

Treatment of infants identified as having severe combined immunodeficiency by means of newborn screening



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Severe combined immunodeficiency (SCID) is characterized by severely impaired T-cell development and is fatal without treatment. Newborn screening (NBS) for SCID permits identification of affected infants before development of opportunistic infections and other complications. Substantial variation exists between treatment centers with regard to pretransplantation care, and transplantation protocols for NBS identified infants with SCID, as well as infants with other T-lymphopenic disorders detected by using NBS. We developed approaches to management based on the study of infants identified by means of NBS for SCID who received care at the University of California, San Francisco (UCSF). From August 2010 through October 2016, 32 patients with NBS-identified SCID and leaky SCID from California and other states were treated, and 42 patients with NBS-identified non-SCID T-cell lymphopenia were followed. Our center's approach supports successful outcomes; systematic review of our practice provides a framework for diagnosis and management, recognizing that more data will continue to shape best practices. (*J Allergy Clin Immunol* 2017;139:733-42.)

Key words: Severe combined immunodeficiency, newborn screening, transplantation, T cells, T-cell lymphopenia

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Severe combined immunodeficiency (SCID) is a genetically heterogeneous group of inherited defects characterized by severely impaired T-cell development combined with an inability to make specific antibodies.¹⁻⁴ Although fatal without treatment, SCID is treatable by allogeneic hematopoietic cell transplantation (HCT) or in certain genotypes by enzyme replacement therapy (ERT) or gene therapy (GT). Unless diagnosed in the neonatal period, affected infants experience severe opportunistic infections early in life. Avoidance of infection was the impetus for population-based SCID newborn screening (NBS) using newborn dried blood spots. Infants with SCID do not generate a diverse repertoire of mature T cells and consequently have absent or very low numbers of T-cell receptor excision circles (TRECs), DNA byproducts of T-cell receptor gene rearrangement.^{5,6} An exception is late-onset adenosine deaminase (ADA)-SCID in which progressive loss of T cells occurs with time. NBS for insufficient TRECs makes possible identification of SCID before infections occur, permitting optimal care of affected infants. Because NBS for SCID has become widespread and in conjunction with establishment of the Primary Immune Deficiency Consortium (PIDTC) funded by the National Institute of Allergy and Infectious Diseases and Office of Rare Diseases, National Center for Advancing Translational Sciences, National Institutes of Health, new definitions for SCID have evolved for healthy-appearing affected infants.^{3,4,7} In contrast to classical descriptions of SCID with infections,¹ the new criteria are based on laboratory parameters. Patients with typical cases of SCID have less than 300 autologous CD3 T cells/ μ L, less than 10% of the lower range of normal proliferation to the mitogen PHA, and/or detectable maternal T-cell engraftment, as well as deleterious mutations in recognized SCID genes (Fig 1).

In addition to typical SCID, TREC screening identifies "leaky" SCID, which is caused by hypomorphic mutations in known SCID genes; 26% of the SCID cases found by means of screening were leaky, as reported in an 11-program study of NBS for SCID in the United States.⁸ Patients with leaky SCID have 300 to 1500 T cells/ μ L or more but lack naive CD4 T cells expressing CD45RA. Their T cells are functionally impaired and have limited diversity, and maternal cells are not detected. A subset of infants with leaky SCID have expansion of oligoclonal dysregulated T cells, leading to adenopathy, erythroderma with

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Abbreviations used

ADA:	Adenosine deaminase
AFP:	α -Fetoprotein
AT:	Ataxia telangiectasia
CMV:	Cytomegalovirus
ERT:	Enzyme replacement therapy
GT:	Gene therapy
GvHD:	Graft-versus-host disease
HCT:	Hematopoietic cell transplantation
NBS:	Newborn screening
NK:	Natural killer
OS:	Omenn syndrome
PAP:	Pulmonary alveolar proteinosis
PIDTC:	Primary Immune Deficiency Consortium
PPD:	Postpartum depression
PTSD:	Posttraumatic stress disorder
RAG:	Recombination-activating gene
RS-SCID:	Radiation-sensitive severe combined immunodeficiency
SCID:	Severe combined immunodeficiency
TCL:	T-cell lymphopenia
TREC:	T-cell receptor excision circle
UCSF:	University of California, San Francisco

cutaneous and intestinal T-cell infiltration, hepatosplenomegaly, eosinophilia, and highly increased IgE levels, features collectively known as Omenn syndrome (OS).³

NBS also identifies infants with low TREC numbers who do not have SCID but nonetheless have few T lymphocytes in the peripheral blood, which is termed T-cell lymphopenia (TCL).^{3,9} Although most of these infants have recognized conditions, such as DiGeorge syndrome, others have TCL with no apparent underlying cause.^{10,11} Establishing a definitive diagnosis and managing these infants are new challenges for physicians, who must recognize the level of TCL that is medically significant, select diagnostic tests, and apply appropriate interventions.

Here we discuss our center's approach to infants with SCID, leaky SCID, and non-SCID TCL identified by using NBS, recognizing that individual state screening programs and providers currently use a spectrum of approaches³ and that more data are needed to identify best practices.

APPROACH TO INFANTS WITH TREC RESULTS THAT ARE NOT NORMAL

In California all infants with nonnormal TREC test results immediately have a CBC, differential, and flow cytometric analysis to distinguish the 43% of infants with confirmed TCL from the 57% with greater than 1500 T cells/ μ L, for whom no further intervention is undertaken as long as naive T cells are observed (Fig 1, right).^{8,9,11} Infants with less than 300 T cells/ μ L or with more T cells per microliter but less than 200 naive CD4 T cells, representing approximately 16% of all those with nonnormal TREC results (Fig 1, left), are immediately hospitalized because of the high likelihood of having typical or leaky SCID, respectively, or complete DiGeorge syndrome. The management of SCID at our center is discussed below. The remaining infants with 300 to 1500 T cells/ μ L but with naive CD4 T cells present (Fig 1, middle right) have multisystem syndromes or secondary and undiagnosed or idiopathic TCL. Diagnosis and management of infants with non-SCID TCL is undertaken on a case-by-case

basis and generally includes (in addition to flow cytometry) proliferative responses to mitogens, measurement of serum immunoglobulin levels, and DNA arrays to reveal genomic copy number variation.

HOW WE TREAT THE INFANT WITH SUSPECTED SCID

Infants suspected of having SCID or leaky SCID based on laboratory testing (and those identified by a family history or clinical features) are admitted to protective isolation in our hospital; any sign of infection or other abnormality is immediately pursued with cultures and treated (Table I and Appendix Table I). Our immune evaluation is listed in Table II.

