NAS Forum on Regenerative Medicine
Hematology and Immunology Session II
Transplant and Gene Therapy for Monogenic Diseases

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Allogeneic Hematopoietic Stem Cell Transplant for Monogenic Diseases

Has become or is becoming the standard of care as curative therapy for:

- ***Hemoglobinopathies and inherited bone marrow failure syndromes***
- ***Primary immune deficiencies***
- ***Lysosomal storage diseases, some metabolic enzyme deficiencies and leukodystrophies***

Allogeneic Hematopoietic Stem Cell Transplant for Monogenic Diseases

Successful transplant requires:

- ***A suitably HLA matched donor***
- ***An adequate and safe conditioning regimen to achieve permanent engraftment***
- ***A preventative regimen to reduce or prevent graft versus host disease***
- ***Prevention, detection and effective treatment of virus, bacterial and fungal infections***

Allogeneic Hematopoietic Stem Cell Transplant for Monogenic Diseases

Current commonly used transplant regimens:

- ***Donor: HLA matched sibling or matched unrelated donor found through National Marrow Donor Program***
- ***Conditioning regimen: Deplete lymphocytes with chemotherapy/radiation/serotherapy; Deplete hematpoietic stem cells with chemotherapy/radiation.***
- ***Prevent GVHD: Immune suppressors or tolerance inducing agents***
- ***Infection: Early virus detection PCR – antivirus agents; Improved bacterial/fungus detection – New antibiotics.***

Allogeneic Hematopoietic Stem Cell Transplant for Monogenic Diseases

Transformative emerging technologies/approaches:

- ***Donor: Expanding the donor pool through approaches allowing use of haploidentical related donors.***
- ***Conditioning regimen: Serotherapy to replace radiation or alkylating agents to target hematpoietic stem cells.***
- ***Prevent GVHD: Eliminating alloreactive T cells.***
- ***Infection: Retaining anti-viral T cells; Treatment with virus specific donor or third party T cells.***

Allogeneic Hematopoietic Stem Cell Transplant for Monogenic Diseases

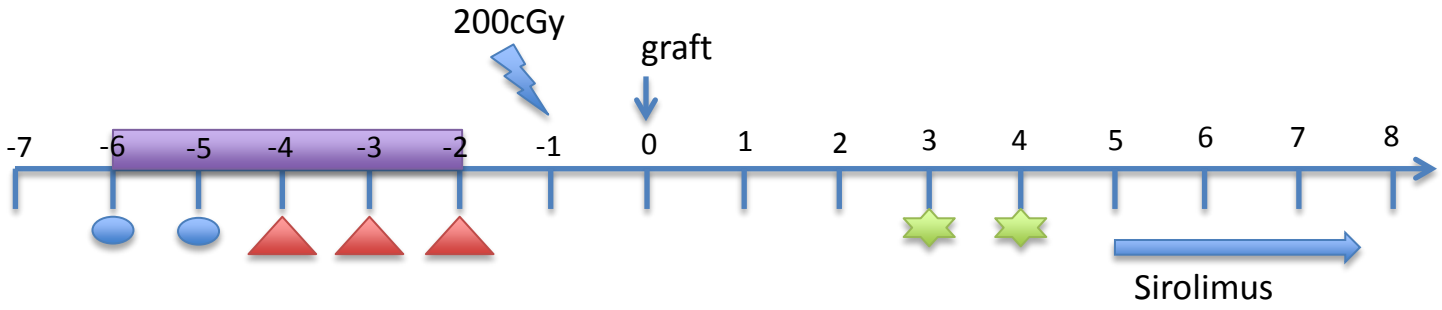
Expanding the donor pool: Haploidentical related donors.

- Depletion of mature T cells carrying α and β chains of the T-cell receptor, permitting maintenance of mature donor-derived Natural Killer cells and γδ(+) T cells in the graft. Locatelli F, et al. Immunol Lett. 2013 155:21.

- High-dose, post-transplantation cyclophosphamide for in vivo elimination of the activated strongly alloreactive T cells to promote tolerance in alloreactive host and donor T cells, leading to suppression of both graft rejection and GVHD after alloHSCT. Luznik L, Fuchs EJ. Immunol Res. 2010 Jul;47(1-3):65; Kanakry CG, Fuchs EJ, Luznik L. Nat Rev Clin Oncol. 2016 13:10.

