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# Pediatric Outcomes in Transplant: Personalising Immunosuppression To Improve Efficacy (POSITIVE Study): The Collaboration and Design of a National Transplant Precision Medicine Program

Tanya Papaz, HBA,<sup>1,2</sup> Upton Allen, MBBS,<sup>2,3</sup> Tom Blydt-Hansen, MD,<sup>2,4</sup> Patricia E. Birk, MD,<sup>5</sup> Sandar Min, MBBS,<sup>1,2</sup> Lorraine Hamiwka, MD,<sup>2,6</sup> Veronique Phan, MD,<sup>7</sup> Tal Schechter, MD,<sup>8</sup> Donna A. Wall, MD,<sup>2,8</sup> Simon Urschel, MD,<sup>2,9</sup> Bethany J. Foster, MD,<sup>2,10</sup> and Seema Mital, MD<sup>1,2</sup>

**Background.** Despite age-related differences in biology, physiology, and behavior, transplant immunosuppression is not tailored by age. This likely contributes to high graft failure and posttransplant complications. We present the aims, design, and methods of the Pediatric Outcomes in Transplant: Personalising Immunosuppression To Improve Efficacy Study aimed at personalizing posttransplant immunosuppression in children and young adults. **Methods.** In this prospective observational cohort study, we recruited pediatric and young adult solid organ transplant, pediatric allogeneic hematopoietic stem cell transplant recipients, and matched living and deceased organ donors from 14 transplant centers across Canada. Clinical data, questionnaires, biospecimens, and pharmacy records were collected at serial time points: (1) to identify genetic and host immune factors that influence immunosuppression dose requirements across different ages and transplant types, (2) to identify viral-host interactions that increase susceptibility to Epstein-Barr virus infection, and (3) to define care processes and structures associated with medication adherence in adolescents and young adults. **Results.** From 2015 to 2018, 1662 new and prevalent transplant recipients were screened, 1166 were recruited for the various aims, including 370 liver, 445 kidney, 277 heart, 19 lung, 19 multiple, and 36 hematopoietic stem cell transplant transplants. Twelve percent were younger than 2 years, 30% were 2 to 10 years, 42% were 10 to 18 years, and 16% were 18 to 24 years at enrollment. Nine hundred thirty-one consented to participation in aims 1 and 2 (90% consent rate), 287 to aim 3 (82% consent rate). Biospecimens collected included 898 for DNA, 276 for immunoassays, and 717 for biomarker studies. Seventy percent participants have completed follow-up; 30% are pending study completion. **Conclusions.** The design of this national multicenter cross-organ network helped maximize recruitment of a large patient cohort for studying age and organ-related differences in immunosuppression needs that would not otherwise be feasible. Leveraging the unique clinical, biological, environmental, and behavioral characteristics of this cohort will help develop precision medicine strategies for individualizing posttransplant immunosuppression.

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<sup>1</sup> Division of Cardiology, Department of Pediatrics, Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada.

<sup>2</sup> Canadian National Transplant Research Program, Edmonton, Alberta, Canada.

<sup>3</sup> Division of Infectious Diseases, Hospital for Sick Children, Toronto, Ontario, Canada.

<sup>4</sup> Division of Pediatric Nephrology, University of British Columbia, Vancouver, Canada.

<sup>5</sup> Department of Pediatrics and Child Health, Health Sciences Centre Winnipeg, Winnipeg, Manitoba, Canada.

<sup>6</sup> Division of Nephrology, Alberta Children's Hospital, University of Calgary, Calgary, Canada.

<sup>7</sup> Division of Nephrology, Department of Pediatrics, Centre Hospitalier Universitaire Sainte-Justine, Montreal, Quebec, Canada.

<sup>8</sup> Division of Hematology Oncology and Bone Marrow Transplantation, Hospital for Sick Children, Toronto, Ontario, Canada.

<sup>9</sup> Division of Pediatric Cardiology, University of Alberta, Edmonton, Alberta, Canada.

<sup>10</sup> Division of Nephrology, Montreal Children's Hospital, McGill University Health Centre, Montreal, Quebec, Canada.

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Correspondence: Seema Mital, MD, Hospital for Sick Children, 555 University Avenue, Toronto, ON, Canada M5G 1X8. (seema.mital@sickkids.ca).

