Published in final edited form as:

Prog Retin Eye Res. 2016 January ; 50: 25–33. doi:10.1016/j.preteyeres.2015.11.001.

Molecular Diagnosis: Implications for Ophthalmology

James T. Rosenbaum, M.D.^{1,2,3}, Cailin H. Sibley, M.D.³, Dongseok Choi, Ph.D.^{2,3,4}, Christina A. Harrington, Ph.D.⁵, and Stephen R. Planck, Ph.D.^{1,2,3}

James T. Rosenbaum: rosenbaj@ohsu.edu; Cailin H. Sibley: sibleyc@ohsu.edu; Dongseok Choi: choid@ohsu.edu; Christina A. Harrington: harrincg@ohsu.edu; Stephen R. Planck: plancks@ohsu.edu

¹Devers Eye Clinic, Legacy Health Systems, 1040 NW 22nd Avenue, Portland, Oregon 97210 USA

²Department of Ophthalmology, Casey Eye Institute, Oregon Health & Science University, 3375 SW Terwilliger Blvd, Portland, OR. 97239, USA

³Department of Medicine, Oregon Health & Science University, 3181 SW Sam Jackson Park Rd, Portland, OR. 97239 USA

⁴OHSU-PSU School of Public Health, Oregon Health & Science University, 3181 SW Sam Jackson Park Road, Portland, OR. 97239

⁵Integrated Genomics Laboratory, Oregon Health & Science University, 3181 SW Sam Jackson Park Rd, Portland, OR 97239 USA

Abstract

The effort to subdivide diseases and to individualize therapies based on characteristics of the patient has been labelled precision medicine. Jameson and Longo define precision medicine as “treatments targeted to the needs of individual patients on the basis of genetic, biomarker, phenotypic or psychosocial characteristics that distinguish a given patient from other patients with similar clinical presentations” (Jameson and Longo, 2015). We illustrate how molecular diagnosis can be applied to orbital inflammatory disease to achieve the goals of precision medicine.

Keywords

molecular diagnosis; transcriptomics; thyroid eye disease; granulomatosis with polyangiitis; sarcoidosis; nonspecific orbital inflammatory disease

1. Introduction

You are evaluating a 48 year old female with bilateral exophthalmos affecting her visual function for the last 6 months. Ten years ago she was treated for hyperthyroidism with

Correspondence to: James T. Rosenbaum, rosenbaj@ohsu.edu.

Conflict of Interest: Dr. Rosenbaum has received honoraria from Genentech which manufactures rituximab. The other authors report no conflicts of interest.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

radioactive iodine and she has been on thyroid supplementation since. She is now euthyroid. You take a thorough history and find that she is in excellent health except she has been having sinus “infections” regularly for the last two years. You decide to obtain laboratory tests. Thinking that this could be sarcoidosis, you obtain a chest x-ray and then a chest computerized tomography (CT) scan. Both show no abnormalities. Thinking that this could be a systemic vasculitis such as granulomatosis with polyangiitis (GPA) and sinus involvement, you obtain an anti-neutrophil cytoplasmic antibody (ANCA) study which is also negative. Then you order CT imaging of the orbits. The radiologist confirms the bilateral proptosis, thinks that the likelihood of either infection or primary or metastatic malignancy is low, but is unwilling to offer a more specific diagnosis as to the cause of inflammation. The radiologist judges that there is no extra-ocular muscle enlargement. Although the patient’s disease is troubling, you decide that the severity does not currently require surgery to relieve pressure on the optic nerve. But you wonder if you should obtain an orbital biopsy, and if you do obtain this biopsy, what is the likelihood that the tissue will provide a specific diagnosis?

Although many clinicians approach the evaluation of orbital inflammatory disease differently, this scenario should be familiar to most ophthalmologists and to all orbital surgeons.

Establishing a precise diagnosis is the initial goal of most therapeutic encounters between a physician and a patient. Often that diagnosis is obvious from the history or the physical examination. In some instances, a serological test such as a fluorescent treponemal antibody titer plays a critical role in establishing the diagnosis. Sometimes imaging as with ultrasound, optical coherence tomography, or magnetic resonance plays a vital role. Within ophthalmology, current efforts are directed toward defining subsets of diseases such as diabetic retinopathy (Cunha-Vaz et al., 2014) and scleritis (Wakefield et al., 2013) such that more accurate prognosis and more tailored therapy can be offered. The last resort in differential diagnosis is often a biopsy so that tissue can be examined histologically. Although intra-ocular biopsies are uncommon, they can be invaluable in assessing some pathologies like lymphoma or melanoma. Biopsy of orbital tissue is a frequent approach to obtain a diagnosis of disease that causes a space-occupying lesion with the orbit.

In this review, we consider specifically the diagnostic value provided by biopsy of orbital tissue. We propose that the information gained from routine histology can be complemented and perhaps even exceeded by an analysis of the genes expressed in the removed tissue. We argue that molecular diagnosis, meaning an analysis of the genes expressed within a tissue, gives clues to pathogenesis, facilitates diagnosis, and might ultimately be fundamental in therapeutic decisions and assessment of prognosis. We use the term, transcriptomics, as a synonym for mRNA profiling since transcriptomics refers to an analysis of the RNA transcripts expressed in a tissue. The studies which we discuss represent an application of precision medicine to an assortment of orbital inflammations, some of which are frequently labelled as idiopathic or nonspecific. The approach that we describe requires refinements and validation before becoming a part of the routine approach to orbital disease. Thus, we offer both a review and a speculation about the future.

1.1 The Differential Diagnosis of Orbital Inflammatory Disease

Orbital diseases can be divided into inflammations, infections, and malignancies. The inflammation can involve primarily extra-ocular muscle, orbital fat, the lacrimal gland, or some combination of these tissues. The most common inflammatory condition that affects the orbit is thyroid eye disease, also known as Graves' ophthalmopathy (Bartalena and Tanda, 2009). Graves' disease affects about 0.5% of the US and European population (Bartalena and Tanda, 2009). Seventy percent of patients with Graves' disease demonstrate extra-ocular muscle enlargement on imaging (Bahn, 2010). About 50% of patients with Graves' disease develop some symptoms of ophthalmopathy, although it is severe in only about ten per cent of those affected (Bartalena and Tanda, 2009). Other non-malignant, non-contagious causes of orbital swelling include sarcoidosis (Rosenbaum et al., 2015b), GPA (Rosenbaum et al., 2015c) (previously known as Wegener's granulomatosis and also known as ANCA associated vasculitis), histiocytoses like Erdheim Chester disease (Hayden et al., 2007), and xanthogranulomas. A common diagnosis for orbital inflammation is nonspecific orbital inflammation (NSOI), which is also known as idiopathic orbital inflammation or orbital pseudotumor (Yuen and Ruin, 2003).

1.2 Molecular diagnosis as applied to cancer

One of the first applications of molecular diagnosis was reported by Louis Staudt and his colleagues in an analysis of lymphomas (Alizadeh et al., 2000). These cancers have distinct subsets clinically, but it is not always easy to distinguish the subset by routine light microscopy. For example, in a report in *Nature* in 2000, Staudt and colleagues analyzed gene expression in diffuse large B cell lymphoma (Alizadeh et al., 2000). They used gene expression profiling to show that some lymphomas, which could not be subdivided on the basis of routine light microscopy, resembled the pattern of expression of germinal center B cells and others resembled the pattern seen with activated peripheral blood B cells. Moreover, the prognosis was much better for the lymphomas with the germinal center pattern.

