Final Report Summary - NEUROPAIN (Neuropathic pain: biomarkers and druggable targets within the endogenous analgesia system)

Executive Summary:
The NeuroPain consortium has provided scientifically-sound new knowledge in the neurobiological mechanisms on how pain is generated, propagated and quenched by studies both in animals and in humans. This project has delivered reliable and fully validated new animal models, employing behavioural and electrophysiological approaches, specifically addressing the cognitive, emotional and behavioural components of neuropathic pain as well as main side effects of treatment. For this purpose, different novel genetically-modified mouse lines targeting the endogenous opioid and cannabinoid system have been generated, fully characterised and employed with the behavioural and electrophysiological approaches mentioned above. We have identified the role of delta opioid receptors in GABAergic forebrain, but not in peripheral, neurons in depressive-like behaviour, anxiogenic effects and cognitive impairment associated with neuropathic pain. Mu opioid receptors in those neuron seem involved in emotional responses to chronic pain, whilst their complete deletion modified the response to chronic neuropathic pain in mice. Enkephalins modulate the nociceptive, emotional and cognitive consequences of neuropathic pain and specific roles have been identified for enkephalins expressed in forebrain GABAergic neurons. On the other hand, we show that CB2 receptors on microglia and macrophages, but not on neurons, modulate neuropathic pain responses, whilst the disruption of CB1-dependent endocannabinoid signalling on GABAergic neurons is accompanied by functional changes in astrocyte activity. The role of endocannabinoid biosynthetic and metabolizing enzymes (DAGL, ABDH12 and MAGL) in pain and its concomitant behaviour is only minor, whereas the study of the generated conditional DAGLalpha knockout mice points at a role of this enzyme in anxiety-related and depressive-like phenotypes. These findings constitute a major step forward in the preclinical research on chronic pain. Moreover, personality traits are included in these constructs, thus allowing gaining further knowledge on interpreting patients’ responses to drug therapies and throwing light on how significant inter-individual variations are relevant in the response to painful stimuli and analgesic drugs.

We have also identified new biomarkers of neuropathic pain and we have developed an open data base (http://bioinf.mcb.uj.edu.pl/NeuroPainMine) for the comparison of mouse and human biomarkers of chronic pain. Alterations of gene expression in the motivational and emotional circuitry have been found in mice, namely, the opioid gene prodynorphin, the transcription factor Npas4 and the neuronal activity sensor, Gadd45. Also, genome-wide transcriptional profiling (RNASeq) identifies several modulated transcripts in a chronic pain model. Moreover, specific genes of susceptibility of proenkephalin neurons to neuropathic pain have been identified. A pharmacogenetic study of novel opioid analgesic revealed that prolonged pain caused transcription alterations of a group of genes; Gdf1, Cfd, Ccdc150, Stap1, Potec, Stk17b, Nagpa, Zscan2 and Rp9, which were diminished by drug treatment and can be considered as putative biomarkers of “therapeutic” effects of the drug. The evaluation of the analgesic potential and suitability from the safety and toxicity of novel plant-based phytocannabinoids and mu-opioid agonist/sigma-1 receptor antagonist bivalent compounds has been carried out. These compounds have also been successfully tested in our preclinical models using the behavioural and electrophysiological approaches shown above. A NeuroPain Central Phenotype Database to standardizing data collection and phenotype definitions across neuropathic pain cohorts has been created and extensively used with GWAS of the various phenotype performed. Common genetic underpinnings to depression and chronic and neuropathic pain have been found. Clinical studies results have been completed and reported for the neuropathic pain, post-menopausal surgery and HIV-1 studies. Genes associated with neuropathic pain
syndromes have been identified. Finally, a clinical trial on the analgesic uses of the phytocannabinoid compound cannabidivarin in patients with HIV-associated painful neuropathy has shown interesting results and the search of genetic markers of cannabinoid responders and non-responders has also been done.

To sum up, specific crucial issues such as pain predisposing genetic polymorphisms, circuitries and processes modulating nociception and endogenous analgesia, understanding the cognitive, emotional and behavioural components of pain, as well as validation of new druggable molecular targets, have been done. The Consortium partners have published forty-two scientific articles and filed forty-one patents related to the project.

Project Context and Objectives:
The NeuroPain project profits from the expertise of several complementary European research groups leading behavioural, electrophysiological, genetic and clinical studies in order to generate new neuropathic pain models of high predictive value for the preclinical evaluation of novel treatments and the identification of biomarkers of inter-individual variation and treatment effectiveness in human patients.

Nearly one in five Europeans (19%) suffers from chronic pain. This represents a significant burden to the whole society and to European economies, and has major consequences for the ability of patients to lead productive working, social and family lives. With an ageing population and greater pressure to increase the retirement age, the problem is set to escalate, and the current estimates of the direct and indirect costs for Europe run into the billions. Yet awareness, understanding and intervention are limited, particularly for those pain syndromes that still lack effective treatment, such as neuropathic pain. Neuropathic pain is defined by the International Association for the Study of Pain (IASP) as a clinical entity initiated or caused by a primary lesion or dysfunction in the nervous system that is often associated with hyperalgesia, allodynia, spontaneous pain and important emotional alterations. The manifestations of neuropathic pain show a very high inter-individual variability that depends on multiple poorly known factors, including among others the different personality traits of the patients (Tang and Gibson, J Pain 6:612, 2005). Several compounds are currently used to treat neuropathic pain (Woold and Mannion, Lancet 353:1959, 1999; Dickenson and Ghandehari, Handb Exp Pharmacol 177:145, 2007). However, these compounds have a limited efficacy and present side effects that can restrict their use. Some of the most relevant side effects are sedative effects and abuse liability that represent serious limitations for the clinical use of multiple analgesic compounds. Nowadays treatment of severe chronic pain remains unresolved and there is an urgent need for more effective drugs and biomarkers of inter-individual variability as well as new animal models with high predictive value to evaluate the analgesic and side effects of novel compounds.

The translational research approach adopted in the NeuroPain project includes cross validation of the findings in animals and humans with the following specific objectives:

1. To develop new reliable methods for evaluating the behavioural, emotional and cognitive components of neuropathic pain in rodents. Novel behavioural mouse operant models based on the ability of a mouse to self-administer an analgesic compound to alleviate chronic pain will be improved to provide a precise evaluation of the emotional and cognitive manifestations of neuropathic pain with the support of sophisticated electrophysiological techniques (WP1, achieved by month 12).

2. To evaluate the influence of emotional traits responsible for the inter-individual variation of neuropathic pain manifestations. Behavioural, emotional, cognitive and electrophysiological manifestations of neuropathic pain will be characterized in mice displaying extreme personality phenotypes (WP2, achieved by month 24).

3. To identify new targets and biomarkers within the endogenous opioid and cannabinoid systems involved in the behavioural, emotional and cognitive manifestations of neuropathic pain. The endogenous opioid and cannabinoid system are excellent targets to identify novel mechanisms, biomarkers and druggable targets for neuropathic pain treatment (WP3 and 4, achieved by month 60).

4. To define the potential for clinical effectiveness of new analgesic compounds and targets relevant within the opioid and cannabinoid systems. These new compounds have been developed by the two pharmaceutical companies involved and will be evaluated in the novel animal models of neuropathic pain (WP5 and 6), achieved by month 60).
To identify biomarkers of neuropathic pain and comorbid states using different human cohorts of neuropathic pain. Through a comprehensive genome-wide search for novel DNA sequence variants associated with various forms of neuropathic pain using methods dissecting out the affective component, we will identify genes harbouring biomarkers associated with neuropathic pain with extensive phenotypic profiles of each variant, charting out biological pathways leading to the development of neuropathic pain (WPs 7 and 8, achieved by month 60).

To evaluate the relevance of these new biomarkers of neuropathic pain for the efficacy of novel analgesic compounds. Combining the human genetics results with the rich phenotypic profiles from corresponding animal studies we hope to identify several new validated drug targets ready to enter clinical trials. We will also assess the effect of the biomarkers associating most strongly with neuropathic pain on treatment outcome in an ongoing clinical trial (WPs 9 and 10, achieved by month 60).

The work is organised in the following work packages:

WP1 - Development of new reliable methods of evaluating the behavioural, emotional and cognitive components of neuropathic pain in rodents. We have recently validated a novel mouse operant model based on the ability of an animal exposed to chronic neuropathic pain to self-administer an analgesic compound (self-medication). The operant responses obtained in this animal model have a high predictive value to estimate the clinical benefit/risk ratio of new analgesic compounds. This WP improves this novel model in order to provide a precise evaluation of the emotional and cognitive manifestations of neuropathic pain with behavioural and electrophysiological correlates.

WP2 - Evaluation of specific personality traits responsible for the inter-individual variation in the behavioural, emotional and cognitive responses to neuropathic pain. The influence of personality traits on the manifestations of neuropathic pain is studied. The behavioural responses of mice are evaluated in different models of emotional, social and aggressive behaviour. Mice displaying extreme phenotypes are selected to study the manifestations of neuropathic pain using the behavioural and electrophysiological models developed in WP1. This WP provides the samples needed for the identification of specific biomarkers on WP5. These personality traits are analogous to those evaluated in humans in WP7, facilitating the validation of results in humans (WP10).

WP3 - Evaluation of the role of specific components of the endogenous opioid system in the behavioural, emotional and cognitive components of neuropathic pain. The opioid system is a well-characterized endogenous analgesia system with enormous research potential to identify novel mechanisms, biomarkers and druggable targets. This WP addresses the role of opioid receptor populations operating at the level of either peripheral or brain sites in the control of neuropathic pain using novel genetically modified mice and the behavioural and electrophysiological models developed in WP1. Together, the new and powerful genetic tools developed in WPs 3 and 4 uncover key elements of the biology behind the mode of action of pain- and/or mood-controlling drugs, either existing or in clinical development, and identify novel biomarkers for neuropathic pain and emotional co-morbidities.

WP5 - Identification of new biomarkers of neuropathic pain and drug effectiveness using the novel animal models and genetically modified mice and of the effectiveness of cannabinoid and opioid compounds using the novel animal models. This WP identifies new gene biomarkers in the neuropathic pain models developed in WP1 using out-bred (WP2) and conditional knockout (KO) mice deficient in specific components of the endogenous opioid (WP3) and endocannabinoid system (WP4). Moreover, it identifies pharmacogenomics biomarkers of efficacy of the novel compounds selected in WP6. A complete transcriptome analysis is performed in specific neural structures in rodent models of neuropathic pain. These candidate genes are compared to those identified in humans (WP7) and subjected to validation studies (WP8), as well as to those identified in WP10 for across validation of animal and human findings.

WP6 - Evaluation of the analgesic and safety profile of new compounds acting on the endocannabinoid and opioid system in the novel animal models of neuropathic pain. Novel compounds acting on the endocannabinoid and opioid system with potential interest for neuropathic pain treatment have been developed by the two pharmaceutical companies involved. The most promising candidates are evaluated in the behavioural and electrophysiological approaches developed in WP1.

WP7 - Identification and characterization of new biomarkers of neuropathic pain in human cohorts. This WP entails the
identification of the key pathways and molecular processes involved in the transition from acute to chronic neuropathic pain by comparison of the genomes of patients developing or not neuropathic pain under similar nerve injury conditions. We utilize large, well-defined cohorts of patients for discovery, cost-effective high throughput next generation sequencing and genotyping technologies and large replication. For discovery we also obtain whole exome sequences for two extremely well phenotyped samples: a PMPS cohort (Finland, Partner 10, HUS) and an HIV-neuropathy cohort (UK, Partner 11, Imperial). Findings are replicated using well characterized samples from Germany, Cambodia, USA, Canada and Israel. Endophenotypes are used as well as data for a subset of patients and controls undergoing extensive re-phenotyping procedures, including quantitative sensory testing. Identified variants are also studied by mining genotype-phenotype correlations in our existing large and well characterized mood disorder samples and the Icelandic migraine cohort that has been extensively phenotyped using detailed questionnaires. The Icelandic re-phenotyping includes assessments of the affective component, as does that in Finland (Partner 10, HUS). Patients at the Charité (Partner 9, Germany) are screened and followed in a multidisciplinary Pain Clinic staffed by specialists trained to detect emotional aspects of chronic pain. Other replication samples have also been extensively characterized with respect to the affective component.

