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GENE THERAPY FOR WISKOTT-ALDRICH SYNDROME IN A SEVERELY AFFECTED ADULT

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KEY POINTS

We describe the first successful use of gene therapy in a severely affected adult with Wiskott-Aldrich syndrome

Gene therapy is a viable strategy for adult WAS patients with severe chronic disease complications where allogeneic transplantation presents

ABSTRACT

Until recently hematopoietic stem cell transplantation was the only curative option for Wiskott-Aldrich syndrome (WAS). The first attempts at gene therapy for WAS using a γ-retroviral vector improved immunological parameters substantially but were complicated by acute leukemia as a result of insertional mutagenesis in a high proportion of patients. More recently treatment of children with a state-of-the-art self-inactivating lentiviral vector (LV-w1.6 WASp) has resulted in significant clinical benefit without inducing selection of clones harbouring integrations near oncogenes. Here, we describe a case of a pre-splenectomised 30 year old patient with severe WAS manifesting as cutaneous vasculitis, inflammatory arthropathy, intermittent polyclonal lymphoproliferation, significant chronic kidney disease and requiring long-term immunosuppressive treatment. Following reduced intensity conditioning there was rapid engraftment and expansion of a polyclonal pool of transgene-positive functional T cells, and sustained gene marking in myeloid and B cell lineages up to 20 months of observation. The patient was able to discontinue immunosuppression and exogenous immunoglobulin support, with improvement in vasculitic disease and pro-inflammatory markers. Autologous gene therapy using a lentiviral vector is a viable strategy for adult WAS patients with severe chronic disease complications and for whom an allogeneic procedure could present an unacceptable risk. This trial was registered at ClinicalTrials.gov #NCT01347242.

INTRODUCTION

Wiskott-Aldrich syndrome (WAS) is a rare, X-linked, complex primary immunodeficiency disease caused by mutations in the *WAS* gene which encodes a 502-amino acid protein called the Wiskott-Aldrich protein (WASp). This regulates the actin cytoskeleton in most hematopoietic lineages and is consequently important for normal function of many immunological processes (1-4). Clinical and biological manifestations of WAS include microthrombocytopenia, recurrent infections and eczema. Patients also display an increased incidence of autoimmunity and are at risk of developing lymphoproliferative disorders and lymphoid malignancies (5). A clinical scoring system grades disease severity. Patients with a score of 3-5 display 'classical' WAS usually as a result of null mutations and without definitive treatment these patients are not normally expected to survive beyond their third decade (6). For many years the only potential curative therapy has been allogeneic hematopoietic stem cell transplantation (HSCT) (7, 8, 9). HLA-matched HSCT is today associated with high rates of survival, but HLA-mismatched HSCT may still in some cases be accompanied by unacceptable risk even though new immune cell-depletion technologies are promising for reduction of graft versus host disease (GvHD) and for accelerating immunological reconstitution (10,11).

Gene therapy for Wiskott-Aldrich syndrome was first attempted using a □-retroviral vector (12). This approach resulted in a sustained increase in the proportion of WASp-corrected cells in all patients. However, 7 of the 9 treated patients developed acute leukemia secondary to viral enhancer-mediated insertional mutagenesis. More recently, gene therapy using a self-inactivating lentiviral vector has shown promise in children (13, 14). Interim analysis of results from both studies has demonstrated significant clinical benefit, and importantly without evidence of oncogenic transformation or sustained clonal dominance.

Here, we describe a case of an adult WAS patient (aged 30) for whom a HLA-matched donor could not be found and for whom a TCR αβ-depleted haploidentical transplant was deemed to be excessively high risk as a result of significant pre-existing disease co-morbidities. The patient was successfully treated on-trial (NCT01347242) with gene therapy using the same self-inactivating vector (LV-w1.6 WASp), which had previously recruited only children (14).

METHODS

Clinical Protocol

The patient was managed under the care of the clinical immunology and adult bone marrow transplant services at the Royal Free London Hospital NHS Foundation Trust (London, England). The patient was treated on trial (NCT01347242) sponsored by Genethon (14). The trial protocol was approved by the UK Medicines and Healthcare Products Regulatory Agency (MHRA), Gene Therapy Advisory Committee (GTAC) and the National Research Ethics Service (NRES). Autologous hematopoietic stem cells were collected from mobilised peripheral blood (after cryopreservation of an unmodified back up stem cell harvest) and were genetically modified *ex vivo* during concurrent reduced intensity conditioning of the patient as described below.

He received methylprednisolone 70mg (1mg/kg) intravenously on days -8 to -4 as prophylaxis against a potential GCSF-induced inflammatory reaction, lenograstim 1.28 million units/kg subcutaneously on days -7 to -5 followed by plerixafor 0.16mg/kg subcutaneously on days -5 to -4. The patient underwent leukapheresis on days -4 and -3. Following confirmation of an adequate starting dose of CD34+ cells, conditioning was commenced as per protocol with fludarabine 40mg/m^2 daily (days -3 to -1) and busulfan 3.2mg/kg daily (days -3 to -1). No adjuvant serotherapy was given. Due to the patient's poor renal function (EDTA glomerular filtration rate 34ml/min), prophylactic haemodialysis was undertaken on day 0. The patient was already established on antibacterial prophylaxis with penicillin V and IVIg following the earlier splenectomy and this was continued. Antifungal prophylaxis with voriconazole was commenced on day +3 and antiviral prophylaxis with acyclovir was started on admission at day -9. Oral trimethoprim-sulfamethoxazole three times a week was used as *Pneumocystis Jirovecii* prophylaxis from day -9.

On day 0 transduced autologous CD34+ haematopoietic stem cells were infused. The patient received a total dose of CD34+ cells of 3.77 x 10^{6}/kg, with a 52% transduction efficiency as determined by flow cytometry (gating on $CD34^{+}$, $CD45^{+}$ cells). The transduced cells products were manufactured at Great Ormond Street Hospital (London, England). Vector production, biological analyses, CD34+ cell gene transfer procedure, integration site analysis and clonality assays were performed as previously described (14). The manufacturing process is further outlined below.

Vector Production

The clinical LV-w1.6 WASp vector is an advanced generation HIV-derived lentiviral vector pseudotyped with VSVg and expressing the full-length WAS cDNA from the WAS gene proximal 1.6kb promoter. The clinical vector batch was manufactured at Genethon (Evry, France). Batches were purified, concentrated and titered for infectious particles (infectious genomes per millilitre) as previously reported (14).

