

# Chronic Graft-versus-host Disease and Late Effects after Hematopoietic Stem Cell Transplantation

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## Abstract

Late effects following HSCT are related to either the transplant process or to the transplant preparative regimen. Problems related to the transplant process include delayed recovery of the immune system and chronic GVHD. Chronic GVHD presents between 3-14 months post-HSCT in approximately 20% of matched sibling transplants and 40% of matched unrelated donor recipients. Most commonly involved sites are skin, mouth, liver, gastrointestinal tract, and eye. Patients with platelet count <100,000/ml and receiving corticosteroid therapy at day 80 with any clinical manifestations of chronic GVHD require prolonged immune suppressive therapy with prednisone, cyclosporine ± other agents. Treatment should be administered until all clinical and pathological signs and symptoms of chronic GVHD have resolved which may take one to several years. Problems related to the transplant preparative regimen include those involving the endocrine system, eyes, lungs, bone, and development of secondary malignancies. Endocrine deficiencies include growth failure with growth hormone (GH) deficiency, overt hypothyroidism, primary gonadal failure, Type 1 or Type 2 diabetes, and exocrine pancreatic insufficiency. These problems develop at any time post-HSCT, but usually occur within the first few years and should be treated with appropriate hormone supplementation. Eye problems are primarily related to development of cataracts secondary to total body irradiation (TBI) or prolonged corticosteroid use. Cataracts developing after fractionated frequently do not require removal. Pulmonary problems may be due to bronchiolitis obliterans (BO) or to restrictive lung disease. BO may be associated with chronic GVHD and may respond to chronic GVHD therapy. Restrictive lung disease does not occur for many years after HSCT. There is no therapy for this problem. Development of decreased bone mineral density (BMD) is related to GH deficiency and/or corticosteroid therapy. Treatment includes withdrawal of corticosteroids, administration of GH and calcium, Vitamin D and antiresorptive agents. All malignant disease survivors are at risk for development of secondary malignancies, including survivors of HSCT. Recipients of TBI are at highest risk as are children. All pediatric and adult survivors of HSCT should be followed for their life-time for development of delayed effects of transplantation.

*Key Words:* Chronic GVHD, Growth Hormone deficiency, Ovarian dysfunction, Thyroid dysfunction, Fertility

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## 1. Introduction

Over the past 3 decades hematopoietic stem cell transplantation (HSCT) has been administered to increasing numbers of patients with malignant and non-malignant disorders. As a result there is an ever-increasing population of children and adults who have survived to develop late complications. A major compli-

cation of the allogeneic transplant process is chronic graft versus host disease (GVHD) and delayed immune reconstitution. The patient's underlying disease determines the probability of recurrent disease. Delayed effects related to the chemotherapy and radiotherapy either administered before the transplant or as the transplant preparative regimen include neuroendocrine dysfunction and damage to target organs such as the lung, teeth,

eye, and skeleton. This manuscript will focus on chronic GVHD and major delayed effects related to chemotherapy and radiotherapy.

## 2. Chronic Graft-versus-Host Disease (GVHD)

When GVHD persists beyond 80-100 days it is usually termed "chronic". This disease process is distinguished from acute GVHD by clinical manifestations of a pelotropic syndrome which resembles several of the autoimmune diseases. Chronic GVHD often involves the skin, liver, eyes, mouth, and gastrointestinal tract. Less frequent organ systems that may also be involved include serosal surfaces, female genital tract and fascia. Chronic GVHD occurs in about 30-60% of allogeneic transplant recipients, with higher frequencies occurring when the patient is older, when the donor is other than HLA matched sibling and when peripheral blood stem cells are utilized as the hematopoietic stem cell product [1,2]. Chronic GVHD has been categorized as **clinical limited** disease or **clinical extensive** disease according to the number of organ systems involved at the time of diagnosis. The two most important high-risk features identified at day 80-100 post-transplant evaluations that are associated with increased morbidity and non-relapse mortality are thrombocytopenia (platelet count <100,000/ $\mu$ L) and progressive onset from acute GVHD (on steroid therapy at day 80-100). Systemic treatment should be given to patients with clinical extensive or clinically limited with high risk features.

## 3. Current Standard Primary Therapy for Chronic GVHD

Initial treatment of patients with chronic GVHD includes prednisone and continued administration of cyclosporine or tacrolimus. The prednisone is administered daily for a period of two weeks to gain control of symptoms before tapering to an alternate day schedule to maintain clinically quiescent manifestations of chronic GVHD. The duration of therapy varies. Koc et al, (unpublished) reviewed the outcome for 330, mostly adult, patients who received methotrexate and cyclosporine as acute GVHD prophylaxis between January 1995 and December 1998. Only 5% of patients were able to discontinue treatment with all systemic immunosuppressive medications during the first year after diagnosis of chronic GVHD without the need for secondary systemic treatment. Approximately 27% of patients had chronic GVHD manifestations resolve and treatment discontinued within 3 years of chronic GVHD diagnosis without the need for additional immunosuppressive therapy. However, within 3 years, 10% died of causes other than recurrent malignancy during primary treatment of chronic GVHD, 20% developed recurrent malignancy and 11% continued treatment beyond 3 years. The remaining 33% developed additional organ system involvement with chronic GVHD and required secondary immunosuppressive agent therapy.