WP8 – Mice and Men, validation of findings across species. Human sequence data for pathways identified under WPs 3-5 are mined for associations with pain, endophenotypes and affective traits. We recruit carriers of mutations influencing gene function, and thus conduct human studies quite analogous to the mouse KO studies. In addition, deep re-phenotyping of carriers of specific mutations, through questionnaires and various electrophysiological, cognitive and neuroimaging tests, represent an extremely effective strategy for target validation. Genes identified in human subjects provide the exciting opportunity to generate KO animals, and to apply the paradigms and batteries developed by the NeuroPain consortium. Central components of pain and effects of drug candidates are examined by behavioural assays in animals (Partner 1) and by neuroimaging techniques at Charité Campus (Partner 9). Follow-up studies of the impact and roles of the gene products identified in WPs 8-9 are also made by recording the activity of sensory neurons to sensory modalities in rodents (Partner 2). Bringing all these data together allows building solid predictions as to whether an agonist or antagonist of each particular target would be needed to reduce neuropathic pain.

WP9 - Evaluation of the effectiveness of cannabinoid and opioid compounds in neuropathic pain in humans. To evaluate analgesic effects of a cannabinoid agonist (developed by Partner 3 GW and selected in WP6) in clinical neuropathic pain, we examine patients with HIV-associated neuropathy. Patients have been randomized to receive either the cannabinoid followed by placebo or active control (gabapentin) followed by placebo. Efficacy and safety endpoints are assessed. To complement WP3 and WP6, the clinical relevance of peripheral opioid receptors and a compound acting on sigma and opioid receptors developed by Partner 4 (GIE-CERBM) is discussed.

WP10 - Pharmacogenomic studies in humans to evaluate the genetic characteristics of responders and non-responders to cannabinoid compounds. Genotypes for biomarkers identified as associating with neuropathic pain in previous WPs are used in a pharmacogenomic study. For this purpose, we condition the arms of an ongoing trial of the effectiveness of a selected phytocannabinoid (cannabidivarin, CBDV) for treatment of neuropathic pain in HIV-1 patients to dissect the effect of biomarkers on treatment outcome.

WP11 - Dissemination and Exploitation plan. To ensure a maximum exploitation of the new findings developed in this project, the three companies add new online marketing tools to its commercialization strategy. Preclinical research laboratories and mainly other pharmaceutical companies are potential customers. The three companies are strongly involved in this WP.

WP12 - Management. The Coordinator is responsible for the management and coordination of all research activities, owing to his previous experience as coordinator of several European projects. A dedicated project manager acts as direct Coordinator support and permanent help for all project participants, together with the Project Office. Relevant bodies are the Executive Steering Committee, the General Assembly and an External Advisory Board.

Project Results:
The scientific-technical work has been distributed in ten work packages, whilst one has been dedicated to the dissemination and exploitation of the results and another one to the management issues. The description of the main scientific and technical results and derived foregrounds is shown below.
WP1. New methods to evaluate the behavioural, emotional and cognitive components of neuropathic pain

These WP1 has yielded new methodologies for the evaluation of neuropathic pain in animal models.

New animal model studies (partner 1, UPF)

The experimental activity was focused on the evaluation of the emotional components of pain, as several reports have demonstrated a frequent association of chronic pain in humans with affective disorders, such as anxiety and depression, and impaired cognitive functions, including memory and decision-making, and motivation for goal-directed behaviours. Preclinical drug discovery for the treatment of chronic pain is at present challenged by the difficulty to study behaviours comparable to the complex human pain experience in animals. In this study we validated different behavioural outcomes to measure the emotional and cognitive manifestations of neuropathic pain induced in mice by partial sciatic nerve ligation (PSNL). In these mice, we evaluated at different time points: nociceptive responses, anxiety- and depressive-like behaviours, anhedonic state, cognitive impairment, operant responding maintained by food and the effects of the repeated administration of pregabalin on these manifestations. Our results demonstrated that the presence of allodynia and hyperalgesia in neuropathic pain mice was associated with increased anxiety- and depressive-like behaviours, reduced memory functions, development of an anhedonic state and impaired motivation to obtain food in the operant task. Chronic pregabalin treatment improved the nociceptive, anxiety-like and anhedonic responses, as well as the memory deficit, but did not modify the depressive-like alterations and the decreased motivation in these mice. These results indicate that some emotional manifestations of chronic pain do not necessarily resolve when pain is relieved, and underline the relevance to evaluate multiple behavioural responses associated with chronic pain, including the affective-motivational and cognitive behaviours, to increase the predictive value of preclinical drug discovery. This model is now ready to be further employed (Deliverable D1.1). Further and detailed description of these results have been published (La Porta et al., 2015 and 2016; Negrete et al., 2016).

Electrophysiological studies (partner 2, UCL)

On the other hand, electrophysiological correlates of the emotional and cognitive consequences of neuropathic pain were provided. Neuronal recordings in vivo were made in the right central nucleus of the amygdala (CeA) in three groups of mice displaying anxiety, sociability and depression. These consisted, within groups, of low and high percentiles and control mice.

WP2. Specific personality traits responsible for the inter-individual variation to neuropathic pain.

In WP2 we have completed the study of the relationship between different personality traits and the manifestations of neuropathic pain.

Behavioural studies (partner 1, UPF)

The behavioural study of the set of animals identified as extreme phenotypic mice for sociability, anxiety and depression confirm our previous results (Deliverable D2.2). Partial sciatic nerve ligated mice showed a significant impairment in nociceptive, emotional and cognitive-like behaviours regardless of the personality trait analysed. Moreover, we identified anxiety trait prior to a nerve injury as an inter-individual vulnerability factor to develop altered neuropathic pain manifestations. With the brain samples extraction of these last animals we completed Deliverable D2.3 allowing partner 5 (IF PAN) to identify specific biomarkers of interindividual vulnerability to neuropathic pain.

Electrophysiology analyses (partner 2, UCL)

We also completed the electrophysiological correlates of the behavioural, emotional and cognitive consequences of neuropathic pain on mice displaying extreme behavioural phenotypes. The recordings in the amygdala revealed a neuronal signature for sociability. Recordings employed showed that in the central nucleus, there were greater firing levels in the high percentile of sociability group. The results reported in this study highlight the interest of the combinatorial use of behavioural and electrophysiological approaches. This approach may help in understanding the mechanisms that may explain the inter-individual variation of the neuropathic pain manifestations. This study represents a relevant step towards the development of highly efficient personalized treatments for chronic pain.
Further and detailed description of these results can be found in the published article (Martinez-Navarro et al., 2019).

WP3. Evaluation of the role of specific components of the endogenous opioid system in neuropathic pain

This WP3 has yielded new and fully characterised mouse lines targeting the endogenous opioid system, ready for the evaluation of neuropathic pain endpoints.

Several mouse lines targeting the mu opioid receptor (MOR) or the delta opioid receptor (DOR) have been generated and further characterised (partner 5, CRBM) or evaluated employing behavioural (partner 1, UPF) and electrophysiology (partner 2, UCL) approaches. These lines are, for the mu receptor: a conditional deletion of mu opioid receptors in peripheral nociceptors (Nav1.8-MOR initially reported in Weibel et al., 2013), a conditional deletion of mu opioid receptors in GABAergic forebrain neurons (Dlx5/6-MOR, Charbogne et al., 2017), a complete deletion of mu opioid receptors (CMV-MOR, initially reported in Weibel et al., 2013); and for the delta opioid receptor: a conditional deletion of delta opioid receptors in peripheral nociceptors (Nav1.8-DOR initially reported in Gavériaux-Ruff et al., 2011), a conditional deletion of delta opioid receptors in GABAergic forebrain neurons (Dlx5/6-DOR, Chu Sin Chung et al., 2015) and a complete deletion of delta opioid receptors (CMV-DOR, initially reported in Gavériaux-Ruff et al., 2011). The evaluation of the role of these receptors in chronic pain and its emotional and cognitive components showed very interesting results. Nerve-injured constitutive KO mice shows the highest mechanical and cold sensitivity, while central and peripheral conditional DOR knockouts present intermediate mechanical and cold sensitivity values between the wild-type and the total KO animals. Mice with a conditional deletion of DOR in GABAergic forebrain neurons exhibit higher heat hypersensitivity regardless chronic pain. Mice lacking DOR in GABAergic forebrain neurons, but not those with DOR deletion in Nav1.8+ nociceptors, show an enhancement of depressive-like behaviour under baseline and neuropathic pain conditions. Therefore, only central DOR contribute to the pro-depressive effect previously described in constitutive DOR KO mice. Peripherally expressed DOR do not contribute to the anxiolytic-like effect previously described. DOR are involved in long-term memory processing according to memory deficits observed in constitutive knockout. This effect seems mediated by DOR expressed in forebrain sites.

Mice lacking MOR in forebrain GABAergic neurons (Dlx-MOR) mice showed no difference to wild-type mice for nociceptive thresholds in mechanical, heat and cold modalities. The ubiquitous CMV-MOR mice displayed a lower threshold than wild-type mice (p<0.001) in the heat test. The targeted deletion of MOR in GABAergic forebrain neurons (Dlx-MOR) reduces alcohol reward and drinking behaviour, bringing additional evidence that reward pathways are modified in these animals; moreover, this phenotype may have implications in emotional responses to chronic pain. The constitutive -but not the conditional in specific neuronal populations- deletion of DOR disrupted long-term memory and enhanced mechanical allodynia (only in males), the peripheral DOR population is not involved in the modulation of depressive-like behaviour, while DOR expressed in GABAergic forebrain neurons may be partially involved in this response in males. The constitutive deletion of MOR reduced mechanical allodynia and increased thermal sensitivity and thermal hyperalgesia, produced an anxiogenic-like phenotype and had no effect on long-term memory. However, male and female central conditional KO mice showed an anxiolytic phenotype in the elevated plus maze and, only for females, also in the black-white test. Preliminary results of CeA and spinal cord neuronal recordings in the different group of genetic altered mice showed differences between neurons (partner 2, UCL). These results are detailed in Deliverables D3.4 and D3.5.

We have also studied the role of enkephalins in the same behaviours. For this purpose, we have generated a constitutive knockout mouse line for the Penk gene (PENK KO) as well as a conditional line where the Penk gene is deleted in GABAergic forebrain neurons (Dlx5/6-Cre mice), using a similar approach to that already described for MOR (Charbogne et al., 2017) and DOR (Chu Sin Chung et al., 2014) conditional KO mice (collaboration between partners 5, CERBM, and 6, UKB). Their behavioural evaluation has shown that enkephalins contribute to the development of mechanical allodynia in neuropathic pain, as well as to basal emotional states (anxiety) and cognition (memory). However, there is no indication that the later roles contribute significantly to emotional and cognitive behavioural alterations produced by neuropathic pain. On the other hand,
enkephalins play a general role in nociceptive, emotional and cognitive consequences of neuropathic pain and specific roles of enkephalins expressed in GABAergic neurons of the forebrain (central enkephalins). These include (i) a critical contribution to reduce mechanical, but not thermal allodynia, as well as depressive-like behaviour induced by neuropathic pain, and (ii) a role in enhancing memory under basal conditions. These results are detailed in Deliverable D3.6.

WP4: Evaluation of the role of specific components of the endocannabinoid system (ECS) in neuropathic pain

WP4 allowed us to perform the generation and molecular characterization of mouse mutants for the different components of the ECS (partner 5, UKB), as well as their behavioural characterisation (basal nociceptive and personality traits) (partners 5, UKB, and 1, UPF). In the further course of the project, the most promising mouse lines were analysed with regard to the long-term consequences of neuropathic pain.

New conditional knockout mouse lines targeting the endocannabinoid system

During the whole NeuroPain project we have generated several knockout mice as planned. These lines include both the cannabinoid receptors CB1 and CB2, as well as mice that are mutant for the enzymes catalysing the biosynthesis and degradation of endocannabinoids (DAGLalpha, DAGLbeta, ABDH12, MAGL).

