

IMI1 Final Project Report Public Summary

Project Acronym: EUROPAIN

Project Title: Understanding chronic pain and improving its treatment

Grant Agreement: 115007

Project Duration: 01/10/2009 - 30/09/2015

1. Executive summary

1.1. Project rationale and overall objectives of the project

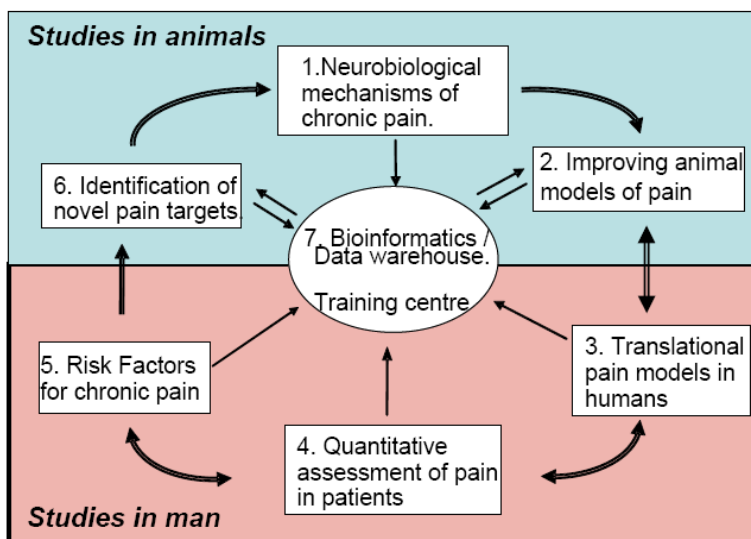
Chronic pain affects one in five European citizens and is one of the major burdens of society, both from an individual suffering perspective as well as a socioeconomic burden for society. Overall, chronic pain impacts society with direct costs corresponding to 0.5-2% of GNP in the EU region as well as in North America (OECD 2012). More specifically, 7-8% of the general population suffer neuropathic pain. With current treatments, only one third of patients overall obtain adequate pain relief. For each available pharmacological treatment option, the Numbers Needed to treat (i.e. how many patients need to be treated to obtain 30 or 50% pain relief) for common treatments vary between 6.4 and 10.6 (Finnerup et al, Lancet Neurol 2015). Chronic pain also contributes to the development of depression and is one of the most important disease conditions for work disability. There have been no new pharmacological treatments developed specifically for the treatment of neuropathic pain in more than 20 years. The latest launches of analgesic treatment for neuropathic pain were in the mid 1990:s, namely the gabapentinoids and antidepressants of the serotonin-norepinephrine dual reuptake inhibitor type (SNRIs). To be noted, these were part of Life Cycle Management programs and not the first indications for these drugs. There are clear bottlenecks in the research and development chain that have hampered the further development of drugs having reached clinical development. The EUROPAIN consortium has aimed at better understanding of the mechanisms and physiology of chronic pain, both from a preclinical and clinical mechanistic perspectives as well as better understanding of predictors and clinical signs and symptoms involved in neuropathic pain. The Europain consortium believes that addressing these bottlenecks in drug development will contribute to the development of better and more efficacious treatment options for this large group of patients. In the end, the project is hoping that their achievements will contribute to reduce the burden of illness of large groups of the European population.

1.2 Overall deliverables of the project

EuroPain, a consortium of international leading pain researchers and pain clinicians from academia and industry, have undertaken a multidisciplinary translational research approach with the objective to increase the understanding of chronic pain mechanisms and to improve tools to do so. The outcome of this project intends to facilitate future development of novel analgesics, thereby ultimately providing better treatments for chronic pain patients. In order to achieve these very extensive goals, a number of work packages were created, each aiming to address a specific area, but all closely interlinked and collaborating.

In the end, these separate work packages have together contributed substantially to an overall progress of the field from clinical and preclinical perspectives, a better understanding of the roles of the peripheral and central nervous systems. This includes development of animal models for neuropathic conditions where these have been lacking. It also includes developing and cross-validating relevant animal readouts of spontaneous pain behavior, which were also lacking. In the clinical space, translational models to bridge between animal models and patients, including relevant biomarkers as well as phenotypic markers of disease were to be developed and validated. EuroPain aimed to identify and validate better biomarkers to predict analgesic response as well as identifying patients at risk of developing chronic pain after surgery. Further, as an added component and very important contribution to obtain the above, improved quality in standards for preclinical and clinical research in the field of neuropathic pain were to be developed, validated and shared with the wider pain community. Together, the overall aim was to create a platform enabling improved tools for the development of new pharmacological treatments for neuropathic pain has been achieved.

The below flow chart cartoon describes the different work packages of EuroPain, designed to obtain the overall objectives described above.



The work packages above included technical deliverables designed to address the above overall objective as listed in more detail below.

To identify novel pain mediators and to understand the mechanisms of excitability changes at both spinal and supraspinal levels in chronic pain states;

To improve and refine animal models of chronic pain, including the clinical relevance of endpoints used in chronic pain models and methodologies to reduce experimental bias ;

To establish and validate mechanism-based human pain models.

To find objective measures of spontaneous pain using microneurography and functional imaging; to collect detailed phenotypic data on chronic pain patients (sensory, psychological including expectancy, electrophysiological) and correlate with sensitivity to pharmacological agents;

To determine psychosocial and clinical risk factors for development of chronic pain using postoperative pain as model and test the role of genetic variation in candidate genes as a contributor to chronic pain development;

To extend existing databases and integrate data from consortium database resources and other data being generated by all other workpackages;

To mine these extended data bases to improve translational knowledge.

To establish and maintain a Training Center for QST and the initiation and external quality assurance of clinical consensus proceedings;

By utilising the above, to develop and validate objective and quantitative biomarkers for improved development of new treatments for neuropathic pain.

To manage and coordinate this integrated research program in an optimal way.

1.3 Summary of progress versus plan since last period

The project has achieved most of the objectives for the period. These include:

1. A meta analysis approach of a multicentre study in rodents, where the publications was selected as Editor's choice as ground breaking. This is the first time that a spontaneous pain behavioural rodent readout has been validated through a multi-center approach. It was demonstrated that the burrowing paradigm can be standardised across a number of different labs, when applying a strict protocol similar to what is done in clinical trials.
2. A meta analysis of human surrogate models was completed, concluding that these can be used to demonstrate target engagement P/ proof of mechanism, but they cannot be surrogates for
3. A clinical study investigating a new non-invasive diagnostic tool, Corneal Confocal Microscopy, for peripheral polyneuropathy was successfully initiated and completed.
4. A new electrophysiological protocol for non-invasive sensory nerve fiber measurements was developed and patient profiles were characterised vs. microneurography. This is an important step of making objective measurements of pain related nerve activity more widely clinically feasible.
5. Scientific advice for four clinical biomarkers of neuropathic pain was obtained at EMA. These included QST for diagnosis, stratification and enrichment in clinical trials, microneurography and the non-invasive threshold tracking as a biomarkers of ongoing pain related activity in sensory nerves and Corneal Confocal Microscopy as a sensitive and specific non-invasive diagnostic of small fiber neuropathy. This was a successful interaction, where several of the biomarkers were accepted and others received good advice for future development in order to qualify for regulatory approval.

There were also some minor deviations. These include that though regulatory scientific advice was obtained by EMA but the timelines eventually did not allow to also obtain feedback from FDA as planned. Further, the non-budgeted task to ensure the public availability and sustainability of the EUROPAIN/Neuropain integrated database was started, but however, due to delays outside of the consortium control, this could not be completed within timelines.

Overall, the WP4/6 clinical database, consisting of the merged Europain/Neuropain databases will be sustained for at least 2-3 years in its present format, and will be available for data mining. Such data mining will be possible albeit only in collaboration with the data owners, since the existing database is anonymized but not de-identified.

None of the deviations had an impact on the overall objectives of the project.

1.4 Significant achievements since last report

During this last year of the project, there have been several major achievements made by the project. The activities in Year 6 have all been building on and refining outcomes of previously performed tasks and have resulted in the following:

- A metaanalysis was performed of the burrowing deficit data, obtained from 11 prospective multicenter blinded experiments in 8 Europain laboratories, on the ethologically relevant rodent outcome measure of spontaneous pain behavior. During the previous years of the project, a burrowing deficit protocol has been developed by the consortium, the endpoint validated across several pain models, and also shown to be sensitive to pharmacological intervention. . This unique multicentre approach provided high-quality evidence, not only validating burrowing as a robust, reproducible outcome measure which could be a useful tool to infer the global effect of pain on rodents, but also informing about the impact of minor protocol variations on burrowing, providing educational value for future investigations and contributing to increasing reproducibility of preclinical research.
- A protocol library of animal experimental protocols has been made publically available at www.imieuropain.org. The protocol library contains a number of ethologically relevant validated outcome measures of rodent pain behaviour: the abovementioned burrowing deficit, elevated maze and social interaction models, which all have been developed and validated across laboratories throughout the course of the project. The library has also been populated by protocols of novel and/or improved and standardised animal pain models closely resembling human pathology of diseases causing neuropathic pain. The models now characterised and validated, include a novel rat model of diabetic polyneuropathic pain, a mouse model of chemotherapy-induced neuropathic pain, two rat models of polyneuropathy due to antiretroviral therapy and the UVB model of inflammatory pain. The intention of this open protocol library is that others should be able to replicate results.
- Comparing data from 20 human experimental studies, it was concluded that experimental protocols for human pain models should include standardized endpoints and that how the assessments of specific endpoints reflecting psychophysical and physiological functions

(sensory fiber type, nociceptor type, endpoints for peripheral and central sensitization) are done would benefit from further standardization.

- A considerable step forward has been achieved in the mechanistic understanding of the translational steps from rodent to healthy subjects to patients in chronic pain research, based on the results of the integrated work across Europain including proteomics, lipidomics, transcriptomics, genomics, as well as electrophysiology, sleep impact on pain and somatosensory assessments. This fulfils one of the major overall objectives of Europain.
- Chemotherapy-induced peripheral neuropathic pain (CINP) responds very poorly if at all to current treatments for neuropathic pain. This is important since CINP is often a dose limiting treatment factor for chemotherapy and the population of cancer survivors suffering CINP is a growing one. A better neurophysiological understanding of CINP has been obtained through rodent models as well as a human volunteer models of CINP, as well as better phenotypic characterisation of CINP patients. This will improve the Probability of succeeding to develop an efficacious treatment for this growing patient population.
- A multicenter trial using corneal confocal microscopy (CCM) as a robust non-invasive diagnostic biomarker of peripheral small fiber neuropathy has demonstrated high correlation between Quantitative Sensory Testing (QST) profiles and nerve corneal fiber density. This opens up the avenue for CCM to become a tool of high sensitivity and specificity for diagnostics of peripheral polyneuropathy compared to current standard methods including the present state-of-the-art, namely skin biopsy, which is not as well standardized. CCM would also improve stratification in clinical trials of neuropathic pain enabling feasible inclusion of a wider range of peripheral polyneuropathies.
- It has been demonstrated that subgrouping the QST profiles of patients with peripheral neuropathic pain into clusters reflecting somatosensory function can be used as a biomarker for patient stratification in early clinical trials. This biomarker may have the potential to both be used to improve clinical trial design by enriching the study population for treatment responders as well as to be used for better diagnosis in medical practice. The QST profiling tool is superior for this purpose due to its high specificity not giving a false positive diagnosis. It has also been demonstrated that questionnaires developed for the purpose of easy diagnostic screening of neuropathic pain are sensitive but not specific. This means that questionnaires cannot exclude other pain diagnoses, which dilutes the inclusion of the proper patient population in clinical trials. For this, a specific tool like QST is needed. .
- It was demonstrated that microneurography, an advanced electrophysiological tool, has a clear value as a biomarker in early clinical trials. A more easy-to-use and non-invasive tool (Threshold tracking) for more wide-spread clinical use is under development by Europain partners but will need more validation studies before it can be used as routine in large late phase clinical trial programs and in medical practice.
- These clinical biomarkers (QST, CCM, microneurography and threshold tracking) were presented to EMA at a Biomarker Qualification Advice meeting and a CHMP opinion was received. Europain claims for biomarker validation level of QST and microneurography are reflected in the updated EMA *Guidelines for the development of medicinal products intended for the treatment of pain*.

