

# In Utero Hematopoietic Stem Cell Therapy

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In utero hematopoietic stem cell transplantation (IUHSCTx) is a promising approach for the treatment of a potentially large number of fetuses affected by congenital hematologic disorders. With technical advances in prenatal diagnosis and fetal intervention, the majority of these diseases can now be diagnosed early in gestation, allowing consideration of prenatal treatment. It, therefore, stands to reason that there is increasing interest in performing in utero hematopoietic stem cell transplantation at many centers around the world. Although the approach remains experimentally promising, expansion of clinical application will depend on improved understanding of the biological barriers to engraftment in the fetus as well as on the development of effective clinical strategies based on the hematopoietic biology of individual disorders.

**Key Words:** In utero, hematopoietic stem cells, transplantation

## INTRODUCTION

In utero hematopoietic stem cell transplantation (IUHSCTx) is a theoretical alternative to postnatal transplantation (SCT) for the treatment of congenital hematologic disorders that can be cured by SCT, and can be diagnosed early in gestation. The early gestational fetal environment is a therapeutic milieu that is rarely considered. However, its potential is staggering when considered from the perspective of converging technologies in modern medicine. The combination of advances in maternal screening for fetal disease, progress with molecular diagnosis of genetic abnormalities, gene chip technology, and rapid progress with the human genome project, make it exceedingly likely

that within the next decade, nearly all human genetic disease will be diagnosed early in gestation from fetal cells or fetal DNA in maternal blood. The early gestational diagnosis of a disease will create management options of termination, treatment in utero, or treatment after birth. For non-anatomic disorders prenatal treatment options will include cellular therapy. Therefore if significant advantages for fetal treatment over postnatal treatment exist, many families that decide to continue pregnancy will be candidates for consideration of prenatal stem cell transplantation.

Realization of the full potential of SCT for the treatment of genetic diseases continues to be limited by a critical shortage of immunologically compatible donor cells, the inability to specifically control the recipient or donor immune response, and the requirement for recipient myeloablation to achieve engraftment. The price of human histocompatibility leukocyte antigen (HLA) mismatch remains high; the greater the mismatch, the greater the incidence of graft failure, graft versus host disease (GVHD), and delayed immunologic reconstitution. In combination, these problems remain prohibitive for the majority of patients who might benefit from SCT. A theoretically attractive alternative, that can potentially address many of the limitations of SCT, is IUHSCTx.

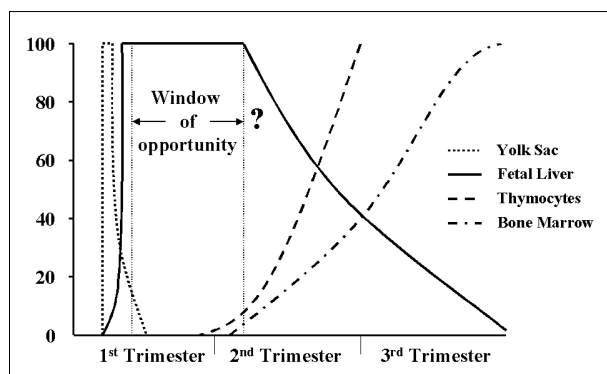
Although increasing experimental and clinical experience exists, expansion of clinical application will depend on improved understanding of fetal transplant biology and the development of new strategic approaches for the therapeutic application of IUHSCTx, in a variety of clinical circumstances.

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## THEORETICAL BASIS FOR IN UTERO HEMATOPOIETIC STEM CELL TRANSPLANTATION

The rationale for IUHSTx is based on normal developmental ontogeny (Fig. 1) and is supported by 2 major assumptions. First, the concept of fetal tolerance is that the early gestational fetus is immunologically immature and uniquely tolerant to foreign antigen. Fetal immunologic tolerance was first experimentally tested in the classic experiments of Billingham and Medawar characterizing the phenomenon of "acquired immunologic tolerance".<sup>1</sup> There is now a large body of experimental evidence for the central role of the fetal thymus in self recognition and tolerance for foreign antigen.<sup>2,3</sup> The details of thymic processing are still being elucidated but the critical events have now been defined. Thymocytes are positively selected for recognition of self Class I or Class II major histocompatibility complex (MHC) antigens which are presented by thymic



**Fig. 1.** Schematic of normal hematopoietic and immunologic ontogeny. The window of opportunity is the optimal theoretical period for engraftment of donor cells. The limits of this window in humans are poorly defined and the late limit may be extended indefinitely in circumstances of genetic T-cell deficiency (SCID).