Starting with the CB2 receptor, we first generated conditional KO mice with a selective deletion of the receptor in nociceptive neurons, in glutamatergic forebrain neurons and in descending serotonergic neurons. The CB2 receptor is an interesting target for neuropathic pain treatments, because compounds acting on this receptor relieve neuropathic pain symptoms without inducing central nervous system side effects. Our conditional mouse lines were subjected to different behavioural tests to determine their nociceptive and affective traits. Most surprisingly, the pain behaviour in mice with a deletion of CB2 in neurons was unaltered. These results indicate that neuronal CB2 receptors do not play a modulatory role in pain sensitivity and in the development of neuropathic pain. Another phenomenon occurring in neuropathic pain is the mirror-image pain: pain induced by a nerve ligation is typically restricted to the side of the injured nerve, but may occasionally spread to the other, contralateral side. To address the question if CB2 receptors on neurons or on microglia/macrophages are involved, we used different genetic CB2 mouse models. First, we show that a GFP reporter protein under control of the CB2 promoter is induced upon partial sciatic nerve ligation in spinal cord, dorsal root ganglia, and sciatic nerve macrophages, but not in neurons. Mice lacking CB2 receptors specifically on myeloid cells (microglia, macrophages) developed a mirror-image allodynia similar to constitutive CB2 receptor KO mice. Such a phenotype was not observed after the deletion of CB2 from neurons. This behavioural pain phenotype was accompanied by an increased activation of microglia in the dorsal horn of the spinal cord and by an up-regulation of leptin receptor in the injured sciatic nerve. We conclude that CB2 receptors on microglia and macrophages, but not on neurons, modulate neuropathic pain responses.

Finally, we aimed to elucidate alternative therapeutic targets for neuropathic pain by modulating the level of endocannabinoid production and the duration of their activity. To approach this, we have generated new mouse mutants for the endocannabinoid biosynthetic and metabolizing enzymes (DAGL, ABDH12 and MAGL). After the first basal tests, we saw that the basal nociceptive and anxiety-like behaviour in DAGLbeta, ABDH12 and MAGL mutant mice remained unchanged. This indicates that the impact of these enzymes on pain and its concomitant behaviour is only minor.

We therefore concentrated our efforts on the characterization of the DAGLalpha KO mutant mouse lines. In contrast to the pain phenotype, where constitutive DAGLalpha KO mice showed no alterations, they displayed an increased anxiety-like behaviour (Jenniches et al, 2015). We therefore generated conditional DAGLalpha KO mice and evaluated them in anxiety- and depressive-like behavioural paradigms. In these lines, DAGLalpha is knocked out under control of the GLAST- or GFAP-promoter, respectively. GFAP-DAGLalpha KO mice showed increased anxiety-related behaviour as well as a depressive-like phenotype. We have observed the latter in a mitigated form also in GLAST-DAGLalpha KO mice.

The lack of an overt nociceptive phenotype in mouse mutants for the ECS enzymes DAGLbeta, ABDH12 and MAGL probably relates to the complex synthesis and degradation pathways of endocannabinoids. This limits the functional consequences of
the blockade of a single enzyme. From a genetic perspective we would thus argue that ECS enzymes are not ideal candidate targets for novel analgesic drugs. Cannabinoid receptors therefore remain the more interesting ECS components as therapeutic targets in ECS-associated pathological conditions.

Self-medication experiments
The potential role of CB1 receptors was also examined, because it is known to modulate neuronal circuits contributing to chronic pain states and affective behaviours. For this purpose, we analysed the consequences of a partial sciatic nerve ligation on anxiety- and depression-related behaviours in mice lacking CB1 receptors. Our results show that the development of mechanical hypersensitivity was similar in CB1 deficient mice and wild type controls. However, CB1 knockouts showed much more pronounced behavioural manifestations of anxiety-related behaviours, sucrose anhedonia, and a disturbed home-cage activity. These results indicate that the endocannabinoid system affects chronic pain-induced mood changes through CB1 receptors (Racz et al., 2015). As a subsequent step we generated conditional CB1 receptor KO mice, allowing to assess potential cell-specific impacts of the CB1 receptor on pain conditions. Initially, conditional knockouts deficient for the CB1 receptor in peripheral sensory neurons were tested in nociceptive paradigms. However, here we did not find any differences between KO and wild type mice, indicating that the selective deletion of CB1 receptors in nociceptors had no effect on acute thermal and tactile pain sensitivity. Thus, we generated further conditional lines with cell-specific CB1 deletions. It was known from our previous studies and from the literature that glia cells play a crucial role in neuropathic pain. That prompted us to hypothesize that the cannabinoid system plays a role in the regulation of glial activity, thus in neuroinflammatory processes key to pathological pain sensitivity. Based on our earlier findings showing that CB1 receptors on GABAergic neurons are critical to glial regulation (Albayram et al., 2011), we now focused on the role of GABAergic neuron-specific CB1 receptors in the regulation of glial activity. We compared microglia density, morphology and cytokine expression in wild type and GABAergic neuron-specific CB1 knockout mice after lipopolysaccharide treatment. Our results suggest that CB1 receptor agonists can modulate microglial activity indirectly, through CB1 receptors on GABAergic neurons. We demonstrated that GABAergic neurons, despite their relatively low density in the hippocampus, have a specific role in the regulation of microglial activity and that cannabinoid signalling plays an important role in this arrangement. Following the role of microglia, we consequently concentrated on astrocytes. Astrocytes are key regulators of brain homeostasis, which are known to significantly influence neuronal activity and pain sensitivity. Our findings demonstrate that the disruption of endocannabinoid signalling on GABAergic neurons is accompanied by functional changes in astrocyte activity.

WP5. Identification of new biomarkers of neuropathic pain and drug effectiveness using the novel animal models and genetically modified mice.

In this WP5 we have identified new biomarkers of neuropathic pain and we have developed a data base for the comparison of mouse and human biomarkers of chronic pain (Partner 7, IF PAN).

Anxiety and mood disorders are frequently associated with chronic neuropathic pain after partial sciatic nerve ligation (PNSL). Neuronal substrate is related to pathological adaption that are poorly understood in key nodes of motivational neural circuitries. We therefore studied potential relationship between behavioural phenotypes (related to depression, anxiety and sociability) on the development of neuropathic pain and alterations of gene expression in the motivational and emotional circuitry. Behavioural studies showed that low sociable, high anxious and low depressive phenotypes developed enhanced nociceptive hypersensitivity after peripheral nerve injury. We have searched for molecular transcriptomic biomarkers associated with cognitive and emotional phenotypes of neuropathic pain. The main finding demonstrated that high expression of opioid gene prodynorphin (Pdyn) in the amygdala appears to be associated with the enhanced nociceptive hypersensitivity. Activation of transcription factor Npas4 as well as a neuronal activity sensor, Gadd45, indicates an amygdala overactivation upon neuropathic pain suggesting the involvement of these biomarkers in emotional component of pain. Furthermore, activation of expression of gene Il6 accompanying by high anxiety and low depression traits may also indicates a relationship between neuroinflammation and nociceptive hypersensitivity as well as affective component of neuropathic pain.
At whole genome level we have searched for genomic biomarkers of PNSL and performed genome-wide transcriptional profiling (RNAseq) at the spinal cord of C57BL/6 mice. We have identified distinct subsets of mRNAs of 28 (FC>2 fold, FDR<5%) highly up-regulated (e.g. Xlrfb4, Tgtl2, Atf3, Prx) and 33 down-regulated transcripts (e.g. Mfsd13b, Thbs4, Foxn1, Rem1, Jph2). At a lower level of expression (FC<2 fold, FDR<5%), over 4000 transcripts have been found to be significantly altered indicating their putative relation to neuropathic pain. Gene-ontology enrichment analysis suggests that genes involved in neurotransmission, calcium signalling, structural modification of neuronal cells, and immune responses and defence, coding for cytokines and chemokines (and their receptors) are affected after PNSL induction of neuropathic pain in mice.

Identification of neuropathic pain genetic biomarkers associated with deficiency in the endogenous opioid systems

We have used three different lines of KO mice deficient in specific components of the endogenous opioid system.

PENK KO mice. We have elucidated the genetic markers of susceptibility of proenkephalin (PENK) neurons to neuropathic pain using RT-PCR in the nucleus accumbens, hippocampus and amygdala of neuropathic PENK knockout mice. PNSL altered expression of genes selected for their role in the neuronal activity (Npas4, Egr1), opioid signaling (Pdyn, Oprk1), stress (Nr3c1) and inflammation (Il-1beta, Aif1, Iba1). Given the importance of endogenous PENK neurons localised in the spinal cord and the forebrain in the regulation of neuropathic pain we have further focused on global gene expression (RNAseq) in mice with constitutive PENK KO and conditional PENK KO in forebrain neurons. Nociceptive data of PNSL mice revealed increased allodynia for mice PENK in the forebrain GABAergic neurons. At whole genome level we have identified in constitutive PENK KO mice spinal cord subsets of mRNAs of 104 (FC>2 fold, FDR<5%) highly up-regulated transcripts (eg. Rsc1a1, H2a1Lk, Sprr2a2, Trim10, Dcstamp, Bcl2a1a, Tldc2, Camp) and 112 down-regulated biomarkers (e.g. Ifnz, Sprr2a1, Fam167b, Phox2a, Mettl7a2, Lmx1a, Trelm1, Tbx21). The results revealed that the upregulated genes were predominantly associated with transmembrane signaling receptor activity, molecular transducer activity and sensory process, G-protein signaling pathway, sensory perception of chemical stimulus pathway. The downregulated genes were involved with catalytic activity, protein and enzyme binding, neuron projection, mylein sheath, postsynapse, psychomotor, cognition and locomotor behaviour. These gene alterations in deficient of PENK mice might be related to enhancement of allodynia after the nerve injury.

DOR opioid receptor KO mice: Behavioural research (Partner 1, UPF) indicated an increase of mechanical allodynia of constitutive opioid receptor delta (DOR) KO as well as conditional DOR KO in forebrain neurons mice. This response was accompanied by decrease of the expression level of PDYN and enhanced expression of proinflammatory cytokine IL-1b. This may indicate that DOR KO affect the expression of PDYN in the spinal cord what is associated with enhancement of pain transmission. Global gene expression revealed in the spinal cord of 207 genes upregulated (FC>2, FDR>5%) (e.g. Top Gvin1, Sycp1, CldnB, Yy2, Myh 1, Acta1, Tuba1c, Serpina3m) and 149 downregulated transcripts (e.g. Igflr1, Pth2r, Tgm3, Kira2, Parg1b, Hbq1b, Tomt). Gene ontology revealed actin filament organization, cellular response to heat, neural crest cell migration, negative regulation of synaptic transmission and negative regulation of NF-kappaB. Thus both deficiency of DOR and prohormon generating endogenous ligands for the receptor, it means PENK, may leads to alteration of set of transcripts might related to enhancement of mechanical allodynia.

MOR opioid receptor KO mice: The constitutive deletion of the MOR gene revealed that the severity of neuropathic pain was accompanied by thermal hyperalgesia (Partner 1, UPF). Enhancement of thermal hyperalgesia was accompanied by increase in the PDYN expression in the spinal cord of KO mice with complete and a partial deletion forebrain neurons deletion of MOR. The behavioural changes were accompanied by increase in expression of IL-1b, C1q and GFAP. Global gene expression revealed in the spinal cord of 50 genes upregulated (FC>2, FDR<5%) (eg. Hmga1b, Khdc3, Hbq1a, Igflr1, Mgl2, Pcdh12, Il31ra, Prtn3) and 98 downregulated transcripts (eg. Pcdhga10, Hist1h2al, Hbq1b, Hist2h3b, Wt1, Tmem37, Gpha2, Ush1c, Nppa, Mmp25, Egr2). Gene ontology revealed among overs, regulation of long-term neuronal activity, proteasome assembly, positive regulation of DNA repair, cellular response to heat, actin filament-based process and cytoskeleton organization. The present study indicated set of transcripts which may be related to the process enhancement of thermal hyperalgesia, enhancement sensitivity to heat stimuli.
Data-base of candidate gene biomarkers

We searched to generate this data base for candidate gene biomarkers found in mice with genes identified in human cohorts. The obtained results in neuropathic mice in a broader context allow comparison with already gather for human genes associated with neuropathic pain diseases. We have decided to set up a copy of HumanMine and extend it by results of our analysis using the following bioinformatics platform developed by us: [http://bioinf.mcb.uj.edu.pl/NeuroPainMine](http://bioinf.mcb.uj.edu.pl/NeuroPainMine). Comparison of mouse and human biomarkers of chronic pain, including neuropathic pain by using the developed database based on degrees within the merged network revealed that the six most highly connected genes were Prx, Folr1, Sic5a7, Pmp22, Snap29 and Scn9a. In the neuropathic pain model we found also altered expression of sodium voltage-gated channels such as Scn3a, Scn5a Dnmt1, Retreg1, Sptlc1 and Sptlc2. Thus, the present research on global gene expression in animal models has detected several genes associated with various kind of human gene variant characteristic for monogenic neuropathy disorders. We have further compare list of human pain genes delivered by Partner 8 with set of transcripts found in our mice study. The study identified the importance of the immune system (Il1a, Il1b) neurotransmission (Comt, Htr2c), calcium and sodium channel (Cacng2, Scn9a), transient receptor-potential M8 channel (Trpm8) and cellular Prkca, which encodes the protein kinase C isozyme alpha. Thus, comparison of mouse and human genetic biomarkers of chronic pain revealed by the Consortium Members, identified the importance of the immune system cytokine signalling genes, pathways associated with neurotransmission, calcium and sodium channel genes and cellular signalling.