1.5 Scientific and technical results/foregrounds of the project

Work package 1: Neurobiological mechanisms of chronic pain

Key overall Work Package 1 achievements

1. *Proteomics, transcriptomics, lipidomics and genomics have emerged as reliable techniques in research. However, their use as robust markers in models of pain and translationally from animal*

to humans have not been robustly validated. Europain has undertaken a huge assay method development task in validating and cross validating these analytical methods and techniques to simplify the -omics field. Europain has for the first time validated the refined technique of Next Generation Sequencing (NGS) vs. older methodologies. This was a necessary step to advance proteomics and transcriptomics in the pain field, also extending from peripheral tissues to brain and spinal cord level. Lipidomics has managed to demonstrate that also platelets may have an important role in mediating pain in peripheral tissues.

- 2. Several new possible drug targets have been identified as a result of these –omics results.*
- 3. Two models of transgenic mice have been developed and characterised.*
- 4. Electrophysiological experiments have increased the knowledge around spinal mechanisms of peripheral pain, so called central sensitisation, considered a substantial contributor to chronification of pain. These experiments have also clarified that noradrenaline reuptake inhibition at spinal level, thereby reducing central sensitisation, is the major analgesic mechanism of action of the recommended first line treatment for neuropathic pain, the tricyclic antidepressant amitriptyline.*

Next generation sequencing has been validated as a means of transcriptional profiling and this has been applied to multiple models of persistent pain in animal and human tissue. A large number of dysregulated transcripts in persistent pain states have been demonstrated and undergone validation. Proteomic analysis of the spinal and supra-spinal level of persistent pain models have hitherto not been possible to perform, but was managed to be undertaken by Europain. Results were very clear and robust and supported the important role of these central relay stations. Lipidomics has demonstrated an important role for pain specific mediators released by platelets in contributing to mechanical hypersensitivity. Electrophysiology at the spinal level has been used to understand the mechanism of hypersensitivity at a spinal level in persistent pain models such as diabetes and to demonstrate the importance of noradrenaline in mediating the analgesic actions of amitriptyline. This has also been used as a translational model as a close correlation of firing properties in wide dynamic range neurons in the rat to pain reports in humans following laser stimulation was observed.

The approach consisting of focused PCR array and genome-wide transcriptional profiling of multiple pain models have gathered robust results. These include rodent models of inflammatory, neuropathic, chemotherapy and antiretroviral-therapy induced pain. Genome-wide transcriptional profiling has been performed on collected tissue from several tissues: skin, joint, DRG and spinal cord.

A number of chemokines are linked to peripheral inflammatory pain models. Doublecortin contributes to the central plasticity and was found expressed in the membrane fraction of the central amygdala of neuropathic rats while LPA 18:0 identifies as novel pain mediator in inflammatory pain. A web database called “Pain Networks” (WP6) has been finalized. It will enable to share Next Generation Sequencing (NGS) data and integrate further genetic data across university, industries in EU and globally.

Data collected by gene expression and lipid mediator analysis in the skin of rats, mice and human healthy volunteers undergoing UVB-induced skin erythema and pain, suggest that there is a high level of correlation in mediator expression in experimental pain models between species. Equally, electrophysiology data based on correlation between the evoked activity of rat spinal neurones and human perceptual responses (psychophysics and EEG recordings) supports the above evidence and highlight that, in some respect, the neurophysiology and pain pathway regulation can be considered comparable between rodents and human. Along this line, e-phys recordings in three animal models of diabetic neuropathy, although only partially overlap, has provided evidence for diabetes-dependent spinal modulation. Overall these findings provide evidence that rodent models can serve

as surrogate for pain research and provide valuable information to further understand the neurophysiology of pain pathways.

Further, two models of transgenic mice were developed with sodium channel point mutations associated with hereditary human pain conditions. Strains were successfully bred and characterized also for transcriptomics of transposed genes and expected behavioural changes.

Work package 2: Improving animal models of pain

Key overall Work Package 2 achievements

Preclinical models of neuropathic pain have previously mainly focused on nerve injury as the sole proxy for neuropathy, and to some extent also on a model of diabetes neuropathy related to hyperglycemia. These show poor resemblance and are poorly predictive as drug efficacy models of common neuropathic pain states associated with Type 2 diabetes, peripheral polyneuropathy, spinal cord injury, HIV-associated neuropathy as well as treatment induced neuropathic pain due to chemotherapy and HIV antiretroviral treatments. Europain has successfully developed and validated more disease relevant models of these clinical pain states. Further, Standard behavioural outcomes in rodent pain experiments all measure response to external stimuli evoked by the experimenter. These outcome measures correspond poorly to human pain assessments and are easily confounded by drug adverse effects or the pain model as such. There was a lack of methods for assessing spontaneous pain related behaviour in rodents. Europain has standardised and validated four such measures, all relevant to rodent innate behaviour. These were not only possible to standardise but also found sensitive to pain models and behavioural changes were attenuated by pharmacological intervention.

New rodent experimental pain models

In all, seven rodent models of pain have been validated: diabetic neuropathy in ZDF and in BB/Wor rat strains, the spinal cord contusion (SCC) rat model of central neuropathic pain, the rat model of nerve growth factor (NGF)-induced hypersensitivity as a proxy for small fiber neuropathy, the Stavudin (d4T) and indinavir rat models of antiretroviral neuropathy and the mouse oxaliplatin model of chemotherapy induced neuropathy.

The lack of an appropriate animal model of diabetic neuropathic pain was a clearly unmet need at the start of the Europain collaboration. The Zucker diabetic fatty rat (ZDF) is a genetically obese rat selectively inbred to exhibit type 2 diabetes. WP2 has characterized the pain phenotype and morphology of the ZDF rat. Results so far indicate that the ZDF rat is the most viable and clinically relevant animal model of Type 2 diabetes and diabetic polyneuropathy in terms of construct validity, pain behaviours, electrophysiology and histopathology painful diabetic neuropathy to have been described to date.

HIV-associated sensory neuropathy afflicts 40–50% of HIV patients and is associated with previous exposure to both nucleoside reverse transcriptase inhibitors including stavudine (d4T) and the protease inhibitor indinavir, both widely used in resource-limited settings. We have reported complex behavioural changes and a distinctive neuropathology in clinically relevant rat models of d4T and indinavir-induced sensory neuropathies, respectively, that are suitable for further pathophysiological investigation and preclinical evaluation of novel analgesics. Both rat models have

the potential to be useful in the development of drugs for the treatment of HIV-related and Anti RetroViral Therapy (ARVT) induced neuropathic pain.

Rat models for chemotherapy induced neuropathy are not feasible due to toxicity. Therefore a model of chemotherapy Induced Neuropathic Pain (CINP) was instead developed in mice. A standardized mouse model of chronic oxaliplatin-induced neuropathy associated with behavioural measures suitable for pharmacological studies has been developed. We showed that following 4 weeks treatment with oxaliplatin the mice develop a distinctive and robust phenotype, including mechanical hypersensitivity, reduced spontaneous locomotion and cool avoidance. The phenotype was still routinely observable up to 7 days after oxaliplatin administration and slowly normalised after 10 or more days, suggesting that the effects of oxaliplatin may be partly reversible. The model is amenable to read-outs based on natural rodent behaviours of exploration of environment and thermal place preference.

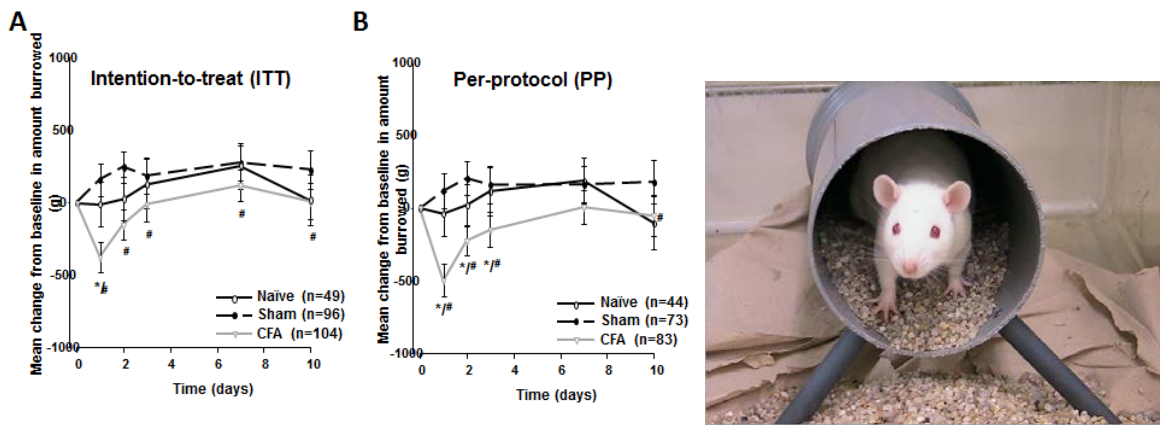
Peripheral nerve morphology was examined in skin biopsies from all of the above rodent models of neuropathic pain, and correlated with known human findings in all cases. This was also accompanied by evidence of injury at the level of the dorsal horn of the spinal cord in some models and microglial activation in others. Differences between models are distinct, emphasizing the need to develop animal models relevant to specific clinical scenarios. Further, in the rat, local administration of NGF to the rat paw causes increased responses to both mechanical and thermal stimuli, which were synergistically amplified when combined with low levels of UVB exposure. This new rat experimental pain model is translatable to human experimental model and potentially also as a clinical proxy.

