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Understanding chronic inflammatory and neuropathic pain

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This meeting report highlights the main topics presented at the conference "Chronic Inflammatory and Neuropathic Pain," convened jointly by the New York Academy of Sciences, MedImmune, and Grünenthal GmbH, on June 2–3, 2011, with the goal of providing a conducive environment for lively, informed, and synergistic conversation among participants from academia, industry, clinical practice, and government to explore new frontiers in our understanding and treatment of chronic and neuropathic pain. The program included leading and emerging investigators studying the pathophysiological mechanisms underlying neuropathic and chronic pain, and experts in the clinical development of pain therapies. Discussion included novel issues, current challenges, and future directions of basic research in pain and preclinical and clinical development of new therapies for chronic pain.

Keynote Lecture

Clifford Woolf (Children's Hospital, Boston, Massachusetts) opened the conference with a keynote lecture on the importance of target selection. Woolf stressed that choice of a wrong target will guarantee failure, therefore successful development of novel analgesics is contingent on a detailed molecular understanding of the mechanisms of pain.[1] He reviewed new technologies which enable this process to shift from the standard hypothesis-based candidate approach used so far with very limited success, to an unbiased genome-wide strategy[2] a change to a discovery science strategy to which Woolf claims will reveal true novelty. This will include genome-wide association studies in patient cohorts, computational genetics in mice, proteomics, transcription profiling and genetic manipulation in model organisms with iterative validation and replication.[3] While each individual strategy may have limitations, combining them increases confidence. To meet this challenge Woolf stressed the importance a cooperative pre-competitive effort, since he believes that no single investigator or company can accomplish this alone.

Session I: Novel Targets and preclinical discovery

Chaired by Iain Chessell (MedImmune, Cambridge, UK), the first session examined recent therapeutic breakthroughs based on small molecules and the emerging role of biologics as potential new therapies.

Frank Rice (Integrated Tissue Dynamics, Rensselaer, New York, and Albany Medical College, Albany, New York) opened the session by discussing

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the role of plasticity in peripheral fibers and epidermal molecular organization. Rice summarized how normal tactile sensation, including acute pain, is perceived through predictable patterns of activity involving a mix of peripheral sensory neuron types whose cutaneous endings are differentially activated by particular physical properties of tactile stimuli. Various neuronal types supply Aβ fibers, Aδ fibers, or C fibers. Subtypes predictably terminate as endings that have a unique combination of morphology, disposition and molecular expression that impart unique albeit overlapping functional properties involving differing proportions and types of mechanical, thermal, and chemical stimuli. The normal variety, proportion and disposition of cutaneous innervation is genetically programmed through complex molecular interactions during development and sustained maintenance. Likewise, a programmed pattern of differential terminations develops in the central nervous system so that a given tactile encounter will produce a predictable pattern of neuronal activity whose correlations provide the basis of normal perception.

Rice demonstrated that the characteristics and distributions of sensory endings can be permanently profoundly altered in association with peripheral neuropathies as documented in numerous immunocytochemical studies particularly of post herpetic neuralgia (PHN), complex regional pain syndrome type 1 (CRPS1), and type 2 diabetic neuropathy (DN2) that have occurred naturally in humans and Rhesus monkeys. Chronic pain-related pathological changes also occur among the neurochemical properties of epidermal keratinocytes that normally participate in tactile sensory transduction and modulation. Importantly Rice also claims that different types of peripheral neuropathies can manifest different combinations of painful symptoms. For example, painful DN2 is commonly associated spontaneous burning pain especially occurring symmetrically in the hands and feet. By contrast, PHN is usually asymmetrical, limited to one dermatome, and typically manifests extremely sharp pain in response to normally non-noxious mechanical contact with the skin (mechanical allodynia) or excessive pain response to normally noxious stimuli (hyperalgesia). Such differences indicate that there are potentially different mechanisms of chronic pain that may warrant different disease-dependent therapeutic strategies.

Interestingly, under a variety of chronic pain conditions in humans, including PHN, CRPS1 and DN2, Rice stressed that a consistent finding has been a seemingly paradoxical loss of epidermal innervation that presumably would be the very nociceptors that would contribute to pain sensation. However, Rice showed that this paradox may be partly explained by electrophysiological detection of increased spontaneous activity and hypersensitivity of remaining cutaneous innervation. Although the neurons in the spinal cord and brainstem also have increased spontaneous activity and hypersensitivity, referred to as central sensitization, increasing evidence indicates that this is driven by aberrant peripheral input. Multi-molecular immunocytochemical analyses indicate that the depletion of epidermal innervation preferentially involves all types of innervation but is most severely a depletion of the C fiber innervation. Most remaining innervation has microfilament properties indicative of Aδ fibers, and their sensory endings can have aberrant branching and neurochemical properties that would be consistent with pathologically increased activity. The epidermal innervation is not only reduced but becomes unevenly distributed with clusters of endings separated by wide, uninnervated gaps. Importantly, Rice suggested that keratinocytes of the epidermis can also manifest an aberrantly increased expression of neural characteristics such as a beta-isoform of CGRP, which may be released, and voltage gated sodium channels that mediate keratinocyte ATP release. This may also contribute to increased activation of sensory endings that remain in the epidermis. For the future, Rice suggested that strategies to facilitate the ability to re-establish stable, predictable patterns of tactile neural activity, even if they are abnormal, may help alleviate chronic pain.

