Introduction: There is a strong clinical need to overcome the increased early non relapse mortality (NRM) associated with delayed neutrophil recovery following cord blood transplant (CBT). Therefore we established a methodology using Notch ligand (Delta1) as a strategy for increasing the absolute number of marrow repopulating CB hematopoietic stem/progenitor cells (HSPC). We previously reported preliminary results of the first 10 patients in 2010 demonstrating the ability of Notch-expanded CB HSPC to provide rapid myeloid recovery post-CBT. Herein we present the updated results on 23 patients accrued to this trial aimed at assessment of efficacy as well as the feasibility of overnight shipment of the expanded cell product to three outside institutions. Methods: Between July 2006 and March 2013, 23 patients with hematologic malignancies were enrolled in this prospective multi-center Phase I trial coordinated by the Fred Hutchinson Cancer Research Center in which one CB unit was ex vivo expanded prior to infusion. Conditioning consisted of Fludarabine (75mg/m²), Cyclophosphamide (120mg/kg) and TBI (13.2 Gy) over 8 days. On day 0, the unmanipulated CB unit was infused first followed 4 hours later by infusion of the freshly harvested expanded CB cells. Graft versus host disease (GVHD) prophylaxis consisted of cyclosporine and MMF beginning on day -3. All CB grafts were 4-6/6 HLA-matched (A/B antigen level, DRB1 allele level) to the recipient. Engraftment, NRM, relapse and GVHD were calculated using cumulative incidence rates to accommodate competing risks. Overall survival was analyzed using Kaplan-Meier estimates. Results: Patient diagnosis was AML (n=16), ALL (n=5) and biphenotypic leukemia (n=2). Nine patients (39%) were ≥CR2 and 5 were MRD+ at the time of transplant. Median age was 28 years (range, 4-43) and weight 70 kg (range, 16-91) with a median follow-up of 614 days (range, 271-2443). 22 patients received the expanded graft with one product not meeting release criteria. The cell doses infused were significantly higher in the expanded CB graft: 2.7 (1.5-6.3) vs 6.9 (0.4-27.6) x10⁷ TNC/kg, p<0.0008; 0.15 (0.02-0.57) vs 7.7 (0.62-49.5) x10⁶ CD34/kg, p<0.0001. HLA-matching and ABO incompatibility of the expanded and unmanipulated products were similar. The incidence of neutrophil recovery was 95% (95% CI, 71-100) at a median of 13 days (range, 6-41 days) among the 22 patients receiving expanded CB cells which is significantly faster than that observed in 40 recipients of two unmanipulated units otherwise treated identically at a median time of 25 days (range, 14 to 45; p<0.0001). The
incidence of platelet recovery (>20 x 10^9/L) was 77% (CI 95%: 53-89) by day 100 at a median of 38 days (range, 19 – 134). There was one case of primary graft failure. Importantly, rate of neutrophil recovery correlated with CD34⁺ cell dose/kg with 8 out of 11 patients receiving greater than 8x10^6 CD34⁺ cells/kg achieved an ANC ≥ 500/µl within 10 days. 21 patients were evaluable for in vivo persistence of the expanded cells. Ten (48%) demonstrated in vivo persistence beyond one month post infusion. The expanded cell graft was persistent at day 180 in 7 patients, and in those that survived to one year, dominance of the expanded cell graft persisted in one patient. The incidences of grade II-IV and III-IV acute GVHD was 77% (95% CI, 53-89) and 18% (95% CI, 5-36%), respectively; mild chronic GVHD was observed in 4 patients and severe chronic GVHD in one. Probability of OS was 62% (95% CI, 37-79%) at 4 years. Notably, the cumulative incidence of NRM at day 100 was 8% (95% CI, 14-24%) and at 4 years was 32% (95% CI, 8-40%). Nine patients died at a median time of 216 days (range, 31-1578 days) with respiratory failure/infection the most common cause (n=6). There were two relapses at day 156 and 365 post-transplant, with one death due to relapse. Secondary malignancy and primary graft failure were the other 2 causes of death. Conclusions: Infusion of Notch-expanded CB progenitors is safe and effective, significantly reducing the time to neutrophil recovery and risks of NRM during the first 100 days. An advantage for infusion of higher numbers of CD34⁺ cells/kg further demonstrates the need to develop methods that reproducible provide even greater expansion than currently achieved of repopulating cells to improve efficacy and potentially cost effectiveness.