CD34+ Cell Gene Transfer Procedure

Cells were cultured at 1 x 10^6 cells / ml in tissue culture treated flasks (NUNC) in serum-free medium (X-vivo 20; Lonza) and animal free stem cell factor (SCF, 300ng/ml), Fms-like tyrosine kinase 3 ligand (Flt3, 300ng/ml), thrombopoietin (TPO, 100ng/ml) and IL-3 (20ng/ml) (all from Peprotech). G-CSF and Plerixafluor mobilised peripheral blood was collected from the patient by apheresis. Immunomagnetic beads and an immunomagnetic separation device were used to positively select CD34+ cells (CliniMACS, Miltenyi Biotec). CD34+ cells were seeded in serum-free medium (X-Vivo20, Lonza), supplemented with 1% human albumin solution (HAS) and cytokines IL-3, SCF, Flt3, TPO. All cytokines (Peprotech) and culture reagents were approved for *ex vivo* clinical use. Cells were then incubated at 37ºC, 5% CO_2. After 17 hours total pre-stimulation, cells were returned to pre-coated tissue culture flasks and transduced twice with LV.w1.6 WASp (2 x 10^8 infectious particles per ml), for 17 hours each time. Washed cells were then resuspended (at 3x10^6 CD34+/ml) in 2% human albumin in a sterile bag for infusion to the patient. Reserved cell aliquots were cultured for 14 days afterwards to assess proviral integration by quantitative PCR and WASp expression by flow cytometry.

Analysis of thymic output

Signal joint T-cell receptor excision circles (sjTREC) were assessed using quantitative real-time polymerase chain reaction (qRT-PCR). sjTREC content was expressed in copies per 10^5 peripheral blood mononuclear cells (PBMCs) (control range, 150-2500/10^5 PBMCs).

T cell repertoire analysis by TCR spectratyping

Clonality of T cells was monitored using T-cell receptor TCR V beta spectratyping. Briefly, the CDR3 region of the beta chain was amplified from patient cDNA using a single constant region primer with 24 variable region primers. A fluorescent run-off reaction was then carried out and the products were run on a 3500xL Genetic Analyzer. Subsequent spectratypes were analysed using GeneMapper 5 software and flow analysis used the IOTest Beta Mark assay (Beckman Coulter).

Analysis of vector copy number

Vector copy number per cell was measured by quantitative PCR detection of the vector's HIV psi sequence with normalisation against the copy number of the albumin gene. Lymphoid and myeloid subpopulations were sorted by flow cytometry using their corresponding fluorescence-labelled monoclonal antibodies.

Analysis of integration site distributions

Detailed methods are as described previously (15-19). Briefly, DNA was randomly sheared via ultrasonication and DNA linkers were ligated to the cleaved ends. DNA fragments were then amplified by ligation-mediated PCR, using primers annealing to the LTR of the vector and to the linker to isolate and enrich for host-vector junctions. PCR was performed in quadruplicate for each sample. Amplicons were sequenced using an Illumina MiSeq instrument. Sequence reads were aligned to the human genome (hg18) to identify vector integration sites. The distance between the vector-host junction and the breakpoint of the DNA produced by shearing can be used to differentiate distinct cells with the same integration site. Since the DNA is randomly sheared via sonication prior to amplification, these DNA fragment lengths, or "sonic lengths" (19), can be used to estimate the abundance of cell clones, avoiding the need to estimate based on read counts acquired after biased amplification and sequencing. Cells harboring the same integration site can be differentiated and directly counted by sonic length and then summed to estimate the abundance ("sonic abundance") of the integration site within the population. DNA sequence reads used in this study are available at the National Center for Biotechnology Information SRA under accession numbers BioProject ID PRJNA387194.

Analysis of WASp protein expression

WASp expression in PBMC and lymphocytes was analysed by flow cytometry on a FACSCalibur cell analyser (BD Biosciences) using the Simultest CD3 FITC/CD16+CD56 PE and CD19PECy7 antibodies (BD Biosciences) for lymphocyte characterisation and the WASp (IgG_{2a}) 5A5 clone (BD Biosciences) for WASp intracellular staining. For Western-blotting, platelets were purified from EDTA-blood by serial centrifugation (15min at 800rpm with no brake followed by 15min at 1800rpm). Protein extracts from the pelleted platelets were separated on a 10% SDS-PAGE gel, and WASp was detected using the WASp (D-1) antibody (Santa Cruz Biotechnology).

RESULTS

Case Presentation

The patient first presented aged 3 years with easy bruising and epistaxis. Thrombocytopenia and small platelets on peripheral blood film examination raised the suspicion of WAS. Western blotting for WASp and gene sequencing were performed later and confirmed the clinical diagnosis. Sanger sequencing confirmed a T>C mutation 2 bases into the 5' splice site of intron9 of the WASP gene (IVS9+2 T to C). This mutation is predicted to disrupt the splicing of exon 9 resulting in the activation of a cryptic splice site and the insertion of the 115bp 5' segment of intron 9 causing a frameshift and a termination codon within the inserted segment (20-22). The patient is of Asian British Pakistani ethnicity and there were no other documented cases in the patient's family. After episodes of spontaneous bleeding secondary to thrombocytopenia later in childhood, a splenectomy was performed, which resulted in normalisation of platelet numbers. In mid-childhood the patient began to develop autoimmune and inflammatory phenomena, including recurrent episodes of uveitis, cutaneous leucocytoclastic vasculitis with skin ulceration, episodic synovitis, thyroiditis and non-malignant lymphoproliferative disease. These episodes were accompanied by gross elevation in acute phase proteins. The vasculitis in particular was increasingly difficult to control and persisted throughout adolescence. Immunosuppression with varying combinations of steroids, colchicine, methotrexate, cyclophosphamide, cyclosporin, mycophenolate mofetil and rituximab failed to fully control the patient's symptoms or to normalise inflammatory markers.

Due to the patient's increasingly aggressive autoinflammatory phenotype he was referred for consideration of HSCT. No HLA matches were identified within his immediate family. A matched unrelated donor was identified but transplant was delayed by recurrent attacks of vasculitis, uveitis and synovitis during which time the donor was deleted from the registry. Further unrelated donor searches proved unsuccessful.

The clinical picture was further complicated by progressive deterioration in renal function. A renal biopsy was performed that showed features of neutrophil-rich infiltrating tubulointerstitial disease, reminiscent of reflux nephropathy, but with no previous history of recurrent urinary tract infections in childhood. No changes consistent with calcineurin inhibitor toxicity were identified and no deposition of immunoproteins observed. Creatinine clearance fell to 24ml/min. Following the recent success of the gene therapy trial in children it was suggested that the patient could benefit from treatment using a similar protocol (14).

WAS severity score prior to gene therapy was 5 reflecting his severe disease phenotype and his Hematopoietic Cell Transplantation-Specific Comorbidity Index (HCT-CI) score was 8 indicating a high-risk of treatment-related mortality (23). Written informed consent was obtained from the patient after explanation of the risks and benefits and discussion of other treatment options available.

