Neuropsychopharmacology (2011) 36, 2375–2394

www.neuropsychopharmacology.org

Review

Functional Biomarkers of Depression: Diagnosis, Treatment, and Pathophysiology

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Major depressive disorder (MDD) is a heterogeneous illness for which there are currently no effective methods to objectively assess severity, endophenotypes, or response to treatment. Increasing evidence suggests that circulating levels of peripheral/serum growth factors and cytokines are altered in patients with MDD, and that antidepressant treatments reverse or normalize these effects. Furthermore, there is a large body of literature demonstrating that MDD is associated with changes in endocrine and metabolic factors. Here we provide a brief overview of the evidence that peripheral growth factors, pro-inflammatory cytokines, endocrine factors, and metabolic markers contribute to the pathophysiology of MDD and antidepressant response. Recent preclinical studies demonstrating that peripheral growth factors and cytokines influence brain function and behavior are also discussed along with their implications for diagnosing and treating patients with MDD. Together, these studies highlight the need to develop a biomarker panel for depression that aims to profile diverse peripheral factors that together provide a biological signature of MDD subtypes as well as treatment response. *Neuropsychopharmacology* (2011) **36,** 2375–2394; doi:10.1038/npp.2011.151; published online 3 August 2011

Keywords: antidepressant; BDNF; IGF-1; IL-1β; serum; mood disorders

INTRODUCTION

Major depressive disorder (MDD) is a prevalent, heterogeneous illness characterized by depressed mood, anhedonia, and altered cognitive function. The lifetime prevalence of MDD is approximately 17% of the population and results in tremendous secondary costs to society (Kessler *et al*, 2005; Wang *et al*, 2003). Diagnosis and treatment of MDD is based on relatively subjective assessments of diverse symptoms representing multiple endophenotypes. To date, the biological bases for the heterogeneity of MDD remain poorly defined. Toward this goal, identification of biological markers could improve the diagnosis and classification of MDD subtypes, as well as stratify patients into more homogeneous, clinically distinct subpopulations. Despite decades of searching, a non-invasive, quantitative clinical test to aid in the diagnosis and treatment of MDD remains elusive (Lakhan *et al*, 2010).

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Received 18 April 2011; revised 13 June 2011; accepted 5 July 2011

However, recent studies of MDD provide renewed hope. While there is no clear single biomarker, there is mounting evidence of multiple dysregulated contributing factors, including growth factors and/or pro-inflammatory cytokines (Castren and Rantamaki, 2010; Krishnan and Nestler, 2008; Miller *et al*, 2009; Schmidt and Duman, 2007). In addition, there is a long history and clear evidence for altered endocrine factors (eg, hypothalamic–pituitary–adrenal (HPA), thyroid, sex steroids) and metabolic dysregulation (eg, insulin resistance) in mood disorders (Hendrickx *et al*, 2005). Thus, a viable alternative to the single-biomarker approach could be the development of biomarker panels that aim to profile a diverse array of peripheral/serum growth factors, cytokines, hormones, and metabolic markers, to provide coverage of multiple biological abnormalities that contribute to the heterogeneity of MDD, as well as treatment response. This endeavor will require a large number of patient samples to define severity, subgroups, and response, but analytical tools are currently available to make biomarker assessment possible. In this review, we provide a brief overview of the key growth factors, cytokines, hormones, and metabolic markers that could be included in an initial multi-analyte biomarker panel of MDD.

Providing further support for this approach are recent preclinical studies demonstrating that serum growth factors and cytokines can influence brain function, including cellular and behavioral responses. These findings indicate that analyses of these factors will not only serve a biomarker function, but will also provide information about the underlying neurobiology of MDD subtypes, which will allow improved diagnosis and individualized treatments.

WHAT IS A BIOMARKER?

The term biomarker can be used in a variety of ways. The most common biomarker concepts include specific features of an individual that are useful in distinguishing the presence or absence of a disease state ('diagnostic biomarkers'), or that predict treatment response ('treatment biomarkers'). With regard to treatment biomarkers, the biomarker may either be present at baseline and predict response to treatment, or, alternatively, may change in the short term in such a way as to predict the ultimate response. In the latter case, the biomarker would be measured at baseline and again early in the course of therapy; ideally, lack of change in the marker would lead to alteration of the course of treatment. In general, biomarkers are measurable features of an individual that represent indicators of a disease state or outcome with treatment. Moreover, biomarkers are typically thought of as a biological feature (eg, genome variation, plasma concentration of a protein, etc), but do not have to be limited in this manner (Perlis, 2011).

Most biomarkers are discovered initially in a type of retrospective analysis of existing data sets. This, for example, was how a variety of gene variants were found to be associated with antidepressant treatment outcome in the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) study (Laje *et al*, 2009). In this case, as in others, the specific genetic variants were assayed in a *post-hoc* manner, demonstrating some degree of factor loading with response. However, alternative prospective designs can be employed by using a type of enrichment strategy. In an enriched design, biomarkers may be used to select people into a clinical trial to maximize response to a given intervention, particularly enhancing drug–placebo differences. Biomarker designs, then, may be used to minimize sample size to test for a therapeutic effect. A similar strategy is the 'biomarker stratified design,' in which there is a randomization in order to balance the distribution of a particular marker (Perlis, 2011). This approach can be used to actually test for the differential usefulness of a biomarker in predicting differential responsiveness to a treatment.

In the case of treatment response, analysis of biomarkers represents a variation of mediator and moderator analyses as proposed by Baron and Kenny (1986). As elaborated by Kraemer *et al* (2002b), treatment moderators are factors that 'specify for whom or under what conditions the treatment works … They also suggest to clinicians which of their patients might be most responsive to the treatment and for which patients other, more appropriate, treatments might be sought.' Treatment biomarkers can serve as a special case of a biomarker that 'labels' the likelihood of responding to a given treatment. A positive moderator, then, indicates the selection of a particular treatment and a negative moderator suggests choosing an alternative. A prescriptive moderator would favor one treatment against another. Again, as stated by Kraemer *et al* (2002b), 'moderators may also provide unique new and valuable information to guide future restructuring of diagnostic classification and treatment decision making.'

A number of pharmacogenomic studies have evaluated the moderating effect of specific genetic variation on response to antidepressant therapies. For example, as summarized recently by Lin and Chen (2008), the STAR*D study found single-nucleotide polymorphisms (SNPs) in several genes associated with response or adverse effects with the SSRI antidepressant citalopram, subsequent antidepressants, or combinations of treatments. These included FK506-binding protein-5 (*FKBP5*), glutamate receptor ionotropic kainate-1 (*GRIK1*) and 4 (*GRIK4*), n-methyl-d-aspartate receptor-2A (*GRIN2A*), 5-hydroxytryptamine receptor-2A (*HTR2A*), potassium channel subfamily-K member-2 (*KCNK2*) (six SNPs), and the serotonin transporter (*SLC6A4*) long/short variants. Several genes were also associated with treatment-emergent suicidality, including, cyclic-AMP response element-binding protein-1 (*CREB1*), glutamate receptor ionotropic AMPA-3 (*GRIA3*), and *GRIK2*.

Other biological factors have been shown to be associated with lesser response to antidepressant therapy (Papakostas and Fava, 2008). For example, greater number or size of white matter hyper-intensities on structural magnetic resonance imaging brain scans (presumably indicating small vessel disease) are associated with reduced response to antidepressants (Alexopoulos *et al*, 2008; Iosifescu *et al*, 2006; Papakostas *et al*, 2005). Higher baseline levels of anxiety and overall depression severity have been shown to be predictors of poorer response to antidepressant therapy (Papakostas and Fava, 2008).

Fournier *et al* (2009) recently reported examples of clinical prescriptive moderators of antidepressant response *vs* cognitive behavioral psychotherapy (CBT) treatment of depression. In the original study, CBT and the antidepressant paroxetine were equally helpful and more effective than placebo in a large MDD sample (DeRubeis *et al*, 2005). However, a secondary moderator analysis found that chronic depression, older age, and lower intelligence each predicted relatively poor response across both treatments. Three prescriptive variables, being married, unemployment, and having experienced a greater number of recent stressful life events, were identified and each predicted superior response to cognitive therapy relative to antidepressant medications.

A mediator, on the other hand, is a factor that changes along with response to a particular intervention (Kraemer *et al*, 2002b). In many cases, a mediator is a fundamental causal mechanism by which a particular treatment produces a change, but this does not have to be the case. There can be special cases of secondary effects of treatments that are affected by the treatment and are associated with response to treatment but may not be the actual causal mechanism that produces improvement on an illness measure. However, ideally, the mediator variable will change in such a way as to indicate subsequent improvement before the change in

the underlying disease state is manifest. In addition, the absence of early change in the mediator should predict lack of subsequent improvement in the disease-specific variable (eg, symptom measure). Therefore, the mediator variable is most useful as an early marker of subsequent improvement in the disease state, and, therefore, would be a practical guide to treatment prediction. A lack of early change in the mediator variable would indicate the need to change the treatment.

