from metastatic infection. Mortality rates of 20%–40% have not changed in the past several decades, indicating that another approach is required [1]. The goal for the field of ID in the 21st century should be to predict and prevent infections in individual patients before they occur by interrogating the patients' immune system using multiomics approaches. We have focused this review on the human immunology because the pathogen virulence and its impact has been discussed elsewhere [2–4].

The need for a preemptive, risk-modifying approach to infections in ID is evident by the increasing number of high-risk individuals due to advances in immunosuppression enabling solid organ transplantation, chemotherapy for cancer, and immunomodulatory therapeutics for autoimmune diseases [5–9]. Evidence-based prophylaxis has reduced ID burden in immune compromised patient (eg, *Pneumocystis jirovecii* pneumonia prophylaxis in human immunodeficiency virus [HIV] patients with CD4 count of <200 cells/ μ L) [10]. However, total T-cell counts do not account for specific effector T-cell populations of function, rendering this approach less precise. The

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future of ID medicine should predict and prevent infection to avoid severe outcomes (eg, delay of therapy for underlying processes and death). As a field, we are poised to leverage our understanding of basic mechanisms in microbiology and immunology to learn how to risk-stratify patients and judiciously deploy prophylactic antimicrobials to prevent infections using a personalized medicine approach. In the near term, multiomics studies can nominate specific molecular and cellular biomarkers that can be measured using platforms currently deployed in patient care that will inform clinical decisions.

Medical mycology would greatly benefit from a precision approach to infection. Invasive fungal infections (IFIs) are dreaded complications in immunocompromised populations, often carrying mortality rates exceeding 50% [7, 11, 12]. Clinical data and current literature implicate certain components of the immune system as critical for swift clearance of fungal pathogens [13–15]. However, the rules governing an inflammatory response that contributes to the broad spectrum of clinical outcomes in immunocompromised patients are not known. For example, individuals that receive a single- or double-lung transplant due to end-stage pulmonary disease are at high-risk of developing and succumbing to IFIs. The opportunistic fungal pathogen *Aspergillus fumigatus* is the most commonly diagnosed fungal pathogen in lung transplant recipients (LTRs), with lower incidence of infection by *Mucor*, *Cryptococcus*, and endemic fungi (*Histoplasma*, *Blastomyces*, *Coccidioides*) [16, 17]. Furthermore, *A. fumigatus* colonization in these patients is associated with accelerated chronic rejection [18, 19]. Understanding the underlying factors that predict development of fungal infection in LTRs, and consequent rejection, will enable targeted preventative strategies (eg, vaccine, prophylactics, and/or alteration in immunosuppression) in those at the greatest risk of poor outcome.

Application of precision ID approaches benefits not only relatively small, highly defined cohorts (~4000 lung transplants annually), but also larger, more heterogeneous at-risk populations. Risk of infection in healthcare settings is amplified by the need for invasive procedures (eg, insertion of central venous catheters) leading to a break in the skin barrier and disruption of commensal fungal populations [20, 21]. *Candida* spp is the seventh most prominent pathogen in healthcare-associated infection ([HAI] <10% of all pathogens) and is a leading cause of bloodstream infection (~22% of all pathogens) [22]. The observation that only a subset of at-risk patients develop candidemia despite ubiquitous exposure indicates that host factors potently modulate risk for this infection. Precision ID enables identification of patients at the highest risk of developing invasive candidiasis so that a targeted prophylaxis approach will lead to less morbidity and improved outcomes. It is not hard to imagine the benefits of a screen to identify hospitalized patients at-risk of not only *Candida* infection, but also common bacterial pathogens contributing to HAIs (eg, *Clostridium difficile*,

S aureus, *Pseudomonas aeruginosa*) [22]. Although the idea of personalized medicine is not new [23–26], technologies and bioinformatic approaches required to advance precision ID are becoming available, and they are broadly applied to other fields of medicine (eg, oncology).

POTENTIAL APPLICATION OF NEXT-GENERATION TECHNOLOGIES IN THE CLINICAL SETTING

The immune system, one of the most complex and dynamic biological systems in mammals, comprises diverse cell types with varying functional states. Advances in high-throughput profiling technologies, particularly single-cell omics platforms, enable comprehensive characterization of immune components at multiple scales. However, immunity is not merely a sum of its components, and its behavior cannot be explained or predicted solely by examining individual components. Therefore, systems biology approaches are essential for decoding the cellular complexity, plasticity, and functional diversity of the immune system. The emerging field of systems immunology enables physician-scientists to better understand how the immune system works in health and disease. Evaluation of clinical samples from known high-risk populations will empower future risk-stratification of these populations and improve our ability to deliver precision ID care. In addition, there is increasing evidence that local immunity provides a better window into immune responses than interrogating peripheral blood alone [27]. Interrogation of tissue resident memory cells has provided significant insight into host responses and autoimmune diseases [28, 29]. Specifically, group 3 innate lymphoid cells and T helper type 17 (Th17) cells may serve as key components of tissue-resident memory cells acquired over time or elicited by mucosal immunization that provides the host with enhanced immunity against specific pathogens [30]. For example, LTRs are often diagnosed with pulmonary infection, and therefore it is critical to interrogate both systemic (ie, peripheral blood) and local (ie, bronchoalveolar lavage fluid and lung biopsies) patient samples to provide a fuller picture of immunity in response to infection. Understanding local and systemic immune response to infection at such a large-scale will enable refinement of future clinical assays and identification of relevant biomarkers in susceptible patient populations. We envision an even closer relationship between the ID/transplant physicians and oncologists/rheumatologists whereby ID physicians assist the treating physician to select appropriate preventative strategies to avoid life-threatening infections in at-risk patients.