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Pediatric transplantation represents up to 15% of all solid organ transplants (SOTs) performed in Canada (14% heart, 10% liver, 3% renal, 1% lung as of 2015 statistics),<sup>1</sup> and approximately 18% of hematopoietic stem cell transplants (HSCT).<sup>2</sup> Immunosuppression management is challenging with the interval from birth to young adulthood seeing profound changes in physiological processes, body size, and immune maturation. Infancy and adolescence are the periods of most rapid and dramatic change.<sup>3-16</sup> Three pivotal factors affect immunosuppression requirements in the young: (1) age-dependent variation in drug metabolism; (2) developmental changes in immune function with increased childhood susceptibility to infections, in particular viruses; and (3) behavioral changes in adolescence and young adulthood leading to poor treatment adherence.

The Pediatric Outcomes in Transplant: PersOnalising ImmuNosuppression To ImProVe Efficacy (POSITIVE) Study is a national collaborative research study that was launched to address the challenges faced by this underrepresented population with unique needs. The Clinical Trials in Organ and Transplantation in Children (CTOT-C) network (ctotc.org) sponsored by the National Institute of Allergy and Infectious Diseases in the US is an example of a multicenter network that supports organ-specific projects.<sup>17</sup> Unlike consortia that are age- or organ-specific, the POSITIVE network includes participants across all ages and transplant types with the goal of developing strategies to individualize immunosuppression tailored to the unique biological and behavioral attributes of the growing child.<sup>18,19</sup> The POSITIVE network was launched as part of the Canadian National Transplant Research Program funded by the Canadian Institutes for Health Research.<sup>20</sup> The purpose of this article is to describe the design of the study and how this unique collaborative will generate a new paradigm for application of precision medicine in the posttransplant care of children and young adults.

The specific aims are to: (1) develop age-appropriate calcineurin inhibitor dosing for pediatric SOT recipients, (2) develop risk prediction tools based on viral genotype and viral-host

interactions that predispose to Epstein-Barr virus (EBV) and/or posttransplant lymphoproliferative disorders (PTLD) in SOT and HSCT recipients, (3) develop healthcare delivery strategies to enhance medication adherence in adolescent and young adult SOT recipients (POSITIVE-Adherence study). Figure 1 shows the various disciplines involved in each study aim.