WP6. Analgesic and safety profile of new compounds evaluated in the new animal models of neuropathic pain

Two leading pharmaceutical companies developed new phytocannabinoids (Partner 3, GW) and sigma-1/mu-opioid dual compounds (Partner 4, ESTEVE), which were evaluated in animal models for their analgesic efficacy using behavioural (Partner 1, UPF) and electrophysiology (Partner 2, UCL) approaches.

Partner 3 (GW Pharma) has identified the reproducible plant (Cannabis sativa) genotype and chemotype most suited to the reproducible production of cannabidivarin (CBDV). To this end, GW Pharma has developed a well-defined, reproducible and established process by which purified CBDV is extracted from CBDV plant material (task 1). In order to guarantee the supply of purified CBDV for the preclinical and clinical phases of this programme, proprietary technology was applied to produce purified compound for conformance with a detailed specification agreed by Regulatory Authorities and which is subject to the same rigorous standards associated with the manufacture of other pharmaceutical products (task 2).

In turn, Partner 4 (ESTEVE) has carried out an extensive discovery programme to identify and select mu-opioid agonist/sigma-1 receptor antagonist bivalent compounds. Modern drug discovery can involve the design and synthesis of compounds, identification of screening hits and a medicinal chemistry approach for the optimization of those hits to increase the affinity, selectivity (to reduce the potential of side effects), metabolic stability (to increase the half-life) and other ADME parameters including oral bioavailability, efficacy/potency, and safety and tolerability. At the end of the discovery process (Lead Optimization phase) a compound that fulfils all of these requirements was identified and nominated as a candidate: SIMU-1.

During the execution of this programme, the vitro pharmacological properties (affinity, selectivity and functionality) of novel plant-based phytocannabinoids and mu-opioid agonist/sigma-1 receptor antagonist bivalent compounds were investigated (task 3).

CBDV and its major metabolites (7-hydroxy-CBDV and 6-hydroxy-CBDV) do not bind to cannabinoid receptors (CB1 or CB2) with high affinity. However, purified CBDV, but not its major metabolites, inhibits at rather high concentrations some endocannabinoid-related enzymes including diacylglycerol lipase and the endocannabinoid membrane transporter. Selectivity was also assessed against a panel (>100) of non-endocannabinoid system molecular targets and significant affinity was only found for some Transient Receptor Potential (TRP) channels. CBDV is an agonist of TRPV1 and TRPA1, a weak agonist of TRPV2 and TRPV4 channels, and an inhibitor/antagonist of TRPV6 and TRPM8 channels.
Similarly, the in vitro pharmacological profile of mu-opioid agonist/sigma-1 receptor antagonist bivalent compounds was assessed for affinity (binding to the two main molecular targets: mu-opioid and sigma-1 receptors), functionality at the mu-opioid (cAMP levels by HTRF) and sigma-1 receptors (binding in the presence of phenytoin), and selectivity (binding assays) in an extensive panel of other molecular targets (task 4). SIMU-1 compound, selected for development, binds to both mu-opioid and sigma-1 receptors with high affinity and behaves functionally as a partial agonist on mu-opioid receptors and as an antagonist on sigma-1 receptors. SIMU-1 shows no significant binding affinity in a panel of more than 180 other molecular targets, including 7-trans-membrane receptors, voltage and ligand-gated ion channels, nuclear hormone receptors, endogenous transporters and enzymes. The affinity profile of the main metabolites detected in plasma samples of animal species was also assayed and no major affinities for the molecular targets tested were observed.

GW Pharma extensively investigated the pharmacokinetic profile of CBDV, in accordance with regulatory pre-clinical requirements. CBDV was subjected to systematic examination in a well-defined programme for the determination of metabolic route, metabolite identification, tissue distribution of parent compound and metabolites and route of elimination. Pharmacokinetic studies were also done from Phase I clinical trials samples.

ESTEVE also performed a battery of in vitro and in vivo studies in different preclinical species and the ADME properties of the SIMU-1 compound were deeply investigated. Allometric scaling to predict the pharmacokinetic behaviour in humans from preclinical interspecies (mouse, rat, monkey, dog and minipig) scaling, as well as pharmacokinetic/pharmacodynamic relationship were performed to estimate dose posology in humans to get the minimum effective concentration and pharmacological active doses.

Importantly, the safety and toxicology of CBDV and dual mu-opioid agonist/sigma-1 receptor antagonist bivalent compounds was investigated (task 5), with no findings precluding further development. Safety/toxicology CBDV and SIMU-1 was assessed in compliance with the requirements of the EMA and FDA. GLP-compliant toxicology studies (i.e. regulatory package) comprising repeated dosing in rodent and non-rodent species, genotoxicity and reproductive toxicology studies were done with CBDV and SIMU-1. The non-clinical toxicological programme evidenced good tolerability, with a favourable safety profile and thus no cause for concern for undergoing clinical trials.

The in vivo pharmacology assessment was performed studying the analgesic profile of the novel plant-based phytocannabinoid and the dual mu-opioid agonist/sigma-1 receptor antagonist was investigated in a battery of pain animal models (task 6), particularly focused on neuropathic pain as these drugs engage key players in the endogenous analgesia system and were primarily intended to treat neuropathic pain. Both phytocannabinoids and SIMU-1 inhibited neuropathic pain-related behaviours (i.e. allodynia and/or hyperalgesia), thus reinforcing an important premise in this project: compounds acting on druggable targets within the endogenous analgesia system may represent a promising therapeutic approach to treat neuropathic pain. Another important, widely-accepted premise was that activity on evoked responses to mechanical or thermal stimulation in animal models poorly translates to real analgesic activity in clinical practice. In WP1, new behavioural approaches to assess the effect on pain and comorbidities taking into consideration its emotional and cognitive dimensions, as well as electrophysiological recordings to analyse the effect on neurophysiological pain transmission were developed by UPF and UCL, respectively. Accordingly, CBDV and SIMU-1 were assayed for their activity in behavioural (UPF) and electrophysiological (UCL) approaches described in WP1 (task 7). The tested compounds were able to reverse not only sensory but also emotional and cognitive deficits/abnormalities associated to neuropathic pain, and both compounds elicited an effect on electrophysiological recordings compatible with and/or potentially underlying its antinociceptive activity.

WP7. Identification and characterization of new biomarkers of neuropathic pain in human cohorts

The main objective of WP7 was the identification of new biomarkers of neuropathic pain in human cohorts and characterization of those biomarkers with respect to the affective component of neuropathic pain in humans and other relevant phenotypes. To this end, Partner 8 (deCODE) focused recruitment, re-recruitment and re-phenotyping efforts on rapidly assembling
neuropathic pain cohorts phenotyped in accordance to international expert consensus guidelines published (van Hecke et al., 2015). Partner 8 (deCODE) and Partner 11 (Imperial) were invited to the expert panel and all clinical partners of NeuroPain agreed on using this core set of criteria to phenotype the various neuropathic pain samples studied in this grant in Iceland (Partner 8, deCODE), Germany (Partner 9, Charité), Finland (Partner 10, HUS) and the UK (Partner 11, Imperial). To this end, Partner 8 (deCODE) created and hosted the NeuroPain Central Phenotype Database to ensure standardized phenotype collection on the samples to be used for replication and meta-analyses.

In 2016, a meta-analysis of genome wide association studies (GWAS) results for 3,135 Icelandic migraine cases and 95,585 controls were published as part of the largest GWAS meta-analysis of migraine to date (with 59,674 cases and 316,078 controls) in collaboration with the International Headache Genetics Consortium (Gormley et al., 2016). The study identified 44 independent single-nucleotide polymorphisms (SNPs) significantly associated with migraine risk (P<5×10−8); the identified loci showed enrichment for genes expressed in vascular and smooth muscle tissues. However, loci were found in two genes that harbour known ion channels (KCNK5 and TRPM8) and three genes (SLC24A3, near ITPK1, and near GJA1) that can be linked to ion homeostasis (Gormley et al., 20162). As phenotyped samples have grown over the study period, Partner 8 (deCODE) has regularly scanned the top markers from the migraine findings for associations with other neuropathy, neuropathic pain and affective phenotypes assembled within the NeuroPain project to seek the genetic underpinnings of these comorbid conditions. While results from these scans have not survived correction for multiple testing, several interesting results require follow up. Results of these phenotype association scans were presented to the NeuroPain Executive Board Meetings in Strasburg (8-9th January 2017) and in Berlin (February 12-14th, 2018), as well as to the Final Meeting in Helsinki (October 15th-16th, 2018), to ensure timely transfer of knowledge from human studies to mouse studies.

Partner 8 (deCODE) focused recruitment efforts on rapidly gaining statistical power by collecting large sample sizes of well characterized migraine, neuropathic and neuropathic pain phenotypes and obtaining appropriate replication cohorts. Over the study period, Partner 8 has run case/control GWAS on over 150 NP phenotypes and scanned top markers in GWAS of various chronic pain and affective phenotypes (anxiety, depression, pain catastrophizing and personality). The sample sizes in these various NP phenotypes range from N=26 (phantom limb pain) to N=12,500 (Migraine). The main NP pain groups were based on recruitment of women undergoing mastectomy operations (1,517 genotyped) and patients diagnosed with diabetes (4,226 genotyped). Following recruitment and re-phenotyping Partner 8 identified N=5,389 pregabalin or gabapentin, non-epileptic subjects, 191 women with chronic post-mastectomy pain (PMPS) and 140 with painful diabetic neuropathy, with most of those not meeting criteria providing pain-free controls for GWAS. Partner 8 also conducted a GWAS of the diabetic neuropathy disability scale scores in diabetics (N=1,788 quantitative trait) and the diabetic neuropathy symptom scale (N= 2029), again without detecting genome-wide significant variants. These GWAS’s are well-powered studies using up to 300,000 population controls given described imputation methods (Kong et al., 2008). Quantitative trait studies include GWAS of neuropathic pain scores DN4 (Bouhassira et al., 2005) (N=4,627), Quantitative Sensory Testing (QST) phenotypes according to the DFNS criteria (N=1,545) (Rolke et al., 2006) and sural nerve-conduction tests to screen for peripheral neuropathy in at risk neuropathic as well as general population samples (N =9,630). From these large efforts, Partner 8 has identified a biomarker involved in the development of sciatica, the excruciating neuropathic pain secondary to a herniated lumbar disc (Bjornsrdottir et al., 2017), and a sensory negative peripheral neuropathy that is described in a manuscript currently in review at Nature Communications (Bjornsrdottir et al., 2019).

By using polygenic risk scores for depression (affective component of chronic and neuropathic pain), calculated from recently published GWAS meta-analyses of depression (Wray et al., 2018, Partner 8 has shown that the polygenic risk scores from depression (not including Icelandic samples) predicts chronic and neuropathic pain in Icelandic data quite strongly and significantly, indicating that there are indeed common genetic underpinnings to depression and chronic and neuropathic pain that warrant further study.