- Examples of reports of high-dose, post-transplant cyclophosphamide for haploidentical related donor transplant of patients with primary immune deficiencies. Parta M, Hilligoss D, Kelly C, Kwatemaa N, Theobald N, Malech H, Kang EM. Haploidentical Hematopoietic Cell Transplantation with Post-Transplant Cyclophosphamide in a Patient with Chronic Granulomatous Disease and Active Infection: A First Report. J Clin Immunol. 2015 35:675; Freeman AF, Shah NN, Parta M, Su HC, Brofferio A, Gradus-Pizlo I, Butty S, Hughes TE, Kleiner DE, Avila D, Heller T, Kong HH, Holland SM, Hickstein DD. Haploidentical related donor hematopoietic stem cell transplantation with post-transplantation cyclophosphamide for DOCK8 deficiency. J Allergy Clin Immunol Pract. 2016 Sep 15. pii: S2213-2198(16)30311.

NIH Haplo Donor Conditioning Regimen



Fludarabine 30mg/m2

Cyclophosphamide 14.5mg/kg

Busulfan 3.2mg/kg

Cyclophosphamide 50mg/kg

Outcome to date 2014-2016 of a haplo donor protocol: Chronic Granulomatous Disease (group of disorders with mutations in subunits of the neutrophil microbicidal oxidase, leading to recurrent life-threatening infections and autoinflammation)

Patient	Donor	GvHD	Current status
#1 - Gp91	Father	Grade 1 gut, resolved with steroids	2 years post, doing well
#2 - Gp91	Father	Grade 1 gut, resolved with steroids	1 year post, doing well, still with PLE*
#3 - P47	Brother	Grade 2 gut and skin including eyes, resolving	100 days post, GvHD resolving with steroids and tacrolimus
#4 - P67	Mother	Grade 1 gut, resolved	100 days post, doing well
#5 - Gp91	Father	Grade 1 gut, resolved with steroids	60 days post, doing well

Allogeneic Hematopoietic Stem Cell Transplant for Monogenic Diseases

Replacing alkylating agents/radiation for marrow stem cell clearance conditioning: Serotherapy.

- Anti-c-Kit monoclonal antibodies to deplete HSCs from bone marrow niches with enhancement of the Fc-mediated antibody effector activity through simultaneous blockade of CD47, a myeloid-specific immune checkpoint, extends anti-c-Kit conditioning to fully immunocompetent mice. Such a targeted conditioning regimen that uses only biologic agents has the potential to transform the practice of HSC transplantation and enable its use in a wider spectrum of patients. Chhabra A, Ring AM, Weiskopf K, Schnorr PJ, Gordon S, Le AC, Kwon HS, Ring NG, Volkmer J, Ho PY, Tseng S, Weissman IL, Shizuru JA. Hematopoietic stem cell transplantation in immunocompetent hosts without radiation or chemotherapy. Sci Transl Med. 2016 Aug 10;8(351):351ra105.

- Further development of the concept such as use of toxin conjugated antibiodies or development of anti-cKit CAR T-cells might improve the efficacy of this approach, though off-target effects must be considered.

Allogeneic Hematopoietic Stem Cell Transplant for Monogenic Diseases

Adding to the already known agents that induce tolerance and prevent GVHD (rapamycin, cyclosporine A, tacrolimus, mycophenolate mofetil, methotrexate); There is a pressing need for development of new targets to prevent GVHD:

- One example of a potential new target: Highly specific Adenosine 2A receptor agonists. Zarek PE, Huang CT, Lutz ER, Kowalski J, Horton MR, Linden J, Drake CG, Powell JD. A2A receptor signaling promotes peripheral tolerance by inducing T-cell anergy and the generation of adaptive regulatory T cells. Blood. 2008 111:251; Lappas CM, Liu PC, Linden J, Kang EM, Malech HL. Adenosine A2A receptor activation limits graft-versus-host disease after allogenic hematopoietic stem cell transplantation. J Leukoc Biol. 2010 Feb;87(2):345-54; Han KL, Thomas SV, Koontz SM, Changpriroa CM, Ha SK, Malech HL, Kang EM. Adenosine A_2A receptor agonist-mediated increase in donor-derived regulatory T cells suppresses development of graft-versus-host disease. J Immunol. 2013 1;190:458

Allogeneic Hematopoietic Stem Cell Transplant for Monogenic Diseases

Highly effective measures to prevent GVHD may be associated with increased risk of virus infection in the early post transplant period: Virus specific T cell therapies

Serious viral infections are a common cause of morbidity and mortality after allogeneic stem cell transplantation, particularly with enhanced approaches to prevent GVHD despite improvements in early detection by digital drop pcr and improved antivirus drugs. Virus specific donor or third party T cells are increasingly being used to reduce mortality and provide a bridge to the re-acquisition of T cell immunity in post-transplant patients. Leen AM1, Heslop HE, Brenner MK. Antiviral T-cell therapy. Immunol Rev. 2014 Mar;258(1):12-29.