Early discussions between the family and the immunology and HCT teams present information about SCID, the diagnostic approach, and expectations for hospitalization. The HCT team works with the family to identify the optimal donor. A social worker versed in SCID is involved to identify needs for support.

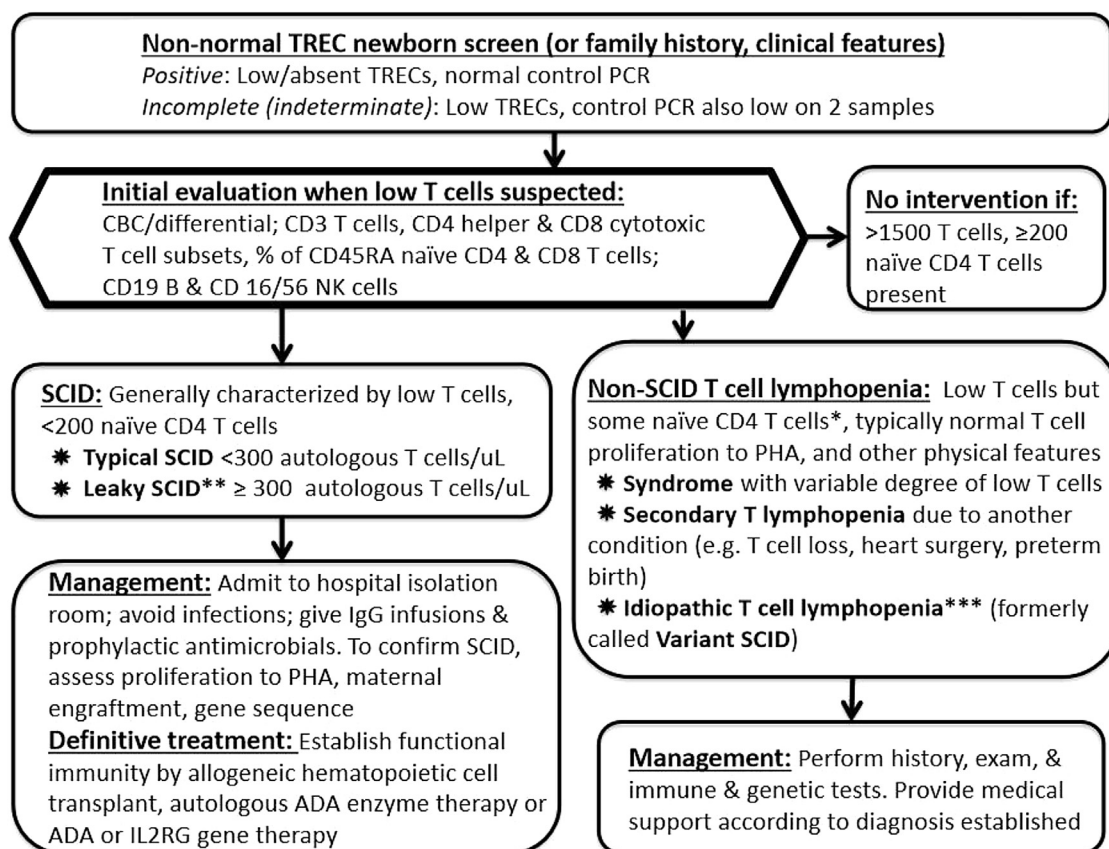
Infectious disease work-up includes assessing for cytomegalovirus (CMV) by using PCR, which can be transmitted through breast milk of CMV-seropositive mothers before confirmation of TCL. Two (6%) infants in our 32-patient cohort were infected by CMV at baseline, with breast milk from a CMV-seropositive mother the only identified potential source. Baseline lymphocyte subset confirmation and functional testing are performed, along with HLA typing of the infant and family members to identify potential HCT donors. Evidence of maternal T-cell engraftment is sought by means of analysis of maternal and infant DNA; maternal chimerism, which was detected in greater than 50% of SCID cases,¹² suggests that a maternal graft can be accepted without conditioning. An infant with CD45RO, but not CD45RA, T cells might have expansion of a limited repertoire of T cells of either autologous or maternal origin. A T⁺B⁺ natural killer (NK)⁺ lymphocyte profile suggests ADA deficiency, which is often accompanied by neutropenia; screening for ADA enzyme activity and metabolite levels is quickly accomplished before pursuing gene sequencing.

SPECIAL CONSIDERATIONS Iatrogenic anemia

Iatrogenic anemia is a concern in small infants with SCID. Blood draws are planned to minimize discomfort and use the smallest possible volume for each test. Transfusions are avoided to minimize the risks of infection and allosensitization. If transfusions are needed, only CMV-negative, irradiated, leukoreduced packed red cells are given. A standard operating procedure developed with our clinical laboratory uses specific minimum volumes for every test (see Appendix Table II).

ADA deficiency

ADA-SCID, which was diagnosed in 6 (19%) of our initial 32 infants with SCID, is a systemic metabolic disorder in which increased concentrations of purine intermediates are particularly toxic to lymphocytes but affect all tissues. Early complications of ADA deficiency included neutropenia, which was seen in 4 of our 6 infants with ADA, and pulmonary alveolar proteinosis (PAP),



*Variable can be < 200 naïve CD4 T cells. ****Omenn syndrome** is a form of leaky SCID with rash; eosinophilia; autoreactive, oligoclonal T cells; and variable CD3 T cell count which can be >1500. ***Some infants never leave this group but some move out of this category when other diagnoses are made. These infants need to be followed over time.

FIG 1. Identification of T-cell immune defects by means of TREC NBS. Primary immune defects can also be diagnosed based on a history of affected family members or clinical features.

which was seen in 2, one of whom required intubation for respiratory distress. Two others with tachypnea at diagnosis were suspected to have PAP. Bronchoscopy to obtain cultures, as well as histology, is required to distinguishable PAP from infection.¹³ Increases in liver function test results can occur with ADA deficiency but also can be a sign of a viral infection. Prompt administration of ERT with PEG-ADA has led to resolution of abnormalities over several days in our patients. Therefore, unless there is an HLA-matched sibling, we start ERT immediately at a full dose of 30 U/kg or more to achieve rapid detoxification. After T cells appear, the infants are cared for at home with intramuscular PEG-ADA injections (125 U) twice weekly. Before HCT without other conditioning, we discontinue ERT, monitoring increasing metabolite levels until approaching pre-PEG-ADA levels.

