

Population-based newborn screening for severe combined immunodeficiency: Steps toward implementation

Jennifer M. Puck, MD,^a on behalf of The SCID Newborn Screening Working Group^b

San Francisco, Calif

Severe combined immune deficiency (SCID) has been identified as a disorder of high priority for population-based newborn screening. Most affected infants are not brought to medical attention until they develop serious infectious complications, and SCID is fatal if untreated. Effective treatment with allogeneic hematopoietic stem cell transplantation is widely established. The best outcome for SCID, as with many other conditions for which newborn screening is now done, is achieved if hematopoietic stem cell

transplantation is performed in the first months of life, ideally before clinical presentation with infections and failure to thrive. A meeting in San Francisco in May 2007 brought together experts from newborn screening programs; the pediatric immunology community; pediatric transplant centers; and federal, state, and nongovernmental agencies to consider obstacles to and implications of developing newborn screening for SCID. Development of an appropriate low-cost, high-throughput screening algorithm has been a challenge,

From ^athe Department of Pediatrics, University of California, San Francisco.

^bThe SCID Newborn Screening Working Group met in San Francisco, May 14–15, 2007. The group includes John C. Baker (Kaiser Permanente, Oakland Medical Center, Oakland, Calif); Mei W. Baker (University of Wisconsin, Madison); Marcia Boyle (Immune Deficiency Foundation, Towson, Md); Amy Brower (Third Wave Technologies, Madison, Wis); Rebecca H. Buckley (Duke University Medical Center, Durham, NC); Fabio Candotti (National Human Genome Research Institute, National Institutes of Health, Bethesda, Md); Anne Marie Comeau (University of Massachusetts Medical School, Jamaica Plain); Morton Cowan (University of California, San Francisco); Joie Davis (National Human Genome Research Institute, National Institutes of Health, Bethesda, Md); Elaine Eastman (Kaiser Permanente, Oakland Medical Center, Oakland, Calif); Gilian Engelson (National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Md); Diana Gonzalez-Espinosa (University of California, San Francisco); Nancy S. Green (Columbia University Medical Center, New York, NY); Howard Grodman (Tufts New England Medical Center, Boston, Mass); William Grossman (Medical College of Wisconsin, Milwaukee); R. Rodney Howell (National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Md); Naynesh R. Kamani (Children's National Medical Center, Washington, DC); Martin Kharrazi (California Department of Public Health, Richmond); Alan P. Knutsen (St Louis University School of Medicine, St Louis, Mo); Ronald H. Laessig (University of Wisconsin/State Lab of Hygiene, Madison); Tonya Lebet (University of California, San Francisco); Howard M. Lederman, Johns Hopkins School of Medicine, Baltimore, Md); David Lewis (Stanford University Medical Center, Palo Alto, Calif); Fred Lorey (California Department of Public Health, Richmond); Fizza Gulamali-Majid (Maryland State Department of Health and Mental Hygiene, Baltimore); Elaine Mansfield (Affymetrix, Santa Clara, Calif); Louis Matis (Immune Tolerance Institute, Inc, Southport, Ct); Sean McGhee (University of California Los Angeles Center for Health Sciences, Los Angeles); Fred Modell (Jeffrey Modell Foundation, New York, NY); Vicki Modell (Jeffrey Modell Foundation, New York, NY); Luigi Notarangelo (Harvard Medical School, Boston, Mass); Richard S. Olney (Centers for Disease Control and Prevention, Atlanta, Ga); Joseph J. Orsini (Wadsworth Center, New York State Department of Health, Albany); Sung-Yun Pai (Harvard Medical School and Childrens Hospital, Boston, Mass); Susan R. Panny (Maryland State Department of Health and Mental Hygiene, Baltimore); Kenneth Pass (Wadsworth Center, New York State Department of Health, Albany, NY); Susan Prockop (Memorial Hospital/Sloan-Kettering, New York, NY); Jennifer M. Puck (University of California, San Francisco);

Michele A. Lloyd-Puryear (Maternal and Child Health Bureau, Health Resources Services Administration, Department of Health and Human Services, Rockville, Md); John M. Routes (Medical College of Wisconsin, Milwaukee); John E. Sherwin (California Department of Public Health, Richmond); Bradford L. Therrell, Jr (National Newborn Screening and Genetics Resource Center, University of Texas Health Science Center at San Antonio); Troy Torgerson (University of Washington and Seattle Childrens Hospital, Seattle); Robert F. Vogt, Jr (Centers for Disease Control and Prevention, Atlanta, Ga); Diane Wara (University of California, San Francisco); and Kenneth Weinberg (Stanford University Medical Center, Palo Alto, Calif).

The meeting from which this report originated was supported by the National Newborn Screening and Genetic Resource Center, Austin, Tex; the Genetic Services Branch, Maternal and Child Health Bureau, Department of Health and Human Services, Rockville, Md; and the Jeffrey Modell Foundation. Dr Puck is supported by a Clinical and Translational Sciences Award to the University of California, San Francisco, National Institutes of Health National Center for Research Resources (UL RR024131-01); the Jeffrey Modell Foundation; and a National Institute of Allergy and Infectious Diseases and National Institute for Child Health and Human Development-sponsored US Immunodeficiency Network research award.

