An Interferon/JAK1-2/MCL1/BCL2A1/BCL-XL signaling axis causes resistance to Venetoclax in human AML

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Methods

Selective amplification of mutated AML driver genes from scRNA-seq derived cDNA samples

To simultaneously analyze AML-related gene mutations and transcriptomic profiles in single cells, we adapted targeted amplicon sequencing to the Drop-seq platform. We identified AML-related mutations in *NPM1*, *FLT3* and *IDH2* that are adequately expressed and detectable in scRNA AML cDNA and located towards to the 3' end of the transcripts. We designed gene-specific primers that are upstream to the mutation site and compatible with the Drop-seq P5-TSO_Hybrid primer (Macosko et al.) for each mutation. This approach preserved the mutation sequence as well as UMI & cell barcodes in the amplicons.

The Targeted Amplification (Step 1) PCR was performed on 1 ng of high-quality cDNA libraries generated from the scRNA-seq workflow described above using gene-specific primers combined with the Drop-seq P5-TSO_Hybrid primer. After purification using AMPure XP Beads

(Beckman Coulter Life Sciences), the products were subject to a NGS Library Prep (Step 2) PCR to add sample index and complete P7 (Illumina) binding sequences to the libraries. Step 2 PCR was performed on 20 µl of purified products from Step 1 using Index primers (**Supplementary Table 12)** and the Drop-seq P5-TSO_Hybrid primer. After purification using AMPure XP Beads, the NGS-ready libraries were sequenced on an Illumina Miseq sequencer (150 or 300-cycle kit, tailored to the nature of specific mutations).

Computational methods for Drop-seq analysis

Preprocessing of Drop-seq data

Read filtering and alignment. Raw paired-end sequence data were converted to queryname-sorted BAM files using *Picard* v2.6.0 FastqToSam (GitHub repository; http://broadinstitute. github.io/picard/) and processed using *Drop-seq tools* v1.13 from the McCarroll laboratory as described before (1, 2). Briefly, the first read is comprised of, from left to right, a 12-base cell-barcode, an 8-base unique molecular index (UMI), and a 6-base poly-T segment. Read pairs with a base quality of less than 10 for any base of the cell barcode were removed. The second read with read length of 58 nt was trimmed at the 5' end to remove any SMART adaptor sequence, and at the 3' end to remove poly-A tails of 6 consecutive bases or greater. The trimmed reads were then aligned to the GRCh37 reference genome for human samples, or a combined mouse (GRCm38) – human mega-reference using *STAR* v2.5.2a (3) with default settings (3). Reads uniquely mapped to the sense strand of gene exons were recorded and grouped by cell barcode. Throughout this study we used the Ensembl transcriptomic annotation (GRCh37 from Ensembl release 75). We corrected for cell barcodes with synthesis errors as described before (1). We obtained digital gene expression matrixes using the standard Drop-seq pipeline.

Downstream analysis for Drop-seq data

Filtering cells from normal human bone marrows (NBM). Among the 5 sequenced NBM donors from 8 replicates in total, including 3 replicates for NBM2 and 1 replicate for each of the other 4 NBM donors, there were in total 4,822 human NBM cells that passed the cell size factor filter of >500 detected genes and the cell integrity filter of <10% mitochondrial transcripts, with an average of 953 detected genes and 1,895 UMIs. There were in total 39,787 detected genes for the filtered human NBM cells. For each cell, we normalized the raw transcript counts by (1) dividing by the total number of UMIs per cell and (2) multiplying by 10,000 to obtain a transcripts-per-10K measure, and then plus 1 and log-transformed by E=ln(transcripts-per-10K+1). The normalized gene expression matrix was standardized for genes by centering and scaling for each gene using (E-mean(E))/sd(E).