Methods for assessing ethologically relevant spontaneous pain behavior

Standard behavioural outcomes in rodent pain experiments all measure response to external stimuli evoked by the experimenter. These outcome measures correspond poorly to human pain assessments and are easily confounded by drug adverse effects or the pain model as such. There was a lack of methods for assessing spontaneous pain related behaviour in rodents. Therefore, Europain partners have directed efforts on new outcome measures to behaviours that are ethologically-relevant to the rodent. Behaviours include those linked to predator avoidance (thigmotaxis, preference for closed arms of elevated plus maze) and natural burrowing behaviour. The reduced burrowing performance has been validated within and across several laboratories in the WP. The natural rat behaviour to avoid open space is accentuated by pain and methods to measure this in both the open field (thigmotaxis) and the elevated plus maze have been developed. For these studies, both new models as well as already established models to induce pain related behaviour have been used.

Burrowing is an ethologically relevant rodent behaviour, proposed as novel outcome measure to assess the global impact of pain in rats. A number of groups (Pfizer, Imperial, UHEI, Lilly, AZ, BI, Grünenthal & AbbVie) have measured functional deficits in burrowing performance in a rat model of inflammatory pain. The readout protocol was harmonised and standardised across laboratories, with some local adaptations. The model was also investigated in neuropathic pain models as well as in mice, with the Complete Freund's Adjuvant (CFA) model being the one that collected most data for an across-laboratories analysis. In a prospective multicentre replication study, the burrowing paradigm was validated in the CFA model. Eleven studies were performed across 8 centres and data were collected and analysed centrally with a restricted maximum likelihood-based mixed model for repeated measures. The Burrowing protocol was co-developed between sites and experiments replicated across centres with baseline burrowing performance. Compared to naïve and sham

(vehicle injection) animals, 24 hr following the insult a significant burrowing deficit was induced, returning to baseline over 9 days. This unique multicentre approach provided high-quality evidence, not only validating burrowing as a robust, reproducible outcome measure which could be a useful tool to infer the global effect of pain on rodents, but also informed about the impact of minor protocol variations on burrowing, providing educational value for future investigations. Pharmacological validation has followed and the results suggest that pain is responsible for the deficit: the burrowing was improved upon treatment with analgesics known to be efficacious in applicable pain models (NSAIDs for inflammatory pain models and gabapentin for models of neuropathic pain), i.e. the standardised burrowing outcome measure was validated as sensitive to change and not due to the insult giving a motor deficit.



Colleagues at Imperial College lead work on thigmotaxis an outcome measure. Thigmotaxis is the innate behaviour of prey animals, such as rodents, to avoid danger of visibility in open spaces. This behaviour was reduced as a result of pain induction, e.g. by antiretroviral treatment induced neuropathy and could be reliably reproduced and quantified.

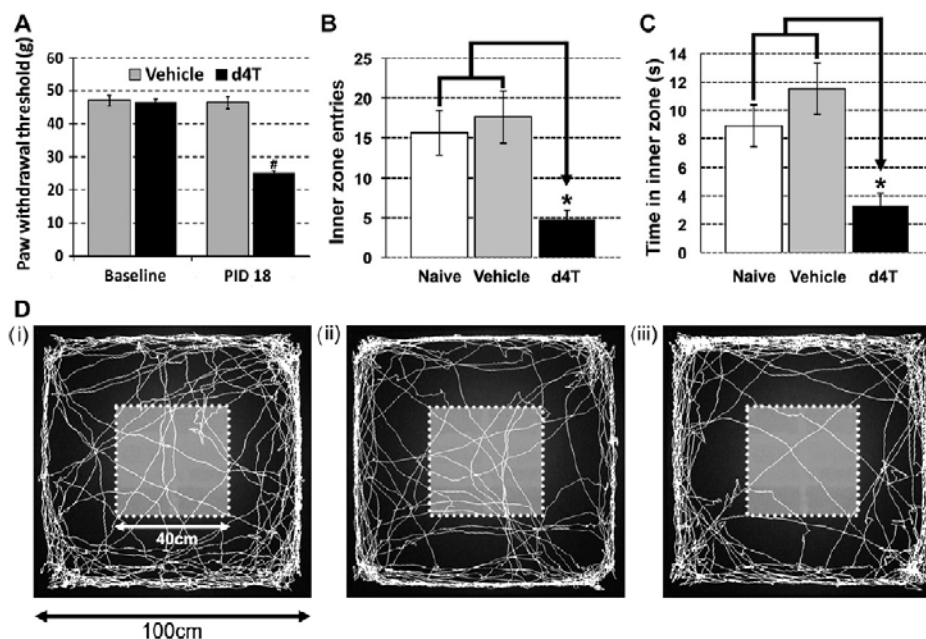


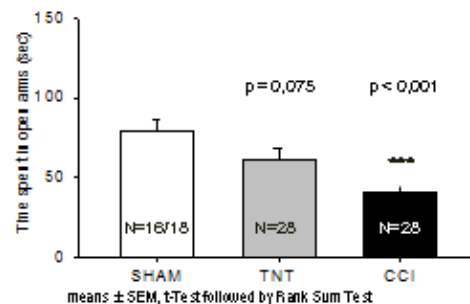
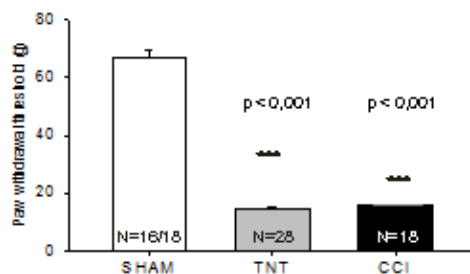
Fig. Thigmotaxis behaviour is reduced in d4T rats (Diii) compared to naïve (Di) and sham treated (Dii) rats. Change in behaviour was parallel to reduced paw withdrawal threshold (A,B,C).

Colleagues at University of Heidelberg (UHEI) have reproduced earlier findings at BI of reduced time spent in the open arms of the elevated plus maze in two rats models of neuropathic pain following peripheral nerve injury.

EPM: 3. Reproducibility:

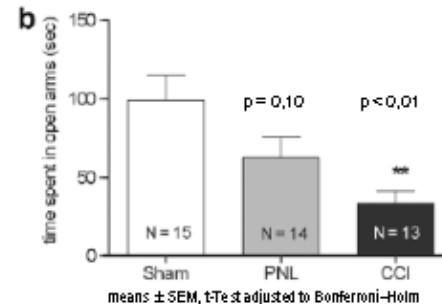
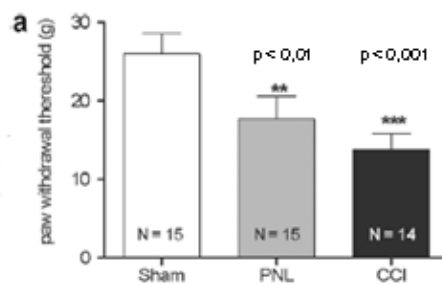
EXPERIMENTS in 2012 at UHEI

- CCI and TNT



EXPERIMENTS in 2008 at BI

- CCI and PNL *2008 Roska et al., Pain



The Elevated Plus Maze (EPM) as a spontaneous pain behaviour was successfully replicated across partners UHEI and BI, using the same protocol, after across laboratory training visits.

Sleep deprivation and pain

Animal experiments (Lilly) and human experiments (UHEI) indicate profound changes in sensitivity to mechanical and thermal nociceptive stimuli after sleep deprivation. Results from animal experiments suggest a pro-inflammatory component in sleep deprivation-induced hypersensitivity. In close collaboration with UHEI pathophysiological aspects were addressed in both rats and humans.

At Lilly UK, sleep architecture, cognitive performance and motivational changes were investigated in inflammatory pain models. Some clinically relevant chronic pain outcomes can be measured in the

animals. A key finding is that after sleep deprivation in the monosodium iodoacetate (MIA) model of chronic joint pain in the rat, a significantly increased sensitivity to evoked pain measures was found.

Studies on pain-related cognitive function were carried out at Sanofi-Aventis. From these, a new outcome measure was developed, based on novelty discrimination in a social context. It involves a direct encounter between an adult rat and two juvenile rats, during which the investigatory behaviour of the adult rat is recorded. Social discrimination is defined by longer durations of investigatory behaviour towards the novel juvenile than towards the familiar one. Nerve injured rats display deficits in social discrimination compared to normal rats. These deficits are sensitive to several analgesic drugs, and insensitive to several non-analgesic drugs, suggesting that they reflect the painful condition.

Electrophysiology studies

Microneurography studies have detected spontaneous C-fiber activity (considered a biomarker of spontaneous pain) in various rat models of neuropathy - focal traumatic nerve injury (crush, suture), chronic constriction injury, tibial nerve transection and diffuse nerve pathology (streptozotocin, ZDF, ddC and d4T). Spontaneous firing measured by microneurography in peripheral afferent C-fibres is a surrogate measure of ongoing pain in rats. The pharmacological sensitivity of this measure to gabapentin, diclofenac and lidocaine was measured in rat models of sciatic crush injury, STZ and ZDF diabetic neuropathy.

Lessons learned: importance of training across partners

Overall, there has been close collaboration between work package 2 and work packages 1 and 3 in order to align workstreams of common interest, e.g. electrophysiology and neuropathology (WP1), and sleep restriction and cold hypersensitivity (WP3).

Training visits proved to be a key factor in successfully reproducing findings within the workpackage. This has been a unique success factor in the consortium, where exchange training visits have been extensively used. Pain outcome measures can be highly sensitive to varying laboratory conditions and small changes in experimental techniques. So, training visits over one or two weeks for intense training have been undertaken by WP2 scientists in order to learn the exact details of new techniques developed elsewhere in the workpackage, in industry as well as in academia. This was also crucial in order to align protocols across labs. Training activities also included the establishment of an electronic library of animal model protocols, peer reviewed before being entered into the library. The library is publically accessible at the Europain website (www.imieuropain.org) and will remain there for 3 years after end of project, *bona fide* for researchers as an important legacy and long lasting contribution to the field of the EUROPAIN project.

Another key success factor and focus of the workpackage has been that of methodology refinement. This was a key objective and a main focus has been to estimate and reduce the influence of experimental bias animal model of pain. A consensus paper has been published to disseminate the result of this work.

Work package 3: Translational pain models in humans

Key overall Work Package 3 achievements

In this work-package several activities were carried out to refine human experimental pain models and to refine endpoints in human pain models that can serve as a bridging comparator between animal data and chronic pain patients. This approach has been very successful for demonstrating target engagement and proof of mechanism in the drug development process.

1. *A meta analysis of the endpoints used in industry trials was performed, supporting the need for further standardisation.*
2. *The UVB UV-light model has been characterised in collaboration with Work Package 1, demonstrating mechanistic characteristics.*
3. *Most human experimental pain models induce increased sensitivity to sharp mechanical and heat stimuli, common in both animal models and neuropathic pain patients. In contrast, sensitivity to cold is the main characteristic of chemotherapy induced neuropathic pain (CINP), a hitherto treatment resistant condition. To fill this gap, a novel model of cold hyperalgesia was developed and validated.*
4. *Microneurography was proven to be a valuable tool for measuring pain, fully comparable from animals to humans.*
5. *Sleep deprivation increases sensitivity to pain.*
6. *Novel models of Functional magnetic resonance imaging (fMRI) techniques have been demonstrated sensitive to pharmacological intervention with standard analgesics.*

[A meta analysis of endpoints used in human experimental pain studies](#)

A meta-analysis of human experimental pain studies comprised original data from 28 clinical trials on 12 different human experimental pain models including central sensitization. These data sets provided by 6 different EFPIA partners were pooled, mapped and harmonized. Ultimately data from 20 studies were compared.