## MATERIALS AND METHODS

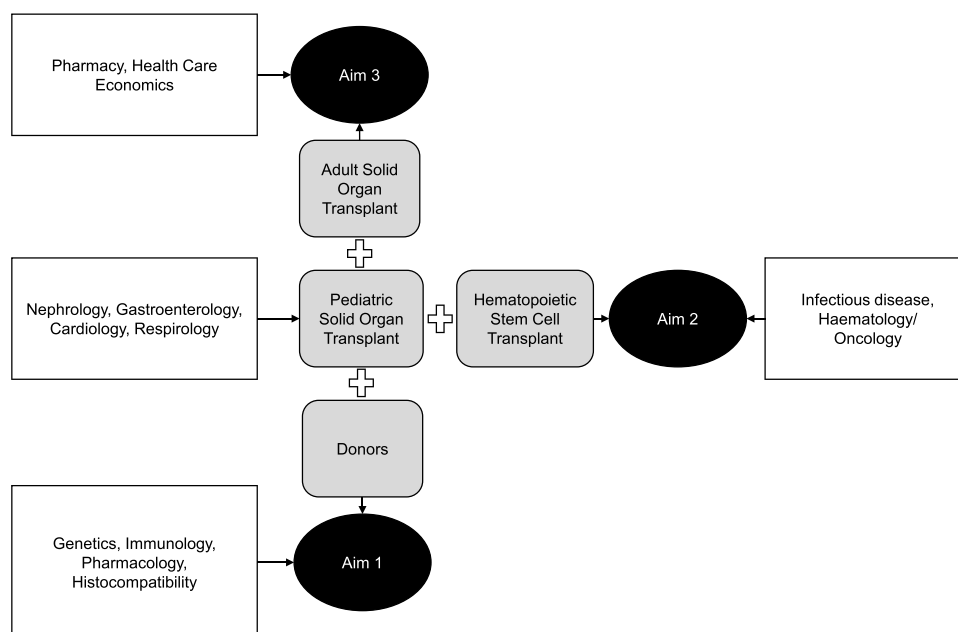
### Study Design

This was a prospective cohort study involving 14 transplant centers across Canada which included 7 pediatric (including 6 HSCT programs) and 7 adult SOT centers. Incident (listed or newly transplanted) and prevalent (previously transplanted) SOTs were recruited. HSCTs were recruited only for study aim 2. Living transplant donors were recruited where applicable. For deceased transplant donors who had consented to research at the time of donation, samples were obtained through participating histocompatibility (HLA) laboratories (Figure 2). The protocol was approved by the local or central Research Ethics Boards at pediatric (Vancouver: H15-02562; Edmonton: Pro00053576; Calgary: REB15-1876; Winnipeg: HS14051 (H2011:321); Toronto: 1000045186; Montreal: MP-CUSM-14-174-PED) and adult transplant centers (Vancouver: H14-03020; Toronto: 14-8220-AE; Ottawa: 20150393-01H; Montreal: 14.126) and local Organ Procurement Organizations. Written informed consent was obtained from each participant and/or parent/legal guardian and study protocols adhered to the Declaration of Helsinki.

### Study Participants

#### Aim 1

For the incident cohort, patients 0 to 18 years of age at the time of listing for SOT (heart, liver, kidney, or lung) were approached for consent to participate which included 2-year prospective data collection, self-reported questionnaires, and blood collection for genetic studies and biobanking, and for serial immune function and future biomarker assays (at pretransplant,



**FIGURE 1.** Clinical and research disciplines involved in project aims.



Pediatric Transplant Centres	Adult Transplant Centres
Hospital for Sick Children, Toronto, ON	Toronto General Hospital, Toronto, ON
Alberta Children's Hospital, Calgary, AB	Vancouver General Hospital, Vancouver, BC
Stollery Children's Hospital, Edmonton, AB	St.Paul's Hospital, Vancouver, BC
CHU Sainte Justine, Montreal, QC	Royal Victoria Hospital, Montreal, QC
Montreal Children's Hospital, Montreal, QC	Centre Hospitalier Universitaire Montreal, Montreal, QC
BC Children's Hospital, Vancouver, BC	Foothills Medical Center, Calgary, AB
Winnipeg Children's Hospital	The Ottawa Hospital, Ottawa, ON

**FIGURE 2.** POSITIVE Study participating sites (pediatric solid organ transplant center only ☆, pediatric solid organ and hematopoietic transplant center ★, adult solid organ transplant center ▲).

3 ± 1 month and 12 ± 3 months posttransplant). Retransplants were excluded. For the prevalent cohort, all patients 0 to 18 years of age who had undergone an SOT were consented for retrospective data collection, and blood or saliva collection for genetic studies. Living donors were approached for blood or saliva collection for genetic studies. For deceased donors who consented to research, leftover DNA available through HLA laboratories was accessed. Target enrollment for incident and prevalent patients was 800.

**Aim 2**

The SOT recipients who developed new EBV infection ie, seronegative patients who became EBV polymerase chain reaction–positive or developed PTLD during 1-year posttransplant follow-up were eligible for this aim. The HSCT recipients who developed new or secondary EBV infection and/or PTLD were eligible. Eligible retransplants were included. Consented patients were administered an EBV illness severity questionnaire and provided a blood sample for EBV sequencing and immune function assays within 3 weeks of diagnosis (acute phase) and 8 ± 2 weeks later (convalescent phase). Target enrollment was 150 patients including 50 EBV+ SOT recipients, 50 EBV+ HSCT recipients and 50 with PTLD. A schedule of study procedures for aims 1 and 2 are shown in Table 1.

**Aim 3 (POSITIVE-Adherence Study)**

Adolescent and young adult kidney, liver, and heart recipients, 14 to 25 years of age, who were at least 3 months posttransplant with intact graft function and receiving maintenance immunosuppression were eligible for participation in Aim 3. Patients who underwent retransplant or multiorgan transplant, or could not independently complete study questionnaires due to neurocognitive disabilities or language barriers were excluded. Study participation included serial questionnaires (at enrollment, 3 months, and 6 months), manual pill count by study coordinator during follow-up visits, and collection of pharmacy refill records. Within 3 months of the first

participant enrolled, transplant directors and nurses were asked to complete questionnaires on care processes and structures in their transplant program. Target enrollment was 300 participants. The schedule of study procedures is detailed in Table 2.