Stephen B. McMahon (King's College London, London, United Kingdom) next went on to discuss how most chronic pain states can be temporarily ameliorated by local anaesthesia of the affected tissues. He suggested that in these states, peripheral pain signaling pathways are being tonically activated. The mediators responsible are for the most part unknown but the limited efficacy of nonsteroidal anti-inflammatory drugs (NSAIDs) suggests the existence of factors other than prostanoids. One factor that has been identified and shown to act as a peripheral pain mediator is the neurotrophin nerve growth factor (NGF). McMahon reviewed a

wealth of preclinical data and more recently clinical trial data has shown the efficacy of peripheral anti-NGF strategies in relieving several forms of chronic pain, most notably pain associated with osteoarthritis. McMahon also described a novel approach being pursued in his laboratory to identify other peripheral pain mediators.[4] His group undertook transcriptional profiling of human biopsy specimens from painful conditions to identify candidate mediators and then tested the role of these candidates and their mechanism of action in preclinical models. They used ultraviolet B (UVB) irradiation to induce persistent, abnormal sensitivity to pain in humans and rats. The expression of more than 90 different inflammatory mediators was measured in treated skin at the peak of UVB-induced hypersensitivity with custom-made polymerase chain reaction arrays. McMahon found a significant positive correlation in the overall expression profiles between the two species. The expression of several genes (interleukin-1β (IL-1β), IL-6, and cyclooxygenase-2 (COX-2)), previously shown to contribute to pain hypersensitivity, was significantly increased after UVB exposure, and there was dysregulation of several chemokines (CCL2, CCL3, CCL4, CCL7, CCL11, CXCL1, CXCL2, CXCL4, CXCL7, and CXCL8). Among the genes measured, CXCL5 was induced to the greatest extent by UVB treatment in human skin; when injected into the skin of rats, CXCL5 recapitulated the mechanical hypersensitivity caused by UVB irradiation. This hypersensitivity was associated with the infiltration of neutrophils and macrophages into the dermis, and neutralizing the effects of CXCL5 attenuated the abnormal pain-like behavior. McMahon's findings demonstrate that the chemokine CXCL5 is a mediator of some inflammatory pain states.

The emerging role of biologics as potential new therapies was then presented by Jane P. Hughes (MedImmune, Cambridge, United Kingdom). Hughes opened with the statement that discovery and development of novel pain therapies remains an imperative, but the ability to genuinely test the efficacy of novel therapies is often limited by effects at targets other than intended, particularly with novel small molecule approaches. As a result, few novel mechanisms are genuinely tested in the clinic with unequivocal evidence of pharmacological interaction with the target. In addition, Hughes discussed how the regulatory environment is increasingly challenging, with reasonable expectations for drugs with highly favorable safety profiles; despite the devastating effects on quality of life, co-morbidities and socioeconomic impact, chronic pain is considered to be non-life threatening, and thus the risk/benefit profile of novel therapies must meet very high expectations. Approaches that limit these off-target activities may provide a greater ability to genuinely test targets of choice clinically.

Hughes went on to suggest that biologic therapeutics, in particular monoclonal antibodies (mAb's) provide such an opportunity. She explained that the attraction of mAb therapeutics is several-fold. Antibodies provide excellent affinity and specificity of target recognition; rarely do mAbs interact with any target other than that selected. In addition, because of their relatively large size, in vivo stability, and their ability to be sequestered and recycled via interaction with the FcRn receptors on endothelial cells, they tend to have extended pharmacokinetic half lives (typically around 7–14 days), which reduces inter-dose frequency.[5] Together, Hughes suggested these properties increase the likelihood of achieving true target engagement, assuming the target is accessible, and thus testing new therapeutic targets without the associated difficulties of managing off-target interactions. However, it was made clear that there are drawbacks to mAb-based therapeutics; immunogenicity, while of low incidence with humanized mAbs,[6] can be an issue with long term administration.

When considering the use of mAbs for the treatment of pain, Hughes stressed that not only must the above benefits and limitations be taken into account, but consideration must also be given to the discoverability and developability of mAbs against various target classes. An optimal target for mAb intervention is a soluble mediator; cytokines, growth factors, and inflammatory mediators generally have distinct identities within their active regions, and outside of the CNS are localized in readily-accessible compartments such as blood or joints. There are numerous soluble factors implicated in the pathogenesis of pain which play a role in the peripheral system, including various cytokines (e.g., IL-6), prostanoid products and kinins, giving potentially an already wide choice of possible targets to investigate further.

Hughes also demonstrated that therapeutic mAbs have been successful in targeting cellular receptors where the ligand binding domain lies on the

extracellular face; here a number of tyrosine kinase and T cell receptors have already been targeted by launched products for oncology and inflammatory indications. The extension here is utilization of mAbs to target G-protein coupled receptors, opening a wealth of target opportunities for the treatment of pain. Coupled with some advances in the targeting of ion channel targets with mAbs, Hughes claimed that the target landscape for analgesic biologics is indeed a broad one, even when considering only peripheral targets. Further development of blood-brain-barrier penetrating platforms will serve to open this landscape still further. Conventionally, mAbs also do not penetrate the blood-brain-barrier readily, with typical concentrations of mAbs within the CNS compartment after chronic dosing being around 0.1–0.5% of that of the systemic circulation.[7] However, Hughes showed that this appears to be a surmountable issue, demonstrating with examples such as antibody fusions to insulin, utilizing the insulin transporter to allow the construct to enter the brain.[8]

Stephen G. Waxman (Yale University School of Medicine, New Haven, Connecticut, and Veterans Affairs Connecticut Healthcare System, West Haven, Connecticut) discussed how sodium channels serve, within the mammalian nervous system, as obligate generators of the upstroke of nerve impulses. Sodium channel blocking drugs have been used, for many years, to treat epilepsy and related disorders with some success. And while the efficacy of existing sodium channel blockers for the treatment of chronic pain has been limited, a number of recent developments suggest, however, that this situation may change. Waxman summarized our current understanding of the nine different genes that encode nine distinct sodium channel isoforms (NaV1.1–NaV1.9), with different amino acid sequences, different physiological properties, and different distributions within the nervous system. Waxman suggested that for a number of years pain research has focused on the following question: are there sodium channels that are preferentially expressed within nociceptors—a pattern of distribution that might permit selective targeting—so that drugs with limited cardiac and/or CNS side effects might be developed?