Disclosure of potential conflict of interest: M. Boyle has received grant support from the Immune Deficiency Foundation. A. Brower is employed by and owns stock in Third Wave Technologies. R. R. Howell has consulting arrangements with Genzyme Corp, is on the speakers' bureau for ZLB-Behring, and is on the advisory board for Cryocell. A. P. Knutsen is on the speakers' bureau for Novartis, ZLB-Behring, and Talecris. E. Mansfield is employed by Affymetrix. L. Matis is employed by the Immune Tolerance Institute. S. R. Panny has received grant support from the Health Resources Services Administration. J. M. Puck is a consultant for the US Immunodeficiency Network and has received research support from the National Newborn Screening and Genetic Resource Center, the Genetic Services Branch, Maternal and Child Health Bureau, and the Jeffrey Modell Foundation, Clinical and Translational Sciences Award to UCSF. The rest of the authors have declared that they have no conflict of interest.

Received for publication July 2, 2007; revised August 2, 2007; accepted for publication August 3, 2007.

Reprint requests: Jennifer M. Puck, MD, Department of Pediatrics, Box 0519, University of California, San Francisco, 513 Parnassus Avenue, HSE 301A, San Francisco, CA 94143-0519. E-mail: puckj@peds.ucsf.edu.

0091-6749

doi:10.1016/j.jaci.2007.08.043

although absence of T-cell receptor gene excision circles is a sensitive marker of profound T lymphocytopenia and currently is the most developed screening method. A consensus was reached on several points: SCID newborn screening should be pursued with pilot studies in key locales, test methodologies need to be optimized, screening programs must be integrated with plans for definitive diagnosis and management, centralized specimen banks and registries are required to foster test validation and track outcomes that will guide future treatment, and SCID newborn screening will lead to important knowledge about human immune disorders as well as better care of patients. (J Allergy Clin Immunol 2007;120:760-8.)

Key words: Public health, primary immunodeficiency, newborn screening, severe combined immunodeficiency (SCID), live vaccine, bone marrow transplantation, hematopoietic stem cell transplantation, early diagnosis, genetic disease

Infants with severe combined immunodeficiency (SCID) are healthy at birth but die of recurrent, severe infections in infancy unless they are provided with a functional immune system.^{1,2} Hematopoietic stem cell transplantation (HSCT) and enzyme replacement (in the case of adenosine deaminase deficiency) have made this previously fatal set of diseases treatable.²⁻¹⁰ Gene therapy is also a promising treatment despite occurrences of leukemia related to retroviral insertional mutagenesis in 1 early trial.¹¹ Currently there are at least 15 known genes that, when mutated, cause SCID; these genes encode cell surface receptors and other activation signaling molecules (IL2RG, JAK3, IL7R, CD45, CD3 components, FOXP1), T-cell and B-cell antigen receptor gene recombinases (RAG1, RAG2, Artemis, DNA ligase 4), and purine pathway enzymes (adenosine deaminase, purine nucleoside phosphorylase). Molecular diagnosis of SCID gene mutations has brought carrier and prenatal diagnosis to some families; infants with SCID identified because of a family history, in some cases after prenatal mutation diagnosis, can be treated early in life, and consequently have better survival, less morbidity, and lower treatment costs than patients recognized only after the onset of serious infections.¹²

Unfortunately, most infants with SCID are not identified in the preinfectious period. A universal newborn screening program could in theory remedy this problem. At a Centers for Disease Control and Prevention conference in 2001 on "Applying Public Health Strategies to Primary Immunodeficiency Diseases," Rebecca Buckley reported that because the majority of infants with SCID have very low absolute lymphocyte counts, a complete blood count and manual differential count could alert health care providers to suspect the diagnosis.^{2,12} At that meeting universal newborn screening for SCID was identified as an important public health goal,¹³ and Jennifer Puck suggested that absence of T-cell receptor gene excision circles (TRECs) could identify infants with SCID by using DNA from the dried blood spots (DBSs) already collected for screening newborns in every state. In 2005, Chan and Puck¹⁴ published a successful DBS TREC assay

Abbreviations used

DBS: Dried blood spot
HSCT: Hematopoietic stem cell transplantation
NK: Natural killer
SCID: Severe combined immunodeficiency
TREC: T-cell receptor gene excision circle

to distinguish T-lymphocytopenic infants with SCID from healthy controls. McGhee et al performed a theoretical cost-benefit analysis that supported SCID newborn screening if a sufficiently inexpensive and accurate test could be found¹⁵ and suggested high IL-7 levels as an alternative or second-tier method in addition to TRECs for increasing specificity of SCID detection.¹⁶

Routine screening of all newborns for genetic disease and other congenital disorders began by the 1960s with testing for phenylketonuria using the Guthrie procedure of infant heel-stick blood spotted onto filter paper. Today all states in the United States use DBS for universal screening; the number of conditions screened for varies between states but is increasing to encompass multiple assays for metabolic disorders, hypothyroidism, hemoglobinopathies, and other genetic diseases such as cystic fibrosis. In 2006, the American College of Medical Genetics report on expanding newborn screening was endorsed by the Advisory Committee to the Secretary for Health and Human Services¹⁷; this report found SCID to meet all criteria for inclusion in newborn screening panels once a screening assay could be validated.¹⁷ A further workshop seeking public health partnerships to advance SCID newborn screening was held in Atlanta, Ga, in November 2006, following which, in early 2007, the Wisconsin newborn screening program announced its intention to pilot SCID newborn screening. In May 2007, a SCID Newborn Screening Working Group was convened in San Francisco, Calif, with the goal of fostering teamwork among experts in newborn screening, trial design, immunologic diagnosis, and SCID transplantation so integrated approaches to SCID screening could be efficiently pursued. Interactive sessions were held on cost-effectiveness, analytes for SCID detection, introduction of new genetic screening tests by means of clinical trials with informed consent, confirmatory diagnostic testing, and treatment strategies. Summarized here are conclusions and action items from these sessions.