One of our ADA-deficient patients received a matched sibling HCT, but 5 others who lacked a matched related or unrelated donor received ERT for several months. After 6 months of age, 4 infants enrolled in the experimental GT protocol of Dr Donald Kohn at the University of California, Los Angeles, all with successful development of functional gene-corrected lymphocytes. GT is planned for a fifth patient.

Leaky SCID and OS

Infants with defects in any SCID gene that permit residual protein function can have more T cells than those with typical SCID; without screening, the diagnosis can be delayed many months or years. Although nearly all are identified by using TREC NBS, the mildest cases might not have low TREC numbers at birth. OS, which is caused by hypomorphic mutations in recombination-activating gene 1 (*RAG1*) and *RAG2*, requires immunosuppression while awaiting HCT, which should be implemented as swiftly as possible.³ Aggressive autoreactive oligoclonal T-cell expansion occurring within a few days to weeks of life can result in a severe scaly rash, diarrhea, and failure to thrive. Systemic corticosteroids, calcineurin inhibitors, and/or antithymocyte globulin or anti-CD52 mAb treat this condition effectively until an HCT can be performed.

Radiation-sensitive SCID

Radiation-sensitive severe combined immunodeficiency (RS-SCID) includes deficiencies of Artemis, DNA Ligase IV, DNA-dependent protein kinase catalytic subunit, Cernunnos-XLF, and nibrin (the protein associated with Nijmegen breakage syndrome). Infants presenting with abnormal NBS results generally

TABLE I. Approach to early management of a new infant with a lymphocyte subset profile consistent with SCID or leaky SCID

- Isolate in a single room in SCID treatment center; prohibit ill contacts
- Diagnose and treat any clinical abnormalities, such as respiratory distress or signs of infectious or autoimmune conditions
- Introduce social worker to help with support and services for family
- Omit live vaccines for patient or household contacts, including rotavirus vaccine
- Avoid infection:
 - Advise mother to suspend nursing while evaluating her prior exposure by using CMV IgG serology
 - If mother is CMV seronegative, encourage her to resume nursing
 - If mother is CMV seropositive and the baby is CMV negative based on blood and urine PCR, advise the mother to avoid nursing, given the risk of breast milk transmission of CMV
 - Obtain infant blood CMV PCR weekly for 4 weeks and periodically thereafter (more often if CMV is clinically suspected, such as with an increase in liver transaminase levels)
- Intravenous access needed for gammaglobulin replacement with coordination of blood draws to decrease frequency of venipuncture; establish more permanent intravenous access just before eventual HCT
- Provide nutritional support, including monitoring for iron deficiency
- Transfuse if symptomatic or hemoglobin <8 mg/dL using only CMV-negative, leukoreduced, irradiated packed red cells
- Administer immunoglobulin to maintain IgG level >800 mg/dL
- Administer palivizumab during the respiratory syncytial virus season
- Begin prophylactic fluconazole, acyclovir, and trimethoprim-sulfamethoxazole (the latter after 30 days of age)*
- Perform HLA typing on parents and full siblings to evaluate as potential HCT donors; in the absence of a matched sibling, initiate search for an unrelated adult or cord blood donor

*See [Appendix Table I](#) for prophylactic medication dosing.

TABLE II. Early clinical and laboratory assessment for a new infant with suspected SCID

- Directed history, including infections, family history, pedigree analysis, consanguinity
- Physical examination, including documentation of any signs of infection, skeletal features, and congenital anomalies associated with certain SCID variants, rash of GVHD or erythroderma of OS, or desaturation associated with PAP in patients with ADA deficiency
- Evaluate lymphocyte subsets, including T-cell CD45RA/RO determined by using flow cytometry, to confirm the initial flow cytometry done by the screening program
- Blood chemistries, albumin, liver function tests, and total bilirubin
- PCR or antigen tests for HIV, HSV, EBV, HepB, parvovirus B19, adenovirus, respiratory viruses, and rotaviruses; do not rely on serologic tests; serial maternal serology and/or PCR might suffice to rule out some of the above
- Quantitative immunoglobulins: IgG (reflects maternal transfer to baby, must be drawn before IVIG administration), IgA, IgM, and IgE
- Lymphocyte proliferation to PHA: send large enough sample to account for low lymphocyte count and inform the testing laboratory of the infant's low T-cell count
- ADA and PNP enzyme and purine metabolites (unless there is a family history or strong suspicion of another genotype)
- Maternal chimerism studies by DNA testing of polymorphic alleles
- Molecular diagnosis directed by sex and lymphocyte profile (T^+B^+ or T^+B^- and whether NK cells are present); sequence single leading candidate gene or panel of SCID genes
- SNP array (or other copy number DNA array) preferred over chromosome 22 fluorescence *in situ* hybridization for infants with cardiac anomalies or any features suggesting DiGeorge syndrome
- Unless concern for radiation-sensitive SCID exists,* perform a chest radiograph to document whether the thymus is visible and view lung parenchyma and osseous structures (In asymptomatic infants imaging can be deferred until documenting central venous line placement.)

HepB, Hepatitis B; HSV, herpes simplex virus; IVIG, intravenous immunoglobulin.

*Microcephaly and bird-like facial appearance; infants with Navajo background.

have a T^- (or T-low) B^-NK^+ phenotype because the process of recombination of T- and B-cell antigen receptor genes require nonhomologous end-joining by the DNA repair complex.¹⁴ Although RS-SCID is treatable with HCT, donor selection and conditioning must be adjusted because of increased sensitivity to typically used alkylating agents, such as busulfan, melphalan, thiopeta, or cyclophosphamide.^{14,15} $T^-B^-NK^+$ lymphocyte profiles and developmental abnormalities, such as microcephaly and bird-like facial appearance, suggest heightened radiation sensitivity, prompting radiation sensitivity testing of patients' fibroblasts, Western blotting, and/or gene sequencing (eg, *LIG4* and *NHEJ1*) to establish the diagnosis. Although these take several weeks to perform, they must be obtained before HCT if conditioning is planned. Despite this delay, it is our practice to hospitalize

these infants while the work-up is performed. Although no absolute contraindication exists for clinically indicated radiography, exposure should be minimized. Ultrasound and magnetic resonance imaging are safe alternatives.

UNIQUE PSYCHOSOCIAL VULNERABILITY OF PARENTS WITH SCID-AFFECTED INFANTS RECEIVING A DIAGNOSIS BASED ON NBS

The combination within the first month of life of the postpartum period and the infant's hospitalization for SCID treatment presents a situation of compounded vulnerability with the potential to destabilize family functioning and parental ability to provide care during and after HCT. At our institution, care

providers create an early and open dialogue about psychosocial health effects in this unique situation. Social workers interact extensively with the families during hospitalization and after discharge.