**Study Procedures, Outcomes, and Planned Analyses**

**Aim 1**

For genetic studies, DNA was extracted from blood from participants for genotyping using the Axiom Transplant Genotyping Array (Thermo Fisher) designed by the iGeneTRiN international consortium.<sup>21,22</sup> The array has 782000 single-nucleotide polymorphisms (SNPs), copy number variants, and insertion/deletion markers that also include content relevant to transplantation, including HLA and killer immunoglobulin-like receptors markers, phenotype associations, expression quantitative trait loci, pharmacogenomics, ancestry markers and markers related to transplant relevant outcomes. The array includes a comprehensive genomewide imputation grid for major populations, including European, Asian, and

**TABLE 1.** Schedule of study procedures for POSITIVE study (aims 1 and 2)

	Pretransplant					Posttransplant				
	T1	T2	T3	T4	T5	T6	T7	E1	E2	E3
<b>Aim 1: pharmacogenetics and immune function</b>										
Enrollment questionnaire	x									
Blood collection	x					x	x			
Data collection	x	x	x	x	x	x	x	x	x	x
<b>Aim 2: EBV-host interactions</b>										
EBV questionnaire								x	x	
PTLD questionnaire										x
Blood sampling								x	x	x

T1, baseline pretransplant; T2, 36-48 hours posttacrolimus initiation; T3, 7 days; T4, 14 days; T5, 30 days; T6, 3(±1) month; T7, 12(±3) months; E1, EBV acute phase (within 3 weeks of diagnosis); E2, EBV convalescent phase (8 ± 2 weeks postdiagnosis); E3, PTLD diagnosis.

**TABLE 2.**  
Schedule of procedures for POSITIVE-adherence (aim 3)

	T1	T2	T3
Care structures and organization questionnaire (transplant directors)	X		
Care processes and team expertise questionnaire (transplant nurses)	X		
Coordinator Administered participant questionnaire (includes BAASIS and manual pill count <sup>a</sup> )	X	X	X
Patient Perspectives Questionnaire (includes ABMS)	X		
Patient Education and Employment Questionnaire (for patients 18 years and older)	X		
Pharmacy refill records	X	X	X
Data collection	X	X	X

BAASIS, Basel Assessment of Adherence to Immunosuppressive Medications; T1, enrollment; T2, 3 months postenrollment; T3, 6 months postenrollment.

<sup>a</sup> Manual pill count at baseline only.

African ancestry. The planned approach is a genomewide association study to identify SNPs in recipients and donors associated with variability in tacrolimus trough concentrations after transplant and to combine genetic-, age- and organ-specific factors to develop individualized tacrolimus dosing to achieve and maintain therapeutic drug levels. We will apply principal component analysis to the genotype data for race and other clinical variables to explore unmeasured subpopulations or population structure further. To account for intersubject variation, a linear mixed-effect model, including subject as a random effect, will be applied to the data. Clinical covariates associated with pharmacological outcome will be first identified using univariate models, tacrolimus dose will also be included along with clinical covariates in the model. In addition, the impact of these factors on clinical outcomes will be assessed as described in Tables 3, 4.

For immune function and maturation assays, peripheral blood mononuclear cells are isolated from heparinized blood samples collected pretransplant, and at 3 and 12 months posttransplant. Immune phenotyping is performed using multicolor flow cytometry with 5 panels of up to 10 surface markers using BD Fortessa and Beckman Coulter Navios flow cytometers. Besides the global phenotyping, T and B cell populations are assessed for specific subtypes. T cells are assessed for naive versus memory/effector, regulatory, recent

thymic emigrant, and “exhausted” phenotypes. B cell subanalysis measures (switched) memory phenotypes, transitional, splenic marginal zone and “B-10” cells. To assess the capability of activation and proliferation, peripheral blood mononuclear cells are stimulated with global mitogens and specific antigens, including EBV and cytomegalovirus surface structures and cell lysates. Activation is measured by fluorescence-activated cell sorting via expression of CD69 and quantification of intracellular cytokines. Proliferation is measured after 5 days of stimulation to determine which cells have proliferated and to which generation they have grown and whether memory or naive cells mounted the response. Antigen stimulation provides information specifically with regard to the response to the respective virus and possibly prediction of the risk for persisting infection and development of PTLD. Immune function assays will be compared between patients in different age groups relative to tacrolimus levels. This will help to define the optimum target range of tacrolimus for patients in different age groups and will assist in developing age-appropriate dosing that is targeted to immune function.