Transcriptional Genomics

Development of systematic transcriptomic profiling and computational analyses have provided meaningful translational insights into various disease states and immunological response in infections [31–37]. In the immune system, cell populations may appear homogeneous, but analysis by single-cell sequencing

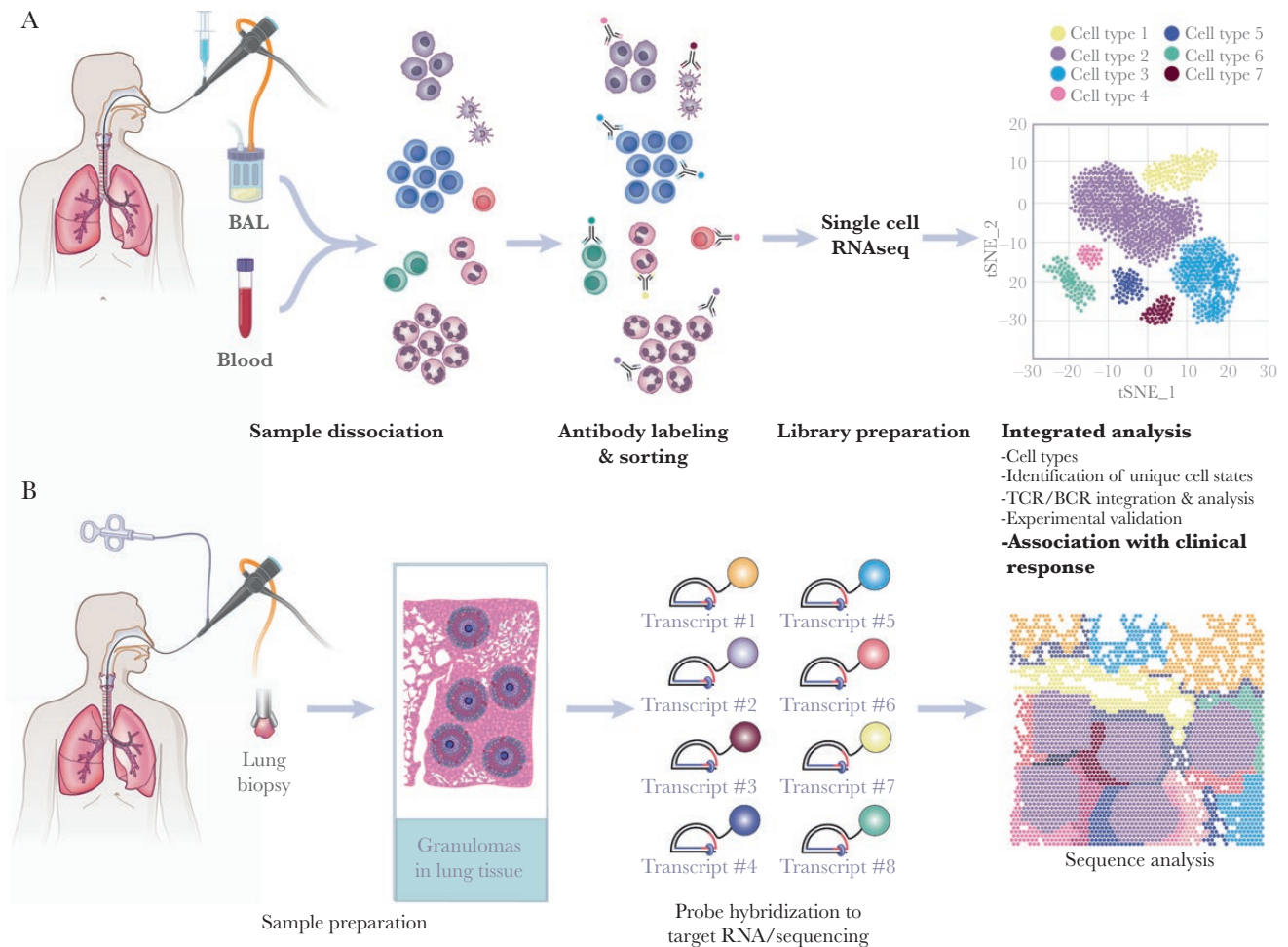


Figure 1. Workflow of transcriptional genomics from patient samples through single-cell ribonucleic acid sequencing (scRNA-seq) (A) and spatially resolved transcriptomics (B). (A) Samples for scRNA-seq require dissociation of cells to ensure cells are not clumped together. Cells are sorted via antibody labeling to sort immune cells and nonimmune cells. Samples undergo reverse transcription and complementary deoxyribonucleic acid amplification and profiled by sequencing through selected sequencing technologies. These libraries often achieve 50 000 reads, providing detailed readouts of cell populations and substates, T-cell receptor (TCR) and B-cell receptor (BCR) profiling, and underlying immune pathways in disease correlating with disease. (B) Tissue samples from infected regions, in this example lung tissue from lung transplant recipients infected with *Aspergillus*, are snap frozen and sectioned into thin slices for spatial transcriptomics. After permeabilization, tissues are exposed to probes designed to target specific RNA sequences followed by amplification that enables visualization of transcripts. Although methodology differs depending on the approach used, the current example of these probes requires a ligation and crosslinking with a fluorescent tag. The resulting data allow analysis of transcripts in their spatial location. BAL, bronchoalveolar lavage. (Printed with permission from Wolf N: 2021).

of ribonucleic acid (scRNA-seq) or examination of epigenetic modifications can reveal cell-to-cell variability that may help subpopulations of cells to rapidly adapt to evolving environments. Single-cell omics approaches, which result in quantitative and high-resolution snapshots of thousands to millions of cells, interrogate human systems and underlying phenotypes that may contribute to disease. Although it is less expensive, bulk RNA-sequencing remains a lower powered approach because results generated reflect an average of all cells, which may lead to critical changes in rare cell populations being overlooked. The scRNA-seq analysis provides an unbiased, data-driven way to systematically detect cellular states to reveal diverse simultaneous facets of cellular identity, from discrete cell types to continuous dynamic transitions, which cannot be defined

by a handful of predefined markers or for which markers are not yet known (Figure 1A). For example, scRNA-seq identified rare epithelial cells, ionocytes, as the major source of cystic fibrosis transmembrane conductance regulator, which reshaped the cystic fibrosis field [38]. In addition, a recent study demonstrated a monocyte phenotype, termed MS1, associated with bacterial sepsis, which appears to be a hallmark of severe infections [39]. In previous studies, after the MS1 marker genes were defined from scRNA-seq, there was evidence of its presence in dozens of bulk transcriptional studies of sepsis with diverse anatomic sources and microbiological causes, underscoring the power of this approach.