The personality trait of neuroticism has been examined as a mediator variable for response to SSRI treatment (Quilty *et al*, 2008). Neuroticism is considered a personality trait (thought by some as an endophenotype), which is characterized by a tendency to experience negative emotions such as anxiety, sadness, embarrassment, anger, guilt, or disgust in face of perceived or anticipated stressors. Therefore, neuroticism is a vulnerability factor to both anxiety and depressive disorders. Quilty *et al* (2008) evaluated two models of the relationship between neuroticism and response to antidepressant therapy, a 'mediation' model (ie, SSRI → Neuroticism Change → Depression Change) and a 'complication' model (ie, SSRI → Depression Change → Neuroticism Change), by using a maximum likelihood of estimation approach. The 'mediation' model best fit the SSRI response data, indicating that overall neuroticism change is associated with change in depression severity.

In the case of both moderators and mediators of treatment effects, the slope of change in the underlying disease state is predicted by the baseline level of the marker in the case of moderators or the rate of change in the marker in the case of mediators (Kraemer *et al*, 2002b). Mediators can serve as moderators and vice versa, but this does not have to be the case.

Diagnostic biomarkers represent a different type of moderator analysis. In this case, the presence of a marker indicates a higher likelihood of an underlying disease state and may be present before a disease is actually present (indicating a 'risk' or 'vulnerability' marker). As with treatment biomarkers, a diagnostic marker may or may not be directly related to the underlying causal mechanism for the condition. However, putative biomarkers must be distinguished from associated features of a particular disease. For example, as noted below in the case of inflammatory factors such as elevations in cytokines, a difference of a particular marker in a disease population in contrast to an unaffected group could be the result of an associated condition. In the case of inflammatory biomarkers, the presence of an elevated cytokine may be a marker for depression, but it also could be associated with other conditions such as obesity, Type-2 diabetes, or cardiovascular disease that are commonly associated with depression (Shelton and Miller, 2010). Therefore, matching of affected and unaffected groups should take co-varying features into consideration.

Another inherent limitation of biomarker identification has to do with the diagnostic accuracy of the typical clinical procedure used for identifying the disease state. In some cases, such as the prostate-specific antigen (PSA) test for prostate cancer, the disease itself can be identified with high accuracy by prostate biopsy (Balk *et al*, 2003) (although, notably, even in this instance, the benefit of the PSA test for identifying prostate cancer has been called into question (Andriole *et al*, 2009)). However, a test is unlikely to be better than whatever method was used to identify the population at risk; in the case of psychiatric disorders, identification of the affected state is usually through a diagnostic interview. Hence, in psychiatry, any biomarker is not likely to be better at identifying the condition than the clinical interview used to diagnose people in a study. Therefore, a diagnostic biomarker test is predominantly useful in situations in which an extensive clinical interview is not feasible, such as in large-scale screenings. Alternatively, a biomarker discovered to be useful in accurately distinguishing affected and unaffected people might be present in the case of people not yet affected and be a risk marker for the disease state before it is actually present.

Although some biomarkers are truly dichotomous, as in the case of the presence of a specific SNP or the repeat region of a gene, many others are actually continuous variables (eg, the plasma concentration of a specific protein). In this case, a criterion cutoff will have to eventually be specified (Kraemer *et al*, 2002a). This process is consistent with a receiver operating characteristic (ROC) analysis, in which different criterion levels can be used to create binary outcomes (eg, presence *vs* absence of a condition or response *vs* non-response to a treatment) based on a classifier—in this case, a biomarker (Kraemer *et al*, 2002a). The ROC expresses the sensitivity (ie, true positive rate *vs* the rate of false positives) and the specificity (true negatives *vs* false negatives) of a specific criterion level. Ideally, the criterion level will reflect a high degree of sensitivity and specificity of the classification threshold, again with classification indicating either the presence (*vs* absence) of the condition or the response (*vs* non-response) to a treatment.

For typical psychiatric diagnoses such as major depression or schizophrenia, the likelihood of any given biomarker achieving a high enough degree of sensitivity and specificity—that is, an ideal ROC curve—to make the biomarker clinically useful is relatively low. We propose that the use of multiple biomarkers may provide a possible solution to this problem. Although individual biomarkers may provide some greater level of true *vs* false positive and negatives, the predictive abilities may improve when several different biomarkers are aggregated into a group, or biopanel, of predictor characteristics. Rather than depending on a high level of predictive power of an individual marker, the biopanel approach would depend on an aggregate score or predictive algorithm for classification. Individual items could then be added or subtracted to identify the best-performing set of predictor characteristics. In addition, the assessment of a panel of markers could potentially aid in the subdivision of a heterogeneous illness that presents with a similar phenotype in a clinical interview. It is possible that individual biomarkers will aggregate in ways to inform the parsing of the MDD phenotype into subtypes that may relate more closely to specific etiological pathways. Inflammatory cytokines and related factors, discussed in greater detail below, appear to more consistently aggregate in individual patients but not in others. This type of clustering is likely to reflect something more closely related to an etiology of a subset of MDD. This, in turn, could lead to more effective, etiologically based therapies for subgroups of patients.

We will review a proposed set of biomarkers that should be considered for inclusion in future biomarker studies, with a focus on growth factors, cytokines, and metabolic factors.

GROWTH FACTORS

A large body of evidence indicates that stress impairs trophic support whereas antidepressants function, in part, to enhance trophic factor expression and neuroplasticity (Schmidt *et al*, 2008; Schmidt and Duman, 2007). Clinical studies demonstrate that patients with MDD have altered blood/serum levels of growth factors. Consistent with these results, increasing evidence indicates that chronic stress exposure, which can precipitate or exacerbate depressive episodes, alters the expression of growth factors, and that antidepressant treatment produces opposing effects. The following sections will discuss several of these key growth factors, and will focus on (1) preclinical studies of stress and antidepressant regulation, and (2) clinical studies of blood of MDD patients. Evidence that peripheral administration of these factors influences neuronal plasticity and behavior will also be discussed.

BRAIN-DERIVED NEUROTROPHIC FACTOR

Brain-derived neurotrophic factor (BDNF) regulates synaptic plasticity in neuronal networks involved in depressive behaviors (Pittenger and Duman, 2007; Schinder and Poo, 2000). Regulation of BDNF may reverse stress-induced deficits in structural and synaptic plasticity in the adult brain, resulting in cognitive flexibility and, subsequently, an increased ability to adapt/cope with environmental challenges that may precipitate or exacerbate depressive episodes. Recent studies demonstrate that BDNF levels are decreased in the blood of MDD patients and reversed with antidepressant treatment (Brunoni *et al*, 2008b; Sen *et al*, 2008).

Influence of Stress and Antidepressants on BDNF

Exposure to physical or psychological stressors leads to rapid downregulation of BDNF expression in the hippocampus, which could contribute to experience-dependent modifications in neural networks that contribute to the pathogenesis of MDD (Nibuya *et al*, 1995, 1999; Rasmusson *et al*, 2002; Russo-Neustadt *et al*, 2001; Smith *et al*, 1995b). By contrast, chronic antidepressant administration increases BDNF expression in the hippocampus (Altar *et al*, 2004; Newton *et al*, 2003; Nibuya *et al*, 1995; Russo-Neustadt *et al*, 1999). Furthermore, recent studies have demonstrated that BDNF (ICV or intra-hippocampal) produces antidepressant behavioral responses in animal models of depression (Hoshaw *et al*, 2005; Shirayama *et al*, 2002; Siuciak *et al*, 1997). Consistent with these findings, transgenic mice expressing a variant BDNF allele (Val66-Met), which decreases the processing and release of BDNF, are more vulnerable to stress-induced behavioral deficits and have an attenuated antidepressant response (Chen *et al*, 2006; Egan *et al*, 2003). BDNF deletion mutants also show a depressive phenotype when exposed to mild stress (Duman *et al*, 2007), although there is no difference in behavior under non-stressed conditions (Chen *et al*, 2006; Monteggia *et al*, 2004; Saarelainen *et al*, 2003). Interestingly, clinical studies have reported a similar increase in stress vulnerability in subjects carrying the BDNF Val66Met polymorphism (Gatt *et al*, 2009). Postmortem studies report that hippocampal BDNF is decreased in MDD suicide subjects, but increased in subjects receiving antidepressant medication at the time of death (Chen *et al*, 2001b; Dwivedi *et al*, 2003; Karege *et al*, 2005).