Four sodium channel isoforms have emerged as attractive molecular targets in this regard.[9,10] NaV1.3 is up-regulated within DRG neurons following injury to their peripheral axons. According to Waxman, this sodium channel isoforms has a number of physiological attributes (rapid recovery from inactivation, robust response to small, slow depolarizations close to resting potential, and production of a persistent current) that increase cell excitability. But whether up-regulated expression of this channel within axotomized DRG neurons will allow selective targeting is currently under exploration.

NaV1.9, originally termed NaN, is specifically expressed within nociceptors, and does not appear to be present within any other types of nerve cells. As a result of its slow kinetics and broad overlap between activation and steady-state inactivation, this channel plays a strong role in modulating the excitability of nociceptors. Waxman explained that development of subtype-specific blockers of NaV1.9 have been limited by modest levels of expression of this channel isoform within heterologous expression systems, and the development of expression systems that will permit high-throughput screening remains a challenge.

Of other sodium channel isoforms, according to Waxman, NaV1.7 is preferentially expressed at high levels within dorsal root ganglion and sympathetic ganglion neurons and NaV1.8 is specifically produced within dorsal root ganglion neurons. These two channel isoforms work in tandem, with NaV1.7 acting as a threshold channel that responds to small, slow stimuli such as generator potentials by producing its own depolarization, bringing membrane potential closer to the threshold for activation of NaV1.8. NaV1.8 produces the majority of the inward membrane current underlying the action potential during repetitive firing of DRG neurons. Waxman mentioned that NaV1.7 has attracted special interest because of its key role in producing human pain syndromes. For example, gain-of-function mutations that enhance the activation of NaV1.7 produce inherited erythromelalgia, a striking disorder characterized by severe burning pain of the hands and feet.[11] On the other hand, gain-of-function mutations that impair inactivation of NaV1.7 produce another disorder, paroxysmal extreme pain disorder, characterized by perirectal, periorbital, and perimandibular pain. Finally, loss-of-function mutations of NaV1.7 produce channelopathy-associated insensitivity to pain, a disorder in which humans lack functional NaV1.7 channels and do not feel

pain in response to stimuli or events that should be painful; individuals with this disorder display painless burns, painless fractures, etc. and undergo dental extractions and surgery without feeling pain.

It has also become clear during the past few years, said Waxman, that polymorphisms of sodium channel can modulate sensitivity to pain. The R1150W polymorphism of NaV1.7, for example, contributes to sensitivity to pain following nerve root compression, limb amputation, and in osteoarthritis. It is highly likely that other sodium channel polymorphisms will also be found to contribute to pain sensitivity. Waxman concluded by discussing studies on human subjects with NaV1.7 mutations that have demonstrated that some mutations can alter sensitivity of the mutant channels to sodium channel blocking agents such as mexiletine and carbamazepine.[12] Together with the recent observations on sodium channel gene polymorphisms, this suggests that the goal of genomically-based personalized pain pharmacotherapeutics may not be unrealistic.

Robert W. Gereau (Washington University School of Medicine, St. Louis, Missouri) next went on discuss how epigenetic modulation may be an important mechanism of analgesia. Several reports indicate that L-acetylcarnitine (LAC) can be considered as a therapeutic agent in neuropathic disorders including painful peripheral neuropathies. His studies aimed at defining the mechanism of LAC analgesia indicated that LAC acts in part by epigenetic regulation of mGlu2 metabotropic glutamate receptor expression. Consistent with this finding, Gereau showed that activation of mGlu2 is robustly analgesic in animal models, though the therapeutic utility of mGlu2 agonists for the treatment of pain is limited by the robust development of analgesic tolerance to mGlu2 agonists. Mechanistic studies suggest that LAC exerts this effect via regulation of p65/RelA acetylation. These findings led Gereau and colleagues to test whether inhibiting deacetylation using HDAC inhibitors might similarly have analgesic effects via upregulation of mGlu2. He found that indeed two separate HDAC inhibitors promote analgesia and upregulation of mGlu2, and that the analgesic effects of HDAC inhibitors are reversed by an mGlu2 antagonist. Importantly, they found no evidence for the development of analgesic tolerance on repeated dosing of HDAC inhibitors. Gereau concluded that "epigenetic" drugs that increase mGlu2 receptor expression, including L-acetylcarnitine and inhibitors of histone deacetylases may have a unique analgesic profile with no tolerance to the therapeutic effect after repeated dosing.

The last speaker in the first session, Linda R. Watkins (University of Colorado at Boulder, Boulder, Colorado), described how her work over the past 18 years has challenged classical views of pain and opioid actions. Watkins summarized how glia (microglia and astrocytes) in the central nervous system are now recognized as key players in: pain amplification, including pathological pain such as neuropathic pain; compromising the ability of opioids, such as morphine, for suppressing pain; causing chronic morphine to lose effect, contributing to opioid tolerance; driving morphine dependence/withdrawal; driving morphine reward, linked to drug craving and drug abuse; and even driving negative side effects such as respiratory depression. It is well documented that glial activation arises under conditions of chronic pain from neuron-to-glia signaling. Intriguingly, Watkins suggests that the glial activation receptor that creates neuroinflammation under conditions of chronic pain is the same receptor that is activated by opioids. Atop this, Watkins stated that opioid effects on glia that create neuroinflammation are via the activation of a non-classical, non-stereoselective opioid receptor distinct from the receptor expressed by neurons that suppresses pain. According to Watkins, this implies that the effects of opioids on glia and neurons should be pharmacologically separable, leading to new drugs for the control of chronic pain increasing the clinical efficacy of pain therapeutics. As such, Watkins claims that drugs in development which target this glial activation receptor have shown efficacy as stand alone treatments for neuropathic pain, by blocking neuron-to-glia signaling, plus blocking unwanted side effects of opioids, as well as other drugs of abuse.

