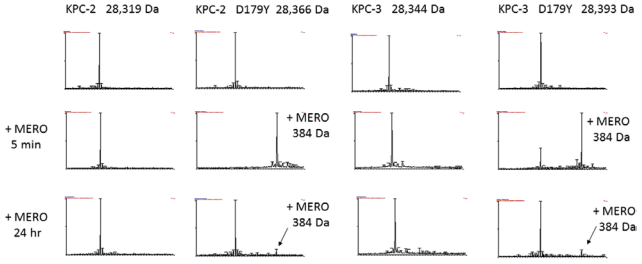
Background. Resistance to CZA is a serious limitation of treatment for KPC bearing Enterobacteriaceae infections. Recently, a single amino acid substitution (D179Y) was described in KPC-2 and KPC-3 bearing CZA-resistant *K. pneumoniae* recovered from patients failing treatment. In class A β-lactamases the D179 residue is located at the neck of the omega loop and is critical for KPC catalytic activity. In attempts to understand the evolution of substrate specificity in KPC-2, the D179Y variant of KPC-2 was shown to be resistant to CZA (ceftazidime forms a long-lived acyl enzyme with in KPC-2), but susceptible to MEM. A similar observation was made in clinical and laboratory-generated *K. pneumoniae* and *E. coli* strains bearing D179Y KPC-3. We were compelled to explore the catalytic mechanisms of susceptibility to MEM of the D179Y variants in KPC-2 vs. KPC-3.

Methods. KPC-2, KPC-3, and D179Y in the respective KPC were cloned into an expression vector and the β-lactamase proteins were purified. 5 mg of each β-lactamase with and without MEM (1:1 molar ratio) was incubated for the time indicated and analyzed using the Quadrupole Time-of-Flight (QTOF) timed mass spectrometry for the reaction intermediates. To assess thermal stability, denaturation melting curves were run for 2 hours using 12 μM β-lactamase.

Results. The D179Y variant forms prolonged acyl-complexes with meropenem in KPC-3 and KPC-2, which can be detected up to 24 hours (Figure 1). This prolonged trapping of meropenem by D179Y variants is not evident with the respective KPCs. Further, the tyrosine substitution at the D179 position (Tm = 48–52°C) destabilizes the KPC β-lactamases (TmKPC-2/3 = 52–56°C).

Conclusion. These data suggest that MEM acts as a covalent β-lactamase inhibitor more than as a substrate for KPC-2 and -3. The mechanistic basis of paradoxical susceptibility to carbapenems provides an impetus to develop better therapeutic approaches to the increasing threat of carbapenem resistance and highlights how the rational design of novel β-lactam/β-lactamase inhibitors must consider mechanistic bases of resistance.

Fig. 1



Disclosures. **All Authors:** No reported Disclosures.

1830. Single-cell Transcriptional Profiling Reveals an Immune Cell State Signature of Bacterial Sepsis

Miguel Reyes, BS[1]; Roby P. Bhattacharyya, MD, PhD[2]; Michael Filbin, MD[2]; Kianna Billman, BS[3]; Thomas Eisenhaure, BS[3]; Deborah T. Hung, MD, PhD[3]; Bruce Levy, MD[4]; Rebecca Baron, MD[4]; Paul Blainey, PhD[3]; Marcia B. Goldberg, MD[2,4] and Nir Hacohen, PhD[2]; [1]Massachusetts Institute of Technology, Cambridge, Massachusetts; [2]Massachusetts General Hospital, Boston, Massachusetts; [3]Broad Institute, Cambridge, Massachusetts; [4]Harvard Medical School, Boston, Massachusetts

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Background. Despite intense efforts to understand the immunopathology of sepsis, no clinically reliable diagnostic biomarkers exist. Multiple whole-blood gene expression studies have sought sepsis-associated molecular signatures, but these have not yet resolved immune phenomena at the cellular level. Using single-cell RNA sequencing (scRNA-Seq) to profile peripheral blood mononuclear cells (PBMCs), we identified a novel cellular state enriched in patients with sepsis.

Methods. We performed scRNA-Seq on PBMCs from 26 patients with sepsis and 47 controls at two hospitals (mean age 57.5 years, SD 16.6; 54% male; 82% white), analyzing >200,000 single cells in total on a 10× Genomics platform. We identified immune cell states by stepwise clustering, first to identify the major immune cell types, then clustering each cell type into substates. Substate abundances were compared between cases and controls using the Wilcoxon rank-sum test.

Results. We identified 18 immune cell substates (Figure 1a), including a novel CD14+ monocyte substate (MS1) that is enriched in patients with sepsis (Figure 1b). The fractional abundance of the MS1 substate alone (ROC AUC 0.88) outperformed published bulk transcriptional signatures in identifying sepsis (AUC 0.68–0.82) across our clinical cohorts. Deconvolution of publicly available bulk transcriptional data to infer the abundance of the MS1 substate externally validated its accuracy in predicting sepsis of various etiologies across diverse geographic locations (Figure 1c), matching the best previously identified bulk signatures. Flow cytometry using cell surface markers unique to MS1 confirmed its marked expansion in sepsis, facilitating quantitation and isolation of this substate for further study.

Conclusion. This study demonstrates the utility of scRNA-Seq in discovering disease-associated cytologic signatures in blood and identifies a cell state signature for sepsis in patients with bacterial infections. This novel monocyte substate matched the performance of the best bulk transcriptional signatures in classifying patients as septic, and pointed to a specific cell state for further molecular and functional characterization of sepsis immunopathogenesis.