epithelial cells of thymic stromal origin. Lack of MHC recognition results in programmed cell death. Thymocytes that recognize self MHC are then negatively selected by high affinity recognition of "self" antigen in association with self MHC. Self-antigen presentation is directed by thymic dendritic cells which are derived from hematopoietic stem cells (HSCs). The end result is a repertoire of single positive (CD4+ or CD8+) functionally competent lymphocytes that recognize foreign antigen and emerge from the thymus into the peripheral circulation of the fetus at around 12 - 14 weeks gestation.<sup>4</sup> Theoretically, transplantation of foreign cells prior to completion of the thymic selection process would result in processing of foreign antigen as "self" with secondary specific tolerance on the basis of clonal deletion. The combined advantages of receptivity for engraftment and specific tolerance induction could avoid most of the current problems related to postnatal bone marrow transplantation. In addition, treatment of the disease prior to its clinical manifestations would avoid many of the quality of life issues that currently exist with postnatal therapies (Table 1). The second major advantage provided by normal developmental ontogeny is a rapid expansion of fetal hematopoiesis, making the fetal recipient receptive to the engraftment of transplanted cells. During development, hematopoiesis goes through a sequence of migrational events, from the yolk sac and/or periaortic splanchnopleure, to the fetal liver, and finally to the bone marrow.<sup>5,6</sup> The transition of hematopoiesis from the fetal liver to the bone marrow is particularly interesting from a potential therapeutic perspective. The fetal liver becomes a hematopoietic organ precipitously at 5 - 7 weeks gestation. There is then a prolonged period of transition from fetal liver to bone mar-

**Table 1.** Advantages of in Utero Hematopoietic Stem Cell Transplantation

Advantage	Effect
Immunological tolerance	No HLA-restriction/Immunosuppression
BM space available	No myeloablation
Sterile "Isolation"	No post-transplant isolation
Proliferative environment	Potential competitive advantage-normal cells
Preempts clinical disease	Avoids complicating morbidity/suffering

row derived hematopoiesis which ultimately becomes the predominant source of definitive hematopoiesis (after 34 weeks gestation). During this time the bone marrow compartment is exponentially expanding in both size and cellularity, suggesting that new sites for hematopoiesis are constantly forming and being occupied by migrating HSC. Thus engraftment of donor HSC should be possible on a competitive basis. This concept of competitive population of forming hematopoietic niches is fundamental to IUHSTx and is one of the conceptual differences that separate prenatal from postnatal bone marrow transplantation (BMT). In postnatal transplantation, myeloablation is generally used with the ultimate aim of replacement of host hematopoiesis. In prenatal transplantation, engraftment occurs by competitive population of available sites. The goal is an adequate level of mixed chimerism to clinically ameliorate a disease.

## EXPERIMENTAL SUPPORT FOR IUHSTx

The best supporting evidence that IUHSTx might work remains an "experiment of nature" first described by Owen in 1945.<sup>7</sup> He observed that dizygotic cattle twins that share cross placental circulation were born chimeric for their sibling's blood elements. This state of "mixed chimerism" persists for life and is associated with donor specific transplantation tolerance. Natural chimerism has been observed in other species as well, most notably, humans<sup>8,9</sup> and cotton top tamarin (primate).<sup>10,11</sup> Interestingly, it has been observed that donor hematopoiesis in some chimeric animals can actually predominate, with the persistence of very high levels of donor-derived cells. This experiment of nature represents "proof in principle" that, under specific circumstances, allogeneic donor cells can competitively populate a hematopoietically normal recipient, with substantial and stable levels of donor cell expression.

Experiments designed to reproduce this phenomenon by the prenatal transplantation of allogeneic or xenogeneic HSC have shown that long-term multilineage hematopoietic chimerism can be achieved without evidence of rejection or the need for immunosuppression. The most successful

animal model remains the sheep. Early gestational transplantation of allogeneic, fetal liver derived, HSC into normal sheep fetuses results in a high rate of sustained multilineage hematopoietic chimerism<sup>12</sup> that persists for many years and is typically in the range of 10 to 15% bone marrow (BM) and peripheral blood donor cell expression.<sup>13</sup> The fetal sheep model is also permissive for widely disparate xenogeneic engraftment. Multilineage hematopoietic chimerism in fetal sheep has been well documented after human fetal liver derived HSC transplantation<sup>14</sup> and after transplantation of a variety of human cord blood and adult BM derived populations.<sup>15-20</sup> In addition, we have shown that chimerism in the human sheep model is caused by the engraftment of pluripotent HSC by documentation of long-term repopulation by donor cells on retransplantation into second-generation fetal sheep recipients.<sup>21</sup> In contrast to the sheep, however, other animal models have shown much greater resistance to engraftment after in utero transplantation. Although chimerism has been achieved in the normal primate,<sup>22</sup> goat,<sup>23</sup> rat,<sup>24</sup> and mouse,<sup>25-28</sup> the levels of engraftment are much lower and well below what might be expected to be therapeutic for most hematologic diseases.