JUSTIFICATIONS FOR POPULATION-BASED NEWBORN SCREENING FOR SCID

Severe combined immunodeficiency is asymptomatic at birth and cannot be identified by clinical examination in the newborn nursery, but affected infants must be diagnosed before onset of infections to maximize opportunity for life-saving treatment. Although the precise incidence of SCID is not known, it affects more than 1/100,000

TABLE I. Interview study of SCID family history, age of diagnosis, and survival*

	Known affected relative (18% of the 39 cases)	Negative family history (82% of the 39 cases)
No. of infants	7	32
Mean age of SCID diagnosis	2 mo	9 mo (1 at autopsy)
Received HSCT or ADA treatment (mean age)	7 (mean age of 3 mo), all had HSCT	24 (mean age of 9.5 mo); 8 died without HSCT or ADA
Survival after BMT or ADA treatment	7/7 (100%)	14/24 (58%); 8 died after HSCT, 2 with ADA
Overall survival to 2.5 y or more	100%	44%

*J. Davis, K. Chan, and J. Puck (unpublished data).

births,¹⁴ and experts in attendance at the meeting estimated a total of around 100 new US cases annually. Certain populations, such as Indians of Athabascan heritage, have an increased incidence of autosomal-recessive SCID, but all ethnicities are at risk. A recognized positive family history affords some families early diagnosis of a subsequent SCID-affected infant. As shown in Table I, summarizing data from a nationwide survey of parents of 39 patients with SCID born since 2000, only 7 (18%) had a known positive family history (Davis et al, unpublished data, May 2007). However, these fortunate infants were diagnosed earlier than their sporadic counterparts and received HSCT at a mean age of 2 months, and all survived. In contrast, 32 cases in this series (82%) were diagnosed with SCID after the onset of infectious complications (including 1 unexpected diagnosis postmortem), and 8 died before definitive treatment could be given. Of the 24 infants with sporadic SCID who received HSCT or polyethylene glycol conjugated to adenosine deaminase treatment (75% of the sporadic cases), only 14 survived, making the overall survival rate for sporadic SCID 44% in this series. A goal of newborn screening for SCID would be to give the sporadic cases the same access to early care and better outcome that known familial cases have.

High human and monetary costs of long hospitalizations, intensive care, and early death were agreed by attendees to result from delayed SCID diagnosis. R. Buckley reported her experience at Duke University, where infants with prenatally diagnosed or suspected SCID have had better survival after much less expensive outpatient HSCT compared with patients diagnosed later, who had higher mortality and required long hospital stays because of life-threatening infections, particularly caused by viral pathogens. Fig 1 shows long-term survival data from the Duke University SCID transplantation program, with infants divided into 2 groups on the basis of age at HSCT. Of 46 infants treated in the first 3.5 months of life, 96% survive, whereas survival is 66% in patients who received HSCT after 3.5 months of age.

Furthermore, public health vaccination programs that mandate use of live, attenuated virus vaccines, such as the new multivalent antirotavirus vaccine (given at 6 weeks of age), as well as varicella and measles-mumps-rubella vaccines pose a danger to infants with SCID.¹⁸ Through early diagnosis, newborn screening would protect

immunocompromised infants from potentially severe disease by avoidance of vaccine strain viruses.

SCREENING TEST METHODS

In the current screening environment, assays for SCID should, if at all possible, take advantage of the DBSs already collected and tracked by state laboratories. Although screening must be highly sensitive so as not to miss true cases, specificity is also important to avoid overwhelming the system with need for follow-up of false-positives. More than at previous meetings, there was a clear consensus in San Francisco that SCID screening will soon become technically feasible. The main barrier to implementation is the rate of false-positive or indeterminate test results. Because of the rarity of disease, the positive predictive value needs to be extremely high. Much of the meeting focused on strategies to reduce rates of false-positive or indeterminate results to make the number of referrals manageable. Screening analytes are based on the inability of all patients with SCID to make normal numbers of T cells, but must take into account the possibility of maternally engrafted T cells and the fact that T⁺B⁺ patients with SCID may have high numbers of B cells. Several laboratories are now investigating screening methods for SCID, which are summarized in Table II. The method originally proposed by Buckley, determination of absolute lymphocyte counts, has not been systematically carried out in a large sample of unselected newborns, but now Alan Knutsen has proposed initiating such a trial.

Quantitation of TRECs as pioneered by Chan and Puck¹⁴ is undergoing refinement to improve specificity. Although blood of newly diagnosed infants with SCID—and, more importantly, actual Guthrie cards recovered from state labs for infants with SCID—uniformly show extremely low to undetectable TRECs, there have been around 1.4% of anonymous, outdated Guthrie cards with indeterminate results caused by poor DNA recovery (as measured by low copy number of a genomic actin segment in a quantitative PCR reaction). Repeat DNA isolation may decrease this rate of indeterminate samples, and fresh samples may yield superior DNA with lower rates of indeterminate results. The TREC test will be implemented on a large scale in Wisconsin by Laessig et al,¹⁹