Integrating Vector Transduced Autologous Hematopoietic Stem Cell Transplant Gene Therapy for Monogenic Diseases

In the last 15 years there have been an accelerating number of published reports of clinical trials demonstrating significant unequivocal long-lasting clinical benefit from integrating vector autologous HSC gene therapy (See: Malech HL, Ochs HD. An emerging era of clinical benefit from gene therapy. JAMA. 2015 313:1522) and many current trials for these and related monogenic disorders that are in progress:

- ***Hemoglobinopathies***
- ***Primary immune deficiencies***
- ***Leukodystrophies***

Integrating Vector Transduced Autologous Hematopoietic Stem Cell Transplant Gene Therapy for Monogenic Diseases

Clinical trial reports demonstrating significant unequivocal long-lasting clinical benefit :

- *Thalassemia (Self-inactivating lentivector; busulfan conditioning):* Cavazzana-Calvo M, et al. Transfusion independence and HMGA2 activation after gene therapy of human β-thalassaemia. Nature. 2010 467:318.

- *ADA SCID (Murine gamma retrovirus vector; busulfan conditioning):* Aiuti A, et al. Correction of ADA-SCID by stem cell gene therapy combined with nonmyeloablative conditioning. Science. 2002 296:2410; Gaspar HB1, et al. Gene therapy of X-linked severe combined immunodeficiency by use of a pseudotyped gammaretroviral vector. Lancet. 2004 364:2181; Candotti F, et al. Gene therapy for adenosine deaminase-deficient severe combined immune deficiency: clinical comparison of retroviral vectors and treatment plans. Blood. 2012 120:3635; Aiuti A, et al. Gene therapy for immunodeficiency due to adenosine deaminase deficiency. N Engl J Med. 2009 360:447:Gaspar HB, et al. Hematopoietic stem cell gene therapy for adenosine deaminase-deficient severe combined immunodeficiency leads to long-term immunological recovery and metabolic correction. Sci Transl Med. 2011 3:97ra80.

Integrating Vector Transduced Autologous Hematopoietic Stem Cell Transplant Gene Therapy for Monogenic Diseases

Clinical trial reports demonstrating significant unequivocal long-lasting clinical benefit:

- *X-linked SCID (Murine Gamma Retrovirus; no conditioning):* Hacein-Bey-Abina S, et al. N Engl J Med. 2002 346:1185. Gaspar HB, et al. Lancet. 2004 364:2181; Hacein-Bey-Abina S, et al. Efficacy of gene therapy for X-linked severe combined immunodeficiency. N Engl J Med. 2010 363:355.

- *X-linked SCID (Murine Gamma Retrovirus; genotoxicity):* Hacein-Bey-Abina S, et al. N Engl J Med. 2002 346:1185. Gaspar HB, et al. Lancet. 2004 364:2181; Howe SJ, et al. Insertional mutagenesis combined with acquired somatic mutations causes leukemogenesis following gene therapy of SCID-X1 patients. J Clin Invest. 2008 118:3143.

- *X-linked SCID (Self-inactivating Murine Gamma RetrovirusX-linked SCID (Self-inactivating Murine Gamma Retrovirus; no conditioning):* Hacein-Bey-Abina S, et al. A modified γ-retrovirus vector for X-linked severe combined immunodeficiency. N Engl J Med. 2014 371:1407.

- *X-linked SCID (Self-inactivating lentivector; Busulfan conditioning):* De Ravin SS, et al. Lentiviral Hematopoietic Stem Cell Gene Therapy for X-linked Severe Combined Immunodeficiency. Sci Translat Med 2016 8:335ra57.

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Integrating Vector Transduced Autologous Hematopoietic Stem Cell Transplant Gene Therapy for Monogenic Diseases

Clinical trial reports demonstrating <u>significant unequivocal long-lasting clinical benefit</u> :

- *Wiskott-Aldrich Syndrome (Murine gamma retrovirus vector; Busulfan conditioning; genotoxicity):* Braun CJ, et al. Gene therapy for Wiskott-Aldrich syndrome--long-term efficacy and genotoxicity. Sci Transl Med. 2014 6:227ra33.

- *Wiskott-Aldrich Syndrome (Self-inactivating lentivector; Busulfan conditioning):* Aiuti A, et al. Lentiviral hematopoietic stem cell gene therapy in patients with Wiskott-Aldrich syndrome. Science. 2013 341:1233151; Hacein-Bey Abina S, et al. Outcomes following gene therapy in patients with severe Wiskott-Aldrich syndrome. JAMA. 2015 313:1550.

