FK506 AND “MINI” METHOTREXATE FOR THE PREVENTION OF GRAFT-VERSUS-HOST DISEASE IN PEDIATRIC UMBILICAL CORD BLOOD TRANSPLANTATION

By

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A THESIS PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE

UNIVERSITY OF FLORIDA

2008

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To all the children and families who I have been fortunate to have the privilege of caring for—
you are my inspiration.

ACKNOWLEDGMENTS

I thank Dr. Stephen Hunger for encouraging my participation in the APPCI program and for his mentorship and advice while the division chair for Pediatric Oncology at the University of Florida. I thank Dr. John Wingard for his mentorship during my time at UF. I thank all the children and families whom I have been privileged to care for. Your willingness to allow us to learn from you is what makes scientific advancement possible. I thank my committee for your guidance and assistance.

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August 2008

Chair: Douglas Barrett
Major: Medical Sciences-Clinical Investigation

An increasing number of stem cell transplants (SCT) are being performed each year, as the non-malignant indications for transplant are expanding. With families having fewer children and complex family structures with half- and step-siblings, there has been a significant decline in the number of children with matched sibling stem cell donors. Unrelated donors are therefore required for these patients. In recent years umbilical cord blood (UCB) has become the leading stem cell source for transplantation in children. A majority of unrelated donor umbilical cord blood transplants (UCBT) to date utilized steroids and cyclosporine for graft-versus-host disease (GVHD) prophylaxis. This combination is associated with numerous complications including hyperglycemia, hypertension, avascular necrosis/osteoporosis, and significant risk of infections.

Here we report our experience with the combination of "mini" methotrexate (5mg/m^2 x 4 doses) and FK506 (Tacrolimus) as a graft-versus-host disease prophylaxis regimen. Thirty-one children (ages four months to 17 years) underwent UCBT at the University of Florida between June 2004 and September 2007. Twenty-seven received cord blood from unrelated donors and four from sibling donors. All units were matched at 6/6(n=9), 5/6(n=11), or 4/6(n=11) Human Leukocyte Antigen (HLA) loci. FK506 was started as a continuous infusion at day -3, targeting levels of 10-15 and changed to twice daily oral dosing when tolerated. All children were

scheduled to receive Methotrexate 5mg/m2 on days 1,3, 6 and 11. A taper of FK506 was begun between 100 and 180 days post transplant.

Twenty-five of the 31 patients engrafted (two died prior to engraftment of transplant related causes, four had autologous recovery or leukemic relapse prior to day 45), with engraftment occurring at a median of 18 (range 14-37) days post transplant. Rates of acute and chronic GVHD were low, with only one case of grade III or IV acute GVHD seen, and no children with extensive chronic GVHD occurring. Mucositis was very similar to other GVHD regimens, and no patients required TPN or routine IV medication after hospital discharge. The average length of hospitalization was 27 days post transplant (range 17-63). Transplant related mortality and infectious complications were as expected or lower, with twenty children (65%) surviving event free for a median of 24 (range 8- 45) months post transplant, (overall survival of 68 %).

FK 506 and "mini" Methotrexate for GVHD prophylaxis in pediatric patients undergoing UCBT is well tolerated. Engraftment was not decreased or delayed and recovery of all cell lines occurred earlier than in historical controls receiving cyclosporine and steroids. Low rates of GHVD were seen with minimal serious infections or adverse events, making this an attractive option for GVHD prophylaxis in the pediatric UCB transplantation.

CHAPTER 1
STEM CELL TRANSPLANT

Overview and Indications

Stem cell transplantation is being used with increasing frequency as a curative therapy for not only children with relapsed and refractory malignancies, but also in a wide array of non-malignant diseases.[1, 2] A decrease in transplant related morbidity and mortality as well as early screening and diagnosis of metabolic diseases has only served to increase the numbers of patients undergoing transplant each year. Allogeneic transplant has over 100 indications in children and young adults.[3, 4] Relapsed or refractory malignancies are still the leading indications for transplant in children. As current chemotherapeutic strategies change in effectiveness, and better classification of malignancies by genetic features and minimal residual disease is possible, the specific disease indications and situations where transplant is indicated are constantly changing. Transplant still remains the treatment of choice for multiply relapsed and refractory leukemias. The transplantation of stem cells allows the use of total body radiation (TBI) which has efficacy in most malignancies, but can not be used without stem cell repopulation therapy. The ability to administer TBI is not the only reason allogeneic transplant is potentially curative in patients with malignancies. Immune surveillance occurs in every individual continuously, and cells with errors or potential for malignancy are usually destroyed. In persons with cancer, the immune surveillance is unable to detect the malignant cells and activate the necessary apoptiotic pathways. From the 1960s the landmark ethics case at the Jewish Chronic Disease Hospital where 22 elderly patients were injected with leukemia cells but did not develop leukemia stemmed the origins of immune surveillance and the concept of and immune system (or graft) versus leukemia effect.[5] Following a stem cell transplant, donor cells reform the immune system,

and should be able to recognize residual cancer cells and destroy them via the graft versus tumor or leukemia effect.

While malignancy is the most frequent indication for allogeneic stem cell transplantation in children, some of the first transplants in both the related and unrelated donor setting were done in children with severe combined immunodeficiency syndrome (SCID).[2, 6] Due to the fact that individuals with SCID already are immunocompromised, they do not have the ability to reject the new transplant, and therefore a preparative regimen (chemotherapy and/or radiation to decrease the host immune system allowing acceptance of the donor cells) was not required or could be given at very low doses. This made for an attractive early setting for stem cell transplant.[7] In order to determine indications for transplant, the benefit of transplant is weighed against the risks. In diseases where if transplant is not performed the child is unlikely to survive, such as relapsed or refractory leukemias, many immune deficiencies such as SCID, or many metabolic diseases such as muccopolysaccharidosis, the decision to proceed to transplant is relatively easy.[3, 4, 8] As the transplant field advances, and the morbidity and mortality decrease, the number of diseases and situations where the benefit of transplant outweighs the risk is increasing. In the past 10-15 years many diseases have been added to the list of transplant indications. Current indications include bone marrow failure syndromes, severe aplastic anemia, immune deficiency syndromes, and recently the addition of metabolic disease such as muccopolysacharidosis and leukodystrophies. Patients with diseases such as sickle cell diseases and thalassemia major, where transplant was previously reserved for persons with a matched related donors, are now also being considered for unrelated transplant.[9-12]

History

The origins of stem cell transplant date to the 1950s when it was shown in a mouse model that a mouse could survive what would be otherwise lethal radiation if the spleen was shielded

during the procedure. This led scientists to the concept of a stem or progenitor cell that could completely repopulate the hematopoitic and immune system.[6, 13] Even a single cell has the potential to repopulate the entire marrow space. In the 1950s and 1960s it was discovered that spleen cells or bone marrow can be taken from one mouse, infused into a lethally irradiated, genotypically identical mouse, and the infused mouse would survive. Thus, stem cell transplants were pioneered.[6] The discovery was then made that cells from one mouse given intravenously could hone to the bone marrow and expand sufficiently. This lead to the first stem cell transplants in humans in 1950s. Unfortunately, once transplants were tried in individuals or mice that were not genotypically identical, the transplants had unseen complications, what we now know as graft versus host disease (GVHD). This led to the discovery of Human Leukocyte Antigens (HLA), and the necessity to match HLA antigens in transplant.