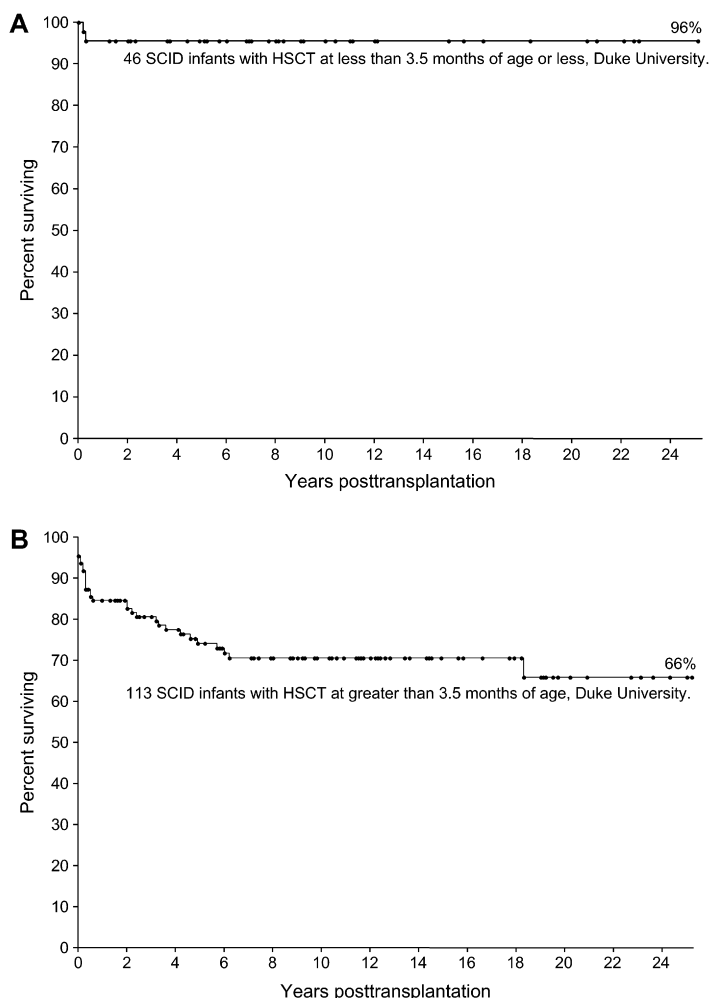


FIG 1. Kaplan-Meier plot of patients with SCID transplanted at Duke University Medical Center. **A**, Forty-six infants diagnosed and treated in the first 3.5 months of life. **B**, One hundred thirteen infants treated after age 3.5 months. Kindly provided by Rebecca Buckley.

where the commitment has been made to pilot SCID screening within the next year.

In Maryland and 11 other states, a routine second DBS is collected from almost all infants at a pediatric visit in the early weeks after nursery discharge. In this setting, an indeterminate TREC test could be followed up automatically with a TREC assay on the second specimen, with a substantial reduction, to below 0.1%, of persistently abnormal or indeterminate readings. A second DBS sample, either routine or requested by the laboratory, represents a strategic solution to the high indeterminate rate of TREC testing. A routine second sample for all newborns is currently being debated in the screening community because it has been postulated to improve detection of hypothyroidism substantially, but double-sampling is costly and not a nationwide standard. Research on the efficacy of a routine second screen is now underway.

Technologic solutions for SCID newborn screening would rely on developing alternative or 2-tier assays. Immunoassays for IL-7 and for T-cell-specific proteins are

being evaluated by McGhee et al¹⁶ and Ken Pass, respectively. High IL-7 levels are associated with T-lymphocytopenic states, but it is not yet known whether this association can be developed into a robust newborn screening tool for SCID. CD3 either alone or in combination with CD45 or other T-cell proteins could be assayed by sensitive, high-throughput Luminex bead capture technology (Panomics, Inc., Fremont, Calif).²⁰ Another new approach is to detect mutations, either previously defined or as yet unreported, in known SCID genes by using resequencing microarray chips, as being investigated by J. Puck and Tonya Lebet in collaboration with Elaine Mansfield and Janet Warrington at Affymetrix, Inc. Although yielding a specific gene diagnosis if positive, this approach may have an unacceptable false-negative rate.

There was consensus among the pediatric immunologists that although indeterminate test results will be problematic, any infant who truly lacks TRECs, has absent CD3, or has any test result that signifies a lack of T cells should be brought to medical attention. Thus, non-

TABLE II. Screening test methods suggested for SCID

Method	Platform	DBS	Preliminary published data	Comment	Investigators
CBC and differential for absolute lymphocyte count (ALC)	Hospital clinical laboratory test on liquid cord blood	No	Most SCIDs have low ALC, ² high false-negatives and false-positives	Expensive, decentralized; manual differential count may be required	A. Knutsen, R. Buckley
Absent TRECs	DNA copy number by quantitative PCR	Yes	Sensitive, but samples with inadequate DNA limit specificity ¹⁴	Inadequate samples have low DNA (low TRECs and genomic actin copies)	J. Puck, K. Chan, D. Gonzalez, S. Panny
Absent TRECs in 2 separate samples	DNA copy number by quantitative PCR	Yes	Sensitive and specific ¹⁴	Maryland, 11 more states now mandate 2 samples	J. Puck, S. Panny, K. Chan
IL-7 immunoassay	ELISA or other method	Yes	Limited data, ¹⁶ blood interference with detection	Possible 2nd tier test with TRECs	S. McGhee
Low CD3 (multiplex) immunoassay	Luminex	Yes	None	Might extend with additional antibodies, define a SCID signature profile	K. Pass
Microarray mutation detection	DNA on Affymetrix custom resequencing array	Yes	None	Possible 2nd tier test; specific gene diagnosis, but may have false-negatives	J. Puck, T. Lebet, E. Mansfield, J. Warrington

SCID false-positive tests may identify infants with serious primary immunodeficiencies other than SCID, such as DiGeorge syndrome or other disorders with increased susceptibility to infections.

