irradiation based (TBI) reduced intensity conditioning regimens for allogeneic hematopoietic cell transplant (alloHCT). We present results of the first 10 patients receiving a novel conditioning regimen comprising fludarabine 40 mg/m$^2$/day × four, melphalan 50 mg/m$^2$ × one and TBI 200 cGy × two fractions. The goal of this reduced intensity regimen was to achieve complete rapid complete donor lymphoid chimerism in order to promote graft vs. malignancy effects without excess regimen related toxicity.

**Methods:** Ten consecutive patients, six male and four female, underwent reduced intensity conditioning followed by alloHCT. The median age was 51.5 (range 5-71). Diagnoses and patient numbers were myelodysplastic syndrome (MDS), n = 3,acute myeloid leukemia (AML), n = 3, Non-Hodgkin lymphoma (NHL), n = 3 and Diamond Blackfan anemia, n = 1. Stem cell sources peripheral blood (PB), n = 8 and bone marrow, n = 2. Donors were related n = 8 of which n were HLA matched and unrelated, n = 4 which × were HLA matched at × loci. Three patients were undergoing second transplants (second allo n = 1) and prior autoHCT (n = 2). Three patients had uncontrolled malignancy at the time of transplant. The extent of donor chimerism was assessed by flow-sorting PB for myeloid (CD33) and lymphoid (CD3) markers followed by short tandem repeat (STR) analysis.

**Results:** At a median follow up 88 days (range 14-171); nine of ten recipients were alive at day 30. One patient died on day fifteen post transplant with progressive disease. Six of nine patients achieved complete donor lymphoid chimerism and seven of nine patients achieved complete donor myeloid chimerism in peripheral blood day 30. This is significantly faster than historical controls using 200 cGy based regimens. Of the three patients who had incomplete donor chimerism, two had refractory malignancy (AML or MDS) at the time of transplant and the third patient developed progressive MDS soon after. Treatment related mortality at day 100 was ten percent. One of the ten patients manifested grade 3-4 Bearman regimen related toxicity.

**Conclusions:** Reduced intensity with Flu/Mel/TBI 400 is well tolerated and produces rapid donor chimerism in allogeneic stem cell recipients.

**Results**

| Patient | Follow-up (days) | Days post BMT | % Donor Lymphoid Chimerism | % Donor Myeloid Chimerism | Grade 3/4 Bearman Toxicity | Uncontrolled Malignancy |
|---|---|---|---|---|---|---|
| #1 | 128 | 30 | 100 | 100 | No | Yes |
| #2 | 58 | 27 | 100 | 100 | No | No |
| #3 | 104 | 30 | 100 | 100 | No | No |
| #4 | 98 | 34 | 100 | 100 | No | No |
| #5 | 77 | 33 | 94 | 75 | No | No |
| #6 | 171 | 27 | 95 | 95 | Yes | No |
| #7 | 78 | 34 | 100 | 100 | No | No |
| #8 | 100 | 30 | 100 | 100 | No | Yes |
| #9 | 30 | 30 | 92 | 100 | No | No |
| #10 | 14 | died day 15 | NA | NA | Yes | Yes |

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### A STEM CELL POTENCY, QUALITY AND RELEASE (PQR) ASSAY FOR UMBILICAL CORD BLOOD THAT IS COMPLIANT WITH REGULATORY GUIDELINES

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New guidelines and regulations in the U.S.A. and Europe will require that the cell potency of cellular and gene therapy products are determined prior to their intended use in the patient. Umbilical cord blood (UCB) is a cellular therapeutic product whose potency and release criteria will fall under these criteria. The present parameters of total nucleated cell (TNC) count, viability, CD34 and CFU not only fall significantly short of what would be required, but are used post-processing, pre-cryopreservation rather than post-thaw prior to transplantation. Furthermore, none of these tests determine stem cell "quality" or potency, which is a measure of stem cell engraftment potential (EP). The CFU assay, as used by the UCB community, measures the differentiation (not proliferation) of GM progenitors, which have no stem cell properties and therefore can only detect engraftment outcome, i.e. time to neutrophil engraftment, but not EP. An in vitro, instrument-based, ATP bioluminescence potency, quality and release (PQR) assay that can be fully validated for the intended use has been developed and tested. 24 UCB samples were tested for proliferation status ("quality") and potential (potency) of 2 stem cell populations, 1 primitive (HPP-SP) and the other mature (CFC-GEMM), each at 3 cell doses, and the response compared to the same cell populations from a UCB reference standard. The results showed that the assay is >90% effective in predicting EP. Testing a single stem cell population (CFC-GEMM) can lead to a false interpretation, but a duel stem cell potency assay can provide greater predictive value for short- and long-term EP. Stem cell "quality" should also be determined using duel populations, since together with potency, acceptance limits for release criteria can be defined. The PQR assay is a powerful and predictive tool that, together with other assays, can provide the transplantation decision-maker with information that has, until now, not been possible.

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### THIOTEPA (TT), BUSULFAN (BU), AND CLOFARABINE (CLO) AS A CONDITIONING THERAPY FOR ALLOGENEIC HEMATOPOETIC STEM CELL TRANSPLANT FOR PATIENTS WITH HIGH RISK MALIGNANCIES: EARLY RESPONSE AND ENGRAFTMENT DATA

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**Introduction:** Fludarabine (F) and Busulfan (Bu) are commonly used together in transplantation conditioning regimens. To improve on this combination, we substituted Clofarabine (Clo) for F for its improved antileukemic activity. In addition, we added Thiotepa (TT) as a polyfunctional alkylating agent recognized for its antileukemic activity and its ability to cross the blood brain barrier. We investigated TT+ (IV) Bu + Clo as a conditioning regimen for advanced malignancies using a variety of stem cell sources.

**Methods:** Patients (pts) received TT 5 mg/kg over 1 hr on d -8. Bu was given IV over 3 h to a daily AUC of 5,000 mcMol-min +/-10% on d -5 to -3. Clo 40 mg/m$^2$ was infused over 1 hr daily on d -6 to -3. All 9 pts are evaluable for engraftment and survival beyond d +30. M/F: 4/5. Median age was 22 (range 6-60). Five pts had AML [relapse refractory/Flt3$^+$/3$^{rd}$ transplant, CR1 h/o leptomeningeal disease, MDS/AML (CR2), CR4 (3$^{rd}$ BMT) and CR7 (3$^{rd}$ BMT)]. Others had T-cell PLL (2), ALL (CR3), CLL (3$^{rd}$ relapse-refr). GVHD prophylaxis was tacrolimus and mini-MTX or tacrolimus and MMF [cord blood (CB) recipients]. Those with one Ag-mism or unrelated grafts received rabbit-ATG. Stem cell sources included cord (4 db CB; 1 single CB); MUD-apheresis (2), MUD-BM (1), MSD-apheresis (1).

**Results:** All pts engrafted neutrophils at a median of 14 d (10-25), and platelets at a median of 42 d (CB) and 12 d (PB/BM), respectively. 8 of the 9 pts are ≥60 days post transplantation, and the remaining pt ~ 30 days. Esophagitis (max grade 2) was the most common regimen related toxicity (RRT). Transient, mild hyperbilirubinemia from RRT occurred in 5 pts. (med 3.6 mg/dl; range 2 – 9.1). Deaths (2) were due to GVHD (d +56) and infection (d +56). Two pts relapsed (d +59, d +87). PB microsatellite chimerism around day +30 showed 100% donor-derived T cell and myeloid cells in 8 pts. One had mixed chimerism (3% recipient T cells).