Correcting for batch effect and clustering for NBM. We corrected for batch effect using the following two ways: 1) our in-house within-batch standardization, and, 2) canonical correlation analysis (CCA) embedded in *R* package *Seurat v2.3.4* (4). Firstly, we corrected batch effects using within-batch standardization. For each individual batch of NBM, we normalized for cells by dividing the total UMIs per cell and multiplying by 10k, plus 1 and log-transformed, standardized for genes by subtracting mean and dividing by standard deviation, and then we merged the standardized data from each of the 8 batches of NBM. The rationale behind this is that by centering and scaling each individual batch to the same extent before merging, we are removing the technical artifact for each batch. For the merged data, we selected highly-variables genes (HVG) (N=2,872) using Seurat2 and performed principal component analysis (PCA) on the within-batch standardized gene expression matrix using HVG. We used elbow points in the scree plot and the distribution of eigenvalues to choose the top PCs. We performed t-SNE using the top 7 selected PCs to further reduce the dimensionality to 2. We visualized the eight batches of NBM samples in the top PCs and t-SNE spaces, which showed that all NBM batches had the same centroids and mingled well. To compare with the

batch correction result using CCA, we performed Louvain-jaccard clustering using the top 7 PCs and obtained nine clusters. We ordered the nine clusters by minimizing Euclidean distance of cluster centroids using the Optimal leaf ordering method in R package *seriation*.

Secondly, we corrected batch effects using CCA as an alternative approach. For each individual batch of NBM, we normalized for cells, plus 1 and log-transformed, and then standardized for genes. We selected HVG (N=5,230) that are present in all batches by combining the top 2k highly variable genes from each batch. We performed dimension reduction to obtain canonical correlation vectors (CCs) with maximal correlation to all eight batches of NBM using the HVG by projecting each dataset into maximally correlated subspaces. Then we aligned the top 14 CCs using dynamic time warping to place all 8 batches on a common aligned scale. We performed Louvain-jaccard clustering using top fourteen aligned CCs (ACCs) and obtained 9 clusters. We ordered the nine clusters by minimizing Euclidean distance of cluster centroids using Optimal leaf ordering. We compared the nine ordered clusters for NBM obtained separately from the above two batch correction methods and found that the two sets of clusters are mostly consistent with very few splitting and merging of cells.

Filtering for cells and clustering for AML pairs procured at diagnosis (Dx) and relapse (Rel). For each of the 20 AML cases sequenced at the Dx and Rel stage of the disease we kept good-quality cells with >500 detected genes or <10% mitochondrial transcripts. We then removed genes that were not detected in the filtered cells and further selected HVG as defined in Seurat. We directly merged the gene expression matrix for the Dx and Rel pair for each of the AML cases, separately. For each Dx-Rel pair, we normalized cells by dividing the total UMIs per cell and multiplying by 10k, then plus 1 and log-transformed. We standardized each gene by subtracting the mean and dividing by the standard deviation. We selected ~3k HVG using Seurat and performed PCA on the standardized gene expression matrix using HVG. We selected top PCs based on the elbow position of the scree plot and did t-SNE to further reduce the dimensionality to two. To assess global cellular heterogeneity, we used the Louvain-Jaccard

clustering using the top PCs to identify cell clusters (5). We then ordered the clusters for each Dx-Rel pair by minimizing the Euclidean distance of cluster centroids using Optimal leaf ordering.

Marker selection: Differentially expressed markers for each cluster were obtained by comparing a given cluster with all other clusters using the binomial likelihood test. Selection criteria are (1) at least a 20% difference in detection rate; (2) a minimum of 2-fold higher mean expression level in the cell type compared to all other cell types.

Classifying cell types for single cells and Inferring Lineage. We annotated cell types for each single cell of the normal bone marrow and AML samples using *singleR*. We first computed spearman's rank correlation for the gene expression levels between the test single cell and each sample in the Blueprint and Encode reference dataset using all variable genes in the reference dataset, which are the top genes (200 – 500 genes, depending on the number of cell types used in the analysis) with a higher median expression in the cell type compared to the other cell types in the reference dataset. The score for each cell type was defined as the 80th percentile of the rank correlation between the test cell and all reference samples in the cell type. The cell type with the highest score was assigned as the predicted cell type for that cell. For a cell mapped to multiple cell types, we did an additional fine-tuning step by iteratively rerunning the correlation analysis for only the top cell types using variable genes specific for the top cell types. For each iteration, the cell type with the lowest score or with a margin of >0.05 below the maximal score was removed, and this step was repeated until only two cell types remained. The cell type corresponding to the top score after the last run was assigned to the single cell. We visualized the classification of cell types in a heatmap of the aggregated scores as well as in t-SNE space for each matched pair at Dx and Rel.

