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

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Summary: Translational systems immunology approaches to infectious diseases will enable the switch from reactive to precision treatment of patients, improving clinical outcomes while reducing the use of prophylactic antibiotics and incidence of infection in high-risk individuals.

Abstract

The field of infectious diseases currently takes a reactive approach, treating infections as they present in patients. Although certain populations are known to be at greater risk of developing infection (*e.g.,* 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 (*i.e.,* prophylaxis). The advances of high-throughput immune profiling by multiomics approaches (*i.e.,* transcriptomics, epigenomics, metabolomics, proteomics) holds 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: infectious diseases, systems immunology, invasive fungal infections, high-throughput technologies

Reactive Versus Precision Medicine

Infectious diseases (ID) currently takes 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. While *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. 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. 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 as the pathogen virulence and its impact has been discussed elsewhere.

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. Evidence based prophylaxis has reduced ID burden in immune compromised patient (*e.g.,* PJP prophylaxis in HIV patient with CD4 count of <200 cells/µL). However, total T cell counts do not account for specific effector T cell populations of function, rendering this approach less precise. The future of ID medicine should predict and prevent infection to avoid severe outcomes (*e.g.,* 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 measure 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 morality rates exceeding 50%. Clinical data and current literature implicate certain components of the immune system as critical for swift clearance of fungal pathogens. Yet, 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*). Furthermore, *A. fumigatus* colonization in these patients is associated with accelerated chronic rejection. Understanding the underlying factors that predict development of fungal infection in LTRs, and consequent rejection, will enable targeted preventative strategies (*e.g.,* 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 (~4,000 lung transplants annually), but also larger, more heterogenous at-risk populations. Risk of infection in healthcare settings is amplified by the need for invasive procedures (*e.g.,* insertion of central venous catheters) leading to a break in the skin barrier and disruption of commensal fungal populations. *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). 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 (e.*g., Clostridium difficile, S. aureus, Pseudomonas aeruginosa*). Although the idea of personalized medicine is not new, technologies and bioinformatic approaches required to advance precision ID are becoming available and broadly applied to other fields of medicine (*e.g.,* 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. Additionally, there is increasing evidence that local immunity provides a better window into immune responses than interrogating peripheral blood alone. Interrogation of tissue resident memory cells has provided significant insight into host responses and autoimmune diseases. 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. For example, LTRs are often diagnosed with pulmonary infection, and therefore it is critical to interrogate both systemic (*i.e.,* peripheral blood) and local (*i.e.,* 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 assists 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. In the immune system, cell populations may appear homogeneous, but analysis by single-cell sequencing of RNA (scRNA-seq) or examination of epigenetic modifications can reveal cell-to-cell variability that may help sub-populations of cells to rapidly adapt to evolving environments. Resulting in quantitative and high-resolution snapshots of thousands to millions of cells, single-cell -omics approaches interrogate human systems and underlying phenotypes that may contribute to disease. While less expensive, bulk RNA-sequencing remains a lower powered approach since results generated reflect an average of all cells, which may lead to critical changes in rare cell populations being overlooked. 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 pre-defined 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 (CFTR), reshaping the cystic fibrosis field. Additionally, a recent study demonstrated a monocyte phenotype, termed MS1, associated with bacterial sepsis which appears to be a hallmark of severe infections. Having defined MS1 marker genes from scRNA-seq studies, 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 HIV patients demonstrate that a loss of T cell immunity is closely tied to incidence of cryptococcal meningitis. Impaired T cell responsiveness in other immunologically vulnerable populations have also been observed in invasive *Aspergillus* and *Candida* infections. Additionally, the importance of B cells in ID has been well documented in viral and bacterial infections, including SARS-CoV-2, *Klebsiella*, and *Haemophilus influenzae*. 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. CITE-seq utilizes oligonucleotide barcoded antibodies to quantitate protein expression through flow cytometry measurements in tandem with mRNA information provided my RNA-seq. Coupled with scRNA-seq, these paired analyses provide a comprehensive look at immune cells and tissues in disease.

While 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. Additional complementary approaches such as multiplexed cytometric imaging (CODEX) and *in situ* hybridization (RNAscope) may be utilized to improve power of spatial transcriptomics. 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 (*e.g., CARD9* mutations in IFIs), translation to the clinic does not provide the whole story for risk and development of infection. 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 DNA methylation and histone modification (*e.g.,* acetylation, methylation, phosphorylation). Methodology to interrogate epigenomic changes utilize chromatin conformation studies (*e.g.,* assay for transposase-accessible chromatin using sequencing; ATAC-seq) and histone modification profiling (*e.g.,* chromatin immunoprecipitation; ChIP) of clinical samples (***Figure 2***). Longitudinal studies examining 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 cART therapy.

Historically, immune memory has been considered a function of the adaptive immune system (*e.g.,* 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 (*e.g.,* neutrophils, monocytes, dendritic cells). 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 a year) protection

against foreign invaders. Histone methylation or acetylation are hallmarks of trained immunity in innate immune cells following stimulation with the fungal cell wall carbohydrate β-1,3-glucan, a major component of *Candida* spp.. 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. Identification of circulating metabolites offers biomarker profiles that precede disease and track severity of disease. Technology platforms for metabolic profiling typically utilize mass spectrometry coupled with chromatographic separation (including liquid chromatography [LC-MS] and gas chromatography [GC-MS]) and/or nuclear magnetic resonance spectroscopy (NMR). Since there is ample diversity of metabolites, priority should be made to process through complementary detection methods. LC-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 utilized (***Figure 3***). While 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, requiring immediate processing to accurately dissect patient profiles. That said, an advantage of metabolomics is the ability to identify metabolites

from small sample volumes (as little as 10 µL), 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. Recent studies have demonstrated a role of metabolism in trained immunity to fungal pathogens. Emerging data suggest an increase in glycolysis and glutaminolysis used in the TCA-cycle and a corresponding decrease in oxidative phosphorylation are important to host defense against fungal pathogens. Unfortunately, these studies primarily utilize *ex vivo* stimulation of peripheral blood samples from healthy individuals. Expansion of large-scale nontargeted metabolomics in high-risk patient populations is warranted. Limited studies into metabolic changes in organ transplantation identified an increase in glycolysis during transplant rejection, which provides rapid ATP generation and biosynthetic intermediates that support anabolic processes. 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. Thus, these profiles could be applied to precision medicine approaches to identify patients most likely to develop infection. Understanding longitudinal changes in infection would license clinicians to better predict the course of disease. Furthermore, examination of the metabolome may be applied to prediction of 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 following antifungal therapy, when compared to healthy

controls. 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 post-translational modifications (*e.g.,* 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 post-translational modification that enables regulation of most biological processes. 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 (*i.e.,* 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. Indeed, novel insights into TLR signaling and T cell activation have been uncovered by phosphoproteomics analysis (***Figure 4A***). 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. 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.

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 (*e.g.,* multi-analyte assay). Multiplex assays allow for simultaneous detection and quantification of multiple proteins (***Figure 4B***). Stimulation of 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 to healthy controls. Multi-analyte assays have been used to investigate numerous infections.

