(higher CT value), whereas the presence of an increased number of symptoms (≥3) was associated with a higher viral load. Lower viral load at initial surveillance testing correlated with an increased likelihood of being asymptomatic initially and remaining asymptomatic throughout the viral shedding episode. These results provide initial support for the use of viral load as a clinical endpoint in interventional trials.

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Therapeutic Drug Monitoring of Tacrolimus in Allogeneic Hematopoietic Transplant Patients in a Single Oncology Center

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Background: Tacrolimus is extensively employed in allogeneic hematopoietic cell transplant (alloHCT) for the prevention of graft-versus-host disease (GvHD). Renal and hepatic toxicities have been associated with increased blood concentrations post alloHCT. Due to the narrow therapeutic index of tacrolimus and variability in blood concentrations, drug monitoring is essential. Difficulties of therapeutic monitoring include dosage forms limited to capsules, drug interactions, and changes in organ function.

Methods: All patients were initiated on 0.005 mg/kg (ideal body weight) intravenously every 12 hours on Day -1 through a central venous catheter. Tacrolimus trough levels were drawn twice weekly by peripheral vein (Tuesday and Fridays). Once levels were stable in the therapeutic range (5 to 15 ng/ml—desired target between 5 to 10 ng/ml), doses were converted to the solution formulation of 1 mg/ml (Elefante et al BBMT 2006). Data were collected from January 2013 to March 2014.

Results: Eighty-seven patients were admitted for alloHCT during this time period. Of these 87 patients, 527 tacrolimus adjustments were performed. These adjustments resulted in 12 out of greater than 10,000 levels drawn to be outside the upper recommended range of 15 ng/ml.

Conclusions: Tacrolimus adjustments utilizing the intravenous and solution dosage forms allowed doses to be adjusted in smaller increments minimizing the frequency of toxic trough levels.

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DNA Methyltransferase-1 (DNMT1): A Flow Cytometric Pharmacodynamic Assay for Azacitidine Hypomethylating Therapy after Allogeneic Hematopoietic Stem Cell Transplantation (AlloSCT)

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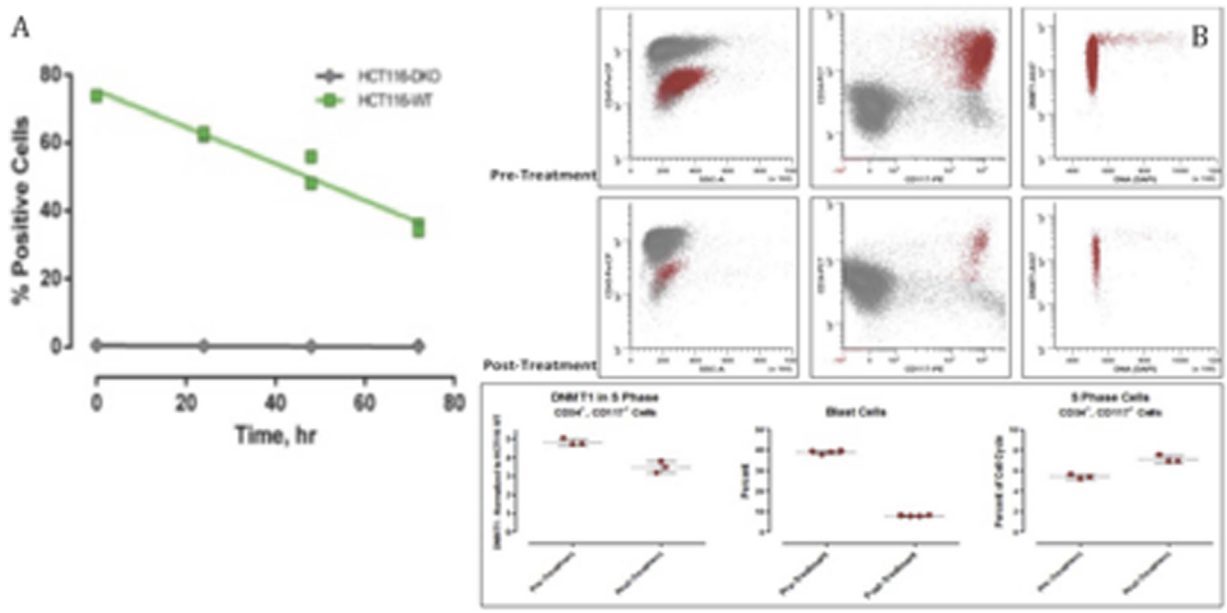

Figure A. Depletion of DNMT1 in HCT116-WT and DKO cells cultured with 0.5μM decitabine. B: Depletion of DNMT1 in immature myeloid cells (CD34+CD117+) by flow cytometry in a patient with relapsed AML post-transplant who was treated with parenteral azacitidine. The pre-treatment peripheral blood sample was collected at T+32 after reduced-intensity allo-HCT and before starting parenteral azacitidine (40 mg/m^2 IV daily for 5 days). The post-treatment sample was collected 45 days after start of azacitidine. The DNMT1 level was determined in CD34+CD117+ and S-phase cells by flow cytometry.