Similar technology has been employed in ophthalmology to identify two subsets of uveal melanoma (van Gils et al., 2008). The more common subset is unlikely to metastasize. But there are distinct gene transcripts associated with those melanomas that are most likely to involve the liver or other distant organs (van Gils et al., 2008). Estrogen receptor positivity is another example of a marker that distinguishes subsets, in this case subsets of breast cancer, but requires a technique other than routine histology for recognition (Yamamoto-Ibusuki et al., 2015).

1.3 Molecular diagnosis and inflammation

The most common application of molecular diagnosis is for malignancies, but inflammatory diseases are also yielding clues as a result of molecular diagnosis. For example, a subset of patients with systemic lupus erythematosus has up regulated genes in peripheral blood that are inducible by type I interferons (Baechler et al., 2003). This realization has led to clinical trials in which neutralization of interferon alpha is the target (Petri et al., 2013). Gene expression in CD8 T cells can aid in forecasting the prognosis in inflammatory bowel disease (Lee et al., 2011), systemic lupus erythematosus (McKinney et al., 2010), and GPA

(McKinney et al., 2010). Synovitis has a broad differential diagnosis that includes rheumatoid arthritis, lupus, and spondyloarthritis. A pathologist has a limited ability to distinguish among these diagnoses, but transcriptomics can potentially provide a more precise differential (Yeremenko et al., 2013). Gene expression profiling has also been employed successfully to subdivide myocarditis (Lassner et al., 2014) and esophagitis (Wen et al., 2013).

1.4 Techniques for molecular diagnosis

In the work that we describe below, we have characterized orbital inflammation on the basis of gene expression profiling using microarray. In this technique, RNA is extracted from tissue. The mRNA is converted to cDNA, amplified in an *in vitro* transcription reaction to produce sense RNA, converted to double-strand cDNA and labeled. The labeled cDNA is then allowed to hybridize to complementary DNA probes which are attached to a glass chip. Thousands of probes or probe sets complementary to gene transcripts can be exactly positioned on a relatively small surface, and the quantity of labelled cDNA that adheres to each position can be measured. In this way, a laboratory can interrogate a tissue using more than 50,000 probe sets simultaneously. Our work has been based on the use of formalin-fixed tissue. We elected to work with formalin-fixed tissue as opposed to frozen tissue or tissue fixed in an alternative fixative because we wanted to collect tissue from multiple centers and needed a broadly used, standardized method that also allowed for inexpensive shipping costs. Formalin fixation and extended specimen storage times, however, lead to RNA degradation. To compensate for this, we utilized a cDNA synthesis and amplification method optimized for highly fragmented RNA.

Although we discuss the limitations of our work more extensively at the conclusion of this report, the tissue collection itself represents a potential source of artefact. Because the background gene expression in lacrimal gland is quite different from gene expression in orbital adipose tissue, we have been careful to compare gene expression in the inflamed lacrimal gland to normal lacrimal gland and inflammation in orbital fat to gene expression in healthy orbital adipose tissue. It clearly makes a difference how the tissue is sectioned since this will influence how much uninflamed tissue is present. Furthermore, the cellular population within the tissue is heterogeneous. Assume, for example, that the mast cell plays a critical role in pathogenesis but represents less than 1% of the cellular infiltrate. This methodology might well obscure the relevance of genes being expressed by mast cells. Despite these potential obstacles, the approach has provided novel insights as described below.

RNA Seq is an alternative method to characterize thousands of transcripts from a single sample (Nagalakshmi et al., 2008). In RNA Seq, the identity and number of mRNA sequences is determined by sequencing of the nucleic acids. A major advantage over microarray is the ability to detect mRNAs which might not be represented on the microarray chip. As the cost of sequencing technology has fallen, many centers are now opting for the RNA Seq technology over microarray. We anticipate that this technology will become the future standard for molecular diagnosis.

A tissue could also be characterized in terms of proteins, lipids, sugars, methylation status of the DNA, micro RNAs (which regulate the expression of other transcripts), or other measurements. For malignancies, scientists have applied drug-eluting microarrays to a single cancer stem cell in an effort to optimize therapeutic choices (Carstens et al., 2015). Our experience is limited to the measurement of mRNA transcripts.

2 Molecular diagnosis and orbital inflammatory disease

2.1 What is orbital pseudotumor?

Our initial interest in molecular diagnosis of orbital inflammation arose from an effort to understand nonspecific orbital inflammation which is often called by its misleading name, orbital pseudotumor. A diagnosis of “nonspecific inflammation” is unsatisfying to both the treating physician and the patient. We hoped that a characterization of the transcriptome would provide some insight.

In order to clarify the nature of NSOI relative to other orbital inflammations, we organized a consortium of orbital surgeons and ophthalmic pathologists. The members were mostly from North America but we also had participants from Saudi Arabia, Taiwan, and Australia. Although the geographic diversity could potentially affect the gene expression, it also made it more likely that our results would have broad extrapolation. For comparators we collected biopsies from patients undergoing enucleations or cosmetic surgeries if there was no history of orbital disease. Our tissue “library” included examples of thyroid eye disease, GPA, NSOI, and sarcoidosis.

A principal coordinate analysis (PCA, or multidimensional scaling) is a method to visualize the level of similarity among cases in a complex data set. In a PCA the distance between two points is proportionate to their similarity. For example, the alleles in your genome make up a complex data set. If the genome of two siblings was represented on a PCA, the two points would presumably be close together. But if you represented the genome of an Inuit from the Arctic Circle and the genome of someone from Saudi Arabia, presumably the two points would be relatively far apart.

Figures 1A and 1B show PCA plots based on RNA transcripts for orbital adipose tissues from healthy controls as well as subjects with sarcoidosis, GPA, and thyroid eye disease. Two separate batches are shown to allow for a discovery set and a validation set. In performing thousands of statistical comparisons, one expects to identify some differences that cannot be reproduced even if the false discovery rate adjusted p value is significant. Inclusion of a validation set helps to reduce the number of statistical artifacts that potentially result from multiple comparisons. As one would predict, the plots show that each entity clusters. For example, the controls tend to form a group; and each disease makes a group as well. The clustering is imperfect. In each specimen, the ratio of diseased to normal tissue undoubtedly varied. In addition, the subjects varied in age, disease duration, medications, and geographic location. Despite all of these variables, the clustering pattern is obvious.

2.2 How would you predict that NSOI would cluster on a PCA plot?

Figures 2A and 2B depict PCA plots for gene expression in orbital fat from patients with NSOI relative to the healthy controls and the known entities. It is apparent that there is more heterogeneity for NSOI compared to the better defined entities. This should not be surprising. From a prognostic or therapeutic perspective, NSOI is clearly heterogeneous. While the NSOI samples are disparate, many of the samples are relatively close to GPA, suggesting that many patients with NSOI actually have a *forme fruste* of GPA. The differences between NSOI and GPA, sarcoidosis, TED, or controls can be appreciated with Venn diagrams (Figure 3). Although NSOI has genes up or down regulated relative to controls, sarcoidosis or TED, from a statistical perspective we were unable to identify gene transcripts that consistently distinguished NSOI from GPA in both our discovery and our validation set (Rosenbaum et al., 2015c).