Postpartum depression

Given the high stress experienced by these new parents, special efforts are made to recognize postpartum depression (PPD) and posttraumatic stress disorder (PTSD). PPD is a significant health concern affecting 10% to 15% of US women after normal births.¹⁶ PPD has also been reported in new fathers.¹⁶ The onset of PPD is typically 2 weeks after childbirth, coinciding with the diagnosis of SCID by using NBS. Mothers who initiate or maintain breast-feeding are at lower risk for PPD, and interruption of breast-feeding can contribute to PPD.¹⁷ Thus interrupting breast-feeding while serologic status for CMV is tested adds to the stress during the postpartum period.

Posttraumatic stress disorder

Parents learning of an abnormal metabolic NBS result have reported shock as if a death in the family occurred.¹⁸ Likewise, the emotional distress experienced by parents of newborns with SCID can stem from shock, grief, guilt, and apprehension regarding losing the child. During the first weeks of hospitalization, confirmation of the SCID diagnosis and characterization of the genotype are sought through genetic testing. Additional testing for HLA typing is conducted on siblings and parents. Genetic testing is highly personal and is often associated with anxiety, fear, and guilt for passing on an inherited condition.^{19,20} Our pilot study in which parents were interviewed revealed the majority of mothers and fathers caring for infants with SCID experience PPD and PTSD (M. Dorsey, unpublished data). Thus involvement of experienced social workers and nurses is a critical component of overall care for families with SCID, with early introduction to available services and support serving as a key to decreasing stress. In addition, routine screening for PPD and PTSD can identify families in need of referral to mental health providers.

TREATMENT

Transplantation: Allogeneic and autologous gene-modified hematopoietic stem cells

Historically, single centers reported transplantation outcomes for SCID in which the contributions of specific gene defects, infections, and variations in treatment protocols could not be isolated. The PIDTC is addressing these issues by enrolling large numbers of patients from centers across North America.^{4,12} In a retrospective report, Pai et al⁴ showed that infants who received transplants before 3.5 months of age had a 5-year survival rate of 94%, as did infants older than 3.5 months but with no history of infection (90%) or whose infections had resolved by the time of HCT (82%). Children older than 3.5 months with active infection at the time of HCT and no HLA-matched sibling had the lowest survival (50%). HLA-matched sibling donors resulted in the best outcomes regardless of age or infection. Although reduced-intensity conditioning or myeloablative conditioning versus no conditioning or serotherapy alone had a higher likelihood of full T-cell reconstitution and no need for immunoglobulin

replacement therapy, the long-term effects of chemotherapy remain to be addressed.⁴ For patients infected at the time of transplantation at any age, the use of haploidentical donors without conditioning had the best 5-year survival. In a prospective PIDTC study, Dvorak et al¹² reported that the time needed for an unrelated adult donor or umbilical cord blood search delayed HCT by approximately a month compared with using a mismatched related donor. Additional studies will determine whether earlier HCT produces superior outcomes or whether closer HLA matching in unrelated donors justifies the prolonged time to transplantation in uninfected infants.

Gene correction of autologous hematopoietic stem cells has been successful for children with ADA and X-linked (*IL2RG*) SCID.^{21–28} The initial success in patients with *IL2RG* SCID was tempered by the development of T-cell leukemia caused by retroviral vector insertional mutagenesis in 5 of the initial 20 recipients.^{23,24} Current trials use self-inactivating retroviral or lentiviral vectors, which appear to have a significantly lower risk of insertional mutagenesis.²⁵ Initial GT attempts without conditioning for ADA-SCID failed; when low-dose busulfan was added as conditioning, T- and sometimes B-cell immunity was reconstituted.²⁶ In early *IL2RG* SCID GT trials without conditioning, T-cell reconstitution occurred, but B-cell and NK cell reconstitution were limited.^{25,27} Results from an initial trial using low-dose busulfan for patients with *IL2RG* SCID suggest that B and NK cells will be reconstituted.²⁸

Treatment outcomes at UCSF

Twenty-six patients with NBS-identified SCID and leaky SCID from California and 6 from other states were treated at UCSF between September 2010 and October 2016. Twenty-nine (94%) are alive and well, 19 of them more than 2 years after HCT. All 26 who are more than 6 months after HCT have reconstituted T cells, and 13 (50%) of 26 have B-cell reconstitution. Only 4 (14%) had a recognized family history of SCID. Gene diagnoses were as follows: *IL2RG*, 7; *ADA*, 6; *DCLRE1C*, 5; *IL7R*, 4; *RAG1*, 4; *RAG2*, 2; *JAK3*, 1; *RMRP*, 1; and *BCL11B*, 1.²⁸ One infant with unknown genotype (despite family exome and genome sequencing) died of refractory CMV infection present on admission. One of 5 Artemis-deficient Navajo infants died of refractory human herpesvirus 6 infection. Four patients with ADA underwent busulfan-conditioned autologous HCT with gene-corrected CD34⁺ cells; all are reconstituted with T cells, and 2 of 4 who are more than 6 months after GT have B-cell reconstitution.

Our approach to selection of the best donor and conditioning regimen for patients with typical SCID is summarized in Fig 2. The guiding principles are to (1) use the donor associated with the lowest risk of graft-versus-host disease (GvHD), (2) minimize the duration of pre-HCT lymphopenia and risk for infections, and (3) use the least amount of chemotherapy possible to ensure donor T-cell engraftment but minimize the risk of short- and long-term toxicity. Unless there is an urgent need to proceed with HCT, such as CMV infection, busulfan is not given to infants less than 3 months old. This approach accepts that booster HCT might be needed and that B-cell immunity will typically not be restored when a donor other than a matched sibling is used, except in patients with intrinsically normal B cells (eg, *IL7R* SCID), leaving many patients dependent on gammaglobulin replacement. In many patients with typical SCID, irrespective of donor type, no chemotherapy is required, although use of serotherapy might be

