Stanford | MEDICINE

Katja G. Weinacht, MD, PhD
Assistant Professor
Department of Pediatrics
Stem Cell Transplantation
Regenerative Medicine

Stanford University
240 Pasteur Drive
Biomedical Innovations
Building
Suite 2500
Stanford, CA 94305

May 11, 2024

Re: **TRAN: TRAN1-16025 – Response to CIRM reviewer comments**

Dear Members of the Independent Citizen's Oversight Committee,

I am writing to express my gratitude for reviewing our application "Translating iPSC-derived Thymic Epithelial Cells into a Cell Therapy for Children with Congenital Athymia". We are thrilled to see that all reviewers unanimously recognized the high impact of our work. While carefully examining the concerns raised, it became clear the reviewer's questions pertained almost exclusively to our HLA-matching approach. As a pediatric stem cell transplanter, I took critical information for granted and failed to convey our carefully thought-out HLA matching strategy in the original submission. I sincerely regret this oversight and would like to take this opportunity to provide critical information for evaluating our proposal in this letter.

Background

High resolution HLA-typing is currently considered standard of care for donors and recipients of hematopoietic stem cell stem cell grafts and provides information for the 8 most important antigen recognition domains on both alleles of HLA-A, -B, -C and -DRB1. As the thymus determines the T cell receptor repertoire of the immune system, the same considerations as for hematopoietic stem cell grafts apply. If a related (i.e., sibling) 8/8 HLA-matched donor is not available, unrelated donors are considered. For bone marrow or peripheral blood stem cell products, an 8/8 match or 7/8 match are desirable/suitable, while for cord blood products, a 4/6 match at HLA-A, -B and DRB1 is still acceptable. Unlike hematopoietic stem cells, the availability of solid donor organs is much more limited. Accordingly, it is usually not feasible to HLA-match solid donor organs in the same way as hematopoietic grafts, although there is overwhelming evidence that better HLA-matching of donor organs greatly benefits graft function and patient survival[1]. Due to lack of adequate HLA-matching in solid organ transplantation, lifelong immunosuppression of the recipient is often required.

Allogeneic thymus transplants are even more limited in their availability, necessitating that physicians forego HLA-matching altogether, which usually results in an 0/8 HLA-match. This practice seems to defy all principles of transplantation tolerance and indeed is only possible because congenital athymia patients have no immune system to reject the thymus transplant. The transplanted thymus then gives rise to a new immune system that is tolerant of the transplanted thymus tissue, but almost always attacks the unmatched host, resulting in autoimmunity. Unlike other epithelial organs, thymic epithelial cells lack effective surface expression of ABO blood group antigens and ABO matching is therefore not required[2-4]. Notably, congenital athymia

patients have normal ABO titers (John Sleasman, Director of the Duke Thymus implantation program, personal communications).

Addressing the Reviewer Comments

The main questions raised by the GWG reviewers were,

(1) how feasible it will be to find a suitable donor for a future clinical trial and, related to this,

(2) *how closely we plan to HLA-match*.

Because we have developed a way to generate thymic epithelial cells from iPSCs and can generate iPSCs from blood cells, our HLA-matching strategy can tap into the resources developed for hematopoietic stem cell transplantation. For patients without a suitable related HLA-matched donor, HLA-unrelated donor registries of adult volunteers and banked umbilical cord–blood units, such as the "Be the Match Registry" of the National Marrow Donor Program (NMDP), represent an invaluable source of donors[5]. In anticipation of a clinical trial testing the safety and efficacy of our therapeutic candidate, we have established a collaboration with the NMDP (letter of support from Heather Stefanski, MD, PhD, Vice President of Medical Affairs at the NMDP is included in the original submission). To answer the reviewer questions, we have examined

(1) the representation of volunteer donors in the NMPD registry broken down by broad and detailed racial/ethnic groups, and

*(2) the racial/ethnic distribution of patients with congenital athymia (**Figure 1**).*

