Critical Review

The Potential Role of Sensory Testing, Skin Biopsy, and Functional Brain Imaging as Biomarkers in Chronic Pain Clinical Trials: IMMPACT Considerations


*Department of Anesthesiology, University of Rochester School of Medicine and Dentistry, Rochester, New York. 
†Departments of Neurology and Psychiatry, and Center for Human Experimental Therapeutics, University of Rochester School of Medicine and Dentistry, Rochester, New York. 
‡Department of Anesthesiology and Pain Medicine, University of Washington, Seattle, Washington. 
§Division of Neurological Pain Research and Therapy, Department of Neurology, University Medical Center Schleswig-Holstein, Kiel, Germany. 
||Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, Maryland. 
|||Nuffield Department of Clinical Neurosciences, University of Oxford, Oxford, United Kingdom. 
***Center for Pain and the Brain, Department of Anesthesia, Boston Children’s Hospital, Harvard Medical School, Boston, Massachusetts. 
****Department of Anesthesiology, Brigham & Women’s Hospital, Boston, Massachusetts. 
*****Department of Anesthesiology and Internal Medicine, University of Michigan, Ann Arbor, Michigan. 
******Departments of Psychology and Neuroscience, University of Colorado, Boulder, Colorado. 
*******Center for Sensory-Motor Interaction, Department of Health Sciences and Technology, School of Medicine, Aalborg University, Aalborg, Denmark. 
******School of Pharmacy, University of Maryland, Baltimore, Maryland. 
*******LORA Group, LLC, Royal Oak, Maryland. 
********Departments of Public Health, Anesthesiology, Medicine, and Molecular Physiology and Pharmacology, Tufts University School of Medicine, Boston, Massachusetts. 
*********Eli Lilly and Company, Indianapolis, Indiana. 
**********Departments of Epidemiology and Anesthesiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania. 

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Abstract: Valid and reliable biomarkers can play an important role in clinical trials as indicators of biological or pathogenic processes or as a signal of treatment response. Currently, there are no biomarkers for pain qualified by the U.S. Food and Drug Administration or the European Medicines Agency for use in clinical trials. This article summarizes an Initiative on Methods, Measurement, and Pain Assessment in Clinical Trials meeting in which 3 potential biomarkers were discussed for use in the development of analgesic treatments: 1) sensory testing, 2) skin punch biopsy, and 3) brain imaging. The empirical evidence supporting the use of these tests is described within the context of the 4 categories of biomarkers: 1) diagnostic, 2) prognostic, 3) predictive, and 4) pharmacodynamic. Although sensory testing, skin punch biopsy, and brain imaging are promising tools for pain in clinical trials, additional evidence is needed to further support and standardize these tests for use as biomarkers in pain clinical trials.

Perspective: The applicability of sensory testing, skin biopsy, and brain imaging as diagnostic, prognostic, predictive, and pharmacodynamic biomarkers for use in analgesic treatment trials is considered. Evidence in support of their use and outlining problems is presented, as well as a call for further standardization and demonstrations of validity and reliability.

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Key words: Biomarkers, IMMPACT, sensory testing, skin punch biopsy, brain imaging.
and 3 types of assessments that have the potential to be used as biomarkers for pain in analgesic trials: 1) sensory testing (R.B.), 2) skin punch biopsy (M.P.), and 3) brain imaging (I.T.). Sensory testing, skin punch biopsy, and brain imaging were selected because the evidence base for the use of these tools in pain RCTs is the greatest; other potential biomarkers (eg, serum markers, cerebrospinal fluid markers) were beyond the scope of the meeting. Although sensory testing is dependent on patients’ evaluations of sensory experiences and therefore does not meet the strict definition of a biomarker in that it is not entirely objective, we have included it in this review because of its use of standardized stimuli and procedures and its prominent role within current clinical pain research. In addition, although sensory testing is subjective, it directly assesses how a patient feels, functions, or survives.

This article presents evidence-informed recommendations (ie, informed by expert-identified representative research) for the development and potential use of these 3 types of assessments as biomarkers for pain for use in clinical trials of analgesic treatments. These recommendations are on the basis of targeted literature reviews prepared by the 3 content experts (R.B., M.P., and I.T.) before the meeting to identify the key empirical data that support the 3 types of assessments for use as biomarkers in the development of analgesic treatments, as well as evidence showing their limitations. The targeted literature reviews were then presented during the meeting (available on the IMMPACT Web site [www.immpact.org; IMMPACT 2012]), followed by discussion and deliberation regarding what content to include and the appropriate conclusions to draw. After the meeting, additional focused reviews of the literature were conducted to identify further evidence regarding the 3 types of assessments, and these results were incorporated into the article. All literature reviews were conducted by content experts in sensory testing, skin biopsy, and brain imaging as they relate to pain. Each reviewer was asked to select the most representative research regarding the use of these 3 types of assessments as biomarkers for use in analgesic RCTs, regardless of whether the research supported or refuted the use of the tools as a pain biomarker. Formal systematic reviews and meta-analyses were not conducted for each of the 3 types of assessments because such efforts would be substantial initiatives in their own right, and the primary intention of this IMMPACT meeting was to achieve consensus among the broad group of experts on the possible benefits and risks in using the 3 types of assessments as biomarkers in pain treatment trials. Revisions to preliminary drafts of the article were made until agreement was achieved among all authors.

Methods

In June, 2012, IMMPACT organized a meeting to identify potential biomarkers that could be used in clinical trials of analgesic medications and other pain treatments. The meeting participants included international representatives from academia, regulatory and other governmental agencies, industry, and a pain patient advocacy group selected on the basis of expertise in pain clinical research and pain biomarkers. To facilitate discussion, background presentations were delivered regarding regulatory perspectives on biomarkers as outcome assessments (L.B.B., Marc Walton), recommendations for developing biomarkers as surrogate outcomes (J.C.M.),

### Table 1. FDA Biomarker Categories

<table>
<thead>
<tr>
<th>BIOMARKER TYPE</th>
<th>DESCRIPTION</th>
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<tr>
<td>Diagnostic</td>
<td>Identifies whether an individual has a particular disease or biological condition</td>
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<tr>
<td>Prognostic</td>
<td>Evaluates individuals' characteristics to determine the “degree of risk for disease occurrence or progression” (pp 14), and can be used in randomized clinical trials to stratify participants or to select a specific subpopulation to be included in the trial</td>
</tr>
<tr>
<td>Predictive</td>
<td>Assesses baseline characteristics in an attempt to predict the likelihood of treatment response or adverse effects</td>
</tr>
<tr>
<td>Pharmacodynamic</td>
<td>Measures whether an individual has shown a biological response to treatment</td>
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<tr>
<td>Surrogate end point</td>
<td>Pharmacodynamic biomarkers that have been thoroughly tested and have shown that treatment-associated changes in the biomarker closely reflect treatment-associated changes in the outcome of interest (can be used in place of clinical efficacy end points to indirectly evaluate treatment benefit)</td>
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brain imaging) for use as biomarkers in analgesic randomized controlled trials (RCTs).