A basic element of WP7 was the identification and characterisation of new biomarkers of neuropathic pain human cohorts. In this context, and as described above, partner 8 (deCODE) laid the foundation and assembled chronic neuropathic pain groups
relying on re-phenotyping of Icelandic discovery cohorts. This discovery effort was strengthened by two extremely well phenotyped patient cohorts from partners 10 (HUS) (post-mastectomy pain) and partner 11 (Imperial) (HIV-neuropathy) as described below. For further replication, Partner 9 (Charité) launched an observational study including German patients with painful and non-painful neuropathy associated with mastectomy, diabetes mellitus and HIV infection. For comparable phenotyping of the international cohorts, a minimum set of pain measures and questionnaires were collected by all clinical partners (partners 8, 9, 10, and 11), using the standardized data framework implemented in the NeuroPain Central phenotype database, as previously described.

Before the observational study was launched at the Charité study site, several administrative steps had to be taken, including the application to the institutional ethics committee (EC). The first authorization was granted by the EC in July 2014. At the same time, partner 9 registered this study in a publicly accessible registry (ClinicalTrials.gov Identifier: NCT02208206). In February 2015, the final protocol including amendments for additional neuropsychology and quantitative sensory testing approved. Standardized German-language questionnaires had to be acquired/purchased and infrastructures had to be established (study personnel, rooms, equipment). For patient recruitment, different strategies were used, mainly direct contact to clinicians and physicians, presentations to lay public, and advertisements in public transportation.

To comply with biosafety regulations in the laboratory of partner 8 (deCODE), special arrangements had to be made regarding processing and shipping of HIV-infected samples for DNA isolation and subsequent genotyping. As no local institutions in Berlin agreed to isolate DNA from knowingly HIV infected samples, the blood samples underwent special procedures to inactivate HIV-virus at the Charité clinical site utilizing reagents proposed by partner 8 (deCODE). This was successful, and all 94 samples were sent to Partner 8 (deCODE) and have been chip-typed using the Infinium Global Screening Array-24 v1.0 (GSA) BeadChip from Illumina. This an advanced genotyping array for population-scale genetic studies, used by Partner 8 for all the foreign samples analyzed under this study. This part of the study is in collaboration with DeCODE, Reykjavik, Iceland (Partner 8), University Hospital Charité, Berlin, Germany (Partner 9), and Imperial College London, UK (Partner 11).

The 94 German samples (Partner 9, Charité) were from 46 diabetes patients (44 of European descent, 1 sample unusable = 43 for combined analyses), 19 HIV-infected individuals (18 of European descent), and 29 subjects with symptoms related to breast cancer surgery or treatment (28 of European descent). These small, but information-rich and well-characterized samples, were appropriately used in meta-analysis with other replication samples from individuals of European descent with comparable phenotypes. The initial analysis searching for association of common markers with response has been performed, and as expected from these small samples, there were no clearly significant signals. The genotype data for these samples will be returned to Partner 9 (Charité) and can be regarded as a foundation on which to build larger GWAS samples and can be used by them in future meta-analyses and collaborations.

Partner 10 (HUS) completed the detailed re-phenotyping of (N=402) from the sample of 1,000 breast cancer operated females who were extensively phenotyped prior to and immediately after their operation. Over the course of the NeuroPain study, Partner 10 has assembled a unique cohort of patients with similar nerve injury, but different phenotypes. “What factors make surgical nerve injury painful?” has been the main research question. As described, the cohort is part of a previous study of 1,000 breast cancer operated women where the patients underwent a cold-water test before the index breast cancer operation. Surgeons registered the handling of the intercostobrachial nerve (ICBN): it was saved, resected partly or totally, or was not visualized. Immediate postoperative analgesia was carefully followed for one week. The patients answered questionnaires about pain, mood, and quality of life preoperatively and at 1 month, 6 months, 1 year, 2 years, 3 years, and 5,5 years after surgery. Partner 10 aimed to study as many patients with a surgeon-defined ICBN resection as possible from the initial cohort of 1,000 patients. Partner 10 also recruited patients with saved ICBN, but reports of pain in the surgical or nearby area in the history. A total of 589 patients were re-contacted, of whom 187 refused or were lost to follow-up. A total of 402 patients were finally re-examined and extensively re-phenotyped.

The main objective was to phenotype patients some years after breast cancer surgery according to stepwise neuropathic pain
grading system and identify and characterize possible new biomarkers of chronic postsurgical neuropathic pain. Partner 10 used the surgeon-defined status of the ICBN as a confirmatory test for the NP grading. Patients were classified accordingly to non-, possible, probable and definite neuropathic pain groups. Part of the patients were pain free and part of them had pain in the operated or nearby area. The detailed phenotyping consisted of tests as follows: structured questionnaires concerning pain, temperament, mood, cognition, and quality of life, a detailed clinical examination of sensory testing with patients’ pain drawings, cold pressor test to study autonomic nervous system function and endogenous analgesia, and fasting blood samples for glucose level, lipids, vitamin D, and inflammatory markers (high-sensitivity CRP [hs-CRP]; orosomucoid were drawn. For definite neuropathic pain patients, QST was conducted. To study cognition, a computer based cognitive test was used. In addition, to understand the genetic regulation of neuropathic pain, DNA and RNA samples were taken.

Preoperative factors that may present risk factors for persistent postsurgical pain were studied and an online risk calculator for clinicians and researches was created. The prediction models were developed and tested using three prospective data sets from Finland, Denmark, and Scotland. Prediction models for persistent pain of moderate to severe intensity at 1 year postoperatively included as follows: preoperative pain in the operative area, high BMI, axillary lymph node dissection, and more severe acute postoperative pain intensity at the seventh postoperative day. These factors form a simple tool to screen patients at high risk of developing persistent pain after breast cancer surgery.

Preoperatively, Partner 10 (HUS) identified differences in the future neuropathic pain and non-neuropathic pain patient groups in terms of chronic pain and psychological factors. Future neuropathic pain patients showed preoperatively higher BMI, reported more pain in the surgical area and elsewhere, had more depressive symptoms and anxiety, and reported insomnia more frequently than future non-neuropathic pain patients. Postoperatively neuropathic pain patients showed signs of pain sensitization in cold pressor test suggesting a role for central nervous system sensitization. Partner 10’s results suggest that inherent patient-related risk factors such as accumulation of pain conditions with multisite pains, biopsychosocial burden with increased anxiety, pain catastrophizing, depressive symptoms, and impaired quality of sleep, and low-grade inflammation play a significant role in the development and maintenance of chronic neuropathic pain after ICBN resection. Chronic postsurgical neuropathic pain patients presented distinct features when studied by Questionnaire-based self-report or examination-based evoked pain measures. Self-reported pain associated with psychological factors and evoked pain with biological markers such as sensitivity to cold pressor test. These pain types may represent new pain phenotypes for future neuropathic pain studies.

Sensory profiles in chronic postsurgical neuropathic pain showed mostly hypoesthesia in the surgical area both in bedside examination and in QST several years after surgery. The handling of the ICBN did not affect the sensory profile in QST, but ICBN resection seemed to lead to larger areas of sensory impairment. The outcomes of clinical examination and QST were in most of the cases inconclusive, which may reflect differences in the nature and conduct of these assessments. In QST, subclinical neuropathy on the lower extremities was a common finding, also in patients without history of chemotherapy.

The Douleur Neuropathique 4 questionnaire (DN4)4 with an interview and a clinical examination part, and a shorter version of DN4 with only the interview part (DN4i) both showed significant accuracy in stratifying possible and definite neuropathic pain, but DN4 showed greater sensitivity and specificity compared with DN4i. Partner 10 (HUS) showed that the total score of DN4i correlated with pain and psychological variables.

Psychological factors play a crucial role in chronic pain. Expecting more severe pain associates with a more severe pain experience. The most important psychological factors associating with persistent pain were analysed with machine-learning to create a set of questions most relevant in chronic pain. Partner 10 (HUS) showed that high psychological resilience associates with better quality of life and less pain interference. Cognitive performance was overall good and it was not affected by either neuropathic pain or previous chemotherapy. Autonomic nervous system reactions to the cold pressure test showed significant interindividual variation and associated with pain tolerance.

To find novel biomarkers associated with neuropathic pain, Partner 10 (HUS) studied extreme phenotypes of chronic
postsurgical neuropathic pain with those having ICBN resection with or without pain (N= 58). By sequencing the miRNome from plasma samples drawn both preoperatively and after 4-9 years from surgery, it was shown that multiple novel miRNAs have a putative role in the development and maintenance of chronic neuropathic pain after surgical nerve injury. With the same extreme phenotypes, Proteomics from plasma samples using the same two time points and could identify novel mediators in this process was also studied. None of the studied inflammatory mediators showed significant intergroup difference preoperatively. This suggests that systemic inflammation plays more important role in maintenance of neuropathic pain after surgical nerve injury rather than predisposing to it. Additionally, there are ongoing analyses of autoantibodies and rare genetic Nav1.7 variants on chronic postsurgical neuropathic pain in collaboration with Partner 8 (deCODE) who has run case-ctrl GWAS on the extreme phenotypes and sequenced the N=58 cohort. While the small GWAS study showed no significant results, the forthcoming sequencing results may give further insight to the neuropathic pain pathophysiology and possible therapeutic targets.

In summary, the results of Partner 10 implicate a role for several biomarkers in chronic postsurgical NP in breast cancer-operated women. NP phenotype may be classified also according to pain sensitivity in CPT with respect to central nervous system sensitization and low-grade inflammation. These may be new ways of looking at the phenomenon of chronic postsurgical NP and have potential in utilizing these phenotypes in future therapeutic interventions.

Psychological factors play a role in chronic NP and we have shown that the expectation of pain has an impact to the future pain experience. This is an important attribute for the clinicians and researchers for patients facing surgical procedures. Other chronic pain conditions, psychological factors, and pain sensitivity in CPT are significant in the NP phenotype. Partner 10 found some potentially promising biomarkers in proteomics and miRNA analyses. These results await further confirmation from future studies in separate cohorts. More information can be found in the related Deliverable D7.14.

The HIV-1 study (POGO, Partner 11, Imperial) completed recruitment and phenotyping (N=198) (WP7). This work resulted in the first detailed description of chronic pain in an HIV cohort with the specific aim of comparing symptoms and associations in those with and without sensory neuropathy. DNA samples from Partner 9 (N=94) and Partner 11 (N=148) have been genotyped by Partner 8 and association studies have been performed. Subsets of samples from Partner 10 (N = 58) and Partner 11 (N = 33) were defined with well-characterized neuropathic pain vs pain-free controls and sequenced by Partner 8 in search for rare variants associating with neuropathic pain and to replicate suggestive findings in Icelandic neuropathic pain samples. An overview of the HIV-1 POGO study is given below.

All ethical and regulatory approvals for the clinical phenotyping and genotyping study for patients with HIV sensory neuropathy at Imperial College were finalized by December 2014. The site investigator, Dr Harriet Kemp (previously known as Wordsworth) has undergone training in Quantitative Sensory Testing with the DFNS, in Mannheim, and Conditioned Pain Modulation (CPM) with Prof Yarnitsky at Ram Bam Medical Centre in 2015. A healthy volunteer study to establish a CPM protocol in the laboratory and to assess the best paradigm in terms of reliability and standard error of measurement was conducted. This study recruited 50 healthy volunteers who were tested twice, one month apart. The results from this project were presented at NeuPSIG 2017 as a poster presentation and to the Royal Society of Medicine in June 2017 as an oral presentation. The work was awarded the trainee prize for the Anaesthetic Section of the RSM.

A total number of 148 subjects with HIV were fully phenotyped to the complete protocol (QST, CPM, clinical examination, demographics, self-report pain profiling, cognitive function, and psychosocial questionnaires). All provided blood samples for genetic analysis. DNA for genetic analyses was extracted on site at Imperial and sent to Partner 8 (DeCODE genetics) where N = 117/148 samples from individuals of European descent could be used in meta-analyses with European descent samples from other partners and the UK Biobank as described above.

Detailed phenotyping data was entered onto a bespoke database specifically designed and constructed for this study by the requirements of Imperial College at Imperial College, the data definitions compatible with the standardized data collection
defined for the multi-site NeuroPain database designed and hosted by Partner 8 (deCODE). These data were shared with Partner 8, to allow for in-depth genetic analysis of pain characteristics and phenotype definitions for the meta-analyses with data from other partners in WP7 and the UKB data. All 148 samples from Partner 11 have been chip-typed by Partner 8, using the Infinium Global Screening Array-24 v1.0 (GSA) BeadChip from Illumina. Several GWAS studies have been performed on phenotypes defined from these data without significant results. Sequencing of 33 samples with extreme NP phenotypes comparable to the case/ctrl definitions of the PMPS sample of Partner 10 are ongoing as described below.