PREVIOUS EXPERIENCE WITH INITIATING NEW SCREENING TESTS

Leaders in the field of newborn screening shared insights on the essential elements of public health screening programs (Table III) and the ways in which programs have recently expanded to screen for additional diseases. Once a condition has been determined to merit newborn screening, an entire sequence from sampling to identification, treatment, and outcome assessment must be designed. In general, pilot programs must be approved by institutional review boards and include informed parental consent for participation. The tests themselves may need US Food and Drug Administration approval. Recent expansions in screening programs in their respective states were described by Anne Comeau, Nancy Green, Ronald Laessig, Fred Lorey, Joseph Orsini, Susan Panny, John Sherwin, and others. A review written by 5 of these experts²¹ relates lessons in developing screening derived from introductions of testing for cystic fibrosis, Krabbe disease, HIV, and hemoglobinopathies. Although some issues are unique to each state and each disease, every new program must meet strict standards. Progressing from conception to implementation requires many questions to be addressed:

1. Analytic validity: How well does the assay detect the analyte?
2. Analytic utility: Does the test perform well on a large scale? What are mean values and appropriate cutoffs? Are there special subpopulations, such as premature infants, whose values are systematically different?
3. Clinical validity: Does the test identify affected infants, and at what cost in terms of false-positives? Do screening algorithms need adjustment to optimize predictive value?
4. Clinical utility: What are the clinical benefits and harms of testing, and how are outcomes changed by screening?

The SCID Newborn Screening Working Group agreed that collaboration on standardization, with sharing of samples for cross-checking, would facilitate adoption of any of the suggested SCID screening tests. True SCID positive control blood spots are necessary, both from time of diagnosis and from newborn nurseries. However, the rarity of SCID, the limited amounts of blood available from affected infants, strict policies covering research with DBS samples, and the fact that many states discard them after completing mandated tests combine to make these critical samples hard to obtain.

RECALLING BABIES WITH ABNORMAL SCREEN RESULTS

John Baker and Elaine Eastman emphasized that community physicians and parents must have confidence

in newborn screening tests. That confidence is based on being fully informed and on having tests with acceptable sensitivity and specificity characteristics. Some screening experts considered a maximum callback rate of 0.1% a benchmark, but J. Baker warned that if more than 90% of these are false alarms, there might be nonacceptance by physicians. In addition to requiring extra clinic visits and heel sticks, callbacks have negative psychological consequences for families whose children screen positive but are unaffected; parents in these families have been shown to experience anxiety and increase their use of health services. Effective, timely follow-up for babies with positive screening results requires detailed planning, comprehensive protocols, and well written educational materials that parents receive before their children are screened. Marcia Boyle indicated that the Immune Deficiency Foundation would like to help prepare information for the public and community health providers.

Plans are needed for patients who will be screen positive and then have follow-up immunologic studies that are not diagnostic for SCID, but are not completely negative. Tandem mass spectroscopy screening has identified infants with unusual biochemical analytes for which the clinical significance is uncertain. In a SCID screening program, some infants are bound to have abnormal immunologic studies that have mild or minimal clinical significance. Most participants felt that the process of screening for T-cell lymphopenia, although intended to identify SCID, would in addition identify children with other disorders. Some, such as complete DiGeorge syndrome or congenital intestinal lymphangiectasia, are predictable, but others will come to attention only in the context of a universal screening program, similar to benign hyperphenylalaninemia, discovered when phenylketonuria screening began.

CONFIRMATORY TESTING

Much discussion centered on what immunologic testing should follow an abnormal screening result and at what point a referral to a sophisticated center of excellence in pediatric immunology should be instituted. Current screening programs have networks of metabolic disease specialists, hematologists, and so forth, throughout their states who are always available to be contacted in addition to the primary care provider. The definitive diagnosis and treatment for SCID will likewise require recruitment of a new group of clinical specialists who have not traditionally been involved with newborn screening. However, local physicians may be able to perform follow-up evaluations and flow cytometry according to structured protocols to assess economically which infants will be normal despite an abnormal screening result. One scheme, with many variations being actively discussed by the group, is outlined in Table IV. Teams from each state will need to establish initial plans for the number of infants anticipated to require follow-up given the screening test characteristics,

TABLE III. Elements of a genetic screening program

1. Universality; all babies should be included (some states require parental permission)
2. Prescreening education of parents, primary care providers
3. Specimen quality control—every nursery, every day (obtain, transport to central lab)
4. Laboratory screening tests, cutoffs, standards, analysis, quality control
5. Data integrity (HIPAA compliance)
6. Timely notification and follow-up
7. Appropriate definitive diagnostic work-up
8. Appropriate referral for treatment; immediate and long-term care of affected infants
9. Education, reassurance for families with false-positive tests
10. Continuous monitoring of all phases of program, including outcomes
11. Cost-effectiveness assessments to justify continuation, suggest improvements
12. Acquisition of new knowledge about the conditions identified by screening

HIPAA, Health Insurance Portability and Accountability Act.

to be adjusted as experience dictates. However, a nationally coordinated work-up for all infants with results deposited in a central databank would be the most efficient and valuable resource for assessing the performance of the screening process as well as providing normative data and population-based rates of abnormal immune parameters.

