polymerase chain reaction using peripheral blood leukocytes, pp65 antigenemia, and viral culture. *Transplantation*. 1997;64: 108-113.
4. Gumá M, Angulo A, Vilches C, Gómez-Lozano N, Malats N, López-Botet M. Imprint of human cytomegalovirus infection on the NK cell receptor repertoire. *Blood*. 2004;104: 3664-3671.
5. Gumá M, Budt M, Sáez A, Brckalo T, Hengel H, Angulo A, López-Botet M. Expansion of CD94/NKG2C+ NK cells in response to human cytomegalovirus-infected fibroblasts. *Blood*. 2006;107:3624-3631.
6. van Stijn A, Rowshani AT, Yong SL, et al. Human cytomegalovirus infection induces a rapid and sustained change in the expression of NK cell receptors on CD8 + T cells. *J Immunol*. 2008;180: 4550-4560.
7. Sáez-Borderías A, Gumá M, Angulo A, et al. Expression and function of NKG2D in CD4 + T cells specific for human cytomegalovirus. *Eur J Immunol*. 2006;36:3198-3206.
8. Battiwalla M, Wu Y, Bajwa RP, et al. Ganciclovir inhibits lymphocyte proliferation by impairing DNA synthesis. *Biol Blood Marrow Transplant*. 2007;13:765-770.
9. Li CR, Greenberg PD, Gilbert MJ, Goodrich JM, Riddell SR. Recovery of HLA-restricted cytomegalovirus (CMV)-specific T-cell responses after allogeneic bone marrow transplant: correlation with CMV disease and effect of ganciclovir prophylaxis. *Blood*. 1994;83:1971-1979.

Carolyn E. Behrendt, PhD
Ryotaro Nakamura, MD
John Zaia, MD
City of Hope National Medical Center, Duarte, California

Biol Blood Marrow Transplant 16: 133-135 (2010)

doi:10.1016/j.bbmt.2009.06.010

Alloimmune Retinopathy Associated with Antibodies to Transducin-α as a Complication of Chronic Graft-Versus-Host Disease

The paraneoplastic retinopathies, first described in 1976 [1], include cancer-associated retinopathy (CAR) and melanoma-associated retinopathy (MAR) [2]. Affected patients present with acute, subacute, or chronic signs and symptoms of retinal dysfunction [3]. Findings on retinal examination include attenuated vessels, "waxy" disc pallor, and retinal pigment epithelial mottling, although at times the exam is normal [4,5], and there are characteristically no signs of inflammation [6]. Electroretinograms (ERGs) are typically abnormal, and negative waveforms are common [3]. Pathogenic retinal antibodies are often found in the serum of affected individuals.

Patients with CAR usually have coexisting solid tumors involving the lung, ovaries, or colon [7]. Serum from affected individuals may contain antibodies to recoverin, a calcium binding protein that controls phosphorylation of rhodopsin in both rod and cone photoreceptors [8,9].

Recently, a series of patients has been identified with antibodies to transducin-α, a 40-kDa photoreceptor G-protein naturally expressed in rods and cones (G. Adamus et al., unpublished data). Visual transducin is activated by rhodopsin following initial photon capture, subsequently activating PDE6, and ultimately photoreceptor membrane hyperpolarization [10]. Transducin is also present in other tissues and associated with tumors, including melanomas and carcinomas [11].

Here, we report the case of a 25-year-old woman found to have new onset retinopathy in the setting of chronic graft-versus-host disease (cGVHD) after allogeneic transplant, with subsequent demonstration of serum anti-40-kDa antibodies that react with retinal transducin-α. To our knowledge, this is the first report of antibodies to transducin associated with retinopathy as a complication of cGVHD.

The patient presented in May 2003 with pancytopenia, and was found to have FAB M4 acute myelogenous leukemia (AML) with a 6;11 translocation. Following successful standard induction chemotherapy and 2 cycles of consolidation, she underwent an HLA-matched, ABO mismatched unrelated donor (MMUD) peripheral blood stem cell transplant (PBSCT) in September 2003. Short-course methotrexate (MTX) and tacrolimus were utilized for GVHD prophylaxis.

In May 2005, early cytogenetic recurrence of leukemia was noted by interphase fluorescein in situ hybridization (FISH). Donor lymphocyte infusion (DLI) was administered twice, followed by reinduction chemotherapy in mid-August 2005. After entering a second complete remission (CR2), the patient underwent a second allogeneic PBSCT in October 2005, from a 10/10 matched unrelated donor (MUD).

