Supplemental Methods

In vitro cell cytotoxicity assays

Decitabine (Selleck Chem) was dissolved in dimethylsulfoxide and stored at – 80°C at a concentration of 50 mM. Doxorubicin (Sigma Aldrich) was dissolved in dimethylsulfoxide and stored at – 20°C at a concentration of 4.25 mM. Drugs were thawed immediately before use and appropriate dilutions were made in PBS. Resazurin (R&D Systems) - based viability assays were used to determine the 50% inhibitory concentration (IC50) values of decitabine (daily dosing for five days) and doxorubicin (first-day dosing, two-day treatment). Apoptotic and total viable cell fractions were quantified based on Annexin V/PI staining followed by flow cytometry.

RNA sequencing and bioinformatics analysis

Total RNA was isolated from uncultured PBMCs of ATLL patients and healthy donors using RNA purelink mini kit (Invitrogen), and the integrity of the total RNA was validated using the Agilent 2100 Bioanalyzer. Libraries were generated following the Illumina protocol for preparing samples for sequencing of mRNA as described previously.[1] Paired-end sequencing was performed on the Illumina NextSeq 500 platform. Sequence reads from RNA-sequencing were aligned to genomic sequences. FPKM (Fragments Per Kilobase of transcript per Million mapped reads) values for the North American samples were obtained from the HTSEQ[2] gene counts of the RNAseq data aligned to the HG19 reference with Tophat[3] and normalized with the R package[4] DESeq2.[5] Gene expression analysis was performed using Cufflinks. To compare the 9 North American RNA-seq profiles to those reported for a Japanese ATLL cohort (n=57, European Genome-phenome Archive, EGAS00001001296),[6] the Ward's method of hierarchical clustering was applied and the FPKM values were used to calculate distance metrics with the distance between intra-cluster samples defined as 1 minus their correlation. GSEA (Gene Set Enrichment Analysis) was used to compare the RNA expression of North American samples to the oncogenic(C6) gene sets of the publicly available MSigDB database to determine if there was a statistically significant concordance in the groupwise differences of the North American ATLL dataset and the gene signatures they are measured against.[7,8] The functional pathways were generated through the use of IPA (QIAGEN Inc., https://www.qiagenbioinformatics.com/products/ingenuity-pathway-analysis).[9]

Analysis of drug interaction

The effect of the combination of decitabine and doxorubicin was evaluated according to the method of Kern et al.[10] The IC10 and IC20 concentrations of decitabine were used along with various concentrations of doxorubicin to assess for synergy in 3 samples. In brief, the expected cell survival (Sexp, defined as the product of the survival observed with drug A alone and the survival observed with drug B alone) and the observed cell survival (Sobs) for the combination of A and B were used to construct an index (R): R = Sexp/Sobs. An R index of 1 (additive effect) or lower indicated the absence of synergism. Synergism was defined as any value of R greater than unity.[11]

Mass spectrometry

Six samples (4 cell lines and 2 primary samples) were treated with decitabine for 48 hours. DNA was prepared from treated and untreated samples and using the PureLink® Genomic DNA mini Kits (Invitrogen). Quantity and quality were assessed with a Nanodrop 1000 instrument (Thermo Scientific). DNA was then degraded using DNA Degradase Plus (ZymoResearch) before submitting to ZymoResearch where an SRM-based mass spectrometry assay was used to quantify 5-hydroxymethyl-

2'-deoxycytidine (5HmdC) and 5-methyl-2'-deoxycytidine (5mdC). The assay was designed to measure 5HmdC concentrations and 5mdC concentrations as a percentage of 2'-deoxyguanosine (dG) (e.g. – [5HmdC]/[dG] and [5mdC]/[dG]). Samples were analyzed in triplicate.

Western Blot

Whole cell lysates were prepared with Laemmli Sample Buffer, and Western blotting was performed as described previously using gradient SDS-PAGE gel.[12] Antibodies against GAPDH (FL-355) (SC-25778) was purchased from Santa Cruz Biotechnology. Antibodies against total p53 (#9282), acetyl p53 lys382 (#2525) and p300 (#70088) were purchased from Cell Signaling Technology (Supplemental Table S15).

Proviral load analysis

HTLV-1 proviral load of patient samples was measured by Digital droplet PCR method as described previously.[13] In brief, DNA from PBMC was isolated and PCR was performed using Bio-Rad 2x ddPCR supermix for probes, with two sets of primers and probes specific for the HTLV- 1 provirus (HTLV-1 tax) and human gene encoding the RNase P enzyme (RPP 30). Droplets were prepared using QX 200 droplet generator and PCR was performed in a 96 well plate and analyzed on a QX 200 droplet reader. The PVL was calculated as: [(copy number of HTLV-tax)/(copy number of RPP 30 / 2)]* 100. All samples were run in duplicate and the PVL is the average of the two measurements (Supplemental Table 1).