While 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 7,000 unique human proteins (***Figure 4C***). Although optimized for a set of core sample types (plasma, serum, urine), the SomaScan assay sample source has the ability to interrogate non-core 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. Indeed, use of Slow Off-rate Modified Aptamers (SOMAmers) in the SomaScan assay and RNA-seq was utilized to identify tissue-specific clinical markers of heart, kidney, liver, and skeletal muscle damage in COVID-19 patients. These biomarkers can be used upon presentation to the hospital using blood samples to detect COVID-driven organ damage. Together, these proteomic approaches identify activated pathways and biomarkers of infection critical for implementation of precision ID medicine into clinical practice.

Precision Medicine in ID

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. Regularized regression methods such as the Elastic Net algorithm have proven useful for selection of key predictive features and development of clinical models. 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. This approach has been successfully implemented for the prediction of various clinical outcomes, including development of insulin resistance, onset of spontaneous labor, survival of persons with pancreatic cancer, and severity of COVID-19 infections. Furthermore, development of effective visualization methods for these integrated machine learning models improves understanding of results. 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 (*e.g.,* 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). 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 employed to understand host response to pathogens, barriers remain that need to be addressed. One major barrier to our understanding is the reliance on animal models to study human disease, as 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. Previous efforts have attempted to understand initial immune responses in *C. albicans* stimulated PBMCs using RNA-seq and unveiled the predominance of interferon responses and activation of major innate cell populations (*e.g.,* neutrophils, macrophages, monocytes). While foundational, the generalizability of these *ex vivo* stimulations of blood from normal volunteers is limited. Additionally, 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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Patient Consent Statement

This study does not include factors necessitating patient consent.

Figure Legends

Figure 1. Workflow of transcriptional genomics from patient samples through single-cell RNA 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 non-immune cells. Samples undergo reverse transcription and cDNA 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 and B cell receptor (TCR & BCR, respectively) profiling, and underlying immune pathways in disease correlating with disease. **(B)** Tissue samples from infected regions, in this example lung tissue from LTRs infected with *Aspergillus*, are snap frozen and sectioned into thin slices for spatial transcriptomics. Following permeabilization, tissues are exposed to probes designed to target specific RNA sequences followed by amplification that enables visualization of transcripts. While 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 allows analysis of transcripts in their spatial location.

Figure 2. Epigenomic approaches utilizing clinical local (*e.g.,* lung tissue and BAL) and systemic (*e.g.,* 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 for fragmentation of open chromatin regions. These fragments are amplified and sequences 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 DNA-binding proteins and their respective DNA targets (*e.g.,* histone modifications by methylation or acetylation). 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. While there are multiple downstream analyses that can

be run on ChIP precipitates, sequencing following DNA purification provides information on genome-wide binding in health and disease.

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 LC-MS procedures to optimize metabolite coverage. These methods utilize different settings for separation via gas (GC) or liquid chromatography (LC) step. Mass spec (MS) analysis in the positive (C8-pos or HILIC-pos) or negative (C18-neg or HILIC-neg) ion mode provides a wide array of metabolic peaks. These peaks can be compared to 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 of 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.

Figure 4. Workflow for mass cytometry **(A)**, multi-analyte 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 min to 6 h) stimulated with pathogen (*e.g., Aspergillus*) of interest as well as with proper controls (*e.g.,* unstimulated and LPS). 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 over 50 simultaneously measured parameters on a cell-by-cell basis. **(B)** Targeted multi-analyte arrays enable measurement of

multiple proteins within a 96- or 384-well plate. Cell supernatants are put in individual wells containing color-coded beads pre-coated with antibodies for multiple analytes of interest. Detection antibodies for each target analyte as well as streptavidin-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 (*e.g.,* SOMAmers) labeled with a fluorophore, photocleavable linker, and biotin to be immobilized on streptavidin-coated beads and incubated with patient samples. Following a biotin-tagging step, these aptamer-protein complexes are released by 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 following incubation with denaturing buffer. A microarray chip is utilized to quantify fluorescence intensity within to total protein amount of the initial sample. Throughout this process, unbound proteins and non-specific interactions are removed.

Figure 5. Workflow of reactive **(A)** and precision **(B)** infectious disease (ID) medicine. A precision approach would enable clinicians to employ 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 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 multi-analyte 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.

References

1. Souli M, Ruffin F, Choi SH, et al. Changing characteristics of *Staphylococcus aureus* *b*acteremia: results from a 21-year, prospective, longitudinal study. *Clin Infect Dis*. Nov 13 2019;69(11):1868-1877. doi:10.1093/cid/ciz112

2. Lee MH, Nuccio SP, Raffatellu M. Pathogen interference: targeting virulence factors to tackle intracellular microbes. *Cell Chem Biol*. Jul 16 2020;27(7):765-767. doi:10.1016/j.chembiol.2020.06.017

3. Jack RS. Evolution of immunity and pathogens. *Results Probl Cell Differ*. 2015;57:1-20. doi:10.1007/978-3-319-20819-0_1

4. Chin VK, Lee TY, Rusliza B, Chong PP. Dissecting *Candida albicans* infection from the perspective of *C. albicans* virulence and omics approaches on host-pathogen anteraction: a review. *Int J Mol Sci*. Oct 18 2016;17(10)doi:10.3390/ijms17101643

5. Baddley JW, Cantini F, Goletti D, et al. ESCMID Study Group for Infections in Compromised Hosts (ESGICH) Consensus Document on the safety of targeted and biological therapies: an infectious diseases perspective (Soluble immune effector molecules [I]: anti-tumor necrosis factor-α agents). *Clinical Microbiology and Infection*. 2018/06/01/ 2018;24:S10-S20. doi:https://doi.org/10.1016/j.cmi.2017.12.025

6. Rawson TM, Moore LSP, Zhu N, et al. Bacterial and fungal coinfection in individuals with coronavirus: a rapid review to support COVID-19 antimicrobial prescribing. *Clin Infect Dis*. Dec 3 2020;71(9):2459-2468. doi:10.1093/cid/ciaa530

7. Rayens E, Norris KA, Cordero JF. Mortality trends in risk conditions and invasive mycotic disease in the United States, 1999-2018. *Clin Infect Dis*. Apr 20 2021;doi:10.1093/cid/ciab336

8. Zembower TR. Epidemiology of infections in cancer patients. *Cancer Treat Res*. 2014;161:43-89. doi:10.1007/978-3-319-04220-6_2

9. Varughese T, Taur Y, Cohen N, et al. Serious infections in patients receiving ibrutinib for treatment of lymphoid cancer. *Clin Infect Dis*. Aug 16 2018;67(5):687-692. doi:10.1093/cid/ciy175

10. Sidhu VK, Foisy MM, Hughes CA. Discontinuing *Pneumocystis jirovecii* pneumonia prophylaxis in HIV-infected patients with a CD4 cell count <200 cells/mm3. *Ann Pharmacother*. Dec 2015;49(12):1343-8. doi:10.1177/1060028015605113

11. Cornely OA, Gachot B, Akan H, et al. Epidemiology and outcome of fungemia in a cancer cohort of the Infectious Diseases Group (IDG) of the European Organization for Research and Treatment of Cancer (EORTC 65031). *Clin Infect Dis*. Aug 1 2015;61(3):324-31. doi:10.1093/cid/civ293

12. Taccone FS, Van den Abeele AM, Bulpa P, et al. Epidemiology of invasive aspergillosis in critically ill patients: clinical presentation, underlying conditions, and outcomes. *Crit Care*. Jan 12 2015;19:7. doi:10.1186/s13054-014-0722-7