In summary, the outcome of this meta-analysis project indicates that experimental protocols for human pain models should include standardized endpoints. This does not preclude further supplemental assessments tailored to a specific study. Experimental pain model protocols will still need to be adapted to allow for specificities depending on study hypothesis and profile of drug tested. However, Especially, how specific endpoints reflecting psychophysical and physiological functions (sensory fiber type, nociceptor type, endpoints for peripheral and central sensitization) are assessed would benefit from standardization (see Quantitative Sensory Testing (QST) WP9 for reference). Standardized endpoints would facilitate enhanced comparison and understanding of data between studies and might subsequently allow a more meaningful comparison of compounds in clinical development.

[UVB model characterization and pain plasticity protocol](#)

A short-form protocol of pain plasticity using QST to be used prior to surgery in prospective clinical trials of postoperative persistent pain has been developed. The short form protocol has been developed at UHEI consisting of pin prick testing before and after conditioning High Frequency Sensory (HFS) electrical stimulation (5 x 1 s at 100Hz) through special surface electrode. This protocol was then used in a prospective trial in 1000 patients undergoing mastectomy (WP5).

Skin biopsies were taken in all studies with UVB lesions and analysed for cytokines, chemokines and lipids.. Analyses were conducted in WP1. UVB inflammation produced very similar sensory changes in both rodents and humans. The mediators released into the inflamed tissue were remarkable similar. We also identified one chemokine, CXCL5 as a novel pain mediator. Further, preoperative skin biopsies from patients in WP5. The latter results were correlated with acute and persistent postoperative pain.

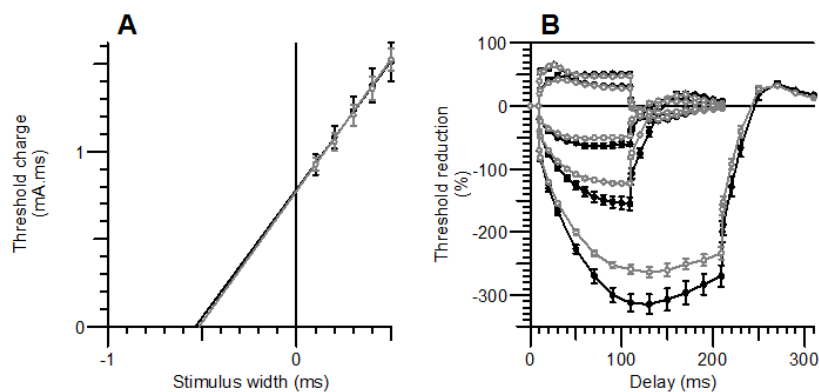
The LC-MS/MS methods have been established to measure lipid mediators from human skin biopsies. In order to measure the expected low concentrations of lipids very sensitively, LC-MS/MS methods have been switched to nano-LC. We used the UVB lesion model of inflammatory pain in healthy human subjects and characterized its effect on sensory perception by Quantitative Sensory Testing (QST, D 3.7). In addition, we measured oxidized lipids (Prostanoids and oxidized linoleic acid metabolites) but without significant findings in UVB-treated vs. normal skin.

Cold hyperalgesia model of CINP

A human experimental model of chemotherapy induced neuropathic pain (CINP)-like sensory profile has been developed and validated: Three studies have established the topical application of high-concentration 40% menthol as a useful stable model for studies of cold hyperalgesia and pinprick hyperalgesia in humans. Stability data up to 4 hours are important for psychophysical and pharmacological research in humans and provide insights on experimental cold and mechanical hyperalgesia. Reproducibility of cold and mechanical hyperalgesia showed good correlation, $r = 0.8$, but not so the area size of hyperalgesia. The topical menthol pain model can be used for pharmacological interventions with study sessions one week apart without carryover effects. The menthol model is reliable in producing cold hyperalgesia and not sensitive to expectation effects, while mechanical hyperalgesia is not as reliable. A familiarisation or training session is recommended before interventional sessions. The model shows high resemblance to sensory changes seen in CINP, suggesting it affects the same neural mechanisms.

Microneurography and in vivo electrophysiology:

The translational work on single nerve excitability has defined strict rules for identification of spontaneous activity by microneurography (μ NeG) both in humans and in rats (Sprague-Dawley). The model has already for use in pharmacological studies of modulation of spontaneous activity.



A novel technique to study excitability of the most sensitive sensory nerve fibers is developed and validated based on psychophysical thresholds - Threshold electrotonus. The excitability measures correlate with heat pain sensitivity in healthy subjects, suggesting a possible switch between C- and Adelta-fibers at about 45°C. Multiple non-invasive measures of excitability were validated. An automatized protocol for peripheral nerve excitability studies was applied in patients with nerve injury, polyneuropathy and in healthy controls. Excitability correlated with "pins and needles" and "tingling" self-reported positive sensory symptoms.

Sleep deprivation and pain

Chronic pain and insomnia are serious health problems that severely impact quality of life and productivity. Experimental protocols can discriminate the pathophysiological link between disturbed sleep and pain. A human pain model was established, contrasting one night of total sleep deprivation (TSD) with one night of habitual sleep (HS). Both psychophysical and electrophysiological methods were used to investigate the impact of TSD on nociception. TSD is able to induce generalized, mechanical and thermal, hyperalgesia but not spontaneous pain. Further, TSD has attention-dependent effects on the signature of pain habituation. This may be the mechanism behind TSD-induced dissociation of perceptual and electrophysiological outcome measures of nociception. Using

a conditioned pain modulation (CPM) paradigm, we found sleep deprivation particularly compromising female endogenous antinocifensive mechanisms while descending pain modulation of sleep-deprived males was unaffected. Particular attention was taken to maximise translational aspect of these studies. UHEI responsible for the human studies (WP3) and Lilly responsible for the animal studies (WP2) worked in close collaboration co-designing the studies in WPs 2 and 3, including visits across laboratories.

Functional imaging (fMRI)

A study in 24 healthy volunteers investigated the utility of functional imaging in conjunction with a central sensitization (CS) model of topical capsaicin as an objective measure for selecting clinically effective drugs in neuropathic pain. CS has been shown to increase activity in the brain stem in response to punctate stimuli. We used gabapentin, ibuprofen and placebo to validate this model. Only gabapentin suppressed the neural response to secondary mechanical hyperalgesia in the right nucleus cuneiformis (rNCF) (an area of brainstem specifically activated by CS), in left posterior insular (pIN) and in secondary somatosensory cortex (SII): nociceptive specific areas. Similarly, only gabapentin suppressed resting state (RS) functional connectivity during CS between the thalamus and SII. A power analysis revealed that when using neural activity from the rNCF area and left pIN, a statistically significant difference between placebo and gabapentin was detected with a probability ≥ 0.8 with a sample size of $N=12$. FMRI with CS can be used as a mechanism-based assay in limited study populations to make clear go/no-go decisions in selecting analgesics effective in NP in early drug development.

Consensus meetings

A first consensus meeting was held together with Wrook package2, agreeing on how to compare endpoints between animal experiments and human experimental studies.

An overall final consensus meeting was held, where it was agreed that the project data results provided a considerable step forward in mechanistic understanding of the step from rodent to man, based on parallel tasks to WP2 and biomarker analyses including skin biopsies, quantitative sensory assessments including the development of a cold allodynia model, electrophysiological measures and sleep studies conducted across species. Further, the translational mechanistic links to patients were investigated with positive outcomes in relation to several of these biomarkers. Thus, human experimental studies would be valid for proof of mechanism studies. However, endpoint assessments would benefit from standardisation in order to be able to compare study outcomes.

Work package 4: Quantitative assessment of pain in patients

Key overall Work Package 4 achievements

The main objectives of WP4 were to assess pain in chronic pain cohorts objectively and quantitatively for use in clinical trials.

- 1. Imaging techniques, such as Arterial Spin Labelling (ASL) and Resting State Brain Activity (RSN) can be used to quantify chronic pain in patients, by visualizing central areas in the brain important for pain control.*
- 2. The to-date largest database collected, of >1,000 healthy volunteers and over 2,500 neuropathic pain patients, has enabled analyses of sensory profiling, clearly suggesting that it makes more sense to classify neuropathic pain based on somatosensory profile than on underlying etiology. This novel finding is of utmost importance for future development of new treatments for neuropathic pain, since some sensory profiles can be expected to respond to treatment while others will not, depending on which mechanism the development candidate has.*
- 3. Roughly, the standardized Quantitative Sensory Testing (QST) differentiates sensory phenotypic profiles into Gain-of-Function (GOF) and Loss-of-Function (LOF) across etiological neuropathic pain diagnoses. It was hypothesized that a GOF profile would predict a better response to a sodium channel blocker analgesic. This hypothesis was tested directly in a first trial ever to do so, and positive results demonstrated that the hypothesis held true.*
- 4. The whole Work Package 6 clinical database was analysed with cluster analysis, hypothesizing that patient sensory phenotypes could be separated into clear clusters of Gain-of-Function (GOF), Loss-of-Function (LOF) or a mix of GOF and LOF sensory profile. This cluster analysis demonstrated that these phenotypes were clear, with very little overlap, and within clusters were identical across etiologies behind the neuropathic pain. This supports a future mechanism based pain treatment paradigm.*
- 5. Placebo effects are often large in pain trials and can even make them fail to show efficacy, requiring very large studies to overcome this factor. A meta-analysis of individual placebo data from >2,000 patients, the main factor predicting the magnitude of placebo response seems to be the patient expectation of treatment efficacy.*
- 6. Electrophysiological measures of pain translate well from animals over volunteers and also into patients, as a direct measure of spontaneous pain activity in nerve fibers.*
- 7. Confocal Corneal Microscopy (CCM) can be used as a sensitive and specific diagnostic biomarker of small fiber neuropathy across etiologies, as a more feasible tool than skin biopsy.*
- 8. Neuropathic pain questionnaires are not able to differentiate neuropathic pain in relation to Gain or Loss of function. Their role is rather to screen for potential neuropathic pain.*

Functional Imaging

Arterial spin labelling (ASL) was evaluated as a novel tool to measure the neural activity underlying pain. The whole brain Pseudo-Continuous Arterial Spin Labeling (pseudo-CASL) method was validated as preferred tool for measuring ongoing pain in mechanical, thermal and capsaicin models. We also evaluated Arterial spin behaviour as a novel tool to measure ongoing pain in patients with neuropathic pain. Conclusions: 1) Multi-TI ASL fMRI offers excellent reproducibility for both within- and across-subject investigations; 2) The dorsal posterior insula is a key biomarker of nociception; 3)

The multi-inversion times integrated (multi-TI) integrated ASL method can be used in chronic pain patients. The improvement vs. standard ASL is a higher resolution in conjunction with a much shorter scanning time, which is of high importance for pain patients.