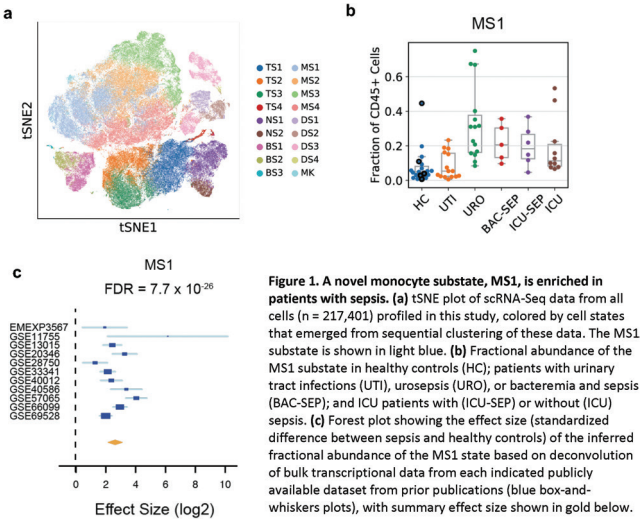

Figure 1. A novel monocyte substate, MS1, is enriched in patients with sepsis. (a) tSNE plot of scRNA-Seq data from all cells (n = 217,401) profiled in this study, colored by cell states that emerged from sequential clustering of these data. The MS1 substate is shown in light blue. **(b)** Fractional abundance of the MS1 substate in healthy controls (HC); patients with urinary tract infections (UTI), urosepsis (URO), or bacteremia and sepsis (BAC-SEP); and ICU patients with (ICU-SEP) or without (ICU) sepsis. **(c)** Forest plot showing the effect size (standardized difference between sepsis and healthy controls) of the inferred fractional abundance of the MS1 state based on deconvolution of bulk transcriptional data from each indicated publicly available dataset from prior publications (blue box-and-whiskers plots), with summary effect size shown in gold below.

Disclosures. **All Authors:** No reported Disclosures.

1831. Machine Learning Approaches to Predicting Resistance in Pseudomonas aeruginosa

David E. Greenberg, MD[1]; Jiwoong Kim, MS[2]; Xiaowei Zhan, PhD[3]; Samuel A. Shelburne, MD, PhD[4]; Samuel A. Shelburne, MD, PhD[4]; Samuel L. Aitken, PharmD[4] and Samuel L. Aitken, PharmD[4]; [1]UT Southwestern Medical Center, Dallas, Texas; [2]UTSouthwestern Medical Center, Dallas, Texas; [3]UT Southwestern, Dallas, Texas; [4]The University of Texas MD Anderson Cancer Center, Houston, Texas

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Background. Multi-drug-resistant (MDR) *P. aeruginosa* (PA) infections continue to cause significant morbidity and mortality in various patient groups including those with malignancies. Predicting antimicrobial resistance (AMR) from whole-genome sequencing data if done rapidly, could aid in providing optimal care to patients.

Methods. To better understand the connections between DNA variation and phenotypic AMR in PA, we developed a new algorithm, variant mapping and prediction of antibiotic resistance (VAMPr), to build association and machine learning prediction models of AMR based on publicly available whole-genome sequencing and antibiotic susceptibility testing (AST) data. A validation cohort of contemporary PA bloodstream isolates was sequenced and AST was performed. Accuracy of predicting AMR for various PA–drug combinations was calculated.

Results. VAMPr was built from 3,393 bacterial isolates (83 PA isolates included) from 9 species that contained AST data for 29 antibiotics. 14,615 variant genotypes were identified within the dataset and 93 association and prediction models were built. 120 PA bloodstream isolates from cancer patients were included for analysis in the validation cohort. ~15% of isolates were carbapenem resistant and ~20% were quinolone resistant. For drug-isolate combinations where >100 isolates were available, machine-learning prediction accuracies ranged from 75.6% (PA and ceftazidime; 90/119 correctly predicted) to 98.1% (PA and amikacin; 105/107 correctly predicted). Machine learning accurately identified known variants that strongly predicted resistance to various antibiotic classes. Examples included specific *gyrA* mutations (T83I; $P < 0.00001$) and quinolone resistance.

Conclusion. Machine learning predicted AMR in *P. aeruginosa* across a number of antibiotics with high accuracy. Given the genomic heterogeneity of PA, increased genomic data for this pathogen will aid in further improving prediction accuracy across all antibiotic classes.

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1832. Development of an Ultrasensitive Field-Applicable Plasmodium falciparum Assay for Malaria Diagnosis and Eradication

Rose Lee, MD, MSPH[1]; Helena De Puig Guixe, PhD[2]; Jeffrey Dvorin, MD PhD[1] and James Collins, PhD[3]; [1]Boston Children's Hospital, Boston, Massachusetts; [2]MIT, Boston, Massachusetts; [3]MIT, Harvard, Cambridge, Massachusetts

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Background. Malaria control and eradication have been hampered by asymptomatic carriage which serves as a parasite reservoir. Low-density infections (< 100 parasites/microliter) frequently fall below the limit of detection (LOD) of microscopy and rapid diagnostic tests (RDT) which are antigen-based tests. Molecular methods such as polymerase chain reaction are capable of higher sensitivity yet remain impractical for resource-limited settings. We describe development of an isothermal assay using the nucleic acid detection platform SHERLOCK (Specific High-Sensitivity Enzymatic Reporter UnLOCKing), which may also be increasingly important as there has been