The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

617.ACUTE MYELOID LEUKEMIAS: BIOMARKERS, MOLECULAR MARKERS AND MINIMAL RESIDUAL DISEASE IN DIAGNOSIS AND PROGNOSIS

Leveraging Custom CRISPR/Cas9 Screens to Identify AraC-Daunorubicin-Etoposide (ADE), Gemtuzumab Ozogamicin (GO), and Bortezomib Response Modulators Association with Clinical Outcomes in Pediatric AML

Nam Nguyen, MS,PharmD [1], Phani Krishna Parcha, PhD [2], Abderrahmane Tagmount, PhD [3], Bailey Gregory [4], Jeffrey E. Rubnitz, MD PhD [5], Raul C. Ribeiro, MD [5], Xueyuan Cao, PhD [6], Todd A. Alonzo, PhD [7], Richard Aplenc, MD PhD [8], Alan S Gamis, MDMPH [9], Todd Cooper, DO [10], E. Anders Kolb, MD [11], Soheil Meshinchi, MDPhD [12], Stanley Pounds, PhD [13], Christopher Vulpe, MDPhD [14], Jatinder K. Lamba, PhD [15]

[1] Department of Pharmacotherapy and Translational Research, University of Florida College of Pharmacy, Gainesville, FL
[2] Department of Pharmacotherapy and Translational Research, College of Pharmacy, University of Florida, Gainesville, FL
[3] University of Florida, Gainesville, FL
[4] University of Florida College of Pharmacy, Gainesville
[5] Department of Oncology, St. Jude Children's Research Hospital, Memphis, TN
[6] Department of Health Promotion and Disease Prevention, University of Tennessee Health Science Center, Memphis, TN
[7] Univ. of Southern California Keck School of Med., Monrovia, CA
[8] Division of Oncology, Children's Hospital of Philadelphia, Philadelphia, PA
[9] Hematology/Oncology/BMT, Children's Mercy Kansas City, Kansas City, MO
[10] Division of Pediatric Hematology, Oncology, Bone Marrow Transplant & Cellular Therapy, Seattle Children's Hospital, Seattle, WA
[11] Leukemia & Lymphoma Society, Bronx, NY
[12] Translational Sciences and Therapeutics, Fred Hutchinson Cancer Research Center, Seattle, WA
[13] Department of Biostatistics, St. Jude Children's Research Hospital, Memphis, TN
[14] University of Florida College of Veterinary Medicine, Gainesville, FL
[15] Department of Pharmacotherapy and Translational Research, University of Florida, Gainesville, FL

Cytarabine, daunorubicin, and etoposide (ADE) remain the standard backbone chemotherapy for pediatric AML. In addition to ADE, gemtuzumab ozogamicin (GO) and bortezomib have been evaluated in the COG's AAML0531 and AAML1031 clinical trials, respectively. Nonetheless, when these experimental agents were combined with ADE, ADE+GO had shown to improve EFS but not OS, while ADE+bortezomib displayed no additional benefit over ADE alone. Thus, treatment options for pediatric AML are still limited, and development of resistance is a major concern, resulting in poor clinical outcomes. To overcome treatment resistance, it is crucial to understand the underlying molecular mechanisms of ADE±GO or bortezomib. Herein, we conducted custom CRISPR/Cas9 synthetic-lethal screens targeting 2440 genes (including AML-relevant genes, pharmacologically relevant genes, and druggable genes), in six AML cell lines with exposure to ara-C, daunorubicin, etoposide, GO and bortezomib followed by integration of the results with outcome data from multiple clinical trials where induction treatment was either standard ADE chemotherapy, ADE+GO or ADE+bortezomib.

Six AML cell lines were transduced with a custom library and puromycin selected. Then pool transduced cell populations were then treated with DMSO, the IC30-IC50 levels of cytarabine, daunorubicin, etoposide, GO, or bortezomib for 7-doubling-times at 500x coverage. All samples were processed with genomic DNA extraction, sgRNAs were amplified by PCR, and were subjected to Illumina NovaSeq 6000 SP 100SR sequencing. The abundance of sgRNA was analyzed using MAGeCK-RRA to estimate the RRA drug response score (drug vs. control conditions at 7-doubling times). Genes with an average RRA score of < -1 across six AML cell lines were defined as drug-resistant genes (negative selection), whereas genes with an average RRA score of > 1 were categorized as sensitive genes (positive selection). Next, we investigated leukemic cell gene expression levels of the drug-resistant and drug-sensitive genes with clinical outcomes.

At the average RRA score cutoff from CRISPR screens, 459 genes met the significance as resistant or sensitive genes to at least one drug from ADE's screens as ADE genes, 244 genes were significant from ADE genes and GO's screen as ADE+GO genes, and 166 genes were significant from ADE genes and bortezomib'screen as ADE+bortezomib genes (**Fig 1a**). ADE