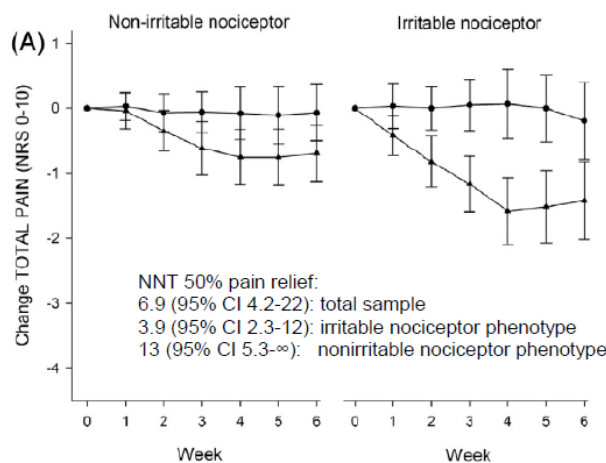
Resting State Brain Activity (RSN) was validated in patients with ongoing chronic pain, with and without pain provocation, to enable visualization of ongoing pain at rest and endogenous pain control by using distraction as an intervention tool. Based on results in healthy volunteers showing that the medial prefrontal cortex (mPFC) is an important structure for impaired pain distraction, it was suggested to have substantial influence on the complex pain syndrome of “chronic somatoform disorder”. In a second step, in patients with somatoform disorder we noted that Default mode networks (DMN’s) and salience networks (SN’s) spatial maps of coherent activity overlapped within the medial prefrontal cortex (mPFC). It was concluded that PFC is an important structure for impaired pain distraction also in somatoform disorder. Patients with pancreas carcinoma and chronic pancreatitis (with and without pain) demonstrated changes in various functionally connected brain regions vs. controls. In addition, the activity in the periaqueductal gray (PAG) increased in patients but decreased in control subjects over time. The PAG is a main component within the endogenous pain inhibitory circuit.

Quantitative Sensory Testing and sensory profiling as a predictor of drug response

Overall, the project has examined patients of different neuropathic pain conditions and healthy controls, characterised them based on Quantitative Sensory Testing (QST) as well as medical history, medication history, and numerous validated questionnaires.

Two first-ever study were conducted to directly test the hypothesis that patients with irritable nociceptor phenotype (GOF) would respond to a greater extent to treatment compared to patients with non-irritable nociceptors (LOF).

In the first study oxcarbazepine, was compared to placebo. Patients were phenotyped at baseline into “irritable nociceptor” (GOF) and “non-irritable nociceptor” (LOF) groups. The irritable nociceptor group (GOF) had substantially greater benefit from oxcarbazepine than their counterparts in the non-irritable nociceptor (LOF) group.



In a similar study using the topical lidocaine patch, also this small study demonstrated a modestly better efficacy in patients with the Gain-of-function (GOF) phenotype vs. Loss-of-function (LOF) phenotype .

A cluster analysis of the sensory phenotype patterns separates these into three clearly disparate patterns: the GOF, the LOF and a mixed form of sensory phenotype, yet to linked to separate neural molecular mechanisms. This opens up for a future paradigm of phenotype mechanism based treatment of neuropathic pain.

Placebo effects

In a meta-analysis, placebo data from 9 EFPIA partner Phase III studies including 2017 adult patients suffering from chronic painful osteoarthritis or low back pain were compared. The primary outcome of trials was pain intensity. Predictors of magnitude of placebo response were, in order: opioid trials, a high number of study visits, and randomization ratio. Based on these results and previous studies, we think that patients' perception of treatment allocation and expectations toward treatment efficacy may predict outcomes of RCTs.

Placebo effects are often substantial in clinical trials of pain. These placebo effects can be studied also in chronic neuropathic pain, both in relation to spontaneous and evoked pain. In the Europain studies, significant nocebo effect were not seen in relation to neuropathic pain, but a meta-analysis of results from the ongoing studies showed that the average magnitude of nocebo hyperalgesia is comparable to the magnitude of placebo analgesia.

To better understand the role of cognitive impairment on nocebo, published data on the general susceptibility for subjective adverse events (AEs) during placebo treatment in donepezil trials for Alzheimer's disease (AD) were reviewed (Ref Amanzio et al, 2012). It was concluded that AD patients were more prone to AEs compared to patients with mild cognitive impairment (MCI), likely related to a higher presence of somatic comorbidity, predisposing to emotional distress again predisposing for increased somatisation. Thus, cognitive processing is a contributing factor to the placebo effect.

Electrophysiological measures of pain:

Measures of peripheral nerve excitability were used to obtain objective indices of axonal membrane excitability in patients with neuropathic pain. The overall aim of this task was to validate the use of peripheral nerve fibre excitability for non-invasive electrophysiological measures in patient stratification and as a read out for spontaneous pain in peripheral neuropathic pain trials. Measures of excitability were obtained through threshold tracking techniques and then correlated to QST phenotypes as well as to ongoing pain. Protocols were tailored to assess different nerve fiber types and ion channels to assess sodium, potassium (fast and slow) and hyperpolarisation-activated cyclic nucleotide gated (HCN) currents. We were able to generate a set of parameters that allow differentiation of neuropathic pain patients into different excitability profiles. Thereby, we have obtained functional indices of sensory axon excitability indices that can be used for subsequent trials. This will enable objective readouts in early Proof of Mechanism studies demonstrating clinical target engagement and reduce sample sizes and shorten treatment periods for Proof of Concept studies, tailored to address the specific target mechanism of the drug under development.

Corneal Confocal Microscopy as a diagnostic biomarker

The utility of Corneal Confocal Microscopy (CCM) as a tool for convenient and non-invasive alternative for improved diagnostic of peripheral polyneuropathy as a cause for peripheral neuropathic pain, as an alternative to the more invasive and resource consuming microscopy of skin biopsy, was investigated via a clinical study conducted via extra funding awarded to the project. The study, named the SECOM study, involved 4 sites (AUH, BGH, Imperial, UHEI) in three countries (DE, DK, UK) including in all 150 patients with peripheral polyneuropathy (PPN) and 41 healthy volunteers who all underwent the same study protocol as in task 3 plus confocal microscopy of the cornea (CCM). Images were centrally read at UCL and BGH. All patient subgroups (Diabetes, CINP, HIV and idiopathic) demonstrated reduction in corneal innervation, measured by nerve fiber density, nerve fiber length and nerve fiber branching. Preliminary data indicate that nerve fiber loss correlated with QST profiles but not with questionnaire results. As advised by the EMA, more subgroups of Painful

Peripheral Polyneuropathy (PPP) need to be investigated in order for CCM to be qualified as a fully etiology-independent diagnostic tool for PPP.

Questionnaires as diagnostic or differentiating biomarkers of neuropathic pain.

The questionnaire previously best validated to differentiate neuropathic from non-neuropathic pain was compared to QST for diagnostic and stratification. The questionnaire was not a reliable tool to diagnose or stratify patients for Loss of gain of function, key purposes of QST.

Work package 5: Risk factors for chronic pain

Key overall Work Package 5 achievements

Three very common and standardized types of surgery were studied, as improved knowledge in large groups would not only generate sufficient data but implementation of results potentially also provide most patient and healthcare benefit. Considering the increasing group of survivors after cancer surgery, improvements for these patient groups is very important from a general health perspective.

- 1. Three major risk factors for persistent post surgery pain at 1 year after surgery were identified: age, psychological factors and nerve injury. Persistent postoperative pain may last for more than 5 years.*
- 2. Incidence of persistent pain after different types of surgical techniques were compared. Certain surgical techniques, such as video-assisted thoracic surgery, nerve sparing techniques and sentinel node technique for breast cancer surgery, significantly reduced the incidence of persistent postoperative pain. In contrast, identifying nerves during surgery was of no benefit.*
- 3. Functional evaluation of specific persistent pain states is important but instruments lacking. Such scales have been developed and validated.*
- 4. QST is a valuable biomarker tool for measuring impact of nerve injury, both after surgery and after adjuvant chemotherapy. Results concur with skin biopsy findings.*
- 5. Proteomics on preoperative skin biopsies predict development of persistent postoperative pain.*
- 6. Gene polymorphisms associated with persistent postoperative pain were identified.*

Risk factors for persistent postsurgical pain

In hernia surgery, the role of surgical technique and surgical treatment for prevention of persistent post-herniorrhaphy pain has been elucidated. In thoracic surgery, studies have been performed to assess the functional consequences of persistent pain after lung cancer surgery, as well as assessment of the role of surgical techniques (use of drain and video-assisted pulmonary surgery) on preventing persistent pain: The functional consequences of persistent pain after lung cancer surgery have been assessed in a nationwide study. Further, a questionnaire to assess the functional consequences on a procedure-specific basis has been developed and validated.. Studies showed that drain insertion may lead to nerve injury and finally that video-assisted thoracic surgery potentially may lower the risk of persistent neuropathic pain. In breast cancer surgery, a major focus has been laid on nationwide analysis of the pain state, validation of a questionnaire on functional consequences, the time course of the problem, the role of different treatment regimens and finally a large, very detailed prospective study on pathogenic mechanisms. The problem of persistent pain after breast cancer has been reviewed and a specific physical functional assessment tool has been developed and validated. The role of different surgical and adjuvant therapies have been described in detail with a focus on the intercostobrachial nerve and as demonstrated less importance of adjuvant therapy. The time course of persistent pain after breast cancer surgery has been assessed in a nationwide study showing a decrease in some, but increase in other patients overall with only a slight decrease over 5 years. One publication on a large (~ 500 patients) prospective study has been published showing that age, psychological factors and nerve injury are the most important factors and that about 15 % of patients have moderate to severe pain 1 year postoperatively. Further analyses on this investigation, to clarify the role of nerve injury assessed by QST, has been completed and published post project. The ways forward reduce persistent postoperative pain are to refine nerve-sparing minimal invasive surgical techniques and to re-study the concept of “preventive analgesia” with newer potent and prolonged multimodal analgesia techniques, including agents to modify the neuroinflammatory response to injury.

Biomarkers of persistent pain after different types of post-surgery adjuvant chemotherapy

In patients undergoing thoracic surgery and in breast cancer surgery, the value and use of QST for diagnosis and as an outcome tool has been assessed, but reproducibility was rather low. Reasons for this low reproducibility is not fully interpreted. However, most of postoperative cancer patients underwent adjuvant chemotherapy, which may have affected long-term follow-up results.

The role of chemotherapy in developing chronic neuropathic pain as a results of treatment for cancer was studied further in well characterized and comparative cohorts. In three studies in 217 patients receiving adjuvant oxaliplatin or docetaxel chronic Chemotherapy Induced Peripheral Neuropathy (CIPN) symptoms were present in 2/3 of patients in the oxaliplatin group and in half of patients in the docetaxel group, while pain in hands and feet was found in one third of patients. There was a clear difference in somatosensory profile during and soon after treatment with the respective adjuvants, but the symptoms and signs caused by chronic polyneuropathy were very similar, QST typically displaying a clear sensory loss especially of largely large-fibre functions. The persistent pain in the docetaxel group was found to have effect on psychological function. Cumulative dose predicted oxaliplatin-induced neuropathy (whereas endocrine therapy predicted peripheral pain in the docetaxel group). Thus these studies indicate that there are important differences in acute neuropathy symptoms and chronic pain profiles in patients following oxaliplatin and docetaxel treatment. An exploratory study to evaluate a new bedside tool to assess increased sensitivity to cold due to oxaliplatin treatment was conducted and shows promising results.