To evaluate the cellular heterogeneity within samples, we visualized the proportion of each cell type as a pedigree tree, where each cell type is either an internal node (stem or progenitor cell types at various levels of hematopoietic lineage hierarchy) or a terminal node

(fully differentiated cell types). We identified relapse patterns based on the shift of major cell type proportions between Dx and Rel for each AML case.

To validate our myeloid progenitors cell type annotations obtained from SingleR annotations (v2.4.1), we implemented a comprehensive methodology. R version 4.3.3 was employed in this analysis. We utilized the celldex package to download the built-in reference ENCODE RNA-seq data ('BlueprintEncodeData') as our benchmark for cell type identification. The logcounts were exponentiated to counts for differential gene expression analysis. Using the limma package, we conducted differential gene expression analysis, comparing each cell type against all others to identify the top 200 genes based on fold change for each cell type. For this analysis, we combined HSCs and MPP cell types.

Our scoring scheme involved extracting gene expression data for each gene per cell using Seurat's (v5.1.0) FetchData function and computing the mean expression of the two hundred differentially expressed genes for each respective cell type. We visualized the results using the ggplot2 package (v3.5.1) and performed Wilcox tests to assess the statistical significance of our scores for each cell type.

To further visualize gene expression patterns, we employed Seurat's AddModuleScore function to compute expression scores for the two hundred genes in each cell. These scores were then displayed on a UMAP embedding in the integrated object space using the FeaturePlot function. This robust approach enabled effective validation and visualization of our cell type annotations, ensuring both accuracy and reliability in our analysis.

Tracking mutations in single cells

Tracking gene mutations in single cells. The mutation tracking detailed above preserved the UMIs and cell barcodes, allowing for pooled sequencing, in silico separation of the reads to cell-specific bins and integration with single-cell expression data. We processed the raw paired-end sequence data using *Drop-seq tools* v1.13 (1, 2) with the following modifications. The

second read with 475 bases for FLT3 and 140 bases for NPM1, respectively, was trimmed at the 5' end to remove any SMART adaptor sequence, and at the 3' end to remove poly-A tails of ten consecutive bases or greater. In addition, the second read was trimmed to keep at most 222 bases from 5' end for FLT3 and 120 bases for NPM1, respectively, and we removed additional bases due to low per-base quality scores as assessed by FastQC. The trimmed reads were then aligned to the GRCh37 reference genome using *STAR* v2.7.1a in 2-pass mode (3). For SNVs, we included the WASP-option using *STAR* with input of known SNVs previously detected from our DNA sequencing data to estimate allele-specific expression. We applied WASP (WASP: allele-specific pipeline for unbiased read mapping and molecular QTL discovery) (6) filtering to eliminate mapping bias for SNVs from mapped allele-specific reads. The uniquely mapped reads were deduplicated using the UMIs and demultiplexed using the cell barcodes. Many of the same cells have been analyzed for single-cell expression profiling, allowing us to compare their lineage and the cell type assignment. We kept cell barcodes within the filtered good-quality cells list with classified cell types.

There were three types of mutations in our AML cases – missense (*IDH2, FLT3*), 4nt insertions (*NPM1*), and internal tandem repeats (*FLT3-ITD*), the latter two of which required additional steps to accurately identify mutant cells. To avoid misaligning to adjacent bases for the small indels (4 nt insertion) of NPM1, we parsed the CIGAR string to identify reads containing four nt insertions at the appropriate position with exact match of 5 bases to the reference before and after. Apart from that, to avoid incorrect alignment of the large size of ITDs in FLT3, we created a contig containing the mutant sequence with the 24 or 72 bp repeat, appended it to the transcriptome reference, and realigned the scRNA-seq data to the expanded reference. Transcripts uniquely mapped to the mutant FLT3 sequence were then extracted and kept as transcripts harboring the mutant allele for FLT3 ITD.

We produced the allele counts for the germline allele and the somatic allele, then used the relative abundance of these two alleles to determine the carrier status of the cell. Cells with

transcripts having only reference allele at the mutation site were labeled as “WT only”, while cells with at least one transcript showing mutant calls at the locus of interest were considered as “mutant” cell. We then tracked the mutation status of cells to their assigned cell types. Cells carrying the same somatic variant were considered clonal, thus allowing us to construct the lineage tree linking the cells. We evaluated the confidence of tracking the mutant cells to the stem cells and progenitors such as HSC and MPP by double-checking the number of cells with UMIs harboring the reference or mutant allele.