In contrast to normal animal models, it is clear that under circumstances where there is a competitive advantage for normal cells, high levels of donor cell engraftment can be expected. This was first shown by Fleischman and Mintz<sup>29</sup> in studies in mutant anemic mouse strains that have a stem cell deficiency based on the absence of c-kit ( $W/W^V$ ). In utero transplantation of normal allogeneic fetal liver cells by transplacental injection at 11 days of gestation results in the rescue of severely anemic mice and reconstitution by donor hematopoiesis correlated with the degree of underlying anemia. Similarly, in the mouse severe combined immunodeficiency (SCID) model in which there is early arrest of T-cell and B-cell development, Blazar et al.<sup>30</sup> have shown lymphoid reconstitution after IUHSTx. In successfully reconstituted animals, T lymphocytes and B lymphocytes were entirely of donor origin. Recent studies in the nonobese diabetic NOD/SCID mouse,<sup>31</sup> in which there are defects in natural killer (NK) cells and antigen presentation in addition to the defects

in T- and B-cell development, confirm and expand upon these observations.<sup>32</sup> Thus, in the presence of a lineage deficiency, IUHSCTx can selectively reconstitute the defective lineage, but it appears that competitive pressure from the normal host lineages prevents high level multilineage donor cell expression.

## CLINICAL EXPERIENCE WITH IUHSCTx

There have now been many reported attempts to transplant human fetuses with a variety of hematologic disorders (Table 2).<sup>26,33</sup> Transplants have been performed by numerous investigators, for many different diseases, using a variety of transplant protocols. The only clear successes, or claims of success, have been in immunodeficiency disorders in which there is a clear selective advantage for donor cells.<sup>34-37</sup> In contrast, efforts to reconstitute fetuses with other immunodeficiency syndromes, hemoglobinopathies, Rh-disease, or glycogen storage disease have resulted in minimal or no detectable engraftment.<sup>38-47</sup> General observations from this collected experience support the presence of significant barriers to engraftment in human fetuses after IUHSCTx in diseases where there is little or no selective advantage for normal cells. The nature of this barrier is poorly understood.

## THE ENGRAFTMENT BARRIER

IUHSCTx differs in 3 major respects from postnatal SCT. First, irradiated systems are biologically different from non-irradiated systems.<sup>48-53</sup> Second, there is competition from a pre-existing, healthy, host hematopoietic compartment. Finally, there is the underlying framework of normal hematopoietic and immunologic ontogeny. Therefore, the paradigm of postnatal BMT cannot be applied to IUHSCTx. However, there are experimental models that are biologically relevant to IUHSCTx. The most relevant is syngeneic transplantation into non-myeloablated hosts.<sup>54</sup> In this model, there is no irradiation effect and host hematopoiesis is intact. However, it is a postnatal model in which donor and recipient cells are

genetically matched. In this system, host and donor elements are absolutely equal from a competitive standpoint. The second analogous model is that of allophenic mice.<sup>55</sup> In this model allogeneic cells coexist, in an immunologically tolerant system, from the embryonic stage forward. As in IUHSCTx, allogeneic cells may have genetic differences in their competitive capacity, however, there is complete developmental mixing of the cells from day 2 of gestation, and more importantly, all cells, including stroma, thymus, and other tissues are chimeric. These two model systems lend important insights into the requirements and barriers for engraftment in normal competitive systems.

### "Space" as a barrier to engraftment after IUHSCTx

Part of the accepted dogma of postnatal BMT is that the engraftment of normal recipients requires myeloablation to create "space" for the engraftment of normal cells. This concept has recently been challenged by experimental studies in syngeneic non-myeloablated hosts that document the ability to engraft donor cells without myeloablation. In this model, stepwise increases in donor cell engraftment can be achieved with repetitive large doses ( $1 - 2 \times 10^9$  cells/Kg) of syngeneic donor BM cells.<sup>54,56</sup> This suggests that there is a steady state of open receptive sites in normal BM. In this model donor cell engraftment appears quantitative at the stem cell level so that peripheral donor cell expression is determined by the ratio of donor to host HSC. This is in keeping with observations on stem cell kinetics in non-irradiated systems. Whereas in the irradiated mouse or large animal, engraftment of a single or few HSC can provide oligoclonal reconstitution, providing an "amplified" readout of engraftment,<sup>57,58</sup> studies of stem cell kinetics in normal mice,<sup>59-61</sup> and in allophenic mice<sup>62-64</sup> suggest that nearly all HSC regularly cycle and that the sum of hematopoiesis is provided by many simultaneously cycling HSC. Thus engraftment in this system of a single or few HSC would be relatively difficult to detect. In the fetus, we have assumed that "space" would be relatively available due to the rapid expansion of the fetal hematopoietic com-

