Published in final edited form as:
Ann N Y Acad Sci. 2012 December ; 1275(1): 101–106. doi:10.1111/j.1749-6632.2012.06787.x.

Biomarker development for myasthenia gravis

Henry J. Kaminski[1,2], **Linda L. Kusner**[2], **Gil I. Wolfe**[3], **Inmaculada Aban**[4], **Greg Minisman**[4], **Robin Conwit**[5], and **Gary Cutter**[4]

[1]Department of Neurology, George Washington University, Washington, DC [2]Department of Pharmacology and Physiology', George Washington University, Washington, DC [3]Department of Neurology, University at Buffalo School of Medicine and Biomedical Sciences, The State University of New York, Buffalo, New York [4]Department of Biostatistics University of Alabama School of Public Health, Birmingham, Alabama [5]Division of Extramural Research, NIH/NINDS, Bethesda, Maryland

Abstract

Biomarkers are defined as characteristics (proteins, RNA, single nucleotide polymorphisms, imaging) that are objectively measured and evaluated as an indicator of pathogenic processes or pharmacologic responses to a therapeutic intervention. Biomarkers are important in clinical trials where the robust biomarker reflects the underlying disease process in a sensitive and reliable manner. For myasthenia gravis (MG), acetylcholine receptor and muscle specific kinase antibodies, as well as single fiber electromyography, serve as excellent biomarkers for diagnosis but do not adequately substitute for clinical evaluations to predict treatment response. New technologies are emerging that enable broad biomarker discovery in biological fluids. Biomarker evaluation is ideally done in the context of longitudinal clinical trials. The MGTX trial has collected plasma and serum for RNA and protein analysis and thymus, which will allow robust biomarker discovery. The ultimate goal will be to identify candidates for a reliable substitute for a clinically meaningful endpoint that is a direct measure of the effectiveness of a therapy in the context of a continuum of disease natural history and a patient's overall well-being.

Keywords

biomarkers; myasthenia gravis; surrogate endpoint; Prentice criteria

The Food and Drug Administration (FDA), National Institutes of Health, and the pharmaceutical/device maker industry have placed a major focus on the identification of biomarkers to assist therapeutic development in preclinical and early phase studies on humans[1, 2]. Why? Despite some remarkable success in discovery of novel treatments, therapeutic development has a high failure rate [3–5]. Numerous forces are now driving limitations on financial support for discovery of new treatments, whether scientists work in the private or public sector. Biomarkers in animal studies, which support efficacy in humans and ones that can robustly support go-no go decisions in preliminary clinical trials, offer promise to decrease the failure rate, shorten the duration, and thereby reduce the cost of therapeutic development.

Correspondence: Henry J. Kaminski, MD Department of Neurology George Washington University 2150 Pennsylvania Avenue Washington DC 20037 HKaminski@mfa.gwu.edu.

Conflicts of interest
The authors declare no conflicts of interest

Biomarker categorization

The FDA of the United States defines biomarkers as characteristics that are objectively measured and evaluated as indicators of normal biologic processes, pathogenic activity, or pharmacologic responses to a therapeutic intervention [6]. Biomarkers may be assessed by a variety of measures from biological specimens, such as molecular genetic characteristics, histology, and serum proteins as well as imaging evaluations. Several varieties of biomarkers exist (Table 1). For example, prognostic biomarkers sort patients according to the likely course of disease (if left untreated), while predictive biomarkers identify subpopulations of patients who are likely to respond to a specific therapy. The drug dosage for responsive individuals is optimized by analysis of pharmacodynamic biomarkers. Biomarkers may predict or identify safety problems related to a therapeutic candidate. In some circumstances, a biomarker may identify a patient population subgroup that becomes the focus for specific clinical trials. These include prognostic biomarkers that identify patients with a disease risk most suitable for an efficient drug development program. In other circumstances, a predictive biomarker may identify a patient subgroup that has a greater potential to benefit from the mechanism of action of the specific drug or a lower risk of an identified adverse effect of the drug. As with any measure, there are variability and specificity issues that must be considered for each specific application. The rigor of the validation process for a biomarker is dependent on its ultimate clinical use.

The surrogate endpoint is of greatest interest for therapeutic development, and their validation as a predictor of efficacy requires fulfillment of strict criteria. The surrogate endpoint is intended to substitute for a primary clinical endpoint and is expected to predict a clinical benefit, lack of benefit, or harm as would the gold standard clinical endpoint. Prentice originally proposed criteria to define expectations for a surrogate (Table 2). To act as a surrogate endpoint a biomarker must fulfill these properties, which are ideally assessed in the context of a clinical trial [7]. Of course, this also assumes that clinical efficacy evaluations have been validated appropriately, which is also a challenge. Only in the case of death as a clinical end point can one consider the clinical measures to be unequivocal.