Currently, there are no biomarkers qualified (ie, considered valid and psychometrically sound) by the FDA or the European Medicines Agency for use in analgesic clinical trials requiring regulatory review, despite their potential usefulness in pain research. If valid and reliable biomarkers were identified, they might aid in the development of analgesic treatments by: 1) identifying additional signs or uncovering pain mechanisms in various pain conditions that could play an important role in the diagnosis and prognosis of pain conditions (ie, diagnostic or prognostic biomarkers), 2) identifying homogeneous subgroups of individuals with specific pain characteristics within a heterogeneous pain condition, potentially identifying patients who may differentially respond to certain treatments (ie, predictive biomarkers), and 3) evaluating response to treatment in clinical trials (ie, pharmacodynamic biomarkers) by providing information regarding biological changes associated with pain and function.

Recommendations

### Sensory Testing

Clinical trials of analgesic treatments typically involve a cohort of participants with a single pain condition.
However, individuals diagnosed with a particular pain condition may not be homogeneous. For example, although the etiology of all neuropathic pain conditions involves damage within the nervous system, the cause and pathogenesis of this damage are generally distinct among neuropathic pain conditions. Furthermore, the pattern of sensory abnormalities in the affected body area varies among neuropathic pain conditions or even within individual patients diagnosed with a specific condition. It has therefore been hypothesized that neuropathic pain is generated not by a single mechanism, but rather by multiple mechanisms operating in concert that lead to phenotypic heterogeneity and potentially to differential treatment response.13 For example, well-characterized neuropathic pain conditions such as postherpetic neuralgia (PHN159), diabetic peripheral neuropathy (DPN160), and trigeminal neuralgia,124 all contain multiple subgroups identified using quantitative sensory testing (QST)-based sensory profiling.

The concept of a phenotype-based methodology for classifying chronic pain has existed for decades, although the terminology has shifted somewhat over time. In a seminal publication, Mitchell Max proposed the potential value of a “physiologically based” categorization system that would identify the specific pathophysiologic mechanisms that account for chronic neuropathic pain and are present to varying degrees in individual patients, which could then be targeted with treatments known to act specifically on those mechanisms.130 Subsequent reviews of neuropathic pain described “mechanism-based” taxonomies on the basis of QST and related assessments of the array of pain mechanisms (eg, sensitization of primary afferents, activation of sympathetic afferents) that contribute to persistent neuropathic pain in some patients.131,183,211,212 More recent overviews of neuropathic pain have emphasized broader terms such as “personalized pain medicine”205 or “patient phenotyping”50 to characterize the interpatient variability in sensory responses, genotypes, psychosocial function, and other domains that have implications for understanding and predicting responses to specific treatments. Importantly, it is not only neuropathic pain conditions that show variability in sensory profiles (eg, Moloney et al,134 and Pfau et al168). In particular, there is substantial heterogeneity across individuals with fibromyalgia (FM) and chronic musculoskeletal pain conditions such as osteoarthritis (OA; eg, Arendt-Nielsen et al,6 Egsgaard et al,52 Frey-Law et al,14 Oudejans et al,142 and Rabey et al157), with similar sensory heterogeneity also observed in patients with systemic inflammatory conditions such as rheumatoid arthritis.92 Moreover, because no particular QST profile is unique to a given pain diagnosis (eg, Freeman et al63), these “trans-etiological” patterns of sensory symptoms and deficits may reflect distinct pain mechanisms, and it may be possible to group together individuals with similar sensory profiles diagnosed with these conditions. Consequently, classifying and treating pain on the basis of either its etiology or symptomatology alone may not be the optimal approach for chronic pain conditions. An alternative approach to classification—analyzing pain on the basis of the underlying mechanisms—may lead to improved treatment responses and outcomes.61 Although the mechanisms of pain cannot be directly examined in humans, the expression of sensory abnormalities (ie, the individual somatosensory phenotype or sensory profile) might provide insights into the pathophysiological dysfunctions of afferent processing. The question for the future is whether sensory testing might reduce the heterogeneity of pain mechanisms within chronic pain conditions by subdividing individuals on the basis of their sensory phenotypes. If this subgrouping approach is successful, novel treatment interventions could be tested in the appropriate, mechanistically homogeneous subgroups of patients, thereby improving assay sensitivity within clinical trials.

**Sensory Testing as a Diagnostic Measure**

QST refers to a set of psychophysical methods used to assess somatosensory function in the central and peripheral nervous systems. These sensory testing procedures use standardized response scales to evaluate responses to calibrated innocuous or noxious stimuli (eg, graded von Frey hairs, pinprick stimuli, pressure algometry, contact thermal testing). Such tests are frequently described as representing an extension of the bedside clinical examination.10 QST has been used for decades in a variety of settings, often for the purpose of diagnosing and monitoring sensory neuropathies and pain disorders, elucidating pain mechanisms, and characterizing individual differences in pain sensitivity and pain modulation.10,50,147 It has been most widely used for testing of cutaneous sensations, to quantify positive sensory symptoms (eg, allodynia and hyperalgesia) or negative sensory deficits (eg, hypoesthesia and hypalgesia), but it has also been adapted to test sensations from deep tissue and viscera, allowing broad application to an array of pain conditions (eg, Arendt-Nielsen et al,5 Farmer et al,150 and van Wanrooij et al194).

A standardized QST protocol was developed by the German Research Network on Neuropathic Pain (DFNS) that includes 13 sensory testing parameters (ie, cold and warm detection thresholds, paradoxical heat sensations, thermal sensory limen procedure, cold and heat pain thresholds, mechanical detection threshold, mechanical pain threshold, mechanical pain sensitivity, dynamic mechanical allodynia, wind-up ratio, vibration detection threshold, pressure pain threshold) for the analysis of the somatosensory phenotype of individuals with neuropathic pain.158 To evaluate pathological ranges of positive or negative signs, an age- and sex-stratified database of healthy individuals was established, including absolute and relative QST reference data.125,158 Comparing the sensory testing data obtained from individuals with chronic pain conditions with a normative database may more accurately identify sensory abnormalities than comparing with a “nonaffected” site within the patient which may also exhibit abnormalities (see Konopka et al,150 and Shenker et al67 for examples). Currently, this multicenter database comprises complete sensory profiles of more than 300 healthy human subjects and more than 2,000...
Sensory Testing as a Prognostic Measure