13. Romani L. Immunity to fungal infections. *Nat Rev Immunol*. Apr 2011;11(4):275-88. doi:10.1038/nri2939

14. Ward RA, Vyas JM. The first line of defense: effector pathways of anti-fungal innate immunity. *Curr Opin Microbiol*. Nov 17 2020;58:160-165. doi:10.1016/j.mib.2020.10.003

15. Köhler JR, Hube B, Puccia R, Casadevall A, Perfect JR. Fungi that infect humans. *Microbiol Spectr*. Jun 2017;5(3)doi:10.1128/microbiolspec.FUNK-0014-2016

16. Baker AW, Maziarz EK, Arnold CJ, et al. Invasive fungal infection after lung transplantation: epidemiology in the setting of antifungal prophylaxis. *Clin Infect Dis*. Jan 1 2020;70(1):30-39. doi:10.1093/cid/ciz156

17. Kennedy CC, Razonable RR. Fungal infections after lung transplantation. *Clin Chest Med*. Sep 2017;38(3):511-520. doi:10.1016/j.ccm.2017.04.011

18. Pasupneti S, Manouvakhova O, Nicolls MR, Hsu JL. *Aspergillus*-related pulmonary diseases in lung transplantation. *Med Mycol*. 2017;55(1):96-102. doi:10.1093/mmy/myw121

19. Weigt SS, Copeland CAF, Derhovanessian A, et al. Colonization with small conidia *Aspergillus* species is associated with bronchiolitis obliterans syndrome: a two-center validation study. *Am J Transplant*. Apr 2013;13(4):919-927. doi:10.1111/ajt.12131

20. Arias S, Denis O, Montesinos I, Cherifi S, Miendje Deyi VY, Zech F. Epidemiology and mortality of candidemia both related and unrelated to the central venous catheter: a retrospective cohort study. *Eur J Clin Microbiol Infect Dis*. Mar 2017;36(3):501-507. doi:10.1007/s10096-016-2825-3

21. Poissy J, Damonti L, Bignon A, et al. Risk factors for candidemia: a prospective matched case-control study. *Crit Care*. Mar 18 2020;24(1):109. doi:10.1186/s13054-020-2766-1

22. Magill SS, Edwards JR, Bamberg W, et al. Multistate point-prevalence survey of health care-associated infections. *N Engl J Med*. Mar 27 2014;370(13):1198-208. doi:10.1056/NEJMoa1306801

23. Carvalho A, Goldman GH. Editorial: an omics perspective on fungal infection: toward next-generation diagnosis and therapy. *Front Microbiol*. 2017;8:85. doi:10.3389/fmicb.2017.00085

24. Moser C, Lerche CJ, Thomsen K, et al. Antibiotic therapy as personalized medicine - general considerations and complicating factors. *Apmis*. May 2019;127(5):361-371. doi:10.1111/apm.12951

25. van de Veerdonk FL, Gresnigt MS, Verweij PE, Netea MG. Personalized medicine in influenza: a bridge too far or the near future? *Curr Opin Pulm Med*. May 2017;23(3):237-240. doi:10.1097/mcp.0000000000000378

26. Al-Mozaini MA, Mansour MK. Personalized medicine. Is it time for infectious diseases? *Saudi Med J*. Dec 2016;37(12):1309-1311. doi:10.15537/smj.2016.12.16837

27. Farber DL. Tissues, not blood, are where immune cells function. *Nature*. May 2021;593(7860):506-509. doi:10.1038/d41586-021-01396-y

28. Quinton LJ, Jones MR, Robson BE, Mizgerd JP. Mechanisms of the hepatic acute-phase response during bacterial pneumonia. *Infect Immun*. Jun 2009;77(6):2417-26. doi:10.1128/IAI.01300-08

29. Woodruff PG, Modrek B, Choy DF, et al. T-helper type 2-driven inflammation defines major subphenotypes of asthma. *Am J Respir Crit Care Med*. Sep 1 2009;180(5):388-95. doi:10.1164/rccm.200903-0392OC

30. Iwanaga N, Kolls JK. Updates on T helper type 17 immunity in respiratory disease. *Immunology*. Jan 2019;156(1):3-8. doi:10.1111/imm.13006

31. Sade-Feldman M, Yizhak K, Bjorgaard SL, et al. Defining T cell states associated with response to checkpoint immunotherapy in melanoma. *Cell*. Nov 1 2018;175(4):998-1013 e20. doi:10.1016/j.cell.2018.10.038

32. Villani AC, Satija R, Reynolds G, et al. Single-cell RNA-seq reveals new types of human blood dendritic cells, monocytes, and progenitors. *Science (New York, NY)*. Apr 21 2017;356(6335)doi:10.1126/science.aah4573

33. Dixit A, Parnas O, Li B, et al. Perturb-seq: dissecting molecular circuits with scalable single-cell RNA profiling of pooled genetic screens. *Cell*. Dec 15 2016;167(7):1853-1866.e17. doi:10.1016/j.cell.2016.11.038

34. Li B, Dewey CN. RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. *BMC Bioinformatics*. Aug 4 2011;12:323. doi:10.1186/1471-2105-12-323

35. Li B, Gould J, Yang Y, et al. Cumulus provides cloud-based data analysis for large-scale single-cell and single-nucleus RNA-seq. *Nat Methods*. Aug 2020;17(8):793-798. doi:10.1038/s41592-020-0905-x

36. Sun Z, Chen L, Xin H, et al. A Bayesian mixture model for clustering droplet-based single-cell transcriptomic data from population studies. *Nat Commun*. Apr 9 2019;10(1):1649. doi:10.1038/s41467-019-09639-3

37. Asp M, Bergenstrahle J, Lundeberg J. Spatially resolved transcriptomes-next generation tools for tissue exploration. *Bioessays*. Oct 2020;42(10):e1900221. doi:10.1002/bies.201900221

38. Montoro DT, Haber AL, Biton M, et al. A revised airway epithelial hierarchy includes CFTR-expressing ionocytes. *Nature*. Aug 2018;560(7718):319-324. doi:10.1038/s41586-018-0393-7

39. Reyes M, Filbin MR, Bhattacharyya RP, et al. An immune-cell signature of bacterial sepsis. *Nat Med*. Mar 2020;26(3):333-340. doi:10.1038/s41591-020-0752-4

40. Jarvis JN, Casazza JP, Stone HH, et al. The phenotype of the *Cryptococcus*-specific CD4+ memory T-cell response is associated with disease severity and outcome in HIV-associated cryptococcal meningitis. *J Infect Dis*. Jun 15 2013;207(12):1817-28. doi:10.1093/infdis/jit099

41. Linyu L, Ali Abuderman AW, Muzaheed, Acharya S, Divakar DD. Modulation of host immune status by *Cryptococcus* co-infection during HIV-1 pathogenesis and its impact on CD+4 cell and cytokines environment. *Microb Pathog*. Feb 2020;139:103864. doi:10.1016/j.micpath.2019.103864

42. Tugume L, Rhein J, Hullsiek KH, et al. HIV-associated cryptococcal meningitis occurring at relatively higher CD4 counts. *J Infect Dis*. Feb 23 2019;219(6):877-883. doi:10.1093/infdis/jiy602