**Table 2.** Clinical Experience with in Utero Hematopoietic Stem Cell Transplantation

Gestational* age (wk)	Donor cell source	Disease treated	No. cases	Postnatal outcome	Reference
11	Fetal BM	Rh disease	1	Postnatal death 32 weeks, no engraftment	38
17	Maternal TCD BM	Rh disease	1	Survived, no engraftment	44
28	Fetal liver and fetal thymus	Bare lymphocyte syndrome	1	Clinically normal, 26% donor HLA expression at 1y	34, 35, 39
26		SCID	1	Alive, engrafted by PCR for Y chromosome	
12		$\beta$ -thalassemia	1	Alive, not engrafted	
19		$\beta$ -thalassemia	1	Intrauterine death	
14	Fetal liver	Nieman-Pick Type A	1	Engraftment without benefit	45
18	Paternal TCD BM Sibling TCD BM	CGD	1	Intrauterine death	
34, 23, 25		MCLD	2	No evidence of engraftment	
		$\beta$ -thalassemia	1	Clinical status consistent with primary disease	
19	Maternal TCD BM	SCID	1	Terminated at 24 wk, No peripheral expression, no autopsy	42
26		Chediak-Higashi	1	No engraftment at birth	
18		$\alpha$ -thalassemia	1	Terminated at 24wk, donor plus cells in extramedullary hematopoiesis	
12	Maternal TCD BM	Rh disease	1	No detectable engraftment, tolerance	46
14	Fetal liver	$\beta$ -thalassemia	1	Septic abortion	41
14	Fetal liver	Hurler's disease	1	Low level engraftment, death at age 2 <sup>†</sup>	47
15	Paternal CD34 enriched	CGD	1	Alive, no detectable engraftment <sup>†</sup>	
15	Cryopreserved Fetal liver	$\alpha$ -thalassemia	1	No detectable engraftment	
13		Sickle cell disease	1	No detectable engraftment	
18		$\beta$ -thalassemia	1	No detectable engraftment	
13	Paternal CD34 enriched	MCLD	1	Intrauterine death at 20 wk, extensive donor cell infiltration	40
16	Paternal CD34 enriched	X-linked SCID	1	Alive at 44 mo, normal immune function, split chimerism	36
20	Paternal CD34 enriched	X-linked SCID	1	Alive at 3.5 mo, split chimerism	37
13	Paternal CD34 enriched	$\alpha$ -thalassemia	1	Alive at 1y, microchimerism, tolerant by MLR	43

BM, bone marrow; Rh, rhesus; SCID, severe combined immunodeficiency disease; CGD, chronic granulomatous disease; PCR, polymerase chain reaction; TCD, T-cell depleted; MCLD, metachromatic leukodystrophy; MLR, mixed lymphocyte reaction.

\*Gestational age at first IUHSTx. In many cases more than 1 transplant was performed.

<sup>†</sup>Flake AW, Zanjani ED, unpublished observations, January 1996.

partment that, by necessity, must include the rapid formation of new niches. In reality however, these niches are rapidly occupied by an excess of host HSC. Thus it appears that space in the fetal microenvironment is not particularly abundant

relative to the postnatal BM microenvironment and that a limited number of receptive sites is at least one component of the barrier to engraftment in normal recipients.

### Host hematopoietic competition as a barrier to engraftment after IUHSCTx

Successful reconstitution is dependent upon the ability of initial inoculums of donor HSC to expand into the host hematopoietic space. If donor cells have a competitive advantage, even the engraftment of a relatively limited number of donor HSC in the initial inoculum could ultimately reconstitute the recipient. In normal animal models after IUHSCTx we have observed little evidence that donor cells can expand their presence in the host milieu, except in the human/sheep model when a competitive advantage is conferred by infusion of donor species specific cytokines.<sup>14,65</sup> In contrast, in circumstances of donor cell competitive advantage, donor cells rapidly expand into the deficient compartment. The high levels of donor hematopoiesis achieved in c-kit deficient mouse strains in which there is a proliferative defect in host HSC supports this hypothesis. Mintz has documented full reconstitution in this model after IUHSCTx by one or two normal HSC.<sup>66</sup> Evidence also supports the ability of limited numbers of donor HSC to fully reconstitute specific defects in host lineage development. In the mouse SCID model<sup>67</sup> as well as in human X-linked severe combined immunodeficiency patients,<sup>36</sup> the number of engrafted HSC in the BM remains relatively low despite full reconstitution of the defective lineage. In this system, there is no selective advantage at the HSC level. Thus, the number of donor HSC does not appear to expand over time, consistent with a mechanism of reconstitution by expansion of lineage committed cells that are replenished from a limited pool of stem cells. In another recent study that is particularly relevant to this application, the syngeneic non-myeloablation model was modified by exposure of the host to minimally myeloablative radiation (100cGy). Syngeneic donor cells showed high levels of donor cell engraftment despite transplantation of relatively low numbers of cells.<sup>68</sup> Transplantation of donor cells receiving the same dose of radiation reduced donor cell engraftment to 14% of that seen with non-irradiated cells, strongly supporting the concept that it is primarily the ability of host cells to compete, rather than space, that determines

ultimate engraftment. Finally, in allophonic mice in which one strain has an HSC pool with relatively rapid cycling kinetics (DBA/2 vs. B6) the early kinetics of engraftment favor DBA/2 HSC until they become senescent and B6 derived hematopoiesis becomes predominant.<sup>69</sup> The allophonic studies demonstrate that in a chimeric microenvironment in which allogeneic cells compete, the level of expression is a function of the genetically defined competitive capacity of the HSC. To summarize, if donor and host cells are truly competitively equal, donor cell expression will quantitatively reflect the ratio of donor to host HSC. Thus a large number of HSC would need to be engrafted to provide clinically significant levels of donor cell expression and the number of receptive sites may be a critical limitation. However, under circumstances of even minimal imbalance favoring host hematopoiesis, the primary barrier to donor cell engraftment and expression will be host cell competition. Thus, it would appear that following IUHSCTx donor hematopoiesis is limited by both an inability to engraft an adequate number of donor cells, due to lack of receptive sites, and the subsequent inability of this limited number of engrafted cells to expand into the host hematopoietic compartment, due to what is probably a competitive disadvantage.