With the identification of HLA types, E. Donnel Thomas pioneered the first human matched sibling bone marrow transplants and therefore received the Nobel Prize in 1990.[6] The first matched unrelated bone marrow (BM) transplant was performed in a six-year-old girl who had no matched family members, so BM from a staff member in the hospital who was HLA compatible was used. It was largely through the lobbying efforts of this family that a bone marrow registry, what is now referred to as the national marrow donor program (NMDP), was established. As our understanding of HLA groups and histocompatibility has increased, and our access to such registries has occurred, our ability to transplant patients successfully has also improved significantly. Currently there are over 15,000 transplants per year performed through NMDP and the international marrow registries that form a worldwide cooperative group. While this has expanded the stem cell donor options tremendously, and opened up transplant as an option for thousands of individuals who lack a matched related donor, only approximately 20%

of individuals will be able to find a donor though the bone marrow registries. This is even lower in minorities or persons with mixed racial backgrounds. One of the biggest obstacles to successful unrelated donor BM or peripheral blood stem cell (PBSC) transplant is graft-versus-host disease which occurs at high rates and with significant morbidity and mortality.[14]

CHAPTER 2
UMIBILICAL CORD BLOOD TRANSPLANT

Overview

In the 1980s it was discovered that umbilical cord blood (UCB), such as that found in the placenta routinely discarded after birth, has a high percentage of circulating stem cells.[15] The discovery of circulating stem cells in high numbers in umbilical cord blood has revolutionized transplant for children. It was first discovered in the 1980 that there were sufficient hematopoietic stem cells in UCB repopulate a persons hematopoietic and immune system.[8, 16, 17] The first cord blood transplant was performed in 1990 in Paris France where a child with Fanconi anemia received a matched cord blood transplant from his sister.[8] The first unrelated UCBT followed in 1993. Cord blood is a stem cell source that is derived from something normally discarded, posing no inconvenience or risk to a donor. It can be stored in liquid nitrogen and retain its viability for at least 20 years, and likely longer.[16] Once early evidence was available that UCB was a viable stem cell source, public banks were established with the help of large grants to prospectively bank units for future use. There are currently over 50,000 cord blood units stored worldwide. Many of banks were initially established in areas with ethnically diverse populations, expanding the HLA representation in the UCB banks. Since 1990, thousands of UCBT have been preformed in adults and children. In recent years UCB has been the leading allogenic stem cell source in children; however, like the other stem cell sources, it has advantages in disadvantages.[4]

Advantages

UCBT has unique advantages that have lead to its current position as the leading stem cell source in pediatric allogeneic transplant. UCB requires less stringent HLA matching. Whereas as a matched unrelated bone marrow donor must match at 11 or 12 of 12 HLA antigens, UCB is

immunologically naive, and does not have to be matched to that same degree to provide similar outcomes.[18, 19] While matching of HLA types does have some effect on engraftment and GVHD, a unit matching only four of the six main HLA antigens, two fewer antigens then required for even a sibling transplant, is considered an acceptable donor. Although less desirable, and subject to more complications, many successful transplants have been performed using a graft matching at only 3of 6 HLA loci. Matching at class II HLA antigens (DRB1) is done at high resolution and is prioritized over class I (HLA A and B) matches which are only does at intermediate resolution.

Umbilical cord blood units are HLA typed prior to banking, and are searchable worldwide. This, combined with the decreased stringency in HLA matching requirements for UCBT, allows the identification of donors for approximately 97% of all children, regardless of race or ethnic background.[18, 20] That is a significant increase form the 20-30% of children who can find an adult bone marrow donor.

Due to the prospective HLA typing and banking of UCB units, the amount of time from start of a search to initiation of transplant can be less than two weeks.[3] Donors in the bone marrow registry have been HLA typed at only a few HLA loci for basic screening. In order to procure an adult BM donor, one needs to search based on the basic HLA typing, identify potential matches and submit a request to the NMDP, after which they contact the donors who are a preliminary match. The potential donors are then requested to have blood drawn and submitted for more extensive (high resolution) HLA typing. This is done at their convenience and once the blood is obtained it takes two to three weeks for the blood to be sent to the transplant center, the HLA typing at high resolution performed and results to be available. If at that point they are indeed a suitable match, the prospective donor is notified of the match. If they

agree to proceed with donation, they must be seen by a local NMDP transplant center, have a history and physical and screening labs including viral testing to be cleared as a donor. This process also takes a few weeks. Once cleared to donate, the donor needs to identify a time in their schedule when donation would be convenient. The whole process from start of search to transplant takes two to three months on average. There are many aggressive malignancies and progressive neurologic diseases where waiting two to thee months could significantly compromise the outcome, making the two weeks it takes to identify and procure a cord blood unit an advantage in these patients. In addition, UCB is screened and free of latent viral contamination (such as CMV) that many adult donors have.

Umbilical cord blood is relatively immunoglogically naïve.[19] Besides allowing the use of donors with lower degree of HLA matching, it also results in lower rates of acute and chronic GVHD. When GVHD does occur it tends to be lower grade and more amenable to treatment with lower morbidity and mortality. The lower GVHD incidence allows immune suppression to be used for shorter periods of time, and with the addition of fewer second line agents. As a result, post transplant lymphoproliferative disease after cord blood transplant is almost unheard of.

Disadvantages

As with any stem cell source, UCB has distinct disadvantages. Due to the number of stem cells infused being on average approximately a log fewer than with BM or PBSC transplants, there is a slightly higher incidence of graft rejection as well as a delay in engraftment (ANC >500). When compared with BM or PBSC grafts where neutrophil engraftment occurs approximately 15 -17 days post transplant, UCB grafts have historically been reported to engraft on average around 24-28 days post transplant.[3, 8, 21, 22] This does provide a longer window for infection risks as well as longer hospitalization.[23] The relative immaturity of the UCB immune system also provides slower recovery of host immune system post transplant, especially in adults, where immune

repertoires may never completely recover.[24] Fortunately in children the delay is less pronounced, and immune recovery is almost always complete.[19]

One of the biggest disadvantages of cord blood is the finite cell dose that is available. As outcomes are linked to cell dose received at least to a threshold level, the size of a UCB unit may limit its usefulness to bigger children or adults. As a single UCB unit may not be of sufficient size, combining units has resulted in improved outcomes on adult UCB recipients.[25] Engraftment rates and immune reconstitution are improved, and UCB is offered to patients where it would not previously have been an option. In addition, the entire cord blood unit is routinely utilized at the time of initial transplant in an effort to obtain maximal engraftment rates and optimize outcomes. As a result, there are no cells remaining for further therapies such as donor lymphocyte infusion or re-transplant in the case of early post transplant relapse graft failure.[26] Groups are beginning to investigate ex vivo expansion a small portion of the UCB unit for cellular therapy post transplant[27, 28], or the use of multiple cord blood units[25]; however, the size of the cord blood unit still remains a significant issue.