Methodological adaptations such as T-cell receptor (TCR) or B-cell receptor (BCR) repertoire profiling and cellular indexing

of transcriptomes and epitopes by sequencing (CITE-seq) further increase the power of scRNA-seq. T-cell responses are essential to adaptive immunity to pathogens, including in IFIs. For example, investigations in patients with HIV demonstrate that a loss of T-cell immunity is closely tied to incidence of cryptococcal meningitis [40–42]. Impaired T-cell responsiveness in other immunologically vulnerable populations have also been observed in invasive *Aspergillus* and *Candida* infections [43, 44]. In addition, the importance of B cells in ID has been well documented in viral and bacterial infections, including severe acute respiratory syndrome coronavirus 2, *Klebsiella*, and *Haemophilus influenzae* [45–48]. The RNA-seq paired with TCR or BCR repertoire analyses enables investigators to interrogate expansion of T- and B-cell populations in disease. In addition to these paired receptor analyses, CITE-seq, which has been implemented in large-scale translational human immunology projects, enables simultaneous single-cell measurements of a predefined array of surface proteins and unbiased gene expression [49]. The CITE-seq utilizes oligonucleotide barcoded antibodies to quantitate protein expression through flow cytometry measurements in tandem with messenger RNA information provided by RNA-seq. Coupled with scRNA-seq, these paired analyses provide a comprehensive look at immune cells and tissues in disease.

Although scRNA-seq provides ample information about the transcriptome in different cell populations, there is a loss of spatial data due to the required step of tissue dissociation. Through advances in sequencing and imaging technologies, understanding expression of transcripts at the single-cell level in their spatial layout provides better understanding within the distinct microenvironment in infected regions and tissues (Figure 1B). Numerous novel spatial transcriptomics approaches have been reviewed previously [37, 50]. Additional complementary approaches such as multiplexed cytometric imaging (CODEX) and in situ hybridization (RNAscope) may be used to improve power of spatial transcriptomics [37, 51, 52]. A key advantage to spatial transcriptomics is the ability to determine the impact of local immune response in tissue, with temporal resolution. In infection, development of a granuloma occurs during infection to contain pathogens. Use of spatial transcriptomics of these granulomas may provide insight into the molecular and cellular mechanisms that govern pathogen containment. Thus, complementary scRNA-seq and spatial transcriptomics approaches may provide ample information on host-pathogen responses during infections and insights into prognosis.

Epigenomics

Although studies investigating genetic susceptibilities have identified polymorphisms associated with infection (eg, CARD9 mutations in IFIs), translation to the clinic does not provide the whole story for risk and development of infection [53]. Common genetic traits do not produce consistent

phenotype, but epigenetic modifications may bridge the gap between phenotype and genes. Epigenetic modifications alter gene expression and function through deoxyribonucleic acid methylation and histone modification (eg, acetylation, methylation, phosphorylation) [54]. Methodology to interrogate epigenomic changes utilize chromatin conformation studies (eg, assay for transposase-accessible chromatin using sequencing [ATAC-seq]) and histone modification profiling (eg, chromatin immunoprecipitation) of clinical samples (Figure 2) [55, 56]. Longitudinal studies that examine epigenetic modifications paired with scRNA-seq in high-risk patients may provide a roadmap to infection susceptibility and ability to effectively clear pathogens. Epigenetic-based treatments are being explored in the context of HIV infections, leading to preclinical and clinical trials for combination antiretroviral therapy [57, 58].

Historically, immune memory has been considered a function of the adaptive immune system (eg, T cells and B cells), providing highly specific, long-lasting protection against invaders. Trained immunity is the concept of long-term functional reprogramming (namely, through epigenetic processes) in early immune responders, also known as innate immune cells (eg, neutrophils, monocytes, dendritic cells) [59]. Interaction with these innate cells and a pathogen leads to altered response during a second challenge with the same pathogen, contributing to innate-mediated short-term (ranging from 3 months to 1 year) protection against foreign invaders [59]. Histone methylation or acetylation are hallmarks of trained immunity in innate immune cells after stimulation with the fungal cell wall carbohydrate β -1,3-glucan, a major component of *Candida* spp [60–62]. Expansion of these studies into individuals with an elevated risk of infection may provide insights into novel preventative and therapeutic strategies to preemptively treat infections.

Metabolite Profiling

Metabolomics systematically quantifies metabolites in biological samples. These metabolites are derived from metabolic processes as well as complex biological interactions within an organism. Clinical metabolomics detects the direct result generated by a biochemical response to a variety of factors, including invading pathogens. The benefit of metabolite profiling is well demonstrated in the fields of cardiovascular disease, kidney disease, cancer metabolism, and emerging in the field of ID [63–71]. Identification of circulating metabolites offers biomarker profiles that precede disease and track severity of disease. Technology platforms for metabolic profiling typically utilize mass spectrometry (MS) coupled with chromatographic separation (including liquid chromatography and gas chromatography) and/or nuclear magnetic resonance spectroscopy (NMR) [72, 73]. Because there is ample diversity of metabolites, priority should be made to process through complementary detection methods.