Recent research by Petersen and colleagues \(^{144}\) showed a positive relationship between the sensory profiles of individuals with painful knee arthrosis before knee replacement and postsurgical pain (ie, <3 on the visual analog scale vs ≥3 on the visual analog scale) 12 months after surgery. In this study, temporal summation of pain, a psychophysical index of pain facilitation evaluated by measuring increase in the perception of pain intensity when applying a series of identical noxious stimuli, \(^{174,175}\) was the strongest QST-based predictor of long-term postsurgical pain outcomes. Similar findings showing that preoperative pain sensitivity is associated with elevated report of pain after joint replacement have also emerged in other research, highlighting the consistency of this effect. \(^{154,213}\) Additionally, prehysterectomy brush-evoked allodynia has been shown to be statistically significantly associated with pelvic pain 4 months postsurgery. \(^{27}\) Martinez and colleagues have further shown that the area of hyperalgesia after iliac crest bone harvest positively predicts chronic postsurgical neuropathic pain. \(^{129}\) Beyond the use of sensory testing to predict postsurgical outcomes, sensory testing may be beneficial as a prognostic indicator of disease progression and severity. For example, in patients with early-stage diabetes, thermal and vibratory sensory deficits have been prospectively linked with subsequent severity and painfulness of the neuropathy that develops. \(^{47,104}\) and in later-stage...
diabetes, QST-assessed sensory deficits predict outcomes such as foot ulcerations, amputations, and health care costs.\textsuperscript{58,166} Similar findings appear within the context of chemotherapeutic administration, because pretreatment mechanical sensory deficits have been shown to predict the severity and painfulness of neuropathy after oxaliplatin treatment for colorectal cancer.\textsuperscript{199} These findings, across an array of settings from orthopedic surgery to oncology, highlight the potential prognostic value of sensory testing to identify profiles that may have implications for future trajectories of pain-related symptoms.

**Sensory Testing as a Predictive Measure**

Using sensory profiling as a predictive biomarker that can identify treatment-responsive subgroups requires a 2-step approach: 1) exploratory (ie, baseline sensory profiling with exploratory analyses of treatment effects to identify likely responders), and 2) confirmatory (ie, prespecification of subgroup analyses or enrichment of the cohort on the basis of sensory profiles in a prospective RCT). In the initial step, baseline sensory profiling is performed in clinical trial participants. Exploratory analyses of treatment effects are then performed to examine differences between subgroups determined using baseline sensory profiling.\textsuperscript{9,51,82,141,155,169,204}

For example, in secondary analyses of a published trial, Edwards and colleagues\textsuperscript{51} reported that among individuals with PHN, high heat pain thresholds predicted responsiveness to opioids, but not to tricyclic antidepressants. It may also be possible to analyze nociceptor function and sensitization in the affected skin of individuals with neuropathic pain by cutaneous application of capsaicin.\textsuperscript{145} In a randomized placebo-controlled trial with topical clonidine in painful DPN, participants’ sensory profiles were assessed during screening using the capsaicin response test (.1% topical capsaicin), in which increases in spontaneous pain presumably indicate intact, but sensitized nociceptors.\textsuperscript{32} Using an intention-to-treat analysis, no statistically significant difference was observed in pain intensity reduction between participants who received clonidine (.65 mg clonidine gel applied to each foot 3 times per day) compared with those who received placebo. In secondary analyses comparing capsaicin responders with nonresponders, clonidine appeared to reduce pain intensity in participants with a capsaicin response (ie, those with presumably functional, sensitized nociceptors).\textsuperscript{25} Sensory profiling has also been shown to predict responses to nonpharmacologic therapies such as acupuncture. FM patients with higher pressure pain thresholds exhibited a differential response to real and sham acupuncture whereas participants with lower thumb pressure pain thresholds did not.\textsuperscript{76}

Sensory profiling can also identify subgroups with altered endogenous pain modulation, helping to predict treatment outcomes of drugs and other interventions that affect a given mechanism.\textsuperscript{6} In a study of pain modulation in DPN, individuals with abnormal conditioned pain modulation (CPM), assessed by measuring the amount of inhibition of pain perception using QST during simultaneous administration of a conditioning painful stimulus at a distant body site, exhibited greater reductions in pain intensity from duloxetine (a serotonin noradrenaline reuptake inhibitor believed to increase activation of descending inhibitory pain pathways) than individuals with normal pain modulation, although there was no comparison with placebo in this open-label study.\textsuperscript{215} In another open-label pilot study, enhanced central sensitization (ie, pain hypersensitivity) assessed using QST as well as decreased CPM at baseline predicted lower pain intensity 3 months after implantation of a spinal cord stimulator.\textsuperscript{31} In contrast to the inverse association between basal CPM function and analgesia in response to these central nervous system (CNS)-focused treatments, a recent open-label study of topical diclofenac for knee OA patients indicated that more robust pretreatment CPM predicted better analgesic responses to this peripherally applied nonsteroidal anti-inflammatory drug.\textsuperscript{199}

After identifying a potential sensory profile that may predict treatment versus placebo differences, the confirmatory step involves conducting a clinical trial in which participants are prospectively stratified according to the information gathered from the initial study regarding the relationship between sensory profiles and treatment efficacy.\textsuperscript{64} Recently, the DFNS approach to QST subgrouping was used in a study by Demant and colleagues\textsuperscript{42} to test the effect of a treatment in individuals with 2 specific pain phenotypes defined a priori. The investigators reported that individuals with an “irritable nociceptor” peripheral neuropathic pain phenotype (ie, hypersensitivity and cold and warmth detection thresholds within the range of normal values, suggesting preserved small nerve fiber function; see Fields et al\textsuperscript{45}) had a significantly better response to oxcarbazepine, a drug with sodium channel blocking properties, versus placebo than individuals with a “nonirritable nociceptor” peripheral neuropathic pain phenotype.\textsuperscript{42} However, a follow-up study of individuals with peripheral neuropathic pain failed to show a greater reduction in pain intensity for those with the “irritable nociceptor” phenotype compared with those without this phenotype who were treated with a lidocaine patch\textsuperscript{61}; this study may not have had sufficient power to detect differences between phenotypes.\textsuperscript{19,61} In a subgroup analysis from a clinical trial, individuals with PHN whose small nerve fiber function was impaired on the basis of sensory testing responded well to lidocaine compared with placebo, whereas there was no statistically significant difference between lidocaine and placebo for those with intact small nerve fiber function, results that were contrary to what was expected.\textsuperscript{204} In addition, Mainka and colleagues\textsuperscript{27} hypothesized, but did not find, a relationship between capsaicin-induced reductions of small fiber function, identified by sensory testing results, and pain relief among individuals with peripheral neuropathic pain. Interestingly, the presence of cold and pinprick hyperalgesia seem to be predictive of response to capsaicin, although this open-label study did not have a control group. A recent placebo-controlled study in patients...
with peripheral neuropathic pain revealed that the presence or severity of allodynia as well as limited thermal deficits on the basis of QST predicts the response to intracutaneous botulinum toxin type A treatment.