Biomarkers in persistent post surgical pain

Extensive QST examinations in persistent post surgical pain clearly demonstrate that QST protocols for these conditions need to be developed, since location of the sensory disturbances vary between surgeries and between patients. The protocol developed for Work package 5 in collaboration with Work Package 3, serves this purpose well. In post-thoracotomy patients, there was an increased mechanical pain sensitivity in patients with persistent postoperative pain vs. those without pain, but no differences between the two patient groups with regard to intradermal nerve fibre density or signs of central sensitization. Thus, the loss of nerve fibers is the only clear postoperative sign of nerve injury and Loss-of-Function, but not pathognomonic for pain to occur. This supports the QST findings in Work Package 4, brought forward to EMA in Work Package 9.

In hernia repair patients extensive characterisation including quantitative sensory testing has been done in open and laparoscopic inguinal hernia repair, including also development of a new technique to detect deep tissue hyperalgesia – these results are of importance for design of future mechanistic and interventional studies.

Pharmacological intervention in persistent postoperative pain

Peripheral nerve injury may result in ectopic neuronal activity in the spinal cord dorsal horn, implying central sensitization. This was studied in a crossover study in 14 well characterized neuropathic pain patients, peripheral nerve block and systemic lidocaine administration were compared with regard to effects on ongoing pain intensity and on evoked responses on QST stimulation. This study demonstrated that regardless of the individual somatosensory phenotype and signs of central sensitization, primary afferent input is critical for maintaining neuropathic pain in peripheral nerve injury and distal polyneuropathy.

Local anesthetics as nerve blocks have been used traditionally for the treatment of post nerve injury pain and persistent postoperative pain, but their role has been questioned based on insufficient evidence. The potential role of nerve blockade techniques in post-thoracotomy pain was reviewed, previous studies critically reviewed, calling for improved study designs. This was followed by preliminary feasibility studies with promising results, calling for larger RCT's. As a comparative control, literature review of radiofrequency treatment was negative. The role of peripheral nerve

blocks has been assessed and recommended. In conclusion, the roles of lidocaine and capsaicin patches have been assessed in RCT's, supporting efficacy in certain hyperalgesic patients. Another small study investigated the effect of a topical lidocaine patch on chronic post herniorrhaphy neuropathic pain including skin biopsy diagnosis of neuropathy. The results of this study indicate a better reponse to topical lidocaine treatment in patients with remaining sensory nerve function, expressing hyperalgesia on QST, supporting the results of studies in WP4.

Genetic risks for development of persistent postoperative pain

In persistent pain and functional impairment after hernia surgery, there was an association of functional variations in COMT and GCH1 genes, gene polymorphisms known from the literature to be involved also in other chronic pain conditions, e.g. fibromyalgia and other types of chronic musculoskeletal pain.

Work package 6: Extending and integrating databases.

Key overall Work Package 6 achievements

A broad understanding of neuropathic pain, genomics, phenotypes and patients characteristics is of utmost importance to obtain adequate knowledge bases in developing new treatments for neuropathic pain.

- 1. A preclinical database of external data warehouse rodent pain genomics and Europain rodent experimental data on genomics, transcriptomics, proteomics and lipidomics were linked in a large database called Painnet and integrated with the database of the London Pain Consortium. This database is fully accessible for data mining.*
- 2. The largest clinical database on neuropathic pain, including 1,000 healthy volunteers and now >2,500 well characterised neuropathic pain patients has been created. Data includes Quantitative Sensory Testing (QST), multiple questionnaires, medical history, medical examination results. This prospective database has been mined to test a number of predefined hypotheses, as also of Work Package 4 tasks.*
- 3. There has been extensive data mining performed in these databases. Results are included as part of Work packages 1,2,3 and 4.*

Preclinical database

In this work package, the UCL team has extended the London Pain Consortium database (LPD) with gene annotations from over 20 publically available databases relevant to the analysis of the genotype data. Additionally we have included many pain specific datasets both publically available and collected in house. For example we have integrated and gathered 15 different pain specific expression datasets from various microarray pain models. In house, we have also collected both SNT and D4T RNA sequencing expression time course datasets. We have also gathered the published known pain genes from the Mogil database. Pain relevant cell type and tissue specific localisation datasets have been integrated to provide further pain specific contexts. The database contains extensive pathway annotation and functional annotation from several other databases. On top of this we have integrated a highly comprehensive protein interaction dataset for human obtained by integrating all major publically available databases. This allows us to use publically available tools like Cytoscape to build protein networks, seeded by proteins from the Mogil database, implicated in pain. We have also integrated multiple drug annotation and drug side effect resources giving further clues as to which genes from genotype screens (once they come available) would make good targets for detailed study. In order to integrate with the genotype data we have stored the datasets in a human centric manner. This is done by building gene trees in order to identify one-to-one orthology relationships between human and model organism genes. The database is available through an open access fully searchable website (<http://www.painnetworks.org>).

We have developed various analysis tools that can look for protein modules by combining interaction expression data and other pain relevant data. In so doing we have looked for models where the genes from the genotype data are found. Modules detection methods provide further supporting evidence and mechanistic explanations for putative pain genes. In summary we have developed a highly comprehensive gene annotation data warehouse with a website front end and analysis tools for integrating with the genotype data in the Europain consortium. We will continue to maintain and update the database. Data sets from WP1 (i.e. RNA sequencing data) and other WP1 and 2 datasets of (i.e. proteomics, lipid-protein networks) have been integrated. The database has been made publically available.

Another goal for this work package was to extend the London Pain Database (LPD) providing the gene annotations with data produced in the EuroPain workpackages, as appropriate and to link the LPD with the (N)euroQuast databases of QST data and patient questionnaires established at BGH, in collaboration with TUM, CAU, UHEI, AUH, SDU, Imperial and NT as well as the genetic samples collected in the blood biobank. When establishing the LPD datawarehouse, there was not yet any (N)euroQuast data to integrate. The database was extended such that once clinical genotype data is available in connection with the (N)EuroQuast QST, as well as clinical and questionnaire data, it would be trivial to determine protein identifiers that would allow for electronic linkage of the LPD and the (N)EuroQuast data-warehouses to give and integrated EuroPain data-warehouse. However, this last step was not completed since there was no clinical genetic analyses performed from the Blood bank for genetic samples. This task was thus modified not to include the linkage between human and animal pain genetics.

Clinical database

A new clinical multicenter international database (N)EUROQUAST has been established. The data quality was ensured, firstly by site qualification of data quality including data interpretation, and by gathering a common healthy volunteer database to obtain normative data using 95% confidential values and z-values. Several updates were also performed for better usability after having received feedback from the participating centers. There were regular database locks, pre-agreed, to ensure clean data for individual deliverables underway. For the SECOM study, added only in the last part of the project, there was yet another update of the database to accommodate for these data.

During the course of the project, the Europain clinical database was merged with the Neuropain Cohort study clinical database, resulting from a parallel clinical study with the exactly the same protocol, and conducted in parallel, but from another funding source (an unrestricted research grant from Pfizer, with Prof Ralf Baron as Principal Investigator and grant recipient, encompassing 12 European sites from Denmark, Finland, France, Germany, Italy, Spain, Sweden and UK). This has resulted in one of the largest cohorts of well characterized neuropathic pain patient populations worldwide, including fully characterised individuals to date containing medical history, questionnaires, clinical examination results and QST data from 1000 healthy volunteers and over 2500 patients with neuropathic pain. The quality of the data has been demonstrated by heterogeneity analyses showing low heterogeneity between sites, both for healthy volunteers as well as neuropathic pain patients.

A new standard for the inclusion and exclusion of healthy subjects in studies using Quantitative Sensory Testing (QST) and other pain studies has been published. In addition, algorithms have been developed in order to increase the diagnostic accuracy, in particular the specificity, of QST. Reference data from different body locations, gender and age have been obtained.

Substantial amounts of data mining has been done to test predefined hypotheses. As an example, it was clear that Neuropathic Pain Questionnaires correlate poorly with QST in diagnosing sensory profiles of Gain and Loss of function, respectively.

The Europain blood sample bank has collected blood for whole genome analysis. Anonymous storage will be sustained by partners TUM and RH.

Work package 7: Training center, clinical consensus and external quality assurance.

Key overall Work Package 7 achievements

When QST is used in clinical trials or clinical routine, results vary too much to be compared between sites and between studies. This training work package was conducted to overcome this.

- 1. Two training centers were set up, training all preclinical and clinical investigators in the consortium, as well as all investigators in the Neuropain study (the database of which was merged with the Clinical database of Work Package 6) before study starts. This resulted in low heterogeneity of results between sites.*
- 2. All clinical site laboratories were certified by an independent process, resulting in high technical quality of incoming data.*
- 3. These rigorous standards resulted in very high quality data from all QST examinations, as also recognised by EMA.*

Wider post project impact: The training and certification procedure has already been implemented in a larger scale by the involved Europain partners, though outside of Europain. The training and certification has now achieved a global reach, enabling QST to be used in late phase clinical trials.

Proper training and data quality assurance are key to the quality of all clinical data, not the least in a multi-center setting. The across-center reliability of assay performance, analysis results, data handling, i.e. quality assurance as well as having not only language but also content and context validity of procedural documents, handling instructions, how to instruct patients/ subjects, patient/ subject questionnaires, etc. are key challenges to any multi-center multi-lingual and cultural clinical trials. Low quality assurance is a common factor for failed clinical trials. Ensuring high quality data across sites makes data reliable and endpoints, e.g. QST, other biomarkers and questionnaires, feasible from a regulatory perspective. This has not been demonstrated for QST before. Therefore, this has been a key focus for Europain. By following this training and rigour in quality assurance, Europain has accomplished, for a first time ever, to demonstrate that it is feasible to accomplish this training and certification and that it is feasible to use QST as a reliable biomarker tool in a multi-center international setting. Publications pertaining to WP6, on low heterogeneity across sites, demonstrates the success in this.

Two QST training centres were established in Bochum (BGH) and Mannheim (UHEI). All individual not having completed training and certification and partner laboratories not previously certified (local laboratory standards) in the German Network on Neuropathic Pain (DFNS) protocol and requiring QST training underwent training and certification (from WP 3,4 and 5). Moreover, the previous German QST instructions were translated into English and modified in consensus between all participating centers, based on the already existing DFNS database. Clinical Report Forms (CRFs) for assessment of medical history and clinical data were defined in cooperation with WP 4, 5 and 6, along with SOPs and technical manuals.

The same training and certification was also performed simultaneously for the participants in the Neuropain study, the database which was later merged with the Europain database, thus the quality of the clinical data was of the same high quality in the whole clinical database.