2.3 What do these observations tell us about diagnosis?

Orbital biopsy analyzed by histopathology is an excellent method to diagnose lymphoma or metastatic disease, but it is not an accurate method to diagnose orbital inflammatory diseases. We tested this contention by asking two expert ophthalmic pathologists to make a diagnosis from an orbital biopsy without the benefit of clinical information (Rosenbaum et al., 2015d). The pathologists accurately identified non-caseating granulomas and thus could correctly diagnose sarcoidosis. Both pathologists, however, struggled to distinguish a biopsy from a healthy control relative to biopsy from a patient with TED (Rosenbaum et al., 2015d). The pathologists were especially poor at diagnosing GPA without clinical information. In the lung, a diagnosis of GPA can be accurately established because medium-sized arteries are included. Since vessels this size are not present within the orbit, the inability to diagnose GPA accurately from orbital tissue is hardly surprising. Pathologists also struggle to identify GPA confidently in tissue such as nasal mucosa or sinus (Borner et al., 2012; Del Buono and Flint, 1991), locations that are classically involved by GPA. We did not ask our pathologists to identify NSOI since virtually by definition, NSOI can mimic other inflammations.

We used gene expression and random forest learning to establish a computerized algorithm to diagnose orbital inflammatory diseases. We then applied this algorithm to orbital adipose biopsies that were diagnosed as healthy, thyroid eye disease, or GPA on the basis of pathology and clinical information from highly regarded ophthalmology centers. The computerized algorithm demonstrated better accuracy (76%) than either of the expert pathologists who scored an accuracy 49% and 58% respectively (Rosenbaum et al., 2015d). Accuracy is defined as the average of sensitivity and specificity.

These observations can and should be further refined. They are based on a relatively small number of examples of GPA (n=6). They are based on quantification of transcripts from more than 20,000 genes. To become a component of routine clinical testing, the algorithm needs to be tested on a greater number of clinical samples. The comparator group needs to be expanded. The differentiating transcripts should be honed to a relatively small number that could be measured accurately and routinely on biopsies so that the results can be applied to clinical decision making. We predict that some form of profiling will ultimately become

routine in the evaluation of orbital inflammation. Profiling on an RNA level is feasible, accurate and fast. Profiling based on proteins (proteomics) represents an alternative approach to a similar goal.

Biopsy of orbital tissue, of course, is an invasive test associated with some morbidity, risk, and expense. Blood is far more accessible than orbital tissue. Could this technology be extrapolated such that a blood sample alone would allow accurate diagnosis of orbital inflammation? We tested this hypothesis as it relates to sarcoidosis involving the orbit (Rosenbaum et al., 2015b). We characterized the genes expressed in the lacrimal gland affected by sarcoidosis in comparison to genes expressed in orbital adipose tissue affected by sarcoidosis. It is critical to compare lacrimal tissue from a patient with sarcoidosis to normal lacrimal gland tissue because the background genes expressed are distinctly different from what is expressed in orbital fat. Similarly we use normal adipose tissue as a comparator to learn what transcripts are up or down regulated in orbital fat affected by sarcoidosis. Our observations indicate that there is a great deal of overlap between genes expressed in the sarcoidosis-affected lacrimal gland compared to the sarcoidosis-affected orbital adipose tissue (Rosenbaum et al., 2015b). More importantly many of the transcripts that are up or down regulated in both lacrimal gland and orbital adipose tissue as a result of sarcoidosis are similarly affected in an assay based on whole blood (Rosenbaum et al., 2015b). Currently serological testing for sarcoidosis as by measurement of angiotensin converting enzyme (ACE) level or lysozyme is woefully deficient in sensitivity and specificity (Ainslie and Benatar, 1985). The potential of RNA profiling is that a blood test based on a relatively small number of transcripts may prove more accurate than either ACE or lysozyme as a method to diagnose sarcoidosis.

Many diagnostic tests appear to have specificity until they are tested using diseases that can be clinically difficult to differentiate. Tuberculosis, for example, like sarcoidosis, is a granulomatous disease that could involve the orbit. Relying on a whole blood test that could not distinguish between TB and sarcoidosis could result in a therapeutic disaster. Although we have not attempted this comparison, a group has studied whole blood profiling of children in Africa and reported that this technology accurately recognizes active tuberculosis (Anderson et al., 2014). Additional studies are indicated to establish more completely and prospectively the sensitivity and specificity of this approach to diagnose sarcoidosis within the orbit or in other tissues.

The diagnosis of sarcoidosis is frequently strongly supported by the finding of hilar and mediastinal adenopathy on chest imaging. But several other forms of orbital inflammation cannot be routinely diagnosed by imaging or serological study. ANCA testing, for example, is most often negative when GPA affects the orbit and only tissues above the clavicle, i.e., no lung or kidney involvement (Rao et al., 1995). NSOI is diagnosed on the basis of biopsy and no other testing is helpful except to exclude additional possibilities. Although we have not attempted to diagnose GPA confined to tissue above the clavicle on the basis of a blood test, we have observed that several of the transcripts that we found to be up regulated in orbital tissue from patients with GPA are also up regulated in leukocytes from patients with GPA based on findings reported by another group (Alcorta et al., 2002; Alcorta et al., 2007) This observation supports the hypothesis that so called limited GPA might be accurately

diagnosed on the basis of a whole blood assay that quantifies gene expression. It remains to be determined whether NSOI, a disease confined usually to the orbit, is characterized by any detectable changes in gene expression in blood. Furthermore, studies will need to include critical comparators such as orbital metastatic disease, lymphoma, Erdheim-Chester disease, or infection like mucormycosis in order to be confident that a blood based assay could replace invasive biopsy.

As noted above, biopsy of tissue such as nasal mucosa, sinus, or subglottis frequently fails to diagnose GPA. If our observations on orbital inflammation can be extrapolated to these and other tissues, molecular diagnosis will become a standard part of the analysis of biopsies from a wide array of tissues.

2.4 What do these observations tell about pathogenesis and therapy?

Functional analysis software such as DAVID (Dennis Jr. et al., 2003; Sherman et al., 2007) and GSEA (Subramanian et al., 2005) now exists to interrogate the relationship among transcripts with altered expression. As noted above, this has led to insights into systemic lupus erythematosus and the contention that a subset of patients with SLE has a disease driven by type I interferon (Baechler et al., 2003). We have applied this type of pathway analysis to sarcoidosis. We find that STAT-1 is consistently up regulated in lacrimal gland, adipose tissue, blood, lymph node and lung from patients with sarcoidosis (Rosenbaum et al., 2015b; Rosenbaum et al., 2009). STAT-1 is an intracellular signaling molecule that can be activated by a variety of cytokines (Kasperkovitz et al., 2004). The prototypical cytokine for STAT-1 activation is gamma interferon. Gamma interferon in mice affects the histology of granulomas (Ehlers et al., 2001) which are characteristic of sarcoidosis. Therapy with gamma interferon has been complicated by granuloma formation (Fiel et al., 2008). Accordingly a role for STAT-1 in the pathogenesis of sarcoidosis is highly plausible. Furthermore, STAT-1 interacts with the Janus family of kinases (JAKs). A JAK inhibitor is already in clinical use as in the treatment of rheumatoid arthritis (van Vollenhoven et al., 2012). Therefore, molecular diagnosis might clarify pathogenesis and concurrently suggest a novel, but untested, approach to therapy.