### The immune system as a barrier after IUHSCTx

The competitive disadvantage for donor cells may be multifactorial including immunologic. Since Billingham and Medawar's classic observations of "acquired immunologic tolerance" the phenomenon of fetal tolerance has been relatively accepted. Evidence is now overwhelming that the fetal thymic microenvironment plays a primary role in determination of self-recognition and repertoire of response to foreign antigen. Pre-T-cells undergo positive and negative selection during a series of maturational steps in the fetal thymus that are controlled by thymic stromal cells. The end result is deletion of T-cell clones with high affinity for self-antigen in association with self-MHC, and preservation of a T-cell repertoire against foreign antigen. Therefore, theoretically at least, introduction of foreign antigen prior to thymic processing should result in presentation of

donor antigen in the thymus with clonal deletion of alloreactive T-cells. It is important to note however that the mechanism of central thymic tolerance has been defined primarily in TCR transgenic mice. In these mice thymic maturation of lymphocytes occurs in an environment of unregulated, high level, expression of TCR with high affinity for a specific self-antigen that is expressed from the earliest to the latest stages of thymic development.<sup>70-72</sup> This is distinct from the clinical situation following IUHSCTx, where there are a large number of circulating antigens interacting with recipient TCRs that vary in affinity for donor antigen. Differences in thymic maturation of lymphocytes in normal mice from the defined mechanisms in TCR transgenic mice have been recognized.<sup>73,74</sup> In addition, there are other mechanisms of rejection including NK or B-cell mediated response, the developmental aspects of which are relatively poorly understood. Finally, it is now clear that thymic deletion is incomplete and that peripheral mechanisms of tolerance are needed to prevent autoreactive T-cells that escape thymic deletion from causing autoimmune disease. Experimental efforts to induce tolerance by prenatal presentation of antigen have had inconsistent

results.<sup>1,26,28,75-79</sup> The role of the intact immune system in the engraftment barrier for IUHSCTx is currently unknown but there is increasing evidence that fetal tolerance requires more than simply the prenatal presence of donor antigen.

### DISEASES THAT MAY CURRENTLY BENEFIT FROM IUHSCTx

It is clear that there are a large number of diseases that might be considered as targets for IUHSCTx. However, it is also clear that each disease must be considered individually and may or may not have a favorable enough biology or an adequate rationale for attempting IUHSCTx. Table 3 categorizes selected candidate diseases by rationale for IUHSCTx. In contrast to a decade ago, there is now adequate clinical and experimental information available to guide rational clinical application of this approach.

In contrast to postnatal SCT, IUHSCTx strives to create a level of mixed chimerism adequate to ameliorate the clinical manifestations of the disease. Therefore, in consideration of various diseases, two important questions must be asked:

**Table 3.** Diseases Potentially Treated by IUHSCTx

(1) Diseases that may benefit from IUHSCTx	
Rationale:	Selective advantage for donor cells
	SCID: X-linked
	ZAP 70
	Jak 3
	Adenosine deaminase deficiency
	Wiskott Aldrich syndrome
	Chromosomal breakage syndromes
	Fanconi anemia
	Bloom syndrome
(2) Diseases that may benefit from IUHSCTx in combination with minimally ablative postnatal strategies	
Rationale:	Successfully treated by mixed chimerism.
	May be a selective advantage for donor erythroic lineage
	Hemoglobinopathies
	$\beta$ -thalassemia/? $\alpha$ -thalassemia (Ed- please complete)
	Sickle cell disease
Rationale:	Minimal engraftment requirement*
	Hyper IgM syndrome
	Chronic granulomatous disease

\*May also be placed in the category (1) of diseases that may benefit from IUHSCTx.

(1) What level of engraftment would be adequate to treat a specific disease? (2) Is there reason to believe a competitive advantage for donor cells is present? From the preceding discussion, it would be unreasonable at present time to expect a conventional protocol for IUHSCTx to be successful, in the absence of either a selective advantage for donor cells or the requirement for a low level of donor cell engraftment. This considerably limits the number of diseases for which IUHSCTx, as currently practiced, can be applied with reasonable expectation of benefit.

The most biologically favorable target diseases are those that offer a prenatal selective advantage for donor cells. The best examples of diseases in this category are the SCID disorders, particularly the characterized mutations encoding the common cytokine receptor gamma chain (X-SCID). Other characterized mutations in cytokine receptor pathway (i.e., Jak 3 or ZAP-70), resulting in SCID, should also be favorable candidate diseases for IUHSCTx.<sup>80</sup> It is important to emphasize that while early postnatal T-cell-depleted haploidentical SCT is highly successful for T-cell reconstitution in these patients, B-cell function often remains deficient.<sup>81</sup> Clinical trials of IUHSCTx for SCID need to be established, and the results compared with early postnatal transplantation protocols to determine whether there is a biologic advantage favoring IUHSCTx.