Session II: Transitioning from preclinical to clinical studies

The second session, moderated by Martin Perkins (AstraZeneca R&D, Montreal, Quebec, Canada) consisted of a series of presentations on the challenge of translating results between clinical and preclinical studies.

Frank Porreca (The University of Arizona, Tucson, Arizona) discussed the translational capacity of reflexive endpoints used in animal models of pain. The majority of animal models of experimental neuropathic pain rely on nerve injuries with consequential various behavioral manifestations believed to reflect the different mechanisms contributing to a pathological pain state (For review see Ref. 13). None of these models, however, capture affective dimensions of pain as they rely on, typically, reflexive evoked responses to external stimuli whereas pain patients complain, primarily, of ongoing pain independent of an external stimulus. There is, therefore, a need to evaluate spontaneous or *stimulus-independent* pain in animal models.[13–15]

Pain has a strong emotional component exemplified by its unpleasantness which may have a protective role.[16,19] Porreca hypothesized that chronic pain produces an aversive state providing behavioral motivation to seek relief that will be rewarding. This concept was explored in the conditioned place-pairing assay, in which pairing pain relief with a distinct context resulted in increased time spent in that context.[16] Importantly, such conditioned place preference (CPP) was only observed in rats with nerve injury, leading to the "unmasking" of spontaneous experimental neuropathic pain.[16–18]

Rats with experimental nerve injury were placed in boxes consisting of two chambers with different visual and textural characteristics. Following preconditioning, a vehicle treatment was paired with one chamber and spinal administration of clonidine or ω-conotoxin in the other chamber, resulting in place preference in these animals where they preferred this chamber where pain relief occurred, revealing the presence of spontaneous pain.[16] The place preference associated with pain relief in rats with SNL was prevented by lesioning the rostral anterior cingulate cortex (rACC)[18] consistent with studies indicating that the rACC mediates the affective component of evoked pain.[19–21] This approach was also used to demonstrate descending pain modulatory pathways are important in mediating nerve-injury induced spontaneous pain in rats with nerve injury following rostral ventromedial medulla (RVM) microinjection of lidocaine.[21]

Whether neuropathic pain results as a consequence of activity of injured or adjacent uninjured nerves has been controversial.[18] Peripheral nerve injury results in increased excitability and ectopic discharge in primary afferents, thought to contribute to pain (for review, see Ref. 22). However, the contribution of either injured or uninjured primary afferent bers, or both, in these processes remains unclear with various studies supporting injured and uninjured bers being important (for review, see [23]). Clinically it appears that injured bers are important in driving pain [24] but it has been difficult to demonstrate this clearly in animal models of axotomy.[25,26]

Porreca then described his studies to determine whether CPP could be demonstrated in animals with either partial or complete hind paw denervation to assess the role of injured bers in promoting spontaneous neuropathic pain. Selective place preference to either spinal clonidine or RVM lidocaine in animals was observed in rats with sciatic or sciatic/saphenous axotomy suggesting spontaneous pain arises from injured nerve fibers.

Further studies were done exploring the role of TRPV1 and/or NK-1 receptors. Systemic treatment with resiniferatoxin (RTX) that produces long-lasting desensitization of TRPV1 receptors blocked nerve injury-induced thermal, but not tactile, hypersensitivity as well as the place preference resulting from pain relief.[17] Ablation of NK-1 receptor–expressing cells in the spinal cord with a substance P–saporin construct blocked nerve injury thermal and tactile hypersensitivity as well as ongoing pain.[17] These data suggest that spontaneous neuropathic pain involves both TRPV1 and NK-1 mediated mechanisms.

Porreca concluded by stressing the need for increased exploration of mechanisms associated with ongoing pain to improve translation from animal studies to human therapeutics leading to improved treatment of pain in humans.

Mark J. Field (Grünenthal GmbH, Aachen, Germany) then discussed the Pharma industry's productivity crisis with ever-increasing costs for research and development and decreasing approvals for new medicines. This is evident in the pain field with few new medicines successfully moving through development to the market to help patients. Over the past 10–15 years compounds with novel mechanisms of actions such as pregabalin, duloxetine and most recently tapentadol have made it to the market but still a large proportion of patients remain refractory to the available treatments. Field stressed that the translation of positive

preclinical data into clinical efficacy is a key area of focus as many promising compounds / mechanisms fail to reach a positive proof of concept study. A major issue is the lack of understanding of the complex clinical chronic pain condition and the simple numeric rating scales used to assess clinical pain that give limited feedback to preclinical scientists. The concept of *translational research* hopes to build bridges between preclinical scientists and clinicians and identify biomarkers to assist in the development of novel pain medicines. Biomarkers can range from complex imaging techniques to simple blood borne markers that allow assessment of target engagement, pharmacology and even efficacy in early clinical trials. Closer co-operation between preclinical and clinical scientists through open innovation and pre-competitive consortia will be essential to ensure greater success in identifying novel medicines to treat the patients.

David A. Seminowicz (University of Maryland School of Dentistry, Baltimore, Maryland) discussed rodent brain imaging and behavior, stressing the importance of neuroimaging studies in humans, because perceptions and behaviors are results of brain processes. We strive to understand brain mechanism associated with acute and chronic pain. There is great potential for brain imaging leading to the identification of brain areas and functional networks that can be targeted to treat chronic pain. Neuroimaging in rodents can be used to supplement human imaging studies by addressing specific questions requiring animal models. For example, in rodent imaging studies we can examine the effects over time of an injury producing long-lasting pain behaviors, and we can perform histological studies examining the cellular correlates of brain imaging results and cellular correlates. Other advantages of neuroimaging in rodents include having tight control over genetic and environmental variables as well as performing invasive interventions such as drug injections and brain lesions. Two major disadvantages, however, for animal neuroimaging are that animals are usually anesthetized during the scan, which alters brain function, and we cannot accurately assess a brain-behavior relationship the way we can in humans by administering pain and asking subjects to rate its intensity.