43. Camargo JF, Bhimji A, Kumar D, et al. Impaired T cell responsiveness to interleukin-6 in hematological patients with invasive aspergillosis. *PLoS One*. 2015;10(4):e0123171. doi:10.1371/journal.pone.0123171

44. Liu F, Fan X, Auclair S, et al. Sequential dysfunction and progressive depletion of *Candida albicans*-specific CD4 T cell response in HIV-1 infection. *PLoS Pathog*. Jun 2016;12(6):e1005663. doi:10.1371/journal.ppat.1005663

45. García-Gil A, Lopez-Bailon LU, Ortiz-Navarrete V. Beyond the antibody: B cells as a target for bacterial infection. *J Leukoc Biol*. May 2019;105(5):905-913. doi:10.1002/jlb.Mr0618-225r

46. Hurwitz JL. B cells, viruses, and the SARS-CoV-2/COVID-19 pandemic of 2020. *Viral Immunol*. May 2020;33(4):251-252. doi:10.1089/vim.2020.0055

47. Zargaran FN, Akya A, Rezaeian S, et al. B Cell Epitopes of Four Fimbriae Antigens of Klebsiella pneumoniae: A Comprehensive In Silico Study for Vaccine Development. *Int J Pept Res Ther*. Nov 24 2020:1-12. doi:10.1007/s10989-020-10134-3

48. Perrett KP, John TM, Jin C, et al. Long-term persistence of immunity and B-cell memory following *Haemophilus influenzae* type B conjugate vaccination in early childhood and response to booster. *Clin Infect Dis*. Apr 2014;58(7):949-59. doi:10.1093/cid/ciu001

49. Stoeckius M, Hafemeister C, Stephenson W, et al. Simultaneous epitope and transcriptome measurement in single cells. *Nat Methods*. Sep 2017;14(9):865-868. doi:10.1038/nmeth.4380

50. Ward RA, Thompson GR, Villani A-C, et al. The known unknowns of the immune response to *Coccidioides*. *Journal of Fungi*. 2021;7(5):377.

51. Wang F, Flanagan J, Su N, et al. RNAscope: a novel *in situ* RNA analysis platform for formalin-fixed, paraffin-embedded tissues. *J Mol Diagn*. Jan 2012;14(1):22-9. doi:10.1016/j.jmoldx.2011.08.002

52. Goltsev Y, Samusik N, Kennedy-Darling J, et al. Deep Profiling of Mouse Splenic Architecture with CODEX Multiplexed Imaging. *Cell*. Aug 9 2018;174(4):968-981.e15. doi:10.1016/j.cell.2018.07.010

53. Maskarinec SA, Johnson MD, Perfect JR. Genetic susceptibility to fungal infections: what is in the genes? *Curr Clin Microbiol Rep*. Jun 2016;3(2):81-91. doi:10.1007/s40588-016-0037-3

54. Berdasco M, Esteller M. Clinical epigenetics: seizing opportunities for translation. *Nat Rev Genet*. Feb 2019;20(2):109-127. doi:10.1038/s41576-018-0074-2

55. Rodríguez-Ubreva J, Ballestar E. Chromatin immunoprecipitation. *Methods Mol Biol*. 2014;1094:309-18. doi:10.1007/978-1-62703-706-8_24

56. Buenrostro JD, Wu B, Chang HY, Greenleaf WJ. ATAC-seq: A Method for Assaying Chromatin Accessibility Genome-Wide. *Curr Protoc Mol Biol*. Jan 5 2015;109:21.29.1-21.29.9. doi:10.1002/0471142727.mb2129s109

57. Archin NM, Liberty AL, Kashuba AD, et al. Administration of vorinostat disrupts HIV-1 latency in patients on antiretroviral therapy. *Nature*. Jul 25 2012;487(7408):482-5. doi:10.1038/nature11286

58. Rasmussen TA, Tolstrup M, Brinkmann CR, et al. Panobinostat, a histone deacetylase inhibitor, for latent-virus reactivation in HIV-infected patients on suppressive antiretroviral therapy: a phase 1/2, single group, clinical trial. *Lancet HIV*. Oct 2014;1(1):e13-21. doi:10.1016/s2352-3018(14)70014-1

59. Netea MG, Domínguez-Andrés J, Barreiro LB, et al. Defining trained immunity and its role in health and disease. *Nat Rev Immunol*. Jun 2020;20(6):375-388. doi:10.1038/s41577-020-0285-6

60. Arts RJ, Novakovic B, Ter Horst R, et al. Glutaminolysis and fumarate accumulation integrate immunometabolic and epigenetic programs in trained immunity. *Cell Metab*. Dec 13 2016;24(6):807-819. doi:10.1016/j.cmet.2016.10.008

61. Bekkering S, Arts RJW, Novakovic B, et al. Metabolic induction of trained immunity through the mevalonate pathway. *Cell*. Jan 11 2018;172(1-2):135-146.e9. doi:10.1016/j.cell.2017.11.025

62. Cheng SC, Quintin J, Cramer RA, et al. mTOR- and HIF-1α-mediated aerobic glycolysis as metabolic basis for trained immunity. *Science (New York, NY)*. Sep 26 2014;345(6204):1250684. doi:10.1126/science.1250684

63. Zhang E, Chai JC, Deik AA, et al. Plasma Lipidomic Profiles and Risk of Diabetes: 2 Prospective Cohorts of HIV-Infected and HIV-Uninfected Individuals. *J Clin Endocrinol Metab*. Mar 25 2021;106(4):999-1010. doi:10.1210/clinem/dgab011

64. Guasch-Ferre M, Hu FB, Ruiz-Canela M, et al. Plasma metabolites from choline pathway and risk of cardiovascular disease in the PREDIMED (prevention with m

editerranean diet) study. *J Am Heart Assoc*. Oct 28 2017;6(11)doi:10.1161/JAHA.117.006524

65. Lloyd-Price J, Arze C, Ananthakrishnan AN, et al. Multi-omics of the gut microbial ecosystem in inflammatory bowel diseases. *Nature*. May 2019;569(7758):655-662. doi:10.1038/s41586-019-1237-9

66. Mayers JR, Wu C, Clish CB, et al. Elevation of circulating branched-chain amino acids is an early event in human pancreatic adenocarcinoma development. *Nat Med*. Oct 2014;20(10):1193-1198. doi:10.1038/nm.3686

67. Paynter NP, Balasubramanian R, Giulianini F, et al. Metabolic predictors of incident coronary heart disease in women. *Circulation*. Feb 20 2018;137(8):841-853. doi:10.1161/CIRCULATIONAHA.117.029468

68. Rhee EP, Waikar SS, Rebholz CM, et al. Variability of two metabolomic platforms in CKD. *Clin J Am Soc Nephrol*. Jan 7 2019;14(1):40-48. doi:10.2215/cjn.07070618

69. Wang TJ, Larson MG, Vasan RS, et al. Metabolite profiles and the risk of developing diabetes. *Nat Med*. Apr 2011;17(4):448-53. doi:10.1038/nm.2307

70. Zeleznik OA, Eliassen AH, Kraft P, et al. A prospective analysis of circulating plasma metabolites associated with ovarian cancer risk. *Cancer Res*. Mar 15 2020;80(6):1357-1367. doi:10.1158/0008-5472.CAN-19-2567