In our analysis of transcripts with increased expression in orbital adipose tissue from patients with GPA, we found that several immunoglobulins or immunoglobulin-related products were among the most up regulated transcripts. Immunoglobulin is synthesized by plasma cells which are derived from B lymphocytes. Rituximab is a monoclonal antibody that recognizes a cell surface marker, CD20, present on B lymphocytes. Rituximab depletes these cells and is effective in the treatment of B cell lymphomas (Bohen et al., 2003) or rheumatoid arthritis (Sellam et al., 2014). More recently two randomized controlled trials demonstrated the efficacy of rituximab in the treatment of GPA (Jones et al., 2010; Stone et al., 2010). On this basis one would predict that rituximab would be effective in the treatment of orbital GPA and in the subset of patients with NSOI that resembles GPA. Indeed we and others have reported that rituximab is effective in the treatment of orbital inflammation secondary to GPA or NSOI (Joshi et al., 2011; Suhler et al., 2014). In our prospective study, we did not perform a molecular characterization prior to initiating therapy. Our results support the hypothesis that those tissues marked by an over expression of B cell related

products would be most likely to benefit from rituximab therapy. In other words, in theory, one could identify prospectively patients with NSOI who would most likely benefit from anti-B cell therapy.

2.5 Potential insights into the therapeutic approach to TED

TED is the most common form of orbital inflammation and it is the one form of orbital inflammation that has been studied in terms of gene expression by multiple groups (Chen et al., 2008; Ezra et al., 2012; Kumar et al., 2005; Lantz et al., 2005; Planck et al., 2011; Rosenbaum et al., 2015e). Graves' hyperthyroidism is an autoimmune disease in which the function of the characteristic autoantibody is to activate the receptor for thyroid stimulating hormone. Thus the autoantibody that typifies Graves' disease relates directly to the pathology indicative of Graves' disease, i.e., hyperthyroidism. Since the hyperthyroidism of Graves' disease is autoimmune, it is plausible to hypothesize that the orbital disease is autoimmune as well. However, if the pathogenesis of the orbital disease is identical to the thyroid disease, one might expect a tighter temporal and clinical correlation between the thyroid disorder and the orbital disease.

Examining the PCA plots shown in Figures 1A and 1B, many of the samples from subjects with TED are adjacent to the control tissue. For example, a heat map based on immunoglobulin gene expression (Figure 4) shows how closely TED resembles the controls for this parameter in contrast to the 3 other diagnoses of GPA, NSOI, or sarcoidosis. Our analysis does show some inflammation in orbital adipose tissue affected by TED, but markers indicative of inflammation such as cytokines are much more highly expressed in NSOI, sarcoidosis, or GPA, in support of the contention that inflammation is not a prominent feature of TED. When our two pathologists were denied clinical information, both consistently struggled to distinguish a biopsy from a healthy control from a biopsy from a patient with TED (Rosenbaum et al., 2015d). Multiple uncontrolled studies including more than 40 subjects have assessed the use of rituximab to treat TED and the vast majority of reports have concluded that it is effective (Salvi et al., 2013). However, a randomized controlled study from the Mayo Clinic showed no benefit from rituximab in TED and some morbidity from the therapy (Stan et al., 2015). The lack of efficacy fits with the relative lack of immunoglobulin gene expression in the affected tissue. A randomized controlled trial in Europe using rituximab to treat TED did have positive results (Salvi et al., 2015). It is difficult to reconcile the differences in the two studies but a potential explanation is that the European study treated patients at an earlier stage of disease when the immune response was contributing more to pathogenesis. On the other hand, the Mayo Clinic study shows that the majority of patients with TED who received rituximab improved, but patients receiving placebo improved as well.

As also noted below, our analysis of gene expression in subjects affected by TED could certainly be skewed by studying predominantly subjects who have experienced chronic inflammation. In a collaboration which we have established with the International Thyroid Eye Disease Society, we hope to obtain tissues early in the disease so that we can assess the role of disease duration on gene expression. We cannot currently exclude the possibility that

TED begins primarily as an immunological disease and then the process is sustained by non-immunological mechanisms.

2.6 Insights based on IgG4 expression or fibrosis

In addition to classifying orbital tissue on the basis of a histological diagnosis, we have also scored the biopsies on the basis of IgG4 expression (Wong et al., 2014) and fibrosis (Rosenbaum et al., 2015a) and then determined how gene expression correlates with these parameters. IgG4 is the least common of all four isoforms of IgG. Since it does not bind complement, some believe that its primary function is anti-inflammatory (Rispen et al., 2009). However, prominence of IgG4 plasma cells in tissue correlates with fibrosis and with a multisystem disease that often responds favorably to rituximab therapy (Deshpande et al., 2012; Khosroshahi et al., 2010). Retroperitoneal fibrosis is an example of a disease that might be IgG4 mediated in some instances (Tzou et al., 2014). IgG4-mediated disease has been reported in both the lacrimal gland (Koizumi et al., 2013) and in orbital adipose tissue (Lindfield et al., 2012). Although guidelines for the diagnosis of IgG4-mediated disease have been proposed, there remains a lack of consensus about what constitutes a sufficient infiltration of IgG4 positive plasma cells to establish a diagnosis. We stained more than 100 orbital biopsies and quantified the expression of IgG4 (Wong et al., 2014). We did not include any tissue from a patient diagnosed with IgG4 disease, meaning that none of our samples came from a subject with a multisystem disease in which IgG4 was increased in serum and was deposited in multiple organs. We found that none of our healthy controls and none of our samples from subjects with TED stained for a substantial amount of IgG4. However, IgG4 staining was prominent, i.e., at least 10 IgG4 positive cells per high power field, in 5 of 6 samples from subjects with GPA and in 42% and 38% respectively of subjects with either sarcoidosis or NSOI (Wong et al., 2014). We then analyzed gene expression as it correlated with IgG4 expression and found an increase in inflammation related transcripts in tissue from subjects with increased IgG4 expression. Many of these transcripts were related to B cells and their products. As noted above, we have found that B cell depletion is an effective treatment modality for many patients with orbital inflammation. An untested hypothesis is that either gene expression or quantification of IgG4 staining might help select a subset of patients who are most likely to benefit from immunotherapy targeted at B cells.

Fibrosis can be a prominent component of the tissue response to injury. This is especially true in the orbit. Fibrosis may represent an ominous prognostic sign, and it might indicate irreversible disease. We scored lacrimal and orbital fat pathology for fibrosis and then correlated gene expression with the fibrosis score. In orbital adipose tissue, fibrosis was associated with gene expression of fibronectin, lumican, thrombospondin, and collagen types I and VIII (Rosenbaum et al., 2015a). These mRNAs have also been implicated in pulmonary fibrosis (DePianto et al., 2014). Several pharmaceutical companies are specifically targeting fibrosis to treat diseases like pulmonary fibrosis or scleroderma (O'Riordan et al., 2015). A plausible speculation is that by recognizing fibrosis with an objective and quantitative technique that calls attention to specific mRNAs, gene expression profiling could help identify a subset of patients who would benefit from similar pharmacotherapy.