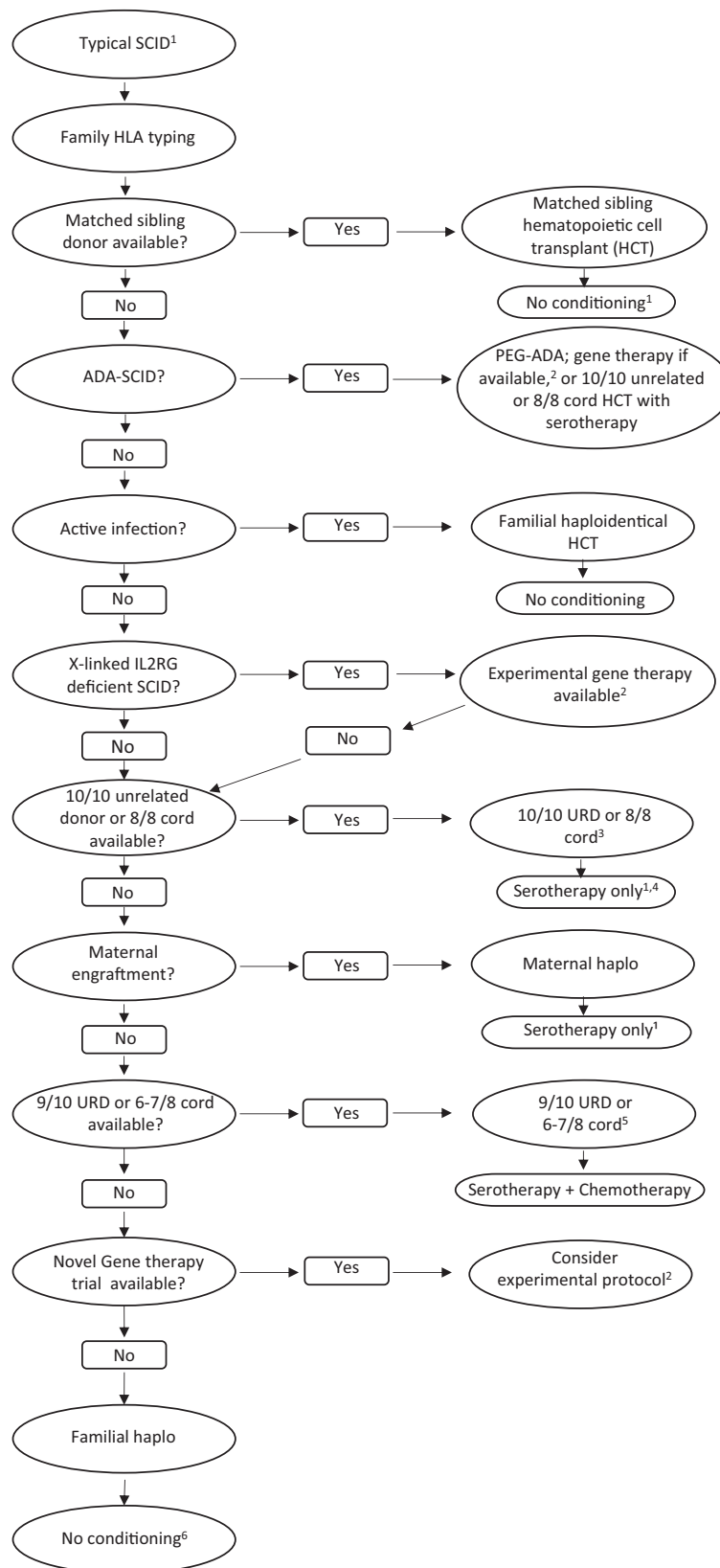
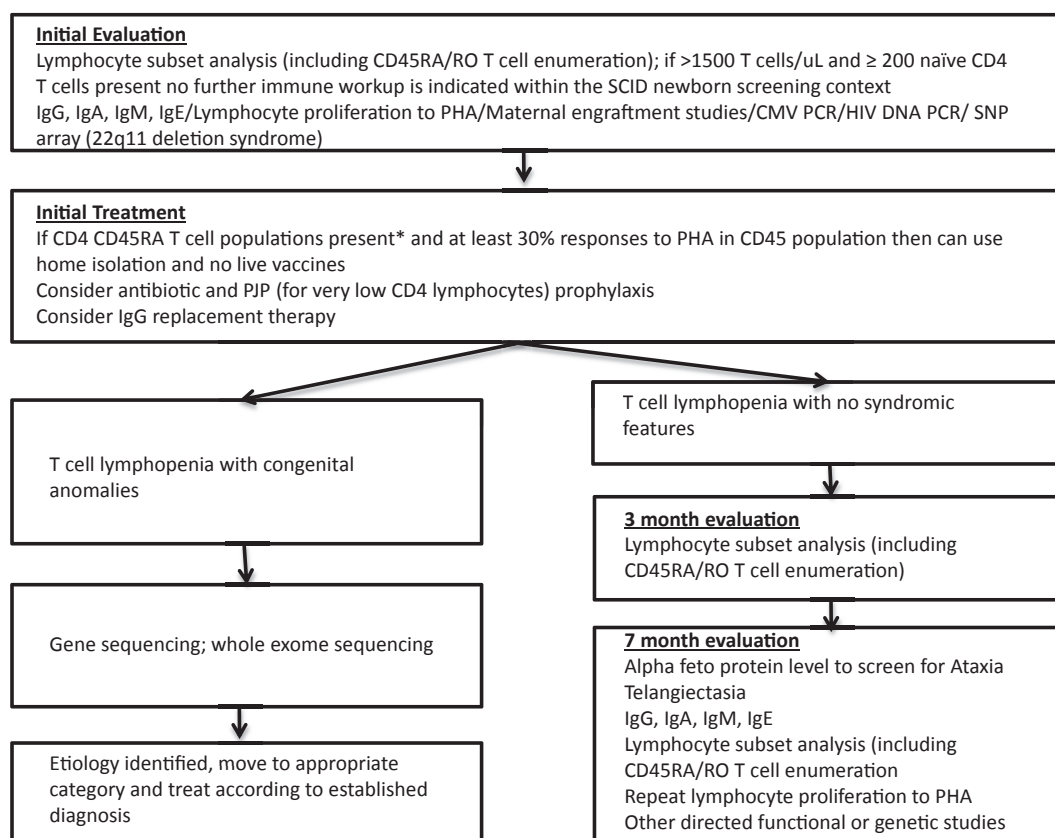


FIG 2. Donor selection and conditioning for typical SCID. ¹Excludes OS and leaky SCID, both of which are classified as atypical SCID for transplant purposes and require at least some conditioning. This also excludes RS-SCID (eg, *DCLRE1* and *LIG4*), for which donor selection and conditioning are individualized to balance risk of rejection versus chemotherapy toxicity. ²GT clinical trials should be considered for X-linked or ADA-SCID when there is no HLA-matched sibling. ³Based on availability, CMV status, donor age, and other variables. ⁴Non-radiation-sensitive T⁺B⁺NK⁺ SCID generally requires chemotherapy plus serotherapy for unrelated donor transplantation. ⁵For X-linked and Janus kinase 3 SCID, a maternal donor with serotherapy alone is preferred over a 9/10 unrelated or 6-7/8 cord donor. ⁶Consider chemotherapy conditioning for enhanced B-cell and/or T-cell reconstitution and to prevent rejection. URD, Unrelated adult donor.



