CLINICAL CELLULAR THERAPY

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CORD BLOOD (CB) APGAR SCORE MAY BE PREDICTIVE OF TRANSPLANT RELATED MORTALITY (TRM), OVERALL SURVIVAL (OS) AND DISEASE-FREE SURVIVAL (DFS) FOR PLASMA DEPLETED/REDUCED CB PRODUCTS

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CB potency is important for engraftment potential prediction and transplantation product selection and Nucleated cell (NC), CD34+ cell (CD34), and colony forming unit (CFU) doses have been used to measure such potency. TNC is widely used for CB selection; however its predictive value is not as robust as the progenitor cell measurements. In contrast, CFU and CD34 suffer from high inter-laboratory coefficient of variance - decreasing their utility as potency measures. Recently, the Duke Group proposed a CB APGAR scoring system composed of (a) a Pre-Cryopreserved Score (PCS) reflecting pre-freeze CFU, CD34, NC, and CB collected volume, as well as a (b) Composite Score (CS) which combines the PCS score with post-thaw NC, CD34, CFU and mononuclear cell dose. Based on single, myeloablative and first (SMF) transplants of largely pediatric patients performed at Duke and using mostly red cell reduced (RCR) CB, the PCS and CS scores were shown to be predictive of graft failure, neutrophil and platelet engraftment. Subsequently, the CB APGAR Score was further validated by us recently for engraftment prediction on a patient population with mostly adults, heavy representation of minority and international patients, and on both SMF transplants, and all transplants (All) using plasma depleted/reduced (PDR) CB products. We next examined if PCS and CS can correlate with relapse, transplant related mortality (TRM), overall (OS) and disease free survival (DFS) on the SMF patients transplanted with PDR CB products. The table below shows K-M probabilities of 100-day and 1-Year TRM, 1-Year OS, DFS and relapse for the various PCS and CS strata that had sufficient sample size. There appears to be no correlation between PCS/CS and relapse; however, both scores appear to be predictive of TRM, OS and DFS. We conclude that in addition to its utility as an easy-to-use engraftment prediction tool, the CB APGAR score may be predictive of TRM, OS and DFS for PDR CB transplanted for mixed adult and pediatric populations and for minority and international patients. This observation may further enhance the value of Duke CB APGAR as a reproducible and practical potency measurement for CB selection by transplant centers.

Table 1. CB APGAR Correlation with TRM, OS and DFS

	1-Year Relapse	100-Day TRM	1-Yr TRM	1-Year OS	1-Year DFS
PCS <4.25	16.7 ± 15.2%	31.9 ± 10.1%	31.9 ± 10.1%	51.6 ± 10.6%	48.9 ± 13.6%
PCS ≥4.25 - <5.5	14.3 ± 13.2%	No Events	11.6 ± 7.8%	79.3 ± 9.3%	71.8 ± 14.0%
PCS ≥5.5 - <7.75	No Events	No Events	No Events	92.3 ± 7.4%	100%
CS Score < 13.5	12.5 ± 11.7%	25.4 ± 9.9%	31.1 ± 10.7%	51.3 ± 11.1%	51.2 ± 14.1%
CS Score ≥ 13.5	33.3 ± 27.2%	No Events	No Events	82.4 ± 9.3%	88.9 ± 10.5%
HR (CS ≥13.5 vs <13.5)	1.26 (0.08 - 20.61)	Not Applicable	Not Applicable	0.32 (0.10 - 1.00)	0.13 (0.02 - 1.06)

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T-RAPA CELL DLI SAFELY BALANCES Th1/Th2 CYTOKINE ACTIVATION AFTER LOW-INTENSITY ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION

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We recently reported that pre-emptive DLI with donor T-rapa cells safely promotes alloengraftment after low-intensity allogeneic HCT (ASH, 2010; multi-center clinical trial NCT 0074490). T-rapa cells were manufactured ex vivo using purified $CD4^+$ T cells, rapamycin, co-stimulation, and IL-4. T-rapa clinical products contained minimal Treg cells and secreted both Th1 (IFN-γ, IL-2) and Th2 cytokines (IL-4, IL-5, IL-10, and IL-13). On one protocol arm, refractory hematologic malignancy patients (n = 40) received EPOCH-FR induction chemotherapy, low-dose fludarabine/cyclophosphamide (Cy) preparative chemotherapy (total Cy dose, 1200 mg/m^2), T cell-replete mobilized allografts, and GVHD prophylaxis of cyclosporine and short-course sirolimus (until day +14 post-HCT). T-rapa cells were administered at day +14 (2.5×10^7 T-rapa cells/kg). This transplant approach was safe, as there was: (1) no engraftment syndrome (0/40 cases); (2) a low rate of grade II-IV acute GVHD (4/40); and (3) no transplant-related mortality (0/40). Complete remission has been sustained in 37.5% (15/40) of patients. Median survival is 28.5 months; 24-month survival probability is 64.4%. T-rapa DLI was associated with conversion of mixed chimerism: median donor $CD3^+$ T cell chimerism values at days +14, +28, and +100 were 61%, 89%, and 93%, respectively. The capacity of T-rapa DLI to safely promote alloengraftment suggested that a balanced pattern of Th1/Th2-type cytokines may have been activated post-HCT. To assess this, post-HCT peripheral blood T cells were co-stimulated; supernatants were then tested for cytokine content.

Table 1. Day +14 Post-HCT DLI With T-rapa Cells Yields Balanced type I/type II Cytokine Secretion

T Cell Supernatant	Prior to T-rapa Infusion		After T-rapa Infusion	
	Day +7	Day +14	Day +28	Day +50
IFN-γ	957 ± 487	246 ± 114	976 ± 722	1165 ± 550
IL-2	2058 ± 1334	124 ± 29	1601 ± 1093	706 ± 214
TNF-α	7617 ± 6475	310 ± 112	413 ± 204	438 ± 118
IL-17	33 ± 1	< 1	108 ± 108	42 ± 36
IL-4	4 ± 4	2 ± 2	20 ± 15	8 ± 5
IL-5	105 ± 10	2 ± 1	157 ± 103	100 ± 57
IL-10	5 ± 2	7 ± 1	24 ± 12	18 ± 7
IL-13	22 ± 15	14 ± 7	218 ± 145	167 ± 57

Ex vivo generated donor T-rapa cells were administered as a pre-emptive DLI at day +14 post-HCT. Post-HCT mononuclear cells were harvested at days +7, +14, +28 (± 2 days) and day +50 (± 4 days) post-HCT; cells were subjected to CD3, CD28 co-stimulation, and 24 h supernatants were tested for cytokine content by multi-plex assay. Values shown are mean ± SEM, in pg/ml. For each measurement, values were available on n = 29 to n = 30 T-rapa cell recipients.

Day +7 T cells secreted large quantities (ng/ml range) of Th1 cytokines IFN-γ and IL-2; Th1 cytokine secretion was blunted by day +14 but increased again after T-rapa DLI. TNF-α secretion was prominent at day +7, normalized prior to T-rapa DLI, and was not increased post-DLI. IL-17 was undetectable at day +14 and was moderately increased after T-rapa DLI. Early post-HCT, Th2 cytokine secretion was modest, particularly at day +14; mean values for IL-4, IL-5, and IL-13 secretion were increased by at least one-log at day +28 and were generally increased at day +50. Mean values for IL-10 were modestly higher after T-rapa DLI. In conclusion, pre-emptive DLI with T-rapa cells was associated with a balanced activation of both Th1- and Th2-type cytokines that appears favorable for the safe promotion of alloengraftment after low-intensity host preparation.