In general standard primary therapy of chronic GVHD

can be considered to have failed if, within 1 month, there is progression of symptoms in a previously involved organ or whenever clinical manifestations appear in an organ system not previously involved. New therapy should be initiated within 2-3 months whenever chronic GVHD does not improve during therapy with the originally prescribed medications or if prednisone at 1 mg/kg/day is needed to control chronic GVHD. Chronic GVHD therapies studied during the past two decades have not demonstrably accelerated the resolution of chronic GVHD or greatly improved survival [3]. Uncontrolled chronic GVHD interferes with immune reconstitution and is strongly associated with increased risks of opportunistic infections and death. In the analysis of Koc et al (above), 104 of the 330 patients with chronic GVHD required secondary treatment. Manifestations resolved and immunosuppressive therapy was able to be discontinued in 8% within two years without the need for additional therapy. Within 2 years after starting secondary therapy, approximately 19% of patients died from non-relapse causes and 13% needed to continue secondary treatment beyond 2 years. The remaining 48% required subsequent therapy with a variety of other immunosuppressive agents. The combined results of primary and secondary therapy indicated room for improvement in the treatment of chronic GVHD.

## 4. Secondary Treatment of Chronic GVHD

There is no standard approach to secondary chronic GVHD therapy. Except where indicated, the approach is to add agents to the "backbone" therapy of alternate day steroids and either cyclosporine or tacrolimus

## 5. Macrolides

### 5.1. Tacrolimus (FK-506)

The macrolide, tacrolimus (FK 506) was originally derived from *Streptomyces tsukubaensis*. It binds to FK-binding protein and, like cyclosporine, the resulting complex inhibits T cell activation by blocking the phosphatase activity of calcineurin. Tacrolimus is capable of rescuing rejecting renal, liver and pancreatic allografts on cyclosporine [4-6]. Based on the hypothesis that replacing cyclosporine with tacrolimus may result in improved control of chronic GVHD, a phase II study of tacrolimus for patients with chronic GVHD failing therapy with alternate day steroids and cyclosporine was conducted [7]. Five (13%) of 39 patients had resolution of chronic GVHD after converting from therapy with cyclosporine to tacrolimus and all immunosuppressive medications were able to be discontinued with no further interventions to control chronic GVHD. This strategy may be of benefit for some patients.

### 5.2. Sirolimus

This macrolide antibiotic forms a complex by binding to a family of tacrolimus-binding proteins at a site dis-

tinct from tacrolimus. The complex binds to and inhibits activation of rapamycin. Inhibition of this key regulatory kinase interferes with transduction of cytokine-induced signals needed for progression from G1 to S phase in the cell cycle. This mechanism is critical for sirolimus to facilitate induction of immunological tolerance. Sirolimus may have additive or synergistic activity with cyclosporine [8-10] or tacrolimus [8,11,12], since calcineurin inhibitors prevent production of cytokines by activated T cells, while sirolimus prevents proliferation in response to stimulation with cytokines. Data from a small Phase I-II clinical trial using sirolimus for patients with steroid refractory acute GVHD trial suggested that sirolimus could be beneficial in controlling GVHD [13]. Among 21 patients treated, 5 complete and 7 partial clinical responses were observed among patients who received a median treatment duration of 36 days. A randomized trial of sirolimus in renal transplant patients compared doses of 2 mg/day to 5 mg/day in conjunction with cyclosporine and glucocorticoids [14,15]. This trial established that sirolimus blood levels of 4.5-14 ng/mL were necessary to prevent rejection of renal allografts and were achievable with a daily oral dose of 2 mg in adults. Higher doses only added more toxicity of neutropenia and thrombocytopenia. Currently, trials to test efficacy in treatment of chronic GVHD are underway.

## 6. Antimetabolites

### 6.1. Mycopholic mofetil (MMF)

Mycopholic mofetil (MMF) is a pro-drug of mycophenolic acid with 94% oral bioavailability and potent immunosuppressive activity. Mycophenolic acid selectively and reversibly inhibits inosine monophosphate dehydrogenase, blocking the de novo pathway of purine synthesis. Since lymphocytes rely exclusively on the de novo purine synthesis pathway for nucleotides necessary for the production of DNA, while other cells can use salvage pathways, mycophenolic acid selectively affects replication of T and B lymphocytes. MMF also inhibits the expression of costimulatory molecules CD28 and CD154, and can induce apoptosis in a large proportion of activated CD4 T cells in vitro [16-19].

Results from 5 phase II studies suggest that MMF may be effective for treatment of chronic GVHD in adults and children when added to the baseline therapy of alternate day prednisone and a calcineurine inhibitor. Three studies that included a total of 84 adults and children demonstrated complete and partial response rates that varied from 32% to 81% [20-22]. Two studies that included a total of 41 children demonstrated complete and partial response rates of 60% to 77% [23,24]. From these results in Phase II trials, it appears that MMF facilitates development of tolerance. One mechanism might involve the MMF-induced apoptosis of alloantigen activated donor T cells. Although the results of these uncontrolled studies are encouraging, a Phase III randomized clinical trial is needed to provide defin-

itive evidence demonstrating the benefit of MMF in treatment of chronic GVHD.

## 7. Topical Therapies

### 7.1. Oral Beclomethasone

The administration of equal ratios of enteric and non-enteric coated oral beclomethasone capsules showed potential as an effective strategy to treat patients with isolated grade II acute GVHD of the gastrointestinal tract [25]. A subsequent randomized trial comparing 1 mg/kg/day prednisone with or without 28 days of oral beclomethasone capsules showed that the combination therapy was more effective than prednisone alone in treating isolated acute GVHD of the upper gastrointestinal tract [26]. This trial also demonstrated that oral beclomethasone allowed prednisone to be more rapidly tapered without recurrent symptoms. Thus it is reasonable to test a 1-2 month trial of oral beclomethasone in patients with upper intestinal chronic GVHD who have been unable to taper prednisone or who have upper gastrointestinal symptoms develop while receiving oral prednisone. The safety of more than 2 consecutive 28 day courses of oral beclomethasone therapy has not been established, so prolonged therapy cannot be advised.