The QST, CPM, pain and demographic information have been analyzed. These data form part of a doctoral thesis that has been submitted for examination by Dr. Harriet Kemp, and is further delineated in the attached Deliverable D.7.16 (according to Annex 1). The data presented in D7.16 comprise the first detailed description of chronic pain in an HIV cohort with the specific aim of comparing symptoms and associations in those with and without sensory neuropathy.

A novel laboratory-based project was proposed using stored serum samples and peripheral blood mononuclear cells (PBMCs) from the phenotyped subjects. The main aim to identify an association between the amount of total viral DNA and certain pain characteristics using quantified PCR. Funding was allocated from within the consortium to complete this extra work. An experimental plan and qPCR protocols were confirmed.

Work on other identified tasks of WP7 was completed according to the plan detailed in Annex 1; namely, sequencing and genotyping, data analysis for gene discovery and evaluation of association of biomarkers with anxiety, depression and personality, were all part of the meta-analyses with data from other partners, described above. The last months of the grant period focused on sequencing of samples from HIV individuals with and without the painful extreme neuropathic pain phenotypes as defined in the Finnish PMPS samples from Partner 10 (HUS).

Replication studies: Replication studies and meta-analyses using the above described samples from NeuroPain partners 8, 9, 10 and 11 have been performed. As the plans outlined in Annex 1, for obtaining larger replication cohorts from Germany, Cambodia, USA, Canada and Israel could not be completed as planned, Partner 8 applied for and obtained replication data from the UK Biobank (N ~ 500,000) and has run meta-analyses on the core set of NP phenotypes defined in Icelandic data and where applicable, with comparably phenotyped cohorts from Partners 9,10 and 11. The initial analyses searching for association of common markers with the core set of comparable neuropathic pain phenotypes has been performed, and there were no clearly significant signals. Larger samples may yet be required as suggested by the meta-analysis of migraine, where ~ 60,000 cases were compared to ~320,000 controls for discovery.

WP8. Mice and men, validation of findings across species.

The selection of targets for study in humans was based on scientific literature (van Hecke et al., 2015), in which Partners 8 (deCODE) and 11 (Imperial), along with renowned neuropathic pain experts, came together under an invitation from the International Association for the Study of Pain (IASP) Special Interest Group (SIG) on Neuropathic Pain (NeuPSIG), and in collaboration with the IASP SIG on Genetics and Pain, with the aim to reach consensus on neuropathic pain phenotyping for human genetic studies.

Partner 8 (deCODE) carried out a comprehensive literature review (Electronic databases MEDLINE, EMBASE, SCOPUS, Science Direct, ISI Web of Science, and CINAHL were searched from January 1966 to April 2014) of genetic studies on neuropathic pain, revealing that they have been inconsistent in their definitions of neuropathic pain and have met with replication difficulties, in part because of differences in phenotypes used for case ascertainment. For genetic research to contribute more fully to furthering knowledge of neuropathic pain, an agreed, valid, and feasible approach to phenotyping is required, to allow collaboration and replication in samples of sufficient size, as per the aims and execution of phenotyping in WP7.

Through this literature review, and from the large migraine GWAS meta-analysis to which Partner 8 (deCODE) contributed
genotype and phenotype data on 99,000 individuals, ~3,000 cases and ~96,000 controls (Gormley et al., 2016), a list of high priority gene targets from human genetics of migraine and neuropathic pain was compiled, based also on the dissection of the effect of genes and pathways. These gene targets were a) shared with other NeuroPain partners as per the aims of WP3 and WP8, and b) used by Partner 8 (deCODE) as gene targets that were routinely scanned for GWAS associations with neuropathic pain phenotypes in Iceland as the sample sizes in WP7 grew. Selection of targets from WP5 for study in humans has been completed with genes and pathways identified under WP3-5 studied by partner 8 (deCODE) in human data for associations with pain and affective trait phenotypes and endo-phenotypes (see poster by Bjornsdottir and Thorgeirsson presented in Helsinki 2018: From mice to men: Studying targets from WP5 in human neuropathic pain).

Partner 8 (deCODE) focused discovery efforts on: a) known neuropathic pain candidate genes from human and mouse studies as well as from syndromic evidence (e.g. SCN9A), b) the list of genes provided by research partners studying mice (WPs3-5), and c) candidate genes identified through studies in WP7.

While no associations from these studies in humans have reached genome-wide significance when correcting for multiple testing, many interesting regions have been identified that will require further follow-up, such as in studies of rare high-impact variants identified in sequencing data. In this regard, under MS41, Partner 8 identified in Icelandic sequencing data, N=90 heterozygote carriers of a rare loss-of-function variant in SCN9A who were alive, over 18 years of age, and ethics committee approval was obtained for recruitment and re-phenotyping to characterize potential effects of this variant in human subjects. Of this small sample, N=19 subjects accepted participation, and of these, only one subject was identified having an aberrant sensory/pain pattern, consistent with somatoform pain disorder. Participants were all middle-aged (the mean age was 54 years), and according to self-report, there is suggestive history of increased pain tolerance among some of these heterozygote carriers in childhood. Partner 8 (deCODE) is increasing sequencing coverage and searching for other rare variants in SCN9A in these carriers that could explain the complex and differing sensory phenotypes observed among these SCN9A carriers.

Recruitment of younger carriers continues to examine closer whether sensory profiles of carriers change with age, and diagnostic and sensory profiles of carriers are still under study.

Partner 8 (deCODE) obtained ethics approval to recruit carriers of the rare loss-of-function variant in PRPH, identified and described in WP7. A PRPH mouse knockout model has been previously developed and studied (Lariviere et al., 2002), supporting the findings presented in the human study in that PRPH null mice have a substantial reduction (approximately 34%) in the number of L5 unmyelinated sensory fibers. While nerve conduction parameters were not measured in the study, axonal loss in general corresponds to lowered nerve conduction amplitude (Tankisi et al., 2005), corresponding to the findings in the human study described in WP7 (Bjornsdottir et al., 2019). These results demonstrate a requirement of peripherin for the proper development of a subset of sensory neurons.


This WP9 was mainly devoted to perform a clinical trial (Partner 9, Charité) to evaluate the potential analgesic effects of a phytocannabinoid compound (Partner 3, GW).

Conventional pain medications are limited by detrimental side effects, inefficacy in certain chronic pain syndromes (such as neuropathic pain) and widespread abuse. This leaves a significant unmet medical need for novel, safe and effective compounds with reduced side effect burden and abuse liability. Therefore, we aimed to identify and characterize novel cannabinoid and opioid targets to effectively reduce clinical neuropathic and inflammatory pain while avoiding unwanted adverse effects.

Partner 3 (GW) performed the preclinical work to select the phytocannabinoid compound cannabidivarin (CBDV), which was tested by us in patients with HIV-associated painful neuropathy. The clinical study is entitled “Oral cannabidivarin (CBDV) solution for treatment of HIV-associated neuropathic pain - a randomized, double-blind, placebo-controlled phase II study”.

Page 17 of 27
Research and Innovation
Upon the beginning of the project the planning process of the clinical trial started and resulted in the described protocol. In this crossover study, each patient received blinded IMP to complete both the active and placebo phases. The first treatment phase with either CBDV or placebo started on day 7 and ended on day 35. Before starting the 2nd treatment phase (day 63-91) each patient changed to the opposite treatment. Inbetween the treatment phases every patient had a 3-week wash-out phase to eliminate a potential carry over effect between both treatment phases. Statistical calculations to meet the primary endpoint [decrease of pain intensity from baseline (on day 7 + 2 or 63 + 2, respectively) by at least 20% at the end of treatment (on day 35 + 2 or 91 + 2, respectively) with CBDV compared to placebo] resulted in a planned sample size of 50 patients to be included.

During the planning phase the beginning of the study had to be postponed due to delivery problems of IMP and delays in the approval procedure by regulatory authorities. This only allowed the inclusion of the first patient in January 2017. The patient recruitment started through contacts to pain- and HIV- specialists in the greater Berlin area, as well as to patient support groups. After the beginning of the study subway advertisement was used and significantly increased the recruitment rate. A budget change in February 2018 again increased the recruitment rate.

To be able to include patients of this clinical trial for the responder-/non-responder-analysis of WP10 we had to request approval for changes in the trial protocol by the regulatory authorities. In this context we also received permission to include patients with non-virulent hepatitis and a history of AIDS-defining diseases, as well as to financially compensate every patient who successfully completed the clinical trial. This also contributed to the increased recruitment rate in the following months. In June 2018 we initiated contact to the collaborating statistics department (´KKS´) for planning the statistical analysis. A data base was developed especially for our trial and our demands in which all data were entered by December 2018. Currently, the KKS is planning the final statistical analysis. Due to these delays the study could not be unblinded yet, although we have already received preliminary results (see also report of Deliverable 10.18).

In total we included 34 patients (33 males, 1 female). Due to the expiry date of the last batch of IMP (November 2018) we could only include patients until end of August 2018. The mean age at inclusion was 50.5 years (31-65 years). The patients were HIV-positive for a mean time of 21.4 years (2-37 years) and the pain emerged about 12.4 years before inclusion (1-30 years). 28 patients finished the whole study. We had 6 drop-outs, with 4 for personal reasons, 1 screening failure and 1 due to adverse events.

The mean pain reduction in the first treatment phase was 0.33 and 1.50 points on an 11-point NRS-scale for treatment group A and B, respectively. In group A 4 patients (26.7%) responded to the treatment (at least 20% pain reduction), whereas 12 (75%) in group B benefitted from treatment. In the second treatment phase there was a mean pain reduction of 0.29 points for group A and 0.31 for group B with 6 responders (42.6%) in group A and 5 responders (35.7%) in treatment group B. In total, there was a mean pain reduction of 0.56 points on an 11-point NRS-scale in treatment group B over group A (p=0.2101).

Even though these results are preliminary and are subject to changes we do not expect any statistically significant results meeting the primary outcome ´Reduction of pain intensity (´baseline´) by at least 20 % determined on an 11-point numerical rating scale (NRS) at the end of CBDV application as compared to placebo´. These results suggest that the promising preclinical data indicating analgesic effects of CBDV, especially for neuropathic pain, could not be confirmed in this cohort of patients suffering from HIV-associated neuropathic pain. However, since the goal of 50 patients to be included could not be reached, further research will be needed for a final conclusion on the efficacy of CBDV for neuropathic pain.

WP10. Pharmacogenomic studies in humans to evaluate the genetic characteristics to cannabinoid compounds.

The objectives of WP10 were the selection of chronic pain patients responding to cannabinoids (Partner 9, Charité) and the assessment of genotypes or biomarkers of neuropathic pain regarding the response to cannabinoids (Partner 8, deCODE).
The main focus was on re-phenotyping patients who developed chronic neuropathic post-mastectomy pain syndrome (PMPS) and are treated with cannabinoids for nausea and/or pain. At the time of writing the original grant proposal, we had anticipated a growing number of patients to be treated with cannabinoids. In the meantime, we came to realize that in Germany, treatment of PMPS patients suffering from nausea and/or pain with cannabinoids is very rare and not well established.

Therefore, we initiated contacts to physicians treating eligible patients in the greater Berlin area for improving the recruitment. However, it was finally decided the inclusion of study participants of WP9 as they were actively recruited for WP9 in any case, so that additional collaborations with other institutions were not necessary. We decided to rely on the much better characterized and more homogeneous group of patients in the clinical trial of WP9. Due to delays in the acceptance of these changes to the trial protocol we were able to start collecting blood samples in May 2017 with patient GW-CBF-003. From May 2017 on every patient who was included for the clinical trial of WP9 was included to the pharmacogenomics analysis of WP10. All data for phenotyping of these patients were collected within the data collection of the clinical trial. By December 2018 all data were collected and entered into the database of the clinical trial. The blood samples were collected during the visits of the clinical trial and processed directly after the visit to inactivate the virus and isolate DNA. Right after processing the samples they were frozen and stored in our labs. Due to the delays in the approval of the changes to the trial protocol and some drop-outs we could include 29 of the 34 patients recruited for the clinical trial for re-phenotyping. The blood-samples of these patients were shipped to DeCode Genetics (Sept. 2018), where the pharmacogenomics analysis was carried out.