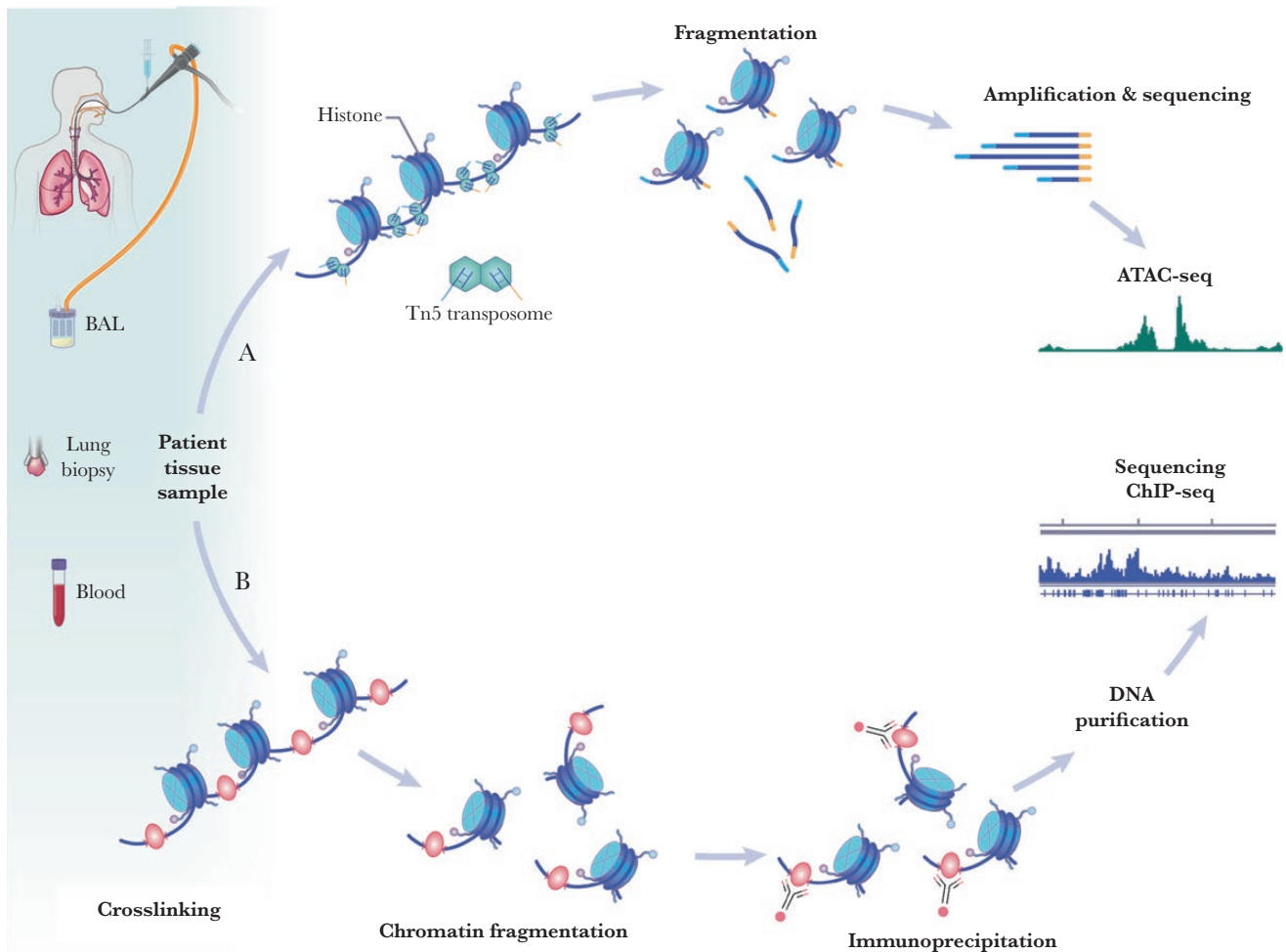


Figure 2. Epigenomic approaches utilizing clinical local (eg, lung tissue and bronchoalveolar lavage [BAL]) and systemic (eg, blood) samples. (A) Assay for transposase-accessible chromatin using sequencing (ATAC-seq) measures chromatin conformation differences. The hyperactive transposase Tn5 loaded with a next-generation sequencing library enables fragmentation of open chromatin regions. These fragments are amplified and sequenced to provide physician-scientists with accessible chromatin regions at the single-cell level. (B) Histone modifications profiling through chromatin immunoprecipitation (ChIP) is an antibody-based technology that selectively enriches deoxyribonucleic acid (DNA)-binding proteins and their respective DNA targets (eg, histone modifications by methylation or acetylation). The DNA and its associated proteins on the chromatin are first crosslinked followed by fragmentation by sonication or a nuclease digestion. These fragments are then immunoprecipitated via antibody selection, which removes remaining cellular debris. Although there are multiple downstream analyses that can be run on ChIP precipitates, sequencing after DNA purification provides information on genome-wide binding in health and disease. (Printed with permission from Wolf N: 2021).

Liquid chromatography-MS, which has emerged as the workhorse for large-scale metabolomics, enables quantification of a broad range of metabolites including lipids, sugars, organic acids, amino acids, amines, nucleotides, bile acids, and acylcarnitines based on the detection method used (Figure 3). Although there are hundreds of known compounds through reference libraries, follow-up protocols (including NMR and other MS approaches) are necessary to identify thousands of unknown peaks. One barrier to keep in mind with respect to comprehensive metabolomic profiling is the dynamic nature of metabolism and metabolite signatures, which requires immediate processing to accurately dissect patient profiles. Furthermore, an advantage of metabolomics is the ability to identify metabolites from small sample volumes (as little as 10 μ L), contributing to the potential to provide early clinical

measurements leading to preventative strategies and treatments of disease.