While there is compelling evidence that BDNF mediates the actions of antidepressants in the hippocampus, recent studies indicate that increased BDNF/TrkB signaling has pro-depressive effects in other brain nuclei. For example, increased BDNF expression in the ventral tegmental area (VTA) promotes depressive-like behaviors (Eisch *et al*, 2003). Consistent with these results, decreased VTA and nucleus accumbens BDNF produces antidepressant responses in a social defeat paradigm (Berton *et al*, 2006; Krishnan *et al*, 2007b). Furthermore, overexpression of a dominant-negative form of TrkB in the nucleus accumbens results in an antidepressant response indicating that increased BDNF signaling has a pro-depressive function in the ventral striatum (Eisch *et al*, 2003). Collectively these data indicate that the behavioral effects of BDNF and TrkB in animal models of depression are region-specific, and that the pathogenesis of MDD is likely to include deficits in multiple brain regions. For these reasons, studies demonstrating antidepressant-like phenotypes in mutant mice overexpressing BDNF or in mice receiving infusions of BDNF into the lateral ventricle may more accurately model the neuropathology of MDD than animal studies examining the role of BDNF in one discrete brain region.

Taken together, these studies indicate that reduced BDNF contributes to depressive behaviors in animal models and in humans, and that antidepressant treatment increases or reverses these behavioral deficits by increasing BDNF. These findings are consistent with the hypothesis that the actions of antidepressants are due, in part, to BDNF-induced neuronal plasticity and/or protection (Pittenger and Duman, 2007).

Blood BDNF Levels are Decreased in Patients with MDD

A large number of clinical studies have reported that BDNF levels in serum (Aydemir *et al*, 2006; Gervasoni *et al*, 2005; Karege *et al*, 2002; Shimizu *et al*, 2003) and plasma (Kim *et al*, 2007a; Lee *et al*, 2006) are significantly decreased in depressed patients, and that this decrease is normalized by antidepressant treatments (Aydemir *et al*, 2005; Bocchio-Chiavetto *et al*, 2006; Gervasoni *et al*, 2005; Gonul *et al*, 2005; Huang *et al*, 2008; Okamoto *et al*, 2008; Yoshimura *et al*, 2007; Zanardini *et al*, 2006), and confirmed by meta-analysis (Brunoni *et al*, 2008a; Sen *et al*, 2008). These findings indicate that blood BDNF may be a useful biomarker, and that blood BDNF could have functional significance in the pathophysiology and/or treatment of mood disorders.

BDNF is transcribed at relatively high levels and expressed in peripheral tissues, including lung, heart, skeletal muscle, spleen, kidney, and blood (Braun *et al*, 1999; Koliatsos

et al, 1993; Lommatzsch *et al*, 1999, 2005b; Nassenstein *et al*, 2003; Scarisbrick *et al*, 1993; Timmusk *et al*, 1993; Yamamoto *et al*, 1996). Although the functional significance of these peripheral sources of BDNF is unknown, it is likely that BDNF in blood is derived from these tissues as well as from brain, and that peripheral sources of BDNF contribute to reductions of blood BDNF in MDD patients.

Functional Significance Peripheral BDNF

The possibility that peripheral growth factors can enter the brain and produce both behavioral and cellular responses is supported by studies of IGF-1 (Aberg *et al*, 2000; Duman *et al*, 2008b), vascular endothelial growth factor (VEGF) (Fabel *et al*, 2003), and more recently of BDNF (Schmidt and Duman, 2010) (Figure 1). Chronic peripheral BDNF

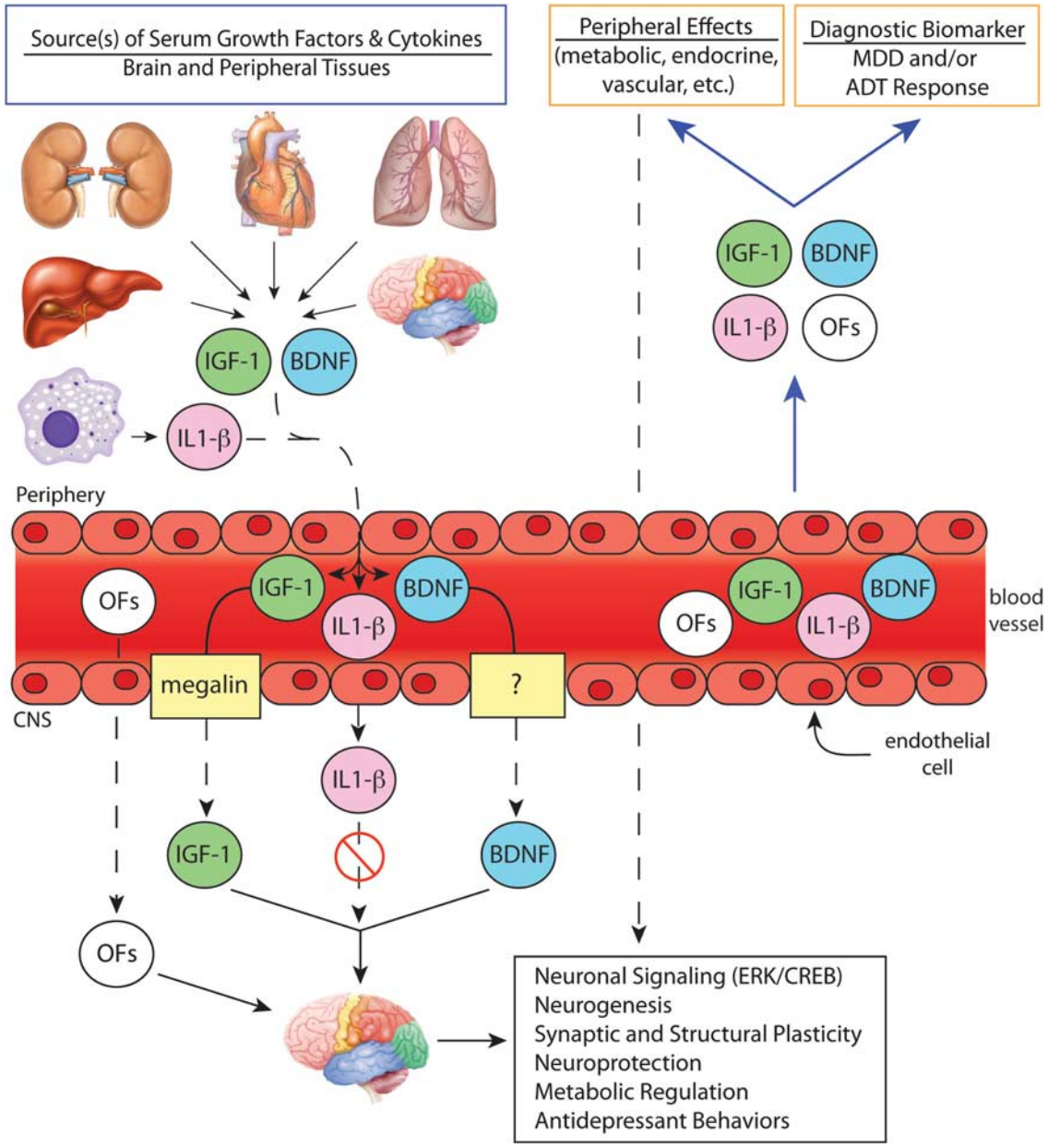

Figure 1 Peripheral growth factors and pro-inflammatory cytokines have central effects and regulate behavioral responses in animal models. Circulating IGF-1 is produced mainly in the liver and it is actively transported into the brain by the endocytic receptor megalin. Peripheral IGF-1 increases adult hippocampal neurogenesis and produces antidepressant-like behavioral responses. A number of peripheral tissues contribute to blood BDNF levels, including heart, kidney, lung, liver, and brain. Peripheral BDNF administration increases adult hippocampal neurogenesis and produces antidepressant-like behavioral responses. However, it remains to be determined whether these effects are mediated by direct (ie, blood BDNF entering the brain) and/or indirect mechanisms. By contrast, stress exposure results in inflammatory processes, including increased cytokine release from macrophages. Circulating cytokines such as IL-1β decrease adult hippocampal neurogenesis and produce depressive-like behaviors. Therefore, peripheral growth factors and pro-inflammatory cytokines exert opposing influences on antidepressant-like cellular (ie, neurogenesis) and behavioral responses. ADT, antidepressant; BDNF, brain-derived neurotrophic factor; IGF-1, insulin-like growth factor-1; IL-1β, interleukin-1β; MDD, major depressive disorder.

administration produces antidepressant-like behavioral responses in animal models (Schmidt and Duman, 2010), effects that are similar to the actions of different classes of chemical antidepressants (Cryan *et al*, 2005), and partially blocks the effects of chronic unpredictable stress (Schmidt and Duman, 2010). These behavioral actions are associated with antidepressant cellular responses, including increased survival of newborn hippocampal neurons (Schmidt and Duman, 2010), consistent with previous studies of central BDNF infusion (Sairanen *et al*, 2005). Peripheral BDNF administration is also associated with increased BDNF levels in the brain and activation of the downstream signaling markers ERK and CREB (Schmidt and Duman, 2010). Despite studies demonstrating a pro-depressive effect of BDNF/CREB in the striatum (Berton *et al*, 2006; Eisch *et al*, 2003; Wallace *et al*, 2009), these results indicate that antidepressant effects predominate in response to peripheral BDNF (Schmidt *et al*, 2008). While the peripheral source(s) of endogenous BDNF and mechanisms for transport into the brain are currently unknown, blood BDNF provides a novel window into brain structure and function that is relevant to MDD (Schmidt and Duman, 2010).