*Variable can be <200 naïve CD4 T cells

FIG 3. Evaluation and management of non-SCID TCL (persistently <1500 CD3 T cells). Continued follow-up is needed to determine the degree and persistence of immune impairment, whether prophylactic therapy is needed, and whether an underlying cause can be found.

beneficial when using T cell–replete stem cell sources or when maternal chimerism is present and the mother is the donor.²⁹ For patients with leaky SCID or OS, a conditioning regimen is required. When using busulfan, we carefully monitor pharmacokinetics, which are highly variable in small infants, and modify divided doses to achieve the target cumulative exposure predicted to allow stem cell engraftment.³⁰ GvHD prophylaxis is aggressive because there is no benefit to alloreactivity. Stem cells from haploidentical donors currently undergo CD34 selection,³¹ although in the near future, alternate *ex vivo* T-cell depletion strategies are planned. With the identification of maternal engraftment as a risk factor for the development of GvHD despite CD34 selection,²⁹ low-dose rabbit anti-thymocyte globulin serotherapy has also been successfully used in the most recent patients. For unrelated donors, we also use pre-HCT serotherapy.³² Serotherapy can abrogate an early (<100 days) wave of homeostatic donor T-cell expansion, but this typically can be tolerated in an uninfected patient identified by using NBS, and naïve T-cell generation occurs at around 3 months after HCT.

POSTTRANSPLANTATION MANAGEMENT

Before hospital discharge, close attention must be given to the availability of immediate medical care, as well as T-cell immunity and infection risk at home. If there are no siblings or if siblings are attending grade school, patients with SCID are considered for

discharge when they have greater than $50 \times 10^6/\text{L}$ CD4⁺ cells and greater than 10% of the lower limit of normal CD45⁺ lymphocyte proliferation to PHA. If preschool-age siblings are in the home, they should not attend daycare or group activities, and patients' T-cell counts and proliferation need to be higher before discharge is considered.

Immune reconstitution is monitored with lymphocyte evaluation, including measurement of naïve and memory T-cell, NK cell, and B-cell subsets every 4 weeks until normal and then every 3 months for the first year. Vaccines are initiated in patients who have B-cell reconstitution when IgG replacement has been discontinued. At our institution, this is defined as total CD19 B cell counts of greater than 150 cells/ μL , normal IgA and IgM levels for age, and IgM isohemagglutinin titers of 1:8 or greater. If one of these criteria is not met, normal percentages of CD27⁺IgM⁺IgD⁺ B cells are considered. Baseline titers against vaccine antigens are obtained 3 months after the last IgG infusion and 3 to 4 weeks after administration of the primary series of inactivated vaccines. If adequate responses are demonstrated, including a normal lymphocyte proliferation to tetanus toxoid, live attenuated vaccines are given.

NON-SCID TCL WORK-UP AND MANAGEMENT

For infants with 300 to 1500 T cells/ μL , reduced but present naïve CD4 T cells (can be variable and <200 cells/ μL), and no

maternal cells (Fig 1, right panel, and Fig 3), initial immune evaluation is similar to that for SCID, but hospitalization might not be required if immunodeficiency is not profound.

Non-SCID TCL conditions evaluated by UCSF immunologists (aside from 10 cases of TCL caused by extreme preterm birth alone, which resolved in survivors) included multisystem syndromes in which T cells are variably reduced. Of 28 syndromic infants, diagnoses were as follows: 17 cases of DiGeorge syndrome/22q11.2 deletion or *TBX1* intragenic mutation (61% of syndromic TCL); 2 cases each of ataxia telangiectasia (AT), coloboma, heart defect, atresia choanae, retarded growth and development, genital abnormality, and ear abnormality (CHARGE syndrome), and trisomy-21; and 1 case each of Noonan syndrome, Kabuki make-up syndrome, Congenital, Lipomatous, Overgrowth, Vascular Malformations, Epidermal Nevi and Spinal/Skeletal Anomalies (CLOVES syndrome), Fryns syndrome, and immuno-osseous dysplasia. Of these, 1 patient each with CHARGE, CLOVES, Fryns, and Noonan syndromes died of severe congenital anomalies. Nine infants have had secondary TCL, in which T cell-generating capacity is normal but circulating T-cell counts were diminished. Causes included 2 cases of hydrops, 3 cases of severe congenital heart disease, and 1 case each of chylothorax, neonatal leukemia, and maternal immunosuppressive medication (fingolimod) taken during pregnancy for multiple sclerosis. An additional 5 infants have had idiopathic lymphopenia, with no underlying cause identified,^{8,11,12} although the syndromic infants with AT and immuno-osseous dysplasia and maternal medication were also in this category at initial presentation. Resolution of idiopathic TCL occurred in 1 infant, a fraternal twin, whereas 2 continue to be followed with low but functional T-cell counts, and 2 have been lost to follow-up. Long-term follow up is important in identifying underlying diagnoses when TCL persists (Fig 3).

It is important to remember that not all serious disorders of T cells are identified by using TREC screening; combined immunodeficiencies that are associated with intact T-cell development beyond the point of T-cell receptor gene recombination in the thymus, including Zap-70 deficiency and MHC class I and II non-expression, can have normal numbers of TRECs, even though T-cell function is severely impaired.³³

α -Fetoprotein (AFP) levels obtained before the age of 6 months are generally not helpful in the diagnosis of AT and should be interpreted with caution. It is known that AFP levels can be increased in healthy infants but trend down with age, unlike AT, in which AFP levels will remain increased.³⁴ Similarly, functional antibody responses cannot reliably be assessed before completion of the primary series of killed vaccines. Those infants with less than 1500 T cells/mL are considered immune deficient and advised to avoid live rotavirus vaccination because of the risk of acquiring diarrheal disease from the vaccine strain rotavirus.³⁵ Need for home isolation, prophylactic antibiotics, and gammaglobulin infusions are made on a case-by-case basis.

CONCLUSION

Early detection of SCID through TREC NBS creates the opportunity to provide immune reconstitution while infants are healthy and uninfected. Although NBS optimizes the chances of successful definitive treatment, newborns with SCID present new issues in management, including parental emotional difficulties, as well as defining best treatment regimens. Early detection of

non-SCID TCL also poses new challenges. Further experience and multicenter studies will establish best practices.