There is extensive neuroimaging evidence that people with chronic have altered brain function and structure, the most common finding being a reduced gray matter volume or density or cortical thickness in widespread brain areas. Yet, two questions remained: (1) are these brain changes the cause or the consequence of chronic pain; and (2) are these brain changes reversible? Seminowicz and colleagues addressed the first question in a rodent model of neuropathic pain using a longitudinal design to follow the onset and progression of brain changes.[27] They examined brain structural changes and behaviors in rats with the spared nerve injury (SNI) model of peripheral neuropathic pain and found that in areas related to the sensory aspect of pain, including the anterior cingulate cortex and primary somatosensory cortex, decreased volume correlated with mechanical hypersensitivity. Furthermore, the volume of the prefrontal cortex decreased several months after the injury and corresponded in time with signs of anxiety-like behavior. They thus dissociated changes in sensory-related areas (S1, ACC), and affect-related areas (PFC) (Fig. 1). An important finding was that the PFC changes occurred only several months after the onset of nerve injury. Most experimental neuropathic pain studies only study animals for days or weeks after injury and thus may miss important aspects of behavioral changes associated with long-term pain. Finally, terminal histological analysis on the brains—about six months after SNI—revealed astrocyte proliferation in the PFC region with volumetric decreases but no obvious changes in neuronal or glial morphology or density.

Seminowicz highlighted some of the recent advances and remaining challenges in the field of neuroimaging in rodents including imaging awake animals, magnetic resonance spectroscopy, real-time fMRI, manganese enhanced MRI, and spinal cord imaging. A major challenge will be linking behavior and brain function in rodents, and translating these correlations to humans. He also described ongoing work with Radi Masri at the University of Maryland Dental School, including studies on electrical stimulation, resting state fMRI, and awake imaging.

Finally, to address the second question—i.e. whether the brain changes seen in chronic pain are reversible—Seminowicz discussed recent human neuroimaging studies suggesting that anatomical changes are reversible,[28–30] as well as his own work suggesting both structural changes and abnormal cognitive-related functional activity are reversible with effective treatment.[31] Together, the imaging

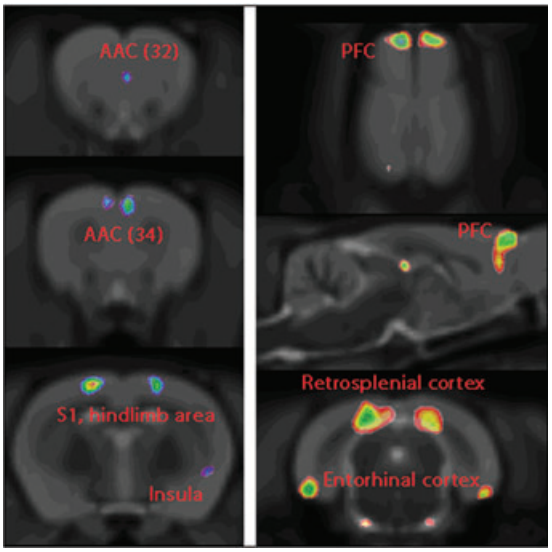

Figure 1. **Sensory-related areas: volume decreases in these areas correlated with increased mechanical sensitivity (left panel). Affect-related areas: volume decreases in these regions corresponded in time with the onset of anxiety-like behavior (right panel).**

data from animal and human studies suggest that pain causes anatomical brain changes and that treatment leads to recovery of normal brain anatomy.

In the first part of her presentation, Katja Wiech (University of Oxford, Oxford, United Kingdom) outlined the motivation to search for an objective readout for pain. Despite its enormous clinical relevance and socio-economic impact, pain and its representation of pain in the brain are only partly understood. Given the pivotal role of the brain for the perception of pain, there is an increasing demand for more detailed insights that could aid the development of new treatments. There is also a need for an objective indicator of pain to guide treatment in clinical settings. Patients' subjective reports are still the gold standard for the assessment of relevant pain features such as intensity or unpleasantness. A more objective measure is desirable if individuals are unable to provide ratings as with unconscious patients or preverbal infants. Finally, there is a growing interest in the use of neuroimaging in compensation claims related to persistent pain following work-related injuries as an objective marker for the presence or absence of pain could help determine the legitimacy of such claims.

Wiech then discussed the conceptual and methodological obstacles hampering the identification of such a biomarker for pain in the brain and recent advances in overcoming these challenges. It is now widely acknowledged that the perception of pain is not a simple reflection of incoming sensory information but a highly subjective experience that is determined by sensory as well as affective and cognitive factors. Hence, measures of pain that could serve as a biomarker has to consider the multidimensionality of pain. While the conventionally adopted univariate analysis approach has proven powerful for inference on highly localized structure-function mappings in the brain, it is insensitive to spatially distributed patterns of neural activity that are characteristic for highly complex phenomena such as pain. Multivariate pattern analysis (MVPA) is an emerging technique in functional brain imaging allowing for the integration of information from an extended network of brain regions.[32] Multivariate decoding models, such as those underlying classification algorithms, can be used to infer, directly, a perceptual state from the activity pattern across voxels—an application that has only recently been implemented in the context of pain.[33,34] In these first studies a classification algorithm was trained to differentiate between several intensity levels of pain induced by different stimulation intensities. The results show that, in principle, decoding of pain from brain images is feasible with prediction accuracies significantly above chance level.