A classification of responders and non-responders was prepared by KKS and sent to DeCode Genetics in February 2019. Due to delays in the work of KKS, the study staff is still blinded at the day of submission of this report. For this reason, the analysis of responders versus non-responders cannot be displayed here. However, blinded and preliminary results can be shown subsequently. Since these are not the final results, small changes may still appear after unblinding.

In our cross-over-study patients were divided into 2 treatment groups (A and B) with 2 treatment phases, respectively. Each group received the active agent and a matching placebo in one of these treatment phases. The succession of these two phases was allocated by chance (for further explanations of the study design please see report of Deliverable 10.18). In the first treatment phase in group A 4 out of 16 patients (26.7%) responded to the treatment, whereas 12 out of 17 patients (75%) in group B responded. In the second treatment phase there were 6 (42.9%) responders out of 14 patients completing the treatment phase in group A and 5 (35.7%) responders out of 14 patients finishing the phase in group A. The initial analysis searching for association of common markers with response has been performed by partner 8, and as expected there were no clearly significant signals. Analysis of sequencing data is forthcoming in 2019.

References:
Negrete et al., Neuropharmacology, 20:1454-1466 (2016)
Weibel et al., PLoS ONE, 8(9):e74706 (2013)
Charboneau et al., Biological Psychiatry, 81:778-788 (2017)
Albayram et al., Proc Natl Acad Sci USA, 2108:11256-61 (2011)

Potential Impact:

DISSEMINATION

The dissemination activities of the project results (Task 4, WP11) has been and will continue to be carried out to all interested stakeholders (scientific community, potential clients, public...) by several means (e.g. Internet by the consortium and the partners’ web pages and commercial leaflets, press releases) as well as publication of scientific articles in peer-reviewed journal and participation to workshops and conferences (e.g. the FENS Meeting and Satellite Workshops, the IASP/EFIC Pain congress or the USA Society for Neuroscience).

The main dissemination items are briefly described below:
- There are 42 scientific publications of the results obtained in this project, both in high-impact international journals (39 articles) and in prestigious books (3 book chapters), so far.
- The Consortium have released more than 157 dissemination items, so far including:
  ✓ Invited lectures, abstracts, posters, oral communications or seminars in International and National scientific conferences;
  ✓ Presentations and talks to the general public;
  ✓ PhD, MSc and BSc theses;
  ✓ Specialised training courses and conferences;
  ✓ Flyers;
  ✓ Articles, press releases, interviews and videos in radio, TV and internet related to the impact of the results of the research in the mass-media;
  ✓ Organisation of conferences, workshops and courses;
  ✓ Patents.

All these publications and other dissemination items have been introduced in the project’s web site (https://www.upf.edu/web/neuropain) as well as in the Funding & Tenders Portal (formerly, the Participants Portal), where all related data are available.

SCIENTIFIC PUBLICATIONS

The 39 scientific articles have been published in several top journals, such as Nature Genetics, Nature Reviews Neuroscience, Nature Communications or Biological Psychiatry (3), in journals covering the topic of pain or its underlying neurobiological mechanisms, such as Pain (9), European Journal of Pain (2), Neuropharmacology (2), Addiction Biology (6), Neuropsychopharmacology (2) Annals of Neurology, Pharmacogenomics Journal, Biochemical Pharmacology, Brain Research Bulletin or Neuroscience, and in journals related to the medical theory or practice, such as the Journal of Clinical Oncology, JAMA Ophthalmology, British Journal of Anaesthesiology, Annual Review of Medicine, Expert Opinion on Investigational Drugs (2) or Der Anästhesist. The 3 book-chapters deal with pain treatments and their clinical application and have been published in high-quality and widely distributed books, thus assuring that they will reach the interested scientific community. In this manner, the dissemination of the results of the project to the scientific community, both basic researchers and clinical practitioners, is guaranteed and we are sure they will be useful in their respective areas of interest. In particular, the
possibility that these results will reach the interested patients remain high.

SCIENTIFIC MEETINGS

The Communications to International and National scientific conferences have represented around 87 items (abstracts, posters, oral communications and invited lectures), so far, whose references have been uploaded in the Participants Portal and in the consortium website.

Among others, it is worth mentioning the 24 items presented at the 16th (Yokohama, Japan, September 2016) and at the 17th (Boston, USA, September 2018) World Congress on Pain organised by the International Association for the Study of Pain (IASP), most probably the top pain research event worldwide; the ones presented at the 9th (Wien, Austria, September 2015) and at the 10th (Copenhagen, Denmark, September 2017) Congress of the European Pain Federation (EFIC), at the 1st European Pain Federation-EFIC Topical Symposium on Acute and Chronic Joint Pain (Dubrovnik, November 2014), the 5th (Nice, France, June 2015) and 6th (Gothenburg, Sweden June 2017) NeuPSiG conferences, the 6th Scientific Congress of the Federation of the European Societies of Neuropsychology (Maastricht, The Netherlands, September 2017) or the 11th FENS Forum of Neuroscience (Berlin, Germany, July 2018); the 24th Annual International Cannabinoid Research Society (ICRS) Symposium (Baveno, Italy, June-July 2014), the Gordon Research Conference (Lucca, Italy, May 2015) or the MED Cannabis (Wien, Austria, June 2018), all three on cannabinoids; the European Collegium of Neuropsychopharmacology (ECNP, Amsterdam, The Netherlands, September 2015), the American Society for Pharmacology & Experimental Therapeutics (ASPET, San Diego, USA, April 2016), the International Narcotics Research Conference (INRC, Bath, UK, July 2016), the European Anaesthesiology Congress (Copenhagen, Denmark, June 2018), or the Annual conference of the Scandinavian Association for the Study of Pain (Reykjavik, Iceland, May 2016, and Tampere, Finland, April 2018).

Moreover, the Consortium has been very active in other dissemination events, such as scientific seminars and lectures in high-academic fora and to the general public (19), as well as the organization of six conferences (workshops and training activities), including Summer schools, among others.

THESIS

The results have also been disseminated by means of dissertation and publication of doctoral (PhD), Master (MSc) and Bachelor (BSc) theses. In particular, namely, ten (10) doctoral thesis related to the project have been completed and successfully defended, whilst other six (6) are on their way. In some cases, the predoctoral students have carried out short stays in another partner’s laboratory, which has been extremely useful to extend the impact of the methodologies used and to learn new techniques.

WEBSITE

The NeuroPain consortium established a dedicated website to the project, which is hosted by partner 1 (https://www.upf.edu/web/neuropain/).

The web page is organised as follows:
- Home page, with Links to relevant Scientific associations, the European Union and International institutions and agencies
- Partners of the consortium, with direct links to the respective institutions
- Objectives of the project
- Models and methods employed to approach the objectives
- Relevance of the topic addressed
- Publications: the complete and updated list of peer-reviewed articles, acknowledging the EC support to the project, published in international scientific journals
- Communication events: posters, oral, invited lectures) to international scientific meetings related to the project and the
This web page has been useful both to the Consortium and to external users, as it has provided information on the goals of our EC-funded research and of the main scientific outputs (including published articles and several dissemination events).

**LEAFLETS/FLYERS**

This action has been a limited activity, mainly driven by specific requests or needs for a successful development of the project’s activities. In particular:
- A specific dissemination leaflet was produced for the EC information hub at the Society for Neuroscience meeting in Washington D.C. (15-19/11/2014). Team members of partners 1 (R. Saravia) and 10 (E. Kalso) took part in the event and were actively present at the EC hub to inform interested participants about the project.
- For the clinical trial of Cannabidivarin for the treatment of HIV-associated neuropathic pain a patient leaflet was designed by partner 9 and handed out to different HIV-specialists, general practitioners and pain specialists in the greater Berlin area, as well as pharmacies and patient support groups (2016). Moreover, ads flyers were disseminated in the Berlin area in the last campaign to boost patient recruitment.

**PRESS RELEASE AND MASS-MEDIA**

Together with the scientific publications described above, the consortium has given wider scale dissemination to the results obtained, thus reaching the general audience. Indeed, all partners have worked in close collaboration with the communication departments of their institutions to contact relevant national and European media for a general diffusion of the main results of the project in the main national and European media including more than 10 TV appearances, more than 20 interventions in radio programs and more than 50 items in newspapers (press and digital), including 34 articles published in the general press with citations of the Consortium PIs and/or the results obtained in the project and their potential impact. A broad selection of these items has been uploaded at the PP.

Moreover, there have been around 20 oral presentations to a wider audience. Thus, specific talks that have been given by the researchers of NeuroPain to the general public, including young students. All these activities, as well as Partners’ web pages and social networks like LinkedIn, have facilitated the dissemination to the general audience and the exploitation of the results. Although a selection of these items have been uploaded at the Participants Portal, it is worth mentioning the following actions.

**STAKEHOLDERS**

A particular attention has been devoted to collaborating with interested stakeholders, including the EC DG Health, National health authorities, patient associations, health professionals and scientific societies, such as the International Association for the Study of Pain (IASP). Indeed, the consortium has maintained regular communication of the derived results with interested stakeholders.

The most relevant ones are listed below:

**Scientific societies:**
- International Association for the Study of Pain (IASP). In particular, the Finnish Association for the Study of Pain (IASP Chapter) has a newsletter “Kipuviesti” for which H. Harno has interviewed several IASP active professors of their career in neuropathic pain research. Interviews of professors A. Rice (4/12/2014), R-D Treede (19/4/2015), T. Dickenson (20/6/2016), and C. Sommer (17/6/2017)
- Federation of European Neuroscience Societies (FENS)
Specific Satellite Workshops and Courses:

- Cold Spring Harbor Laboratory Summer Course “Cellular Biology of Addiction”, Rafael Maldonado (partner 1) organised and was Scientific Instructor and speaker in 2014 (13-20 July, 2014, University Pompeu Fabra, PRBB, Barcelona, Spain), and has participated as speaker in 2016 (July 31-August 7, 2018, Gonville & Caius College, Cambridge, UK).
- EFIC Pain School “Translational pain research: from lab to clinic”, organised by Ryszard Przewlocki (partner 7) in 2017 (25-29 June 2017) in Krakow, Poland. Anthony D. Dickerson (partner 2) and Eija Kalso (partner 10) served as teachers.
- The European Pain School (EPS) (IASP Educational Project) in Siena, Italy 4.-11.6.2017. L. Mustonen attended and had an oral presentation on “Intercostobrachial nerve resection in breast cancer operated patients with and without neuropathic pain 4-9 years after treatment” (Partner 10).
- Tuohilampi Pain Summer School (organized by the Finnish Association for the Study of Pain (IASP Chapter)) in Vihti, Finland (7-9/6/2017). S. Liesto had an oral presentation on “Pain, psychological resilience, and quality of life” (partner 10).

Patients associations:


Public health authorities:

- Presentation updates over the course of the NeuroPain study have been provided by Partner 8 (deCODE) to clinical collaborators of the NeuroPain study in Iceland, and the Clinical Pain Speciality Team at the Landspitali, National University Hospital in Reykjavik, Iceland.
- Partner 10 introduced the DFNS QST (German Research Network on Neuropathic Pain, quantitative sensory testing) to the Helsinki University Hospital, when NeuroPain-study started. Two nurses were certified in Mannheim, Germany for the protocol.

Private companies:

- Partner 1 (R. Maldonado) has initiated contacts with a Spanish pharmaceutical industry to collaborate in the R+D of a new phytocannabinoid compound for the treatment of pain (confidential).
- Partner 1 (R. Maldonado) established a research agreement with the USA pharmaceutical company Rhodes Technologies to carry out research on potential new pharmaceutical compounds able to maintain the analgesic properties of psychoactive cannabinoids, whilst avoiding (negative) side-effects (2015-2017).
- Partner 9 (Stein et al.) established contacts with ca. 30 pharmaceutical companies and investors in Europe, USA and Asia to initiate development of novel pain medication. The group won prizes in a Berlin competition for business development (2017) and from the German Federal Ministry for Education and Research (BMBF, 2019).
- Partner 8 (deCODE Genetics) is a subsidiary of Amgen Inc, a USA pharmaceutical company. Amgen has a long-standing drug discovery program for chronic and neuropathic pain conditions. One of these programs is aimed at Nav1.7 encoded by the SCN9A gene (https://www.amgenscience.com/features/the-passionate-pursuit-of-nav-1-7/) which is one of the main genes of
interest within the NeuroPain project.