Biomarkers in myasthenia gravis

The MG research field lags behind other areas of medicine in the development of biomarkers, and existing biomarkers are severely limited in their ability to predict a response to treatment, assess susceptibility to adverse effects of treatment, or correlate with disease severity. This lack of validated biomarkers is a glaring deficiency for therapeutic development for MG, especially when novel treatments are being considered for application. This state is all the more surprising because MG is one of the best characterized autoimmune disorders from a biological perspective and is among the few that fulfills strict criteria for autoimmunity.

Biomarkers that presently exist in MG fall primarily in the diagnostic category. Detection of acetylcholine receptor (AChR) or muscle specific kinase (MuSK) antibodies is highly specific for confirming the diagnosis of MG; however, their absolute levels do not correlate with disease severity [8–10]. Although levels of AChR and MuSK antibodies tend to fall with treatment, this is highly variable and does not correlate well with disease severity or clinical response. The therapeutic usefulness of biomarkers in guiding treatment decisions is illustrated by the identification of MuSK antibodies, which correlate with poor response to cholinesterase inhibitor treatment and often predict refractoriness to other treatment approaches. However, such observations have been drawn from retrospective analyses.

The MG Foundation of America Task Force for evaluation of clinical endpoints, of which three of the authors (HK,GW,GC) were members, reviewed potential biomarkers used in

MG. None fulfill criteria sufficiently to serve as surrogate endpoints. Acetylcholine receptor (AChR) antibody titers have been used as a marker for therapeutic response [11–13], but there is no basis to use them as a potential substitute for a clinical outcome measure. Single fiber EMG, in expert hands, appears to correlate well to clinical state; however, it is of limited use as a predictor of of clinical outcome, especially due to the likelihood of significant inter-observer variability[14]. Of relevance to MG treatment is the thiopurine *S*-methyltransferase activity or identification of the TPMT gene mutation as a marker of toxicity for azathioprine use.[15]

Genetic markers may one day serve as biomarkers important for therapeutic targeting. A genetic association for MG is supported [16] by: 1) MG occurrence in up to 4% of family members of patients with MG, while the risk of generalized MG in the population is 0.01%; 2) twin studies showing a heritability index of 0.65, a level that places MG in the range of Alzheimer's disease and epilepsy and above multiple sclerosis for genetic predisposition; 3) HLA-B8 and DR3 alleles are increased in patients with MG when compared with the general population; 4) The MYSA1 locus is associated with MG and thymic hyperplasia. MYSA1 lies within the central region of the HLA region and the biological basis for it leading to susceptibility to MG has not been determined; 5) linkage dysequilibrium analysis identified an association with a marker in the CHRNA1 gene, which codes for the alpha subunit of the ACHR, that has been closely associated with MG; 6) and a polymorphism identified in the promoter region of the decay accelerating factor (DAF) gene, a complement regulator associated with a severe form of MG that produces irreversible ophthalmoparesis[17].

Preclinical evaluation

Development of therapeutics is dependent on exploratory evaluations in animals, and the field of MG benefits greatly from the existence of robust animal models. Patrick and Lindstrom demonstrated that immunization of rabbits with purified acetylcholine receptor leads to development of disease that mimics the human disorder [18]. Subsequent studies have demonstrated that experimental autoimmune MG (EAMG) can be induced in several mammalian species by immunization with AChR of mammals, the electric organ of eels or rays, and peptide fragments of AChR subunits [19]. These models reproduce aspects of human MG, including a breakdown in tolerance with production of autoantibodies, the neuromuscular transmission defect, response to anti-cholinesterase therapy, and moderation of the disease by treatments used in patients. EAMG produced by administration of antibodies to the acetylcholine receptor fails to mimic the breakdown in tolerance and induces significant inflammation, which is not present in muscles of patient, but does mimic the final common pathway of injury to the neuromuscular junction that occurs in humans, and therefore, also serves as an appropriate model to evaluate certain therapeutics. These models have been used to delineate autoimmune mechanisms and to evaluate therapeutics [20, 21] but to date have not been a focus for biomarker discovery.