At a follow up of 20 months post gene therapy the patient is well, with normal peripheral blood counts, stable renal function (estimated GFR 41ml/min), no significant episodes of vasculitis or arthritis and free of major immunosuppression. Importantly, he has now completed his education and works part time, neither of which were possible prior to treatment because of illness. Normal CD4+, CD8+ and NK cell counts were reached at 12 months post therapy (Table 1). T cell proliferation in response to phytohemagglutinin (PHA) and anti-CD3/anti-CD28 antibody stimulation also normalised. It should be noted that in addition to an intrinsic suppression as a result of long-standing disease, the patient's pre-GT PHA responses were likely also blunted by the anti-proliferative immunosuppressant mycophenylate mofetil (MMF). Long term IVIg supplementation was withdrawn 10 months after gene-therapy as endogenous IgG, A and M (5.7, 1.4 and 0.2 g/L respectively) levels were rising. There was an excellent serological response to tetanus vaccine (Table 1). Response to a single conjugated pneumococcal vaccine (at 22 months post GT) was partial with a 10-fold increase in total anti-pneumococcal specific IgG after 2 vaccinations, but to a restricted repertoire (3/13 serotypes). Platelet counts remained stable, within the normal range after recovery from myelosuppression.

The patient demonstrated robust and multi-lineage engraftment in peripheral blood of gene-corrected cells, which was sustained to the latest time point analysed (20 months post gene therapy). T cells exhibited the highest level of gene marking at 12 months compared to other lineages as observed in previous studies, although gene marking in myeloid cells was also sustained at approximately 10% suggesting that transduced HSCs were successfully engrafted (Figure 1). This was supported by evidence for persistent gene marking in purified bone marrow $CD34^+$ cells analysed 16 months after treatment, with an observed vector copy number of 0.34 (Figure 1). B cell marking was also very robust as observed in some patients in previous studies (14 [see patients 2, 4 and 6]). All lineages exhibited restoration of WASp expression (Figure 2A). Western blot analysis confirmed low level expression of WASp in the platelet fraction at 12 months post gene therapy suggesting that a contribution to the platelet compartment was also now derived from engrafted HSCs (Figure 2B). No platelet transfusions were given at any time. Prominent WASp expression was detected in purified naïve $CD4^+$ and $CD8^+$ T cells at 20 months post GT

(Figure 2C). In addition, a sustained improvement in T cell repertoire and thymic output were observed in both CD4+ and CD8+ T cell compartments post GT (Figure 3A and B).

The distributions of integration site frequencies are summarized in stacked bar graphs in Figure 4. Both the statistics summarized in the Table 2 and the stacked bar graphs indicate highly polyclonal cell populations. Low Gini coefficients, high Shannon indices, and high UC50 values (15, 16) are reported across samples, indicating polyclonality (Table 2). In Figure 4, most of the integration sites sampled are low abundance (colored grey in the figure). No unique clone accounts for greater than 2.5% of the total data for any given sample. Further, no integration site within 100 kb of a gene previously associated with adverse events in gene therapy (LMO2, CCND2, MECOM) accounts for greater than 0.4% of the total abundance in any cell type at any time point. The polyclonality of integration sites identified in this adult patient is comparable to populations seen in pediatric patients treated with the same vector (LV-w1.6 WASp) at corresponding time points (14). More detailed analysis of integration site distributions and relative clonal abundance is included in the supplementary data (supplementary data, Figure S2).

Several short-lived adverse events were noted post gene therapy including disseminated shingles (6 months post therapy), transient lymphadenopathy and pyrexia of unknown origin (7 months post therapy), scleritis requiring topical steroids (10 months post therapy), acute acne associated with an inflammatory ulcer on the jawline (Supplementary data, Figure S1) and transient alopecia areata (fungal culture negative). A lymph node biopsy performed at the time when lymphadenopathy was present showed reactive changes only. Histology of the inflammatory facial lesion was suggestive of pseudoepitheliomatous hyperplasia with a dense inflammatory infiltrate of lymphocytes, plasma cells, eosinophils and neutrophils. No atypical mycobacteria or other pathogens were identified, and the lesion resolved with broad-spectrum oral antimicrobial therapy.

In view of his inflammatory disease manifestations, we measured serum cytokine levels pre- and 18 months post- gene therapy. Compared with healthy controls, significantly elevated lymphoid and myeloid-derived pro-inflammatory cytokines were observed prior to treatment (Figure 5A and 5B). Almost all were substantially lower post therapy, although some remained elevated, notably the pro-inflammatory cytokines IL-18 and IFN-g which are primarily derived from myeloid cells. Serum C-reactive protein (CRP) levels, which were very elevated prior to treatment despite MMF and steroids, also improved over time, possibly enhanced by addition of the anti-inflammatory drug, colchicine (Figure 6).

DISCUSSION

It is reassuring that in this adult patient the same pattern of reconstitution was observed as seen in paediatric patients (LV-w1.6 WASp) (13, 14), suggesting that potential for T cell repertoire recovery in older patients is well-preserved. In keeping with results of previous gene therapy trials gene marking was higher in the lymphoid compared to the myeloid compartment, which is likely due to the previously described survival advantage conferred by WASp expression in mature lymphoid cells in particular (14, 24). This phenomenon has also been observed in WAS patients who have undergone allogeneic HSCT (7).

The re-occurrence of transitory inflammatory complications post gene therapy is interesting as lymphoid reconstitution appears excellent. One episode of acute acne was associated with pseudoepitheliomatous hyperplasia in a facial lesion, and bore some resemblance to features of the rare inherited autoinflammatory PAPA syndrome caused by mutations in *PSTPIP1*, a cytoskeletal adaptor known to regulate WASp in macrophages (25). Both pseudoepitheliomatous hyperplasia and PAPA syndrome are associated with increased production of IL1B family pro-inflammatory cytokines including IL-18 (26, 27). It therefore seems likely that there is a persistent inflammasome–mediated mechanism for residual inflammation which may therefore reflect presence of significant numbers of non-transduced myeloid cells. Interestingly, addition of colchicine successfully suppressed further inflammation.

This is the first report of successful gene therapy in an adult with severe WAS. There was rapid engraftment and expansion of a polyclonal pool of functional T cells, and sustained gene marking in myeloid and B cell lineages up to 20 months of observation. As described, apart from colchicine the patient was able to discontinue all immunosuppression and has recently been withdrawn from exogenous immunoglobulin support. Longer follow-up and testing in other patients will be required to confirm these early results. Whislt we recognise that recent developments in reducing the toxicities associated with mismatched Allo HSCT may impact on the choice between gene therapy or Allo HSCT, the limited side effects associated with the gene therapy procedure and the benefits seen in this case demonstrate that gene therapy using a lentiviral vector may be a viable alternative strategy for adult WAS patients with severe chronic disease-related complications, for whom an allogeneic HSCT procedure presents an unacceptable risk.

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AUTHORSHIP

Contribution: ECM, SB, TF and AT analysed the data and wrote the manuscript. ECM and AT were clinical trial principal and chief investigators. SW, SG, RChee, AS, RC, ECM and SOB provided clinical care of the patient. RT was trial coordinator. HH, CD, KS, CR, KG, KButler, KB, HBG and AT manufactured the gene therapy product. AG and FMulvio were representatives of the vector manufacturer and of the study sponsor. RB and FM performed integration site analysis. AG, HBG and AT were involved in trial design.