Other commonly used indirect measures of brain activity such as blood oxygenation level

dependent (BOLD) contrasts are partly ill-suited for the investigation of prolonged pain states due to methodological constraints such as the reduced sensitivity to events exceed a duration of approximately one minute. The development of fMRI-based arterial spin labeling (ASL) that measures changes in cerebral blood flow directly is less prone to time-sensitive effects and has opened up new possibilities to investigate chronic pain for which objective measures are urgently needed with promising results from the first studies in prolonged pain states.[35,36]

Results of functional neuroimaging studies on pain are commonly based on group data. Conclusions on individual cases as, for instance, required in the legal context, are often compromised by a relatively low signal-to-noise ratio (SNR). Ultra high field MR systems that are increasingly available could provide a better SNR due to increased sensitivity to the BOLD effect allowing for smaller sample sizes or even the identification of an objective measure for pain in the individual.

Märta Segerdahl (AstraZeneca, Södertälje, Sweden) discussed how abundant human pain models have been developed to understand pain physiology. Models range from assessments in normal skin to models inducing increased sensitivity to pain upon provocation, i.e. evoked pain. Lately also models of ongoing pain have been characterized. Pain has been induced by ischemia, heat, UV irradiation, chemical agents, electricity, etc. Most models have been pharmacologically validated with well-known analgesics, such as opioids, ketamine and non-steroidal anti-inflammatory drugs (NSAIDs), and have been used in drug development, mainly in single dose settings. Advantages are the possibility to conduct small studies with low variability under controlled conditions. However, their role in predicting later stage efficacy has been disappointing. Is there then still a role for such models in drug development? When developing new analgesics for new and unprecedented targets, there may be a place for these models. Models involving well understood mechanisms of action, such as capsaicin injection have been used for candidate drug selection for TRPV1 compounds, as a first step in demonstrating human targets engagement. Lately, models of inflammation, such as UV irradiation, have been characterized, linking models to known and new targets. There is a great advantage in being able to build confidence in the target mechanism by local testing avoiding systemic exposure, before traditional first time in man safety and tolerability studies. In this respect, these models well deserve their place in the drug development process of new analgesics.

Laura K. Richman (MedImmune, Gaithersburg, Maryland) began her talk by stating that chronic pain remains a significant problem that has few effective therapies. Choosing the right patients to receive a targeted therapeutic is critical from both the efficacy and safety perspective. There is a great need to reduce the cost of developing new pharmaceuticals, now approaching 1 billion per drug. The bulk of this cost is attributed to failed drugs. The FDA estimates that a 10% improvement in predicting clinical trial failures could reduce the average cost of drug development by nearly $100 million. An effort to generate more discriminatory biomarkers of efficacy and toxicity should eliminate suboptimal compounds earlier in development. Traditional preclinical animal studies often do not predict human-specific metabolic and toxic effects. Currently, the most commonly used toxicity biomarkers generate safety signals only when substantial organ damage has occurred. Ongoing efforts at finding efficacy and safety biomarkers that can predict or anticipate either desirable or undesirable effects in chronic pain and other indications were discussed.

Session III: Post-candidate clinical development

The third session was chaired by Richard Malamut (AstraZeneca R&D, Wilmington, Delaware) and focused on the challenges of clinical development of analgesic treatments.

Miroslav Bačkonja (LifeTree Research, Salt Lake City, Utah, and University of Wisconsin-Madison, Madison, Wisconsin) began the session with a presentation summarizing the current standard in the treatment of pain. Backonja noted that chronic pain is a complex disorder, manifesting with persistent pain and many other somatosensory symptoms, disturbance of sleep and mood. It results in negative impact on work, function and quality of life. He went on to state that both multimodal and multidisciplinary approaches have been accepted and promoted by professional pain societies and organizations as the standard for managing chronic pain. Elements of multimodal approach to pain management include pharmacotherapy,

interventional treatments, physical medicine and rehabilitation, psychological counseling and the development of appropriate coping skills. Drugs proven in randomized clinical trials to be effective in providing partial pain relief are the basis of pharmacotherapy of pain. These drugs are most commonly used in combination thereby taking advantage of diverse mechanisms of action. Interventional approaches include injections at anatomic sites presumed to be sources of pain and usually in combination with local anesthetics and steroids. Backonja then moved beyond pharmacologic approaches for treatment of pain, stating that neurostimulation therapy with implanted devices are frequently used as a last resource for pain relief. Physical and rehabilitation medicine treatments include a wide range of physical modalities but most critical is the patients' engagement into an active exercise program. Psychological therapies are important for the development of strategies to diminish both psychological co-morbidities and catastrophizing as well as for the development of coping skills that allow patients to deal with chronic pain more efficiently. In conclusion, Backonja firmly stated that an optimal multimodal approach is best achieved when trained clinicians from all disciplines, equipped to administer their respective therapies, work together in coordinated fashion.

Ralf Baron (University of Kiel, Kiel, Germany) then spoke about novel ways of segmenting patients with pain and the potential impact upon both clinical studies and clinical practice. In the past the classification of chronic pain syndromes had been based on disease entities, anatomical localization or histological observations. Over the past decade there has been a dramatic increased understanding of the pathophysiological mechanisms leading to the generation of chronic pain. Exciting advances in basic science have occurred in parallel with a growing awareness by clinical investigators that chronic pain is not a monolithic entity, but rather presents as a composite of different pain qualities and other sensory symptoms. Baron believed that the traditional classification of chronic pain may be supplemented with a new classification in which pain is analyzed on the basis of underlying neurobiological mechanisms rather than on the basis of the etiology.[37,38] This mechanism- or symptom-based classification includes pain symptoms such as burning or shooting sensations as well as negative and positive sensory signs. He reviewed his innovative research in which the characteristic profile of sensory symptoms (a combination of negative and positive signs) can be elucidated in each patient by utilizing a battery of several standardized quantitative sensory tests (QST). Verbal descriptors from validated questionnaires can then depict the quality and intensity of the individual pain. Baron is a member of the German Research Network on Neuropathic Pain which has established a large data-base of $>$ 2000 patients with diverse neuropathic pain states that includes epidemiological and clinical data as well as standardized quantitative sensory testing (QST).[39] He informed the audience that epidemiological and clinical data on the symptomatology of 4200 patients with painful diabetic neuropathy, postherpetic neuralgia and radicular pain from a cross sectional survey (painDETECT) is also available (Fig. 2).[40]

Baron went on to say that within disease entities, different subgroups of patients can be distinguished on the basis of the individual sensory profile (sensory phenotype). These subgroups are present across etiologies but occur in different frequencies. By comparing the sensory patterns of human surrogate pain models (e.g., the capsaicin model or the menthol model) with patient subgroups, more information can be learned about the underlying pain mechanisms that operate in chronic pain.