### 7.2. Tacrolimus Ointment

It has been reported that 72% of 18 patients with chronic GVHD had resolution of pruritis and/or erythema in response to twice to three times daily application of 0.1% tacrolimus ointment [27]. The therapy was well tolerated, however, all patients eventually developed recurrent symptoms and needed to receive more aggressive therapy for localized skin GVHD.

## 8. Other Therapies

### 8.1. Methoxalen and Ultraviolet A Light

Psoralen (methoxalen) plus ultraviolet A irradiation (PUVA) has immunomodulatory effects and is used to treat a variety of immune mediated dermatologic diseases. PUVA therapy has been administered to 103 patients for treatment of steroid-resistant acute GVHD of the skin at the Fred Hutchinson Cancer Research Center [28]. After starting PUVA therapy, 57% did not require additional therapy and, in patients able to complete 6 weeks of therapy, 37% had complete resolution of skin manifestations. The mean prednisone dose was 1.6 mg/kg/day before and was 0.7 mg/kg/day after 6 weeks of PUVA therapy. This form of therapy has a role in the treatment of chronic GVHD of the skin.

### 8.2. Extracorporeal Photopheresis (ECP)

ECP is a technique in which lymphocytes collected by apheresis are exposed to PUVA. The mechanism of

action in GVHD is unclear by may include interference with the function of antigen presenting cells, altered production of inflammatory cytokines, and direct interaction of PUVA with effector cells to induce T-cell anergy or apoptosis. Greinex and colleagues have reported their experience of multiple cycles (1 cycle = 2 consecutive days of ECP) for treatment of chronic GVHD [29]. The ECP was well tolerated by individuals more than 40 kg. Among 15 patients with chronic GVHD, a median of 18 (range 7-47) cycles of ECP were administered. Complete responses were reported for 12/15 patients with cutaneous, 7 of 10 with liver, 11 of 11 with oral mucosal ulcerations. Of particular interest were 4 of 4 patients with scleroderma who had partial resolution of joint contractures. Conclusions about the efficacy of ECP in chronic GVHD are difficult to draw from the literature since studies use different measures of response and different treatment regimens. A controlled trial is needed to define the role of ECP in conjunction with standard immunosuppressive therapy in the treatment of chronic GVHD.

## 9. Delayed Effects of Chemotherapy and Irradiation Therapy

### 9.1. Thyroid Dysfunction

Normal thyroid hormone production is necessary for normal linear height growth in young children. Consequently subnormal thyroid hormone production contributes to decreased growth in height. Thyroid function is not impaired following conventional chemotherapy, but irradiation to the thyroid gland has been associated with development of compensated hypothyroidism, overt hypothyroidism, thyroiditis, and thyroid neoplasms [30]. Irradiation of the thyroid causes subsequent hyperplasia and induction of nodules and malignancies. Following irradiation, thyroid dysfunction onset usually begins as asymptomatic compensated hypothyroidism with elevated thyroid stimulating hormone (TSH) and normal thyroid hormone production within the first year and may progress to overt hypothyroidism with elevated TSH and subnormal thyroid hormone production over the next several decades.

### 9.2. Chemotherapy Only Preparative Regimens

After HCT, thyroid function has usually been evaluated with TSH and thyroxine ( $T_4$ ), ant triiodothyrodine ( $T_3$ ) plasma levels. Abnormal values often prompts further study with free  $T_4$ ,  $T_3$ , resin  $T_3$  uptake ( $RT_3U$ ), and TSH response to thyrotropin-stimulating hormone. The majority of results from reported studies (Table 1) show that chemotherapy regimens including CY, BU plus CY, and other chemotherapy agents plus CY have not resulted in thyroid function abnormalities in the majority of patients 13 [31-37]. Idiopathic thyroiditis developed at age 14 (10 years after HCT) in one child of 100 transplanted for severe aplastic anemia after a preparative regimen of CY (200 mg/kg) [31], and inci-

dence which is not different from the expected 1% observed in a normal population of school age children. Two studies of children given BUCY preparative regimen and marrow transplantation for acute myelogenous leukemia (AML) have shown a 9% incidence of hypothyroidism occurring at 6 years, suggesting that patients who received BUCY must be considered at risk for development of hypothyroidism and careful post-transplant evaluation of thyroid function is necessary [35]. While thyroid malignancies have not yet been observed after BUCY, longer follow-up is needed to be able to determine whether thyroid malignancies will occur.

### 9.3. Preparative Regimens Containing Irradiation

Compensated hypothyroidism and overt hypothyroidism have often occurred following TBI or TLI preparative regimens [37-42]. Among 171 children given 7.5 Gy single-exposure TLI or 7.8-10.0 Gy single-exposure TBI, compensated hypothyroidism developed in 35%, and overt hypothyroidism developed in 8.7%. These findings are in contrast to a 12% incidence of compensated hypothyroidism and 4.2% incidence of overt hypothyroidism observed among 351 children after 12.0-15.74 Gy fractionated TBI. The patients followed in these longitudinal studies where single-exposure TBI was administered have usually been followed more than 8 years after irradiation exposure, whereas those given fractionated exposure TBI have usually been followed a median of 4-6 years after irradiation exposure. Although fractionated TBI appears to result in a lower incidence of thyroid abnormalities, data from non-transplant irradiation studies has shown that a risk for development of thyroid dysfunction and thyroid malignancies may be delayed several decades. Studies have shown that the major effect of irradiation is at the level of the thyroid gland and not at the level of the hypothalamus or pituitary gland [31,34].