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What is known on this subject?

- In the new era of NBS for SCID, establishing a diagnosis and managing these infants are new challenges for pediatricians.

What does this study add?

- This article offers the first systematic approach to infants with SCID, leaky SCID, and TCL identified by using NBS.

REFERENCES

- Buckley RH. Molecular defects in human severe combined immunodeficiency and approaches to immune reconstitution. *Annu Rev Immunol* 2004;22:625-55.
- Ochs HD, Puck JM, editors. Primary immunodeficiency diseases: a molecular and genetic approach. Oxford: Oxford University Press; 2013.
- Shearer WT, Dunn LD, Notarangelo G, Dvorak CC, Puck JM, Logan BR, et al. Establishing diagnostic criteria for severe combined immunodeficiency disease (SCID), leaky SCID, and Omenn syndrome: the Primary Immune Deficiency Treatment Consortium experience. *J Allergy Clin Immunol* 2014;133:1092-8.
- Pai SY, Logan BR, Griffith LM, Buckley RH, Parrott RE, Dvorak CC, et al. Transplantation outcomes for severe combined immunodeficiency, 2000-2009. *N Engl J Med* 2014;371:434-46.
- Chan K, Puck JM. Development of population-based newborn screening for severe combined immunodeficiency. *J Allergy Clin Immunol* 2005;115:391-8.
- Morinishi Y, Imai K, Nakagawa N, Sato H, Horiuchi K, Ohtsuka Y, et al. Identification of severe combined immunodeficiency by T-cell receptor excision circles quantification using neonatal Guthrie cards. *J Pediatr* 2009;155:829-33.
- Griffith LM, Cowan MJ, Notarangelo LD, Kohn DB, Puck JM, Shearer WT, et al. Primary Immune Deficiency Treatment Consortium (PIDTC) update. *J Allergy Clin Immunol* 2016;138:375-85.
- Kwan A, Abraham RS, Currier A, Brower A, Andruszewski K, Abbott JK, et al. Newborn screening for severe combined immunodeficiency in 11 screening programs in the United States. *JAMA* 2014;312:729-38.
- Kwan A, Puck JM. History and current status of newborn screening for severe combined immunodeficiency. *Semin Perinatol* 2015;39:194-205.
- Vogel BH, Bonagura V, Weinberg GA, Ballou M, Isabelle J, DiAntonio L, et al. Newborn screening for SCID in New York State: experience from the first two years. *J Clin Immunol* 2014;34:289-303.
- Kwan A, Church JA, Cowan MJ, Agarwal R, Kapoor N, Kohn D, et al. Newborn screening for severe combined immunodeficiency and T-cell lymphopenia in California: results of the first 2 years. *J Allergy Clin Immunol* 2013;132:140-50.
- Dvorak CC, Cowan MJ, Logan BR, Notarangelo LD, Griffith LM, Puck JM, et al. The natural history of children with severe combined immunodeficiency: baseline features of the first fifty patients of the primary immune deficiency treatment consortium prospective study 6901. *J Clin Immunol* 2013;33:1156-64.
- Grunebaum E, Cutz E, Roifman CM. Pulmonary alveolar proteinosis in patients with adenosine deaminase deficiency. *J Allergy Clin Immunol* 2012;129:1588-93.
- Schuetz C, Neven B, Dvorak CC, Leroy S, Ege M, Pannicke U, et al. SCID patients with ARTEMIS vs RAG deficiencies following HCT: increased risk of late toxicity in ARTEMIS-deficient SCID. *Blood* 2014;123:281-9.
- Cowan MJ, Gennery AR. Radiation-sensitive severe combined immunodeficiency: the arguments for and against conditioning before hematopoietic cell transplantation—what to do? *J Allergy Clin Immunol* 2015;136:1178-85.
- Mezzacappa ES, Endicott J. Parity mediates the association between infant feeding method and maternal depressive symptoms in the postpartum. *Arch Womens Ment Health* 2007;10:259-66.
- Ystrom E. Breastfeeding cessation and symptoms of anxiety and depression: a longitudinal cohort study. *BMC Pregnancy Childbirth* 2012;12:1.
- DeLuca JM, Kearney MH, Norton SA. Parents' experiences of expanded newborn screening evaluations. *Pediatrics* 2011;128:53-61.

19. Heller K. Genetic counseling: DNA testing for the patient. *Proc (Bayl Univ Med Cent)* 2005;18:134-7.
20. Fanos JH, Puck JM. Family pictures: growing up with a brother with X-linked severe combined immunodeficiency. *Am J Med Genet* 2001;98:57-63.
21. Kuo CY, Kohn DB. Gene therapy for the treatment of primary immune deficiencies. *Curr Allergy Asthma Rep* 2016;16:1-8.
22. Kohn D. Gene therapy outpaces haplo for X-SCID. *Blood* 2015;25:3521-2.
23. Hacein-Bey-Abina S, Von Kalle C, Schmidt M, McCormack MP, Wulffraat N, Leboulch P, et al. LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1. *Science* 2003;302:415-9.
24. Howe SJ, Mansour MR, Schwarzwaelder K, Bartholomae C, Hubank M, Kempinski H, et al. Insertional mutagenesis combined with acquired somatic mutations causes leukemogenesis following gene therapy of SCID-X1 patients. *J Clin Invest* 2008;118:3143-50.
25. Hacein-Bey-Abina S, Pai SY, Gaspar HB, Armant M, Berry CC, Blanche S, et al. A modified γ -retrovirus vector for X-linked severe combined immunodeficiency. *N Engl J Med* 2014;371:1407-17.
26. Hassan A, Booth C, Brightwell A, Allwood Z, Veys P, Rao K, et al. Outcome of hematopoietic stem cell transplantation for adenosine deaminase-deficient severe combined immunodeficiency. *Blood* 2012;120:3615-24.
27. Gene transfer for severe combined immunodeficiency, X-linked (SCID-X1) using a self-inactivating (SIN) gammaretroviral vector. Available at: <https://clinicaltrials.gov/ct2/show/NCT01129544?term=SCID&rank=18>. Accessed February 1, 2017.
28. De Ravin SS, Wu X, Moir S, Kardava L, Anaya-O'Brien S, Kwatema N, et al. Lentiviral hematopoietic stem cell gene therapy for X-linked severe combined immunodeficiency. *Sci Transl Med* 2016;8:335-57.
29. Wahlstrom J, Patel K, Eckhart E, Kong D, Horn B, Cowan MJ, et al. Transplacental maternal engraftment and posttransplantation graft-versus-host disease in children with severe combined immunodeficiency. *J Allergy Clin Immunol* 2017;139:628-33.e10.
30. Savic RM, Cowan MJ, Dvorak CC, Pai SY, Pereira L, Bartelnik IH, et al. Effect of weight and maturation on busulfan clearance in infants and small children undergoing hematopoietic cell transplantation. *Biol Blood Marrow Transplant* 2013;19:1608-14.
31. Dvorak CC, Hassan A, Slatter MA, Hönig M, Lankester AC, Buckley RH, et al. Comparison of outcomes of hematopoietic stem cell transplantation without chemotherapy conditioning by using matched sibling and unrelated donors for treatment of severe combined immunodeficiency. *J Allergy Clin Immunol* 2014;134:935-43.
32. Dvorak CC, Hung GY, Horn B, Dunn E, Oon CY, Cowan MJ. Megadose CD34+ cell grafts improve recovery of T cell engraftment but not B cell immunity in patients with severe combined immunodeficiency disease undergoing haplocompatible non-myeloablative transplantation. *Biol Blood Marrow Transplant* 2008;14:1125-33.
33. Dimitrova D, Ong PY, O'Gorman MR, Church JA. Major histocompatibility complex class II deficiency complicated by *Mycobacterium avium* complex in a boy of mixed ethnicity. *J Clin Immunol* 2014;34:677-80.
34. Wu JT, Roan Y, Knight JA. Serum AFP levels in normal infants: their clinical and physiological significance. *Alpha-fetoprotein and congenital disorders*. New York: Academic Press; 1985:111-23.
35. Patel NC, Hertel PM, Hanson IC, Krance RA, Crawford SE, Estes M, et al. Chronic rotavirus infection in an infant with severe combined immunodeficiency: successful treatment by hematopoietic stem cell transplantation. *Clin Immunol* 2012;142:399-401.