71. Sindelar M, Stancliffe E, Schwaiger-Haber M, et al. Longitudinal metabolomics of human plasma reveals prognostic markers of COVID-19 disease severity. *Cell reports Medicine*. Jul 21 2021:100369. doi:10.1016/j.xcrm.2021.100369

72. Markley JL, Brüschweiler R, Edison AS, et al. The future of NMR-based metabolomics. *Curr Opin Biotechnol*. Feb 2017;43:34-40. doi:10.1016/j.copbio.2016.08.001

73. Zhou B, Xiao JF, Tuli L, Ressom HW. LC-MS-based metabolomics. *Mol Biosyst*. Feb 2012;8(2):470-81. doi:10.1039/c1mb05350g

74. Pearce EL, Pearce EJ. Metabolic pathways in immune cell activation and quiescence. *Immunity*. Apr 18 2013;38(4):633-43. doi:10.1016/j.immuni.2013.04.005

75. Pellon A, Sadeghi Nasab SD, Moyes DL. New insights in *Candida albicans* innate immunity at the mucosa: toxins, epithelium, metabolism, and beyond. *Front Cell Infect Microbiol*. 2020;10:81. doi:10.3389/fcimb.2020.00081

76. Traven A, Naderer T. Central metabolic interactions of immune cells and microbes: prospects for defeating infections. *EMBO Rep*. Jul 2019;20(7):e47995. doi:10.15252/embr.201947995

77. Dominguez-Andres J, Arts RJW, Ter Horst R, et al. Rewiring monocyte glucose metabolism via C-type lectin signaling protects against disseminated candidiasis. *PLoS Pathog*. Sep 2017;13(9):e1006632. doi:10.1371/journal.ppat.1006632

78. Ochando J, Fayad ZA, Madsen JC, Netea MG, Mulder WJM. Trained immunity in organ transplantation. *Am J Transplant*. Jan 2020;20(1):10-18. doi:10.1111/ajt.15620

79. Braza MS, van Leent MMT, Lameijer M, et al. Inhibiting Inflammation with Myeloid Cell-Specific Nanobiologics Promotes Organ Transplant Acceptance. *Immunity*. Nov 20 2018;49(5):819-828 e6. doi:10.1016/j.immuni.2018.09.008

80. Blaise BJ, Gouel-Cheron A, Floccard B, Monneret G, Allaouchiche B. Metabolic phenotyping of traumatized patients reveals a susceptibility to sepsis. *Anal Chem*. Nov 19 2013;85(22):10850-5. doi:10.1021/ac402235q

81. Dessi A, Liori B, Caboni P, et al. Monitoring neonatal fungal infection with metabolomics. *J Matern Fetal Neonatal Med*. Oct 2014;27 Suppl 2:34-8. doi:10.3109/14767058.2014.954787

82. Candia J, Cheung F, Kotliarov Y, et al. Assessment of variability in the SOMAscan assay. *Sci Rep*. Oct 27 2017;7(1):14248. doi:10.1038/s41598-017-14755-5

83. DeBoer EM, Wagner BD, Popler J, et al. Novel application of aptamer proteomic analysis in cystic fibrosis bronchoalveolar lavage fluid. *Proteomics Clin Appl*. May 2019;13(3):e1800085. doi:10.1002/prca.201800085

84. Aghaeepour N, Lehallier B, Baca Q, et al. A proteomic clock of human pregnancy. *Am J Obstet Gynecol*. 2018;218(3):347.e1-347.e14. doi:10.1016/j.ajog.2017.12.208

85. Feyaerts D, Hedou J, Gillard J, et al. Integrated plasma proteomic and single-cell immune signaling network signatures demarcate mild, moderate, and severe COVID-19. *bioRxiv*. Feb 10 2021;doi:10.1101/2021.02.09.430269

86. Ghaemi MS, DiGiulio DB, Contrepois K, et al. Multiomics modeling of the immunome, transcriptome, microbiome, proteome and metabolome adaptations during human pregnancy. *Bioinformatics*. Jan 1 2019;35(1):95-103. doi:10.1093/bioinformatics/bty537

87. Bendall SC, Simonds EF, Qiu P, et al. Single-cell mass cytometry of differential immune and drug responses across a human hematopoietic continuum. *Science (New York, NY)*. May 6 2011;332(6030):687-96. doi:10.1126/science.1198704

88. Joshi RN, Binai NA, Marabita F, et al. Phosphoproteomics reveals regulatory T cell-mediated DEF6 dephosphorylation that affects cytokine expression in human conventional T cells. *Front Immunol*. 2017;8:1163. doi:10.3389/fimmu.2017.01163

89. Sjoelund V, Smelkinson M, Nita-Lazar A. Phosphoproteome profiling of the macrophage response to different toll-like receptor ligands identifies differences in global phosphorylation dynamics. *J Proteome Res*. Nov 7 2014;13(11):5185-97. doi:10.1021/pr5002466

90. Feyaerts D, Hédou J, Gillard J, et al. Integrated plasma proteomic and single-cell immune signaling network signatures demarcate mild, moderate, and severe COVID-19. *bioRxiv*. 2021:2021.02.09.430269. doi:10.1101/2021.02.09.430269

91. Leigh JE, McNulty KM, Fidel PL, Jr. Characterization of the immune status of CD8+ T cells in oral lesions of human immunodeficiency virus-infected persons with oropharyngeal candidiasis. *Clin Vaccine Immunol*. Jun 2006;13(6):678-83. doi:10.1128/CVI.00015-06

92. Suram S, Silveira LJ, Mahaffey S, et al. Cytosolic phospholipase A(2)alpha and eicosanoids regulate expression of genes in macrophages involved in host defense and inflammation. *PLoS One*. 2013;8(7):e69002. doi:10.1371/journal.pone.0069002

93. Terayama Y, Matsuura T, Ozaki K. Induction of severe chronic hyperplastic candidiasis in rat by opportunistic infection of *C. albicans* through combination of diabetes

and intermittent prednisolone administration. *Toxicol Pathol*. Aug 2017;45(6):745-755. doi:10.1177/0192623317726193

94. Won EJ, Choi JH, Cho YN, et al. Biomarkers for discrimination between latent tuberculosis infection and active tuberculosis disease. *J Infect*. Mar 2017;74(3):281-293. doi:10.1016/j.jinf.2016.11.010

95. Gómez-Escobar LG, Hoffman KL, Choi JJ, et al. Cytokine signatures of end organ injury in COVID-19. *Sci Rep*. Jun 15 2021;11(1):12606. doi:10.1038/s41598-021-91859-z

96. Beardsley J, Hoang NLT, Kibengo FM, et al. Do intracerebral cytokine responses explain the harmful effects of dexamethasone in human immunodeficiency virus-associated cryptococcal meningitis? *Clin Infect Dis*. Apr 24 2019;68(9):1494-1501. doi:10.1093/cid/ciy725

97. Akilimali NA, Chang CC, Muema DM, et al. Plasma but not cerebrospinal fluid interleukin 7 and interleukin 5 levels pre-antiretroviral therapy commencement predict cryptococcosis-associated immune reconstitution inflammatory syndrome. *Clin Infect Dis*. Oct 16 2017;65(9):1551-1559. doi:10.1093/cid/cix598