Integrating Vector Transduced Autologous Hematopoietic Stem Cell Transplant Gene Therapy for Monogenic Diseases

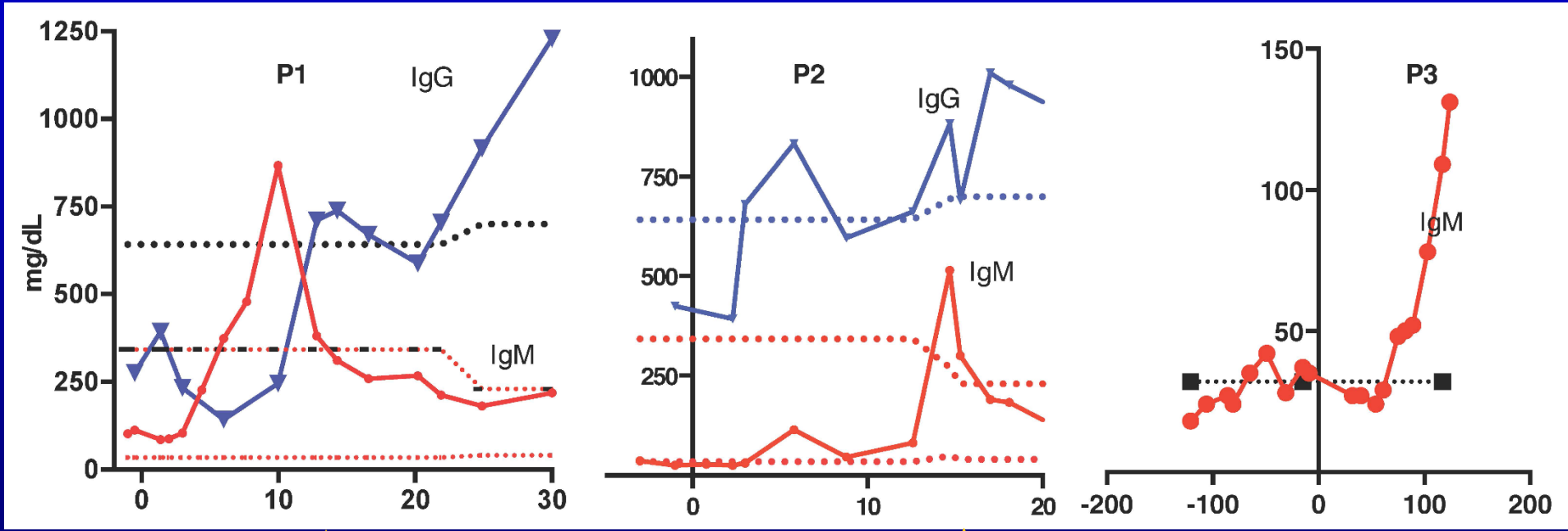
Clinical trial reports demonstrating <u>significant unequivocal long-lasting clinical benefit</u> :

- *Metachromatic leukodystrophy (Self-inactivating lentivector; busulfan conditioning):* Sessa M, et al. Lentiviral haemopoietic stem-cell gene therapy in early-onset metachromatic leukodystrophy: an ad-hoc analysis of a non-randomised, open-label, phase 1/2 trial. Lancet. 2016 388:476.

- *X-lined Adrenoleukodystrophy (Self-inactivating lentivector; busulfan conditioning):* Cartier N, et al. Hematopoietic stem cell gene therapy with a lentiviral vector in X-linked adrenoleukodystrophy. Science. 2009 326:818.

Restoration of immunoglobulin production in older X-SCID patients treated with lentivector transduced autologous HSC with prior busulfan conditioning.

P1, P2 & P3: Serum IgG and IgM (from De Ravin SS, et al. Sci Translat Med 2016 8:335ra57)



Subjects 1 & 2 became supplemental IgG independent and responded with protective titers to tetanus, diphtheria, menigococcus & influenza immunization. Significant restoration of humoral immunity was also seen in the Wiskott-Aldrich studies using busulfan conditioning and lentivector transduction.

Integrating Vector Transduced Autologous Hematopoietic Stem Cell Transplant Gene Therapy for Monogenic Diseases

Self-inactivating lentivirus vectors appear to be the current lead agent, but related integrating vectors need to be explored and there is a need for transformative emerging technologies/approaches in the following areas:

- ***Enhancing receptivity of HSC to transduction.***
- ***Less toxic marrow HSC conditioning regimens.***
- ***Can lentivector or related integrating vectors (foamy virus vectors for example) be developed that can be delivered and targeted to HSC* in vivo *(i.e. "drugable" versions of integrating vectors)?***