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REFERENCES

(1) Thrasher AJ and Burns SO. WASP: a key immunological multitasker. *Nat Rev Immunol.* 2010;10(3):182-92.

(2) Baptista MA, Keszei M, et al. Deletion of Wiskott-Aldrich syndrome protein triggers Rac2 activity and increased cross-presentation by dendritic cells. *Nat Commun.* 2016;7:12175.

(3) Burns S, Cory G, et al. Mechanisms of WASp-mediated hematologic and immunologic disease. *Blood.* 2004;104(12):3454-3462.

(4) Meyer-Bahlburg A, Becker-Herman S, et al. Wiskott-Aldrich syndrome protein deficiency in B cells results in impaired peripheral homeostasis. *Blood.* 2008;112(10):4158-4169.

(5) Worth AJ and Thrasher AJ. Current and emerging treatment options for Wiskott –Aldrich syndrome. *Expert Rev Clin Immunol.* 2015;11(9):1015-32.

(6) Zhu Q, Zhang M, et al. The Wiskott-Aldrich syndrome and X-linked congenital thrombocytopenia are caused by mutations of the same gene. *Blood.* 1995;86(10): 3797-3804.

(7) Pai SY, DeMartiis D, et al. Stem cell transplantation for the Wiskott-Aldrich syndrome: a single-center experience confirms efficacy of matched unrelated donor transplantation. *Bone Marrow Transplant.* 2006 Nov;38(10):671-9.

(8) Pai S and Notarangelo L. Hematopoietic cell transplantation for Wiskott-Aldrich syndrome: advances in biology and future directions for treatment. *Immunol Allergy Clin North Am.* 2010; 30(2):179-194.

(9) Moratto D, Giliani S, et al. Long-term outcome and lineage-specific chimerism in 194 patients with Wiskott-Aldrich syndrome treated by hematopoietic cell transplantation in the period 1980-2009: An international collaborative study. *Blood.* 2011;118:1675-1684.

(10) Kharya G, Nademi Z, et al. Haploidentical T-cell alpha beta receptor and CD19-depleted stem cell transplant for Wiskott-Aldrich syndrome. *J Allergy Clin Immunol.* 2014;134(5):1199-201.

(11) Locatelli F, Bauquet A, et al. Negative depletion of α/β+ T cells and of CD19+ B lymphocytes: a novel frontier to optimize the effect of innate immunity in HLA-mismatched hematopoietic stem cell transplantation. *Immunol Lett*. 2013:155(1-2): 21-23.

(12) Braun CJ, Boztug K, et al. Gene therapy for Wiskott-Aldrich syndrome – long-term efficacy and genotoxicity. *Sci Transl Med.* 2014;6(227):227ra33. doi: 10.1126

(13) Aiuti A, Biasco L, et al. Lentiviral hematopoietic stem cell gene therapy in patients with Wiskott-Aldrich syndrome. *Science.* 2013;341(6148):1233151.

(14) Hacein-Bey Abina S, Gaspar B, et al. Outcomes Following Gene Therapy in Patients With Severe Wiskott-Aldrich Syndrome. *JAMA.* 2015;313(15):1550-1563.

(15) Sherman EA, et al. INSPIIRED: a pipeline for quantitative analysis of sites of new DNA integration in cellular genomes. *Mol Ther Methods Clin Dev.* 2017;4:39-49. (in press)

(16) Berry CC, et al. INSPIIRED: quantification and visualization tools for analyzing integration site distributions. *Mol Ther Methods Clin Dev.* 2017;4:17-26. (in press).

(17) Berry CC, et al. Selection of target sites for mobile DNA integration in the human genome. *PLoS Comput Biol.* 2006;2:e157.

(18) Berry CC, et al. Comparing DNA integration site clusters with scan statistics. *Bioinformatics.* 2014;30:1493-1500.

(19) Berry CC, et al. Estimating abundances of retroviral insertion sites from DNA fragment length data. *Bioinformatics*. 2012;28:755-762.

(20) Zhu Q, et al. The WIskott-Aldrich Syndrome and X-linked Congenital Thrombocytopenia are caused by mutations of the same gene. *Blood.* 1995; 86: 3797-3804. (patient MB).

(21) Zhu Q, et al. Wiskott-Aldrich Syndrome/X-Linked Thrombocytopenia: WASP gene mutations, protein expression, and phenotype. *Blood.* 1997; 90: 2680-2689.

(22) Jin Y, et al. Mutations of the WIskott-Aldrich Suyndrome Protein (WASP): hotspots, effect on transcription, and translation and phenotype/genotype correlation. *Blood.* 2004. 104: 4010-4019.

(23) Sorror ML, Maris MB, et al. Hematopoietic cell transplantation (HCT)-specific comorbidity index: a new tool for risk assessment before allogeneic HCT. *Blood.* 2005; 106:2912-2919.

(24) Westerberg L, de la Fuente M, et al. WASP confers selective advantage for specific hematopoietic cell populations and serves a unique role in marginal zone B-cell homeostasis and function. *Blood.* 2008;112(10):4139-4147.

(25) Starnes TW, Bennin DA, et al. The F-BAR protein PSTPIP1 controls extracellular matrix degradation and filopodia formation in macrophages. *Blood.* 2014;123(17):2703-14.

(26) Ristow HJ. A major factor contributing to epidermal proliferation in inflammatory skin diseases appears to be interleukin 1 or a related protein. *PNAS.* 1987;84(7):1940-1944.

(27) Marzano AV, Borghi A, et al. Pyoderma gangrenosum and its syndromic forms: evidence for a link with autoinflammation. *Br J Dermatol.* 2016;175(5):882-891.

(28) Chao A. Estimating the population size for capture-recapture data with unequal catchability. *Biometrics.* 1987;43(4):783-91.

TABLES

		Pre Gene Therapy	***16 months post***	***Healthy control ranges for 3 day stimulation***	
T Cell Proliferation	Spontaneous (cpm)	103	115		
	PHA (cpm)	2490	50349	18906 – 417388	
	aCD3 (cpm)	226	22699	187 – 122682	
	aCD3/aCD28 (cpm)	2186	52068	1202 – 213904	
		Pre Gene Therapy	***6 months post***	***11 months post***	***16 months post***
Lymphocyte Subsets	Total lymphocytes (x 10^9/ml) [Normal range: 1.0-2.8]	0.572	2.251	1.469	1.11
	CD3 (x 10^9/ml) [Normal range: 0.7-2.1]	0.518	1.389	0.789	0.723
	CD4 (x 10^9/ml) [Normal range: 0.3-1.4]	0.414	0.62	0.407	0.409
	CD8 (x 10^9/ml) [Normal range: 0.2-0.9]	0.105	0.408	0.252	0.23
	CD19 (x 10^9/ml) [Normal range: 0.1-0.5]	0.027	0.408	0.407	0.409
		Pre Gene Therapy	***Post GT Pre-vaccination***	***Post GT Post-vaccination***	
Vaccination Responses	Total Pneumococcus titres (mg/L) [protective levels >20mg/L]	On immunoglobulin replacement therapy	4	43	
	Pneumococcus serotype specific responses [protective levels > 0.35mg/L]	On immunoglobulin replacement therapy	ND	Protective responses against 3/13 serotypes	
	Tetanus Toxoid IU/ml [protective levels 0.1 -0.7 IU/ml]	On immunoglobulin replacement therapy	0.14	2.51	

Table 1: T cell proliferation assays, lymphocyte subsets and vaccination responses pre and post gene therapy.