The final question posed by Baron was whether the different phenotypes (which are presumably related to different mechanisms) are associated with different treatment outcomes. To date several small QST trials have been performed to identify predictive factors of the response to medical treatments. A retrospective analysis of the treatment response in phenotypic subgroups of patients in large clinical trials could demonstrate a differential effect of the study medication between these subgroups.

In summary, Baron stated that this approach of classifying and sub-grouping patients with chronic pain on the basis of symptoms or sign provides the opportunity to stratify patients. The study population can be enriched prospectively in proof of concept studies on the basis of *a priori* defined entry criteria. Enrichment with patients who may be predicted to respond to a specific treatment should increase the likelihood for positive trial outcomes. The implications for clinical practice are an increased capability to design an individualized therapy, i.e. to

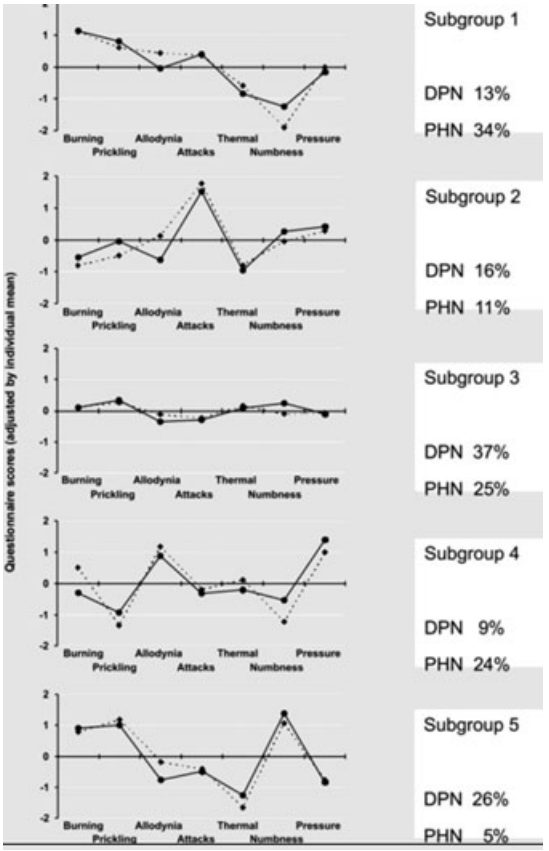

Figure 2. Subgrouping of patients according to sensory profiles using patient reported outcomes (PainDETECT questionnaire). To identify relevant subgroups of patients who are characterized by a characteristic symptom constellation a hierarchical cluster analysis was performed in a cohort of 2100 patients with painful diabetic neuropathy and postherpetic neuralgia. The clusters are represented by the patterns of questionnaire scores, thus showing the typical pathological structure of the respecting group. By using this approach five clusters (subgroups) with distinct symptom profiles could be detected. Sensory profiles show remarkable differences in the expression of the symptoms.% = frequency of occurrence, DPN = diabetic painful neuropathy, PHN = postherpetic neuralgia. Adjusted individual mean: in order to eliminate inter-individual differences of the general perception of sensory stimuli (differences individual pain perception thresholds) a score was calculated in which the given 0–5 score of each question was subtracted by the mean of all values marked in the seven questions. In this individual score values above 0 indicate a sensation which is more intense than the individual mean pain perception, values below 0 indicate a sensation which is less intense than the individual mean pain perception. From Baron *et al.*[4]

identify the patients who would be predicted to respond best to a specific treatment option.

Ian Gilron (Queens University, Kingston, Ontario, Canada) spoke of the challenges of designing clinical studies in neuropathic pain which will provide both valid and reliable results in order to identify beneficial interventions and will provide rigorous evidence which help patients and their caregivers to balance treatment benefits with the costs and risks of those interventions.[41] Optimal design of neuropathic pain clinical trials requires careful consideration of the specific research goals intended which is often dictated by the phase of drug development. Gilron then went on to discuss four components that contribute to a clinical study in patients with pain: study treatments, study populations, outcome measures, and trial designs.

Study treatments. With respect to pharmacological investigations, Gilron stated that design of clinical trials must carefully consider the optimal route of administration, duration of treatment, dosage formulation, and dose amount/frequency as determined by prior pharmacokinetic evaluations (for example, see Ref. 42). The use of placebos requires careful ethical consideration and, in situations where placebos are not appropriate or valid, alternatives to a placebo-controlled design are sometimes considered such as dose-ranging studies.[43,44] Active placebos (e.g., non-analgesic drugs mimicking study drug side effects) have been used in order to improve subject blinding to treatment.[45]

Study populations. Gilron explained that defining the study population is extremely important to the generalizability of trial results as well as to feasibility and conduct of the trial. He defined important considerations which included methods to recruit patients, diagnostic categories (e.g., the broad *peripheral neuropathic pain* vs. a specific entity such as postherpetic neuralgia), use of pain mechanism classifications, such as presence of allodynia,[46,47] disease/pain duration, pain severity/variability, concomitant analgesics and response to prior treatment. He stated that future research is needed to determine if and how these characteristics should dictate eligibility into various trials.[48]

Outcome measures. Gilron communicated that, while measures of pain intensity are the most commonly used primary outcome in neuropathic pain trials, there has been a growing appreciation that chronic pain profoundly impairs various aspects

of quality of life. This has led to the development of IMMPACT (the Initiative on Methods, Measurement, and Pain Assessment in Clinical Trials) consensus recommendations that various multiple core domains,[49] and specific measures for these domains,[50] be considered in clinical pain treatment trials including: (1) pain, (2) physical functioning, (3) emotional functioning, (4) participant global ratings of improvement and satisfaction with treatment, (5) symptoms and adverse events, and (6) participant disposition.