An agreement on internationally valid certification criteria for all QST laboratories was reached. The certification of laboratories was performed using required structures and procedures implemented in the certification criteria of Certkom e.V. ©. As a result and in addition to and simultaneously with the Europain project, training has been completed also outside of Europain and at present over 200 sites have been certified world wide. (This extended training program was not funded as a part of the Europain project but is a fee-for-service training. The extent of this wide training effort demonstrates that the Europain work has been further implemented outside the

consortium increasing the feasibility of larger scale global multicenter clinical trials using the methodology).

Work package 9: To obtain regulatory scientific advice on biomarkers for the use in clinical trials in chronic pain.

Key overall Work Package 9 achievements

The lack of regulatory validated biomarkers of diagnosis, stratification and enrichment of neuropathic pain conditions as well as the fact that categorization of neuropathic pain based on etiology are two important reasons for the lack of successful development of new treatments for neuropathic pains. Europain has developed, refined and validated such biomarkers and has successfully sought EMA Biomarker Qualification advice. Biomarkers brought forward were QST for stratification and enrichment of neuropathic pain populations, electrophysiology (microneurography and Threshold tracking) and Corneal Confocal Microscopy (CCM) as a standardized and more sensitive and specific tool for diagnosing small fiber neuropathy. The outcome of the CHMP response to the Qualification Advice meeting has had an influence on the new regulatory guidelines for the development of new drugs for the treatment of pain.

The WP 9 activities were all part of the extension of the project funded via additional funding awarded by IMI to project, covering the period from M52 to M72. The consortium agreed that the overall results from the project has added substantial further knowledge on new biomarkers and methodologies in neuropathic pain which could be of regulatory relevance. After thorough discussions and presubmission consultations with EMA, the project decided to limit the Qualification Advice submission on four clinical topics: Quantitative Sensory Testing (QST), Electrophysiology: microneurography (μ NeG) and Nerve Excitability (NE), all as functional biomarkers and also Corneal Confocal Microscopy (CCM) as a diagnostic biomarker for peripheral polyneuropathy. Therefore the task on imaging tools as biomarkers of pain was abandoned. Due to the difficulty in understanding timelines for regulatory biomarker advice with FDA, it was decided not to pursue this due to the high risk of not completing before end of project. Thus, the Biomarker Qualification Advice was sought with EMA/ CHMP. A letter of intent was submitted in April 2015 and a final submission package was submitted in May 2015, followed by a qualification advice meeting at EMA in London on September 4, 2015.

Final EMA/ CHMP feedback was received early October 2015. In summary, the EMA feedback states the following:

Overall, the research work into the development of biomarkers for stratification of patients with neuropathic pain was acknowledged.

1. Quantitative Sensory Testing (QST) is adequate for stratification of patients according to sensory phenotype in early clinical trials, while the development of a simpler tool is warranted for PhIII and clinical practice. Further, EMA suggested the use of questionnaires as a simpler tool.
2. Microneurography (μ NeG) can be used as a reliable correlate of spontaneous pain and can be used for stratification in PhII. In the future, it might have the potential as efficacy endpoint in Proof of Clinical Concept (POCC) trials.
3. Nerve excitability (NE) will need further evidence before it can be proposed as a stratification biomarker in POCC and/ or PhII trials.

4. Corneal Confocal Microscopy (CCM) is a promising tool to support diagnosis in small fiber neuropathy. CCM could be used as a diagnostic biomarker in POCC but only in those small fiber neuropathy conditions of etiologies where there is sufficient data, e.g. diabetes. For other etiologies, more data is needed.

To a large extent, the implementation of the EMA feedback advice was already ongoing at the time of receipt of the feedback. For QST, further validation work has been done as part of the D6.1, Data mining (Results reported in WP4). The Questionnaire hypothesis was tested and found not to be of value for stratification (See WP 4). The other suggestions by EMA are under planning. For μ NeG, this tool is already being used in early clinical trials in drug development, where validation data will emerge. In addition, primarily the NE methodology but also μ NeG protocols are currently being further developed as part of a H2020 project. For CCM, the SECOM study (WP4.7) has been completed, providing more robust data on non-diabetic indications compared to the preliminary data available at the time of the EMA Qualification Advice meeting. Further investigations in other indications will be considered as part of future research activities.

The Qualification Advice dossier, EMA feedback and implementations and actions thereof will result in a number of already planned publications, posters and invited oral presentations during the dissemination year.

1.6 Potential impact and main dissemination activities and exploitation of results

Overall, the results of the project have been widely disseminated within the scientific community, both as approximately 200 publications in peer reviewed scientific journals as well as poster presentations and oral presentations at scientific meetings focusing on pain and neuroscience as well as those directed more towards the pharmaceutical development community. In addition, non-project partners have contributed in different collaborations with Europain, both across Europe and in Japan, during the project and as a result of dissemination of results, by co-validating models, contributing with tissues and corresponding clinical data, and by providing clinical results for meta-analyses.

Europain results have contributed to increasing the competitiveness of Europe and have helped to establish Europe as the most attractive place for biopharmaceutical research and development, as exemplified below:

- We have developed, refined and validated across labs, four quantitative outcome measures for spontaneous pain behavior in rodents – burrowing, elevated plus maze, thigmotaxis behavior and social interaction. These models impact a heretofore unaddressed gap in preclinical pain behavioural research, and protocols have been written and are available for others to use. One of these models (burrowing behavior) is already in use in drug development inside as well as outside of Europain partners.
- The meta-analysis of the rat burrowing model experiments showed that prospective randomised and blinded multicentre designs, normally used only for clinical trials, also can be applied in the pre-clinical setting to yield robust data that can both accelerate the validation of outcome measures, pain models and pharmacological interventions as well as help inform the design and conduct of similar multicentre studies.
- We have developed and validated several novel and more relevant rodent models ready for direct use in studying neuropathy due to diabetes, antiretroviral treatment, and chemotherapy.
- Microneurography has been validated as a truly translational tool for pain measurements from rodents to healthy volunteers to patients. This has been acknowledged by EMA. Several Pharma and Biotech companies, both members and non-members of Europain, have already started to implement microneurography for decision making in Proof of Clinical Mechanism and Proof of Clinical Concept. This has been an important validation result for the SME member of Europain that developed the tool, and has allowed the company to extend its business.
- The validation of Next Generation sequencing as a means of transcriptional profiling and its demonstrated wide applicability in multiple models of persistent pain in animal and human tissue has contributed to a more rational and translational use of transcriptomics. The LC-MS/MS and nano-LC methods established to measure lipid mediators in very low concentrations such as from human skin biopsies also has substantially improved the possibilities for lipidomics to become implemented as a routine methodology in preclinical and clinical research.

- A new preclinical model of chemotherapy induced neuropathic pain (CINP) has been validated and is ready to use in drug development for Proof of Mechanism.
- fMRI, using the ASL technique, has demonstrated its usefulness as a readout model for early phase clinical pain trials designed to demonstrate proof of mechanism. The calculation of minimal relevant effect and the following sample sizes needed to show efficacy for Proof of Mechanism and Proof of Concept is an important step forward in the implementation of fMRI in clinical drug development.
- Analysis of Quantitative Sensory Testing (QST) across 10 different laboratories, has demonstrated that this measure shows low heterogeneity both among healthy volunteers, enabling the collection of high quality normative data, as well as in neuropathic pain patients, leading to higher rates of specific recruitment.
- The largest clinical database of well characterised patients with neuropathic pain and healthy controls reported to-date has been created with the Europain/Neuropain database, including 1000 healthy volunteers and >2300 neuropathic pain patients and providing normative data across gender, age, body location. These data show low heterogeneity vs. non-Europain ethnicity, thus demonstrating high generalizability.
- The training activities and certification of QST laboratories to provide operational excellence has spread globally and is now being implemented in pharmaceutical PhII and PhIII development programs and corroborated by non-Europain PhII results presented at scientific congresses. In our opinion, Europain has demonstrated that QST is fully feasible for establishing well-stratified/enriched large scale multi-center global patient populations in PhII/III patient populations.
- We have shown using QST that it is possible to distinguish distinct somatosensory profiles that can be separated into clearly defined clusters that mirror the degree of remaining peripheral innervation across, and irrespective of, underlying nerve injury etiologies. EMA has agreed that QST sensory profiling is feasible for early clinical program stratification. This is a significant step change, and work will continue to qualify QST as an enrichment biomarker along with efforts to create a simpler tool for use in primary health practice. We will seek renewed Biomarker Qualification Advice from EMA when these goals are accomplished and as well from FDA.
- The data obtained on Confocal Corneal Microscopy (CCM) show that it is a very sensitive and specific diagnostic tool for determining small fiber loss. Being shown as sufficient to clearly separate disease from healthy will support not only its application in clinical drug development but also its potential benefit for medical practice, since there is today no non-invasive diagnostic tool to confirm small fiber neuropathy. In agreement with EMA views, more specific subgroups of peripheral polyneuropathy need to be investigated in order for CCM to become a general enrichment tool for peripheral polyneuropathy trials.
- Europain results on catastrophising as a predictor for chronic postoperative pain have been implemented in a large PhIV clinical trial. This trial has demonstrated that perioperative Selective Serotonin Reuptake Inhibitors (SSRI) treatment of catastrophisers reduced the postoperative pain level in elderly patients undergoing knee replacement vs. placebo. Thus, this is another example where Europain results have been exploited for the direct benefit of European patients.

The process of approaching EMA for regulatory Biomarker Qualification Advice was something that

was not originally planned but came out of the overall results of the Europain project; hence, the late start of the process. However, we believe that this extra task has added significantly to the impact of the project by providing a direct translational manifestation of consortium efforts in addition to increased competitiveness of Europe and in so doing help establish Europe as a most attractive place for biopharmaceutical research and development. One major result of the EMA regulatory Biomarker Qualification Advice was that the data put forward was implemented in the new EMA “Guideline on the clinical development of medicinal products intended for the treatment of pain”. As an outcome of improved opportunities to develop better drugs for the treatment of neuropathic pain, such outputs from the Europain project have the potential to contribute to the health of European citizens and to provide socio-economic benefits for European citizens, an ultimate goal of the project from its inception.

1.7 Lessons learned and further opportunities for research

EUROPAIN was one of the first projects under the IMI initiative and brought together expert scientists from leading academic institutions and industry active in the field of pain research. Collaboration in a private public partnership was a new experience for all partners, although academic partners have been collaborating successfully before IMI. From the industry perspective, a precompetitive consortium within pain had been under consideration with rather advanced plans prior to IMI but was ultimately deemed too difficult to set up because of the need for legal, IP and confidentiality agreement support, all of which were found too complex for one single project. The joint framework of the IMI JU provided the environment to enable creation of such a collaborative environment. Of course, there have been in the past numerous smaller collaborations between a single industrial partner and one or a few academic partners, including both those limited to industry funding within an agreed scope as well as those involving substantial industry in-kind work combined with funding of the academic collaborators. While these have enjoyed tangible successes over the years, the unique, multi-partner consortium as defined by the IMI call in our view has far surpassed these more traditional collaboration formats, due to the entirely pre-competitive nature of the work and transparent sharing of ideas and data between industry and academic partners that resulted in greater benefit for all, and in particular, neuropathic pain patients in need.