References

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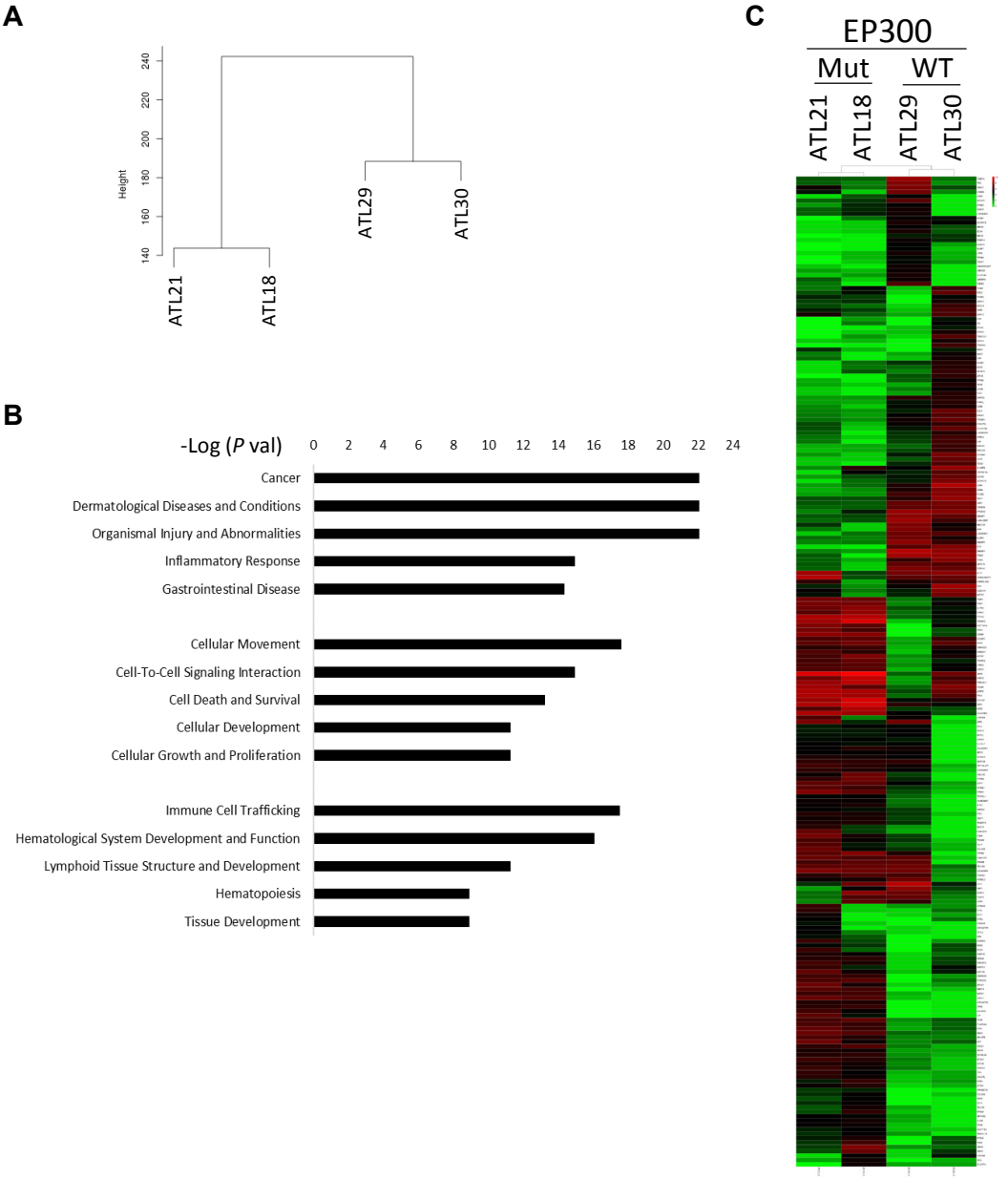
Supplementary Figure 1. (related to Figure 2)
Clustering analysis (**A**), functional pathways enriched in differentially expressed genes (**B**), and heatmap of differentially expressed genes (**C**) between cultured ATLL cells of EP300 Mut and WT status.



Supplementary Figure 2. (related to Figure 3)
Clustering analysis (**A**), volcano plot (**B**), and heatmap of differentially expressed genes (**C**) between normal control PBMCs (n=4) and PBMCs from ATLL patients (n=9).