It has become evident that immune cells rely on changes in cellular metabolism to mount effective antimicrobial responses, with glucose metabolism being a central player in immune cell function, although data from actively infected human patients remain limited [74]. In recent studies, researchers have demonstrated the role of metabolism in trained immunity to fungal pathogens. Emerging data suggest that an increase in glycolysis and glutaminolysis used in the tricarboxylic acid cycle and a corresponding decrease in oxidative phosphorylation are important to the host defense against fungal pathogens [60, 75–77]. It is unfortunate that these studies primarily utilize ex vivo stimulation of peripheral blood samples from healthy individuals. Expansion of large-scale, nontargeted metabolomics

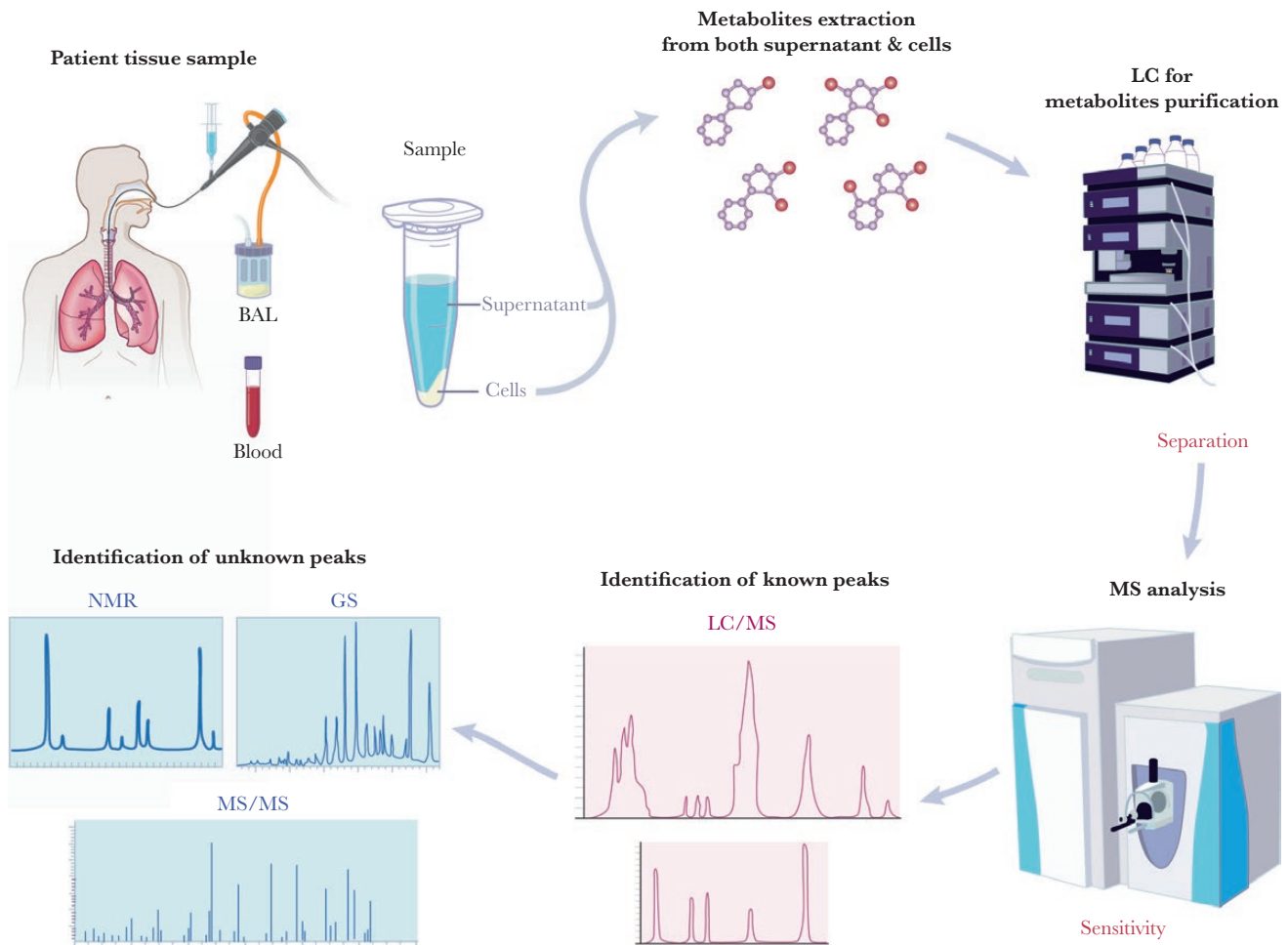


Figure 3. Metabolite profiling through nontargeted approaches of known and unknown peaks. Patient samples for metabolomics require quick processing to extract metabolites prior to them being changed by biological mechanisms. Because the metabolome consists of molecules with very different physical properties, for example, both cationic and anionic compounds ranging from very polar to very nonpolar, it is necessary to devise distinct sample preparation and liquid chromatography-mass spectrometry (LC/MS) procedures to optimize metabolite coverage. These methods utilize different settings for separation via gas (gas chromatography [GC]) or LC step. Mass spectrometry analysis in the positive (C8-pos or hydrophilic interaction chromatography [HILIC]-pos) or negative (C18-neg or HILIC-neg) ion mode provides a wide array of metabolic peaks. These peaks can be compared with known metabolite library for identification. In addition to matches to known metabolites, there are often thousands of unknown peaks, which requires rigorous methodology to identify and authenticate metabolites. Identification and authentication approaches rely on tandem mass spectrometry (MS/MS)-based structure prediction as well as compound isolation and subsequent processing through GC or nuclear magnetic resonance spectroscopy (NMR) methodologies. Interrogation of the metabolome loops back to the patient by identification of metabolic biomarkers of disease. (Printed with permission from Wolf N: 2021).

in high-risk patient populations is warranted. In limited studies into metabolic changes in organ transplantation, researchers identified an increase in glycolysis during transplant rejection, which provides rapid adenosine triphosphate generation and biosynthetic intermediates that support anabolic processes [78, 79]. Expansion of these data in primary clinical samples (both systemic and locally) will provide novel monitoring strategies for LTRs for rejection and development of IFIs.

Considerations for timing of clinical sample collection, particularly in ID medicine, is critical and further studies are needed. In sepsis, metabolism rapidly changes throughout progression of disease. Metabolic profiles differentiated patients who developed sepsis in adults admitted to the intensive care unit due to traumatic injury [80]. Thus, these profiles could be applied to

precision medicine approaches to identify patients most likely to develop infection. Understanding longitudinal changes in infection would enable clinicians to better predict the course of disease. Furthermore, examination of the metabolome may be applied to predict drug response. Investigation into novel therapeutics and treatment strategies is essential with the emergence of more multidrug-resistant pathogens. Metabolomics could be applied as an indicator for the pathogen, which currently relies on culture methods. A study of fungal infections in neonates identified elevations of the amino acid, serine, during active infection, which declined after antifungal therapy, when compared with healthy controls [81]. Thus, large-scale metabolomics to identify diagnostic biomarkers, monitor progress of infection, and development of new treatment is warranted.