3 Summary, limitations, unanswered questions, future directions

In summary, a variety of new insights have arisen from the application of molecular diagnosis to orbital inflammatory disease. First, and probably least surprising, NSOI is a heterogeneous collection of diseases (Rosenbaum et al., 2015c). Second, GPA, TED, and sarcoidosis in the orbit show distinctive molecular signatures (Rosenbaum et al., 2015c). Third, genes with increased expression in orbital adipose tissue affected by sarcoidosis overlap with the transcripts with increased expression in the lacrimal gland and in the blood (Rosenbaum et al., 2015b). Fourth, B cell depletion is a rational approach to treat orbital GPA based on an extrapolation from studies of systemic GPA and based on immunoglobulin gene expression in the orbit from subjects with GPA (Rosenbaum et al., 2015c). Fifth, although NSOI displays heterogeneity, often the molecular signature detected in orbital adipose tissue affected by NSOI is very similar to that detected in tissue affected by GPA (Rosenbaum et al., 2015c). Sixth, surprisingly the “inflammatory signature” detectable in orbital adipose tissue affected by TED is marked by much less inflammation than is characteristic of sarcoidosis, GPA, or NSOI (Rosenbaum et al., 2015e). Seventh, the detection of IgG4+ cells or fibrosis in an orbital biopsy can be correlated with distinct patterns of gene expression (Rosenbaum et al., 2015a; Wong et al., 2014). Eighth, an algorithm based on gene expression shows promise in enhancing diagnostic accuracy from an orbital biopsy (Rosenbaum et al., 2015d).

These observations also have limitations and implications for future studies. While we have characterized gene expression in more than 100 orbital adipose or lacrimal biopsies, some diseases like GPA and sarcoidosis are represented by relatively few samples. Although we employed the strategy of a discovery and validation set, we recognize the critical need to evaluate many more tissues. The statistical analysis is based on multiple statistical comparisons. Additional validation might eliminate some candidate transcripts and it should allow future diagnostic distinctions on the basis of a much smaller set of transcripts. We need many more samples and ideally samples collected over time to address questions such as the effect of medications like corticosteroids, the effect of disease duration, the effect of patient age or gender, and the effect of geographic location. TED is a prime example of a disease which might have an inflammatory phase and a later, non-inflammatory phase. Testing samples when the disease begins would greatly help in testing this hypothesis. RNA Seq and/or analysis of frozen tissue might be superior approaches to answer a variety of questions. While the PCA plot suggests that NSOI can resemble sarcoidosis, TED or NSOI, we remain uncertain as to whether some NSOI is representative of a fourth disease distinct from the other three. Again further tissue collection and analysis offers the ability to test this hypothesis.

Despite these limitations, we are confident that some form of gene expression profiling will ultimately be a part of the pathological characterization of orbital inflammatory diseases. This technology offers an avenue to gain insights into the pathogenesis of diseases such as TED. It allows NSOI to be subdivided and our work strongly suggests that this subdivision has therapeutic implications. The conclusions derived from these studies would seem to have a natural extrapolation to the biopsy of tissues such as nasal mucosa, sinus, or subglottis, i.e., tissue affected by GPA but tissue that often does not yield a diagnostic biopsy. The studies

offer hope that a blood test rather than a biopsy might become the standard for diagnosis of orbital inflammatory disease. The studies have implications for subdividing inflammation in other body sites. And the studies illustrate the value of precision medicine in formulating a therapeutic strategy based on an analysis of the gene expression in orbital tissue.

Acknowledgments

The authors are grateful for funding support from the National Eye Institute (Grants EY020249 and P30 EY 010572), and funding from the Stan and Madelle Rosenfeld Family Trust, the William and Mary Bauman Foundation, and Research to Prevent Blindness, New York. This work would not be possible without the efforts of the Orbital Inflammatory Disease Consortium which includes David J Wilson, Hans Grossniklaus, Roger Dailey, John Ng, Eric Steele, Craig Czyz, Jill Foster, David Tse, Chris Alabiad, Sander Dubovy, Prashant Parekh, Gerald Harris, Michael Kazim, Payal Patel, Valerie White, Peter Dolman, Bobby Korn, Don Kikkawa, Deepak Edward, Hind Alkatan, Hailah Al-Hussain, R. Patrick Yeatts, Dinesh Selva D, and Patrick Stauffer.

References

- Ainslie GM, Benatar SR. Serum angiotensin converting enzyme in sarcoidosis: Sensitivity and specificity in diagnosis: Correlations with disease activity, duration, extra-thoracic involvement, radiographic type and therapy. *Quart J Med*. 1985; 55:253–270. [PubMed: 2991971]
- Alcorta D, Preston G, Munger W, Sullivan P, Yang JJ, Waga I, Jennette JC, Falk R. Microarray studies of gene expression in circulating leukocytes in kidney diseases. *Exp Nephrol*. 2002; 10:139–149. [PubMed: 11937761]
- Alcorta DA, Barnes DA, Dooley MA, Sullivan P, Jonas B, Liu Y, Lionaki S, Reddy CB, Chin H, Dempsey AA, Jennette JC, Falk RJ. Leukocyte gene expression signatures in antineutrophil cytoplasmic autoantibody and lupus glomerulonephritis. *Kidney Int*. 2007; 72:853–864. [PubMed: 17667990]
- Alizadeh AA, Eisen MB, Davis RE, Ma C, Lossos IS, Rosenwald A, Boldrick JC, Sabet H, Tran T, Yu X, Powell JI, Yang L, Marti GE, Moore T, Hudson J Jr, Lu L, Lewis DB, Tibshirani R, Sherlock G, Chan WC, Greiner TC, Weisenburger DD, Armitage JO, Warnke R, Levy R, Wilson W, Grever MR, Byrd JC, Botstein D, Brown PO, Staudt LM. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature*. 2000; 403:503–511. [PubMed: 10676951]
- Anderson ST, Kaforou M, Brent AJ, Wright VJ, Banwell CM, Chagaluka G, Crampin AC, Dockrell HM, French N, Hamilton MS, Hibberd ML, Kern F, Langford PR, Ling L, Mlotha R, Ottenhoff TH, Pienaar S, Pillay V, Scott JA, Twahir H, Wilkinson RJ, Coin LJ, Heyderman RS, Levin M, Eley B. Consortium I, Group KTS. Diagnosis of childhood tuberculosis and host RNA expression in Africa. *N Engl J Med*. 2014; 370:1712–1723. [PubMed: 24785206]
- Baechler EC, Batliwalla FM, Karypis G, Gaffney PM, Ortmann WA, Espe KJ, Shark KB, Grande WJ, Hughes KM, Kapur V, Gregersen PK, Behrens TW. Interferon-inducible gene expression signature in peripheral blood cells of patients with severe lupus. *Proc Natl Acad Sci USA*. 2003; 100:2610–2615. [PubMed: 12604793]
- Bahn RS. Graves' ophthalmopathy. *N Engl J Med*. 2010; 362:726–738. [PubMed: 20181974]
- Bartalena L, Tanda ML. Clinical practice. Graves' ophthalmopathy. *N Engl J Med*. 2009; 360:994–1001. [PubMed: 19264688]
- Bohen SP, Troyanskaya OG, Alter O, Warnke R, Botstein D, Brown PO, Levy R. Variation in gene expression patterns in follicular lymphoma and the response to rituximab. *Proc Natl Acad Sci USA*. 2003; 100:1926–1930. [PubMed: 12571354]
- Borner U, Landis BN, Banz Y, Villiger P, Ballinari P, Caversaccio M, Dubach P. Diagnostic value of biopsies in identifying cytoplasmic antineutrophil cytoplasmic antibody-negative localized Wegener's granulomatosis presenting primarily with sinonasal disease. *Am J Rhinol Allergy*. 2012; 26:475–480. [PubMed: 23232198]
- Carstens MR, Fisher RC, Acharya AP, Butterworth EA, Scott E, Huang EH, Keselowsky BG. Drug-eluting microarrays to identify effective chemotherapeutic combinations targeting patient-derived cancer stem cells. *Proc Natl Acad Sci USA*. 2015; 112:8732–8737. [PubMed: 26124098]