CHAPTER 3
GRAFT VERSUS HOST DISEASE

Overview

Trying to expand transplant beyond identical siblings led to serious transplant failure, and thus the discovery of human leukocyte antigen (HLA) groups in the 1960s and the discovery of what we now refer to as graft versus host disease.[6] Immunocompetent cells from a genotypically non-identical animal were found to view the host specific antigens as foreign and mount an immune response toward the host. This was first described by Barnes and Louttit in mice when mice were given allogeneic spleen cells after radiation and developed a combination of symptoms that were distinct from radiation injury.[29] The combination of fur loss and skin disease, weight loss, diarrhea and eventually death was named “runt” disease, and was the manifestations of what we now know as graft versus host disease. Criteria for GVHD were first delineated in 1966 by Billingham who noted that for GVHD to occur the graft must contain immunocompetent cells, that the host must have antigens that are lacking in the donor and are therefore antigenically stimulating to donor cells, and the host must be incapable at that point of mounting a sufficient immune response against the donor immune system. During transplant a state where the host can not reject the graft is intentionally induced; however, it is this immunocompromised state that allows GVHD to occur. Once transplant was tried in genotypically disparate individuals, the complications and poor results lead to the identification of HLA types and GVHD.[6] The genes of the HLA loci encode class I and class II molecules. Class I molecules are on essentially every nucleated cell. Class II molecules are primarily expressed in the immune system, with highest density on B lymphocytes and monocytes. HLA molecules provide the mechanism to recognize foreign antigens from self.

Pathophysiology

One of the more accepted conceptual pathways of GVHD pathophysiology is that GVHD is a three phase process. First, an insult to host tissues allows expression of host antigens. Conditioning for transplant is damaging to tissues, and both radiation and chemotherapy are injurious to many organs, including skin, liver, GI tract, and other organs. Organs that are affected more by these insults and with high numbers of antigen presenting cells are the organs in which we see GVHD manifestations.[30] The host tissues, once injured, allow production of an inflammatory milieu including Interleukin 1, tissue necrosis factor alpha and interferon gamma. This is the start of an inflammatory cascade that ends in inflammation and cell death, GVHD. The second phase occurs in response to the inflammatory molecules and cytokines produced by the first phase. The recipient and donor derived antigen presenting cells present antigens to T cells. The donor cells view host antigens as foreign and donor derived T cells are triggered to respond. The T cells then expand and differentiate and become effector cells as a Th1 response is generated. The transcription of genes for Interleukin 2 and interferon gamma are activated. In the third phase of the cascade, activated donor T cells mediate cytotoxicity against the host using the FAS-FAS ligand pathway and peforin, resulting in cell death. The CD8 or cytotoxic T cells are the primary cell involved in the cytotoxicity of GVHD, however the CD4 + cells assist by sustaining CD 8 expansion. TNF alpha activates apotosis, activates macrophages, esosinphils and even B and T cells, and allows inflammatory cytokine production to escalate including IL-1, IL-6 10 and 12. This inflammatory cascade further propagates the cycle.[31]

Hematopoeitc stem cell transplant recipients who get reduced GVHD prophylaxis and those who have greater HLA disparity between graft and host have higher rates of GVHD, as do older patients and individuals with older donors.[14, 32] This is felt to be due to the increase in damage to tissues of older persons undergoing transplant as well as the decrease in immune

plasticity that occurs over time. Female donor are believed to be associated with higher rates of GVHD due to maternal exposure to alloantigens and thus sensitization to alloantigens during pregnancies.

Classification

Acute GVHD was historically categorized as any GVHD occurring prior to day 100 post transplant, whereas chronic GVHD occurred following day 100.[33, 34] As transplant has progressed, and less intense conditioning regimens in older individuals or previously transplanted individuals have resulted in more gradual engraftment and changing over to donor chimerism, GVHD timing has also changed. This has required a reclassification of GVHD. The new classification is based on the rapidity of onset and the specific manifestations. Acute GVHD is usually limited to skin (a spectrum of an erythematous, itchy rash to generalized erythromderma or desquamation), enteritis and hepatitis (Table 3-1). Chronic GVHD targets skin, liver, lungs, eyes and mouth and tends to be more indolent and diverse[30] (Table 3-2).

Acute and chronic GVHD are both graded by very established criteria, although there are many clinical scenarios that may confound the diagnosis, for example drug rash may be indistinguishable from acute GVHD even with a biopsy, so best clinical judgment must be used. Acute GVHD has stages that are assigned based on each organ involvement, then the cumulative stages are compiled to provide the overall acute GVHD grade[33] (Tables 3-3, 3-4). Chronic GVHD has a non-numerical system of classification in which it is broken down into only two categories: limited and extensive[34] (Table 3-5).

Incidence and Onset

Acute GVHD has a variable incidence that is reported with wide ranges. GVHD can be difficult to classify and often confounding clinical issues may make the diagnosis difficult. In HLA matched siblings, acute GVHD is reported at 19-66%, in HLA mismatched related, or

unrelated donors the rates of acute GVHD are reported at 70-90%.[35] UCBT has favorable acute GVHD rates in comparison, reported at 25-50%.[14] Onset occurs at a median of 19-25 days post transplant. Chronic GVHD is equally or more variable in the reported incidence. HLA matched siblings have an incidence that ranges from 20-33%, whereas HLA mismatched related and unrelated donors from 40-85%. Once again, UCBT falls somewhere in between with chronic GVHD occurring in 20-50% of recipients. If acute GVHD occurred, chronic GVHD is more likely, with only 25% of chronic GVHD cases arising de-novo.[36] The median onset is 130-200 days post transplant, which coincides with tapering of immune suppression in most cases.

Survival

The highest mortality is seen in those with grade IV acute GVHD. The response to steroids, the gold standard treatment, is very predictive of survival. If response is limited, mortality is between 85-100%. If there is a response, then mortality is 20-25%. In chronic GVHD those with progressive disease despite treatment or extensive disease with thrombocytopenia the survival is very poor. The organ damage form GVHD is often not the cause of death, but the immune suppression and resulting infections that occurs when treating the GVDH accounts for a substantial proportion of the mortality. The chronic GVHD seen in UCBT is primarily confirmed to limited skin disease and rarely is fatal.[37]

Prophylaxis

Upon the realization that GVHD was caused by primarily donor T cells attacking the host, the idea of manipulating the stem cell product and removing the T Cells was utilized.[32] This resulted in a high degree of graft failure, delayed engraftment, incomplete and extremely delayed immune reconstitution with poor survival. Following this, the use of pharmacologic agents to diminish but not completely debilitate the donor immune system came into use.