## Aim 2

Infants and children after SOT or HSCT transplant are at high risk for EBV/PTLD because they are often EBV naive

**TABLE 3.**  
Clinical outcomes definitions

Clinical outcomes	Definition
Rejection	Renal <sup>23</sup> : acute or chronic cell and antibody-mediated rejection Heart <sup>24</sup> : rejection grade of 2R or higher or AMR = 1 Liver <sup>25</sup> : Banff ACR score of 4/9 or higher Lung <sup>26</sup> : Acute rejection grade $\geq$ A2 or chronic airway rejection C1
Graft failure	Graft dysfunction resulting in transplant relisting or retransplantation
CVS complications	New diagnosis of hypertension, coronary artery disease or graft vasculopathy
CNS complications	New diagnosis of stroke, seizures or PRES
Cancer	Diagnosis of PTLD or other cancer(s)
New EBV infection	First positive EBV PCR posttransplant in seronegative patient
New CMV infection	First positive CMV PCR posttransplant in seronegative patient
Other infections	Toxoplasma infection, polyoma/BK virus nephropathy, <i>Pneumocystis carinii</i> Pneumonia, Aspergillus infection, HIV, Hep B, Hep C
Tonsillectomy	
Death	
Renal dysfunction	eGFR using updated Schwartz bedside formula, dysfunction defined as $<90$ mL/min per 1.73 m <sup>2.27</sup>

AMR, antibody-mediated rejection; ACR, acute cellular rejection; CVS, cardiovascular; CNS: central nervous system; PRES: posterior reversible encephalopathy syndrome; PCR, polymerase chain reaction; CMV, cytomegalovirus; HIV, human immunodeficiency virus; Hep B, hepatitis B; Hep C, hepatitis C.

at the time of transplant and therefore lack immunity to EBV.<sup>28,29</sup> Higher intensity of immunosuppression also predisposes to EBV. However, little is known about how EBV strains may interact with an immature host system to predispose to disease.<sup>30-32</sup> In this aim, blood samples from patients with new and secondary EBV infection will be analyzed using next-generation sequencing to identify EBV genotypes circulating in peripheral blood during acute and convalescent phases. Known EBV subtypes, based on the major latent genes, will be determined in addition to novel subtypes.<sup>33</sup> Our primary analysis will be the relationship between major EBV subtypes and clinical and virologic outcomes (illness severity, viral loads, PTLD), evaluated in different age groups. We will assess the interaction between EBV genetic diversity and host immunologic factors (age, immunologic maturation, lymphocyte memory, EBV-specific T cells) that influence host susceptibility to EBV. We will identify whether there is a correlation between lack of immune memory and a higher risk of PTLD in patients with low T cell proliferative capacity. The results will be used to develop an EBV genotyping panel to screen patients with EBV infections to determine which patients exposed should receive EBV therapy.

### Aim 3

In addition to biological factors being investigated in aims 1 and 2, psychosocial and organizational factors may influence transplant outcomes in adolescence and young adulthood. To assess this, eligible participants complete a questionnaire assessing factors associated with adherence including health literacy, self-efficacy, trust in the care team, social supports, attitudes toward taking immunosuppression medications, and intention to adhere. Participants also complete the standardized Adolescent Medication Barriers Scale questionnaire.<sup>34</sup> A separate set of questions is administered to participants by coordinators to assess socioeconomic status and family structure and support, accessibility to care, and treatment characteristics. Participants from adult centers (>18 years) are also asked to report individual sociocultural, education and labor market activity-related questions. A validated adherence self-report tool, the Basel Assessment of Adherence to Immunosuppressive Medications, is administered at enrollment, 3 and 6 months.<sup>35</sup> Medication adherence is also assessed using the combination of a pill count at baseline and pill count and pharmacy refill records over the 6 months of observation. Transplant program directors at each site completed a questionnaire about the characteristics of their program, including factors such as program size, care team composition, care organization, and frequency of routine follow-up. Similarly, transplant nurses at each site complete a questionnaire assessing care processes such as average time spent with patients during clinic visits, methods of assessing and supporting adherence, expertise and competency of team members, and chronic illness management strategies used.<sup>36,37</sup>