Proteomic Analyses

The proteome encompasses the overall protein content within a cell, including protein-protein interactions and posttranslational modifications (eg, phosphorylation) at a particular time point. Measurements of global protein expression within cells or tissues encompass a variety of approaches ranging from targeted, high-dimensional panels to large-scale, unbiased techniques. Protein phosphorylation is an essential posttranslational modification that enables regulation of most biological processes [82–86]. In one sense, the recipe for a successful immune response to an invading pathogen is timely activation and inhibition of distinct

signaling pathways in immune cells to provide efficient antimicrobial effect without inducing excessive tissue damage. Correlating these changes by monitoring phosphorylation status of key intracellular signaling molecules with clinical outcome may provide critical insights into the mechanism of protective immunity. Mass cytometry-based (ie, cytometry by time of flight [CyTOF]) phosphoproteomics methodology provides a platform to globally study activation or inhibition of multiple signaling pathways across the entire immune system with single-cell resolution [87]. Indeed, novel insights into TLR signaling and T-cell activation have been uncovered by phosphoproteomics analysis (Figure 4A)

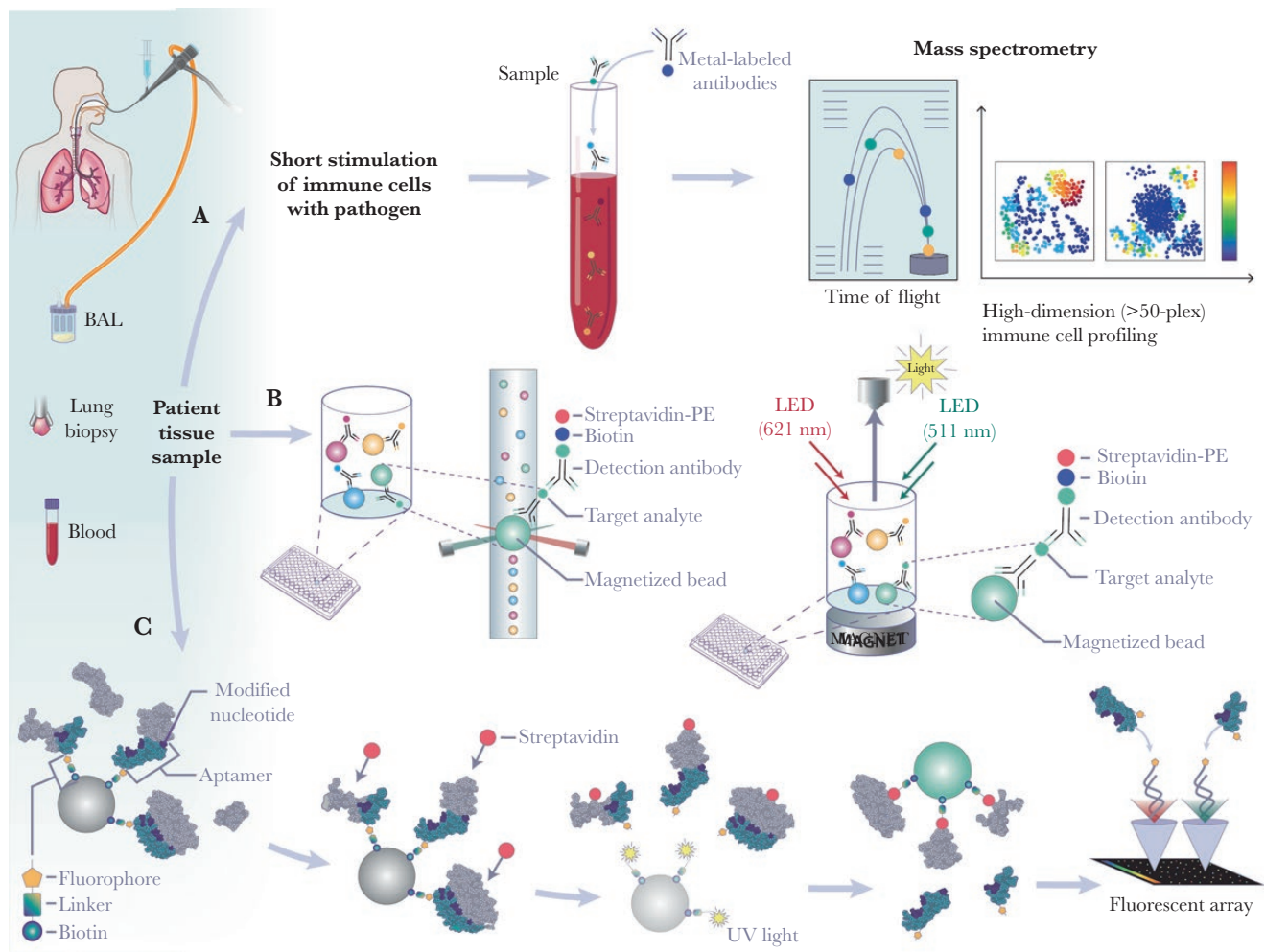


Figure 4. Workflow for mass cytometry (A), multianalyte array (B), and aptamer-based assay (C) proteomic approaches utilizing human patient samples. (A) To interrogate activated and inhibited pathways through phosphoproteomics, biological samples are briefly (15 minutes to 6 hours) stimulated with pathogen (eg, *Aspergillus*) of interest as well as with proper controls (eg, unstimulated and lipopolysaccharide). Stimulated samples are then incubated with metal-labeled antibodies targeting immune cells (cell surface antibodies), intracellular signaling proteins (phosphor-specific antibodies), and/or cytokines (intracellular cytokine antibodies). Cytometry by time-of-flight mass spectrometry (CyTOF) merges traditional flow cytometry with inductively coupled mass spectrometry to assess more than 50 simultaneously measured parameters on a cell-by-cell basis. (B) Targeted multianalyte arrays enable measurement of multiple proteins within a 96- or 384-well plate. Cell supernatants are put in individual wells containing color-coded beads precoated with antibodies for multiple analytes of interest. Detection antibodies for each target analyte as well as streptavidin-phycoerythrin (PE) are added for biotinylated detection. Detection and quantification of each analyte can be determined using a flow-based instrument or magnetic beads. Panels can be created to target specific secreted proteins. (C) Aptamer-based proteomics enables aptamers (eg, Slow Off-rate Modified Aptamers [SOMAmers]) labeled with a fluorophore, photocleavable linker, and biotin to be immobilized on streptavidin-coated beads and incubated with patient samples. After a biotin-tagging step, these aptamer-protein complexes are released by ultraviolet (UV) light-mediated photocleavage of the linker. The biotin labeled- aptamer-protein complexes are captured by a second set of streptavidin-coated beads and aptamers are released after incubation with denaturing buffer. A microarray chip is used to quantify fluorescence intensity within to total protein amount of the initial sample. Throughout this process, unbound proteins and nonspecific interactions are removed. BAL, bronchoalveolar lavage; LED, light-emitting diode. (Printed with permission from Wolf N: 2021).

[88, 89]. Insights into the distribution and activation or inhibition of intracellular signaling pathways by targeting phosphorylation states of known signaling molecules in immune cells provides mechanistic understanding of disease states and infection [85]. Targeted profiling via the CyTOF platform enables investigators to tailor a panel of ~50 markers to dissect specific cell subsets and activation states of different proteins. For example, the CyTOF panel examining differences in innate immunity from invasive candidiasis HAI may include proteins associated with *Candida* immunity, such as iNOS, Arg1, Ym1, Ym2, IL-4, and Egr2 [90–92].