For infants confirmed to have SCID, another immune deficiency, or equivocal results on the confirmatory test panel, additional evaluation would be required at a reference immunology laboratory, in many cases associated with a treatment center. Studies at this level might include chest x-ray to determine thymic shadow and whether infiltrates are present, serum immunoglobulin levels, additional flow-cytometric markers, *in vitro* lymphocyte proliferation, other *in vitro* functional studies, and molecular diagnosis. As indicated by Ken Weinberg, Howard Lederman, and others, certain tests might be widely agreed on, but discretion in testing and referral for treatment must be allowed.

As soon as an immunodeficiency is suspected on the basis of evaluation at this level, measures must be taken to protect the infant without delay. No live vaccines should be given. Avoidance of nonirradiated or cytomegalovirus-positive blood products and administration of intravenous immunoglobulin and prophylactic antibiotics to prevent pneumocystis are important stabilizing measures while definitive treatment is being planned.

TREATMENT OF SCID IN A SETTING OF PRESYMPTOMATIC DIAGNOSIS

The pediatric immunology community around the country eagerly anticipates the launching of newborn screening for SCID. Currently, the therapy of choice is allogeneic HSCT, and the donor of choice is an HLA-matched sibling. For infants without a matched, related donor, alternative donors and cell types have been used,

TABLE IV. Example of a minimal evaluation to be performed after abnormal SCID screening result

Personal history (expected to be benign, but infections and rash should be recorded)
Family history consistent with immune disorder; consanguinity
Physical examination (length, weight, lymph nodes, dysmorphism)
Complete and differential white blood cell counts
HIV evaluation
Blood spot for TRECs (to screening lab for correlation with initial test performance)
Flow cytometric determination* of CD3/DR,† CD4, CD8,‡ CD19, CD56

*Delineation of T⁺B⁺NK⁻, T⁺B⁻NK⁺, T⁺B⁺NK⁻, T⁺B⁻NK⁺ SCID phenotypes; some immunologists also advocate CD45RA to look for naive T cells and diagnose CD45 deficiency.

†Double-staining to reveal MHC class II deficiency and activated T cells that may be a result of maternal engraftment.

‡Absent CD8 cells would suggest Zap70 or Lck deficiency.

including T-cell-depleted, haploidentical, related marrow or peripheral blood stem cells; matched unrelated donor stem cells; and matched unrelated cord blood cells. Fabio Candotti reported on the status of SCID gene therapy, consisting of addition of a correct gene copy to autologous hematopoietic stem cells. Retroviral-mediated gene therapy has been successful in curing X-linked and ADA-deficient SCID.^{11,22} In the first X-linked SCID trial, an unacceptably high 40% of enrolled infants developed clonal proliferations of T cells because of retroviral insertional mutagenesis; however, other trials for patients with X-linked and ADA-deficient SCID, the latter requiring cessation of ADA enzyme replacement therapy and low-dose cytoablative busulfan pretreatment, have achieved impressive reconstitution. These currently experimental gene therapies are being refined and may become widely available for patients with SCID in the future.

The experience with HSCT from several SCID transplant programs, as stated by R. Buckley from Duke University, Susan Prockop from Memorial Hospital/Sloan Kettering, and others, indicates that the best results of allogeneic HSCT are seen when transplantation is performed within the first 2 to 3 months of life, or before the onset of viral or opportunistic infections. Survival data from many different investigators summarized by Naynesh Kamani showed that the chances of a successful outcome after HSCT diminish from more than 90% down to 55% to 75% when transplantation is delayed.

By making possible presymptomatic diagnosis for patients with SCID and prompt definitive therapy with HSCT, newborn screening is anticipated to result in significantly higher survival rates. Definition of the true incidence of SCID, clarification of genetic versus environmental contributions to phenotypes, and discovery of additional gene defects causing SCID are anticipated. An exciting development is the prospect of establishing multicenter collaborations, possibly including prospective randomized trials, to answer important questions in the field efficiently and ethically, such as the following: What are the best donor stem cell sources and enrichment

methods? Are there benefits of pretransplant conditioning that outweigh the risks for very young infants, and if so, what are the optimal preparative regimens? How should genotypic or phenotypic variables (eg, radiosensitivity, presence of natural killer [NK] cells) be translated into tailored HSCT?

LONG-TERM OUTCOME MEASUREMENTS

Assessment of performance should be applied to each element of the screening chain in Table III as well as survival and long-term quality of life of patients with SCID. Registries to track this type of data do not currently exist in the United States. The European Stem Cell Transplantation for Immunodeficiencies in Europe Registry has more than 600 records of HSCT for SCID since 1968. As communicated by Luigi Notarangelo, analysis of registry data has demonstrated higher survival for patients with SCID transplanted at younger ages and for B⁺ versus B⁻ patients with SCID after mismatched related donor HSCT. He also reported on post-HSCT quality of life in the Italian series of survivors of HSCT for severe T-cell immunodeficiency, who for the most part have normal growth and development and lead normal lives.²³

Two US database efforts have been started that could be linked to grow into a comprehensive SCID outcome tracking resource. Diagnostic information can be collected through the US Immunodeficiency Network database, and HSCT information through the Center for International Blood and Marrow Transplant Research (Table V).