Methotrexate

The first agent used to treat and prevent GVHD was methotrexate. Methorexate is an antimetabolite (folate antagonist) which, through the folic acid pathway, blocks proliferation and clonal T Cell expansion and demonstrates cytotoxic function.[38] First used as a chemotherapeutic agent, methorexate is still widely used as both a chemotherapeutic agent and an immune suppressant for not only GVHD prophylaxis but also autoimmune disorders. The first combination GVHD prophylaxis included cyclospirine and methotrexate.[39] The doses used were diminutive compared with chemotherapy doses, as to limit effect on stem cell engraftment. Traditionally 15mg per m^2 is used on day 1 after transplant, followed by 10 mg per m^2 on days 3, 6 and 11. Common side effects include mucosits and renal and hepatic toxicity. The use of MTX is limited by impaired renal or hepatic function as well as mucositis. Doses are held or reduced for these indications. Due to concerns that methotrexate delays engraftment and could severely exacerbate mucositis, and due to the reduced incidence and severity of GVHD in UCBT, we elected to use a lower dose or "mini" methotrexate[40] in our patient series. The dose is 5mg per m^2 on days 1, 3, 6, and 11 post transplant. Lekovorin was given after dose on days 3, 6 and 11.

Corticosteroids

Corticosteriods have been widely used in cord blood transplant and were substituted for methotrexate in the first major cord blood trial (COBLT).[21, 41] In the COBLT study, cyclosporine and corticosteroids where used for GVHD prophylaxis. The concern at the time was that methotrexate would delay the already prolonged engraftment and that mucositis might be severe especially in the infants receiving melphalan instead of radiation in their preparative regimens. The exact mechanism of action of corticosteroids, despite their widespread use, is still poorly understood. The belief is that they suppress pro-inflammatory cytokine production and have some level of cytotoxicity against lymphocyctes. Steroids can also reduce capillary permeability

which causes many of the side effects associated with engraftment syndrome and acute GVHD. The dose required for GVHD prophylaxis is traditionally 2mg per m^2 per day until engraftment followed by a taper over 10 weeks. The side effects of such doses are numerous. Cushinoid features, hyperglycemia, osteoperosis and osteionecorsis, cataracts, hypertension, myopathy and behavior issues are common. In addition the immunosuppressive effect is accompanied by a high rate of infections in the peri-transplant period especially viral reactivations such as CMV. Due to the side effect profile many centers are moving away form steroids for GVHD prophylaxis, and this is the reason we hypothesized a GVHD prophylaxis regimen that spared steroids and relied on the combination of FK506 and mini methotrexate. Steroids remain the first line treatment for GVHD that develops despite prophylaxis.

Cyclosporine

Cyclosporine (CSA), a caclineurin inhibitor, has historically been the mainstay of immunosuppression for both solid organ and stem cell transplant. The immunosuppressive properties stem from its binding to cyclophylin, and the resulting inhibition of calcineurin and the Nuclear Factor of Activated T cells (NF-AT) pathway.[42] By inhibiting the calcium dependent signal transduction pathways in T cells, it prevents T cell activation and the transcription of IL-2 gene. Nephrotoxicity and hepatotoxicity are major complications associated with CSA use. In addition, alteration at baseline of either liver of renal function can alter CSA metabolism. Other side effects include hypertension, hyperglycemia, gingival hyperplasia, histutism, peripheral neuropathy, PRES/seizures, HUS and TTP. CSA can be given orally or IV. Absorption can be erratic, especially when absorption is inconsistent in the early post transplant period. The hyperglycemia and hypertension are also seen with corticosterids, and their cumulative effect can be difficult to manage. This combination of CSA and MTX was the backbone GVHD suppression of the 500 children transplanted on the COBLT protocols.[21, 41]

FK506

FK506, also known as tacrolimus, is also a calineurin inhibitor. FK506 binds to the FK binding protein (FKBP-12).[43] Like CSA, it inhibits T cell signaling and IL-2 production via the NF-AT pathway, thus reducing immunoreactivity of the graft.[44, 45] FK506 can be given orally or IV. It is primarily metabolized by the liver. It can cause elevation on bilirubin and creatinine. In addition, nausea, anorexia, tremors, paresthesias can occur. TTP has also been reported. Hypertension is less than with CSA, and while PRES syndrome can still result, it is less frequent. Hypomagensemia is a complication experienced by a vast majority of the patients, and hyperkalemia can occur as well, especially in infants and toddlers. There have been no direct comparisons; however, there is some data to suggest that as an imunosurpessant, FK506 may be slightly superior, as one can recue patients who failed CSA with FK506.[46, 47] In addition, the side effect profile may be slightly better tolerated than cyclosporine and its absorption and metabolism are more consistent. For this reason, we elected to use FK506 in our GVHD prophylaxis regimen.

Table 3-1. Organ specific manifestations of acute GVHD

Organ	Acute GVHD manifestations
Skin	Pruritic or painful macular/papular rash, red or violaceous Often appear on palms/soles, cheeks, chest, or ears first, can be generalized In severe cases have vesicles or bullae
Liver	Bilirubin rise, often greater than AST/ALT rise Pruritis, jaundice
GI	Diarrhea (voluminous, secretory diarrhea) Bleeding, cramping, pain, ileus, anorexia, vomiting, dyspepsia Abdominal tenderness, hyperactive bowel sounds
Other	Pericardial effusion, pleural effusion, cytopenias

Table 3-2. Organ specific manifestations of chronic GVHD

Organ	Chronic GVHD manifestations
Skin	Erythematous rash, dry or flaky skin
	Lichenoid/sclerodermatous changes, contractures
	Cradle cap-like scalp rash
Eyes	Blurry vision, dry eyes, photophobia, pain
	Hemorrhagic conjunctivitis
	Keratoconjunctivitis/Sicca syndrome
Oral	Dry/painful mouth and lips, odynophagia, mouth sores
	Erythema, lichenoid changes, atrophy of mucosa
GI	Dysphagia, weight loss, nausea, delayed motility
Lungs	Bronchiolitis obliterans (with/without organizing pneumonia)
	Obstructive lung dz, wheezing, cough, hypoxia
Other	Systemic symptoms like systemic sclerosis/ SLE/ Sjogren's/ rheumatoid arthritis
	Cytopenias

Table 3-3. Acute GVHD grading

Stage	Skin	Liver	Gut
I	Maculopapular rash on <25% of body surface	2-3 mg/dL	Diarrhea 500-1000 mL/d or persistent nausea
II	Maculopapular rash on 25-50% of body surface	3-6 mg/dL	Diarrhea 1000-1500 mL/d
III	Generalized erythroderma	6-15 mg/dL	Diarrhea >1500 mL/d
IV	Desquamation and bullae	>15 mg/dL	Pain, ileus