Another immunodeficiency disorder in which a selective advantage for normal cells exists is Wiskott-Aldrich syndrome (WAS).<sup>82</sup> The combination of a selective advantage for normal hematopoietic progenitors and a proliferative defect in host T cells should provide favorable biology for successful IUHSCTx. This disease has been successfully treated with matched sibling bone marrow transplant,<sup>83,84</sup> but the results of mismatched marrow transplants have been poor, adding to the justification for attempting IUHSCTx.

A selective advantage for normal cells would also be expected in diseases in which somatic mosaicism and spontaneous reversion have been documented to occur. In these diseases, there is presumably a survival advantage for spontaneously corrected cells.<sup>85</sup> Such correction has been noted in adenosine deaminase deficient (ADA) SCID,<sup>86</sup> Fanconi anemia,<sup>87</sup> and Bloom's syndrome,

<sup>88</sup> the latter two of which are chromosomal breakage syndromes. This experiment of nature shows the potential for selective expansion of a small number or single clone of normal cells with correction of the disease and suggests that low level engraftment achieved in utero could eventually replace host hematopoiesis as progressive bone marrow failure occurred.

As experimental IUHSCTx has achieved at least minimal engraftment in multiple species, it would follow that diseases requiring only minimal engraftment for significant clinical amelioration might be candidates for IUHSCTx. Therapeutic or near-therapeutic levels of engraftment might be achievable by "standard" protocols of IUHSCTx in diseases such as chronic granulomatous disease (CGD) and hyper IgM syndrome. It has been well documented in animal models that the immune deficit in CGD can be corrected by a level as low as 5% of normal.<sup>89</sup> In X-linked hyper IgM syndrome, a disease caused by a mutation in the CD40 ligand on T cells,<sup>90</sup> carriers have been identified in which the normal gene has been predominantly silenced.<sup>91</sup> In these carriers even a few percent of T cells expressing the normal gene can result in normal class switching and IgG production. As discussed above, two recent attempts to treat fetuses affected by CGD with enriched paternal BM have resulted in no detectable engraftment, suggesting that to effectively treat these diseases further optimization of current IUHSCTx protocols, for donor cell subpopulations, dose, and number of transplants will be needed.

## DISEASES THAT MAY BENEFIT FROM IUHSCTx IN COMBINATION WITH POSTNATAL STRATEGIES

Although in the absence of a selective advantage only low-level chimerism can be reasonably expected after IUHSCTx, even this level of chimerism may carry with it the tremendous advantage of donor specific transplantation tolerance. This would have the clinical effect of providing a donor without antigenicity after birth. As discussed above, in the presence of immune response, there is increasing evidence that engraftment can be achieved with minimally myeloablative strat-



egies. Particularly for diseases that have been shown to be treatable by stable mixed chimerism, postnatal "booster" transplants could be performed to augment the minimal chimerism achieved in utero with relatively minimal toxicity (Fig. 2).

The hemoglobinopathies are the primary candidate diseases for this approach.<sup>92</sup> Although the hemoglobinopathies can be cured by postnatal SCT, they are rarely treated by SCT because of the associated morbidity and high mortality rates. The early gestational prenatal diagnosis of the hemoglobinopathies is well established and could potentially be applied to all pregnancies known to be at risk.<sup>93</sup> Thus, prenatal strategies warrant consideration.

The  $\alpha$ - and  $\beta$ -thalassemias and sickle cell disease (SCD), as candidate disorders for IUHSCTx, are biologically distinct and deserve separate consideration. In the case of  $\alpha$ -thalassemia-1,  $\alpha$ -globin dependent hemoglobin production (HbF, fetal hemoglobin) begins at 8 weeks' gestation.<sup>94</sup> By 10 weeks, physiological evidence of fetal anemia can be observed by ultrasound (placentomegaly). By 12 to 14 weeks, fetal hydrops (high output cardiac failure) may be observed.<sup>95</sup> During this time the fetus develops ineffective erythropoiesis in the fetal liver and abnormal sites of extramedullary hematopoiesis. The fetal hematopoietic microenvironment is hypercellular with multiple sites of extramedullary hematopoiesis and may not be optimal in terms of available receptive sites or donor cell competition. On the other hand, if normal HSC could be engrafted, they might have both a competitive advantage in