INSULIN-LIKE GROWTH FACTOR-1

Insulin-like growth factor-1 (IGF-1) regulates cell growth and metabolism in peripheral tissues (Stewart and Rotwein, 1996). Although IGF-1 is produced primarily by the liver and circulates in the bloodstream, it is also produced in the central nervous system, where it has a critical role in nerve growth and differentiation, as well as neurotransmitter synthesis and release (Anlar *et al*, 1999; Bondy and Lee, 1993; D'Ercole *et al*, 1996; Werther *et al*, 1990). Interestingly, the adult brain contains high levels of IGF-1 receptors, but unlike the developing brain, the expression levels of IGF-1 are low, suggesting that the adult brain may utilize IGF-1 from peripheral sources (Bondy and Lee, 1993).

Influence of Stress and Antidepressants on IGF-1

Chronic antidepressant administration increases IGF-1 expression in the rat brain (Khawaja *et al*, 2004), and IGF-1 regulates adult hippocampal neurogenesis (Anderson *et al*, 2002) and produces antidepressant behavioral responses (Hoshaw *et al*, 2005; Malberg *et al*, 2007). Moreover, IGF signaling is altered in postmortem brain tissue in subjects with bipolar disorder (Bezchlibnyk *et al*, 2007). These results suggest that IGF-1 could contribute to the cellular and behavioral responses to antidepressant treatments, as well as the pathophysiology of mood disorders.

Peripheral IGF-1 crosses the blood–brain barrier through a transporter-mediated mechanism (Carro *et al*, 2005; Pan and Kastin, 2000) and influences neuronal function (Pulford and Ishii, 2001; Reinhardt and Bondy, 1994). Physical exercise stimulates the expression and release of liver IGF-1, and results in elevated brain uptake (Carro *et al*, 2000). Peripheral IGF-1 administration increases hippocampal neurogenesis (Aberg *et al*, 2000), and blockade of peripheral IGF-1 reduces exercise-induced neurogenesis (Duman *et al*, 2008a; Trejo *et al*, 2001). These findings indicate that peripheral IGF-1 is transported into the brain, where it produces cellular and behavioral responses.

Blood IGF-1 Levels in Patients with Altered Mood

There have not been sufficient studies to determine whether peripheral IGF-1 is altered in depressed patients or following antidepressant administration. However, exercise is associated with improved mood and increased serum IGF-1 expression in naïve elderly subjects (Cassilhas *et al*, 2010). Additional studies are needed to determine the role of IGF-1 in MDD and response to antidepressants.

Functional Significance of Peripheral IGF-1

Peripheral IGF-1 administration has been shown to alter behavior independent of exercise and produce antidepressant-like behavioral responses. Peripheral IGF-1 administration reduces immobility in the FST (Duman *et al*, 2009), and produces antidepressant behavioral responses in mice exposed to chronic unpredictable stress (Duman *et al*, 2009). Elevated blood IGF-1 levels are also associated with increased adult hippocampal neurogenesis, improved cognition, and some of the beneficial effects of exercise, including reduced anxiety (Trejo *et al*, 2008, 2007). The behavioral effects of peripherally administered IGF-1 are associated with increased levels of exogenous IGF-1 in the brain (Duman *et al*, 2009). Although speculative, some cases of MDD could result from dysfunction of the peripheral expression and/or the transport of IGF-1 into the brain.

VASCULAR ENDOTHELIAL GROWTH FACTOR

VEGF is an endothelial cell mitogen and survival factor that regulates vascular function (Leung *et al*, 1989), but is also expressed in the brain and has neuroprotective and neurogenic effects (Jin *et al*, 2002; Storkebaum *et al*, 2004; Warner-Schmidt and Duman, 2007).

Influence of Stress and Antidepressants on VEGF

Chronic stress exposure has been shown to decrease (Heine *et al*, 2005) and antidepressant administration to increase hippocampal VEGF (Altar *et al*, 2004; Warner-Schmidt and Duman, 2007). Furthermore, impaired VEGF signaling in the brain blocks the effects of chemical antidepressants (Warner-Schmidt and Duman, 2007) and exercise (Fabel *et al*, 2003) on hippocampal neurogenesis. Pharmacological antagonism of VEGF-mediated signaling in the brain blocks the behavioral effects of antidepressants in rodent models (Greene *et al*, 2009; Lee *et al*, 2009; Warner-Schmidt and Duman, 2007). Peripheral VEGF also has a critical role in the neurogenic effects of exercise, which demonstrates that blood VEGF has functional effects in the brain (Fabel *et al*, 2003). Taken together, these results indicate that VEGF is necessary and sufficient for the neurogenic and behavioral actions of antidepressants.

Blood VEGF Levels in Patients with Altered Mood

Clinical studies of peripheral VEGF in MDD are mixed. One study reports that VEGF expression is increased in peripheral leukocytes of patients with MDD and that antidepressant treatment reverses these effects (Iga *et al*, 2006). Consistent with these results, blood VEGF levels are increased in patients with MDD (Kahl *et al*, 2009).

By contrast, another study found no significant differences in blood VEGF levels between patients with MDD and healthy controls, and following antidepressant treatment (Ventriglia *et al*, 2009). Moreover, preclinical findings indicate that serum VEGF levels are not different in a genetic rat model of depression (Elfving *et al*, 2010). These divergent clinical findings are likely due to significant differences in patient populations, including age, gender, total number of depressive episodes (ie, recurrent *vs* acute), and comorbid disorders. However, these clinical findings suggest that blood VEGF levels may be differentially altered depending upon the endophenotype of MDD studied, but further studies are needed and warranted.

OTHER GROWTH FACTORS

Other growth factors that may also serve as biomarkers of MDD and/or antidepressant response include glial cell line-derived neurotrophic factor (GDNF) and fibroblast growth factor-2 (FGF-2), both of which are altered in humans with MDD (Kahl *et al*, 2009; Rosa *et al*, 2006; Takebayashi *et al*, 2006). FGF-2, FGF receptors, and the GDNF receptor are altered by antidepressant treatment (Chen *et al*, 2001a; Evans *et al*, 2004; Gaughran *et al*, 2006). FGF-2, neurotrophin-3 (NT-3), and nerve growth factor (NGF) influence adult hippocampal neurogenesis and/or are regulated by stress and antidepressant treatments, and could contribute to stress-induced cellular and behavioral deficits, and antidepressant responses (Dwivedi *et al*, 2005; Hock *et al*, 2000; Lu *et al*, 2005; Mallei *et al*, 2002; Molteni *et al*, 2001; Smith *et al*, 1995a). Finally, peripheral VGF expression is decreased in patients with MDD (Cattaneo *et al*, 2010) and administration of recombinant VGF produces antidepressant behavioral responses in mice (Hunsberger *et al*, 2007). While the exact role of GDNF, FGF-2, NT-3, NGF, and VGF in the pathogenesis of MDD and/or antidepressant behavioral and cellular responses is unclear, there is sufficient evidence to implicate these growth factors in MDD.

CYTOKINES AND INFLAMMATORY MARKERS

Increasing evidence indicates that inflammation may have a critical role in the pathophysiology of MDD (Miller *et al*, 2009). Clinical studies demonstrate that patients with MDD have elevated blood/serum levels of inflammatory markers, including pro-inflammatory cytokines. Consistent with these results, inhibiting pro-inflammatory cytokine signaling in patients with inflammatory disorders, as well as patients with MDD, improves mood and facilitates antidepressant treatment response. Furthermore, chronic stress exposure alters the expression of cytokines, and antidepressant treatment neutralizes these effects. The following sections will discuss preclinical and clinical studies of several of these key cytokines in rodent models and MDD patients.

TNF-α AND IL-6

MDD is also accompanied by altered immune function and activation of the inflammatory response system (Dinan, 2009). Activated macrophages secrete pro-inflammatory cytokines, which may contribute to MDD. Cytokine activation produces sickness behaviors, which share features with depression (Dunn *et al*, 2005; Koo and Duman, 2008). Moreover, chronic stress exposure produces changes in immune function that may influence the pathophysiology of MDD (Miller *et al*, 2009).

Cellular and Behavioral Actions of TNF-α and IL-6

The pro-inflammatory cytokines interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) have direct inhibitory effects on adult hippocampal neurogenesis (Iosif *et al*, 2006; Monje *et al*, 2003), and, therefore, may attenuate antidepressant efficacy by decreasing hippocampal neurogenesis or interfering with the neurogenic properties of antidepressants. In addition, mutant mice lacking TNF-α receptors show antidepressant behavioral phenotypes (Simen *et al*, 2006). Taken together, these preclinical findings suggest that TNF-α and IL-6 may block the behavioral and cellular responses to antidepressants and/or facilitate depressive phenotypes.