Table 3-4. Acute GVHD staging

Grade	Stage			
	Skin	Liver	Gut	Functional impairment
I	I to II	0	0	0
II	I to II	I	I	I
III	II to III	II to III	II to III	II
IV	II to IV	II to IV	II to IV	III

Table 3-5. Chronic GVHD classification

Classification	Clinical findings
Limited	Either or both of the following:
	Localized skin involvement
	Hepatic dysfunction due to chronic GVHD
Extensive	Either of the following:
	Localized or Generalized skin involvement
	Hepatic dysfunction due to chronic GVHD
	Plus one of the following:
	Liver histology showing chronic aggressive hepatitis, bridging necrosis, or cirrhosis
	Involvement of eye (Schirmer test with <5-mm wetting)
	Involvement of salivary glands or oral mucosa
	Involvement of deep connective tissue (joint contractures, scleroderma)
	Involvement of any other target organ

CHAPTER 4
FK506 AND ""MINI" METHOTREXATE FOR THE PREVENTION OF GRAFT-VERSUS-HOST DISEASE IN PEDIATRIC UMBILICAL CORD BLOOD TRANSPLANTATION

Introduction

Unrelated donor cord blood transplantation (UCBT) is being used with increased frequency as a stem cell source for children with malignant and nonmalignant disease, due in part to rapid availability, less stringent HLA matching requirements and lower rates of GVHD when compared with other stem cell sources.[2] While GVHD rates following UCBT appear to be lower than rates seen with bone marrow or peripheral blood stem cells, it is still one of the major factors contributing to transplant related morbidity and mortality.

The GVHD prophylaxis regimen historically utilized for UCBT is cyclosporine and prednisone.[21] Initial concerns over the added toxicities of melphalan and methotrexate in the first large trial of UCB transplantation, with additional concerns that methotrexate would prolong the time until neutrophil and platelet engraftment, lead to the avoidance of methotrexate containing GVHD regimens. In addition, when methotrexate was used at a dose of 15mg/m2 on day 1, and 10mg/m2 on days 3, 6 and 11 post stem cell transplant, several institutions reported delayed engraftment of myeloid cells and platelets as well as an increase in severity of mucositis.[44] "Mini" methotrexate, or 5mg/m2 on days 1, 3, 6 and 11 has been used in the non-myeloablative setting, and does not seem to be associated with the same degree of delayed engraftment or mucositis.[40] With all methotrexate regimens, there are only four doses required and all are given IV, making administration and compliance easy while being cost effective.

A higher than expected rate of opportunistic infections and an apparent delay in immune reconstitution have been seen in persons undergoing UCBT.[23, 24] It is unclear if these finding are due to an intrinsic difference in the umbilical cord blood stem cells, or if the steroids that are utilized in the preparative regimen for UCBT delay immune recovery and promote infection. In

addition, steroid containing preparative regimens have been associated with a significant risk of hypertension and hypertension related complications, osteopenia cataracts and other long term sequelae. In an effort to favorably improve the toxicity profile associated with the GVHD preparative regimen for UCBT, we chose to treat children with a GVHD prophylaxis regimen of "mini" methotrexate and FK506.

Methods

Patients

Between June 2004 and June 2005, 12 children at the University of Florida received "mini" methotrexate (5mg/m2 on days 1,3, 6 and 11 post transplant) and FK506 for GVHD prophylaxis following UCBT. All children were enrolled on IRB-approved treatment plans and parents signed written informed consent and children of sufficient age signed assent before enrollment. The diagnoses included high risk and relapsed leukemia, bone marrow failure syndromes and metabolic disease.

Selection of Donors

Patients who had matched sibling donor UCB units stored previously received those units. Searches for UCB units from unrelated donors were conducted utilizing intermediate resolution typing for HLA class I (A and B) and high resolution for HLA-DRB1. The cord blood with the highest nucleated cell count was selected (minimum 3 x 10e7/kg). Each donor and patient pair matched at minimum of 4 of 6 HLA loci. Donors were able to be identified for all patients referred to our center.

Transplantation Procedure

Cryopreserved units of umbilical cord blood were thawed and processed in standard fashion (reference). The total number of nucleated and hematopoietic stem cells were counted,

viability was assessed and cultures were obtained before the infusion. The units were infused over 30 to 60 minutes with hydration and pre-medication per institutional guidelines.

Conditioning Regimen

Children with malignant disease were prepared for transplant with one of three regimens based on disease and study enrollment. Eighteen children received cytoxan (60mg/kg daily x 2 days) and total body irradiation (TBI) (1350 cGy delivered in 9 fractions of 150cGy pr fraction) with or without 3mg/kg rabbit anti-thymocyte globulin (ATG) per kg x 3 days. Only one child enrolled received ATG with cytoxan and TBI, due to study requirements. Three children received etoposide (dose) and TBI (1350 cGy delivered in 9 fractions of 150cGy pr fraction). Of these patients, two had sibling donors, one had secondary AML with a history of Ewings Sarcoma. Children with non-malignant disease received 16 doses of busulfan (4mg/kg q 6 hours), cytoxan 50mg/kg daily x 4 days and 3mg/kg rabbit anti-thymocyte globulin (ATG) daily x 3 days. One infant with AML in whom we were avoiding radiation also received the busulfan, cytoxan and ATG preparative regimen. The patient with HLH was prepared with busulfan, cytoxan and ATG with additional Etoposide. All patients receiving busulfan had pharmacokinetic levels drawn with the first dose and subsequent doses were adjusted to target a steady state level of 600-900 nanograms per milliliter and received phenytoin for seizure prophylaxis during busulfan administration. Patients receiving cytoxan also received mesna for hemorrhagic cystitis prophylaxis during cytoxan infusion.

Graft-Versus-Host Disease Prophylaxis and Treatment

All children were to receive methotrexate 5mg/m2 on days 1, 3, 6 and 11 post UCBT. FK506 was started three days prior to transplant at a dose of 0.04mg/kg/day, administered as a continuous infusion. Levels were drawn and the dose was adjusted targeting a goal of 10-15ng/dl. FK506 was changed to BID oral dosing when patients were eating and drinking and

tolerating oral medications. FK506 was continued for a minimum of 100 days. After day 100, children with high risk malignancies began a dose taper, with a reduction of approximately 25% every 2-4 weeks. In children without malignant disease, the taper was begun approximately 180 days post transplant. Acute GVHD was graded according to the consensus criteria[33] and chronic GVHD categorized using standard criteria described by Shulman et al.[34] Biopsies were done as necessary but were not required to confirm diagnosis in all patients. Patients with grade I acute GVHD were treated with topical FK506 or steroid cream, an increase in their FK506 or both. Patients with moderate acute GVHD were treated with steroids (oral or IV converted to oral dosing rapidly) beginning at 2mg/kg divided into twice daily dosing with a gradual taper over the next 4-6 weeks. Patients who developed chronic GVHD during a taper of FK506 had their FK506 dose increased. If the was skin was involved they were also prescribed topical FK506 or steroid cream. If steroids could not be weaned fairly rapidly, cellcept was added in those children. In the case of relapse of malignancy, all immune suppression was stopped in an effort to enhance graft versus leukemia effect.

