Supplementary Methods

Isolation and DNA extraction of bone marrow cell populations

Bone marrow mononuclear cells (BMMCs) were isolated at screening and within 96 hours prior to Day 1 of Cycles 3, 5 and 7, and were cryopreserved initially at -80C for at least 4 hours then stored or transferred on dry ice for subsequent processing. To isolate $CD3^+$ cells (T cells), CD34+ cells, and the CD3- and CD34-depleted population, we performed sequential magnetic bead-based separation with Human CD3 Microbeads (Miltenyi Biotec, 130-050-101) and CD34 UltraPure Microbead kit (Miltenyi Biotec, 130-100-453) using MACS LS columns (Miltenyi Biotec, 130-042-401) to isolate $CD3^+$ and $CD34^+$ cells respectively. We followed the manufacturer's recommendations with one exception: we performed one additional column purification to increase the purity of the cell population of interest. The flow-through ($CD3^-$ and $CD34^-$depleted population) cells and $CD3^+$ cells were pelleted and processed for genomic DNA extraction through the phenol-chloroform:isoamyl alcohol (Invitrogen, 15-593-031) and ethanol precipitation method.

Targeted DNA sequencing

Targeted sequencing of DNA was performed using hybridization capture with Twist BioScience reagents. DNA from BMMCs (depleted of CD3+ and CD34+ cells) and BM CD3+ T cells was included from patients who had sufficient amount of DNA and had received at least 1 cycle of treatment. First, whole genome libraries were prepared from 100 ng genomic DNA with the Twist Library Preparation EF Kit 1.0 using Universal Adapter ligation and 7 cycles of PCR for Unique Dual Indexing and amplification. Libraries were quantified with the Qubit dsDNA BR kit and pooled by mass in multiplex. Next, target enrichment was performed with a custom Twist panel consisting of 145 genes related to myeloid cancer. The panel capture probe design was performed by a Twist proprietary algorithm with 1x tiling resulting in 6,190 probes synthesized as 120-bp dsDNA baits. Capture pools with enriched libraries were sequenced on an Illumina NextSeq 500 instrument with a PE75 read configuration to obtain an average target coverage of 600x-1000x per sample.

We focused on the 40 genes most commonly mutated in MDS for analysis (Supplementary Table 1). The lower level of detection in the BMMCs was set to a variant allele frequency (VAF) of 2% for all genes, except TP53, for which we used a cut-off of 1%. In the separated CD3+ T cell population we confirmed 90% or greater purity in selected cases. To avoid any false positivity due to contamination by myeloid cells after cell sorting, we only report mutations with a VAF$\geq$ 10% in the sorted T-cells.

Peripheral blood mononuclear cell (PBMC) staining and flow cytometry analysis

PBMC samples were analyzed using multicolor flow cytometry as previously described[12] to evaluate immune activation and correlation of treatment outcome with established T cell and NK cell phenotype and activation panels. Phenotype panels of T and NK cells consist of fluorophore-labelled antibodies recognizing cell surface markers of CD4+, CD8+, and NK cells (Panel 1 and Panel 2), and the T cell activation panel included fluorophore-labelled antibodies specific to cell surface and intracellular T cell activation markers (Panel 3). Based on sample availability, we evaluated up to three different time points from each of 21 patients: pre-treatment (defined as "PRE"), during the second treatment cycle (C2D1 or C2D8; defined as "EARLY"), and after the third or fourth treatment cycle (C4D1, C4D8 or C5D1; defined as "LATE"). PBMCs were thawed and washed twice in RPMI + 10% fetal calf serum (FCS) supplemented with 100 ug/mL DNase and resuspended in flow cytometry buffer (PBS+2% FCS). For each time point 1–2 × 10^6 cells were incubated for 30 minutes at 4˚C with respective flow cytometry panels together with a dead cell marker (LIVE/DEAD Fixable Near-IR; Invitrogen L10119) (final dilution 1/1000) in a 96-well plate (100 µL reaction volume). Cells

were then washed twice in FACS buffer (PBS+2% FCS) and fixed in 1% paraformaldehyde (PFA). Cells stained with T cell activation panel were first incubated with cell surface marker-specific antibodies and processed for intracellular marker staining using Foxp3/Transcription Factor Staining reagents (eBioscience, 00-5523-00) and fixed according to the supplier's instructions. All cells were acquired manually or using an automated 96-well plate reader on a flow cytometer (Fortessa, Becton Dickinson). Data were analyzed using FlowJo analysis software (FlowJo LLC). Frequencies of individual cell population were determined using the gating scheme shown in Supplementary Figure 1A.