Several earlier studies in patients with chronic, peripheral, post-traumatic neuropathic pain have suggested that thermal sensory tests are predictive of long-term responses to sympathetic blockade, and may be especially useful in identifying mechanisms such as sympathetically-maintained pain. Although these trials were exploratory and did not include control groups, strong relationships were observed between the degree of pain relief produced by sympathetic block and the presence of baseline cold hyperalgesia in the painful area, as well as between the degree of pain relief produced by sympathetic block and the block-related degree of change in cold hyperalgesia.

The results highlighted in this report suggest the potential value of using sensory profiling to determine which individuals may obtain substantial benefit from a given treatment, although replication is needed to confirm the results of exploratory and nonplacebo controlled trials. In addition, although the "irritable nociceptor" phenotype as a predictor of treatment response is theoretically plausible, its value in identifying individuals who will benefit from a treatment has not been solidly established. Furthermore, we are aware of only a small number of studies supporting the use of sensory testing as a predictor of treatment response for non-neuropathic pain conditions. In addition to those previously described in FM and OA, one pharmacologic trial in low back pain appears to be ongoing, and several exploratory studies in migraine have linked a phenotype of persistent mechanical allodynia with a reduced analgesic response to triptans, but these results await replication in controlled studies.

Sensory Testing as a Pharmacodynamic Measure

Sensory assessment has the potential to be used as an indicator of treatment response. Using QST as an outcome parameter, one RCT of intravenous lidocaine in individuals with peripheral nerve injury showed decreases in mechanical hyperalgesia and mechanical allodynia from baseline to end of treatment. Another RCT reported decreased mechanical allodynia and decreased cold pain thresholds among individuals with neuropathic pain 14 weeks after intradermal administration of botulinum toxin type A. Yarnitsky and colleagues reported a statistically significant negative association between improved CPM and participant reports of the effectiveness of open-label duloxetine among individuals with DPN.

Interestingly, several recent studies have followed up on these CPM findings using placebo-controlled designs. In one trial, patients with DPN were randomized to receive either sustained-release tapentadol or placebo for 4 weeks. At baseline these patients did not show a significant CPM response, but patients randomized to tapentadol subsequently developed significant CPM (ie, activation of the descending inhibitory pain pathways), the magnitude of which corresponded to the degree and temporal course of patients' reduction in their neuropathic pain. Similarly, a trial of pregabalin in patients with chronic pancreatitis reported that although pregabalin did not produce a mean increase in CPM relative to placebo, CPM did show a selective treatment-related improvement for pregabalin responders. That is, patients whose pain was reduced significantly by pregabalin also showed a significant increase in CPM. These findings highlight the potential for changes in CPM to serve as a biomarker of analgesic response in diverse pharmacologic trials and suggest that, particularly for individuals with neuropathic pain, sensory testing may serve as a useful pharmacodynamic measure of target engagement or treatment efficacy, although further confirmation of these results is necessary. Additionally, the value of sensory testing in showing biological treatment responses should be explored in other chronic pain conditions to determine the generalizability of the findings.

Sensory Testing as a Biomarker in Analgesic Trials?

Although sensory profiling involves patient reports of their experiences rather than being a purely objective test, it has the potential to yield a stratified treatment approach for individuals with chronic pain conditions and ultimately to personalized pain medicine. Individuals' sensory profiles may aid in the diagnosis of chronic pain conditions, in addition to identifying subgroups within neuropathic pain conditions that could then be used to select patients who may respond well to a particular pain treatment. Further, sensory profiling may provide outcome measures for evaluating treatment efficacy. Additional research is needed to show the validity and reliability of sensory profiling for these purposes and to expand the understanding of the role of sensory testing in non-neuropathic pain conditions. In addition, it would be aided by the development and application of a well defined taxonomy of sensory phenotypes. For example, the "irritable nociceptor" phenotype of neuropathic pain, generally applied to subgroups of patients with evidence of sensitization and preserved small fiber function in the area of pain, was originally defined using capsaicin application, but more recently it has been used to describe subgroups showing evidence of thermal and/or mechanical hyperalgesia. Greater precision in terminology will likely help facilitate the application of sensory testing.

Skin Biopsy

Skin punch biopsy as usually performed involves 3-mm wide skin biopsies that are sectioned at intervals of 50 μm, yielding approximately 55 sections per sample. Of these, 3 to 4 sections are systematically selected throughout the skin biopsy to provide a representative sample. Sections are immunohistochemically stained with a panaxonal marker directed against ubiquitin carboxy terminal hydrolase (ie, protein gene product [PGP] 9.5 antibody), a highly specific and abundant protein in neurons, to

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reveal intraepidermal nerve fibers. A Nerve fibers are quantified using a standard methodology that has robust sensitivity, specificity, and positive predictive value. This analysis of nerve fibers resulting from skin punch biopsy will hereafter be referred to as “skin biopsy.” Skin biopsy assessments of epidermal innervation can identify neuronal loss, which may serve as a biomarker for neuropathy and neuropathic pain.

### Skin Biopsy as a Diagnostic Measure

Skin biopsy may be a useful tool to diagnose small fiber neuropathy (SFN), which is a common source of chronic neuropathic pain that is often difficult to diagnose. The clinical presentation of SFN typically involves pain in the feet and distal legs, as well as symptoms such as burning, stinging, and electric shock. However, ankle reflexes, vibratory thresholds at the toe, and normal toe proprioception are often preserved. Distal pinprick sensation and temperature sensation can be reduced, but it is common for individuals with SFN to have near-normal examination and normal nerve conduction velocity test results; therefore, SFN often escapes detection. Skin is more likely to be affected in distal length-dependent processes, making skin punch biopsy a potentially useful tool to identify distal neuronal abnormalities, such as SFN.

Comparing intraepidermal nerve fiber density (IENFD) among individuals with SFN with established reference values in the distal leg has allowed these individuals to be routinely diagnosed.

Several studies have investigated skin biopsy as a diagnostic tool for SFN. McArthur et al developed reference standards for nerve fiber density among normal control participants and individuals with sensory neuropathy. Using a cutoff of the fifth percentile in normal control participants, IENFD in the distal leg had a positive predictive value of 75%, a negative predictive value of 90%, and correctly identified individuals with SFN to have near-normal examination and normal toe proprioception. These findings have been supported by other studies, showing that skin biopsy alone more accurately identifies neuropathy and neuropathic pain conditions as well. The loss of small, unmyelinated nerve fibers can be seen in individuals with transthyretin familial amyloid polyneuropathy before large fiber involvement becomes evident. In patients followed longitudinally before, during, and after oxaliplatin chemotherapy, IENFD testing performed as well as a composite score of signs, symptoms, and nerve conduction results in assessing the progression of chemotherapy-induced peripheral neuropathy.