Despite improving outcomes of HSCT for SCID in many centers, questions about optimal treatment remain to be worked out with the aid of more complete data. There is no current consensus regarding a single optimal HSCT protocol for infants without an HLA-matched sibling donor. Delayed or incomplete immune reconstitution can leave patients requiring lifelong intravenous immunoglobulin, remaining at risk for lethal viral infections, and having poor nutritional status and autoimmunity. Sequellae of chemotherapy include infertility; osteopenia; endocrine, renal, and pulmonary dysfunction; and impaired tooth development. Graft-versus-host disease is not beneficial in HSCT for SCID and can lead to organ dysfunction and autoimmunity. Collaborative studies based on information in shared registries could be used to weigh these factors and arrive at best practices for treatment of very young presymptomatic infants with SCID.

CONCLUSION AND NEXT STEPS FORWARD

Although much remains to be accomplished, momentum is growing for newborn screening for SCID. There are funds and a commitment to start a pilot SCID screening trial in the newborn screening program of the state of Wisconsin. TREC-based and other technical approaches to population-based screening for SCID are under active development, and unprecedented steps are

TABLE V. Resource organizations to assist in SCID screening efforts

Organization	Description
Advisory Committee on Heritable Disorders and Genetic Diseases in Newborns and Children	Chaired by R. Rodney Howell, this Committee (http://mchb.hrsa.gov/programs/genetics/committee/) reports to the Secretary of the Department of Health and Human Services regarding childhood and newborn screening. The committee provides advice on specific grant programs and is finalizing its process for evaluating and recommending new screening tests for newborns and children; nomination of SCID is already pending.
CIBMTR	This center networks transplant physicians with donor registries and clinical trials. In response to 2005 federal legislation, the Bill Young Cell Transplantation Program will mandate tracking of HSCT outcomes, an initiative that can help to establish a database of SCID transplant outcomes. CIBMTR (http://www.ibmtr.org/) coordinates the Blood and Marrow Transplant Clinical Trials Network, funded by the National Heart, Lung, and Blood Institute and the National Cancer Institute. Although most hematopoietic transplantation is cancer-related, pediatric specialists who perform HSCT for SCID and other immunodeficiencies are organizing future meetings and planning standardized reporting forms in cooperation with USIDNET.
Department of Health and Human Services	A multiagency network is planned for research, epidemiologic studies and care of children identified by abnormal screens in the newborn period. Participating agencies include the Center for Disease Control and Prevention (http://www.cdc.gov/nceh/dls/newborn.htm), National Institute for Child Health and Human Development (http://www.nichd.nih.gov/), Maternal and Child Health Bureau of the Health Resources Services Administration (http://mchb.hrsa.gov/about/default.htm), and Office for Rare Diseases (http://rarediseases.info.nih.gov/).
Immune Deficiency Foundation	The Immune Deficiency Foundation (http://www.primaryimmune.org/) seeks to improve diagnosis and treatment of patients with primary immune deficiency diseases through research, education, and advocacy.
ITI	A free-standing, nonprofit corporation, established in affiliation with the University of California, San Francisco. Initially conceived to provide fee-for-service support for the National Institute of Allergy and Infectious Diseases–funded Immune Tolerance Network, the ITI (http://medschool.ucsf.edu/news/features/PowerPoint/2007LeadershipRetreat/INI_ITI/ITN_ITI_files/frame.htm) will offer low-cost, rapid turn-around, high-throughput analyses for large-scale patient trials. ITI could pilot SCID screening assays such as the TREC assay, conduct standardization, assist with US Food and Drug Administration approval, and help state labs learn and set up SCID screening procedures.
Jeffrey Modell Foundation	A public, nonprofit, charitable organization for primary immunodeficiencies, dedicated to research, physician education, patient support, advocacy, and public awareness. The Jeffrey Modell Foundation (http://www.info4pi.org/) currently provides support for SCID newborn screening.
USIDNET	USIDNET (http://www.usidnet.org/) is a National Institute of Allergy and Infectious Diseases–sponsored and National Institute for Child Health and Human Development–sponsored contractor with (1) a patient registry that could track data in SCID newborn screening trials, and (2) a sample repository that could collect and distribute DBSs donated from patients with SCID. In collaboration with CIBMTR, it could make coordinated tracking forms to collect uniform data on diagnosed patients with SCID, SCID transplants, patients with SCID found by newborn screening, and infants who have abnormal newborn screening results but do not have classic SCID.

CIBMTR, Center for International Blood and Marrow Transplant Research; *ITI*, Immune Tolerance Institute; *USIDNET*, US Immunodeficiency Network.

being taken to set up interinstitutional collaborations, registries, and repositories to pool clinical data and samples. Pursuit of the best treatments for SCID diagnosed in the newborn period will bring together translational investigators from several SCID transplantation centers for the first time. Many organizations, some of them listed and described in Table V, are interested in helping the community of SCID families and care providers achieve the goal of healthier lives for infants

with SCID through newborn screening and early treatment. Technological, organizational, and political hurdles remain, but the SCID Newborn Screening Working Group in San Francisco developed a joint vision in which these will soon be overcome.

Many thanks to Lynn Goldman, Cory Fergus, Lisa Woldin, and AnnaMarie McCoy for assistance with meeting logistics and preparation of the manuscript.