Because of inherent differences between humans and animals, no animal model, thus far, fully mimics the human disease. Despite common features of mammalian immune systems, there are differences in basic regulatory proteins of human, mouse and rat systems. EAMG differs from human MG in the need to administer exogenous and repeated antigenic stimulation to produce and maintain disease. Fluctuations of weakness and autoimmune activity are not observed over time. Therefore, caution is necessary when extrapolating positive results from animals to humans. This is not just a problem for MG. A glaring example is over 300 preclinical studies of Alzheimer's disease suggesting efficacy of therapy in mouse models, none of which have led to a therapeutic effect in humans [22]. Therefore, there is a great need for biomarker discovery to be integrated coherently into both preclinical

and clinical efforts. The long term benefit that would result from validated markers of efficacy in humans would be enormous. And if these discoveries have parallels in animal studies the potential to translate novel treatments from preclinical studies to the bedside would be far more efficient. Of course, there is also benefit in the early termination of preclinical efforts that have little chance for success in order to save the hundreds of millions of dollars that are expended on human early phase trials.

MGTX study and biomarker discovery opportunity

In 2005 the National Institutes of Health funded a multicenter, international, single-blinded, randomized trial (MGTX) to determine whether extended transsternal thymectomy for patients receiving the prednisone protocol confers added benefits to the prednisone protocol alone [23, 24]. As part of the investigation, an ancillary study was supported with the intent to collect thymus, plasma, serum and blood-derived RNA with the intent of developing the first biomarker discovery assessment in the history of MG. As of this writing, 82 subjects have provided blood specimens at baseline and 6-month follow-up with collection ongoing at 1, 2, and 3 years post randomization. Over 50 thymic specimens have been collected, and analysis for histological evaluation has demonstrated good to excellent quality of specimen integrity. The opportunity exists now to launch a state-of-the-art, unbiased biomarker discovery program utilizing these specimens. To that end, the authors are moving toward the use of independent -omics assays (proteomics, microRNA, RNA profiling, metabolomics) coupled with antigen-specific IgG subclass definition on samples obtained through MGTX and to perform similar analyses on samples obtained from mice with EAMG. Below we briefly describe the methods to be used.

Nanoparticle proteomics

Body fluids such as serum represent a valuable source of biomarker information. Identification and monitoring of circulating biomarkers enable early disease detection, disease/morbidity risk stratification, and help assess disease progression and thus responsiveness to interventions [25]. Despite recent progress in the field of proteomics, identification of novel plasma biomarkers such as proteins has been difficult due to low quantities relative to larger and more abundant plasma proteins, such as albumin [26]. Nanoparticle proteomic technology will allow identification and quantitation of less abundant proteins within patient samples.

MicroRNA profiling

Micro RNA (miRNA) has emerged as a new and important class of cellular regulators. Experimental studies have provided strong evidence that aberrant expression of miRNA is associated with a broad spectrum of human diseases, including cancer, diabetes, and cardiovascular and psychological disorders. The relatively small numbers of miRNAs discovered in humans (~800 miRNAs, miRBase12.0) are involved in regulation of a large number of human genes (up to 80% of known genes). miRNAs have exceptional potential as biomarkers because of their relative abundance, highly specific expression, and stable presence in serum and plasma. In fact, circulating miRNAs demonstrate reasonable sensitivity in a small number of Duchenne muscular dystrophy patients as biomarkers for disease progression and severity and also correlate with circulating miRNA in mice with dystrophin deficiency [27].

Metabolomics profiling

Metabolomics is a rapidly developing field that aims to identify and quantify the concentration changes of all the metabolites (i.e., the metabolome) in a given biofluid from a subject and support targeting and developing therapeutics [28]. The anticipated contribution of

metabolomics to the field of science and to health care is highlighted by its presence in the current NIH Roadmap. The application of metabolomics to understand the manifestation and progression of complex neurological diseases represents a powerful means to identify the earliest markers associated with disease progression and treatment response.

MG-focused assessments

The final effector mechanism in most patients withMG is the AChR antibody [29]. A fundamental challenge for the MG field is that the level of the autoantibody does not correlate with disease severity. Numerous investigations have demonstrated that there is a significant role of complement as a driver of disease pathology in experimental animals and humans with MG. In concert with the broad-based assessments, we will specifically evaluate IgG subclasses, that are specific to the human AChR autoantigen. It is also well appreciated that cytokines regulate the cell responses of the immune system; multiplex cytometric bead assays can be used to measure levels of serum cytokines [30] to determine the treatment effect on these biomarkers, although these may be too volatile to be used as effective biomarkers.