The primary outcome is “taking adherence,” defined as the proportion of prescribed immunosuppressive medication doses taken. The secondary outcomes are “timing adherence,” defined as the proportion of doses taken late, and the rate of “drug holidays,” defined as a period during which 2 or more consecutive doses were missed. Variability in tacrolimus/sirolimus trough levels are assessed as a measure of timing adherence.<sup>38-42</sup> Clinic attendance and clinical

monitoring tests attended versus expected (based on routine follow-up protocols) are captured and used as an additional predictor of adherence. Graft outcomes and other clinical complications are also collected as secondary outcome measures (Table 3). Although not sufficiently powered due to incomplete overlap of participants in aims 1 and 3, secondary analysis will be performed to assess the relationship between adherence (aim 3) and immunosuppression levels and outcomes (aim 1).

### Power Considerations

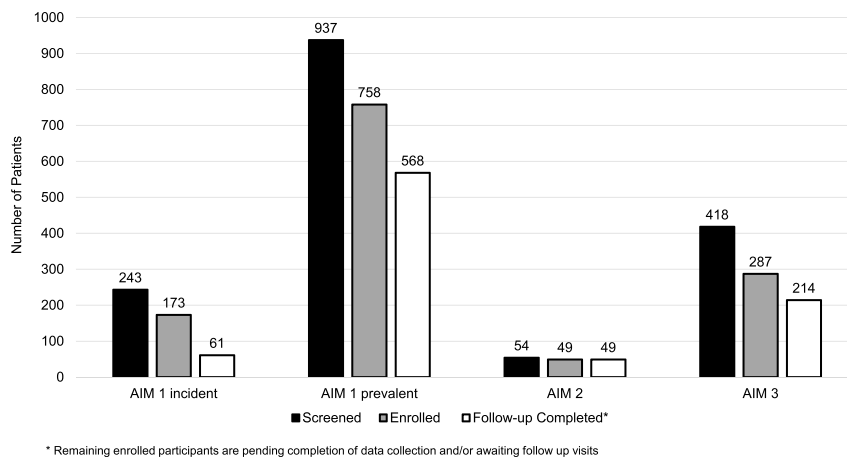
For genomewide association study analysis in aim 1, assuming minor allele frequency of significant SNPs ranges from 0.04 to 0.23, a sample size of 500 is sufficient to detect a mean additive SNP effect of 1.7 for a SNP having a minor allele frequency of 0.17 in controlling an error rate of 0.05 with 80% power. For aim 2, in a group of 50 participants (100 samples), precision levels from whole genome shotgun sequencing are  $\pm 3.5\%$  with 95% confidence to accurately reflect EBV subtypes associated with disease with a minimum prevalence of 5% in the proposed study cohort of 300 samples. For aim 3, based on pilot studies indicating ~78% taking adherence with a SD of 30, we estimate that 300 patients will provide 80% power to detect a correlation between adherence and any continuous variable accounting for 2.5% or greater of the variance in adherence. For categorical variables with a frequency of 10%, we will have 80% power to detect a 20% difference in adherence for that factor. These sample size estimates assume that the small amount of between-program variability in adherence will be explained by between program differences in measured healthcare systems factors.

### STUDY PROGRESS

Enrollment for aims 1 and 3 was completed in September 2017 and December 2017 respectively. Enrollment for aim 2 and follow-up of enrolled patients will continue until December 2018. From 2015 to 2018, 1662 new and prevalent transplant recipients were screened, 1166 were recruited across the various aims. This included 370 liver, 445 kidney, 277 heart, 19 lung, 19 multiple, and 36 HSCT transplants. Twelve percent were younger than 2 years, 30% were 2 to 10 years, 42% were 10 to 18 years, and 16% were 18 to 24 years at enrollment. Nine hundred thirty-one consented to participation in aims 1 and 2 (90% consent rate), 287 in aim 3 (82% consent rate). The number of biospecimens collected included 898 for DNA, 276 for immunoassays, and 717 for biomarker studies. Approximately 70% participants have completed the follow-up, and 30% are pending study completion. Figure 3 shows the number of incident, and prevalent patients who were screened, enrolled, and completed 1 year follow-up. Patient characteristics at enrollment and at 1-year follow-up are shown in Table 4.

