Supplemental Figure 1: Supplemental information related to Figure 1. (A) Flow cytometry analyses on NK cell populations (enriched by antibody-mediated lineage-negative selection) derived from additional patients with advanced ENKTL derived from PB or BM. (B) Immunofluorescence staining of NKp80 in primary tumor tissue from Stage 4- and 5-like ENKTL along with CD56 and DAPI as controls. Higher magnification is displayed in the right panels. (C) Average DNA methylation levels of CpGs located less than +/-50 bp to T-box sequence motifs within the NKDI developmental methylation signature.
Supplemental Figure 2: Mutations detected by targeted sequencing panel of 164 commonly mutated genes in hematological malignancies (HemeSTAMP) in samples with available material. Mutated genes are arranged by functional category. Individual ENKTL patient samples are listed below and those used for PDX models are indicated (*).
Supplemental Figure 3: Copy number heatmaps of two novel recurrent deletions on chromosomes 1p and Xp. Enlargement of the minimally-deleted regions reveals the involvement of a limited number of genes, including ARID1A (A) and BCOR (B) deleted on chromosomes 1p and Xp, respectively. Quantification of genomic copy number was derived from Illumina 850K array data.
Supplemental Figure 4: Analysis of DNA hypomethylation in ENKTL. (A) The location of hypomethylated CpGs (<20% versus NKDIs) relative to gene segment (left panel) and CpG islands (right panel), separated by ENKTL subgroup. Changes that occurred during NK development are also indicated for reference. (B) Enrichment of hypomethylated CpGs in chromatin states of NK, T, and HSC cells. Fold enrichment/depletion of overlapping differential methylation is indicated on the x-axis and the bubble size represents the proportion of the total CpGs either enriched or depleted (prevalence). (C) Enrichment of transcription factor motifs in hypomethylated regions in ENKTL proximal (+/-100 bp) to hypomethylated CpGs. Bubble size represents P-value.
Supplemental Figure 5: Supplemental information related to Figure 3. (A) The proportion of CpG islands that are hypermethylated in ENKTL and NKLGL patients compared to EBV+ CIMP gastric adenocarcinoma patients. Statistical significance was assessed by t-test. (B) Enrichment of hypomethylated CpGs in regions with specific histone marks in NK cells isolated from cord or peripheral blood (n=2 samples each). Fold enrichment/depletion of overlapping differential methylation is indicated on the x-axis and the bubble size represents the proportion of the total CpGs either enriched or depleted (prevalence). (C) Promoter DNA methylation levels of selected genes showing additional hypermethylation in ENKTL patients displaying gain of chromosome 7q. Statistical significance was assessed by t-test. (D) Scatterplot displaying the relationship between the total number of copy-number alterations and the percent of hypermethylated CpG islands across all samples. Primary ENKTL, NKLGL and cell line samples are indicated separately. (E) Proportional Venn diagram showing the overlap of genes with hypermethylated promoter CpG islands among three ENKTL samples with RNA-sequencing from fresh (non-FFPE-derived) material. Pie chart below indicated the proportion of genes hypermethylated among the three patients that were expressed in either ENKTL or stage 4b NKDIs.
Supplemental Figure 6: Additional information related to ENKTL PDX models. (A) The percent of CpG islands that were found to be hypermethylated relative to stage 4b NKDIs in ENKTL-PDX models. (B) Flow cytometry analysis of DFTL-85005 showing dim CD16 expression and the lack of NKp80 expression. (C) Representative image of spleens derived from non-engrafted (top) and ENKTL1-PDX-engrafted (bottom) NSG mice. (D) Blood smear from ENKTL1-PDX-engrafted NSG mouse showing representative circulating lymphoma cells. x600, Wright-Giemsa. (E,F) Representative histological sections of liver tissue derived from an ENKTL-engrafted NSG mouse. The sections were stained with human CD45 (E) or EBV-encoded RNA (EBER) (F). x200 for E and F. (G) Stability of DNA methylation patterns in ENKTL PDX mice. The total number of differentially-methylated CpGs (+/-20% change compared to Stage 4a NKDIs) was determined in the primary patient-derived FACS-sorted ENKTL1 cells and also in ENKTL1-PDX cells collected at moribund from spleen and bone marrow. DNA hypermethylation remained largely stable (approximate gain of additional 5% of CpGs and loss of 2.5%). Existing DNA hypomethylation was stable (approximate loss of 2.5% of CpGs). An additional 20% hypomethylation was observed through one passage in mice. (H) Box and whisker plot of DNA methylation change in ex vivo ENKTL1-PDX samples after 5-aza treatment separating CpGs into those that were hypermethylated (>50%) in the primary patient ENKTL1 sample versus NKDIs, and CpGs that were not hypermethylated (≤10% gain) in the primary patient sample. Boxes represent interquartile range, line represents median value, and whiskers represent the total range of values.
Supplemental Figure 7: Supplemental information related to EZH2 inhibition in ENKTL. (A) EZH2 expression levels in ENKTL cells used for the PDX models and normal stage 4 NKDIs. Levels derived from RNA-sequencing of freshly isolated and FACS-purified cells. (B) Fold enrichment of the 10,000 most hypermethylated CpGs in ENKTL cells used for the PDX models in H3K27me3-marked regions in NK cells. Adjusted enrichment P-value was <1e-300 for both models. (C) In vitro 6 day treatment of ex vivo ENKTL1-PDX cells with the EZH2 inhibitor (EZH2i), tazemetostat alone and in combination with 1.0 uM 5-aza. EZH2 inhibition fails to induce KIR expression alone or synergize with 5-aza. (D) Survival of ENKTL1-PDX mice following treatment with tazemetostat, alone and in combination with 5-aza versus vehicle-treated mice. Mice were treated continuously starting at day 7 with tazemetostat and two weeks on drug with one week drug-free intervals for 5-aza.
Supplemental Figure 8: Individual KIR gene expression during normal NK development in tonsil and blood, demonstrating the majority of KIR upregulation during differentiation occurs between NKDI stages 4a/b and 5. Expression was determined using RNA-sequencing.