IENFD also had the greatest sensitivity in identifying chemotherapy-induced peripheral neuropathy compared with QST and nerve conduction studies. However, the combination of IENFD, QST, and a clinical neurological examination has been shown to more accurately identify HIV sensory neuropathy (on the basis of 2 of 3 abnormal test results: clinical examination, DFNS QST protocol, and skin biopsy) than the individual test components or combinations of 2 test components.

Compared with healthy control subjects, individuals with FM exhibited decreased IENFD, although increased innervation in arteriole-venule shunts, in which C and A fibers are dense, has been reported within palm skin biopsies. A recent study reported that individuals with pachyonychia congenita, a dermatologic condition characterized by thick, abnormally shaped fingernails and toenails and pain in the feet, had significantly higher Merkel cell densities, as well as significantly lower mechanical pressure pain threshold, in the affected skin (ie, ball of the foot) compared with a nonaffected area (ie, arch of the foot), as well as compared with the ball of the foot in healthy control subjects.

Karlsson and colleagues reported that in individuals with painful distal symmetrical polyneuropathies, decreases were seen in IENFD compared with healthy control participants, as well as decreased epidermal and dural nerve fiber length densities and increases in the number of nerve fiber swellings. They also reported high sensitivity and specificity in diagnosing polyneuropathy using a clinician diagnosis as the reference standard when IENFD and epidermal nerve fiber length densities were combined. However, recent results from an observational study showed no differences in IENFD between individuals with painful and painless DPN.

Although IENFD has promise as a diagnostic tool, it is important to recognize that in many of the data presented, IENFD was used to diagnose peripheral neuropathy alone or in combination with other test components. Future research is needed to further evaluate the role of IENFD in the diagnosis and management of neuropathic pain.
neuropathies that may or may not involve pain, rather than specifically to diagnose pain conditions themselves. In order to utilize IENFD as a diagnostic biomarker, additional research is needed that focuses specifically on the identification of pain conditions. Further research should also seek to validate the use of IENFD as a diagnostic tool for FM.

Skin Biopsy as a Prognostic Measure

An advantage of the skin biopsy technique is that it offers a direct window into nerve fiber morphology. Nerve fiber swellings are often observed in areas proximal to regions experiencing neuropathy and pain symptoms, and may represent a predegenerative change.80 In fact, Ebenezer and colleagues48 reported that swellings often contain abnormal mitochondria and watery axoplasm, signs that are linked to degeneration. Swellings have been associated with decreases in IENFD over time among individuals with neuropathic pain,68,117 as well as with decreased time to development of symptomatic neuropathy for individuals with HIV.81 Wendelschafer-Crabb and colleagues206 observed that a significantly greater number of subjects in both the painful and painless groups had nerve fiber swellings in the thigh than did healthy control subjects, and that individuals with SFN had significantly more swellings in the distal leg than did healthy control subjects. Together, these studies indicate that the assessment of nerve fiber morphology may predict future neuropathy, and in some cases, neuropathic pain. To advance IENFD as a prognostic measure for chronic pain conditions, additional research is necessary to identify the specific features that predict chronic pain or chronic pain progression (eg, number of swellings, location of swellings).

Skin Biopsy as a Predictive Measure

There is some evidence that skin biopsy results may be used to predict an individual’s likelihood of treatment benefit. In an early study, Rowbotham and colleagues160 assessed the degree of epidermal innervation among individuals with PHN, and reported that those with neuropathic pain and preserved sensation had less severe epidermal denervation assessed with skin biopsy. Rowbotham and Fields159 have also shown that PHN subjects with preserved sensation respond well to topical treatments. Taken together, the results of these 2 studies suggest that IENFD assessment may predict treatment response. However, in a retrospective review of individuals with painful neuropathies treated on an open-label basis with the 5% lidocaine patch, no relationship was seen between the response to lidocaine and IENFD.82 The investigators also noted that several individuals with denervated distal leg biopsies still reported an analgesic effect, suggesting that epidermal innervation may not play a role in response to lidocaine,82 although this interpretation of the data is limited by the absence of a placebo group. These conflicting results indicate that there is insufficient evidence to support the use of IENFD to predict treatment response.

Skin Biopsy as a Pharmacodynamic Measure

Several studies have assessed the association between IENFD and the experience of pain. Individuals with HIV-associated peripheral neuropathy who exhibited lower IENFD at the distal leg experienced greater pain than those who had greater IENFD.150,218 In Fabry disease, individuals who never reported pain were statistically significantly more likely to have normal distal leg IENFD than those with Fabry-related pain.185 Others have reported similar findings in individuals with SFN and diabetes.152,173 Epidermal nerve fiber length in individuals with diabetes was statistically significantly reduced in those with painful compared with painless neuropathy.152 Surprisingly, Schley and colleagues161 reported that greater IENFD was positively correlated with neuropathic pain, although this was in a small number of individuals with neuropathic pain (n = 36). In contrast, others have noted more severe epidermal nerve fiber loss only in those with DPN pain and few objective neuropathy signs; less difference was observed in IENFD between individuals with more severe neuropathy who did or did not experience pain.174 In addition, Cheng and colleagues36 recently observed no differences in IENFD between individuals with DPN who did or did not have pain, although other differences were seen in the biopsy results, such as higher ratios of markers of axonal regeneration (growth-associated protein 43) and higher numbers of swellings in subjects with pain compared with those without pain. Studies of neuropathy caused by trauma84 and peripheral neuropathy184 reported no differences in IENFD between individuals who did and did not have neuropathic pain. Although IENFD has been shown in some studies to be associated with neuropathic pain, a well characterized, direct relationship between IENFD and pain severity has not been established.

SFN is a common complication of Fabry disease. Studies in individuals with Fabry disease have assessed the effect of enzyme replacement using skin biopsies. In baseline skin biopsies from participants in clinical trials of a recombinant human enzyme (α-galactosidase A [r-haGalA]), accumulations of globotriaosylceramide (GL-3; a neutral glycosphingolipid that leads to neuronal dysfunction when it accumulates) are seen in multiple dermal cell types in individuals with Fabry disease.180,185 Five months of r-haGalA treatment resulted in complete clearance of GL-3 from the superficial capillary endothelium in all individuals in the treatment group and in only 1 individual (3%) in the placebo group, a statistically significant difference. The placebo group achieved similar results after 6 months of r-haGalA in the open-label extension and the capillary endothelium remained free of GL-3 for up to 30 months into the extension study among 98% of those who underwent biopsies.180 These findings suggest that skin biopsy can assess the physiologic effect of treatments for SFN in Fabry disease, although it is important to acknowledge that this evidence supports skin biopsy in evaluating treatment effects on peripheral neuropathy in general, rather than pain specifically. In a separate but similarly treated
cohort, there was some evidence that improvements in IENFD may occur after enzyme replacement in individuals with normal renal function; once again, this finding is not specific to identifying a treatment effect on pain. Among individuals with peripheral neuropathy and DPN pain, exercise interventions were associated with increased IENFD and decreased pain, suggesting that IENFD counts may be useful as indicators of analgesic efficacy.