### DISCUSSION

The goals of precision medicine are to individualize management based on the unique clinical and biological profiles of individuals or groups of individuals. The management of transplant recipients centers around standardized immunosuppression protocols that try to optimize dosing while minimizing immunosuppression-related side effects. However, transplant recipients are clinically and biologically heterogeneous. Yet, individualized approaches are applied very selectively in



**FIGURE 3.** Screening and enrollment summary for POSITIVE study aims.

the transplant field. For example, HLA matching of donor and recipient is used to individualize donor choice.<sup>37</sup> There are recommendations to individualize tacrolimus dosing by the recipient *CYP3A5* genotype published by the Clinical Pharmacogenetics Implementation Consortium.<sup>43</sup> However, these have not had wide uptake and these guidelines do not adjust for age-related differences in pharmacogenetic influences.<sup>15</sup>

**TABLE 4.**

**Baseline characteristics of participants at enrollment and 1 year after transplant**

	Consented (n = 931), n (%)	Completed 1 year follow-up <sup>a</sup> (n = 629), n (%)
Male (%)	493 (53%)	341 (54%)
Median age (IQR), y	11 (5-15)	11 (6-11)
≤2 y	128 (14%)	58 (9%)
2-10 y	331 (36%)	236 (38%)
≥11 y	470 (51%)	334 (53%)
Race (%)		
White	566 (61%)	382 (61%)
Black	46 (5%)	31 (5%)
Asian	175 (19%)	121 (19%)
Aboriginal	36 (4%)	18 (3%)
Mixed race (>1 race)	65 (7%)	46 (7%)
Other	18 (2%)	11 (2%)
Unknown	25 (3%)	20 (3%)
Transplant type (%)		
Liver	323 (35%)	222 (35%)
Kidney	305 (33%)	199 (32%)
Heart	265 (28%)	186 (30%)
Lung	19 (2%)	14 (2%)
Multiple	19 (2%)	8 (1%)
Clinical outcomes		
Rejection		186 (30%)
New EBV infection		124 (20%)
New CMV infection		78 (12%)
PTLD		24 (4%)
Seizures		22 (4%)
Graft vasculopathy		10 (2%)
New-onset hypertension		61 (10%)
Tonsillectomy		12 (2%)

<sup>a</sup> As of April 25, 2018.

Developing a precision medicine approach that incorporates the clinical and biological heterogeneity of different organ groups across different ages requires access to large patient cohorts to achieve sufficient power. The POSITIVE study enabled us to recruit a large patient cohort to achieve power for these analyses by unifying the various transplant populations for this study. Preliminary results from the various aims are being generated that attest to the power to achieve the study goals.<sup>44-48</sup>

At completion of analyses, the POSITIVE study will deliver tacrolimus dosing guidelines based on age, organ type and pharmaco-genotype for pediatric SOT patients. This will be complemented by the development of age-appropriate immunosuppression targets based on immune function and maturation at different ages including a better understanding of the specific aspects of the immature immune system that contribute to better graft tolerance in infants. Another important deliverable is an EBV genotype screening panel that will identify EBV strains that are likely to lead to disease through interaction with the host immune system. It will identify high risk patients who would benefit from timely treatment with antiviral therapies or lowering of immunosuppression. In adolescents and young adults who are at high risk for nonadherence, it will identify healthcare processes and structures that can be modified to improve medication adherence. Finally, collaboration with health economists will be used to analyze cost effectiveness of proposed changes to care organization to support advocacy for these changes as part of public health policy.

Several features make the POSITIVE collaboration unique. Leveraging commonalities across SOT and HSCT recipients, we were able to prospectively recruit patients across Canada to achieve larger sample sizes than otherwise possible. Recruiting not just children but also young adults allowed us to compare variability in care processes and structures between pediatric and adult centers, whereas donor recruitment provided us with the ability to incorporate donor characteristics into precision medicine approaches for recipients. Finally, including researchers and clinicians with expertise in transplant medicine, immunology, genetics, pharmacogenetics, precision medicine, virology, epidemiology and healthcare economics will help us to translate discoveries from this study into changes in clinical practice. The longer-term impact of these changes will be assessed through linkage with

external administrative databases such as provincial health databases, Canadian Blood Services or the Canadian Institute for Health Information to access long-term outcomes. This is made possible because participants in POSITIVE consented to participate in the Canadian National Transplant Research Program Patient Registration Database. Thus, our efforts at unifying the various transplant communities will help us to change our approach to the care of the transplanted patient across all ages and organ groups.

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