Following her second transplant, the patient developed grade III GVHD involving the skin and liver. She was treated successfully with prednisone. A baseline ophthalmologic evaluation in February 2006 disclosed mild exposure keratopathy and buried optic nerve drusen. For persistent pancytopenia, the patient received a CD34-selected marrow boost in May 2006. Subsequent to this, tacrolimus was switched to sirolimus because of hyperkalemia, renal insufficiency, and microangiopathy. Diarrhea developed in August 2006, and after a month of persistent symptoms sirolimus was discontinued, with prednisone and hydroxychloroquine used for GVHD treatment. In March 2007, worsening liver GVHD was successfully treated with daclizumab, rituximab, and IVIg, followed by a return to glucocorticoids and hydroxychloroquine for GVHD control.

The patient was seen by an ophthalmologist in November 2007, and found to have continued

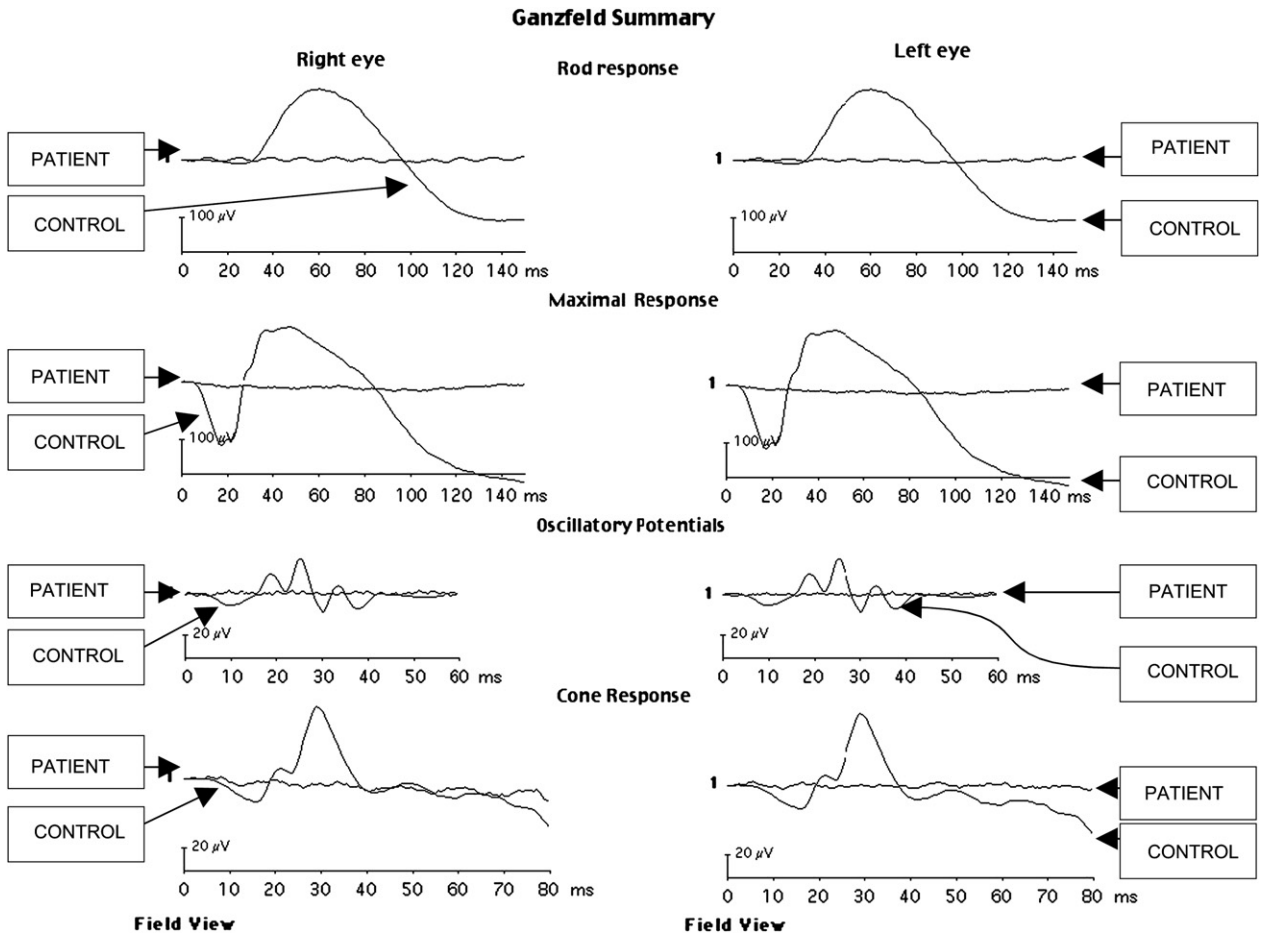

Figure 1. "Full-field" electroretinogram (ff-ERG) of the patient (flat line) compared to normals (contoured line). Each of the tracings demonstrates the patient's markedly diminished summed electrical retinal activity in response to a light stimulus. These findings represent marked loss of function of the retinal cells, including photoreceptors (rods and cones). The oscillatory potentials measure the activity of amacrine cells in the inner retina.

lagophthalmos (incomplete eyelid closure) and secondary exposure keratopathy. In the next 2 months, the patient developed rapid onset of nyctalopia (night blindness) and photopsia (perception of flashing lights). Repeat examination in January 2008 now disclosed abnormal fields with peripheral constriction, in addition to severe arteriolar attenuation, peripheral retinal pigment epithelial mottling and marked retinal thinning. Visual field loss occurred in a ring scotoma pattern. An electroretinogram performed in January 2008 revealed marked reduction in rod and cone amplitudes (Figure 1). The patient's serum was tested for the presence of antiretinal antibodies by Western blot analysis according to a previously published and standardized method [12], and subsequently by ELISA for transducin immunogenic alpha peptide. The patient's peripheral blood was subsequently found to be positive for a 40-kDa protein identified as a transducin-α retinal antibody.

The serum was negative for antibodies to recoverin and other retinal proteins. The patient's symptoms and findings were ascribed to an antibody-mediated retinopathy as a manifestation of GVHD.