Secretion of proteins to the external environment is critical in maintaining cell-cell communication and recruitment of immune cells in response to pathogens. These secreted proteins include hormones, cytokines, chemokines, and growth factors. Measurement of these secreted proteins (secretome) can be done through a targeted multiplex array approach (eg, multianalyte assay). Multiplex assays allow for simultaneous detection and quantification of multiple proteins (Figure 4B). Stimulation of peripheral blood mononuclear cells (PBMCs) or tissues from patients with a pathogen may provide insights in the variations of the secretome in patients with high-risk of infection compared with healthy controls. Multianalyte assays have been used to investigate numerous infections [93–96].

Although a targeted approach may provide single-cell resolution and insights into known immune factors, application of large-scale, unbiased proteomic approaches contributes to identification of novel proteins associated with disease. Furthermore, this type of approach may identify biomarkers of disease and clinical severity. The SomaScan assay developed by SomaLogic is an aptamer-based approach that measures up to 7000 unique human proteins (Figure 4C). Although it is optimized for a set of core sample types (plasma, serum, urine), the SomaScan assay sample source has the ability to interrogate noncore sample sources (bronchoalveolar lavage, cell-conditioned media, cerebrospinal fluid, exosomes, sputum, tissue homogenates). Aptamer-based proteomics is widely used in neurodegenerative disease, cardiovascular diseases, and infections [97–100]. Indeed, use of Slow Off-rate Modified Aptamers (SOMAmers) in the SomaScan assay and RNA-seq was used to identify tissue-specific clinical markers of heart, kidney, liver, and skeletal muscle damage in patients with coronavirus disease 2019 (COVID-19) [101]. These biomarkers can be used upon presentation to the hospital using blood samples to detect COVID-19-driven organ damage. Taken together, these proteomic approaches identify activated pathways and biomarkers of infection critical for implementation of precision ID medicine into clinical practice.

PRECISION MEDICINE IN INFECTIOUS DISEASES

Transcriptional expression, epigenetic forms, metabolites, and protein expression are all interwoven to directly impact immune responses in disease. Individually, high-throughput

omics approaches provide a window into immune responses and phenotypes that portend increased risk of infection and may foretell clinical course. Given the complex interplay among different aspects of the immune system, combining multiomics modalities into a computational framework increases predictive power and reveals crosstalk between different layers of biological profiling (Figure 5). However, merging transcriptomic, epigenomic, metabolomic, and proteomic features into predictive models will require continued development of new statistical tools designed to study high-dimensional datasets. Recent advances in bioinformatic processes overcome many analytic challenges that previously prevented the development of models to accurately predict patient outcomes [86, 102, 103]. Regularized regression methods such as the Elastic Net algorithm have proven useful for selection of key predictive features and development of clinical models [104]. A stacked generalization method that combines multiple regularized regression models developed from individual omic datasets has also been recognized as a valuable approach for data integration and can improve overall model performance [86]. This approach has been successfully implemented for the prediction of various clinical outcomes, including development of insulin resistance [105], onset of spontaneous labor [106], survival of persons with pancreatic cancer [107], and severity of COVID-19 infections [85]. Furthermore, development of effective visualization methods for these integrated machine learning models improves understanding of results [108, 109]. Taken together, these analytic approaches provide mechanistic information regarding the crosstalk between various biological systems that could not otherwise be identified from each assay individually.

Implementation of precision ID medicine requires immune profiling of well phenotyped human cohorts, particularly patients with known risk factors (eg, solid organ transplantation, cancer, invasive procedures). Although recent advances have improved cost effectiveness and availability of these omics technologies, there is still a heavy burden on resources. To realize the full potential of these technologies, there needs to be significant reduction in cost of processing and analyzing each sample to ensure access to all patients. Major funding agencies acknowledge this need as demonstrated by new funding mechanisms through the National Institutes of Health (Human Immunology Project Consortium) and European Research Council (Horizon Program) [110, 111]. Further multiomics studies are warranted to make precision ID a clinical reality.