Timepoint	*Cell Type*	*Total Reads*	*Inferred Cells*	*Unique Sites*	*S.chao1*	*Gini*	*Shannon*	*UC50*
6 months	***B cells***	1036782	4639	3891	19855	0.1204	8.2045	1647
	Monocytes	222125	329	248	893	0.1950	5.4176	86
	Neutrophils	803235	2018	1522	6981	0.1866	7.2162	552
	NK cells	1111841	3000	2128	7757	0.1751	7.5657	795
	PBMC	684175	2115	1386	4844	0.2668	7.0254	381
	T cells	1038680	5685	3089	10815	0.3937	7.4591	539
12 months	***B cells***	925356	2197	1745	7702	0.1438	7.3907	701
	Monocytes	347061	351	242	1970	0.2517	5.3341	69
	Neutrophils	558321	729	492	1866	0.2496	6.0383	139
	NK cells	507007	697	469	1865	0.2413	6.0123	135
	PBMC	463071	2076	1744	7513	0.1387	7.3578	720
	T cells	981471	3142	2089	9696	0.2748	7.3678	574

Table 2. Table summarizing the distribution and relative abundance of integration sites isolated from several cell types at 6 and 12 months after therapy.

The table summarizes the sample metadata and sequencing data, and reports summary statistics for each sample. To summarize the data, the total number of sequence reads (TotalReads), the inferred number of cells with integrated vector identified (InferredCells), and number of unique integration sites identified (UniqueSites) are shown. Statistics to summarize population structure are also presented. The Chao1 analysis (S.chao1) is an estimate of minimum population size (28). The Gini coefficient is a measure to represent distribution. Here, a value of 0 indicates even distribution of integration sites while values increasing towards 1 demonstrate increasing inequality of integration site abundance within the population. The Shannon index summarizes diversity by accounting for both abundance and evenness among the integration sites in the cell population. Lastly, the UC50, a new metric, indicates the number of unique clones contributing the top 50% of the sample's total abundance (16).

FIGURE LEGENDS

Figure 1. Persistent gene-marking post gene therapy.

Longitudinal results of gene marking in peripheral blood and bone marrow after gene therapy as expressed by vector copy number per peripheral blood mononuclear cell, peripheral blood purified cell lineage or bone marrow derived $CD34^+$ cell.

Figure 2. Correction of WASp expression post gene therapy.

(A) WASp protein expression in T cells, B cells and NK cells; (B) Western blot demonstrating WASp expression in patient's platelets compared to healthy control [con = healthy control]. NB, patient received no platelet transfusions at any time; (C) WASp protein expression in purified naïve $CD4^+$ and $CD8^+$ T cells at 20 months post GT.

Figure 3. Improvement of T cell repertoire and thymic output post gene therapy.

(A) T cell repertoire analysis by TCR spectratyping pre and post GT in $CD4^+$ and $CD8^+$ T cells; (B) sjTREC content in $CD4^+$ (red line) and $CD8^+$ (blue line) T cells (dotted line corresponds to 10^{th} centile for patient's age group).

Figure 4. Relative abundance of cell clones.

Stacked bar graphs showing the relative sonic abundance, scaled as a proportion of the total. Data are separated by cell type with side-by-side comparison of time points for visualization of longitudinal changes. The 10 most abundant integration sites for each cell type are emphasized by uniquely colored bars and named by the nearest gene, indicated in the 'GeneNames' key. The genes are marked with symbols to indicate further information about the integration site/gene: * (asterisk) indicates the site is within a transcription unit, ~ (tilde) indicates the site is within 50kb of a cancer related gene, ! (exclamation point) indicates the gene is associated with lymphoid cancers in humans. The remaining low abundance integration sites are indicated in grey (LowAbund). The cutoff values for binning clones as low abundance are indicated at the top of each cell type panel.

Figure 5. Reduction in serum cytokine levels post gene therapy.

Multiple cytokines associated with predominantly lymphoid (A, upper panel) and myeloid (B, lower panel) cells were measured by luminex in the patient pre- and 18 months post- gene therapy (Pt Pre and Pt Post) and compared with healthy controls (HC1 and HC2).

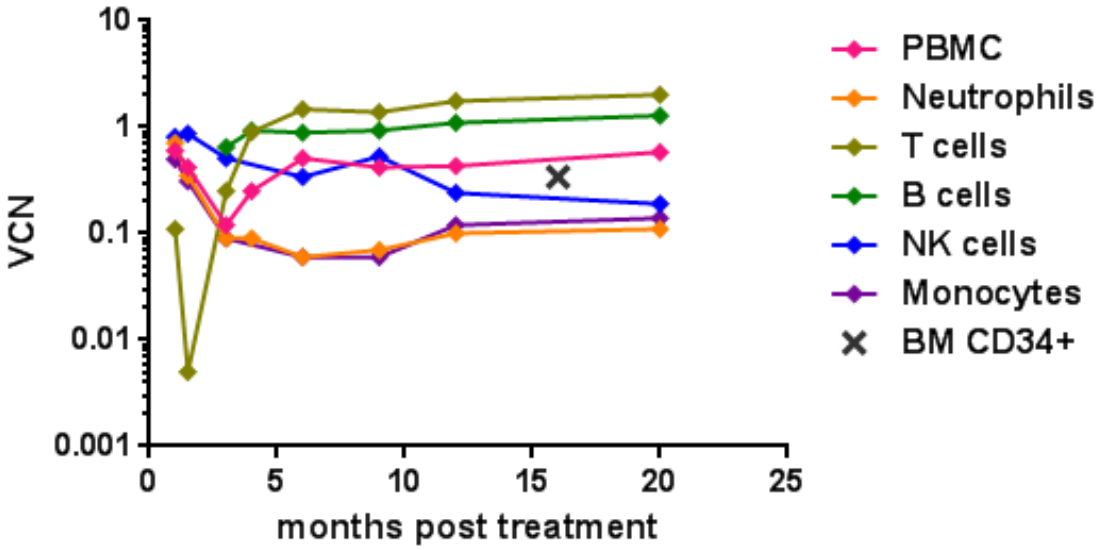
Figure 6. Clinical course pre and post gene therapy showing a reduction in serum C-reactive protein (CRP) over time.