### 9.4. Treatment

All patients in whom overt hypothyroidism develops should receive treatment with thyroxine, but the benefit of thyroid replacement in patients with compensated hypothyroidism is controversial. Although the carcinogenic potential of thyroid irradiation has been well documented, the ability of thyroid replacement to reduce the incidence of radiation-associated thyroid carcinoma remains unproven [30,43]. Among patients in whom benign thyroid nodules developed after conventional irradiation therapy, treatment with thyroxine decreases the risk of recurrence of these nodules but does not decrease the risk of thyroid carcinoma [44]. Papillary carcinoma, toxic goiter and an adenoma have been observed between 4 to 14 years in 6 children after 10.0 Gy single exposure TBI and in at least 5 after exposure to 12-15.75 Gy fractionated TBI. All of these children had abnormal thyroid function and none had received therapy prior to discovery of the thyroid mass. The ade-

noma was found at autopsy, but the other patients were treated successfully with thyroidectomy or radioactive iodine thyroid ablation. Thus, all patients who have received TBI, TLI or TAI should be examined annually with physical examinations and tests of thyroid function and perhaps ultrasound.

### 9.5. Height Growth

Linear growth is a continuous, finely regulated process that in infancy is largely determined by nutrition, in childhood by growth hormone (GH) and in puberty by the synergistic action of GH and sex steroids [45]. The child who receives HSCT has growth disturbances that are most likely related to total body irradiation (TBI). Short stature has been defined as height below the 3<sup>rd</sup> percentile for age, however, linear growth failure may be present when a child's height growth decreases from the pre-HSCT established height channel percentile. The height standard deviation score, SD score, is the best expression of the gain or loss in height because the SD score (height minus mean height for age and sex divided by the SD height for age and sex) considers patient sex and age. Conventional chemotherapy regimens may result in subnormal growth. Studies have suggested that the intensity and duration of combination antineoplastic therapy as well as central nervous system (CNS) irradiation and patient age influence patterns of growth [46-48]. The younger the child at the time of CNS irradiation, the greater the loss of growth.

Because GH secretion is episodic, determination of GH involves use of a stimulus to enhance pituitary GH secretion followed by multiple venous blood samplings. Commonly used stimulants include exercise, sleep, or pharmacological agents such as clonidine, levodopa, arginine, and insulin. A failure to attain a normal circulating GH level after two different stimuli tests, accompanied by decreased growth rates or decreased height SD score defines classic GH deficiency. Although the 24 hour spontaneous pulsatile GH secretion test is considered by many endocrinologists to be the most physiological assessment of GH secretion, it is impractical due to the large volume of blood required. The 12-hour overnight sampling schedule is well tolerated, reliable and reproducible, but is cumbersome and requires hospitalization. Use of two different provocative stimuli, such as clonidine and arginine is most convenient and produce reliable and reproducible results in children. In adults, the most widely used provocative stimuli are insulin and arginine.

### 9.6. Chemotherapy Only Preparative Regimens

Following preparative regimens with high-dose cyclophosphamide (CY) and HSCT for aplastic anemia, normal growth rates and height SD scores have been observed in 91 children. When tested, these children had normal levels of GH. Some children with chronic GVHD treated with steroids had temporary decrease in growth velocity, but once steroid therapy was dis-

continued catch-up growth was observed.

The reported data of height growth of children treated with busulfan (BU) and CY preparative regimens is limited. In general, normal growth rates have been observed for up to eight years after HSCT [35,49-52]. In one series, 10 children have been reported who have achieved normal final adult height [53]. We have followed 36 children who received HSCT for malignant disease at a median age of 10.4 years (range 1.4-17.0). The median age of these children is currently 17.3 years (7-28.4 years). Final adult height, evaluable in 16, demonstrates that 7 girls and 9 boys achieved a median final adult height at the 50<sup>th</sup> percentile and 25<sup>th</sup> percentile respectively.

### 9.7. Total Body Irradiation Containing Preparative Regimens

Growth impairment and GH deficiency after TBI has been well documented [50,52-60]. The incidence of GH deficiency after HSCT varies widely (20%-85%) due to differences in the time of testing after HSCT, differences in preparative regimens received, inclusion of patients with and without cranial irradiation and differences in GH testing methods used. Decreased growth velocity has been observed following either single exposure or fractionated exposure TBI. Post-HSCT growth rates vary, with some observing the poorest growth rates during the first year after TBI while others observe greatest height losses occurring several years after TBI. The data demonstrate that the poorest growth rates occur among very young children and those given prior CNS irradiation. The significant decrease in patient final height SD compared to height SD at HSCT was associated with receiving a TBI preparative regimen. Children who received TBI±CNS irradiation were 6.76 times as likely to have shorter final height than those who received chemotherapy only [53].

Even though GH deficiency has been observed in up to 85% of HSCT children, less than half have received GH therapy. Retrospective studies have found final height SD of HSCT children to be significantly lower than their height at HSCT [54]. These same studies also reported that the HSCT children's final height was significantly lower than their predicted genetic height based on corrected mid-parental height [53]. Both studies concluded that GH therapy was not needed because final height SD was not more than 2 SD below the mean, or because GH treated children did not have significantly improved growth. A third retrospective study's conclusion that GH does not benefit patients is questionable because most patients received less than one year of GH therapy [60]. We have observed that once GH deficiency is diagnosed, the time lapse between diagnosis and initiation of therapy is months to over a year due to a variety of factors. Because height lost during this delay is almost never regained, timely treatment of GH deficiency is necessary to improve final height. Our studies of treatment of 42 GH deficient children suggest that GH therapy should improve

the final adult height achieved by these children with GH deficiency following HSCT. Multiple linear regression analysis adjusted for age at HSCT, gender and height SD score at GH deficiency diagnosis demonstrated that GH therapy was associated with statistically significant gains in height SD compared to GH deficient children that did not receive GH therapy.