Whole exome sequencing

Bone marrow aspirate or peripheral blood was obtained from patients following Informed consent at time of diagnosis and at relapse. On the day of collection, the specimen underwent density gradient centrifugation with Ficoll-Paque Plus (GE Healthcare). The interface layer containing mononuclear cells was then washed twice in PBS (HyClone, GE LifeSciences) with 1% FBS (Sigma-Aldrich). Cells were counted and cryopreserved in aliquots in liquid nitrogen in 90% FBS (Sigma-Aldrich) with 10% DMSO (Sigma-Aldrich).

To enrich myeloblasts in the specimens, thawed mononuclear cells underwent MACS cell separation using Miltenyi columns and microbeads (Miltenyi Biotec) per manufacturer’s protocol. Negative selection was performed to remove B lymphocytes, T lymphocytes, and erythrocytes/erythroid precursors using anti-CD19, anti-CD3, and anti-Glycophorin A microbeads, respectively. Marked enrichment of myeloblasts in the column effluent was confirmed via light microscopy. The enriched myeloblast fraction was digested overnight in lysis buffer containing proteinase K. Genomic DNA was precipitated with ammonium acetate, washed twice in ice-cold 75% ethanol, and re-dissolved in TE buffer for storage at -20°C. Genomic DNA yield was quantified using Nanodrop (Nanodrop One, Thermo Scientific).
The genomic DNA (myeloblast DNA from Dx and Re and paired buccal DNA) was submitted to the Advanced Genomics Sequencing Core at the University of Michigan for whole exome

sequencing (WES) at ~100x coverage. Following standard QC, genomic DNA was processed using the IDT Exome Research Panel kit followed by sequencing on a high-output S4 flowcell of the NovaSeq 6000 (Illumina) platform. The bioinformatics analysis was performed by the University of Michigan Bioinformatics Core (Weisheng Wu, PhD and Karthik Padmanabhan, PhD) using established WES pipelines (7). The WES Sequence files have been deposited in dbGAP under submission # phs002426.v1.p1.

Survival Analysis

To analyze the impact of the DE genes on survival rates of patients with AML, we downloaded the AML TCGA study (referred to as TCGA herein) (8). The TCGA RNAseq datasets and associated clinical datasets were downloaded from https://gdc.cancer.gov/about-data/publications/laml_2012. Survival analysis was performed using TCGA RNA seq and available patient survival data by dividing the AML cohort into expression level quartiles for individual genes (q1 = low expression, q4 = high expression) and by employing the KM method. We conducted a log-rank test to compare survival curves and corrected p-values by FDR. The TCGA study compromised 179 samples with RNA seq data of which 169 have paired survival data.

Supplementary Figure and Table Legends

Supplementary Figure S1: SingleR annotation validation in myeloid progenitor cells.
(A) Each facet shows the expression of a cell-type signature (Signature expression) from sorted CMP, GMP, HSC_MPP, and MEP cells (Y-axis, facet labels), across SingleR annotated single-cells (individual boxes). (B) Same data as in A, visualized at the single-cell level depicting the similarity of cells with high respective signature expression levels. (C) Integrated UMAP with cells colored by SingleR-annotated cell types, highlighting regions enriched in cells annotated with a specific type.

Supplementary Figure S2: Cell type assignment in pooled normal human bone marrow based on single cell gene expression correlation analyses. Left: t-SNE displays of cell clusters of pooled scRNA-seq data from five normal human bone marrows and cell type assignment. Cell type assignment as described in the *methods* using SingleR and sorted progenitor fractions as in Blueprint and ENCODE data. Right: Heatmap of statistical gene expression correlation analyses and cell type assignment. Red: high identity; Blue: low identity. The t-SNE clusters assignment for individual cells is at the top and the color code is on the right.

Supplementary Figure S3: Examples of cell type assignment of two mature monoblast/ monocyte-rich AML cases using statistical gene expression correlation analyses and resulting heatmaps. The stage of the disease is color-coded at the top. A color-coded cluster assignment is provided. Red: high identity; Blue: low identity. The t-SNE cluster assignment for individual cells is at the top and the color code is on the right.