APPENDIX TABLE I. Prophylactic medications for infants with SCID

Medication	Dose	Notes
IVIG	Initial loading dose of 1 g/kg; measure trough IgG level at 1-2 wk; after loading, give at least 0.5 g/kg every 3-4 wk (use full vial [eg, 2.5 or 5 g])	Maintain IgG trough >800 mg/dL, usually 0.5 g/kg every 3 wk
TMP/SMX for PJP prophylaxis*	2.5 mg/kg TMP component BID PO 2 days/wk	Start when >30 d old; monitor bilirubin and CBC
Leucovorin (folinic acid)	1.25 mg of TMP/SMX 2 d/wk with TMP/SMX	Minimizes TMP/SMX neutropenia
Atovaquone for PJP prophylaxis	30 mg/kg PO once daily, 1-3 mo	Alternative to TMP/SMX
	45 mg/kg PO once daily, 4 mo-2 y	
Dapsone	4 mg/kg PO weekly	Alternative to TMP/SMX
Acyclovir for herpesvirus prophylaxis	12.5 mg/kg PO BID or 8 mg/kg IV every 12 h (<12 kg), 400 mg/m ² PO BID or 250 mg/m ² IV every 12 h (>12 kg)	
Fluconazole for fungal prophylaxis	6 mg/kg every 48 h (full term, <1 month) 6 mg/kg/d ≥1 mo	Ensure normal LFTs before starting; follow LFTs
Palivizumab	Per institutional protocol, every 4 weeks	During RSV season
PEG-ADA (Adagen)	At least 30 U/kg (up to half of a vial) twice weekly; start with full dose	Start for ADA-deficient patients while awaiting HCT or GT

BID, Twice daily; *IVIG*, intravenous immunoglobulin; *LFTs*, liver function tests; *PJP*, *Pneumocystis jiroveci* pneumonia; *PO*, by mouth; *RSV*, respiratory syncytial virus; *TMP/SMX*, trimethoprim/sulfamethoxazole.

*Inhaled pentamidine is not recommended in children 5 years of age and younger. There are no recommendations for intravenous pentamidine for prophylaxis in infants, and we would avoid this in newly diagnosed babies given the risk of nephrotoxicity and possible hepatotoxicity.

APPENDIX TABLE II. Workup outline for infants with SCID to prepare for hematopoietic cell transplantation

Blood tests for infant	Amount of blood*	Tube type, notes
CBC w/differential	0.25 mL	EDTA
Lymphocyte subsets and lymphocyte T-cell subsets, naive and memory (CD45 RA/RO)	0.5 mL	Purple Microtainer ordered as 2 separate tests
IgM, IgA, IgG, IgE	2 mL (1 mL without IgE)	SST
Blood chemistries, albumin, total bilirubin, LFTs	0.5 mL	Light green Microtainer
HLA typing (prefer buccal swab if maternal engraftment suspected)	(2.6 mL if blood)	Two buccal swabs or small ACD yellow-top tube
Maternal engraftment by STR markers on CD3 selected cells	2.6 mL	2.6 mL (small) ACD
Serum CMV PCR	1.5 mL	EDTA
Lymphocyte proliferation to PHA (and other mitogens)	5 mL	Na heparin
ABO, Rh, antibody screen	Microtainer	EDTA
SCID gene molecular diagnosis (sequencing)	1 mL	Blood or buccal swabs
ADA and PNP enzyme studies (if appropriate SCID)	1 mL	EDTA
Infectious disease markers (should be done within 30 d of admission to BMT)		
HIV DNA PCR- immunology laboratory	1 mL	EDTA
HBsAg	0.6 mL	SST
EBV PCR	1.5 mL	EDTA
HCV DNA PCR	2 mL	EDTA
Other pre-HCT procedures		
Cardiac echocardiogram		
Audiology examination		
Other tests and procedures to consider		
Central line placement for infusions and blood draws		
BAL only if indicated to be sent for PJP, respiratory virus panel, cultures for bacteria, fungi, AFB, and CMV; pertussis PCR		
Stool for cultures, rotavirus antigen, norovirus, <i>Clostridium difficile</i> if diarrhea present, O&P		
Respiratory virus PCR panel		

ABO, Group A, group B, group O red blood cell type; *ACD*, acid citrate dextrose; *HBsAg*, hepatitis B surface antigen; *HCV*, hepatitis C virus; *LFTs*, liver function tests; *PJP*, *Pneumocystis jiroveci* pneumonia; *PNP*, purine nucleotide phosphorylase; *SST*, serum separator tube; *STR*, short tandem repeat.

*Minimum amounts by special arrangement with our laboratories. We suggest working with a local laboratory to achieve acceptable minimum amounts that are institution specific.