Key: *prednisolone 30mg daily x 3 days; ankle swelling and cutaneous tenderness, ?vasculitis

**prednisolone 30mg daily x 4 days; admitted with lymphadenopathy, pyrexia of unknown origin

***prednisolone 30mg daily x 3 days; foot swelling and cutaneous tenderness (self medicated)

****prednisolone 30mg daily x 3 days; cutaneous tenderness and nodules (self medicated)



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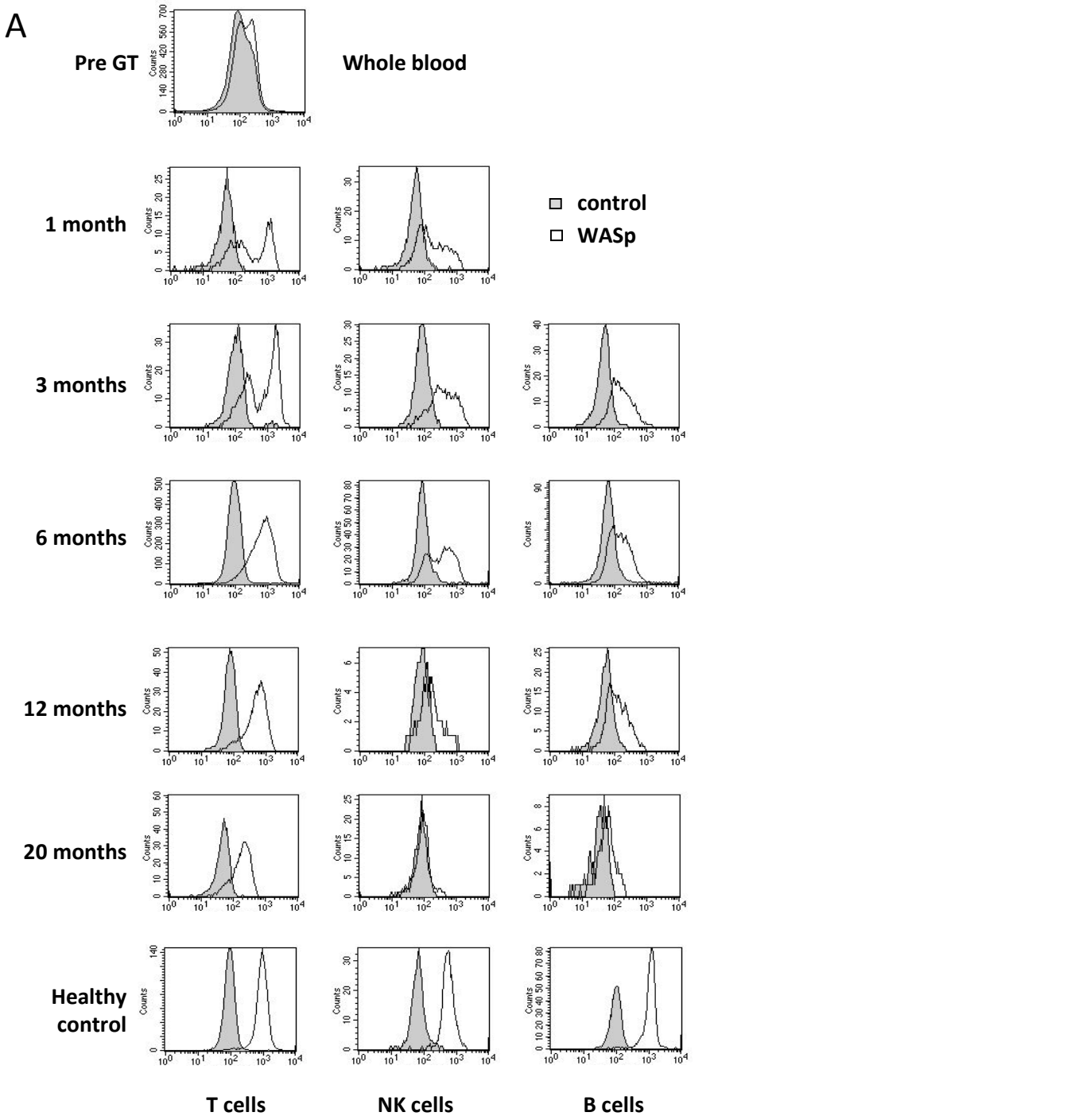

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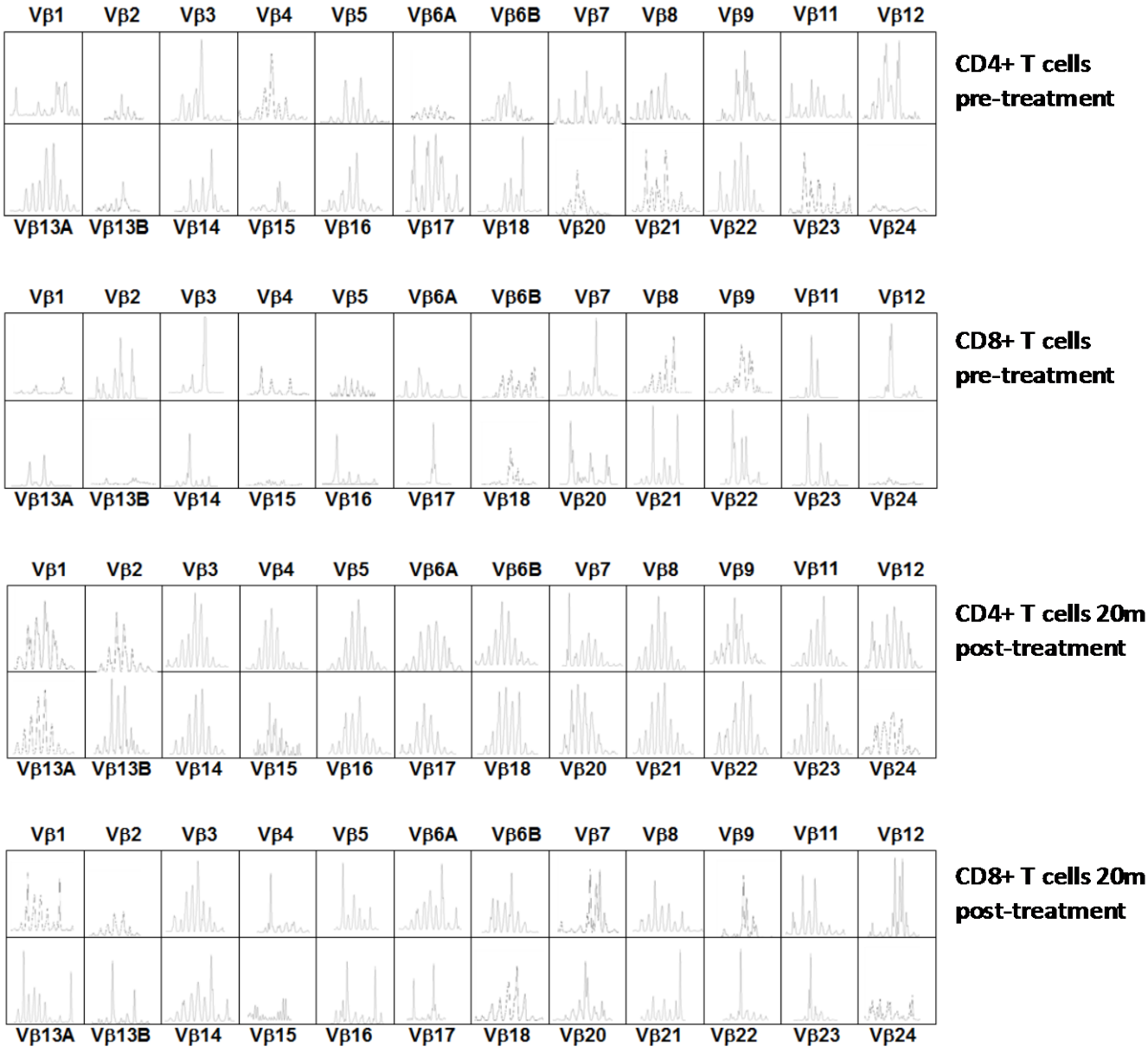