Detailed Racial/Ethnic Group	Effective Registry Size (Donors of Any Age), n (%)	Effective Registry Size (Donor Age ≤35 yr), n (%)	Broad Racial/ Ethnic Group	Effective Registry Size (Donors of Any Age), n (%)	Effective Registry Size (Donor Age ≤35 yr), n (%)
African American	364,670 (3.42)	152,179 (3.39)	African American	433,724 (4.06)	185,822 (4.14)
African	31,510 (0.3)	15,895 (0.35)			
Caribbean Black	33,058 (0.31)	16,293 (0.36)			
Black–South or Central American	4486 (0.04)	1455 (0.03)			
South Asian Indian	345,250 (3.23)	131,675 (2.93)	Asian or Pacific Islander	909,662 (8.52)	380,760 (8.48)
Filipino	87,989 (0.82)	38,273 (0.85)			
Hawaiian or Other Pacific Islander	18,928 (0.18)	7486 (0.17)			
Japanese	34,637 (0.32)	7464 (0.17)			
Korean	118,003 (1.11)	44,258 (0.99)			
Chinese	176,462 (1.65)	86,092 (1.92)			
Southeast Asian	53,331 (0.5)	26,925 (0.6)			
Vietnamese	75,062 (0.7)	38,587 (0.86)			
Middle Eastern or N. Coast of Africa	440,319 (4.13)	177,556 (3.95)	Caucasian	8,378,155 (78.5)	3,576,144 (79.61)
European Caucasian	7,937,836 (74.37)	3,398,588 (75.66)			
Caribbean Hispanic	271,006 (2.54)	122,947 (2.74)	Hispanic	879,156 (8.24)	327,676 (7.29)
Mexican or Chicano	214,696 (2.01)	5420 (0.12)			
Hispanic–South or Central American	393,454 (3.69)	199,309 (4.44)			
American Indian–South or Central American	10,166 (0.1)	4573 (0.1)	Native American	72,582 (0.68)	21,803 (0.49)
Alaska Native or Aleut	5135 (0.05)	2483 (0.06)			
North American Indian	53,407 (0.5)	12,399 (0.28)			
Caribbean Indian	3874 (0.04)	2348 (0.05)			

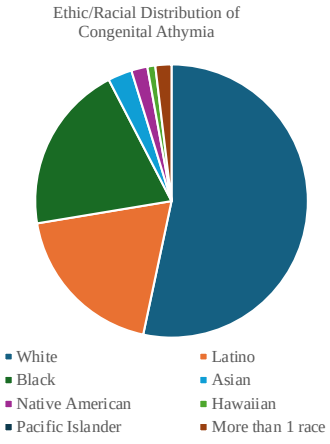

Figure 1. Donor Statistics of Detailed and Broad Racial/ Ethnic Groups in the NMDP Registry (left) and Racial/ Ethnic Distribution of Congenital Athymia (right). Data table (left) from Chowdhury et al., Existence of HLA-Mismatched Unrelated Donors Closes the Gap in Donor Availability Regardless of Recipient Ancestry, Transplantation and Cellular Therapy, 2023. Official data of the NMPD. Graph (right) based on data from Markert et. al, Experience with cultured thymus tissue in 105 children, J All Clin Immunol, 2022.

Extrapolating from the hematopoietic stem cell transplantation experience of the last 60 years, an 8/8 HLA-match for iPSC-derived thymic tissues would be most desirable, however, a based on the allogenic thymus transplantation experience with now >150 patients[2,3], a 7/8 match or even a 6/8 match is beyond doubt far superior in reducing autoimmune complications compared to an 0/8 match which is the current standard of care.

The NMDP has quantified the impact of less restrictive matching in the framework of previously established registry models[6]. In this work, the NMPD calculated HLA-match likelihoods using a population-level genetic model for all detailed and broad racial/ethnic groups available in the "Be The Match" registry for high-resolution HLA-A,-B,-C, and DRB1 typing at 8/8, 7/8, 6/8, and 5/8 match levels[6]. As depicted in **Figure 2**, almost all patients, including underrepresented racial/ethnic groups, will have an > 80% likelihood of finding a 7/8 HLA-match (orange bars),

while 99% of all patients from all racial/ethnic groups will be able to find a donor if match stringency is reduced to a 6/8 HLA-match (green bars). We therefore propose to strive for an 8/8 HLA-match but deem a 6/8 HLA-match acceptable, which will be feasible in 99% of patients from all racial/ethnic groups.