Blood TNF-α and IL-6 in Patients with Altered Mood

Peripheral/serum levels of IL-6 and TNF-α are increased in patients with MDD (Dowlati *et al*, 2010; Kahl *et al*, 2006), and these effects are normalized following antidepressant treatment (Levine *et al*, 1999; Raison *et al*, 2006; Sluzewska *et al*, 1996; Steptoe *et al*, 2007; Thomas *et al*, 2005; Tuglu *et al*, 2003). These consistent clinical findings indicate that TNF-α and IL-6 are putative biomarkers of depressive episodes and treatment response.

Interestingly, treatment-resistant patients with MDD have elevated blood IL-6 levels compared with treatment-responsive patients (Maes *et al*, 1997). Therefore, changes in blood IL-6 levels may serve as a marker to track those patient populations that respond to a given antidepressant treatment.

Functional Significance of TNF-α and IL-6

Preclinical studies of cytokines and depressive behaviors correlate with clinical studies of depression (Khairova *et al*, 2009). For example, immunotherapy using IL-2 or interferon-α (IFN-α) is associated with cognitive impairments and depressed mood that correlate with elevated blood levels of IFN-α, IL-6, IL-8, and IL-10 (Bonaccorso *et al*, 2002, 2001; Capuron *et al*, 2001a, 2001b; Dieperink *et al*, 2000). Depression, anxiety, and memory impairments are also associated with immune activation by the bacterial endotoxin LPS in healthy subjects and are associated with increased blood IL-1 and TNF-α (Yirmiya *et al*, 2000).

Increasing evidence suggests that patients with MDD have an imbalance between pro- and anti-inflammatory cytokines that can be normalized following antidepressant treatment (Kim *et al*, 2007b; Sutcigil *et al*, 2007; Taler *et al*, 2007). Some patients with MDD also have abnormal allelic variants of the genes for IL-1 and TNF-α, and those with elevated levels of TNF-α have an attenuated therapeutic response to antidepressant treatment (Eller *et al*, 2008; Fertuzinhos *et al*, 2004; Khairova *et al*, 2009). Clinical studies also

demonstrate that cytokine antagonists have antidepressant behavioral effects, even in the absence of an immune challenge. The TNF-α antagonists etanercept and infliximab attenuate depressive symptoms induced by immune activation during psoriasis (Krishnan *et al*, 2007a; Tyring *et al*, 2006). There is also a report that the cyclooxygenase-2 (COX2) inhibitor celexocib, which inhibits the production of pro-inflammatory cytokines, including TNF-α and IL-1β, produces a rapid antidepressant response in patients with MDD (Muller *et al*, 2006).

Taken together, these findings raise the possibility that reductions in inflammatory processes might contribute to treatment response, and that inhibiting pro-inflammatory signaling may be a promising strategy to treat depressed patients with increased blood cytokine profiles.

INTERLEUKIN-1β

Effects of Stress and Antidepressants on IL-1β

Recent evidence indicates that dysregulation of pro-inflammatory cytokines, including IL-1β, influences the etiology and/or pathophysiology of MDD (Raison *et al*, 2006). Elevated levels of pro-inflammatory cytokines may also contribute to the damaging effects of stress. Stress exposure increases IL-1β in the hippocampus (Johnson *et al*, 2005; Nguyen *et al*, 1998), IL-1β inhibits adult hippocampal neurogenesis, and blockade of IL-1 inhibits the effects of stress on neurogenesis (Koo and Duman, 2008). Increased IL-1β in the hippocampus is also associated with stress-induced impairments in synaptic plasticity (Murray and Lynch, 1998) as well as activation of the HPA axis (Linthorst *et al*, 1994; Rivier, 1993; Sapolsky *et al*, 1987). Administration of an IL-1β receptor antagonist into the hippocampus blocks the BDNF decrease caused by stress, suggesting that the anti-neurogenic effects of cytokines may be mediated, in part, through regulation of BDNF (Barrientos *et al*, 2003) and/or IGF-1 (O'Connor *et al*, 2008). Thus, stress-induced deficits and hippocampal plasticity are regulated by complex mechanisms involving cytokines and growth factors.

Blood IL-1β Levels in Patients with Altered Mood

Blood IL-1β is increased in patients with MDD (Diniz *et al*, 2010; Thomas *et al*, 2005) and antidepressant treatment may reverse this effect (Himmerich *et al*, 2010; Song *et al*, 2009). However, not all clinical studies demonstrate increased circulating levels of IL-1β in patients with MDD (Jazayeri *et al*, 2010) and these changes are not as consistent as those observed when examining IL-6, TNF-α, and C-reactive protein (CRP) (Howren *et al*, 2009b). These mixed clinical results are likely due to heterogeneity of MDD.

Functional Significance of Peripheral IL-1β

Peripheral and central IL-1β administration induces sickness behaviors, including anorexia, weight loss, anhedonia, fatigue, impaired social interaction, and memory dysfunction, symptoms that are also observed in patients with MDD (Goshen and Yirmiya, 2009; Koo and Duman, 2008). By contrast, inhibition of IL-1β signaling blocks depressive and sickness-related behaviors (Goshen and Yirmiya, 2009; Koo and Duman, 2008). Recent studies demonstrate that impaired IL-1 receptor signaling blocks stress-induced anhedonia (Goshen and Yirmiya, 2008; Koo and Duman, 2008) and produces antidepressant effects in an animal model of behavioral despair (Maier and Watkins, 1995). Future studies are required to identify the precise mechanism(s) by which peripheral/serum IL-1β activates HPA function and produces anhedonic and anxiogenic behavioral responses.

OTHER CYTOKINES/INFLAMMATORY MARKERS

The risk of developing MDD is increased in patients undergoing cytokine or IFN therapy for the treatment of cancer or viral infection such as hepatitis-C (Capuron and Dantzer, 2003). A recent study demonstrated that IFN therapy-induced depressive episodes are associated with decreased blood BDNF levels, suggesting a point of intersection with stress and antidepressant treatments (Kenis *et al*, 2010). These results indicate that cytokines and IFNs significantly contribute to the effects of stress, as well as the precipitation and maintenance of MDD, and conversely that neutralization of these cytokines could have antidepressant effects (Dantzer *et al*, 2008). Additional work is needed to determine the role of other cytokines, including IL-4, IL-2, IL-8, IL-10, and/or IFN-γ, in MDD.

High-sensitivity CRP (hs-CRP), a marker of low-grade inflammation, is a cardiovascular disease risk factor and a potential biomarker of immunological activation (De Berardis *et al*, 2006). Coronary artery disease is associated with a high incidence of MDD (Nemeroff *et al*, 1998) and with higher levels of circulating hs-CRP (Pearson *et al*, 2003), which is synthesized in the liver in response to stimulation from IL-6 and IL-1. A meta-analysis reveals positive associations between MDD and hs-CRP, IL-6, and, to a lesser extent, IL-1 (Howren *et al*, 2009a). These findings highlight a role for hs-CRP and its precursors as mediator/moderator factors of depression, although its precise role remains unclear.

Mood disorders could also result from acquired immune disorders. Prolonged activation of the peripheral immune system as occurs during systemic infections, cancer, or autoimmune disorders results in immune signaling in the brain that can lead to the development of depressive episodes (Dantzer *et al*, 2008). Recent findings indicate that soluble IL-2 receptor levels (a marker of T-cell activation) are increased in patients with MDD (Mossner *et al*, 2007). Collectively these results suggest that both acquired (eg, T- and B-cell) and innate (eg, macrophage) immune response may have critical roles in the pathophysiology of MDD. However, it remains unclear whether activation of inflammatory signaling during depression is an indirect result of peripheral processes and/or whether stress exposure induces inflammatory responses directly within the brain (Miller *et al*, 2009).

DIRECT *VS* INDIRECT EFFECTS OF PERIPHERAL FACTORS ON NEURONAL FUNCTION

It remains to be determined whether the behavioral and cellular actions of peripheral BDNF, as well as other growth

factors and cytokines, are mediated by direct actions on the brain and/or indirect mechanisms through regulation of peripheral endocrine or metabolic actions. There are reports that peripheral BDNF can cross the blood–brain barrier, possibly through active transport similar to IGF-1 (Carro *et al*, 2005; Trejo *et al*, 2007), although this remains controversial (Pan *et al*, 1998; Pardridge, 2002; Poduslo and Curran, 1996). In addition, saturable transport systems from blood to the brain have been described for cytokines including IL-1β, IL-6, and TNF-α (Banks, 2005). Therefore, circulating BDNF and other growth factors may be transported into the brain and have direct effects on neuronal as well as glial function.