- Chen MH, Liao SL, Chen MH, Tsou PL, Shih MJ, Chang TC, Chuang LM. Lysosome-related genes are regulated in the orbital fat of patients with graves' ophthalmopathy. *Invest Ophthalmol Vis Sci*. 2008; 49:4760–4764. [PubMed: 18552385]
- Cunha-Vaz J, Ribeiro L, Lobo C. Phenotypes and biomarkers of diabetic retinopathy. *Prog Retin Eye Res*. 2014; 41:90–111. [PubMed: 24680929]
- Del Buono EA, Flint A. Diagnostic usefulness of nasal biopsy in Wegener's granulomatosis. *Hum Pathol*. 1991; 22:107–110. [PubMed: 2001873]
- Dennis G Jr, Sherman BT, Hosack DA, Gao JY, Lane HC, Lempicki RA. DAVID: Database for annotation, visualization, and integrated discovery. *Genome Biol*. 2003; 4:P3. [PubMed: 12734009]
- DePianto DJ, Chandriani S, Abbas AR, Jia G, N'Diaye EN, Caplazi P, Kauder SE, Biswas S, Karnik SK, Ha C, Modrusan Z, Matthay MA, Kukreja J, Collard HR, Egen JG, Wolters PJ, Arron JR. Heterogeneous gene expression signatures correspond to distinct lung pathologies and biomarkers of disease severity in idiopathic pulmonary fibrosis. *Thorax*. 2014; 70:48–56. [PubMed: 25217476]
- Deshpande V, Zen Y, Chan JK, Yi EE, Sato Y, Yoshino T, Kloppel G, Heathcote JG, Khosroshahi A, Ferry JA, Aalberse RC, Bloch DB, Brugge WR, Bateman AC, Carruthers MN, Chari ST, Cheuk W, Cornell LD, Fernandez-Del Castillo C, Forcione DG, Hamilos DL, Kamisawa T, Kasashima S, Kawa S, Kawano M, Lauwers GY, Masaki Y, Nakanuma Y, Notohara K, Okazaki K, Ryu JK, Saeki T, Sahani DV, Smyrk TC, Stone JR, Takahira M, Webster GJ, Yamamoto M, Zamboni G, Umehara H, Stone JH. Consensus statement on the pathology of IgG4-related disease. *Mod Pathol*. 2012; 25:1181–1192. [PubMed: 22596100]
- Ehlers S, Benini J, Held HD, Roeck C, Alber G, Uhlig S. Alphabeta T cell receptor-positive cells and interferon-gamma, but not inducible nitric oxide synthase, are critical for granuloma necrosis in a mouse model of mycobacteria-induced pulmonary immunopathology. *J Exp Med*. 2001; 194:1847–1859. [PubMed: 11748285]
- Ezra DG, Krell J, Rose GE, Bailly M, Stebbing J, Castellano L. Transcriptome-level microarray expression profiling implicates IGF-1 and Wnt signalling dysregulation in the pathogenesis of thyroid-associated orbitopathy. *J Clin Pathol*. 2012; 65:608–613. [PubMed: 22554965]
- Fiel MI, Shukla D, Saraf N, Xu R, Shciano TD. Development of hepatic granulomas in patients receiving pegylated interferon therapy for recurrent hepatitis C virus post liver transplantation. *Transpl Infect Dis*. 2008; 10:184–189. [PubMed: 17916116]
- Hayden A, Wilson DJ, Rosenbaum JT. Management of orbital xanthogranuloma with methotrexate. *Br J Ophthalmol*. 2007; 91:434–436. [PubMed: 17135338]
- Jameson JL, Longo DL. Precision medicine--personalized, problematic, and promising. *N Engl J Med*. 2015; 372:2229–2234. [PubMed: 26014593]
- Jones RB, Tervaert JW, Hauser T, Luqmani R, Morgan MD, Peh CA, Savage CO, Segelmark M, Tesar V, van Paassen P, Walsh D, Walsh M, Westman K, Jayne DR. European Vasculitis Study G. Rituximab versus cyclophosphamide in ANCA-associated renal vasculitis. *N Engl J Med*. 2010; 363:211–220. [PubMed: 20647198]
- Joshi L, Lightman SL, Salama AD, Shirodkar AL, Pusey CD, Taylor SR. Rituximab in refractory ophthalmic Wegener's granulomatosis: PR3 titers may predict relapse, but repeat treatment can be effective. *Ophthalmology*. 2011; 118:2498–2503. [PubMed: 21907416]
- Kasperkovitz PV, Verbeet NL, Smeets TJ, van Rietschoten JG, Kraan MC, van der Pouw Kraan TC. Activation of the STAT1 pathway in rheumatoid arthritis. *Ann Rheum Dis*. 2004; 63:233–239. [PubMed: 14962955]
- Khosroshahi A, Bloch DB, Deshpande V, Stone JH. Rituximab therapy leads to rapid decline of serum IgG4 levels and prompt clinical improvement in IgG4-related systemic disease. *Arthritis Rheum*. 2010; 62:1755–1762. [PubMed: 20191576]
- Koizumi S, Kamisawa T, Kuruma S, Tabata T, Iwasaki S, Chiba K, Setoguchi K, Horiguchi S, Ozaki N. Clinical features of IgG4-related dacryoadenitis. *Graefes Arch Clin Exp Ophthalmol*. 2013; 252:491–497. [PubMed: 24318531]