EXPLOITATION

The exploitation of the results derived from the project takes into account two main areas, namely the management of intellectual property (Task 2 of WP11) and the Preparation to market uptake (Task 3 of WP11).

MANAGEMENT OF INTELLECTUAL PROPERTY

According to the Consortium Agreement signed by all partners, knowledge (including achieved assets, technical improvements and compounds) shall be the property of the contractor carrying out the work leading to that knowledge. Moreover, when several partners have jointly carried out the work generating the knowledge and when their respective share of the work cannot be ascertained, they shall have joint ownership of such knowledge. Prior to dissemination, decisions have been made as to whether the knowledge should be protected and whether a patent should be filed. The terms and conditions for access to pre-existing and new knowledge by the Partners and third parties have also been detailed in the Consortium Agreement. Thus, patenting strategies have been implemented to secure intellectual property (IP) protection of the chemical structures, chemical synthesis pathways, pharmacological properties and profiles, and their use for both pain syndromes and other relevant clinical conditions. Other IP generated in other WPs, may also substantially enhance the business perspectives for Partner companies and, thus, represents an integral part of our IP portfolio.

This task is directly related to the appropriate exploitation of the results, which includes patent filing, agreements on ownership, licenses, royalties and specific exploitation plans, ensuring adequate protection of the SMEs and companies interests.

The exploitation of the NeuroPain consortium results will be shared between the participants. Pre-clinical research partners (Partner 1, UPF; Partner 2, UCL; Partner 5, GIE-CERBM; Partner 6, UKB; and Partner 7, IF-PAN) will benefit from the reliable and fully validated new animal models. Indeed, new genetically-modified animals have been generated and characterised by GIE-CERBM (Partner 5) and UKB (Partner 6), which have been employed to set up new models of chronic pain, that have been validated and employed by means of behavioural (UPF, Partner 1), electrophysiological (UCL, Partner 2) and molecular (IF-PAN, Partner 7) methodologies. Thus, these five partners have contributed to generate new knowledge, have shared it in a profitable way and are ready to exploit it in new paradigms which will enhance their research capabilities in pre-clinical chronic pain studies, contributing both to joint publications in high-impact scientific journals and to strengthened collaborations with the pharmaceutical industry in new studies. The exploitation of chronic pain treatments is assured by the three private companies (GW, Esteve and deCODE) and by the three clinical partners (Charité, HUS and Imperial). New molecules, namely, phytocannabinoids such as cannabidivarin (CBDV, from GW (Partner 3), and mu-opioid/sigma-1 dual compounds (SIMU), from Esteve (Partner 4), have been successfully obtained, characterised and tested for their analgesic potential. Moreover, new and more reliable biomarkers, as available from deCODE (Partner 8) and IF PAN (Partner 7), will lead to new and more effective treatments as assayed in patient cohorts of specific pain syndromes available from Charité (Partner 9), HUS (Partner 10) and Imperial (Partner 11). The Project Office has coordinated research activities in order to ensure the implementation of research priorities determined by both the industry and the academic partners.

Forty-one (41) different patents related to the project have been filed by different partners of the NeuroPain consortium, holders are the two private pharmaceutical companies, partner 3, GW Pharma (19) and partner 4, Esteve (19), and 3 patents whose holder is partner 9 (Charité). Several team members of different partners (UPF, GW, ESTEVE, Charité) are co-inventors. Detailed public information on those patents has been uploaded at and can be retrieved from the Participants Portal.

GW Pharmaceuticals (partner 3) patents comprise several phytocannabinoids’ formulations and the use of phytocannabinoids (including cannabidivarin, CBDV) for the treatment of neuropathic pain or arthritis, but also of several neuropsychiatric or
neurological disorders (e.g. epilepsy, schizophrenia, neurodegenerative disease, autism spectrum disorder and associated disorders) and other diseases (e.g. inflammatory skin diseases, tuberous sclerosis complex degenerative skeletal muscle diseases). GW has previously submitted patents on CBDV in a variety of indications prior to the beginning of the NeuroPain project, and hence holds background intellectual property on this product and its medical use. Many of these patents pre-date the findings discovered in this programme, and remain in force. In addition, GW Pharmaceuticals have in parallel, independently of this project, continued their development of their CBDV-containing medicines as pipeline asset. On the other hand, the Intellectual Property derived directly from the clinical study of CBDV in patients with neuropathic pain due to HIV-induced neuropathy (WP9 and WP10) should also be taken into account. Esteve (partner 4) has filed several patents protecting new chemical entities of novel sigma-1 receptor dual compounds, including modulators of the opioid system (opiod receptors) having multimodal activity against pain. Finally, Charité (partner 9) is the holder of three patents on the compounds acting within the endogenous opioid system (receptors, agonist and nanocarriers). The potential impact of those patents in terms of an eventual exploitation is very high, as the different partners are fully committed and the results aligned with the companies’ portfolio priorities. In summary, a total of 42 patents have been filed and all them are under further exploitation, either by the owner company itself or by licensing them to an external company. Royalties (if applicable) have been also obtained or are foreseen.

On the other hand, given that the clinical study with CBDV was not significantly positive, the possibility for patenting the findings cannot yet be ascertained and it remains open. Notwithstanding, a final evaluation of this point will be done once the final statistical analysis of the results will have been finished.

In conclusion, the technological innovation conducted within the project has demonstrated a high potential for delivering patentable items (e.g. new targets, new compounds, new uses of existing compounds, new diagnostic kits). Patents covering inventions associated with the results have been filed throughout the project, first in Europe and, thereafter, will be extended to the USA and Japan.

The results of the CBDV clinical trial undertaken in this project showed a trend towards pain reduction in one treatment phase, however, pain reduction following either treatment did not reach statistical significance for relief of neuropathic pain by this formulation of CBDV in the current study. More studies with bigger sample sizes investigating CBDV as a treatment for neuropathic pain will be carried out. Although, from this study, CBDV has not been shown to be an effective analgesic for the relief of neuropathic pain due to HIV-induced neuropathy, the possibility that such a novel finding will be confirmed, leaves open that a patent will be submitted by GW to protect this. As CBDV analgesic effect was not statistically significant in our clinical trial for the relief of neuropathic pain due to HIV-induced neuropathy, a number of possible explanations can be discussed.

To date, there is nothing in the medical literature showing that CBDV is effective in the relief of pain (particularly neuropathic pain) humans. This finding that CBDV could be effective in reducing pain due to neuropathies, may offer the opportunity to further investigate the effects of CBDV in other pain syndromes, particularly those due to neuropathy. CBDV has been shown to be anticonvulsant in preclinical models of seizure/epilepsy and other anticonvulsants have been shown to have analgesic potential (e.g. gabapentin). The full market potential for CBDV may be realised once it is clear which specific pain syndromes CBDV may be effective in. Alternatively, if CBDV is subsequently found only to be effective in specific neuropathic pain syndromes, rather than both neuropathic and non-neuropathic conditions, then the market potential may be limited to specific syndromes, where benefit: risk has been assessed and approved by medicines regulators.

At this moment, phase I studies of the novel dual mu-opioid agonist/sigma-1 receptor antagonist compound (SIMU-1) are ongoing. The evaluation of the market potential has just initiated, and a thorough evaluation will be performed once the indication for the compound is clearly defined and the decision to move to Phase II clinical studies is taken.

In Europe, 19% of the adult population suffers from moderate to severe chronic pain. On average, one in five European adults suffer from chronic pain, being moderate in 2/3 of cases and intense in 1/3 of cases (Breivik et al., 2006). In order to calculate
the market volume, we will focus the analysis on neuropathic pain (NP) and on painful diabetic neuropathy (DPN) in particular, although SIMU-1 has, based on preclinical data in pain animal models (see WP6 reporting), therapeutic potential to treat pain indications others that NP, including osteoarthritic pain or cancer pain, which are among the most prevalent indications in the context of moderate to severe chronic pain.

Major drivers of the growth of the NP market over the forecast period include: i) the increasing NP incidence due to the growing elderly population.; ii) The increase in the prevalence and diagnosis of type 2 diabetes, which will lead to growth of the painful diabetic neuropathy (PDN) market, which is the largest NP segment. On the other hand, major barriers to the growth of the NP market include: i) the NP market will be dominated by generics, which raises the barrier to entry for novel drug therapies and, thus, needs to deal with the impact by the patent expirations and subsequent generic erosion of the key leading brands, Lyrica, Cymbalta, and Lidoderm (lidocaine patch 5%); iii) an incomplete understanding of the disease mechanisms underlying NP has made appropriate target selection for drug development challenging; iv) off-label prescribing of most drugs in some NP indications; and v) pharmaceutical therapies are facing competition from interventional therapies.

The main classes of drugs used in the treatment of NP have traditionally consisted of antidepressants, anticonvulsants, opioid analgesics, and topical analgesics. Each of these classes contains several established drugs that are now widely available in generic form. Although many of the available drugs offer some degree of efficacy in terms of pain relief, there still remains vast room for improvement in efficacy, safety, drug delivery, and dosing convenience. Current treatments leave a high level of unmet need in the NP market.

Therefore, there is thus still room in the market for therapies that can differentiate themselves in terms of increased efficacy, along with good tolerability. In this context, the possible use of phytocannabinoid compounds and dual sigma-1/mu-opioid receptor compounds for the development of new pharmaceutical drugs for the treatment of chronic pain remains a very good option, particularly talking into account he results obtained in the present project.

It can also be stated that in line with deCODE’s ongoing emphasis on publishing new discoveries, any biomarkers significantly associating with NeuroPain phenotypes and discovered by deCODE Genetics under the NeuroPain project have already or will be published in high impact scientific journals as per objective 1 as deCODE Genetics is a subsidiary of the pharmaceutical company Amgen, significant and confirmed deCODE findings from the NeuroPain study have also been relayed directly to the research and discovery branches of Amgen’s Neuroscience drug discovery team. The potential clinical relevance and eventual therapeutic application of the biomarkers discovered remains open for future research, as described below for some of the main deCODE findings published under the NeuroPain grant. These discoveries comprise new loci in migraine (Gormley et al., 2016), 2 loci were found in ion channel genes (KCNK5 and TRPM8) and 3 in genes involved in ion homeostasis (SLC24A3, ITPK1, GJA1) (Gormley et al., 2016) and the, loss-of-function variant in PRPH (Bjornsdottir et al., 2019, manuscript in review), found to associate with reduced sural nerve conduction amplitude, among others.

Overall, results with great translational potential have been generated within the NeuroPain project. The migraine GWAS results represent a list of potential drug targets, and all pharmaceutical companies are currently scrutinizing the results. While our sciatica-associated finding is not readily translatable into a drug discovery program at present, neurologists reviewing the clinical implications of the finding have noted that the work highlights a genetic marker that may help clinicians to determine which patients will require surgery for persistent sciatica compared with those who will have a natural resolution of their symptoms (Moses and Chi, 2017). Furthermore, the implication of neuroimmune-based mechanisms opens avenues of inquiry for research into this most common and costly pain condition, lower back pain. The PRPH finding has great potential when it comes to translation, as it implicates changes in neuronal interfilamentous structure in neuropathy and neuropathic pain, and PRPH itself as a key player. Finally, the handle we now have on the underlying genetic basis through use of polygenic risk scores, can immediately be leveraged to analysis of clinical trial data, and in future for design and execution of information-rich clinical trials. To this end, Partner 8 is using both the migraine and depression polygenic scores to study existing data from AMGEN’s migraine clinical trials.
References:


List of Websites:
https://www.upf.edu/web/neuropain/

Coordinator: Rafael Maldonado (rafael.maldonado@upf.edu)
Project manager: Miquel-Ángel Serra (miquel.serra@upf.edu)
LEAR: Eva Martín (projectes.recerca.upf.edu)

Reported by

UNIVERSITAT POMPEU FABRA
Spain

Subjects

Life Sciences

Last updated on 2019-05-21
Retrieved on 2019-08-20

© European Union, 2019