Case reports have also shown the utility of skin biopsy to detect clinical changes. In one case report of acute diabetic truncal neuropathy, onset of symptoms was associated with denervation on the symptomatic side, whereas resolution of symptoms was associated with reinnervation of the ipsilateral side to a degree similar to that in the unaffected contralateral side. Similarly, an individual with burning feet and SFN due to an autoimmune condition experienced concurrent improvement in IENFD and SFN symptoms with steroid therapy. It is important to replicate these findings and expand them to other conditions to determine the specific changes occurring within the epidermis that are associated with neuropathy. Identifying the degree of neuronal regeneration that is associated with clinically meaningful improvements is critical as well. Furthermore, the current evidence is mixed regarding whether IENFD can be used as a pharmacodynamic indicator of chronic pain.

**Skin Biopsy as a Biomarker in Analgesic Trials?**

Skin biopsy with IENFD assessment has been used in different capacities in neuropathy and neuropathic pain clinical trials. IENFD has emerged as a sensitive and efficient diagnostic tool to identify individuals with SFN, and it may be useful in the early diagnosis of other neuropathic conditions. Observations of nerve fiber swellings may indicate the development of neuropathy as well. Research also suggests that skin biopsy can identify features in the neural environment (eg, swellings, accumulations of glycosphingolipids), as well as changes in those features, that are related to neuropathy. However, the available research does not provide firm evidence supporting the use of skin biopsy to predict treatment benefit or to distinguish between individuals with neuropathy who will or will not experience pain. Future research is needed to show the value of skin biopsy as a biomarker for chronic pain conditions, as well as to replicate and further validate the findings presented.

**Brain Imaging**

Developments in neuroimaging over the past 2 decades have given us insight into the human CNS in healthy and diseased states. Many tools to interrogate the neurochemistry, structure, wiring, and function of the preclinical and human CNS now exist that can unpack spatial, temporal, and molecular processes. In this section we discuss functional magnetic resonance imaging (fMRI), positron emission tomography (PET), and proton magnetic resonance spectroscopy (1H-MRS) because these are widely available and provide complementary information on brain function and underlying molecular action.

Neuroimaging provides neurochemical, structural, or functional information that can measure how the brain processes and modulates nociceptive inputs to produce pain perceptions. Neuroimaging outcomes can be distinct from, as well as related to, patient-reported descriptions of pain. Indeed, many factors contributing to subjective pain ratings are unrelated to the nociceptive input or the presence of strong analgesics. For example, chronic pain is often accompanied by other nonpain comorbidities such as depression, fatigue, sleep disturbances, and poor cognition, as well as psychological processes such as catastrophizing, which may affect patients’ reporting of their chronic pain and the processing of pain in the brain. Because of this, complex relationships exist between patients’ pain self-reports and their concurrent regional brain activity. Although there is not necessarily a straightforward 1-to-1 mapping between neuroimaging and subjective pain reports, neuroimaging can provide insights about the neurophysiologic basis for pain experience and pain’s physiological effects on the brain. It is important to determine specific brain targets that are relevant to pain across individuals because modulation of activation in these target areas may provide evidence that a compound has target engagement or is attenuating nociceptive processing (eg, Harris et al, Tracey, and Wanigasekera et al).

A major challenge in identifying brain predictors of specific outcomes such as pain is that the smallest unit of analysis in fMRI, the “voxel” (approximately 1/100,000 of the volume of the brain), integrates activity across hundreds of thousands to millions of neurons with distinct functional properties. Therefore, there is a many-to-one mapping between physiological and psychological processes and activity in each voxel, which complicates the use of voxelwise activation as a marker for pain. Recent magnetic resonance imaging approaches have capitalized on this highly connected network by using the “interconnectedness” of the brain as a marker in and of itself to provide insights into chronic pain and responses to treatment. Because brain images are a complex source of information, consisting of 50,000 to 200,000 voxels reflecting activity in spatially distributed systems across the brain, corrections need to be made for multiple comparisons across these interconnected voxel activities to avoid false positive results.

Furthermore, what constitutes replication in neuroimaging studies has been unclear. Regions that are often treated as units of analysis in publications (eg, amygdala, dorsal anterior cingulate) include hundreds or thousands of voxels, and therefore replicating relationships between activation of general brain regions and self-reported pain experiences may make it difficult to find sufficient sensitivity or specificity. However, large meta-analyses have successfully combined multiple brain imaging trials, showing consistent results for pain conditions like FM. In addition, analyses of pooled fMRI data from studies using different imaging equipment and
analysis procedures have produced informative and consistent inferences about brain structure and function in chronic pain disorders.\textsuperscript{80,93,99} Emerging approaches to imaging analysis, such as support vector machines, may also prove useful in addressing concerns about replication.\textsuperscript{77,123,196} These statistical approaches typically divide participant data into 2 groups: 1) ‘training’ groups, used to estimate a distributed pattern that serves as a provisional brain marker, and 2) ‘test’ groups, used to evaluate its predictive accuracy. “Cross-validation” is a technique that repeatedly and systematically splits a sample into training and testing groups to provide minimally biased estimates of predictive accuracy for new samples (eg, Brodersen et al,\textsuperscript{28} and Wager et al\textsuperscript{196}). In addition, patterns that are trained to generalize across individuals can be tested prospectively in new individuals and samples (eg, Wager et al\textsuperscript{196}). Because all participants undergo the same procedures, this strategy provides a replication of the brain marker in a different group of subjects. Such an approach was effectively used in a recent proof of concept investigation showing that brain neuroimaging could be used as a sensitive marker of pharmacologic treatment of pain.\textsuperscript{46}

In addition to using fMRI to identify brain targets associated with the functions of sensing, processing, and modulating pain, other neuroimaging techniques can noninvasively assess the relative contribution of brain neurotransmitters and their receptors. For example, \textsuperscript{1}H-MRS is the only current approach that can assess the relative concentrations of neurotransmitters such as gamma aminobutyric acid and glutamate (through glutamate-glutamine). This suggests that \textsuperscript{1}H-MRS may be beneficial in evaluating analgesic compounds thought to work on a specific class of neurotransmitters. In addition, PET analyzes how strongly mu-opioid receptors bind to endogenous opioids using radioactively labeled carfentanil, and this methodology has the potential to identify whether opioid analgesics are binding to opioid receptors (eg, Greenwald et al\textsuperscript{69} and Zubieta et al\textsuperscript{219}). Moreover, PET imaging may provide information about the consequences of pharmacologic opioid use such as habituation, desensitization, and opioid-induced hyperalgesia. The value of PET and \textsuperscript{1}H-MRS lies in their assessment of molecular processes, and as such, these techniques may be informative in developing analgesic drugs and identifying treatment response.