Identification is not enough

Identifying putative biomarkers is a major endeavor in all diseases in the era of personalized medicine, but the challenge in establishing a biomarker should not be underestimated. The Prentice criteria are theoretical and have not been achieved even where biomarkers are felt to exist (e.g., blood pressure for cardiovascular disease or CD4 counts for HIV). Although the path to validation of a biomarker is long and arduous, the payoff is enormous for patients, the field of research, and is now a reasonable undertaking with the tools that are at hand.

Conclusion

The time has come for further breakthroughs in treatment of MG. The only path forward lies in exploiting approaches that the field of cancer therapeutics are beginning to leverage. These include rigorously evaluated clinical endpoints and identification of biomarkers for subcategorization of neoplasms on a molecular level indicative of potential therapeutic targets in clinical trials. One such success story is that of chronic myelogenous leukemia which began with discovery of the Philadelphia chromosome and finally led to targeting an antibody to inhibit a tyrosine kinase [31]. The drug developed is Gleevec, a highly effective treatment for this subset of leukemia patients. MG faces particular challenges in that universally accepted clinical endpoints have only recently been rigorously defined [32, 33] and research consortiums to perform robust clinical trials are only a decade old. The field also faces the challenge of a lack of investigators trained in biomarker discovery. However, it is expected the MGTX supported biological specimen bank will offer many investigators a unique opportunity to move the field forward for the benefit of generations of patients to come.

Acknowledgments

This work was supported by NINDS Grant U01 NS042685.

References

1. Butterfield LH, Palucka AK, Britten CM, et al. Recommendations from the iSBTc-SITC/FDA/NCI Workshop on Immunotherapy Biomarkers. Clin Cancer Res. 2011; 17:3064–3076. [PubMed: 21558394]

2. [Sept 4, 2012] http://www.biomarkersconsortium.org.

3. Mendell JR, Csimma C, McDonald CM, et al. Challenges in drug development for muscle disease: a stakeholders' meeting. Muscle Nerve. 2007; 35:8–16. [PubMed: 17068768]
4. Kola I. The State of Innovation in Drug Development. Clinical pharmacology and therapeutics. 2008; 83:227–231. [PubMed: 18202690]
5. Paul SM, Mytelka DS, Dunwiddie CT, et al. How to improve R&D productivity: the pharmaceutical industry's grand challenge. Nature reviews Drug discovery. 2010; 9:203–214.
6. US Food and Drug Administration. [September 4, 2012] http://www.fda.gov/ScienceResearch/SpecialTopics/CriticalPathInitiative/ucm076689.htm
7. Prentice RL. Surrogate endpoints in clinical trials: definition and operational criteria. Stat Med. 1989; 8:431–440. [PubMed: 2727467]
8. Howard FJ, Lennon V, Finley J, et al. Clinical correlations of antibodies that bind, block, or modulate human acetylcholine receptors in myasthenia gravis. Ann NY Acad Sci. 1987; 505:526–538. [PubMed: 3479935]
9. Lennon V. Serologic profile of myasthenia gravis and distinction from Lambert-Eaton myasthenic syndrome. Neurology. 1997; 48(Suppl 5):S23–27.
10. Guptill JT, Sanders DB. Update on muscle-specific tyrosine kinase antibody positive myasthenia gravis. Curr Opin Neurol. 2010; 23:530–535. [PubMed: 20613516]
11. Palace J, Newsom-Davis J, Lecky B. A randomized double-blind trial of prednisolone alone or with azathioprine in myasthenia gravis. Myasthenia Gravis Study Group. Neurology. 1998; 50:1778–1783. [PubMed: 9633727]
12. Muscle Study G. A trial of mycophenolate mofetil with prednisone as initial immunotherapy in myasthenia gravis. Neurology. 2008; 71:394–399. [PubMed: 18434639]
13. Sanders DB, Hart IK, Mantegazza R, et al. An international, phase III, randomized trial of mycophenolate mofetil in myasthenia gravis. Neurology. 2008; 71:400–406. [PubMed: 18434638]
14. Sanders DB. Electrophysiologic tests of neuromuscular transmission. Suppl Clin Neurophysiol. 2004; 57:167–169. [PubMed: 16106617]
15. Colleoni L, Kapetis D, Maggi L, et al. A New Thiopurine S-Methyltransferase Haplotype Associated With Intolerance to Azathioprine. J Clini Pharmacol. 2012 (in press).
16. Lanford, JW.; Phillips, LH. Epidemiology and Genetics of Myasthenia Gravis. Kaminski, HJ., editor. Springer; New York: 2009. p. 71-78.
17. Heckmann JM, Uwimpuhwe H, Ballo R, et al. A functional SNP in the regulatory region of the decay-accelerating factor gene associates with extraocular muscle pareses in myasthenia gravis. Genes Immun. 2010; 11:1–10. [PubMed: 19675582]
18. Patrick J, Lindstrom J. Autoimmune response to acetylcholine receptor. Science. 1973; 180:871–872. [PubMed: 4706680]
19. Christadoss P, Poussin M, Deng C. Animal models of myasthenia gravis. Clin Immunol. 2000; 94:75–87. [PubMed: 10637092]
20. Vincent A. Autoimmune channelopathies: well-established and emerging immunotherapy-responsive diseases of the peripheral and central nervous systems. J Clin Immunol. 2010; 30(Suppl 1):S97–102. [PubMed: 20387102]
21. Conti-Fine BM, Milani M, Kaminski HJ. Myasthenia gravis: past, present, and future. J Clin Invest. 2006; 116:2843–2854. [PubMed: 17080188]
22. Shineman DW, Basi GS, Bizon JL, et al. Accelerating drug discovery for Alzheimer's disease: best practices for preclinical animal studies. Alzheimer's research & therapy. 2011; 3:28.
23. Minisman G, Bhanushali M, Conwit R, et al. Implementing clinical trials on an international platform: challenges and perspectives. J Neurol Sci. 2012; 313:1–6. [PubMed: 22047648]
24. Newsom-Davis J, Cutter G, Wolfe GI, et al. Status of the thymectomy trial for nonthymomatous myasthenia gravis patients receiving prednisone. Ann N Y Acad Sci. 2008; 1132:344–347. [PubMed: 18567886]
25. Hanash S. Disease proteomics. Nature. 2003; 422:226–232. [PubMed: 12634796]
26. Longo C, Patanarut A, George T, et al. Core-shell hydrogel particles harvest, concentrate and preserve labile low abundance biomarkers. PLoS One. 2009; 4:e4763. [PubMed: 19274087]