### 9.8 Oral-Facial Growth

Irradiation to bone produces epiphyseal, metaphyseal, and diaphyseal injury which affects subsequent bone growth [61]. The effect is related to patient age at the time of irradiation as well as the site irradiated, the dose schedule, and the total dose of irradiation [62]. Young children, especially children less than 6 years of age, at the time of irradiation to the head and neck have the greatest risk for development of subsequent craniofacial and dental disturbances [63-66]. Enamel and dentin formation are disturbed by TBI due to destruction of cells during mitotic phase. Since radiotherapy destroys cells during their mitotic phase, enamel and dentin formation can be disturbed during TBI. Chemotherapy drugs are selectively toxic to actively proliferating cells by disruption of DNA synthesis and replication, RNA transcription and cytoplasmic transport mechanisms [67]. Chemotherapy and irradiation effects on dental development include tooth agenesis, complete or partial arrest of root development with thin, tapered roots, early apical closure, globular and conical crowns, dentin and enamel opacities and defects, microdontia, enlarged pulp chambers, taurodontism, and abnormal occlusion [65,68,69]. Hence, the development of secondary teeth is often affected, with delayed or arrested tooth formation, shortening and blunting of tooth roots, incomplete calcification, premature closure of apices, and dental caries. However, some children with hematological malignancies who received preparative regimens with chemotherapy alone do not have abnormalities in dental maturity or eruption of their permanent dentition [70,71].

Reduction in lower face height in HSCT patients has correlated with impaired dental development [66]. Vertical condyle growth and the alveolar and molar heights were adversely affected by pre-transplant preparative regimens. Cephalometric measurements of facial bones to evaluate facial growth before and after 10 Gy TBI and HSCT have resulted in a significant reduction in the maxilla length and mandible growth compared with measurements obtained from healthy age-matched non-transplant children. These differences were most pronounced in mandibular growth [66]. Compared with the control group, the children and adolescents in the HSCT group also had significantly reduced mouth opening capacity with reduced translation movement of the condyles diagnosed in 53% of children treated with TBI, compared with 5% in the control group [64]. Signs of craniomandibular dysfunction were found in 84% of children in the HSCT children, compared with 58% in the control group. The long-term alterations in con-

nective and muscle tissues result in changes in tissue inflammation and eventually fibrosis.

An evaluation of cranial-facial development in 16 prepubertal children (age range 1.7-11.0 years) with growth failure and GH deficiency following CY plus 10.0 Gy TBI and HSCT demonstrated a significant positive effect on growth among the 9 GH treated patients compared to 7 non-GH treated patients [63]. Another study demonstrated improvement in vertical growth of the condyles, suggesting that condylar cartilage is the most likely site of mandibular growth activity [72]. These observations support the hypothesis that GH most likely encourages longitudinal bone growth both directly, by stimulating differentiation of epiphyseal growth plate precursor cells, and indirectly, by increasing the responsiveness to IGF-I [73]. Treatment with GH, however, did not improve the disturbed root development of the teeth. Thus, there does not appear to be a stimulating effect of dental development on growth of the alveolar process.

Little data are available for the use of orthodontic treatment for children who have dental growth disturbances after TBI and HSCT. A retrospective study of 10 children has demonstrated that orthodontic treatment plans were modified to reflect the patient's medical condition, but in general, the orthodontic treatment did not produce any harmful side effects, even though most treated children exhibited severe preexisting disturbances in dental development [74]. Nine of the 10 patients had severe disturbances in dental development with short v-shaped roots, premature apical closure, enamel disturbances, microdontia and aplasia. The most severe disturbances were found in children less than 5 years of age at the time of 10 Gy TBI. The strategies used to cope with the severe problems of dental growth disturbances included using appliances that minimized the risk of root resorption, using weaker forciers, terminating treatment earlier than normal and choosing the simplest method for treatment needs. In general the lower jaw was not treated. The treatment was judged as unsatisfactory in 4 of the 10 patients. The orthodontists compromised by leaving untreated residual proximal spaces, proclinated upper incisors, and overbite, and terminated an activator treatment, leaving a residual overjet. Further studies of orthodontic outcomes are needed.

### 9.9. Puberty

Puberty is a transitional stage from a sexually immature to a sexually mature state which is accompanied by significant changes in gonadal and growth hormonal activity, development of secondary sexual characteristics and increased growth velocity. Pubertal development correlates with osseous maturation measured by bone age. Normal pubertal growth rate is 1.5-2 times greater than prepubertal growth rates [75]. An intact hypothalamic-pituitary-gonadal axis is required for initiation and completion of puberty. In the absence of pubertal sex hormone secretion, the increased growth velocity associated with the pubertal growth spurt is substantially

blunted, and development of secondary sexual characteristics is delayed or absent. Gonadal hormone production and germ-cell viability are affected by high doses of alkylating agents and irradiation, with variables related to patient age, sex, and type and dose of therapy [76]. Azoospermia develops in prepubertal boys who have received a cumulative dose of more than 350 mg/kg CY, whereas doses of 200 mg/kg or less result in minimal alteration of spermatogenesis. The total dose of BU on the prepubertal testes is not known, but is likely to be at least 12 mg/kg. Irradiation to the prepubertal testes results in damage to the germinal epithelium that does not become apparent until after puberty. Boys who have received more than 24 Gy testicular irradiation have delayed or arrested development of secondary sexual characteristics, with elevated gonadotropin and low testosterone values. Primary ovarian failure usually occurs following total cumulative doses of more than 500 mg/kg CY to prepubertal girls [50]. No data are available regarding BU or irradiation on the prepubertal ovary.