CONCLUSIONS

Translational systems immunology provides the framework to shift from reactive to proactive precision ID medicine, improving the quality of life and dampening complications in high-risk populations (Figure 5). Although many omics approaches have been used to understand host response to

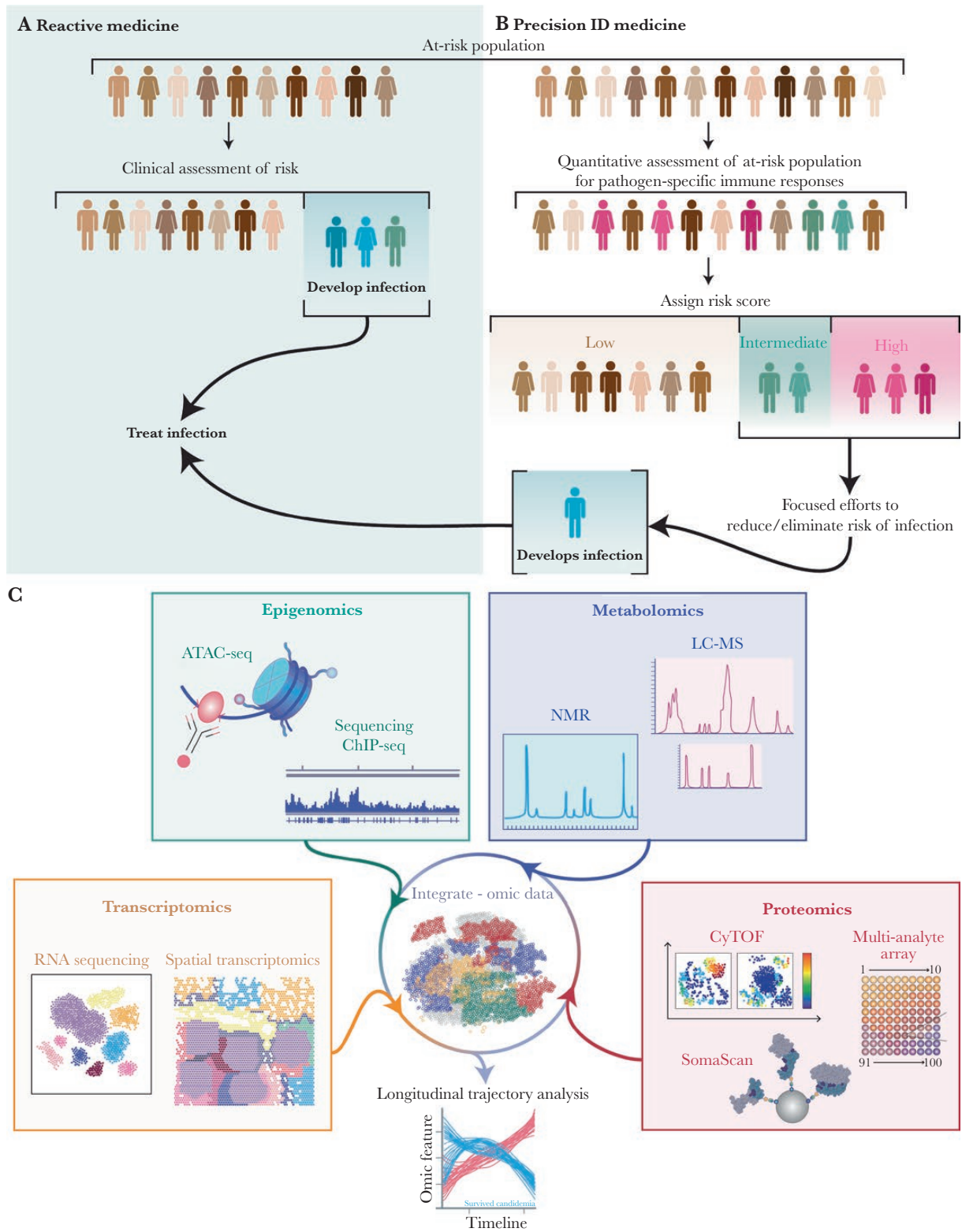


Figure 5. Workflow of reactive (A) and precision (B) infectious diseases (ID) medicine. A precision approach would enable clinicians to use preventative strategies, leading to fewer infections and infectious complications, including targeted prophylaxis or therapies that enhance immunity to specific pathogens. (C) Incorporation of multiomics approaches into an integrative machine learning model more accurately predicts clinical outcomes. Multiomics include transcriptomics (bulk or single-cell ribonucleic acid [RNA] sequencing [with or without paired analyses] and spatial transcriptomics), epigenomics (chromatin immunoprecipitation [ChIP] and assay for transposase-accessible chromatin using sequencing [ATAC-seq]), metabolomics (liquid chromatography tandem mass spectrometry [LC-MS] and one-dimensional proton nuclear magnetic resonance spectroscopy [NMR]), and proteomics (cytometry by time-of-flight [CyTOF], aptamer-based methods [SomaScan], and multianalyte array). Combining these multiomics methodologies across longitudinal samples of local and peripheral immune responses provides insight into relevant pathways and predicts clinical outcomes in disease. (Printed with permission from Wolf N: 2021).

pathogens, barriers remain that need to be addressed. One major barrier to our understanding is the reliance on animal models to study human disease, because these often do not recapitulate the complexity of disease. Furthermore, many reports using these omics approaches utilize samples from healthy patients exposed *ex vivo* to pathogens. In previous studies, researchers have attempted to understand initial immune responses in *C albicans*-stimulated PBMCs using RNA-seq, and they unveiled the predominance of interferon responses and activation of major innate cell populations (eg, neutrophils, macrophages, monocytes). Although the assumptions are foundational, the generalizability of these *ex vivo* stimulations of blood from normal volunteers is limited. In addition, single-cell resolution permits identification of the precise cell types driving observed transcriptional changes, thus suggesting candidate immune pathways/circuits for precision diagnostics and therapeutic targeting. Thus, there is a need for multiomics investigations using well defined clinical cohorts at greater risk of infections. Integration of these data will result in the necessary information to apply risk stratification to high-risk populations, preventive strategies to reduce the burden of infection, and allow for targeted rather than prophylactic antibiotic strategies.

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