- Kumar S, Leontovich A, Coenen MJ, Bahn RS. Gene expression profiling of orbital adipose tissue from patients with Graves' ophthalmopathy: a potential role for secreted frizzled-related protein-1 in orbital adipogenesis. *J Clin Endocrinol Metab.* 2005; 90:4730–4735. [PubMed: 15886250]
- Lantz M, Vondrichova T, Parikh H, Frenander C, Ridderstrale M, Asman P, Aberg M, Groop L, Hallengren B. Overexpression of immediate early genes in active Graves' ophthalmopathy. *J Clin Endocrinol Metab.* 2005; 90:4784–4791. [PubMed: 15928252]
- Lassner D, Kuhl U, Siegismund CS, Rohde M, Elezkurtaj S, Escher F, Tschöpe C, Gross UM, Poller W, Schultheiss HP. Improved diagnosis of idiopathic giant cell myocarditis and cardiac sarcoidosis by myocardial gene expression profiling. *Eur Heart J.* 2014; 35:2186–2195. [PubMed: 24667923]
- Lee JC, Lyons PA, McKinney EF, Sowerby JM, Carr EJ, Bredin F, Rickman HM, Ratlamwala H, Hatton A, Rayner TF, Parkes M, Smith KG. Gene expression profiling of CD8+ T cells predicts prognosis in patients with Crohn disease and ulcerative colitis. *J Clin Invest.* 2011; 121:4170–4179. [PubMed: 21946256]
- Lindfield D, Attfield K, McElvanney A. Systemic immunoglobulin G4 (IgG4) disease and idiopathic orbital inflammation; removing 'idiopathic' from the nomenclature? *Eye (Lond).* 2012; 26:623–629. [PubMed: 22302065]
- McKinney EF, Lyons PA, Carr EJ, Hollis JL, Jayne DR, Willcocks LC, Koukoulaki M, Brazma A, Jovanovic V, Kemeny DM, Pollard AJ, Macary PA, Chaudhry AN, Smith KG. A CD8+ T cell transcription signature predicts prognosis in autoimmune disease. *Nat Med.* 2010; 16:586–592. [PubMed: 20400961]
- Nagalakshmi U, Wang Z, Waern K, Shou C, Raha D, Gerstein M, Snyder M. The transcriptional landscape of the yeast genome defined by RNA sequencing. *Science.* 2008; 320:1344–1349. [PubMed: 18451266]
- O'Riordan TG, Smith V, Raghu G. Development of Novel Agents for Idiopathic Pulmonary Fibrosis: Progress in Target Selection and Clinical Trial Design. *Chest.* 2015; 148:1083–1092. [PubMed: 26020856]
- Petri M, Wallace DJ, Spindler A, Chindalore V, Kalunian K, Mysler E, Neuwelt CM, Robbie G, White WI, Higgs BW, Yao Y, Wang L, Ethgen D, Greth W. Sifalimumab, a human anti-interferon-alpha monoclonal antibody, in systemic lupus erythematosus: a phase I randomized, controlled, dose-escalation study. *Arthritis Rheum.* 2013; 65:1011–1021. [PubMed: 23400715]
- Planck T, Parikh H, Brorson H, Martensson T, Asman P, Groop L, Hallengren B, Lantz M. Gene expression in Graves' ophthalmopathy and arm lymphedema: similarities and differences. *Thyroid.* 2011; 21:663–674. [PubMed: 21510802]
- Rao JK, Weinberger M, Oddone EZ, Allen NB, Landsman P, Feussner JR. The role of antineutrophil cytoplasmic antibody (c-ANCA) testing in the diagnosis of Wegener granulomatosis. *Ann Intern Med.* 1995; 123:925–932. [PubMed: 7486487]
- Rispens T, Ooievaar-De Heer P, Vermeulen E, Schuurman J, van der Neut Kolfschoten M, Aalberse RC. Human IgG4 binds to IgG4 and conformationally altered IgG1 via Fc-Fc interactions. *J Immunol.* 2009; 182:4275–4281. [PubMed: 19299726]
- Rosenbaum JT, Choi D, Wilson DJ, Grossniklaus HE, Harrington CA, Dailey RA, Ng JD, Steele EA, Czyz CN, Foster JA, Tse D, Alabiad C, Dubovy S, Parekh P, Harris GJ, Kazim M, Patel P, White V, Dolman P, Edward DP, Alkatan H, Al Hussain H, Selva D, Yeatts P, Korn B, Kikkawa D, Stauffer P, Planck SR. Fibrosis, gene expression and orbital inflammatory disease. *Br J Ophthalmol.* 2015a; 99:1424–1429. [PubMed: 26038391]
- Rosenbaum JT, Choi D, Wilson DJ, Grossniklaus HE, Harrington CA, Sibley CH, Dailey RA, Ng JD, Steele EA, Czyz CN, Foster JA, Tse D, Alabiad C, Dubovy S, Parekh P, Harris GJ, Kazim M, Patel P, White V, Dolman P, Korn BS, Kikkawa D, Edward DP, Alkatan H, Al-Hussain H, Yeatts RP, Selva D, Stauffer P, Planck SR. Parallel Gene Expression Changes in Sarcoidosis Involving the Lacrimal Gland, Orbital Tissue, or Blood. *JAMA Ophthalmol.* 2015b; 133:770–777. [PubMed: 25880323]
- Rosenbaum JT, Choi D, Wilson DJ, Grossniklaus HE, Harrington CA, Sibley CH, Dailey RA, Ng JD, Steele EA, Czyz CN, Foster JA, Tse D, Alabiad C, Dubovy S, Parekh PK, Harris GJ, Kazim M, Patel PJ, White VA, Dolman PJ, Korn BS, Kikkawa DO, Edward DP, Alkatan HM, Al-Hussain H, Yeatts RP, Selva D, Stauffer P, Planck SR. Orbital pseudotumor can be a localized form of