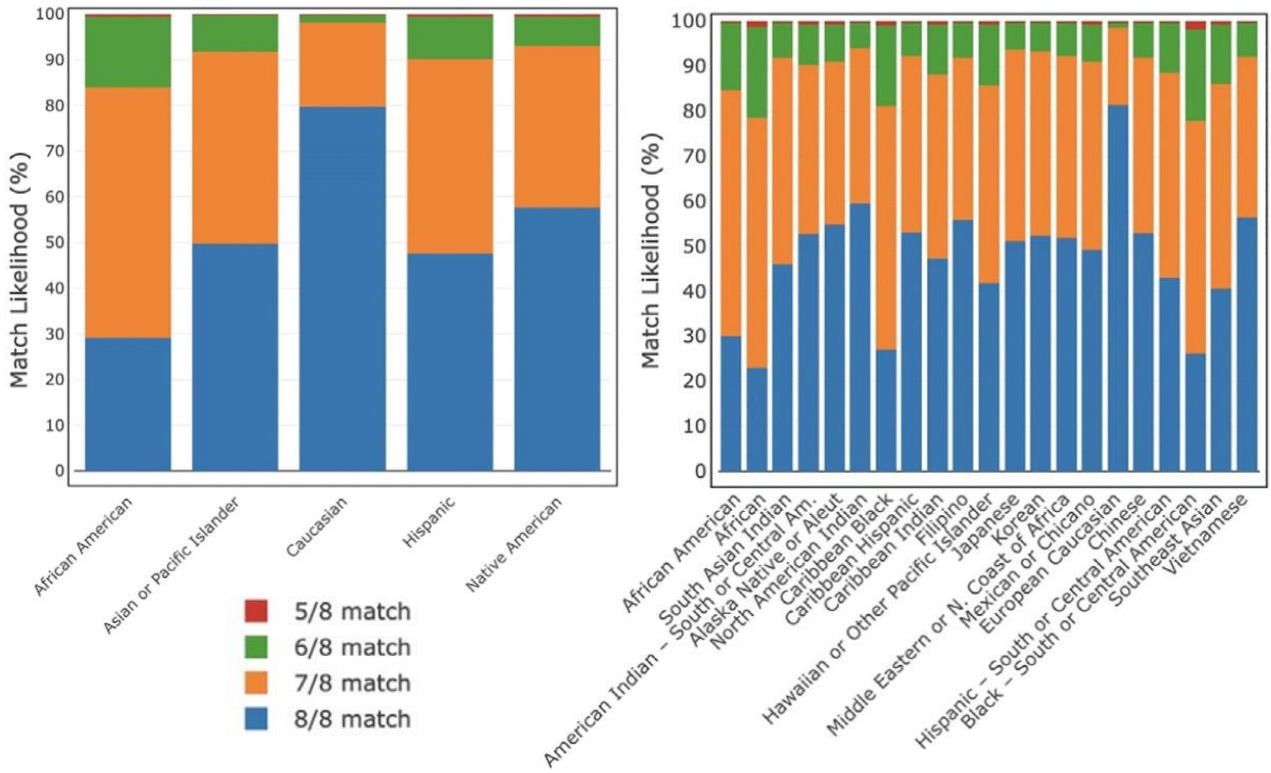

Figure 2. HLA-match Likelihood (%) at 5/8 to 8/8 levels. HLA-match likelihood is represented for for 5 broad ethic groups (left) and 21 more refined ethnic groups (right). At a 6/8 HLA-match level, almost 99% of patients find a suitable donor. Images from Chowdhury et al., Transplantation and Cellular Therapy, 2023. Official data of the NMPD.

Planning ahead to fulfill the value proposition of our work

While this proposal seeks to run the first clinical trial in patients with congenital athymia which have the greatest unmet medical need, we would like to underscore that the value proposition of iPSC-derived thymic epithelial cells is of a much larger magnitude. Our long term goal is to make iPSC-derived thymic tissues available to a much broader population of patients in need of better T cell immunity. These include patients after hematopoietic stem cell transplantation; patients receiving other forms of cancer directed therapies, i.e. check point blockade, cancer vaccines; HIV patients; and recipients of solid donor organs in need of tolerance induction. Lastly, we would like to highlight that the aging California population at large would benefit from improved T cell immunity, as immune competence declines with age (immune senescence). The impact of this phenomenon was tragically exposed during the COVID-19 pandemic. Promoting T cell reconstitution "from within", without the need for laborious and costly T cell manufacturing, could transform the immunotherapy landscape and begin the next chapter of cell therapy. As such, our ultimate goal is to take iPSC-derived thymic tissues from a "bespoke" to an allogenic "off the shelf" approach.

In preparation of expanding the indication for our therapeutic candidate and move to an "off the shelf" approach *in the future*, the NMPD modeled the cumulative population coverage for the

most common HLA-A, -B and -DRB1 alleles. Based on a homozygous expression of HLA-A, -B and -DRB1 alleles, the top 10 haplotypes cover 30% of the US population, the top 45 haplotypes cover 50% of the population, and 361 haplotypes cover 80% of the US population (**Figure 3**). This means, if one were to create a repository of HLA-A, -B and -DRB1 haplo-identical GMP-grade iPSC lines, as few as 10, 45 and 381 iPSC lines would cover 30%, 50% and 80% of the US population, respectively. As such, fulfilling the value proposition of our therapeutic candidate is very feasible.