Hydroxychloroquine was discontinued at this time. Of note, the hydroxychloroquine was not thought to be responsible for the observed findings, but there was concern that continuation of this agent could cause additional retinal dysfunction. The retinopathy of hydroxychloroquine is a maculopathy that produces central visual deficits, manifest as pigmentary mottling in the macula (central retina), with a completely normal standard ERG. In contrast, this patient's central vision was spared and her peripheral vision was compromised, with an ERG that showed widespread dysfunction consistent with the funduscopic findings of severe arteriolar attenuation and peripheral pigmentary mottling.

Therapies subsequently administered to the patient included IVIg (1 g/kg) for 2 days and repeated monthly for 3 months; rituximab (375 mg/kg) weekly for 4 weeks and repeated every other month for a total of 4 cycles; plasmapheresis twice in March 2008; and increased doses of systemic glucocorticoids. With these treatments, the patient's visual symptoms stabilized. Currently, the patient has stable visual symptoms and controlled cGVHD on low doses of

systemic corticosteroids only. There has been no evidence of AML recurrence to date.

Following stabilization of her visual symptoms, the patient's serum was retested for retinal antibodies by Western blot in February of 2009, and found to no longer be positive for the previously identified 40-kDa protein. At this point, the patient's original serum sample from 2008, and repeat sample from 2009, were tested for transducin immunogenic alpha peptide; these were found to be positive in titers of 1:16,000 and 1:2,000, respectively, confirming a decrease in response to treatment.

There is little information available in the literature regarding treatment of antibody-mediated retinopathy. Some, but not all, patients respond to prednisone, and several local and systemic immunosuppressants have been used with varying degrees of success for other patients. Visual fields and ERG can be monitored over time, with tapering of immunosuppressants when stabilization is achieved; this corresponds with disappearance of bands representing pathogenic antibodies on Western blot [2].

It is possible that the transducin-α antibodies identified in this patient were not responsible for her retinal findings. Some antiretinal antibodies can be found in patients with cancer who do not have accompanying visual symptoms [12-14]. Additionally, we did not have donor serum available for testing, nor did we have patient serum from a time point previous to either of her 2 transplants. However, the appearance of highly suggestive signs and symptoms of antibody-mediated retinopathy in our patient following stem cell transplant, the demonstration of antibodies in her serum to a critically important photoreceptor protein, transducin-α, and her response to immunosuppressive therapy in the setting of cGVHD, which paralleled a substantial decrease in the titer of the antitransducin-α antibody, suggests that transducin-α is a target protein for immune reactivity during GVHD.