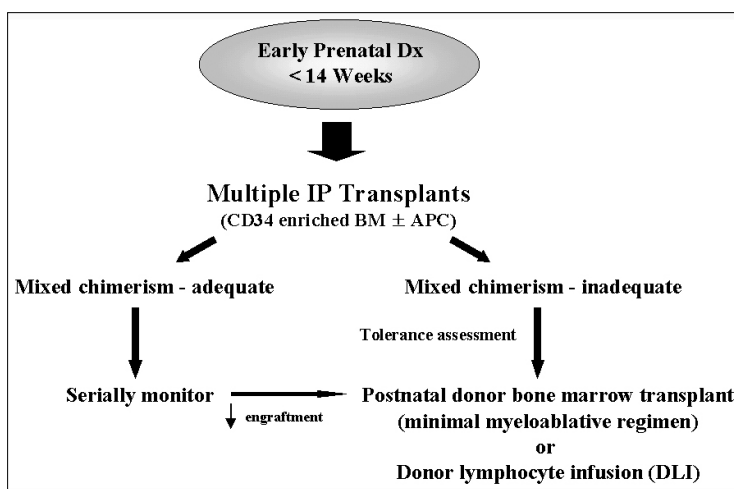
utero and an amplified contribution to peripheral circulating elements, allowing this lethal condition to be rescued before birth.

In contrast,  $\beta$ -globin dependent hemoglobin production does not occur until after birth. Production of HbF is normal in  $\beta$ -thalassemia major and SCD during fetal life; therefore, the fetal microenvironment is relatively normal in its competitive capacity. This differs dramatically from the prenatal environment present in  $\alpha$ -thalassemia described above. Ineffective erythropoiesis begins with the switch to adult hemoglobin after birth and may provide a postnatal selective advantage for donor derived erythropoiesis that begins to become apparent at 3 to 6 months of life.

There are experimental data available that support the concept of thalassemia treatment by the creation of mixed chimerism.<sup>96-99</sup> Clinical observations on patients with  $\beta$ -thalassemia<sup>100,101</sup> and SCD<sup>102</sup> who have mixed hematopoietic chimerism after postnatal SCT support the presence of a selective advantage for donor cells manifested by a peripheral amplification of donor erythroid expression in excess of donor engraftment in the bone marrow. In addition, in  $\beta$ -thalassemia there is evidence that second transplants using minimally myeloablative regimens can augment the presence of minimal chimerism.<sup>103</sup>

## DISEASES THAT ARE CURRENTLY UNLIKELY TO BENEFIT FROM IUHSCTx

This category of diseases includes hematologic



**Fig. 2.** Strategy of combined IUHSCTx and postnatal boosting (minimal ablative regimen or DLI). This strategy presupposes the use of adult-derived donor cells and is dependent on specific tolerance induction and, presumably, the establishment of at least minimal donor HSC engraftment. Methods to improve the efficacy of tolerance induction such as cotransplantation of donor-derived antigen-presenting cells (APCs) may prove useful in the future. If effective, this strategy could be rationally applied to diseases ameliorated by low or moderate levels of mixed hematopoietic chimerism and, potentially, to any disease effectively treated by postnatal SCT in which a matched sibling donor is not available.

disorders not cured by postnatal BMT, diseases that have no selective advantage and require near complete hematopoietic replacement to effect a cure, and diseases that have central nervous system (CNS) manifestations requiring large scale CNS repopulation for prevention of clinical disease. The storage diseases are a heterogeneous group of diseases caused by a deficiency of a specific lysosomal hydrolase, which results in the accumulation of substrates such as mucopolysaccharide, glycogen, or sphingolipid.<sup>104</sup> In some storage diseases, there has been no benefit or improvement in their CNS manifestations after BMT (i.e., Sanfilippo and Hunter syndromes), and there is no rationale for IUHSCTx.<sup>105</sup> Those storage diseases that have been corrected by postnatal BMT, such as Gaucher's disease<sup>106</sup> or Maroteaux-Lamy syndrome,<sup>107</sup> (minimal CNS involvement) may be candidate disorders if there is no matched donor available and if the object is minimal engraftment for tolerance induction and postnatal boosting of engraftment. Another group comprises those in which BMT has corrected the peripheral manifestations of the disease and arrested the neurologic deterioration, but not reversed the preexisting neurologic injury, i.e., adrenoleukodystrophy and Hurler's disease.<sup>105,108</sup> In these diseases, the neurological injury may begin before birth. The primary question is whether donor HSC-derived microglial elements will populate the CNS, thereby providing the necessary metabolic correction within the blood-brain barrier. In a sheep model of Ceroid-Lipofuscinosis, IUHSCTx resulting in levels of donor cell chimerism of approximately 10% in peripheral blood failed to affect the clinical course of the disease.<sup>109</sup> Maturation of the blood-brain barrier restricts access to the CNS of transplanted cells or the deficient enzyme. Although there is good evidence to suggest that CNS glial cells originate from BM, the timing of their differentiation and migration to the CNS is unclear and probably quite early.<sup>110</sup> Thus, even with relatively high levels of systemic engraftment, there may be inadequate enzyme production within the CNS to affect a cure. At the present time, it would seem that this group of diseases is the least biologically attractive group for IUHSCTx. In the future, new approaches such as CNS-directed gene therapy, or

cellular therapy<sup>111</sup> with CNS "stem cells" or "mesenchymal stem cells" in combination with IUHSCTx may be useful in the treatment of some of these diseases.