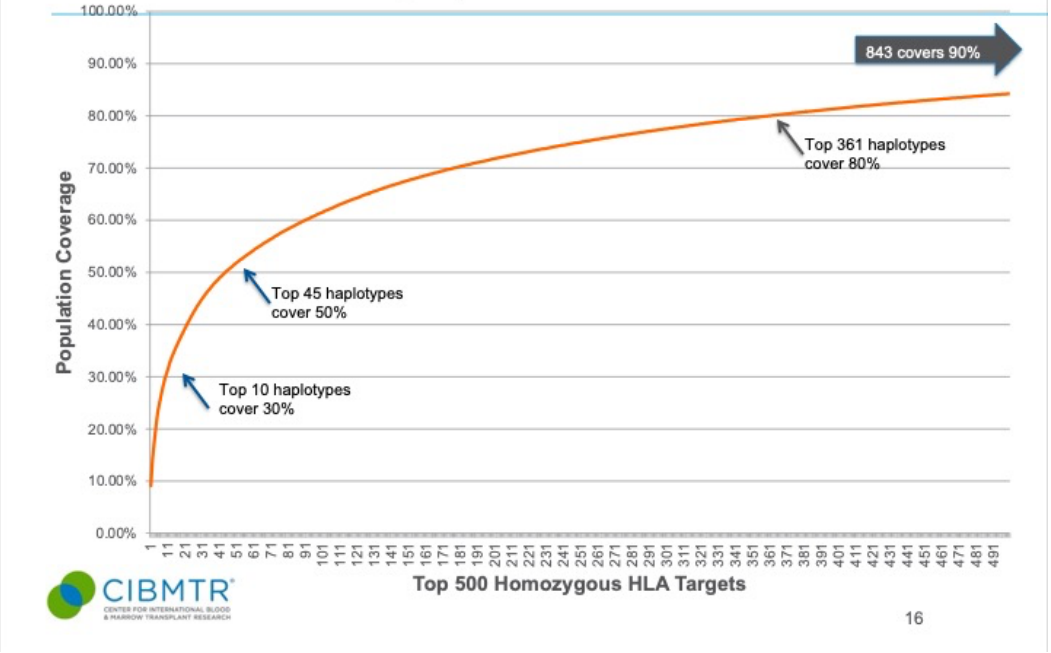

Figure 3. Cumulative Population Coverage Based on the US Census Proportions and NMDP Registry for HLA-A, -B and –DRB1 alleles. Internal NMPD 2024 data courtesy of Heather Stefanski, MD, PhD, VP of Medical Affairs, NMDP.

Once again, I would like to thank the ICOC members for giving us the opportunity to address the reviewer comments and for considering our responses. Based on this information, we hope the reviewers will reconsider funding this uniquely novel and powerful proposal at the upcoming ICOC meeting.

Warmest regards,

Katja G. Weinacht, MD, PhD
Assistant Professor of Pediatrics
Department of Hematology/Oncology and Hematopoietic Stem Cell Transplantation
Stanford School of Medicine

References:

1. Zachary AA, Leffell MS. HLA Mismatching Strategies for Solid Organ Transplantation - A Balancing Act. *Frontiers in immunology*. 2016;7:575. doi:10.3389/fimmu.2016.00575
2. Markert ML, Gupton SE, McCarthy EA. Experience with cultured thymus tissue in 105 children. *J Allergy Clin Immunol*. Feb 2022;149(2):747-757. doi:10.1016/j.jaci.2021.06.028
3. Davies EG, Cheung M, Gilmour K, et al. Thymus transplantation for complete DiGeorge syndrome: European experience. *J Allergy Clin Immunol*. Dec 2017;140(6):1660-1670 e16. doi:10.1016/j.jaci.2017.03.020
4. Sano R, Nakajima T, Takahashi Y, et al. Epithelial Expression of Human ABO Blood Group Genes Is Dependent upon a Downstream Regulatory Element Functioning through an Epithelial Cell-specific Transcription Factor, Elf5. *J Biol Chem*. Oct 21 2016;291(43):22594-22606. doi:10.1074/jbc.M116.730655
5. Gragert L, Eapen M, Williams E, et al. HLA match likelihoods for hematopoietic stem-cell grafts in the U.S. registry. *N Engl J Med*. Jul 24 2014;371(4):339-48. doi:10.1056/NEJMsa1311707
6. Chowdhury AS, Maiers M, Spellman SR, Deshpande T, Bolon YT, Devine SM. Existence of HLA-Mismatched Unrelated Donors Closes the Gap in Donor Availability Regardless of Recipient Ancestry. *Transplant Cell Ther*. Nov 2023;29(11):686 e1-686 e8. doi:10.1016/j.jtct.2023.08.014