Supportive Care

During transplant all patients were kept in rooms with reverse isolation and high energy particulate air filtration. Standard prophylaxis against PCP, viral, bacterial and fungal infections were utilized. With the first fever, patients were started on broad spectrum antibiotics, which were continued until the time of engraftment. IVIG was given as need for IgG <500. Galactomannan was checked weekly beginning on the day of transplant (day 0) until day 100. Adenovirus PCR was checked weekly also from day 0 until engraftment. Twice weekly a CMV PCR was sent beginning with engraftment until day 100. Leukocyte depleted and irradiated blood products were used for all transfusions. If the recipient was CMV negative, CMV negative blood products were used. Filgrastim 5mcg/kg/day was used beginning 6 days post transplant

and continued through engraftment. Engraftment was defined as the first of three consecutive days with an absolute neutrophil count (ANC) exceeding 500.

Post Transplant Evaluation

While in the hospital all children were assessed daily with a physical exam and complete blood counts. GVHD scoring was charted daily. Following discharge from the hospital, all patients were evaluated at least weekly through day 100 post UCBT. Subsequently, they were seen at 6, 9 and 12 month time post transplant, then yearly thereafter. At each visit GVHD scoring and laboratory work was assessed. Chimerism was assessed at the 3, 6, 9 and 12 month visits, then yearly. If any child who had returned home demonstrated symptoms potentially attributable to GVHD, they were seen at the transplant center. Any diagnostic procedure for GVHD such as skin biopsy was performed at the transplant center.

Statistical Analysis

The probability of event free survival was calculated using Kaplan-Meier method. Adverse events included death, relapse and graft failure. Overall survival was also calculated using the Kaplan-Meier method. The probability of other events was calculated individually using cumulative incidence. The cut-off date for data analysis was June 1, 2008.

Results

Patients and Conditioning

From June 2004 through September 2007, 31 children (18 boys, 13 girls) received umbilical cord blood transplants at the University of Florida. The median age of the children was 5 years (range 3 months – 17 years). Indications for transplant included high risk or relapsed malignancies (n=22) including ALL, AML/MDS, undifferentiated leukemia, JMML, Anaplastic Large Cell Lymphoma and CML. Non-malignant indications for transplant (n=9) included familial HLH, leukocyte adhesion deficiency, congenital amegakaryocytic thrombocytopenia,

muccopolysaccharidosis, adrenal leukodystrophy, severe aplastic anemia, and Thalassemia major. Of the children with ALL, four were in complete remission two (CR2) (including one infant ALL) and four were in CR3. Of the children with AML or MDS/monosomy 7, three had secondary or treatment related disease and two were primary induction failures. Not all of the patients with AML were in remission at the time of transplant, but all had demonstrated some sensitivity to chemotherapy. The individual with CML had gleevec resistant disease and was in chronic phase. The conditioning regimens for malignant disease included Cytoxan/TBI (n=17), Cytoxan/TBI/ATG (n=1), Etoposide/TBI (n=3, two sibling donors, one secondary AML with history of Ewings sarcoma), and Busulfan /ctoxan/ATG (n=1, infant AML). The patients with non-malignant disease received Bulsulfan/Cytoxan/ATG, with the addition of etoposide in the infant with HLH.

Donors

Units of umbilical cord blood were matched to the patients HLA phenotype at a minimum of four to six HLA loci. Nine units were matched at 6/6 HLA antigens, with four of those being matched sibling donors, five unrelated donors. Eleven were a 5/6 match and 11 were a 4/6 match. UCB cell dose infused ranged from 2.44- 23.7 (median 6.38) x 10^7nucleated cells/kg. Two units after processing were less than 3 x 10^7nucleated cells/kg, however had been greater than 3 x 10^7nucleated cells/kg at the time of selection. Both of these patients engrafted successfully. Interestingly, the sibling donors had a median cell dose of 4.4x 10^7 nucleated cells/kg, whereas the median cell dose for unrelated donors was 6.9 x 10^7nucleated cells/kg.

Engraftment

Neutrophil engraftment occurred at a median of 18 days post transplant (range 14-37 days) (Figure 4-1). Twenty-five patients engrafted with the first UCBT. Of the six6 who did not engraft, one relapsed day +22, one died prior of multi-organ system failure prior to engraftment,

one engrafted after a second transplant, and three had autologous recovery. Of the three with autologous recovery, one relapsed after a second transplant, one also autorecovered following a second transplant and one was not retransplanted. Two patients who had a cell dose less than 3 x 10^7nucleated cells/kg both engrafted without issues. Patients who received matched sibling donors engrafted at a median of day 21, whereas the unrelated donors engrafted at day 16. Packed red blood cell (PRBC) transfusions were no longer required at a median of 38 days. A platelet count of greater than 50,000 was achieved at a median of 42 days post transplant. Of the children who engrafted, three had mixed chimerism initially, and all three have persistent stable or increasing chimerism (all with nonmalignant disease). All other engrafted patients maintained 100% donor chimerism at every time point assessed.

Graft-Versus-Host Disease

Four patients developed engraftment syndrome requiring steroids. Three of those patients later developed grade II acute GVHD (aGVHD), the fourth relapsed very early post transplant. Seven patients developed grade II – IV acute GVHD (22%) at a median of day 27 post transplant (Figure 4-2). Of these seven patients, six developed grade II aGVHD, with two having skin and gut involvement, four having skin only. No patients developed grade III aGVHD, however one child developed grade IV aGVHD and died of complications including GVHD. All patients with aGVHD of grade II or higher responded to steroids except the patient with grade IV disease who also received daclizumab and etnaercept. No aGVHD greater than Grade I occurred in matched sibling pairs. Limited chronic skin GVHD developed in eight patients (36% of evaluable patients), however no child developed extensive chronic GVHD. All were treated with an in crease in their FK506, steroids or steroids and cellcept. There were no infections seen after day 100 except 1 case of zoster after stopping acyclovir. No children were hospitalized or died due to chronic GVHD.

Infectious Complications

Prior to day 100 clostridium dificile colitis and bacteremia were relatively frequent; however, no deaths were attributable to either. Five patients developed CMV reactivation. Of those five patients, two had only CMV PCR positivity with no symptoms, where as three had disease (two with pneumonia, one with colitis andpneumonia). Two of those patients died; however, both had multiple other complications that could also be implicated in their deaths. Adenovirus viermia was seen in two children (6%), however both were treated successfully with cidofovir without renal toxicity. Fungal disease was limited to two patients who developed aspergillus, both of whom had failed engraftment with their initial UCBT. One had eventual autologous marrow recovery and survived with treatment with voriconazole, the other died after a second transplant but also had nocardia, CMV, and other bacteremias and a very prolonged neutropenic state. After day 100 there were no significant infectious except one case of zoster which occurred after stopping acyclovir prophylaxis.