#### *9.10. Chemotherapy Preparative Regimens*

Following 200 mg/kg CY and BMT for aplastic anemia, 29 girls and 27 boys who were prepubertal at time of administration of CY have now been followed long enough to be more than 12 years of age and evaluable for pubertal development. Age-appropriate normal pubertal development occurred in 26 girls and 24 boys, and 3 girls and 3 boys had delayed pubertal development. Among the 26 girls with normal pubertal development, menarche occurred at a median of 12.5 (11-16) years of age and the three with delayed development had menarche occur between 16-19 years of age. Twenty of these formerly prepubertal girls have given birth to 31 normal children. The three boys with delayed pubertal development had chronic GVHD and did ultimately develop normal secondary sexual characteristics with normal LH, FSH and testosterone. Eight of these formerly prepubertal boys have fathered 20 normal children.

Gonadal function after 14-16 mg/kg BU plus 200 mg/kg Cy and allogeneic BMT for thalassemia has been reported for 30 prepubertal patients (15 girls, 15 boys) who ranged in age from 9.3-17.2 years [52,77]. Thirteen girls had evidence of primary ovarian failure, with elevated gonadotropin levels, and two patients had hypogonadotropic hypogonadism. All girls had low estradiol levels both before and after transplantation. Among the 15 boys, post-transplant LH and FSH concentrations were within normal limits. However, after gonadotropin-releasing hormone stimulation, three had normal responses, 2 had elevated FSH responses, and 10 had low responses. These gonadal function results must be interpreted with caution, however, because patients with thalassemia treated with chelation and transfusion therapy frequently show delayed or absent puberty.

Pubertal development has been recently evaluated among leukemia children who received 16 mg/kg BU

plus 120-200 mg/kg CY [35,51,52,78,79]. Combined data from these several studies demonstrates that among 29 prepubertal girls now evaluable, 22 (76%) have had delay or non-progression through puberty and required hormone supplementation for pubertal development. The studies also demonstrate that among prepubertal boys now evaluable approximately 50% have developed elevated serum gonadotropin levels but have not required sex steroid therapy to promote development of secondary sexual characteristics [51,52,80]. Continued careful follow-up of leukemia children who have received transplant preparation with BU plus CY is needed before conclusions can be reached regarding the effect of BU on the prepubertal gonad.

#### *9.11. TBI Preparative Regimens*

Development of secondary sexual characteristics among children who were prepubertal at the time of TBI administration who are now more than 12 years of age has been evaluated in 24 girls and 31 boys after 10 Gy single fraction TBI and 77 girls and 77 boys after 12-15.75 Gy fractionated exposure TBI. Following 10.0 Gy single exposure TBI, 61% of girls and 81% of boys have delayed development of secondary sexual characteristics and elevated LH, FSH and low sex hormone levels. After fractionated exposure TBI, 40% of girls and 52% of boys have delayed development. All 30 boys who had received 18-24 Gy testicular irradiation for testicular leukemia in addition to TBI have primary gonadal failure and required testosterone therapy to promote development of secondary sexual characteristics. However, 68% of the boys (13 of 25) who receive 400 cGy prophylactic testicular irradiation in addition to fractionated TBI develop normally through puberty [52,81].

Development of secondary sexual characteristics must be monitored carefully after patients reach 10-11 years of age and Tanner Developmental Scores must be determined annually. Because production of sex hormones is necessary for promotion of the pubertal growth spurt in addition to promoting sexual maturation, children with evidence of gonadal failure and delayed development of secondary sexual characteristics may benefit from supplemental hormones. This supplementation should be administered under the guidance of a pediatric endocrinologist and doses of sex hormone treatment should begin low with gradual increase to simulate natural hormone production and to prevent premature advancement of bone age. Patients with normal pubertal development, normal gonadotropins and sex hormone production should receive appropriate sexual behavior counseling as pregnancy may occur.

#### *9.12. Gonadal Function after Puberty*

Alkylating agent therapy administered to adult women may impair reproductive function [82]. Ovarian atrophy has been observed following treatment with BU [83]. After CY, ovarian biopsies have demonstrated loss of

ova, which suggests that CY acts directly on the oocyte [84]. The reversibility of this loss of ova is related to patient age and the total dose of CY received. Because CY acts by first-order kinetics and the number of oocytes normally decreases steadily with increasing age, equivalent drug doses in older patients whose ova are more depleted than those of younger patients may explain why the likelihood of infertility is increased in older women. A cumulative total dose of 5.2 Gm CY given to a 40 year old woman will result in ovarian failure, whereas a cumulative total dose of 20 gm given to a 25 year old woman is needed to produce ovarian failure [84].

The predominant gonadal lesion after alkylating agent therapy in adult men is localized to the germinal epithelium [85]. Testicular biopsies from men treated with CY have demonstrated Sertoli cell damage, with germinal aplasia and absent spermatogonia and spermatozoa [86,87]. This level of damage is usually reflected in an elevated FSH level and azoospermia. Leydig cell function is spared, as evidenced by normal LH and testosterone levels. The degree of testicular function compromise is related to total dose of CY, but age does not appear to be a factor. Azoospermia develops in patients who receive more than a cumulative total dose of 18 gm CY, but oligospermia develops in those who receive less than 250 mg/kg CY given as low doses of CY for short periods. This condition is often reversible. Recovery of spermatogenesis may occur after a period of a year or more.

Impairment of ovarian function following irradiation to the ovary is related to the age of the woman at irradiation, more precisely, the number of oocytes remaining at the time of irradiation [88]. In women less than 40 years of age, doses of 800 cGy result in 70% becoming permanently sterilized. Fractionated irradiation doses of up to 20.0 Gy results in more than 50% of women 20-30 years of age developing ovarian failure [89].