Brain Imaging as a Diagnostic Measure

Pain neuroimaging has been increasingly used in clinical trials and there has been greater focus on diagnostic properties. However, as neuroimaging has been increasingly used in clinical studies across medicine, there has been greater focus on the diagnostic value of brain patterns for clinical pain conditions (eg, Baliki et al\textsuperscript{11}) and treatment responses (eg, Harris et al\textsuperscript{71} and Wanigasekera et al,\textsuperscript{201,205}). For example, reduced gray matter volume and abnormalities in white matter and brain connectivity have been observed in individuals with chronic pain conditions, but not among healthy controls\textsuperscript{12,13,57,87,97,107,165} with changes reversing upon resolution of the pain condition.\textsuperscript{17,24,44,53,71,79,117,136,156} In comparing brain activity in individuals with CLBP, pelvic pain, OA, and PHN, different regions were reported to be activated in the experience of spontaneous pain between the distinct chronic pain conditions. These regions of brain activation may also distinguish chronic pain conditions from the acute pain experienced by individuals without chronic pain.\textsuperscript{2} In addition, imaging of human experimental medicine models of chronic pain symptoms as well as small cohort patient studies have the potential to provide clues to the underlying mechanisms sustaining and exacerbating a chronic pain state (eg, central sensitization, alterations in descending pain modulation, anxiety, catastrophizing, depression [for reviews, see Lee and Tracey,\textsuperscript{116} Mansour et al,\textsuperscript{128} and Tracey\textsuperscript{181}; for examples, see Gwilym et al,\textsuperscript{72} Lee et al,\textsuperscript{115} and Schweinhardt et al\textsuperscript{163,164}]). In addition, patterns of connectivity within the somatosensory cortex as well as between the somatosensory cortex and sensory-discriminative and affective pain processing regions of the brain have been shown to differ between individuals with FM and healthy control subjects.\textsuperscript{100} Loggia and colleagues\textsuperscript{123} similarly reported distinctions between individuals with CLBP and healthy control subjects in the connectivity patterns of the default mode network (DMN) and regions within the insula. \textsuperscript{1}H-MRS has been used to show increased levels of the excitatory neurotransmitter glutamate-glutamine and decreased concentrations of the inhibitory neurotransmitter gamma aminobutyric acid in the insula of FM patients compared with healthy control subjects,\textsuperscript{62,74} implying that this may be an “overreactive” pain-promoting region within the brain. Heightened activity in the insula may in turn influence its connectivity to networks such as the DMN.\textsuperscript{7} Indeed, there is a striking association between the magnitude of self-reported clinical pain and connectivity between the insula and the DMN across multiple chronic pain conditions (eg, FM, CLBP, pelvic pain),\textsuperscript{7,121,136} Emerging work has also begun to explore the relationship between connectivity in different brain networks and chronic pain expression (eg, Hemington et al\textsuperscript{179}). Although these findings are promising, more research is needed to replicate these results and to determine whether brain imaging can also reliably distinguish between different chronic pain conditions.

Brain Imaging as a Prognostic Measure

There are a growing number of studies deploying imaging-based measures to ascertain whether a subject displays a CNS-related ‘vulnerability’ toward or ‘resilience’ against developing a persistent pain state after injury, infection, or other factors that trigger the onset of chronic pain. Measures of connectivity between the medial prefrontal cortex and nucleus accumbens have identified individuals with subacute back pain who developed CLBP with moderate accuracy (81% discrimination between those who did and did not develop CLBP in a holdout sample\textsuperscript{11}). A positive relationship has
been reported between DMN and insula connectivity and increases in chronic FM pain and CLBP. In addition, Wasan and colleagues showed an increase in blood flow in sensory-discriminative and affective pain processing regions was associated with worsening of CLBP during a painful task. Neural systems that appear relevant to prognosis include reward, learning, and motivational networks, as well as aberrations in the descending pain modulatory system (see Denk et al for a review of pain vulnerability and resilience). Brain connectivity may aid in determining the prognosis of chronic pain conditions. Confirmation of these results, particularly showing that the specific patterns of brain connectivity uniquely predict increases in pain, is needed.

**Brain Imaging as a Predictive Measure**

A challenge for neuroimaging is to deliver information at an individual level that can predict treatment response on the basis of neuroimaging markers related to known pain mechanisms, rather than group averages of neural responses. Recent studies highlight how neuroimaging measures (ie, functional, network, and neurological) at baseline and before treatment can contribute to the prediction of treatment responses (including placebo) on behavioral as well as neuroimaging assessments. These studies suggest that neuroimaging may allow for participant stratification that could improve assay sensitivity in clinical trials, as well as advance the development of personalized pain medicine. Going forward, it will be important to replicate these results to determine the precise patterns of functional, neurochemical, and structural findings that can be used to phenotype patients as a way to predict treatment response.

**Brain Imaging as a Pharmacodynamic Measure**

Brain imaging can be used to identify areas of the brain in which there are changes when an individual experiences pain, as well as to evaluate CNS penetration or to define the regions or circuits upon which an analgesic treatment works. Becerra and colleagues have shown similar brain responses to pain across species using fMRI, but additional studies are needed to further define the utility of this approach. Pharmacological magnetic resonance imaging (phMRI) examines changes in the brain produced by a medication and has been used to evaluate analgesic medications in preclinical research (see Jenkins for a review). Furthermore, phMRI in rats and humans has shown activation of similar brain regions in response to buprenorphine. Additional analgesic medications have been evaluated using phMRI in rats to study chronic pain models and the effects of the medication on brain systems (eg, gabapentin in a spinal nerve ligation model; celecoxib in an OA model; buprenorphine in healthy rats; ketamine in healthy rats; and remifentanil in healthy rats). Using phMRI in preclinical models of chronic pain may also assist in identifying the circuits involved in adverse events.

The use of imaging in the early phases of clinical drug evaluation is increasing as well, and may enhance the information gathered from the trial. For example, imaging in clinical research may provide a number of novel insights regarding: 1) CNS penetration and effectiveness of drug dosing alongside pharmacokinetic information; 2) definition of targeted circuits; and 3) potential efficacy, on the basis of neural circuit activation and connectivity during drug administration compared with placebo. Brain imaging in early-phase clinical trials may be beneficial in that it could help to identify individuals who respond to treatments, and it may contribute to the understanding of a treatment’s temporal course (functional and morphological), potential long-term negative effects (eg, opioids), and disease modification. In addition, when individuals with long-term chronic pain involving measurable changes in neurochemistry and the neural structure undergo treatment for their pain, these changes have been seen to reverse, resulting in brains that appear “normal” upon imaging. Interestingly, research using PET has shown a nonpharmacologic pain treatment for FM is associated with a “resetting” of the opioid receptor binding ability to levels comparable with pain-free control subjects. It is important to consider the challenges inherent in implementing imaging in large multinational multicenter trials (eg, standardization across sites), although emerging research suggests that standardization is possible across sites. Although promising, at this stage there is limited evidence supporting brain imaging as a pharmacodynamic biomarker for pain in clinical trials.