While much is known about the roles of peripheral IGF-1 in metabolic processes and peripheral cytokines in inflammatory processes, the functional significance of blood BDNF derived from peripheral tissues is unclear. Moreover, the mechanisms that regulate blood BDNF, IGF-1, and cytokines during MDD have not been identified. Future studies to identify the mechanisms (ie, transcriptional, synthesis, release, clearance, etc) underlying the regulation of peripheral as well as central expression of growth factors and cytokines will further elucidate the neurobiology of mood disorders.

An often-overlooked question with regard to putative biomarkers is the relationship between peripheral and central changes in biomarker levels. It is not clear whether altered levels of putative biomarkers in peripheral tissues must mirror changes in the brain and vice versa. Future studies directly addressing this question will aid in classifying biomarkers as moderators, mediators, diagnostic markers, or a combination of these roles.

ENDOCRINE AND METABOLIC MARKERS

Analyses of stress-induced changes of peripheral endocrine and metabolic markers will also aid in the diagnosis and treatment of MDD. An extensive literature now demonstrates that neuroendocrine and metabolic functions are altered in patients with MDD.

Neuroendocrine Function and MDD

Depression is associated with altered regulation of the HPA axis that results in increased release of corticotropin-releasing hormone (CRH) and in some cases sustained elevation of cortisol (Nestler *et al*, 2002). Glucocorticoids (cortisol in humans and corticosterone in rodents) bind to their receptors in the HPA axis and act as negative regulators of HPA axis activity. Increased activity of the HPA axis in MDD is due, in part, to altered feedback inhibition of the HPA axis by endogenous glucocorticoids (for further review see, Pariante, 2009). Impaired negative feedback of the HPA axis by glucocorticoids is mediated, in part, by altered expression of the glucocorticoid receptor (Pariante and Miller, 2001). It has been proposed that elevated cortisol in patients with MDD is a compensatory mechanism in response to decreased glucocorticoid receptor function and expression in the brain (Raison and Miller, 2003). Preclinical studies demonstrate that chronic antidepressant administration leads to the upregulation of glucocorticoid receptor expression and function, and thus increased negative feedback regulation of the HPA axis (Pariante and Miller, 2001). Biomarker panels that monitor changes in cortisol, as well as other HPA axis factors (eg, CRF), will provide important information for characterization of MDD subtypes.

Cortisol, however, is not elevated in all persons with MDD. Some data indicate that persons with the melancholic subtype of MDD may be more likely to have increased HPA axis activity than non-melancholic patients (Gold and Chrousos, 2002; Wong *et al*, 2000). Melancholia is a distinct form of depression characterized by consistently down and non-reactive mood, anhedonia, decreased sleep and appetite, and weight loss (Fink and Taylor, 2007). Persons with melancholia are more likely to have elevations in plasma cortisol and lack of dexamethasone suppression relative to non-melancholic patients (Gold and Chrousos, 2002), which tend to normalize with effective treatment (Fink and Taylor, 2007).

Inflammatory markers, including cytokines, regulate neuroendocrine function. Acute cytokine administration is associated with increased expression and release of CRH, adrenocorticotropic hormone (ACTH), and cortisol (Besedovsky and del Rey, 1996). Cytokines may impair neuroendocrine function by interfering with the negative feedback regulation of the HPA axis, a hallmark of MDD that is reflected by decreased responsiveness to glucocorticoids (Miller *et al*, 2009). Increased cytokine signaling inhibits glucocorticoid receptor function and increases the expression of the relatively inert β-isoform, while decreasing the expression of the active α-isoform, of the glucocorticoid receptor (Pace *et al*, 2007). In addition, glucocorticoids have clear inhibitory effects on inflammation (Rhen and Cidlowski, 2005). Dysregulation of the exquisite balance between HPA axis sensitivity to glucocorticoids and the innate immune system (Miller *et al*, 2009) can be readily monitored in MDD patients. Therefore, biomarker panels of MDD should target pathways by which the immune system impacts the brain, including cytokines, inflammatory mediators (eg, COX-2, prostaglandin), reactive nitrogen and oxygen species (eg, nitric oxide, hydrogen peroxide), monoamines, neurotrophic factors, and HPA axis hormones (eg, CRH, cortisol) and receptors (eg, glucocorticoid receptors). Monitoring these putative biomarkers during antidepressant treatment may aid in identifying patient populations that are responsive to inflammation-targeted therapies (Miller *et al*, 2009).

Metabolic Function and MDD

Circulating hormones such as leptin and ghrelin relay information pertaining to peripheral energy homeostatic levels to the brain (Lutter and Nestler, 2009). Low levels of leptin have been found to be associated with depressive behaviors in humans and rodents (Lu, 2007), and chronic stress exposure decreases serum leptin (Lu *et al*, 2006). Consistent with these results, acute leptin administration produces antidepressant responses (Liu *et al*, 2010) and leptin administration blocks depressive behavior in leptin-deficient mice, effects that are associated with increased hippocampal BDNF expression (Yamada *et al*, 2011). By contrast, chronic stress exposure increases serum ghrelin levels (Lutter *et al*, 2008). Calorie restriction produces

antidepressant responses that are associated with increased circulating ghrelin levels (Lutter *et al*, 2008). Collectively, these results suggest that ghrelin counteracts stress-induced behavioral deficits by promoting antidepressant responses. Thus, leptin and ghrelin may serve as putative biomarkers for MDD in general or in depressed patients with altered metabolic function.

Metabolic syndrome is a cluster of cardiovascular risk factors that are associated with increased incidence of cardiovascular disease and diabetes. Metabolic syndrome is also associated with MDD (Skilton *et al*, 2007). Antidepressants exert variable effects on the constituent components of metabolic syndrome (McIntyre *et al*, 2010). A recent study suggests that decreased HDL cholesterol levels, but not other markers of metabolic syndrome, may predict the development of new-onset MDD in pre-elderly populations (Akbaraly *et al*, 2011). This finding is consistent with the hypothesis that dyslipidemia mediates depressive episodes in the elderly (Ancelin *et al*, 2010). While future studies are required to determine the exact role of dyslipidemia in the etiology of MDD, HDL levels may predict the onset of an MDD endophenotype that manifests later in life.

Depression is frequently associated with comorbid disorders, including diabetes, a metabolic disorder that is associated with the damaging effects of inflammation and oxidative stress in the brain (Hendrickx *et al*, 2005). Type-2 diabetes is characterized by hyperglycemia and the inability of the body to control blood glucose levels. Type-2 diabetes usually begins as insulin resistance, a disorder in which glucose uptake by peripheral cells is impaired, which leads to a compensatory increase in insulin secretion by the pancreas. Eventually, the pancreas can no longer produce enough insulin to maintain euglycemia and Type-2 diabetes occurs. However, the relationship between MDD and insulin resistance is not clear (Adriaanse *et al*, 2006; Lawlor *et al*, 2003; Pan *et al*, 2008; Qiuhua *et al*, 2011; Timonen *et al*, 2005, 2006). These studies varied significantly in patient demographics, gender, depression ratings, and insulin resistance measurements. Diabetes-induced elevations in blood glucose and insulin levels produce inflammatory effects in the brain and may contribute to the development of MDD (Hendrickx *et al*, 2005). Therefore, a biomarker panel of MDD should track insulin resistance and glucose levels as potential mediators of MDD in pre-diabetic and diabetic patients, respectively. Changes in serum lipid profiles and free radicals should also be considered as future studies determine the extent of these changes in metabolic disorders and the concurrence of depressive episodes.

Further evidence for a role of metabolic dysregulation is provided by studies demonstrating that impaired peripheral glucose regulation is associated with cognitive decline and depression, especially in obese subjects and patients with Type-2 diabetes (Hendrickx *et al*, 2005). The negative consequences of aberrant glycemic control on brain function are mediated, in part, by insulin, glucose, growth factors, cortisol, cytokines, and reactive oxygen species (Hendrickx *et al*, 2005). Specifically, diabetes and metabolic syndrome are associated with increased HPA axis activity, and some of the factors that regulate diabetes-related cognitive decline include peripheral IGF-1 and cortisol (Hendrickx *et al*, 2005). Thus, the etiology and pathophysiology of MDD appear to be tightly regulated by complex interplays between endocrine, immune, and metabolic systems. Although there is not a clear understanding of how these systems function together to mediate depressive episodes, biomarker panels that monitor these peripheral factors will provide descriptive evidence toward this goal.

NON-PROTEOMIC BIOMARKERS OF MDD

Genetic factors have a critical role in the development of MDD and provide insights into the mechanisms underlying depression. Candidate gene studies have implicated polymorphisms in the genes encoding the serotonin transporter, serotonin receptor-2A, BDNF, and tryptophan hydroxylase in MDD (Lohoff, 2010). These studies along with genome-wide association studies have not identified a single common gene variant that increases the risk of MDD substantially (Lohoff, 2010). Instead, depression is likely to result from complex interactions between multiple genetic and environmental factors. Thus, tracking genetic variants in patient blood may serve to compliment biomarker panels by providing more information relating genotype to MDD and treatment response.