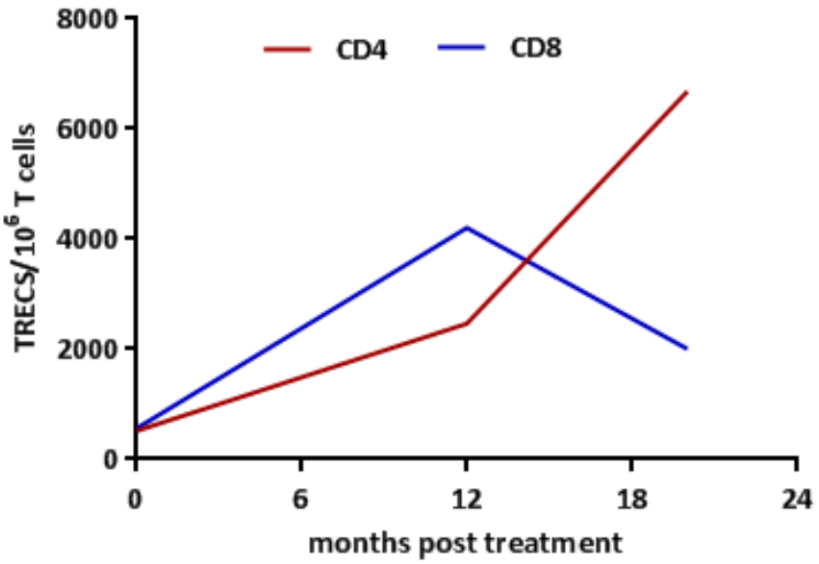

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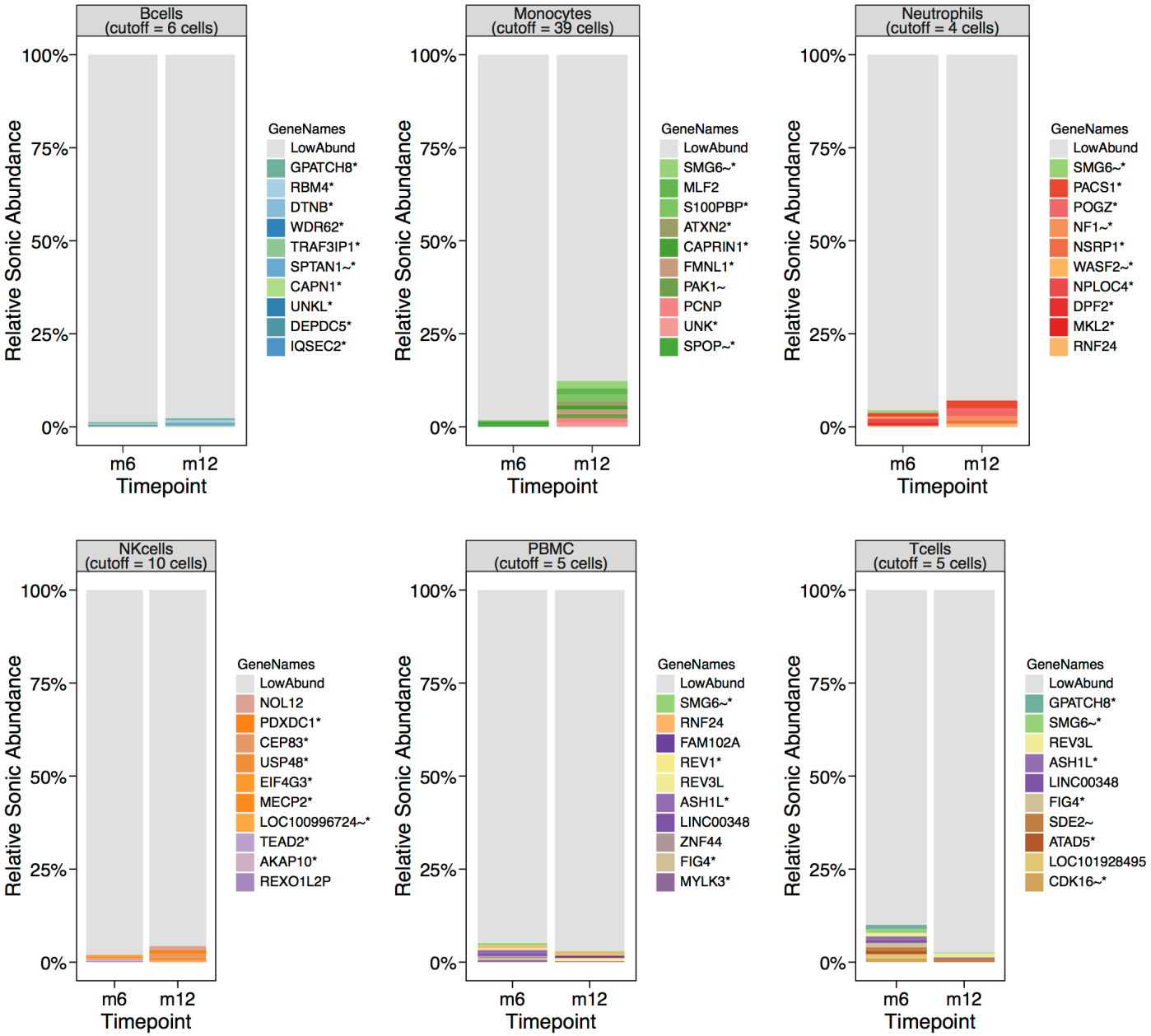