Supplementary Figure S4: Expression of inflammation associated genes in AML TCGA RNA sequencing data grouped by FAB types M0-M5.

Supplementary Figure S5: Selected target gene expression in primary human CD34+ cells after IFN stimulation (qPCR). Purified human CD34+ cells were treated for 24h with 1,000 I.U. IFNα or IFNγ in parallel to untreated control samples, respectively. Log_2 fold change of target gene expression of IFN stimulation normalized to internal control genes (GAPDH and PGK1) are displayed as mean values ± SEM. Stars indicate the results of a paired two-tailed t-test (*p<0.05, **p<0.01, ***p<0.001).

Supplementary Figure S6: Poor survival of AML patients expressing IFN-induced genes (KM-plots). RNA-seq data for individual genes from the AML TCGA dataset were partitioned into quartiles (q1 lowest, q4 highest) and the survival of AML patients in the quartiles was computed using the KM method. Very adverse day 1,000 survival for patients with the highest gene expression (q4, purple) is evident. log-rank test FDR corrected.

Supplementary Figure S7: Analysis of LINE/ERV/IFN expression in fourteen primary human AML. qPCR data for L1PA10, MSTB2, HERV9 and MER34-1 are shown as examples for LINE and ERV expression in AML, respectively. Displayed are mean values ± SEM of target gene expression normalized to internal control genes (GAPDH and PGK1) in different AML cases with WT or MUT *TP53* and/or *TET2*. IFNB and IFNG mRNA expression was measured for the same AML cases. Stars indicate the results of an unpaired two-tailed t-test (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001).

Supplementary Figure S8: High correlation of AUC and IC50 values as read-outs for Venetoclax-mediated cell death in AML. (A) Correlation of IC50 values with AUC for 19 primary human AML cases. Linear regression analysis is shown in blue. **(B)** AUC in AML FAB M0-M2 versus M4-M5 at baseline (AML control) and after IFN treatment.

Supplementary Figure S9: MCL1, BCL2A1, BCL-XL and BCL2 protein expression in primary human AML following IFN stimulation identify patterns in relation to Venetoclax dose-responses. Dose-response curves: AML cases were pre-stimulated for 4h with 1,000 I.U. IFNα and IFNγ each in parallel to untreated control AML samples. Samples were then incubated with varying concentrations of Venetoclax for 24h while IFN was still present. Immunoblots: Primary AMLs were treated for 24h, 48h and 72h with 1,000 I.U. IFNα and IFNγ in parallel to untreated control AML samples and data for various BCL2 family members are shown. Stars indicate the results of a two-way ANOVA analysis (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001). Pattern 2: Venetoclax-sensitive AML with absent BCL2A1 and low MCL1 expression. Pattern 3: Venetoclax sensitive AML with low BCL2A1 and MCL1 expression.

Supplementary Figure S10: MCL1, BCL2A1, BCL-XL and BCL2 protein expression in primary human AML following IFN stimulation identify patterns in relation to Venetoclax dose-responses. Dose-response curves: AML cases were pre-stimulated for 4h with 1,000 I.U. IFNα and IFNγ each in parallel to untreated control AML samples. Samples were then incubated with varying concentrations of Venetoclax for 24h while IFN was still present. Immunoblots: Primary AMLs were treated for 24h, 48h and 72h with 1,000 I.U. IFNα and IFNγ in parallel to untreated control AML samples and data for various BCL2 family members are shown. Stars indicate the results of a two-way ANOVA analysis (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001). Pattern 5: Venetoclax-resistant AML with high BCL2A1 and moderate MCL1 expression. Pattern 6: Venetoclax-resistant AML with high MCL1 and IFN-inducible BCL-XL expression.

Supplementary Figure S11: Venetoclax dose-response curves for three additional primary human AML without immunoblot data. AML cases were pre-stimulated for 4h with 1,000 I.U. IFNα and IFNγ each in parallel to untreated control AML samples. Samples were then incubated

with varying concentrations of Venetoclax for 24h while IFN was still present. Three examples for the shift in cell viability of IFN treated samples (blue) versus untreated AML (black) are displayed. Due to low cell amounts these AML cases are shown without immunoblot data. Stars indicate the results of a two-way ANOVA analysis (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001).