Adverse Events

The most common of all adverse events occurring in the population was hypomagnesaemia with 22 children requiring oral magnesium replacement (70%). Renal dysfunction (described as a creatnine greater than 2 times their baseline) was also fairy common, occurring in 11 patients (35%). None of the children had permanent renal dysfunction or required dialysis for medication related renal disease. Hypertension was uncommon with only two patients (6%) requiring antihypertensive therapy. Outside of the ICU setting no children had insulin requirements for hyperglycemia. Veno-occlusive disease of the liver (VOD) was seen in six patients (19%), five of whom had received busulfan. four of those were moderate, two were considered severe. Of the severe patients one received defibrotide and recovered completely, the other had a more indolent course and died of complications including VOD. Seizures or Posterior Reversible

Encephalopathic Syndrome (PRES) was seen in Two patients (6%), both who had elevated FK506 levels at the time and resolved rapidly with withholding and subsequent decrease of the FK506. Thrombotic Thrombocytopenic Puprpura (TTP) and Hemolytic Uremic Syndrome (HUS) were not seen in our patient population.

Survival

As of June 1, 2008, 19 children (65%) are surviving event free for a media of 24 months post transplant (range 8-45 months) (Figure 4-3). The overall survival is 68%, as two children failed to engraft but are surviving (Figure 4-4). The cause of death included relapse (n=6, 60%), GVHD with VOD (n=1, 10%) and multi-organ system (MOS) failure (n=3, 30%). Of the children with MOS failure, all had multiple infectious organisms, renal failure, were in an ICU setting and one also had VOD. Treatment related mortality was 13%, with the deaths after day 100 all due to relapse. In the non-malignant setting the event free survival is 75%, overall survival 88%.

Discussion

Steroid based GVHD regimens have been associated with significant incidence of hypertension, glucose intolerance, osteopenia and opportunistic infection. In an attempt to decrease the toxicity associated with the GVHD prophylaxis, we utilized FK506 and “mini” methotrexate in 31 children undergoing UCBT. Despite previous concerns with delayed engraftment or reduced engraftment rates with methotrexate, 83% of evaluable patients engrafted at a median of day +18. In the COBLT trial 78% engrafted at a median of 25 days in patients with non-malignant disease[21] and 31 days in young children with leukemia.[41] In similar studies of UCBT where steroids and cyclosporine were utilized for GVHD prophylaxis engraftment occurred at a median of 24[22] and 29 days.[48] The patients in our series achieved a platelet count greater then 50,000 by 41 days post transplant compared with 104 days in COBLT patients.[21] All

surviving children engrafted their platelets. Of patients with malignant disorders, all patients who engrafted achieved 100% donor chimerism at initial check and at all times surveyed, unless they suffered overt leukemic relapse. Of children with non-malignant disease, three have mixed chimerism that is either stable or rising. In those children, all have greater than 80% donor chimerism and are free of symptoms or complications from their underlying disease.

GHVD rates were not adversely affected by our preparative regimen, and compare favorably to all previously published GVHD rates in UCBT. Acute GVHD grades II-IV occurred in seven patients (22%). Previous studies report rates of 29-35%.[21, 22, 41, 48] Only one patient (2%) developed grade III or IV GVHD accounting for 10% of deaths, compared with 7-24% and 25% of deaths.[21, 22, 41, 48] In our patient population no extensive chronic GVHD occurred, and there were no deaths due to chronic GVHD or its treatment. Limited GVHD was seen in 8 patients (36%). This is similar to reported rates of 33-45%.[21, 22, 41, 48] Our survival rates are comparable to those reported in pediatric transplant literature.[21, 22, 41, 48]

Adverse events were infrequent and mild in most cases. Hypomagnesemia was the most common adverse event. The use of antihypertensives was only required in 6% of patients, compared with greater than 50% in COBLT study.[21] No children had TTP, HUS or Post Transplant Lymphoproliferative Disease. Mucositis was as expected, with only four patients missing a dose of methotrexate due to mucositis, and there was no increase in GVHD in these patients. The average hospital stay was 27 days post transplant, with all children being discharged on all oral medications, with no TPN or NG feeds required at time of discharge. No significant infections occurred after day 100 and two deaths were attributable to infection. Event-free survival was 65%, comparable to 47-62% in other studies[3, 21, 22, 41, 48] with EFS in non-malignant patients was 75%, 68% in COBLT patients.[21] Treatment-related mortality was low for

the number of high risk patients at 13%. Other UCBT studies TRM ranged form 23-34%.[22, 41, 48] The only deaths after 100 days were due to malignancy recurrence.

Conclusion

The results from our single center retrospective analysis suggest that the combination of FK506 and "mini" methotrexate is an effective and well tolerated GVHD prophylaxis regimen. No individuals had to change therapy. Rates of engraftment appear to be at least as rapid as when compared with other GVHD prophylaxis regimens, and potentially superior. The rates of acute and chronic GVHD were comparable to or lower than previously reported rates. In addition, the toxicity profile is favorable, with minimal hypertension and survival rates that are equivalent or superior to previously reported date. This regimen includes oral FK506 and IV methotrexate, is cost effective, offers ease of administration and guarantees compliance. We therefore determine that FK506 and "mini" methotrexate is a reasonable and attractive option for GVHD prophylaxis in pediatric UCBT.