An emerging literature indicates that stress exposure induces epigenetic mechanisms such as histone modifications and DNA methylation that promote maladaptive behaviors. Chronic social stress decreases hippocampal BDNF through long-lasting dimethlyation of histones at the level of BDNF promoters and is associated with a pro-depressive phenotype (Tsankova *et al*, 2006). By contrast, chronic antidepressant administration reverses stress-induced BDNF repression through epigenetic mechanisms involving histone-3 acetylation and histone-3 lysine-4 methylation (Tsankova *et al*, 2006). Moreover, systemic administration of a DNA methylation inhibitor produces antidepressant behavioral responses that are associated with decreased DNA methylation and increased BDNF expression in the hippocampus (Sales *et al*, 2011). Stressful events in early life also produce long-lasting epigenetic marks that influence affect and mood. Offspring of mothers with low levels of nurturing behavior had increased methylation of the glucocorticoid receptor variant $GR1_7$ promoter, which leads to decreased $GR1_7$ expression in adulthood (Weaver *et al*, 2004). Thus, long-lasting epigenetic modifications have a critical role in stress-induced and antidepressant behavioral responses. However, these studies to date have focused on the transcriptional regulation of BDNF and glucocorticoid receptor genes in the hippocampus. It remains to be determined whether epigenetic changes in response to stress or antidepressant treatment can be monitored from components of blood and cerebral spinal fluid to aid in the diagnosis of MDD. In addition to comprehensive proteomic screens, future biomarkers of MDD and antidepressant response are likely to include epigenetic and genetic factors.

Recently, more comprehensive approaches to identifying diagnostic biomarkers of mood disorders including MDD have been described. Convergent Functional Genomics is a multidisciplinary method that integrates animal model gene

expression data with human genetic linkage/association data, as well as human tissue (ie, postmortem brain, blood, etc) data, to identify and prioritize candidate genes and molecular substrates for subsequent hypothesis-driven research. Using gene arrays to examine blood biomarker genes, Convergent Functional Genomics has identified genes associated specifically with high or low mood states (Le-Niculescu *et al*, 2009). These results are consistent with previous studies demonstrating differential expression of these genes in postmortem brain tissue from mood disorder subjects (Le-Niculescu *et al*, 2009). Identifying genetic and proteomic biomarkers for psychiatric disorders including MDD is limited by cost, lack of predictability, and unreliability due to polygenetic inheritance and environmental influences (Lakhan *et al*, 2010). It remains to be determined whether any of the genetic biomarker panels identified using Convergent Functional Genetics and other techniques correlate with treatment response and whether these methods could be used to differentiate MDD severity and/or subtypes.

SPECIFICITY OF BIOMARKERS FOR MOOD DISORDERS

Altered blood levels of BDNF, IGF-1, and cytokines are not specific to MDD. Peripheral BDNF and IGF-1 levels are decreased in several psychiatric illnesses, including eating disorders (Nakazato *et al*, 2003; Saito *et al*, 2009), schizophrenia (Green *et al*, 2010; Toyooka *et al*, 2002), and/or panic (Kobayashi *et al*, 2005). Furthermore, there is a high incidence of comorbid or coincident diseases, including Type-2 diabetes and MDD (Katon, 2008), as well as strong associations between MDD and metabolic syndrome (Dunbar *et al*, 2008). Alterations of serum growth factors and cytokines have also been demonstrated in cardiovascular (Ejiri *et al*, 2005; Kaplan *et al*, 2005; von der Thusen *et al*, 2003), inflammatory (Katsanos *et al*, 2001; Lee *et al*, 2010; Lommatzsch *et al*, 2005a; Schulte-Herbruggen *et al*, 2005), and metabolic diseases (Dunger *et al*, 2003; Han *et al*, 2010; Kaldunski *et al*, 2010), all of which are more common in depressed patients than the general population (Shelton and Miller, 2010). However, patients with these conditions but without depression (ie, persons with cardiovascular disease or Type-2 diabetes) will have altered levels of the putative biomarkers described above. These findings suggest that altered peripheral systems contribute to a broader disease state. Monitoring multiple factors will provide a more complete assessment and thereby identify a spectrum of factors that better characterize disease state as well as specific disease symptoms. This information can also be used for targeted treatment to augment or neutralize altered growth factor or cytokine levels. Stated simply, whereas single biomarkers are unlikely to adequately distinguish depressed from non-depressed subjects, panels of multiple biomarkers may work significantly better.

Biomarker panels for simultaneous detection of peripheral cytokines, growth factors, hormones, and other protein markers will allow the identification of a peripheral signature that differentiates MDD subtypes and distinguishes MDD from other disorders (Figure 2). Identifying proteomic biomarkers for psychiatric disorders will require a large sample size in order to demonstrate that these methods are both predictable and reliable. Furthermore, it will be necessary to demonstrate that biomarker panels correlate with antidepressant efficacy, severity, and/or endophenotypes of MDD in independent cohorts of patients. Nevertheless, the information provided by such panels will be invaluable for showing imbalances of multiple systems and will aid in the treatment and management of illness.

Clinical studies examining putative biomarkers have compared changes in patients with MDD *vs* matched, healthy control subjects. Therefore, an important limitation to biomarker selection for depression is the lack of direct comparisons between MDD and other disorders with comorbid depression. Identifying novel candidate biological markers for MDD by using proteomic profiling methods will enable simultaneous detection of cytokines, growth factors, hormones, and other protein markers in plasma samples in order to determine a peripheral signature for MDD. Furthermore, multi-analyte biomarker panels may provide differential diagnoses between MDD and other disorders with depression as a symptom. However, more clinical studies directly comparing changes in peripheral biomarkers in MDD compared with other comorbid disorders are warranted to determine whether changes in putative biomarkers are specific to MDD. Interestingly, a recent study demonstrated that plasma biomarker profiling has the potential to differentiate psychiatric disorders by identifying unique biomarkers for each condition (Domenici *et al*, 2010). Thus, comprehensive biomarker panels provide peripheral signatures that differentiate psychiatric disorders and may facilitate differential diagnoses of MDD subtypes and comorbid disorders.

Predictive algorithms may be successfully derived from multiple biological variables of MDD. Algorithms that predict inclusion in depressed and control groups can be thought of as a set of partial differential equations that aim to achieve maximum separation between these two defined groups. Individual biomarkers of a predictor set might be unlikely to provide sufficient predictive power to produce adequate separation between groups. However, group identity may be optimized by using equation modeling in which each individual variable has a designated weight, depending on its positive predictive value (PPV) (Altman and Bland, 1994). While the use of multiple biomarkers for diagnosis and treatment prediction is not generally done in psychiatry, it is a common strategy in other fields such as oncology (Dunn *et al*, 2011; Malinowski, 2007a, b; Marrero *et al*, 2010; Vauthey *et al*, 2010; Yurkovetsky *et al*, 2010; Zhu *et al*, 2011). A simple clinical example would be methods for staging of various cancers that may use multiple predictive characteristics (Vauthey *et al*, 2010).

It remains unclear, however, as to how comorbid psychiatric disorders, including substance use disorders, will affect prediction algorithms for the diagnosis of MDD. PPV (also known as precision rate) is the proportion of tested individuals with positive test results who are correctly diagnosed (Altman and Bland, 1994). In order for a diagnostic test to be clinically relevant, it will have to have a PPV that is robust to comorbidity. While a test might be developed in a non-comorbid group, it would ultimately have to be tested in samples specifically selected for high rates of medical and psychiatric comorbidity.

