Influence of Drug Absorption, Distribution, Metabolism, and Excretion (ADME) Variants on Sirolimus Blood Levels in Patients Following Allogeneic Hematopoietic Stem Cell Transplantation

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Allelic variants implicated in drug absorption, distribution, metabolism, and excretion (ADME) affect drug pharmacokinetic variability and have been increasingly recognized as important factors in medical therapy. Our recently published study found an association of thrombotic microangiopathy (TMA) with high sirolimus serum levels in a cohort of 177 patients who received sirolimus and tacrolimus as GVHD prophylaxis following allogeneic transplantation. By multivariable analysis, increased risk of TMA was associated with day 14 sirolimus levels, prior aGVHD, and myeloablative conditioning. In the current study we explore the possible influence of ADME variants on sirolimus levels and development of TMA in the same patient cohort. We obtained archived DNA samples from the patients on our TMA study and analyzed them using the iPLEX ADME PGx panel and the MassARRAY® Compact Analyzer. This panel is based on the PharmADME Working Group list and covers >99% of the Pharma ADME Core list; it interrogates 188 mutations and 12 copy number variation assays (in 36 pharmacogenetically relevant genes). Sirolimus levels were measured at least weekly until day 100 with dose adjustments made for target levels and/or clinical toxicity. Possible associations between early sirolimus serum levels (day +14) and assays were evaluated by the Kruskal-Wallis (non-parametric) test and the false discovery rate was used to control for multiple comparisons. Using this panel, 179 samples were genotyped, of which 173 showed high quality data. The average call rate for these samples was 98.85% over 200 assays, with a median call rate of 99%. Of these assays, 66 variants were identified that may be of relevance to sirolimus metabolism; other assays were shown to be homzygosity or >10% missing data. Using this panel, we found 3 assays showing an association with sirolimus drug level, rs1057910 (CYP2C9’3) at P = 0.04, rs1799931 (NAT2’7) at P = 0.03, and rs2032582 (ABCB1 267TG>A/T) at P = 0.007. In addition, the 0-copy haplotype of UGT2B17 also showed higher levels of sirolimus (P = 0.02). These assays were also tested for an association with TMA, showing a trend for increased TMA in the rs1799931 (NAT2’7) (P = 0.08). However, after adjustment for multiple testing, only rs2032582 maintained statistical significance due to the small sample size. In conclusion, this pilot study of the iPLEX ADME PGx panel showed feasibility and provided high quality data. Despite the limitation of small sample size, several genetic variants were implicated in sirolimus levels and may warrant further investigation. Future analysis should focus on specific gene clusters or pathways and will require a large cohort to power validation and training sets.

CD38 Bright Effecter Memory CD8+ T Cell Populations Predict Acute Graft Versus Host Disease

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Introduction: Acute graft versus host disease (aGVHD) is mediated by allogeneic T cell responses. We hypothesized that peripheral blood expansion of activated effector memory T cell populations (TEM) following allogeneic hematopoietic cell transplantation (HCT) would serve as a useful predictor of aGVHD.

Methods: T cells were characterized in peripheral blood samples from 16 consecutive pediatric allogeneic hematopoietic cell transplant (HCT) recipients. Samples were collected prior to transplant and weekly following HCT until day +42. Samples were incubated with fluorochrome-conjugated monoclonal antibodies directed against CD3, CD8, CD38, CD45RA andCCR7, followed by red cell lysis and fixation. Samples were analyzed by flow cytometry on a FACS Canto II flow cytometer (BD Biosciences). Data was analyzed using FCS Express (De Novo Software). TEM were defined as CD3+ lymphocytes which lacked expression of CD45RA and CCR7. CD38 was used as a marker of activation.

Results: Patients had less than 65% of CD8+ CD38 bright TEMs prior to transplant except for 1 patient with HLH who had 99.8%, and was excluded from analysis. Patients who developed aGVHD (n = 5), engraftment syndrome (n = 2), or neither (n = 8) were observed to develop median maximum expansions of CD8+ CD38 bright TEM prior to or on the day of diagnosis of aGVHD, or before day +42, of 49 cells/mcl (range 21-87), 5 cells/mcl (range 0.3-34) and 98 cells/mcl (range 0.04-197), respectively (P = 0.02, GVHD versus neither GVHD nor engraftment syndrome). We observed that an absolute number of CD8+ CD38 bright TEM greater than 20 cells/mcl was predictive of aGVHD with sensitivity of 100%, specificity of 80%, and a negative predictive value of 100%. The cumulative incidence of aGVHD in patients with greater than 20 CD8+ CD38 bright TEMs/mcl was 71%, and in patients with less than 20 cells/mcl, 0% (P < 0.01).

Conclusion: Quantification of peripheral blood CD8+ CD38 bright TEM is a novel predictor of aGVHD.

Novel Strategy for Non-Invasive Sampling of Epidermal Cytokines Using a Skin Sampling Disc in Acute Skin Graft Versus Host Disease and Engraftment Syndrome

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