98. Ahmad S, Milan MdC, Hansson O, et al. CDH6 and HAGH protein levels in plasma associate with Alzheimer's disease in APOE ε4 carriers. *Scientific Reports*. 2020/05/19 2020;10(1):8233. doi:10.1038/s41598-020-65038-5

99. Ngo D, Sinha S, Shen D, et al. Aptamer-based proteomic profiling reveals novel candidate biomarkers and pathways in cardiovascular disease. *Circulation*. Jul 26 2016;134(4):270-85. doi:10.1161/CIRCULATIONAHA.116.021803

100. Penn-Nicholson A, Hraha T, Thompson EG, et al. Discovery and validation of a prognostic proteomic signature for tuberculosis progression: A prospective cohort study. *PLOS Medicine*. 2019;16(4):e1002781. doi:10.1371/journal.pmed.1002781

101. Dong L, Watson J, Cao S, et al. Aptamer based proteomic pilot study reveals a urine signature indicative of pediatric urinary tract infections. *PLOS ONE*. 2020;15(7):e0235328. doi:10.1371/journal.pone.0235328

102. Filbin MR, Mehta A, Schneider AM, et al. Longitudinal proteomic analysis of plasma from patients with severe COVID-19 reveal patient survival-associated signatures, tissue-specific cell death, and cell-cell interactions. *Cell reports Medicine*. May 3 2021:100287. doi:10.1016/j.xcrm.2021.100287

103. Jehan F, Sazawal S, Baqui AH, et al. Multiomics characterization of preterm birth in low- and middle-income countries. *JAMA Netw Open*. Dec 1 2020;3(12):e2029655. doi:10.1001/jamanetworkopen.2020.29655

104. Culos A, Tsai AS, Stanley N, et al. Integration of mechanistic immunological knowledge into a machine learning pipeline improves predictions. *Nat Mach Intell*. Oct 2020;2(10):619-628. doi:10.1038/s42256-020-00232-8

105. Aghaeepour N, Kin C, Ganio EA, et al. Deep immune profiling of an arginine-enriched nutritional intervention in patients undergoing surgery. *The Journal of Immunology*. 2017;199(6):2171-2180. doi:10.4049/jimmunol.1700421

106. Schussler-Fiorenza Rose SM, Contrepois K, Moneghetti KJ, et al. A longitudinal big data approach for precision health. *Nat Med*. May 2019;25(5):792-804. doi:10.1038/s41591-019-0414-6

107. Stelzer IA, Ghaemi MS, Han X, et al. Integrated trajectories of the maternal metabolome, proteome, and immunome predict labor onset. *Sci Transl Med*. May 5 2021;13(592)doi:10.1126/scitranslmed.abd9898

108. Baek B, Lee H. Prediction of survival and recurrence in patients with pancreatic cancer by integrating multi-omics data. *Sci Rep*. Nov 3 2020;10(1):18951. doi:10.1038/s41598-020-76025-1

109. Ding H, Sharpnack M, Wang C, Huang K, Machiraju R. Integrative cancer patient stratification via subspace merging. *Bioinformatics*. May 15 2019;35(10):1653-1659. doi:10.1093/bioinformatics/bty866

110. Gallivan KA, Srivastava A, Xiuwen L, Dooren PV. Efficient algorithms for inferences on Grassmann manifolds. 2003:315-318.

111. NIAID. Human Immunology Project Consortium (HIPC). National Institutes of Health. Accessed 07/06/2021, 2021. https://www.niaid.nih.gov/research/human-immunology-project-consortium