Studies of the irradiated adult testes demonstrate that the magnitude and duration of suppression of spermatogenesis depends on the dose administered [89,90]. As little as 0.3 Gy to the testes has resulted in germinal epithelial damage, decreased sperm counts, and increased FSH levels. Leydig cell function is usually spared with normal LH and testosterone levels. Leydig cell damage does not occur until higher doses of irradiation, and even then the testosterone levels remain near normal. After doses of 200-300 cGy, FSH levels and sperm counts return to normal after 3 years, but after doses of 400-600 cGy, testicular function does not return to normal until 5 years later. When irradiation is administered in fractionated exposures, the effect may be more profound than when it is administered as a single exposure. The total irradiation dose above which recovery never occurs has not been established, but few patients have been documented to have recovery above doses of 800 cGy.

## 10. Chemotherapy Preparative Regimens

### 10.1. Women

Ovarian function has been evaluated in 103 women between ages 13 and 48 years at time of receiving 200 mg/kg CY and BMT for aplastic anemia [52,91-96]. All women had normal menstrual periods prior to CY administration. Follow-up studies with measurements of LH, FSH and estradiol as well as histories of menstruation, constitutional symptoms related to menopause, and hormone replacement therapy administered were obtained. All women developed amenorrhea for varying lengths of time after CY administration. Ovarian function recovery occurred in 54% at a median of 9 (3-36) months with a return of normal gonadotropin and estrogen levels and normal spontaneous menstruation. Patient age at the time of receiving CY appears to be an important factor with nearly all women less than 26 years of age having evidence of ovarian function recovery. Those who do not recover normal estradiol levels benefit from receiving estrogen/progesterone cyclic hormone supplementation. Chronic GVHD was not a factor associated with ovarian recovery, but may be a factor in normal sexual function due to dry vaginal mucosa [97].

Following BU plus CY preparative regimen 73 women have been evaluated for return of ovarian function [52,98]. One of these women has had return of normal gonadotropin and estradiol levels and menstruation. The remainder have primary ovarian failure with LH and FSH levels elevated in the menopausal range and have low estradiol levels. Many of these women have symptoms of ovarian failure which may be controlled with cyclic estrogen/progesterone therapy.

### 10.2. Men

Testicular function has been evaluated in 109 men between 13 and 52 years of age when given 200 mg/kg CY and BMT [52,92,98]. Follow-up studies demonstrated that Leydig cell function was normal in more than 95% with normal LH and normal testosterone values. Sertoli cell function was normal in 61% with normal FSH levels suggesting normal spermatogenesis. Documentation of the probability of spermatogenesis and quality of the sperm was not able to be evaluated due to lack of specimen submission. In the 12% who did submit semen for analysis, sperm motility was normal as were sperm counts.

Following a preparative regimen of BU 16 mg/kg and CY 120 or 200 mg/kg, 46 men have had testicular function evaluated between 1-5 years after BMT [52,92,98]. Leydig cell function was normal for the majority with normal LH and testosterone levels, but Sertoli cell function was impaired with elevated FSH levels and azoospermia for 75%. Eight of the men have had return of testicular function defined as high normal FSH levels and low normal sperm counts.

## 11. Total Body Irradiation Preparative Regimens

### 11.1. Women

A total of 532 women who were between 13-50 years of age at the time of exposure to 10 Gy TBI in a single setting or 12-15.75 Gy fractionated TBI have had ovarian function evaluated from 1-14 years (median 4) after BMT [52,92,93,98-103]. All of these women were menstruating prior to initiation of the preparative regimen. After TBI, all women developed primary ovarian failure with elevated LH and FSH levels, low estradiol levels and amenorrhea. Between 3 to 7 years (median 5 years) after TBI, 53 women demonstrated ovarian recovery with spontaneous return of normal LH, FSH and estradiol levels and spontaneous menstruation. Sixteen of the 59 women given 10.0 Gy TBI, 26 of 270 women given 12.0 Gy TBI and 11 of 203 given 14-15.75 Gy TBI have recovered. In order to minimize the contribution of lack of estrogen to osteoporosis and to control systemic symptoms, it is recommended that all women receive cyclic hormone therapy beginning approximately three months after TBI.

### 11.2. Men

After BMT with TBI-containing preparative regimens, a total of 463 men have been evaluated for return of testicular function between 1-12 years [50,92,98]. In general, Leydig cell function was preserved, with normal LH and testosterone levels, but Sertoli cell function was damaged as evidenced by elevated FSH levels in the majority of men. Among the 71 men evaluated after 10 Gy TBI, 14 recovered testicular function as did 37 of those given 12.0 Gy TBI and 30 of the 166 given 14-15.75 Gy TBI. A high incidence of sexual dysfunction following TBI for malignancy in 51 men showed that more than half had chronic GVHD [104].

## 12. Pregnancies

Both alkylating agents and irradiation are mutagenic with the potential of injury to germ cell chromosomes. Fertility has been shown to be reduced when alkylating agents are combined with irradiation below the diaphragm [105,106]. Thus children born to patients who recover gonadal function and who may be fertile, may also be at increased risk for development of genetic diseases and congenital anomalies [107,108]. However, children born to long-term survivors of conventional chemotherapy for childhood cancer do not have an increased incidence of congenital anomalies, but survivors who received abdominal irradiation have a higher risk of spontaneous abortions and the babies tend to have lower birth weights [106-109]. Irradiation may reduce the elasticity of the uterine musculature and/or produce uterine vascular damage [110,111].