### IMPORTANT CLINICAL CONSIDERATIONS (maternal and fetal risk; ethical considerations of the fetus as a patient)

The risks of IUHSCTx can be divided into procedural and biological risks for the mother and fetus. The procedural risks are relatively well characterized and can be extrapolated from extensive obstetric experience with chorionic villus sampling (CVS), amniocentesis, and fetal transfusion and blood sampling. The maternal risk, independent of fetal loss, from these procedures (i.e., infection, hemorrhage, infertility) are negligible. The risk of fetal loss or other fetal complications from CVS has been well documented and is less than 1%.<sup>112</sup> The procedural risk of IUHSCTx before 14 weeks' gestation has been previously analyzed and is probably also less than 1% per transplant.<sup>39</sup> Therefore, using our current protocol of 3 transplants, we would anticipate a procedural fetal loss rate of no more than 4%. The biological risks to the mother and fetus include infection with bacterial, fungal, or viral pathogens from the donor cells, fetal GVHD, Rh sensitization for future pregnancies (if the donor cells are Rh-positive and mother and fetus are Rh-negative), and maternal graft-versus-host phenomenon ("autoimmune" disease) if donor lymphocytes cross the placental barrier and survive in the mother.<sup>113</sup> Many of these risks can be minimized by using adult sources of donor cells (rather than fetal liver) with careful screening for infectious disease, and scrupulous T-cell depletion. We currently limit the T-cell dose to less than  $1 \times 10^5$  CD3<sup>+</sup> cells/Kg estimated fetal weight, as a precaution against GVHD. The risk of Rh sensitization can be avoided by the use of Rh-negative donor cells, if possible; if not, sensitization can be prevented by administration of Rh-immune globulin. The risk of donor cells crossing the placenta and surviving in the maternal circulation is probably remote, but no data exist. An important concern is whether IUHSCTx would in any way

prohibit what is currently considered the optimal standard of care for a given disease after birth. At the present time there is no rationale to expect that it will, and in fact there is good reason to think that it can potentially facilitate postnatal SCT if tolerance is achieved.

Because of the experimental nature of IUHSCTx and considerations unique to fetal therapy in general, it is essential to develop an ethical framework for nondirective counseling of patients and families. Fortunately, the ethical framework for the field of fetal therapy has been relatively well developed.<sup>114</sup> The concept of the fetus as a patient, and the time when the fetus has achieved independent moral status, has been rationally addressed by McCullough and Chervenek.<sup>115</sup> They argue that the pre-viable fetus has no independent moral status, and that all arguments to that effect on religious, philosophic, or moral grounds are impossible to bring to closure. Rather, the pre-viable fetus should be considered to have "dependent" moral status. The status of the pre-viable fetus is totally dependent on the mother's autonomous decisions. It follows that only she can present the fetus as a patient for treatment. However, because of the experimental nature of IUHSCTx, she has no moral obligation to present her fetus for experimentation. Thus, the moral imperative for the investigator is to respect the pivotal status of the mother in the decision to treat her fetus. Implicit in this moral imperative is the necessity for accurate, honest, and nondirective counseling in the informed consent process. A key component of counseling is to assure that the parents understand the options, including postnatal treatment, and potential outcomes. This requires discussion with pediatric bone marrow transplant physicians or immunologists regarding conventional postnatal treatment. It is inappropriate to convince a patient who would otherwise terminate the pregnancy that an unproven, experimental procedure (i.e., IUHSCTx) is her best option.

The ethical questions in case selection are also similar in many respects to those of other fetal therapeutic endeavors and can be discussed in the context of benefit and risk to the subjects involved. Determination of risk and benefit may be somewhat arbitrary and may be influenced by a mother's experience with previous children or

family members. The assessment of benefit is complicated by the variable phenotypic expression of some diseases based on known genotype and previous family history. If risks are low, the required benefit can be relatively small and a wider spectrum of candidate diseases could be treated. If, on the other hand, risk is high, then the required expectation of benefit should be high and only a few diseases would be reasonable candidates. Until wider experience is gained with IUHSCTx, this risk-to-benefit analysis will remain imperfect, but at the present time the risks appear small relative to the potential benefits. Therefore, it seems premature to make absolute statements about appropriate candidate diseases for prenatal therapy, although application should be based on rational expectation of success based on a thorough understanding of the field. Further insights into the biology of prenatal transplantation will undoubtedly yield more successful therapeutic strategies based on IUHSCTx in the future.

## CONCLUSION

At this point in the evolution of IUHSCTx, there are more questions than answers. Widespread clinical application is premature based on the extremely limited clinical success that has been achieved. Attempts to treat fetuses based on faulty rationale, inappropriate selection, or lack of understanding of the known biology of IUHSCTx must be avoided. The biology of each disease is unique and expectations of success or failure can only be based on sound clinical investigation guided by an understanding of the relevant issues and careful selection and evaluation of patients. Clinical centers must make an active research effort to solve the remaining problems with this potentially valuable therapeutic approach. In the near future, advances in our understanding of stem cell biology, developmental ontogeny, and gene therapy may allow prenatal stem cell and gene therapy to achieve their full potential.

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