112. EuropeanCommission. Horizon 2020. Accessed 07/06/2021, 2021. https://ec.europa.eu/programmes/horizon2020/en/home

Figure 1

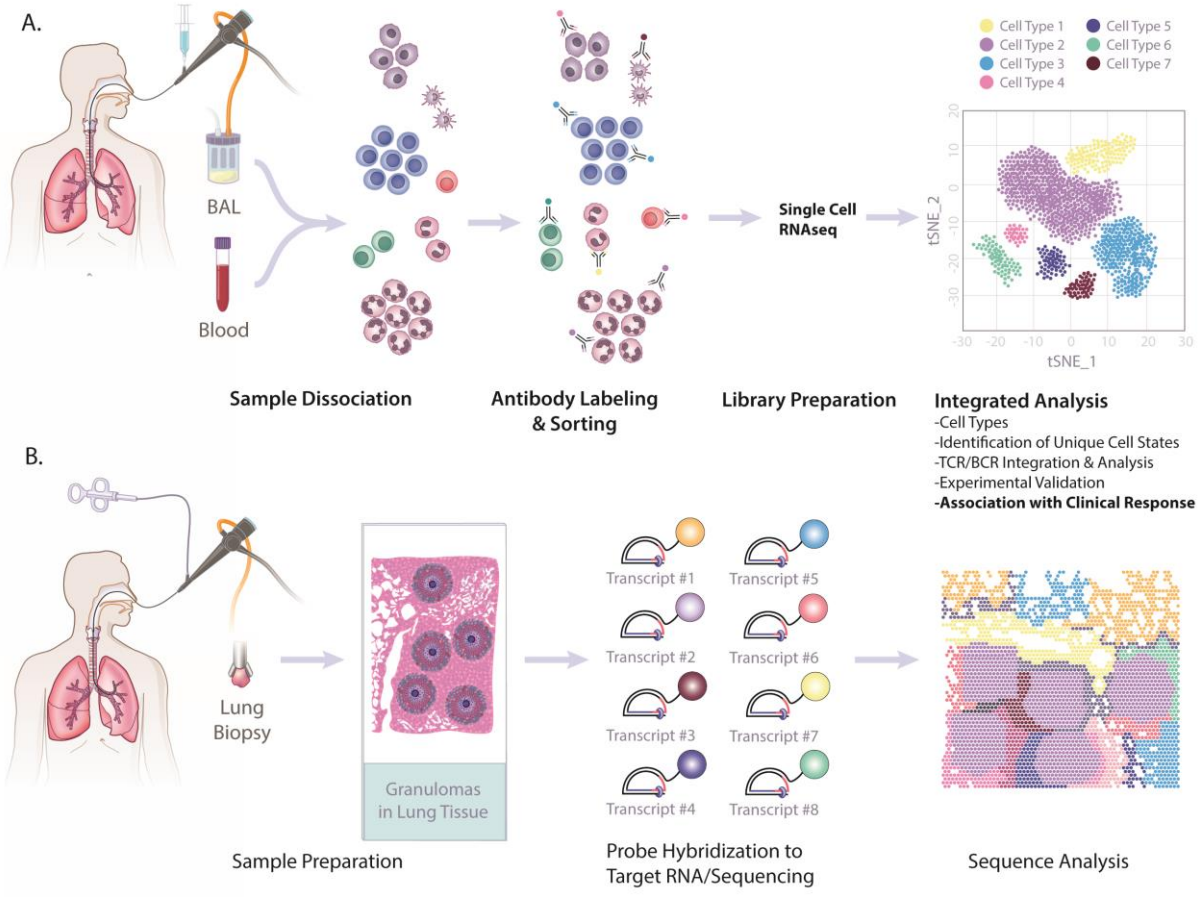


Figure 2

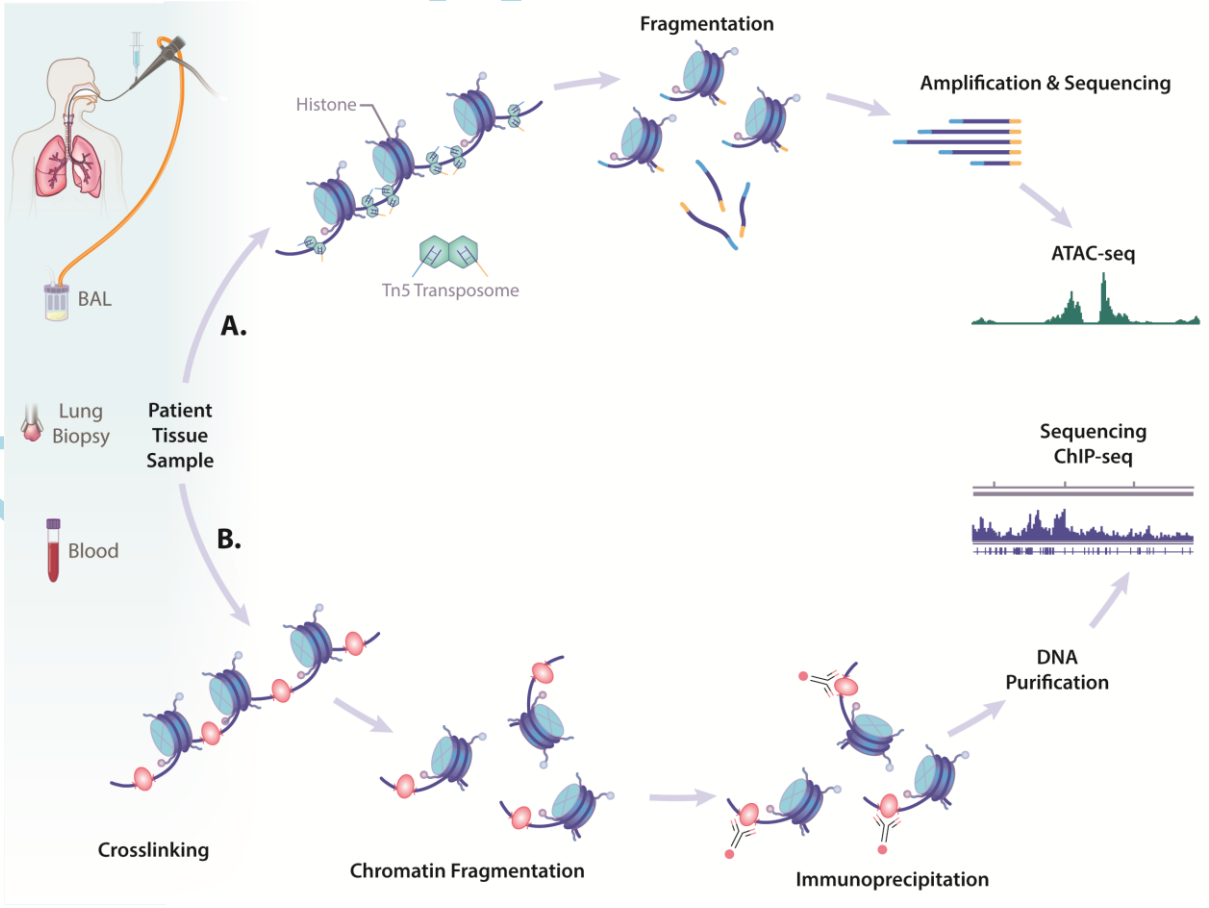