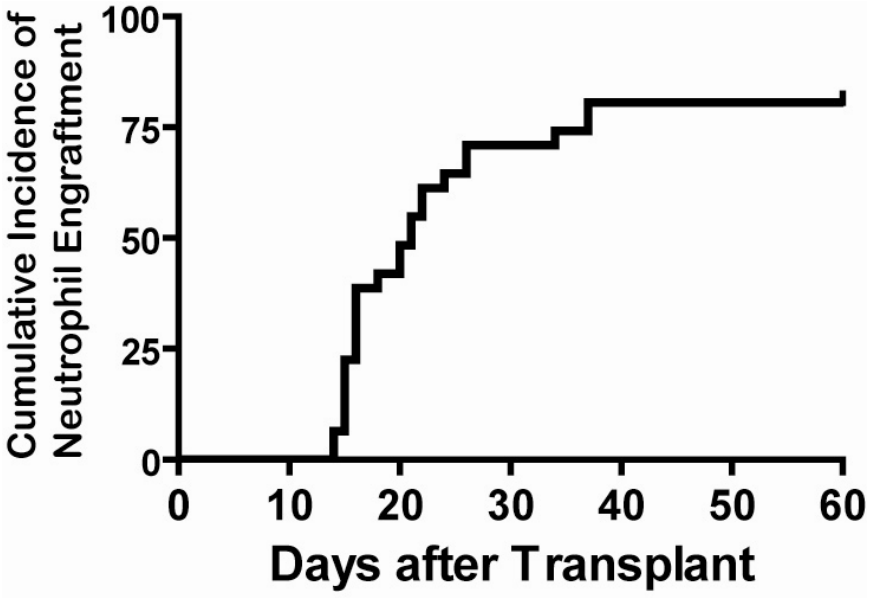

Figure 4-1. Cumulative incidence of neutrophil engraftment

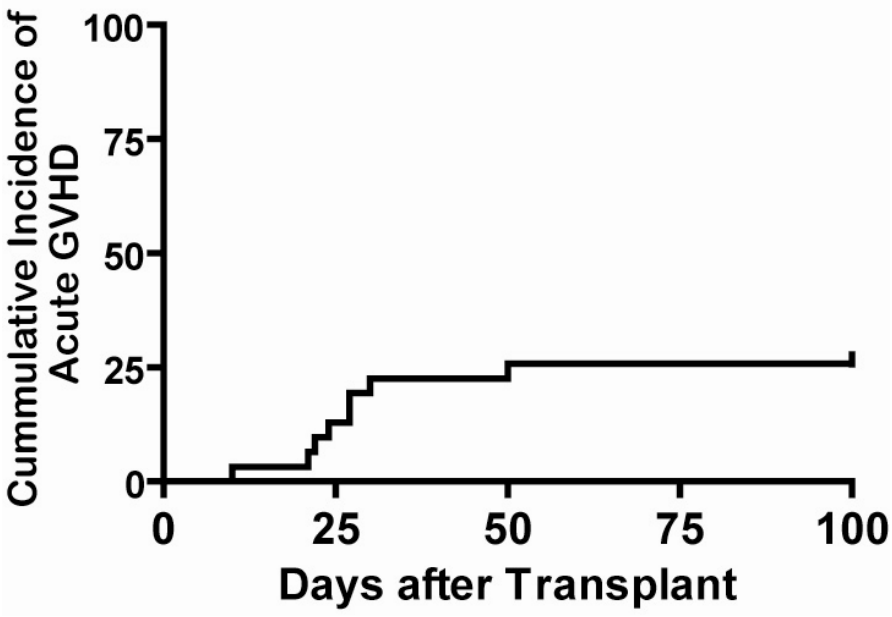

Figure 4-2. Cumulative incidence of Grade II – IV Acute GVHD

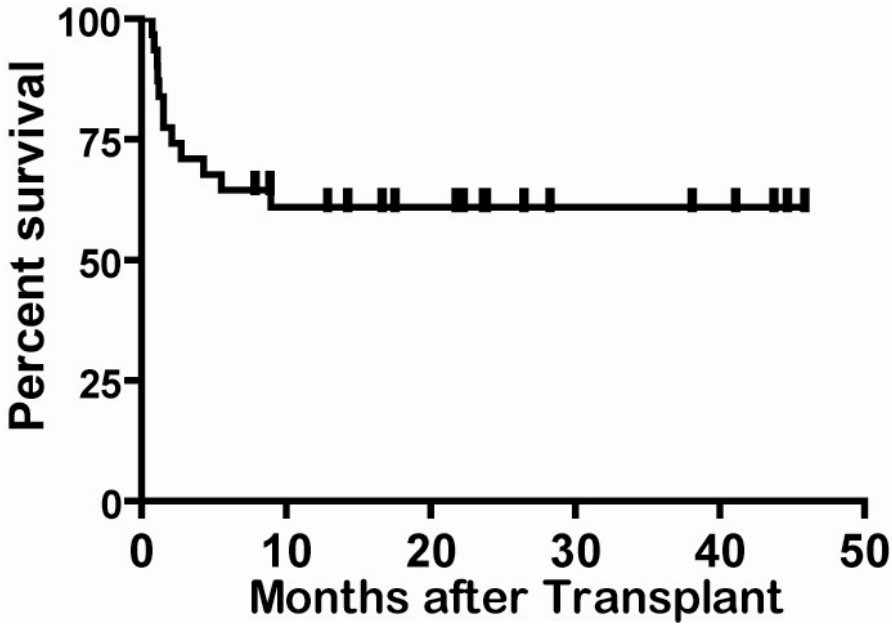

Figure 4-3. Event free survival

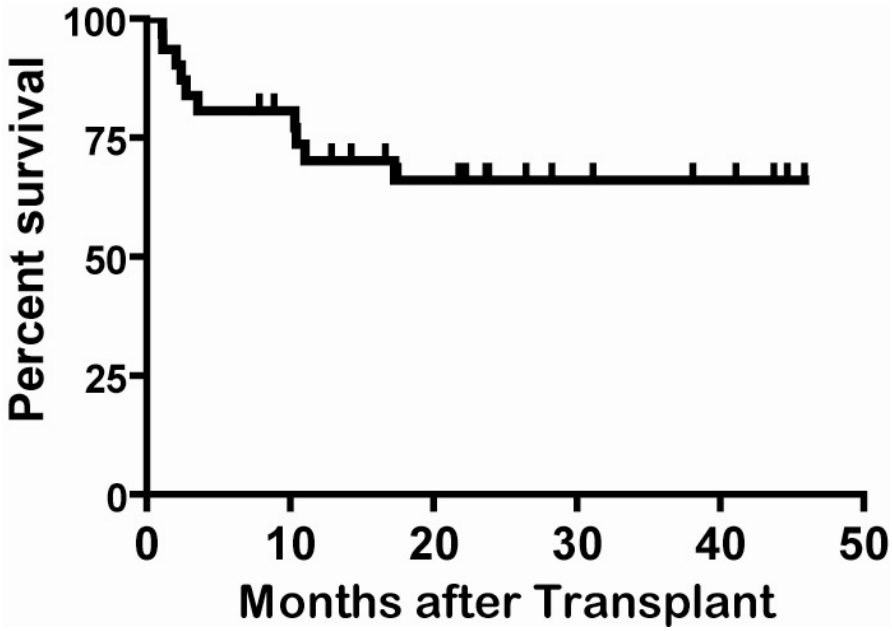

Figure 4-4. Overall Survival

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BIOGRAPHICAL SKETCH

Susan Staba Kelly was born in Hartford, CT, as Susan Lynn Staba. She graduated with highest honors from the University of Florida in 1994 with the degree Bachelor of Science in interdisciplinary medical sciences as part of the Junior Honors Medical Program. Next, She then proceeded to graduate from the University of Florida College of Medicine in 1997. Next, she completed her pediatric residency at the University of Florida prior to attending Duke University for her pediatric hematology/oncology fellowship as well as a pediatric stem cell transplant fellowship. Upon completion of training, Susan returned to the University of Florida to join the Division of Pediatric Oncology. In 2007 she was married to Dr. Patrick Kelly. She is currently an assistant professor in The Department of Pediatrics and Director of the Pediatric Stem Cell Transplant Program at the University of Florida.