In summary, we describe a novel form of "alloimmune retinopathy" in a patient with extensive cGVHD characterized by antibodies specific for the retinal protein transducin-α. Further work is necessary to determine if the presence of single-nucleotide polymorphisms (SNPs) in this protein predisposed to the generation of immune responses in the setting of cGVHD.

AUTHORSHIP CONTRIBUTIONS AND DISCLOSURE OF CONFLICTS OF INTEREST

Contribution: W.W., S.G., G.A., D.G., T.C., and J.S. performed research, analyzed data, and wrote the paper. S.G. obtained and contributed the electroretinogram. G.A. performed research demonstrating the transducin-α antibody in the patient's serum. All authors approved the manuscript.

Financial disclosure: The authors have nothing to disclose.

REFERENCES

1. Sawyer RA, Selhorst JB, Zimmerman LE, Hoyt WF. Blindness caused by photoreceptor degeneration as a remote effect of cancer. *Am J Ophthalmol*. 1976;81:606-613.
2. Heckenlively JR, Ferreyra HA. Autoimmune retinopathy: a review and summary. *Semin Immunopathol*. 2008;30:127-134.
3. Weleber RG, Watzke RC, Shults WT, et al. Clinical and electrophysiologic characterization of paraneoplastic and autoimmune retinopathies associated with antienolase antibodies. *Am J Ophthalmol*. 2005;139:780-794.
4. Jacobson DM, Thirkill CE, Tipping SJ. A clinical triad to diagnose paraneoplastic retinopathy. *Ann Neurol*. 1990;28: 162-167.
5. Eltabbakh GH, Hoogerland DL, Kay MC. Paraneoplastic retinopathy associated with uterine sarcoma. *Gynecol Oncol*. 1995;58:120-123.
6. Adamus G. Autoantibody-induced apoptosis as a possible mechanism of autoimmune retinopathy. *Autoimmun Rev*. 2003;2: 63-68.
7. Chan JW. Paraneoplastic retinopathies and optic neuropathies. *Surv Ophthalmol*. 2003;48:12-38.
8. Polans AS, Burton MD, Haley TL, Crabb JW, Palczewski K. Recoverin, but not visinin, is an autoantigen in the human retina identified with a cancer-associated retinopathy. *Invest Ophthalmol Vis Sci*. 1993;34:81-90.
9. Jankowska R, Witkowska D, Porebska I, Kuropatwa M, Kurowska E, Gorczyca WA. Serum antibodies to retinal antigens in lung cancer and sarcoidosis. *Pathobiology*. 2004;71:323-328.
10. Bazhin AV, Schadendorf D, Willner N, et al. Photoreceptor proteins as cancer-retina antigens. *Int J Cancer*. 2007;120: 1268-1276.
11. Zhu B, Strada SJ. The novel functions of cGMP-specific phosphodiesterase 5 and its inhibitors in carcinoma cells and pulmonary/cardiovascular vessels. *Curr Top Med Chem*. 2007;7: 437-454.
12. Adamus G, Ren G, Weleber RG. Autoantibodies against retinal proteins in paraneoplastic and autoimmune retinopathy. *BMC Ophthalmol*. 2004;4:5.
13. Bazhin AV, Shifrina ON, Savchenko MS, et al. Low titre autoantibodies against recoverin in sera of patients with small cell lung cancer but without a loss of vision. *Lung Cancer*. 2001;34: 99-104.
14. Savchenko MS, Bazhin AV, Shifrina ON, et al. Antirecoverin autoantibodies in the patient with non-small cell lung cancer but without cancer-associated retinopathy. *Lung Cancer*. 2003; 41:363-367.

William Wood
Seema Garg
Grazyna Adamus
Donald Gabriel
Thomas Shea
Jonathan Serody
[1]*Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina*
[2]*Department of Ophthalmology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina;*
[3]*Casey Eye Institute, Department of Ophthalmology, Oregon Health Sciences University, Portland, Oregon; and*

[4]Department of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina
E-mail address: wwood@unch.unc.edu

Biol Blood Marrow Transplant 16: 135-138 (2010)

doi:10.1016/j.bbmt.2009.06.012

High-Dose Chemotherapy with Autotransplantation in AL Amyloidosis: A Flawed Meta-analysis

The conclusions of the meta-analysis by Mhaskar et al. [1] evaluating high-dose chemotherapy with autotransplantation in immunoglobulin light chain amyloidosis are compromised by the omission of a key publication [2] and flawed analytical techniques. The meta-analysis included 1 prospective randomized study of 100 patients and 2 nonrandomized studies containing a total of 49 patients. The pooled results of these 3 studies showed superior overall survival (OS) with conventional chemotherapy (CC) compared to autologous hematopoietic stem cell transplantation (AHSCT) (hazard ratio 1.79; $P = .018$).