Figure 3

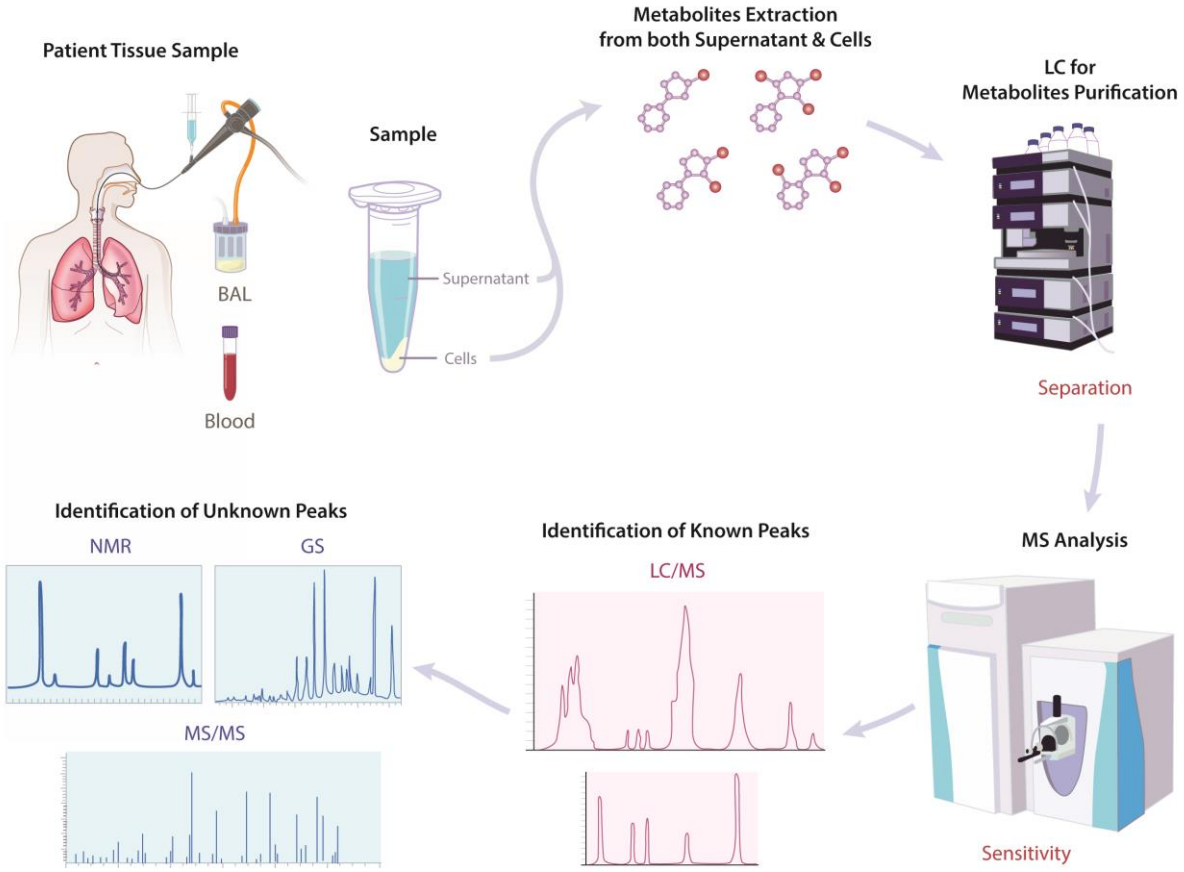


Figure 4

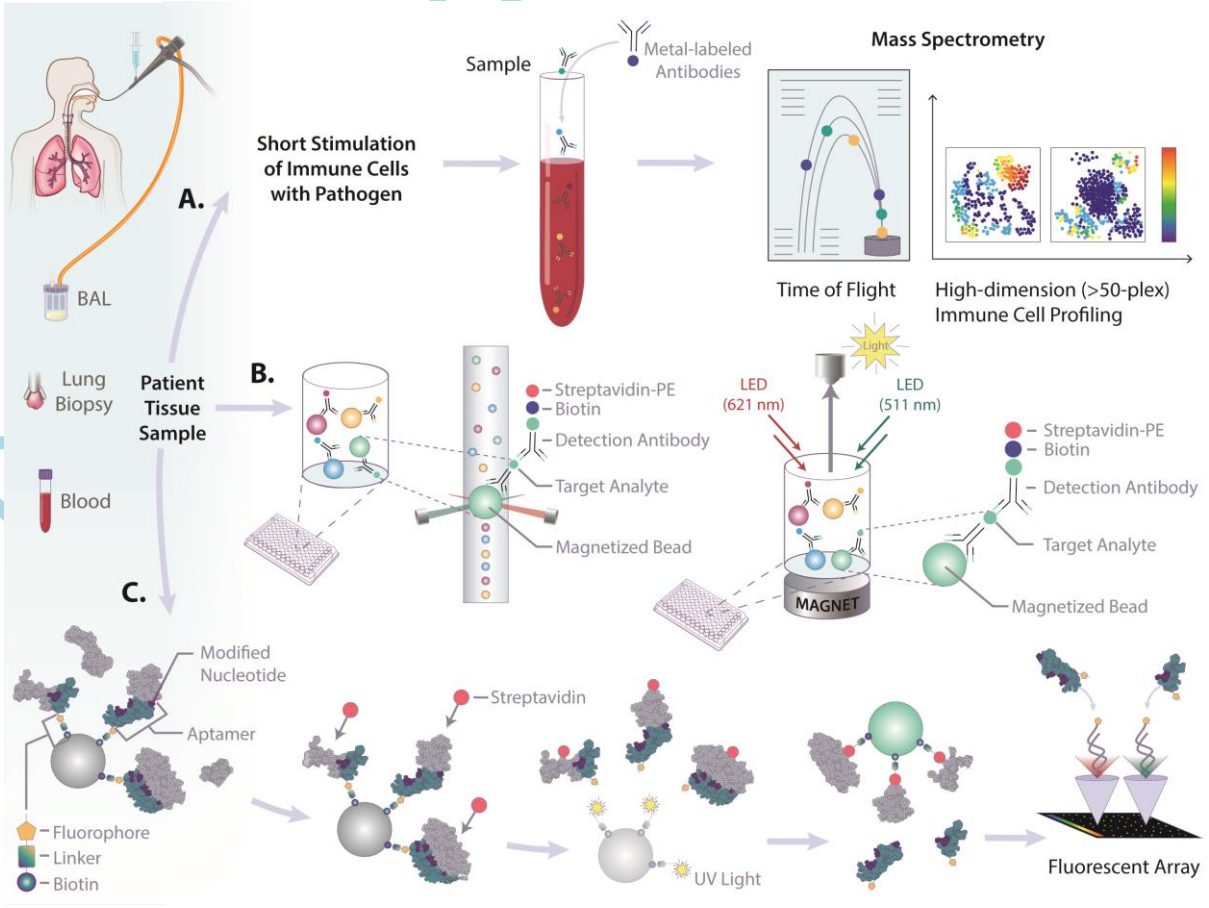
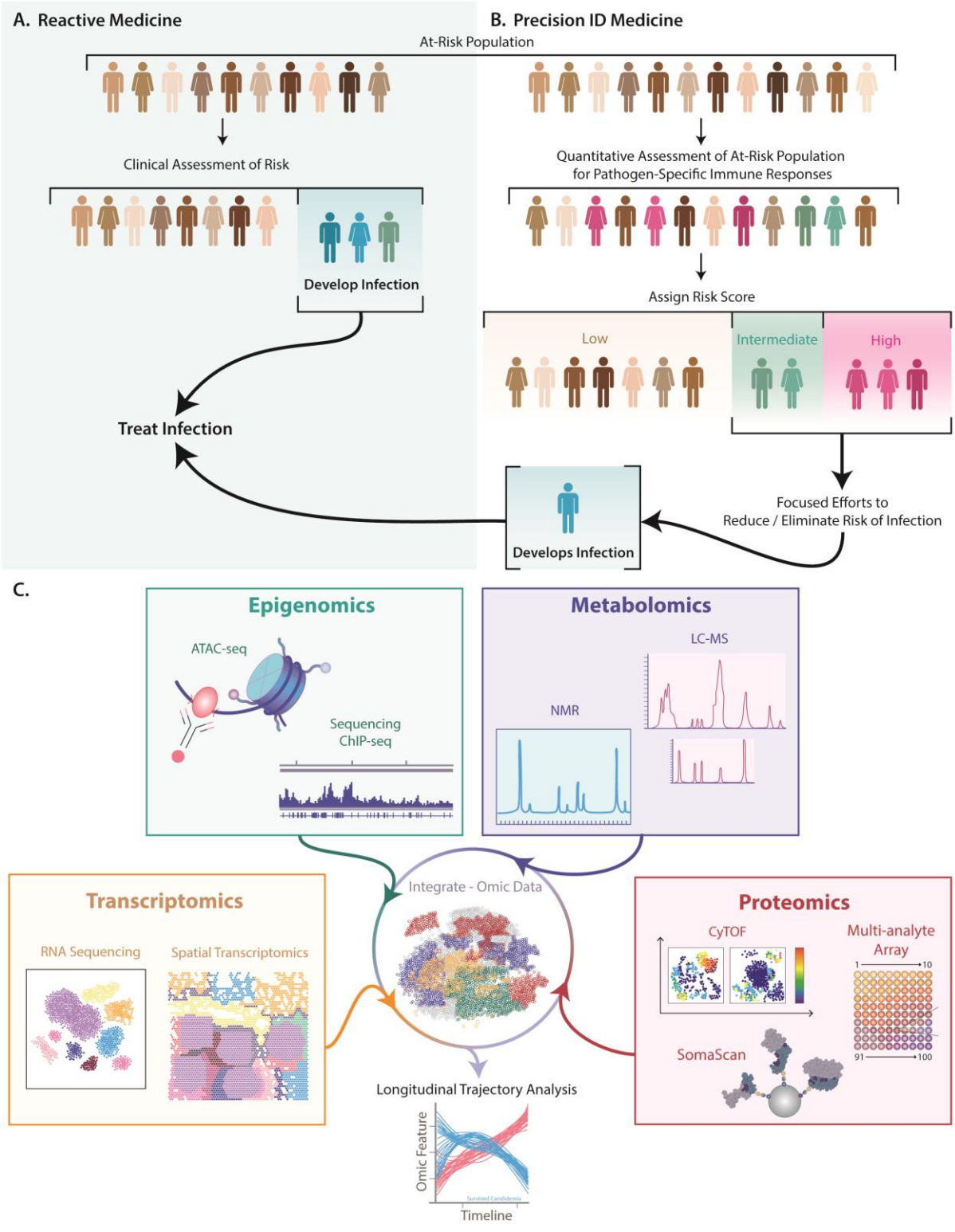


Figure 5



Integrative Machine Learning Models to Predict Clinical Outcomes