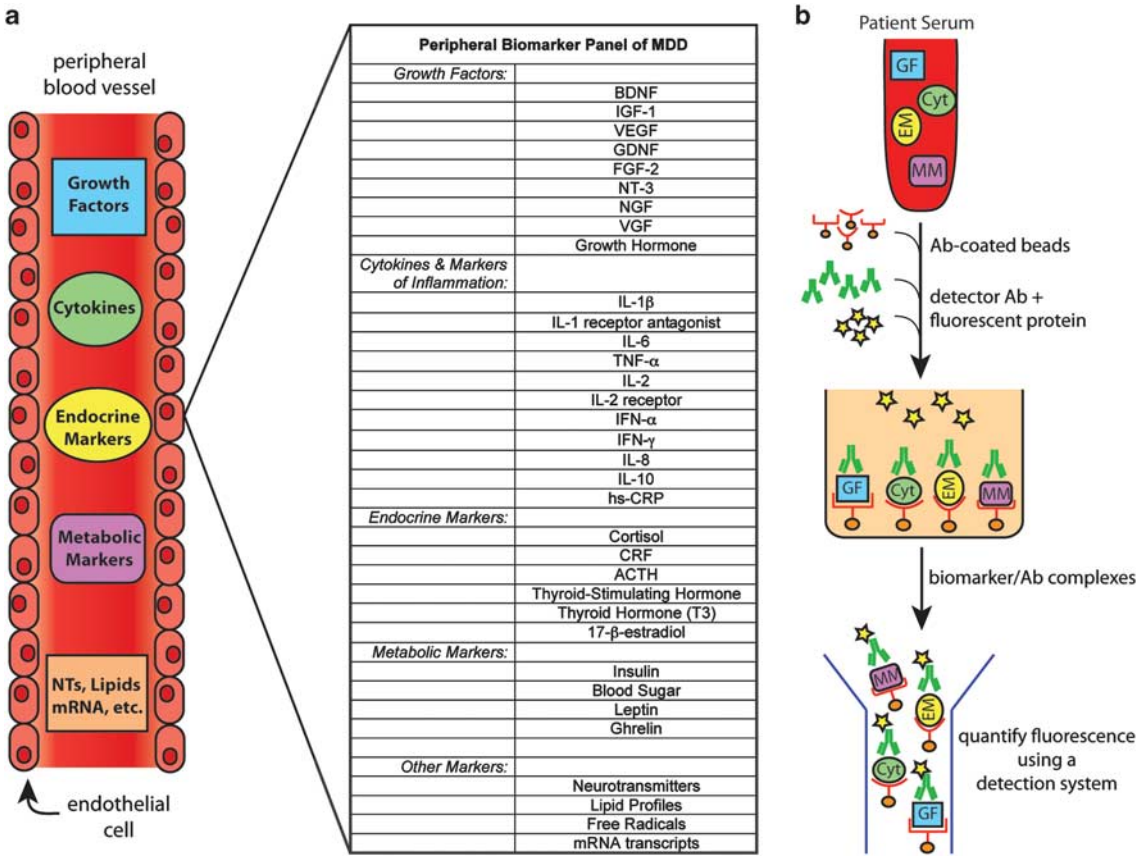

Figure 2 Biomarker panels for MDD and/or antidepressant efficacy. Preclinical and clinical studies have demonstrated a number of putative biomarkers for the diagnosis and/or treatment of MDD. (a) Development of a biomarker panel that quantifies changes in the peripheral levels of growth factors, cytokines, hormones, and metabolic markers will aid in diagnosing MDD, identifying heterogeneous MDD patient populations, and/or measuring and tracking antidepressant efficacy and clinical outcomes (see text for more details). (b) A simplified protocol for assaying patient blood using a biomarker panel of MDD. Sera from medicated or non-medicated patients with MDD is isolated, purified, and added to a multi-well, filter bottom microplate along with standards and control samples. Primary antibodies (Ab) that are conjugated to beads with defined spectral properties are added to each well. Subsequent steps involve adding a biotinylated detector antibody and a streptavidin-conjugated fluorescent protein. Protein/antibody complexes are eluted and biomarkers are quantified by using the spectral properties of the beads and the amount of associated fluorescence. Therefore, multiple growth factors (GF), cytokines (Cyt), endocrine markers (EM), and metabolic markers (MM) can be assayed simultaneously from a patient's blood. (This proposed biomarker panel is based on Invitrogen's Luminex assay protocol: http://www.invitrogen.com/site/us/en/home/Products-and-Services/Applications/Cell-and-Tissue-Analysis/Immunoassays/Luminex-Assays.html).

CONCLUSIONS

Clinical and preclinical studies have identified a number of factors that may serve as putative biomarkers for diagnosing and treating MDD. However, the utility of any given growth factor, cytokine, endocrine factor, or metabolic marker to serve as a clinically useful biomarker of MDD is limited by a lack of sensitivity and specificity. Therefore, we propose a panel of multiple biomarkers to improve the predictive power of these factors as measured using an aggregate score or predictive algorithm to diagnose and classify MDD subtypes as well as measure treatment response.

A number of questions regarding peripheral/blood biomarkers and MDD remain. First, the optimal time point at which peripheral/blood biomarkers should be measured during the day and during treatment is not clear. There are also potential confounds in interpreting changes in biomarkers during antidepressant treatment. For example, it remains uncertain whether clear distinctions in biomarker levels will differentiate antidepressant efficacy or remission. Finally, it is not clear whether putative biomarkers for MDD have sufficient sensitivity, specificity, and reproducibility for predicting therapeutic responses and remission rates that are reliable to diagnose and treat patients with MDD (Leuchter *et al*, 2010).

One approach that could address these issues is the use of a stress, immune, and/or metabolic challenge test in MDD, to reveal altered regulation of peripheral biomarkers. This would be analogous to a stress test used for cardiovascular disease or glucose tolerance/insulin resistance for diabetes. By comparing pre- and post-test levels of blood biomarkers, this type of challenge could reveal more robust abnormalities in the regulation of growth factors, cytokines, endocrine, and metabolic markers. Challenge paradigms are routinely used for other medical conditions and could provide an important approach for the diagnosis and treatment of mood disorders.

Developing an operational biomarker panel of MDD will require significant effort and resources. Successful implementation of a biomarker panel capable of tracking endophenotype signatures and treatment response must

provide comprehensive coverage of multiple biological systems. While putative biomarkers of MDD have been identified, further studies are needed to classify these factors as mediators, moderators, or diagnostic markers. Large network collaborations will be key to obtaining sufficient power as large sample sizes will be essential to define severity and parse MDD into identifiable subtypes. Results obtained from biomarker panels will need to be standardized such that clear associations between these signatures and current clinical definitions of heterogenous subtypes of MDD are readily apparent. In line with these goals, operational definitions of MDD as set forth by the DSM-V and future terminology must recognize and classify depression as multiple disorders. Significant financial resources and sustained investigation will be required as initial biomarker panels are updated and better-performing measures are introduced. As new biomarkers are identified, it is likely that multiple panels will be needed to diagnosis MDD, monitor disease progression/severity, and select an appropriate treatment.

While acute or chronic stressors may induce depressive episodes in some individuals, most people are resilient to these effects. Therefore, it is conceivable that markers of stress resilience may be identified. Recent studies have begun to investigate the biological bases underlying stress resilience with the hope of identifying protective factors that may promote resilience in individuals who cannot successfully adapt to stress (Feder *et al*, 2009). Critical individual differences in resilience to the behavioral and neurochemical effects of stress have been reported (Feder *et al*, 2009). For example, a recent study demonstrated that increased hippocampal BDNF mediates resilience in rodents exposed to chronic stress (Taliaz *et al*, 2011). Moreover, peripheral BDNF administration partially attenuates stress-induced behavioral deficits (Schmidt and Duman, 2010). Taken together, these results suggest that BDNF may serve as a putative resilience marker. Resilience is regulated by neuroadaptations in neural circuits that regulate fear (Bush *et al*, 2007), reward (Cao *et al*, 2010), social behavior (Elliott *et al*, 2010), affect, and mood (Wager *et al*, 2008). Future studies are required to identify molecular substrates that may serve as resilience biomarkers and pharmacotherapeutic targets to promote resilient phenotypes.

In summary, a growing body of evidence indicates that MDD is associated with decreased expression of peripheral/serum growth factors as well as increased levels of circulating cytokines. Antidepressant treatment normalizes or reverses many of these effects. In addition, recent evidence indicates that peripheral/serum BDNF and IGF-1 produce antidepressant effects in behavioral and cellular models of depression, and that blocking IL-1β- and TNF-α-mediated signaling attenuates stress-induced behavioral and cellular deficits in rodents and humans. Therefore, peripheral/serum BDNF, IGF-1, and cytokines may serve not only as biomarkers of MDD and treatment response, but also have functional consequences. The heterogeneity of MDD and concurrent changes in the expression of these peripheral proteins in comorbid psychiatric, immune, inflammatory, and metabolic disorders renders the selection of one individual biomarker for MDD outdated and impractical. Instead, new methods that simultaneously profile a diversity of peripheral biomarkers will inform the diagnosis of MDD, including heterogeneous subtypes and the response to antidepressant treatments.

ACKNOWLEDGEMENTS

We thank Dr Andrew H Miller for critical reading of the manuscript.

DISCLOSURE

Drs Heath D Schmidt and Ronald S Duman report no biomedical financial interests or potential conflicts of interest. Richard C Shelton, MD, has served as a consultant for Eli Lilly & Company, Evotec AG, Forest Pharmaceuticals, Janssen Pharmaceutica, Medtronic Inc., Otsuka Pharmaceuticals, Pamlab Inc., Pfizer Inc., Repligen Inc., and Ridge Diagnostics. He has received research support from Eli Lilly & Company, Euthymics Bioscience, Forest Pharmaceuticals, Janssen Pharmaceutica, Novartis Pharmaceuticals, Otsuka Pharmaceuticals, Pamlab, Pfizer Inc., Repligen Inc., Ridge Diagnostics, and St Jude Medical.

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