Trial designs. Gilron noted that methods for allocating study subjects to various treatment conditions within a trial are critical to the conduct and interpretation of trial results.[41] In a parallel groups design, enrolled trial participants are randomized to one of two or more treatment groups (i.e., study treatment, placebo, active comparator). Study drug may be evaluated as monotherapy or as adjunct to existing analgesic therapy. In a crossover design, subjects are randomized to one of two or more sequences of treatment groups (i.e., study treatment, placebo, active comparator).[51] Crossover designs are considerably more powerful and efficient because each subject serves as his/her control that thus eliminates many sources of variability. However, Gilron cautioned that a crossover design requires stability of the underlying condition throughout all treatment periods, need to account for the potential of *carryover effect* from one treatment period to the next and may require a washout period.

Gilron concluded by describing potential future directions in neuropathic pain study design. He summarized that investigators continue to grapple with several ongoing challenges and priorities including: (1) internal validity (freedom from bias), (2) external validity (generalizability), (3) assay sensitivity (reduced variability/better precision), and (4) feasibility of subject recruitment/retention. Ongoing efforts to address these challenges include: (1) proposals for innovation in pain measurement methods (e.g., identifying the ideal primary outcome, electronic data capture, optimal timing & frequency,[52] (2) systematic review of clinical trial characteristics with a view to evidence-based trial design,[53] (3) trial design modifications for the improvement of assay sensitivity,[41,48] advancement of academia-industry-regulatory collaborations to strengthen analgesic research.[54] But as a final message suggesting hope for the future of developing better medications for patients, Gilron firmly stated that with the growing interest and effort into the improvement of various aspects of clinical trial design, it is anticipated that promising new treatments will be more optimally evaluated with respect to costs, risks and the healthcare benefits that they may provide to individuals suffering from neuropathic pain.

John T. Farrar (University of Pennsylvania, Philadelphia, Pennsylvania) provided a nice segue from the previous presentation in his discussion of outcome measurements in analgesia studies. He noted that the subjective nature of pain and pain measurements have often been sighted as a reason why clinical trials of potential therapies are difficult to conduct and interpret. However, beginning in the middle of the last century with simple parallel, two group, short term, randomized trials of analgesics (by Ray Houde, Henry Beecher, and others) consistent and valid results have been obtained for a variety of analgesics including non-steroidal anti-inflammatories, opioids, and adjuvant therapy. The underlying principals of random allocation, blinding, and a priori hypotheses based on primary pain outcomes remain the underpinnings necessary to produce valid clinical trial results. Standardization of patient reported pain outcomes (including pain intensity, pain interference, and global perception of change) have been validated in hundreds of studies. Farrar highlighted that an expanded conceptual framework for the pain process has provided a greater level of understanding of physiologic components underlying the pain experience and how they influence self reports of pain. There has been an improved understanding of the appropriate use of measures and analysis techniques and how to provide clinically useful interpretations of pain studies. Farrar went on to state that an improved understanding of the physiologic processes that underlie the pain response have also resulted in the development of a number of new pain therapeutics which underscores the need to create standard approaches to pain studies to allow for comparisons across disease states and types of therapies. He closed by confirming for the audience that improved efficiency of these studies has, in fact, become an important focus of current research.

Bob A. Rappaport (U.S. Food and Drug Administration (FDA), Center for Drug Evaluation and Research (CDER), Division of Anesthesia, Analgesia, and Addiction Products (DAAAP), White

Oak, Maryland) was the final speaker of the session. He discussed regulatory considerations in the development of analgesic medications. Rappaport stated that the FDA has long been concerned about the paucity of safe and effective analgesic drug products. To rectify this problem, the FDA has worked closely with the academic community and pharmaceutical industry to better understand the impediments that have led to a limited supply of novel analgesic drugs in the development pipeline. Representatives from the FDA have worked with the IMMPACT consensus organization for nearly a decade to provide recommendations for improving the design, conduct, and analysis of clinical studies of analgesic treatments. The clinical pain team in DAAAP has interacted with numerous stakeholders over many years to develop a guidance document for industry that will clarify the regulatory requirements for analgesic drug product development. Recent efforts to assure the continued availability of certain potent opioid drug products have included the development of risk, evaluation, and mitigation strategies designed to assure the safe use, storage, and prescribing of these products. Rappaport informed the audience that recently the FDA has taken the proactive step of creating a public–private partnership (ACTION) under the Critical Path Initiative, which is intended to advance the field of analgesic drug development at a more rapid pace by bringing together the best minds to address the problems associated with clinical studies of analgesics, and to find the funding necessary to perform the research to achieve that goal.

Summary

The conference "Chronic Inflammatory and Neuropathic Pain" provided an excellent environment for lively, informed, and synergistic conversation among participants. Although a great deal of superb science is taking place, if the overwhelming goal of research is to improve patient health, the conference highlighted the need for a cooperative, precompetitive effort from academia, industry, clinical practice, and government to explore new frontiers in our understanding and treatment of chronic pain.

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Conflicts of interest

Jane Hughes, Iain Chessell, and Laura K. Richman are employed by MedImmune. Richard Malamut, Martin Perkins, and Märta Segerdahl are employed by AstraZeneca.

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