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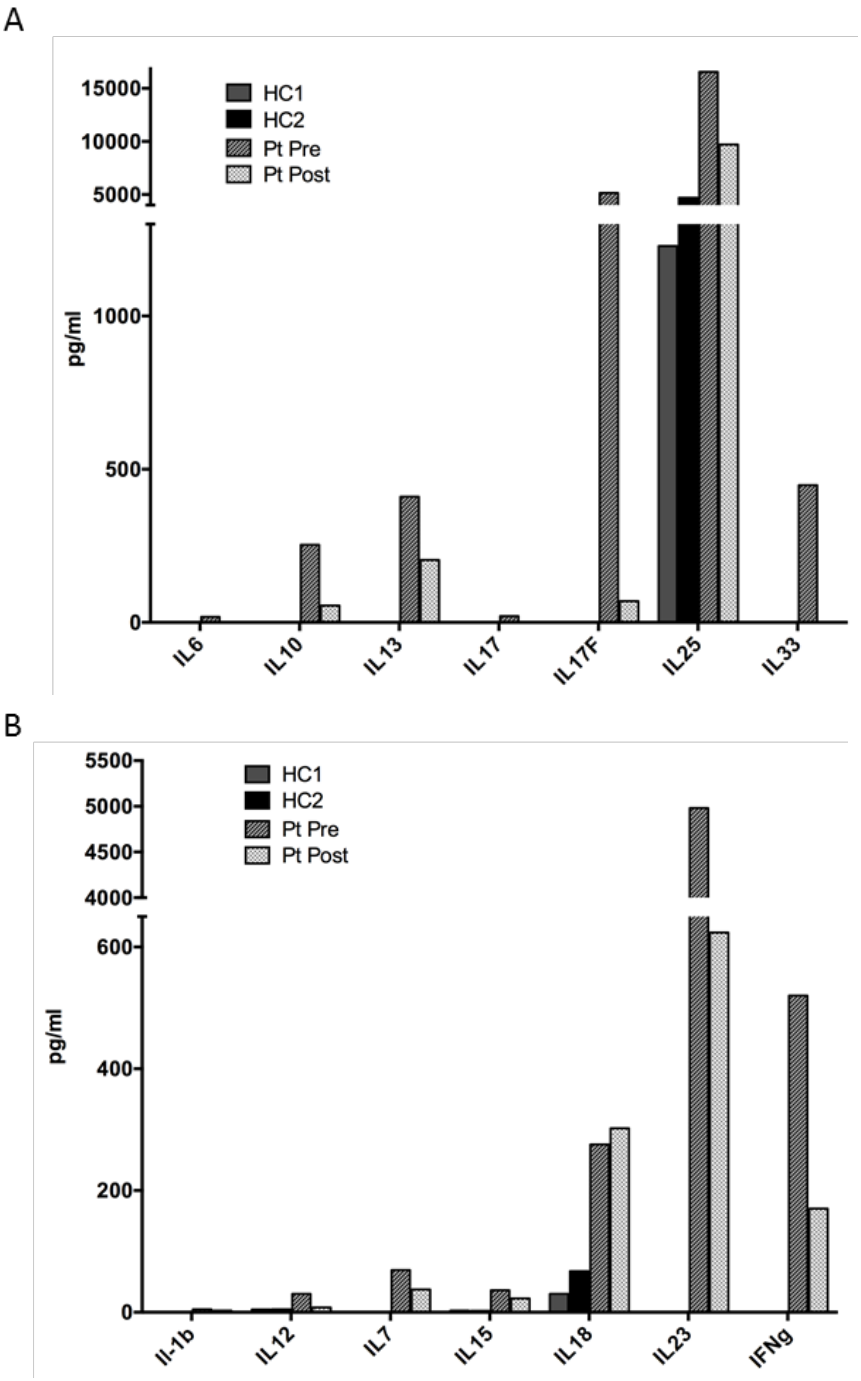

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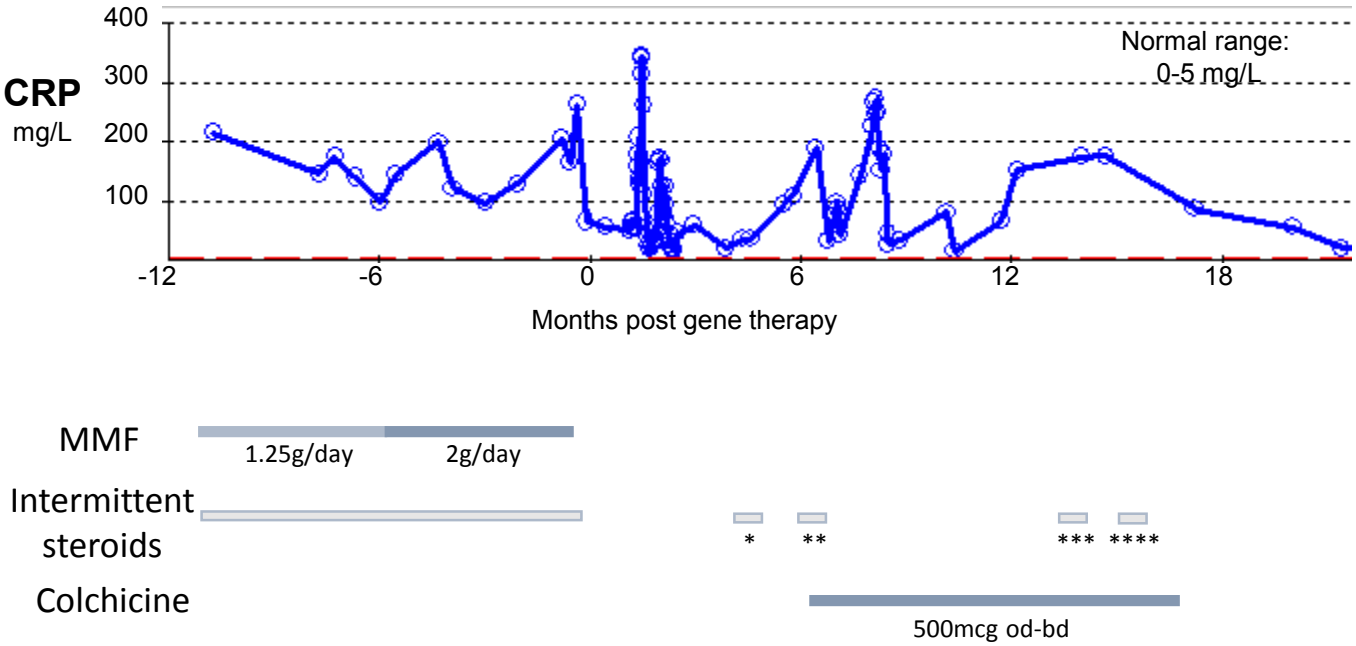

* prednisilone 30mg daily for 3 days; ankle swelling and cutaneous tenderness, ?vasculitis

** prednisilone 30mg daily for 4 days; admitted with lymphadenopathy, infection work up negative

*** prednisilone 30mg daily for 3 days; foot swelling and cutaneous tenderness (self medicated)

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Gene therapy for Wiskott-Aldrich syndrome in a severely affected adult

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