- granulomatosis with polyangiitis as revealed by gene expression profiling. *Exp Mol Pathol*. 2015c; 99:271–278. [PubMed: 26163757]
- Rosenbaum JT, Choi D, Wilson DJ, Grossniklaus HE, Sibley CH, Harrington CA, Planck SR. Orbital Disease, C. Molecular diagnosis of orbital inflammatory disease. *Exp Mol Pathol*. 2015d; 98:225–229. [PubMed: 25595914]
- Rosenbaum JT, Choi D, Wong A, Wilson DJ, Grossniklaus HE, Harrington CA, Dailey RA, Ng JD, Steele EA, Czyz CN, Foster JA, Tse D, Alabiad C, Dubovy S, Parekh PK, Harris GJ, Kazim M, Patel PJ, White VA, Dolman PJ, Edward DP, Alkatan HM, Al Hussain H, Selva D, Yeatts RP, Korn BS, Kikkawa DO, Stauffer P, Planck SR. The Role of the Immune Response in the Pathogenesis of Thyroid Eye Disease: A Reassessment. *PLoS One*. 2015e; 10:e0137654. [PubMed: 26371757]
- Rosenbaum JT, Pasadhika S, Crouser ED, Choi D, Harrington CA, Lewis JA, Austin CR, Diebel TN, Vance EE, Brazier RM, Smith JR, Planck SR. Hypothesis: sarcoidosis is a STAT1-mediated disease. *Clin Immunol*. 2009; 132:174–183. [PubMed: 19464956]
- Salvi M, Vannucchi G, Beck-Peccoz P. Potential utility of rituximab for Graves' orbitopathy. *J Clin Endocrinol Metab*. 2013; 98:4291–4299. [PubMed: 24009135]
- Salvi M, Vannucchi G, Curro N, Campi I, Covelli D, Dazzi D, Simonetta S, Guastella C, Pignataro L, Avignone S, Beck-Peccoz P. Efficacy of B-cell targeted therapy with rituximab in patients with active moderate to severe Graves' orbitopathy: a randomized controlled study. *J Clin Endocrinol Metab*. 2015; 100:422–431. [PubMed: 25494967]
- Sellam J, Marion-Thore S, Dumont F, Jacques S, Garchon HJ, Rouanet S, Taoufik Y, Hendel-Chavez H, Sibilia J, Tebib J, Le Loet X, Combe B, Dougados M, Mariette X, Chiocchia G. Use of whole-blood transcriptomic profiling to highlight several pathophysiologic pathways associated with response to rituximab in patients with rheumatoid arthritis: data from a randomized, controlled, open-label trial. *Arthritis Rheumatol*. 2014; 66:2015–2025. [PubMed: 24756903]
- Sherman BT, Huang da W, Tan Q, Guo Y, Bour S, Liu D, Stephens R, Baseler MW, Lane HC, Lempicki RA. DAVID Knowledgebase: a gene-centered database integrating heterogeneous gene annotation resources to facilitate high-throughput gene functional analysis. *BMC Bioinformatics*. 2007; 8:426. [PubMed: 17980028]
- Stan MN, Garrity JA, Carranza Leon BG, Prabin T, Bradley EA, Bahn RS. Randomized controlled trial of rituximab in patients with Graves' orbitopathy. *J Clin Endocrinol Metab*. 2015; 100:432–441. [PubMed: 25343233]
- Stone JH, Merkel PA, Spiera R, Seo P, et al. Rituximab versus cyclophosphamide for ANCA-Associated vasculitis. *N Engl J Med*. 2010; 363:221–232. [PubMed: 20647199]
- Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES, Mesirov JP. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci USA*. 2005; 102:15545–15550. [PubMed: 16199517]
- Suhler EB, Lim LL, Beardsley RM, Giles TR, Pasadhika S, Lee ST, de Saint Sardos A, Butler NJ, Smith JR, Rosenbaum JT. Rituximab Therapy for Refractory Orbital Inflammation: Results of a Phase 1/2, Dose-Ranging, Randomized Clinical Trial. *JAMA Ophthalmol*. 2014; 132:572–578. [PubMed: 24652467]
- Tzou M, Gazeley DJ, Mason PJ. Retroperitoneal fibrosis. *Vasc Med*. 2014; 19:407–414. [PubMed: 25161213]
- van Gils W, Lodder EM, Mensink HW, Kilic E, Naus NC, Bruggenwirth H, van Ijcken W, Paridaens D, Luyten GP, DeKlein A. Expression profiling in uveal melanoma provides a strong marker for survival and reveals two regions on chromosome 3p related to prognosis. *Invest Ophthalmol Vis Sci*. 2008; 49:4254–4262. [PubMed: 18552379]
- van Vollenhoven RF, Fleischmann R, Cohen S, Lee EB, Garcia Mejjide JA, Wagner S, Forejtova S, Zwillich SH, Gruben D, Koncz T, Wallenstein GV, Krishnaswami S, Bradley JD, Wilkinson B, Investigators OS. Tofacitinib or adalimumab versus placebo in rheumatoid arthritis. *N Engl J Med*. 2012; 367:508–519. [PubMed: 22873531]
- Wakefield D, Di Girolamo N, Thureau S, Wildner G, McCluskey P. Scleritis: Immunopathogenesis and molecular basis for therapy. *Prog Retin Eye Res*. 2013; 35:44–62. [PubMed: 23454614]

- Wen T, Stucke EM, Grotjan TM, Kemme KA, Abonia JP, Putnam PE, Franciosi JP, Garza JM, Kaul A, King EC, Collins MH, Kushner JP, Rothenberg ME. Molecular diagnosis of eosinophilic esophagitis by gene expression profiling. *Gastroenterology*. 2013; 145:1289–1299. [PubMed: 23978633]
- Wong AJ, Planck SR, Choi D, Harrington CA, Troxell ML, Houghton DC, Stauffer P, Wilson DJ, Grossniklaus HE, Dailey RA, Ng JD, Steele EA, Harris GJ, Czyz C, Foster JA, White VA, Dolman PJ, Kazim M, Patel PJ, Edward DP, al Katan H, al Hussain H, Selva D, Yeatts RP, Korn BS, Kikkawa DO, Rosenbaum JT. IgG4 immunostaining and its implications in orbital inflammatory disease. *PLoS One*. 2014; 9:e109847. [PubMed: 25303270]
- Yamamoto-Ibusuki M, Arnedos M, Andre F. Targeted therapies for ER+/HER2– metastatic breast cancer. *BMC medicine*. 2015; 13:137. [PubMed: 26059247]
- Yeremenko N, Noordenbos T, Cantaert T, van Tok M, van de Sande M, Canete JD, Tak PP, Baeten D. Disease-specific and inflammation-independent stromal alterations in spondylarthritis synovitis. *Arthritis Rheum*. 2013; 65:174–185. [PubMed: 22972410]
- Yuen SJ, Ruin PA. Idiopathic orbital inflammation: distribution, clinical features, and treatment outcome. *Arch Ophthalmol*. 2003; 121:491–499. [PubMed: 12695246]

Article highlights

1. Molecular diagnosis can distinguish among causes of orbital inflammation which include thyroid eye disease, granulomatosis with polyangiitis, and sarcoidosis.
2. Molecular diagnosis confirms the heterogeneity of nonspecific orbital inflammation but suggests that a subset of these patients has disease which is indistinguishable from granulomatosis with polyangiitis.
3. Molecular analysis of tissue (often late in disease course) from patients with thyroid eye disease indicates that this tissue has fewer markers of inflammation compared to tissue from patients with sarcoidosis or granulomatosis with polyangiitis.

Figure 1.

Principal coordinate analysis based on the gene expression from subjects with T (thyroid eye disease), S (sarcoidosis), G (granulomatosis with polyangiitis), or C (controls). Panel A is the discovery set; Panel B is the validation set. Each diagnosis tends to cluster indicating similarity in gene expression. The subjects with thyroid eye disease closely resemble the controls who have no known orbital disease.

Figure 2.

Principal coordinate analysis similar to Figure 1 above except that subjects with nonspecific orbital inflammatory disease (N) are also included in panel A (discovery set) or panel B (validation set). The gene expression from tissue from subjects with NSOI is heterogeneous. A subset of subjects with NSOI have a pattern of gene expression that is very similar to the gene expression from subjects with GPA.

Figure 3.

Venn diagram comparing the gene expression from subjects with NSOI compared to those with GPA. A total of 53,798 probe sets were studied. In the discovery set 832 probe sets were differentially expressed comparing the two diagnoses. In the validation set, 45 probe sets were differentially expressed. However, none of these 45 probe sets were also detected in the discovery set as differentially expressed. Thus there were no consistent statistically significant differences between the two diagnoses.

Figure 4. Heat map comparing gene expression for selected immunoglobulin related genes for various orbital inflammatory diseases. Red tones indicate increased gene expression; blue tones indicate reduced gene expression. For these selected transcripts, subjects with thyroid eye disease closely resemble the healthy controls. Subjects with GPA closely resemble the subjects with sarcoidosis. Most of the NSOI subjects resemble subjects with GPA and sarcoidosis, although some subjects with NSOI have low expression of immunoglobulin related genes similar to the controls or subjects with thyroid eye disease.