The meta-analysis excluded a study that compared 63 consecutive amyloidosis patients receiving high-dose chemotherapy to 63 matched controls who received conventional-intensity treatment [2]. The groups were comparable in terms of age, left ventricular ejection fraction, peripheral nerve involvement, interventricular septal wall thickness, kidney function, and bone marrow (BM) plasmacytosis; factors known to influence OS. The 1-, 2-, and 4-year OS rates from diagnosis were 89% and 71%, 81% and 55%, and 71% and 41%, respectively, favoring AHSCT ($P < .001$). The 1-, 2-, and 4-year OS rates from date of transplantation (cases) or initiation of therapy (controls) were 82% and 68%, 81% and 53%, and 70% and 40% favoring AHSCT ($P < .001$). These findings remained unchanged when 7 patients receiving nonstandard treatment in the conventional-intensity cohort were excluded. The outcome of unmatched historic patients was even worse, confirming previous observations from the Mayo Clinic that eligibility for AHSCT (the control group) improved outcome [3]. However, the magnitude of the difference in outcomes between the control and AHSCT groups provided strong suggestive evidence of the benefit of AHSCT in appropriately selected patients with amyloidosis [4].

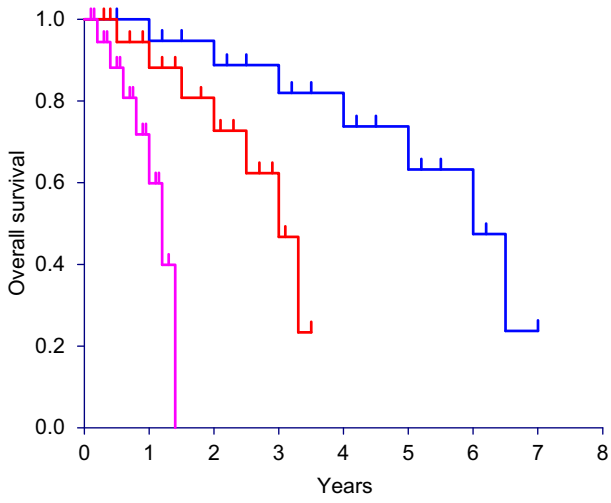

Figure 1. A hypothetical scenario showing the survival of 3 different populations of patients. There are 20 patients in each arm and 7 (35%) have died in each arm.

The size and outcome of the Mayo study [2], had it been included, would have changed the findings of the meta-analysis completely, very likely showing benefit of AHSCT over CC in amyloidosis. It would certainly have undermined the current conclusion of the meta-analysis—that outcome of AHSCT is inferior to CC.

The exclusion of the Mayo study on the grounds that it was retrospective is not justifiable because a number of the other single-arm AHSCT studies included in the meta-analysis are not prospective studies. The authors must clarify whether the exclusion was deliberate. A deliberate exclusion should have been accompanied by a substantial discussion in the meta-analysis on the totally contradictory findings of the 2 studies. If the Mayo study was overlooked by the authors, it suggests inadequate methodology and quality control.

There are other serious methodologic and analytical errors in the manuscript.

Figure 3 in the paper apparently shows a forest plot for the proportion of deaths with CC and AHSCT. The plot, in fact, shows the combined proportion of survivors in 7 of the 10 single-arm AHSCT studies included in the analysis. Depiction of a time-dependent event such as survival in this manner is misleading and statistically inappropriate because it ignores the time to event and the variable duration of follow-up time in survivors. Figure 1 illustrates why the analytical technique chosen by the authors to depict survival is incorrect. In this hypothetical scenario, each of the 3 survival curves represents 20 patients, 7 of whom have died at the time of analysis. Despite the dramatic outcome differences between the groups, the technique employed by the authors would simply show the proportion of deaths in each arm as 0.35, suggesting equivalence of outcome. This would not be a statistically valid conclusion in a meta-analysis