REFERENCES

1. Puck JM. X-linked severe combined immunodeficiency. In: Ochs H, Smith CIE, Puck JM, editors. *Primary immunodeficiency diseases: a molecular and genetic approach*. 2nd ed. New York: Oxford University Press; 2007. p. 123-36.
2. Buckley RH. Molecular defects in human severe combined immunodeficiency and approaches to immune reconstitution. *Annu Rev Immunol* 2004;22:625-55.
3. O'Reilly RJ, Friedrich W, Small TN. Transplantation approaches for severe combined immunodeficiency disease, Wiskott-Aldrich syndrome, and other lethal genetic combine immunodeficiency disorders. In: Forman SJ, Blume KG, Thomas ED, editors. *Bone marrow transplantation*. Boston: Blackwell Scientific Publications; 1994. p. 849-67.
4. Knutsen AP, Wall DA. Umbilical cord blood transplantation in severe T-cell immunodeficiency disorders: two-year experience. *J Clin Immunol* 2000;20:466-76.
5. Smogorzewska EM, Brooks J, Annett G, Kapoor N, Crooks GM, Kohn DB, et al. T cell depleted haploidentical bone marrow transplantation for the treatment of children with severe combined immunodeficiency. *Arch Immunol Ther Exp (Warsz)* 2000;48:111-8.
6. Antoine C, Muller S, Cant A, Cavazzana-Calvo M, Veys P, Vossen J, et al. Long-term survival and transplantation of haemopoietic stem cells for immunodeficiencies: report of the European experience 1968-99. *Lancet* 2003;361:553-60.
7. Grunebaum E, Mazzolari E, Porta F, Dallera D, Atkinson A, Reid B, et al. Bone marrow transplantation for severe combined immune deficiency. *JAMA* 2006;295:508-18.
8. Buckley RH, Fischer A. Bone marrow transplantation for primary immunodeficiency diseases. In: Ochs H, Smith CIE, Puck JM, editors. *Primary immunodeficiency diseases: a molecular and genetic approach*. 2nd ed. New York: Oxford University Press; 2007. p. 669-87.
9. Cavazzana-Calvo M, Carlier F, Le Deist F, Morillon E, Taupin P, Gauthier D, et al. Long-term T-cell reconstitution after hematopoietic stem-cell transplantation in primary T-cell-immunodeficient patients is associated with myeloid chimerism and possibly the primary disease phenotype. *Blood* 2007;109:4575-81.
10. Chan B, Wara D, Bastian J, Hershfield MS, Bohnsack J, Azen CG, et al. Long-term efficacy of enzyme replacement therapy for adenosine deaminase (ADA)-deficient severe combined immunodeficiency (SCID). *Clin Immunol* 2005;117:133-43.
11. Candotti F, Fischer A. Gene therapy. In: Ochs H, Smith CIE, Puck JM, editors. *Primary immunodeficiency diseases: a molecular and genetic approach*. 2nd ed. New York: Oxford University Press; 2007. p. 688-705.
12. Myers LA, Patel DD, Puck JM, Buckley RH. Hematopoietic stem cell transplantation for severe combined immunodeficiency in the neonatal period leads to superior thymic output and improved survival. *Blood* 2002;99:872-8.
13. Centers for Disease Control and Prevention. Applying public health strategies to primary immunodeficiency diseases: a potential approach to genetic disorders. *Morbidity Mortality Wkly Rep* 2004;52:1-29.
14. Chan K, Puck JM. Development of population-based newborn screening for severe combined immunodeficiency. *J Allergy Clin Immunol* 2005;115:391-8.
15. McGhee SA, Stiehm ER, McCabe ER. Potential costs and benefits of newborn screening for severe combined immunodeficiency. *J Pediatr* 2005;147:603-8.
16. McGhee SA, Stiehm ER, Cowan M, Krogstad P, McCabe ER. Two-tiered universal newborn screening strategy for severe combined immunodeficiency. *Mol Genet Metab* 2005;86:427-30.
17. Watson MS, Mann MY, Lloyd-Puryear MA, Rinaldo P, Howell RR, editors, and Newborn Screening Advisory Groups. *Newborn Screening: towards a uniform screening panel and system*. *Genet Med* 2006;8:1s-250s.
18. Centers for Disease Control and Prevention. General recommendations on immunization: recommendations of the Advisory Committee on Immunization Practices (ACIP) and the American Academy of Family Physicians (AAFP). *Morbidity Mortality Wkly Rep* 2002;51:1-35.
19. Laessig RH, Baker MW, Hoffman GL, Routes JM, Grossman WJ. Piloting SCID newborn screening in Wisconsin using the experience from cystic fibrosis. In: *Proceedings of the 2007 Newborn Screening and Genetic Testing Symposium*, Minneapolis, MN. Silver Spring (MD): Association of Public Health Laboratories; 2007. In press.
20. Calado RT, Garcia AB, Falcao RP. Age-related changes of immunophenotypically immature lymphocytes in normal human peripheral blood. *Cytometry* 1999;38:133-7.
21. Pass K, Green NS, Lorey F, Sherwin J, Comeau AM. Pilot programs in newborn screening. *Ment Retard Dev Disabil Res Rev* 2006;12:293-300.
22. Cavazzana-Calvo M, Fischer A. Gene therapy for severe combined immunodeficiency: are we there yet? *J Clin Invest* 2007;117:1457-65.
23. Mazzolari E, Forino C, Guerici S, Imberti L, Lanfranchi A, Porta F, et al. Long-term immune reconstitution and clinical outcome after stem cell transplantation for severe T-cell immunodeficiency. *J Allergy Clin Immunol* 2007;120:892-9.