genes were evaluated with clinical outcomes with patients treated with ADE AML02 (n=163), ADE arm of AAML0531 (n=201) and AAML1031 (n=411); while ADE+GO genes were evaluated in ADE+GO arm of AAML0531 (n=205) and ADE+bortezomib genes were evaluated in ADE+bortezomib arm of AAML1031 (n=436). Genes showing drug resistance in CRISPR screens and associated with detrimental outcomes (EFS, OS, or MRD1) at high expression were deemed clinically and biologically important drug genes and vice versa. Our preliminary results showed 21 met the criteria as clinically and biologically important drug-resistant or drug-sensitive genes, where genes were significant in multiple CRISPR screens and multiple pediatric AML cohorts (**Fig 1b**). Six genes were determined to resist multiple drugs, with higher expression associated with worse outcomes across multiple cohorts. Particularly, *ABCC1* encodes the ATP Binding Cassette C1-mediated drug efflux transporter; therefore, knocking out the efflux transporter causing drug resistance, would increase the lethality of AML cells, resulting in poor outcomes in multiple cohorts in patients treated with ADE alone and ADE with GO or bortezomib. Five genes showed significant as sensitive genes for multiple drugs in our CRISPR screens with high expression association with favorable outcomes in multiple cohorts. Interestingly, two genes were identified as bortezomib-sensitive genes with high expression in patients treated with ADE+bortezemib in AAML1031 cohort had better outcomes. Other genes were revealed to be sensitive to one drug but resistant to another, indicating that antagonistic pleiotropy effects between genes and drugs may affect clinical outcomes. Current research focuses on developing a pipeline to predict different drug combination outcomes for prioritizing drug treatments and identifying novel drug targets to overcome drug resistance to ADE+GO or bortezomib.

Disclosures Rubnitz: *Biomea, Inc:* Consultancy.
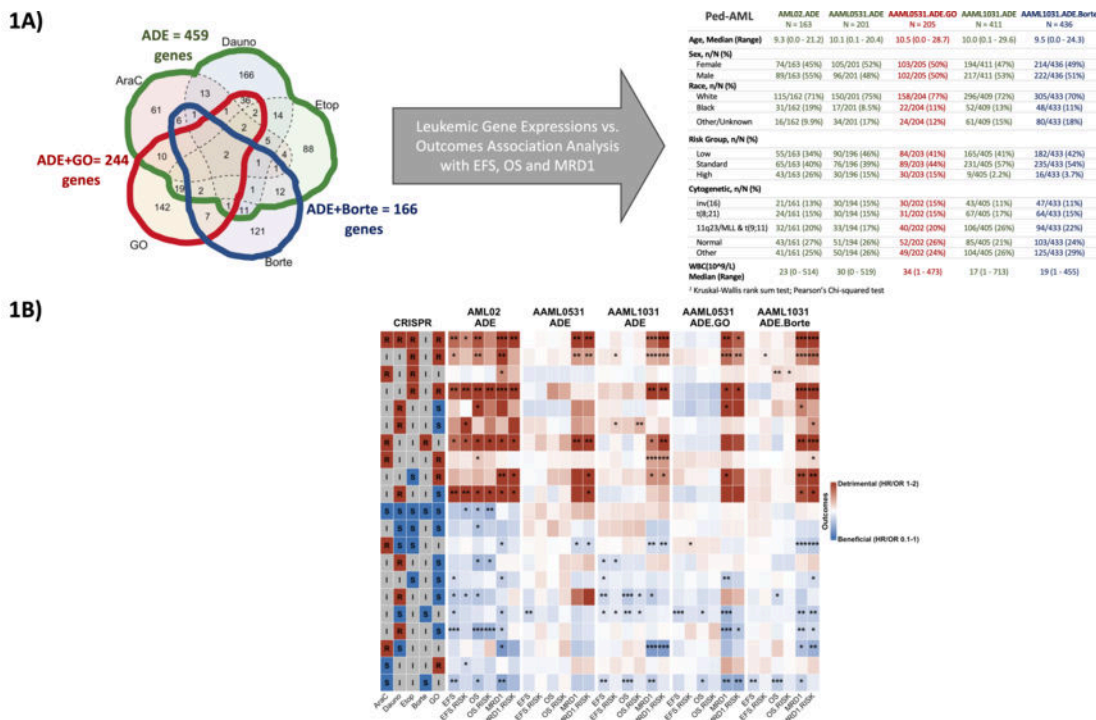
Figure 1: A) Significant genes hit from cytarabine (AraC), daunorubicin (Dauno), etoposide (Etop), gemtuzumab ozogamicin (GO), and bortezomib (Borte) CRISPR screens. Significant genes were classified as either sensitive or resistant in a Venn diagram for three major groups (1) ADE genes (green) included genes that were significant from AraC, Dauno, or Etop CRISPR screens; ADE+GO genes (red) included ADE genes and genes with a significant CRISPR hit for GO; and ADE+Borte genes (blue) included ADE genes and genes with a significant CRISPR hit for bortezomib. ADE genes were analyzed using association tests with AML02, AAML0531, and AAML1031 patients treated with only ADE, ADE+GO genes were analyzed with AAML0531 patients treated with ADE+GO, and ADE+Borte genes were analyzed with AAML1031 patients treated with ADE+Borte. **B)** Heatmap showed significant results in genes with at least 2 CRISPR hits across five drugs and association outcomes results with five mentioned clinical cohorts with EFS, OS, and MRD1 either unadjusted for risk or risk-adjusted (RISK) where each row is a gene with match attribute results from CRISPR and clinical cohort's groups: CRISPR results with R = resistant gene, S = sensitive gene, I = inert gene (non-significant); AML02, AAML0531 and AAML1031 with ADE arms; ADE+GO, and ADE+Borte

Figure 1

https://doi.org/10.1182/blood-2023-178832