Considering the lack of successful development of new treatment modalities for neuropathic pain over the preceding 20 years, despite substantial advancement in our understanding of involved molecular mechanisms, including demonstrated genetic links to disease, the launch of IMI was very timely. The fact that the successful launches of the gabapentinoids and Serotonin Norepinephrine Reuptake Inhibitors (SNRIs) for the treatment of neuropathic pain were actually both the result of life cycle management development clearly underscored the need to better understand how to specifically address neuropathic pain. One major hurdle for successful drug development in the area was the lack of a translational understanding, enabling promising new therapeutic agents to cross the so-called “Valley of Death”. There was growing appreciation among basic researchers, clinicians and industry that there was a need for not only better animal models but also, and not least, for a better understanding of disease mechanisms in patients.

When EFPIA partners convened to assemble the call text, it was clear that a broad scope of activities was warranted to address the overall objective of overcoming the bottlenecks in the development of new treatments against chronic pain. The project focused on neuropathic pain for two main reasons – the very low treatment success rate of clinical treatment and the very high burden of illness of patients with neuropathic pain.

The academic applicant consortium consisted of broad and deep expertise in all areas, ranging from molecular assays to characterisation of nerve function in the periphery as well as in the brain, with partners coming from across Europe, and they very well fit with the full call text, which was quite extensive.

The combination of partners from industry and academia working together in a precompetitive PPP has truly enabled some much needed efforts both preclinically and clinically to be realized at a faster pace, much more efficiently and with higher quality and generalizability than would have been possible without Europain. What must be highlighted as keys to the overall success of this

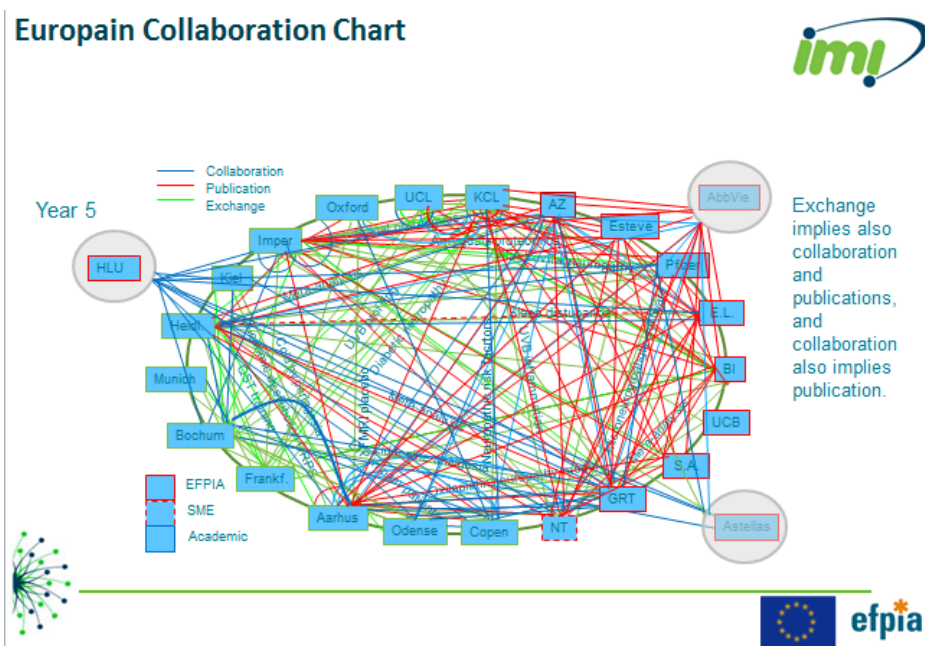
consortium are the high level of cross-partner close collaboration, transparency and collegiality leading to cross-site training and visits, and the continued focus toward keeping the translational thread intact. All of these, and other attributes, were extremely important toward enabling improved disease understanding and for method development of highest value spanning all stages of the drug development process related to animals, biomarkers and clinical science. And above all, this has engendered a mutual trust and increased understanding between academia and industry, a critical success that cannot be overstated, as we recognize the need to work together now and in the future to defeat chronic pain as well as other diseases with high unmet need.

From your experience, please propose any recommendations/ solutions which could be useful for a PPP.

- 1) A clear common goal
- 2) Reiterate successes and visualise them
- 3) Establish realistic PPP project timelines/duration vs. proposed work. There was a strong sense among European members that 5 years was not sufficient time to fully implement and realize all the potential of the work proposed. With any collaboration, there is an inevitable/unavoidable period of start-up inertia, for example, as postdocs are recruited, internal resources are realigned specifically to the project, and clear/transparent lines of communication and, importantly, trust are established. Another 2 years would have enabled proportionally even greater output, as most of the most tangible successes were realized during the latter half of the timeline.
- 4) Be sure that EFPIA partner upper management is fully engaged so that it is possible to inform them about project progress as a natural part of the internal updating process. Keep them informed in order to generate enthusiasm and full buy-in that will help ensure the project receives proper prioritization relative to other activities..
- 5) A clear, strong and dynamic leadership
 - a. An efficient and reliable project management office
 - b. A clear and agreed-upon (upfront) framework regarding legal, patent and financial terms
 - c. A consortium leadership team consisting of the Coordinator, the Managing entity, their deputies and supporting administrative support working well together is key to a smooth working environment in a consortium, not only in the day-to-day problem solving but also to “live the talk” of the commonly agreed-upon project strategy as well as to jointly encourage further and enhanced collaboration between partners and to identify new within-project opportunities.
- 6) A common strategic focus
 - a. As one important example, the common goal of finding a standardised and valid readout for spontaneous pain behaviour (as opposed to the norm of evoked assessments) resulted in the validation of the rat burrowing model, making use of an innate rodent behaviour and standardising the protocol and measurement. This work of validating new models across independent labs, with a common protocol taking two years of work to get the models validated, including pharmacological validation, has resulted in a model that is now ready to use in the drug development process. This important result could not have been achieved outside a PPP and lack of a

common goal and strategy. Such successes resulting from consortium-shared goals and efforts again builds trust between participants.

- 7) Internal collaborations and an interactive environment
 - a. One of the most valuable but intangible outputs of IMI projects is the scientific network and development of greater understanding between EFPIA and academic scientists. The impact of the collaborative aspect can be made more visible by, for example, specific diagrams or alike, similar to what is presented below. It must be emphasized that numbers of joint publications in bibliometric numbers only accounts for a part of these joint efforts.



- b.
 - 1) Within project transparency – document sharing:
 - a. Including a common repository for project related information, Meeting Minutes, Working documents, etc. at a place easily accessible for all partners. In Europain, this was provided as in-kind from the deputy coordinator EFPIA partners. The type of solution available was not optimal due to restrictions and data security issues on the side of the providing partner or the contributing partner. One suggestion could be that the IMI JU provides a repository, for a fee, with the same accessibility as in SOFIA or perhaps a cloud-based solution. This is a critical need as document and data sharing are the life-blood of the consortium.
 - 2) Communication
 - a. Close communication between the consortium leadership team
 - b. Close collaboration between work package leaders and the consortium leadership team
 - c. Close collaboration and frequent group discussions between work package leaders and work package members. This serves many purposes, including maintaining transparency and sharing, keeping work timelines on-track, and helps to maintain and continue building trust and collegiality among the team.

- d. Prioritize internal communication and work rather than external visibility. Though external visibility is of importance, focus on actual research activities needs to be emphasized, particularly in the early days of the projects, in order to meet timelines.

In view of your project achievements, please provide your views on potential new research to further advance the field

Identification of new druggable targets

By combination of implementing newly developed molecular and cellular tools and behavioural models along with an improved understanding of disease mechanisms in different subgroups of neuropathic pain, eventually leading to the possibility of disease modification.

Broader implementation of animal model refinement

More meta-analysis of preclinical data to enhance standards of protocols, design, analysis and reporting. Better alignment would improve how animal models can be predictive of clinical efficacy. The Burrowing protocol and meta-analysis, following the consensus meeting is a good example of this. A similar initiative, led by Dr Malcolm McLeod, University of Edinburgh, UK, aims at a vast meta-analysis of published and unpublished preclinical studies on gabapentin in order to elucidate how well models actually perform work using this standard of care and common active controls. That study, however, is retrospective and does not align protocols, which are often quite variable. Regardless, in the interest of the 3Rs of animal research and aiming at reducing the number of animals needed for research purposes, this is valuable research. Further, not only academia and industry, but also CROs providing animal work should be better aligned when it comes to experimental protocols of models and readouts. While these activities are perhaps better characterized as implementation tasks as opposed to research, it has become clear in recent years that devoting effort to improving standards of experimental conduct and reporting can help address, and perhaps overcome, the currently poor level of experimental reproducibility frequently noted in the preclinical literature

Human experimental models:

Implement the experimental pain models and readouts in pain patients to improve predictability of success by early Proof of Clinical Concept. Work to better align these models, methods and endpoints in a bidirectional way with those used preclinically to improve translational success.

Qualification of clinical biomarkers

Further work on qualification of the biomarkers put forward to EMA in the Qualification Advice is highly warranted. Conducting the necessary validation studies to qualify these biomarkers would likely be a paradigm shift in how we understand chronic pain, and specifically neuropathic pain, and how we can develop new drug treatments that can be personalised to the intended neurofunctional impairment, as described by somatosensory profiles and electrophysiology. Improving diagnostics for neuropathic pain and peripheral polyneuropathy would improve the chances of successful development of new treatments for neuropathic pain patients.

This includes:

- QST qualification
- Development of a simple bedside examination tool kit for late phase clinical trial and medical practice use – with the aim of seeking qualification advice from EMA and FDA.
- CCM further validation and qualification - with the aim of seeking qualification advice from EMA and FDA.
- EPhys further validation and qualification – ongoing in DoloRisk (H2020 program) - with the aim of seeking qualification advice from EMA and FDA.
- Further, given the qualification of the above biomarkers across current etiological diagnoses, this may constitute a step change in the definition and, eventually, treatment of neuropathic pain indications by moving away from the current etiology-based paradigm classifying neuropathic pain by the somatic disease causing the nerve injury toward diagnosis and treatment based on the nature of the nerve injury or dysfunction itself causing the pain.

Improved platforms for readouts in clinical pain trials

By simplified automated reading, personalised devices and simplified examination tools

Prevention of chronic pain by addressing risk factors, related to patient or intervention:

An improved understanding of factors underlying post-surgical neuropathic pain, affecting 5-10% of patients undergoing surgery, is of great clinical importance. Large cohorts of well characterized patients have demonstrated risks that can be mitigated at the stage when surgical treatment is already being planned. Factors like more intense postoperative pain treatment in high-risk patients, should be studied more intensely, and such studies have, as a consequence of Europain results, in fact already begun, as exemplified by SSRI/SNRI perioperative treatment in patients with a high risk of intense postoperative pain. While positive results have been observed as a first step, these need to be confirmed also with long term follow-up data. The importance of optimized surgical technique has been clearly demonstrated as a topic that should be pursued further as an important outcome variable in addition to chronic sequelae such as neuropathic pain as part of recovery. This can be implemented into clinical practise for the benefit of patients and health care immediately.