In general pregnancies occurring among patients who have received marrow transplantation have been limited

to case reports which include limited information regarding the actual pregnancy and few details other than a live birth [50]. An analysis of 41 women who had 72 pregnancies and 35 men whose partners had 63 pregnancies has shown that former BMT women, especially those who received TBI, are at high risk for spontaneous abortion, preterm labor and delivery of low birth weight infants. Minor congenital anomalies were observed in 2 of 44 (4.5%) infants of CY female patients (ventricular septal defect and congenital nevi), 6 of 51 (11.8%) infants of partners of CY male patients (ventricular septal defect, congenital hip disease, eczema at birth) and none of the infants of TBI patients [98]. This incidence is not different than the minor congenital anomalies identified at birth in the general population of 3.8% to 14.8% nor the 8.7% and 11% frequency observed among children of patients treated for childhood cancer. These data regarding congenital anomalies in the offspring born to former BMT patients are limited by the small numbers of children born and the few observed congenital anomalies which may preclude the observance of a biologically significant change in mutation rate as manifest by a change in recognizable single-gene defects.

## 13. Ocular Late Effects

The most common late ocular complication of HSCT include cataracts and ocular sicca syndrome, sequelae of TBI and chronic GVHD.

## 14. Cataracts

The lens of children who have receive HSCT has been exposed to a variety of agents that have the potential to cause cataracts including antineoplastic therapy [112], irradiation to the head and neck [113], steroids [114,115]. Following TBI, posterior subcapsular cataracts are first evident by slit lamp evaluation about 1 year after HSCT and most are visible by 3-4 years [116]. Following 9.0-10.0 Gy single exposure TBI, 80% of patients develop cataracts at 5-6 years, however after  $\geq 12.0$  Gy fractionated TBI, 30%-50% develop cataracts. A recent large study from the European Bone Marrow Transplant group showed that the incidence of cataracts after TBI was related to the number of fractions received. Patients who received TBI administered in 6 or more fractions had a cataract incidence of approximately 10% [117]. Following chemotherapy only preparative regimens and no prior cranial irradiation, the incidence of cataracts is 12-20%, almost exclusively related to the use of steroid therapy [116,118].

## 15. Ocular Sicca Syndrome

The development of dry eyes associated with insufficient tear production is seen in about 33% of patients with and about 10% of patients without chronic GVHD. Late onset sicca syndrome in patients without chronic GVHD is usually a result of TBI and occurs

more frequently in older patients, females and after single fraction TBI [119]. Untreated sicca syndrome can be associated with infection, scar formation and corneal damage. In patients with clinical extensive chronic GVHD, systemic immunosuppression is essential. Topical glucocorticoids or immunosuppressive eye drops are infrequently used. Interruption of nasolacrimal drainage to prolong exposure of the cornea to lacrimal fluid should be utilized for patients with significant sicca syndrome. Nasolacrimal duct puncta can be plugged temporarily or permanently to improve symptoms of dry eyes.

## 16. Secondary Malignancies

Many steps in the developmental pathway for malignant tumors remain elusive, but etiological factors have been identified from the study of genetic disorders (Fanconi anemia, iatrogenic or inherited immunodeficiency) viral infections (Epstein-Barr virus), and epidemiological studies of survivors of Hiroshima, Nagasaki and Chernobyl nuclear exposure [120-123]. Initial preclinical marrow transplant studies in dogs and monkeys demonstrate that malignancies occur significantly more frequently in irradiated animals rescued with autologous or allogeneic marrow relative to non-transplant controls [124,125]. Thus, it should not be surprising that new neoplasms occur in patients after HSCT when one or more of these potentially overlapping risk factors are present.

## 17. Solid Tumors

Two large studies reported the incidence of solid tumors and post-transplant lymphoproliferative disease based on data collected from more than 14,000 patients by the IBMTR and over 4,000 patients by the Seattle group [126,127]. Using these combined data a separate analysis of 3,182 children transplanted before age 17 years for acute leukemia has been reported [128]. TBI-based preparative regimens were given to 87% of children and BUCY regimens were given to 10%. A 34-fold increased risk of solid tumors was found in these children. There were 25 children who develop solid tumors at a median of 6 (0.3 to 14.3) years after HSCT. The cumulative incidence was 1.7% at 10 years and 3.9% at 15 years. Multivariate analyses showed an almost 4 fold greater risk for children 0-9 years of age compared to children 10-16 years of age ( $P=0.0005$ ). Children who received high dose TBI were 3 fold more likely to develop solid tumors than children who received no or low dose TBI ( $P=0.03$ ). Brain and thyroid tumors accounted for more than half of the solid tumors.

## 18. Summary

The successful use of HSCT as a definitive therapy for adult and childhood malignancies, bone marrow failure syndromes and inherited metabolic and immuno-

deficiency disorders has led to an increasing number of patient who survive long-term. The longest survivors have now reached 30 years after HSCT. These long-term survivors have taught us about the many delayed effects that may be observed, particularly those effects related to transplantation biology and those related to the preparative regimens. Chronic GVHD is unique to allogeneic transplant recipients and is an important cause of late morbidity. Attention to details of evaluation of patients at 3-4 months after transplant is important to making early diagnosis and the timely institution of therapy. Prolonged treatment courses must be anticipated. The increasing use of alternate donors (donors other than HLA matched siblings) and the use of peripheral blood stem cells has resulted an in increased incidence of chronic GVHD. Hence, attention to this problem and development of new therapeutic approaches is necessary. Delayed effects of the preparative regimen on the growth and development of children, gonadal function among post-pubertal patients and the impact of secondary malignancies are becoming more apparent with increasing numbers of individuals surviving longer after HSCT. Patients must be aware that long-term follow-up for years after HSCT is necessary to effectively diagnose and treat the delayed complications as they arise. With this attention to detail, the lives of our surviving patients can be immeasurably improved.

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