There are several promising neuroimaging techniques emerging for identifying the experience of pain. For acute nociceptive pain, a distributed pattern of regions (the “neurologic pain signature” [NPS]) that matches well with prototypical ‘pain processing’ regions in humans and animals can track the magnitude of pain. When 2 stimuli are moderately different in pain intensity (eg, 2 points on a 10-point numerical rating scale), NPS activity predicts which stimulus is more painful for an individual with 90 to 100% accuracy. Recent research has shown that the NPS generalizes across distinct types of pain (ie, heat, mechanical, and shock). In addition, activation of the NPS was shown to have 90% or greater sensitivity and specificity in distinguishing between somatic pain and nonpainful warmth, pain anticipation, pain recall, or emotionally evocative images, and it responded to remifentanil administration with a time course predicted by a pharmacokinetic model of remifentanil. The NPS is likely to be sensitive only to certain aspects of pain. In initial tests, it was not affected by a placebo treatment or cognitive self-regulation. In addition, a brain pattern that was optimized to predict placebo analgesia relied on very different systems than the NPS, including frontal cortical activity during pain anticipation. Research on FM patients has shown that fMRI responses to painful and nonpainful stimuli can distinguish between FM patients and healthy control subjects, and that an aversive visual stimulus can distinguish between FM patients who are receiving pregabalin versus placebo at greater than 80% accuracy. Importantly, neuroimaging may highlight patterns of pathological brain activity that may be the...
target of a successful pharmacologic analgesic. These findings suggest that brain imaging may be beneficial in identifying the presence of acute and chronic pain although replication is necessary before it can be used as a biomarker for pain in clinical trials.

**Brain Imaging as a Biomarker in Analgesic Trials?**

Brain imaging is slowly being adopted into analgesic drug development in translational as well as clinical trials. Preliminary evidence suggests that imaging may serve as a diagnostic biomarker distinguishing individuals with different chronic pain conditions from each other, as well as from healthy control subjects. Imaging may also allow for the prediction of the development of chronic pain, as well as who will respond to specific treatment interventions. Of particular interest, research on imaging as an indicator of treatment benefit in preclinical models and clinical trials has begun to show regions of brain activation that are associated with analgesic administration as well as patient self-reports of their pain experience.73,77 Development of brain imaging biomarkers requires additional research, specifically focusing on standardizing outcomes, validation, reproducibility, and the evaluation of diagnostic properties for the outcomes identified.

**Conclusions**

As presented previously, sensory testing, skin biopsy, and brain imaging have the potential to accelerate the development of analgesic drugs and other pain treatments. Preliminary findings for these 3 types of assessments suggest their applicability as diagnostic, prognostic, predictive, and pharmacodynamic biomarkers. Moving forward, standardized administration and analysis methods are needed for these 3 types of assessments that can then be evaluated to establish their reliability and validity. For example, for sensory testing, the DFNS QST protocol is a standardized method of conducting sensory profiling, with good test-retest and interobserver reliability when raters have been trained.66 Although the DFNS protocol is quite comprehensive, it does not include CPM assessment, which requires additional methodologic testing and standardization.214 Furthermore, research must show that these tools can identify subgroups of individuals with chronic pain who may benefit from a particular treatment, and then replicate these results in prospective RCTs. It may also be the case that combinations of these assessments will better serve as biomarkers in the development of analgesic treatments than the individual components, and future research should continue to explore this possibility.

We have not made specific recommendations about the integration of sensory testing, skin biopsy, and imaging into pain treatment clinical trials. However, on the basis of discussions during the meeting and the literature we have reviewed, we conclude that sensory testing, skin biopsy, and brain imaging have promise as pain biomarkers and should be carefully considered for possible inclusion when designing clinical trials of pain treatments. There are, of course, limitations to acknowledge. This article is not on the basis of formal systematic reviews and meta-analyses, but is rather evidence-informed. Conducting formal systemic reviews of the literature on these 3 types of assessments was beyond the scope of one IMMPACT meeting, the primary objective of which was to achieve consensus on the possible role of these tools as biomarkers in pain research. In addition, systematic reviews and meta-analyses of the 3 types of assessments are unlikely to alter our conclusion that although potential benefits exist, the usefulness biomarkers remains a controversial topic. More targeted research is needed because of heterogeneity in their application (eg, the exact features of each tool that uniquely diagnose a given chronic pain condition, indicate prognosis, predict treatment effect, or show pharmacodynamic effects), the chronic pain conditions examined, reference standards (for diagnostic biomarkers), outcomes predicted (for prognostic biomarkers), and time points used for assessments. The relevant content experts and all other authors have identified and reviewed the most relevant research regarding the 3 types of assessments as potential biomarkers for use in developing analgesic treatments. Although we have not included all studies using these 3 tools, this article adequately and accurately reflects the evidence base currently available.

It is important to recognize that many of the studies described were exploratory and conducted multiple statistical analyses that inflate the probability of a type I error and could thereby lead to false positive results. As we have indicated throughout the article, prespecified replication is necessary to provide further support for the tools. Additionally, much of the research supporting the use of sensory testing and skin biopsy as biomarkers comes from studies of neuropathic pain or peripheral neuropathy. This is reflective of the studies that are available in the literature, rather than a limit we imposed on this review and recommendations, and indicates that research on these tools should be expanded to other chronic pain conditions, and in the case of skin biopsy, focused on identifying whether a relationship exists between biopsy results and pain. Finally, this article concentrates solely on the evidence supporting the use of sensory testing, skin biopsy, and brain imaging as biomarkers in the development of analgesic treatments because they have been the most frequently investigated. We have not evaluated other tools, such as electrophysiology for neuropathic pain and plasma levels of cytokines for other types of pain because these were beyond the scope of the meeting.

In implementing sensory testing, skin biopsy, and brain imaging, researchers should consider the specific objective that the biomarker would address (eg, predictive, pharmacodynamic), the mechanism of action of the treatment, and the pain condition being studied. It is also important to evaluate the best approach to integrating sensory profiling, skin punch biopsy, and brain imaging into clinical trials while still complying with regulatory requirements and mitigating additional risks to
the trial, including increased complexity, cost, and enrollment challenges (eg, participant burden). However, if these 3 types of assessments are shown to be valid and reliable biomarkers to diagnose chronic pain conditions, determine risk of progression, predict treatment benefit, or show treatment effect, they may increase assay sensitivity in clinical trials, thereby exposing fewer individuals to the risks of new drugs. Efforts to standardize these tools and gather data on their measurement properties are essential steps toward qualification by regulatory agencies for use in clinical trials.\textsuperscript{55,188}

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