Supplementary Figure S12: Venetoclax dose-response curves for AML cell lines and primary human AML treated with IFNα and IFNγ, or IL1β or combined. AML cell lines and primary AML cases were pre-stimulated for 4h with 1,000 I.U. IFNα and IFNγ, or IL1β or combined each in parallel to untreated control samples. Samples were then incubated with varying concentrations of Venetoclax for 24h while the stimulants were still present. The shift in cell viability of IFN treated samples (dark blue), or IFN + IL1β treated samples (light blue), or IL1β treated samples (green) versus untreated AML (black) are displayed.

Supplementary Figure S13: MCL1, BCL2A1, BCL-XL and BCL2 mRNA expression in 14 primary human AML after IFN stimulation (qPCR). Primary AML were treated for 4h with 1,000 I.U. IFNα and IFNγ each in parallel to untreated control AML samples. qPCR data of target gene expression normalized to internal control genes (GAPDH and PGK1) are displayed as mean values ± SD of quadruplicates on a $\log_2$ y-axis scale.

Supplementary Figure S14: Correlation of normalized ratios of (BCL2A1+MCL1):BCL2 or (BCL2A1+MCL1+BCL-XL):BCL2 mRNA expression with Venetoclax-induced responses in primary human AML (Area Under the Curves; AUCs). BCL2 family mRNA expression was normalized to internal control genes (GAPDH and PGK1). mRNA scores of different ratios have been calculated by dividing the normalized expression value for a target gene of a sample by the mean expression value of all samples for the same gene followed by the calculation of the score of interest. Correlation of mRNA ratios with Venetoclax responses at baseline (top) and after IFN-

treatment (bottom) are displayed and linear regression analysis is shown in blue. Please note the log_{10} x-axis scale used for Venetoclax AUC values.

Supplementary Figure S15: Analysis of LINE/ERV/IFN expression in seven primary human AML. qPCR data for L1P1, L1PBa1, HUERS and ERV-Fb1 are displayed as mean values ± SEM of target gene expression normalized to internal control genes (GAPDH and PGK1) in sensitive/ intermediate and resistant AML cases. IFNB mRNA expression was measured for the same AML cases and correlated with L1P1 and HUERS expression as one example for retroelements and ERVs, respectively. Linear regression analysis is shown in blue. Stars indicate the results of an unpaired two-tailed t-test (ns = p>0.05, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001).

Supplementary Figure S16: Analysis of IFN response gene expression in seven primary human AML. qPCR data for MX1, IFI6 and XAF1 are displayed as mean values ± SEM of target gene expression normalized to internal control genes (GAPDH and PGK1) in sensitive/ intermediate and resistant AML cases. IFNB mRNA expression was measured for the same AML cases and correlated with the expression of IFN response genes. Linear regression analysis is shown in blue. Stars indicate the results of an unpaired two-tailed t-test (ns = p>0.05, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001).

Supplementary Figure S17: MCL1, BCL2A1, BCL-XL, BCL2 and P-/STAT1 protein expression in primary human AML samples at baseline and following IFN stimulation. Immunoblot data for human AML that were sensitive/ intermediate clinically to Decitabine/Venetoclax therapy are displayed. The corresponding post HMA/Ven cycle 1-2 ddPCR MRD VAF measurements (upper table right) and their genomic DNA mutations (lower table right) are displayed. Primary AMLs were treated for 24h, 48h and 72h with 1,000 I.U. IFNα and IFNγ in parallel to untreated control AML samples and immunoblot data for various BCL2 family members

and p-Y701-STAT1/STAT1 are shown. BM – bone marrow, PB – peripheral blood, MRD – minimal residual disease

Supplementary Figure S18: MCL1, BCL2A1, BCL-XL, BCL2 and P-/STAT1 protein expression in primary human AML samples at baseline and following IFN stimulation. Immunoblot data for human AML that were resistant clinically to Decitabine/Venetoclax therapy are displayed. The corresponding post HMA/Ven cycle 1-2 ddPCR MRD VAF measurements (upper table right) and their genomic DNA mutations (lower table right) are displayed. Primary AMLs were treated for 24h, 48h and 72h with 1,000 I.U. IFNα and IFNγ in parallel to untreated control AML samples and immunoblot data for various BCL2 family members and p-Y701-STAT1/STAT1 are shown. BM – bone marrow, PB – peripheral blood, MRD – minimal residual disease