27. Cacchiarelli D, Legnini I, Martone J, et al. miRNAs as serum biomarkers for Duchenne muscular dystrophy. EMBO molecular medicine. 2011; 3:258–265. [PubMed: 21425469]
28. Kaddurah-Daouk R, Kristal BS, Weinshilboum RM. Metabolomics: a global biochemical approach to drug response and disease. Annu Rev Pharmacol Toxicol. 2008; 48:653–683. [PubMed: 18184107]
29. Kusner LL, Kaminski HJ, Soltys J. Effect of complement and its regulation on myasthenia gravis pathogenesis. Expert review of clinical immunology. 2008; 4:43–52. [PubMed: 20477586]
30. Tuzun E, Huda R, Christadoss P. Complement and cytokine based therapeutic strategies in myasthenia gravis. J Autoimmun. 2011; 37:136–143. [PubMed: 21636248]
31. Capdeville R, Buchdunger E, Zimmermann J, et al. Glivec (STI571, imatinib), a rationally developed, targeted anticancer drug. Nature reviews Drug discovery. 2002; 1:493–502.
32. Benatar M, Sanders DB, Burns TM, et al. Recommendations for myasthenia gravis clinical trials. Muscle Nerve. 2012; 45:909–917. [PubMed: 22581550]
33. Jaretzki A 3rd, Barohn RJ, Ernstoff RM, et al. Myasthenia gravis: recommendations for clinical research standards. Task Force of the Medical Scientific Advisory Board of the Myasthenia Gravis Foundation of America. Neurology. 2000; 55:16–23. [PubMed: 10891897]

Table 1

Categorization of biomarkers

Biomarker	Example	Disease
Diagnostic	Elevated fasting blood sugar Acetylcholine receptor and muscle specific antibody	Diabetes mellitus Myasthenia gravis
Disease Extent/severity	Lesion burden on magnetic resonance imaging Tumor size	Multiple sclerosis Various neoplasms
Pharmacodynamic, marker	Serum cyclo-oxygenase (COX)-2 inhibition	Pain relief
Prognostic marker	Estrogen receptor status	Breast cancer
Predictive marker	Serum cholesterol Blood pressure	Cardio- and cerebrovascular disease
Drug characterization	Complement inhibition (by drug eculizumab)	Paroxysmal hemaglobinuria

Table 2

Prentice Criteria for Surrogate Endpoint Validation

Treatment must have an effect on the surrogate
Treatment must have an effect on the clinical outcome
Surrogate and the clinical outcome must be correlated
Treatment effect on the true clinical outcome must disappear when adjusting for the surrogate