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Disease relapse is a major cause of treatment failure following alloSCT for acute myeloid leukemia (AML). Azacitidine has shown efficacy in treating, and preventing, post-transplant relapse in patients with AML. Post-SCT azacitidine administration is challenging due to the possibility of myelosuppression and an incomplete understanding of the optimal dose and schedule. DNMT1 is responsible for genome methylation in S phase and degraded after bonding irreversibly to substituted DNA. Hence, the DNMT1 level may be an attractive pharmacodynamic (PD) endpoint for azacitidine therapy.

We developed a novel DNMT1 assay by flow cytometry as a PD endpoint for hypomethylating agent therapy. To validate the assay, HCT116-wild type (WT) and DNMT1-double-knockout (DKO) cells were incubated with 0.5 μM decitabine, and were harvested, fixed, and processed for flow cytometry using DNMT1 antibody in an indirect assay and co-stained with DAPI to measure DNA content. We assessed the time-dependent depletion of DNMT1 with decitabine incubation (Fig. A).

We subsequently assessed DNMT1 in fixed peripheral blood leukocytes using the same method in 4 AML patients treated with post-alloSCT azacitidine; either parenterally for post-SCT relapse (n=2) or after oral azacitidine maintenance on clinical protocol NCT 01835587 (n=2). Peripheral blood samples were drawn before and 2-6 weeks after starting azacitidine. Immature myeloblasts were identified by co-staining for CD34 and CD117.

We demonstrated depletion in DNMT1 in S-phase CD34+CD117+ cells after azacitidine in the 4 studied paired samples. The degree of depletion was more pronounced with parenteral versus oral azacitidine. In one patient who responded favorably to azacitidine, depletion of DNMT1 correlated with reduction in peripheral blood blasts (CD34+117+ cells) as shown below (Fig. B).

These preliminary data suggest that the DNMT1 assay is a robust single cell approach to assess PD of hypomethylating agents. After further validation, this DNMT1 assay may be a valuable tool to optimize the dose and schedule, and hence the safety and efficacy of post-alloSCT hypomethylating therapies.

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Novel Imaging Analysis of the Marrow Compartment after Myeloablative HSCT Reveals the Kinetics and Degree of Myeloablation and Cell Recovery

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There are no tests that diagnose graft failure early after hematopoietic stem cell transplantation (HSCT); currently, biopsies of single sites are used, thereby limited by small volume of HSCs. To enhance evaluation of the entire marrow space, we have developed a new methodology using an imaging probe, 3'-deoxy-3 ^{18}F-fluorothymidine (^{18}FLT) PET/CT. Previously, by drawing regions of interest on ^{18}FLT PET-CTs, uptake correlated with rate of engraftment after HSCT. This approach is limited by subjective choice of regions within bones. To enhance objectivity and sensitivity, we developed a computer-based algorithm to isolate the entire medullary space and determine standard uptake value (SUV). This method was applied to images from 17 patients

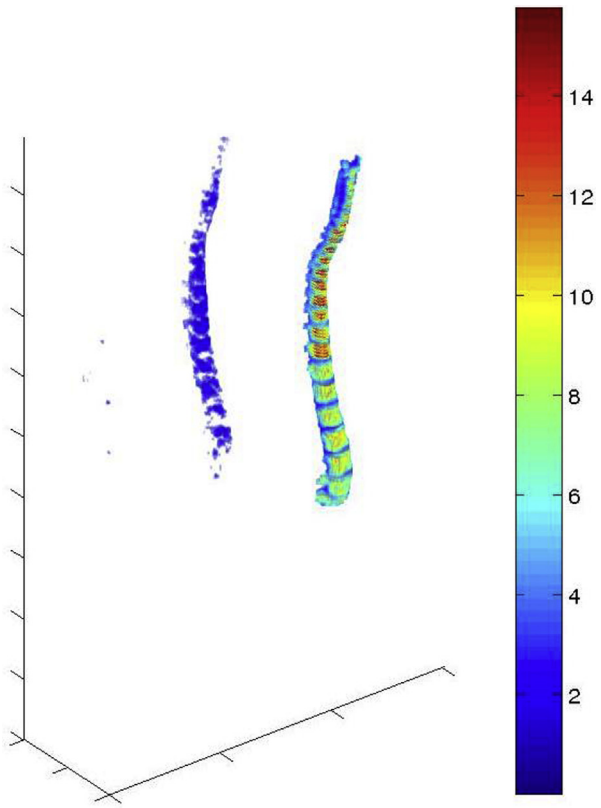

Figure 1. FLT image reconstruction axial spine