Supplementary Figure S19: Analysis of BCL2 family member expression in primary human AML. Primary AML cases were grouped into sensitive/ intermediate and resistant to Decitabine/Venetoclax therapy based on post HMA/Ven cycle 1-2 ddPCR MRD VAF measurements. **(A)** Plots of various BCL2 family members based on scores of protein expression at baseline and after IFN treatment for 24h, 48h and 72h with 1,000 I.U. IFNα and IFNγ (Supplementary Table 8). Mean values ± SEM of AML cases within one group were calculated for all BCL2 family members and analyzed using an unpaired two-tailed t-test. Based on individual protein semiquantitative assessment coded as 0 (absent expression), 1 (low), 2 (intermediate) and 3 (high). **(B)** qPCR data for BCL2 family members are displayed as mean values ± SEM of target gene expression normalized to internal control genes (GAPDH and PGK1) in sensitive/ intermediate and resistant AML cases. Stars indicate the results of an unpaired two-tailed t-test (ns = $p>0.05$, *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$).

Supplementary Figure S20: Plots of MCL1, BCL2A1, BCL-XL and BCL2 protein expression in primary human AML at baseline and following IFN stimulation. Individual plots of various BCL2 family members based on scores of protein expression at baseline and after IFN treatment for 24h, 48h and 72h with 1,000 I.U. IFNα and IFNγ (Supplementary Table 8). Primary AML cases were grouped into sensitive/ intermediate and resistant to Decitabine/Venetoclax therapy based on post HMA/Ven cycle 1-2 ddPCR MRD VAF measurements. Mean values ± SEM of AML cases within one group were calculated for all BCL2 family members and for the sum of MCL1+BCL2A1+BCL-XL and are displayed as summary plots (last plots right). Based on individual protein semiquantitative assessment coded as 0 (absent expression), 1 (low), 2 (intermediate) and 3 (high).

Supplementary Figure S21: JAK inhibition reverses resistance induction to Venetoclax in IFN-treated AML cells. Four primary human AML were pre-treated with 500 nM or 2 μM of Ruxolitinib or Baricitinib, respectively, followed by 4h treatment with 1,000 I.U. IFNα and IFNγ each in parallel to untreated controls. Samples were incubated with varying concentrations of Venetoclax for 24h while IFN and JAK-inhibitors were still present. Dose-response curves are shown and color coded. Immunoblotting results for BCL2 family members and p-Y701-STAT1/STAT1 with and without IFN treatment +/- JAK-inhibitors. Stars indicate the results of a two-way ANOVA analysis (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001).

Supplementary Table S1: Detailed clinical and other data for 20 AML cases analyzed via scRNA-seq.

Supplementary Table S2: Summary of QC metrics for scRNA-seq of 20 paired AML.

Supplementary Table S3: Results from WES of paired AML samples (Diagnosis and Relapse).

Supplementary Table S4: Gene membership in inflammatory signatures in this study compared with GSEA hallmark gene sets.

Supplementary Table S5: Gene membership in inflammatory signatures in this study compared with signatures described by Lasry et al, 2023 (9) and the parsimonious 47 gene signature by Wang et al, 2024 (10).

Supplementary Table S6: Characteristics, experimental and clinical data of primary human AML cases used for Venetoclax dose-response and LINE / ERV expression studies.

Supplementary Table S7: Characteristics of patients treated with Decitabine/Venetoclax clinically. Samples used for immunoblotting, ddPCR and q-RT-PCR.

Supplementary Table S8: Scores of MCL1, BCL2A1, BCL-XL and BCL2 protein expression from immunoblots of primary human AML at baseline and following IFN stimulation.

Supplementary Table S9: Reagent and Supplier information.

Supplementary Table S10: Primer sequences used in Q-PCR (ERV and LINE detection)

Supplementary Table S11: Statistical methods used itemized by figures.

Supplementary Table S12: Primer sequences used in single cell mutation tracking

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