Trends in Pharmacological Sciences

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Opinion

Enhancing Choice and Outcomes for Therapeutic Trials in Chronic Pain: N-of-1 + Imaging (+ i)

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The attrition of novel analgesic drugs in the clinic can be attributed in the main to two factors: failure of preclinical research findings translating into human pain conditions, and a drop-off of efficacy between proof-of-concept (i.e., Phase II trials) and pivotal, confirmatory (Phase III trials) testing. In order to enhance the efficiency of the clinical drug evaluation process and determine rapidly whether a potential therapeutic candidate gives pain relief, by modulating central pain neurobiology, we propose a 'pre-proof-of-concept' approach, in which an efficacy assessment is performed in a single individual (N-of-1) using subjective clinical pain assessments supported by objective validated functional neuroimaging measures. Using an N-of-1 + i methodology, clinical- and neuroimaging-based metrics can be quantified under conditions of drug versus placebo or drug versus current standard of care conditions.

N-of-1 Trials

Current clinical trial programs in analgesic drug development are inefficient given the uncertainty of finding clinical efficacy based on preclinical data. Expensive, large clinical studies with molecules of uncertain efficacy in poorly defined pain indications often fail in late-stage development, draining resources and reducing the funding available for innovation. The challenge for the analgesic drug development field is to find more efficient ways of identifying effective new candidates matched to patients and clinical indications in early proof-of-concept clinical testing paradigms. Traditional analgesic drug trials are often confounded by patient heterogeneity and the use of subjective response rating scales that can be influenced by many factors other than drug efficacy. Subjective responses to analgesics can be influenced by patients prior medication experience [1,2], high placebo responses [3], and incomplete or insufficient efficacy [4,5], all of which can be further confounded by side effects [6].

Here, we have assessed the potential for an N-of-1 + imaging (N-of-1 + i) paradigm consisting of clinical assessments in conjunction with pain neuroimaging to accelerate the identification of analgesic drug candidates with a high probability of clinical success. We hypothesize that by using the N-of-1 + i paradigm to evaluate the efficacy of candidate drugs in a small cohort of individuals, we can improve the speed and efficiency of analgesic drug development by providing clear proof-of-principle prior to testing the drug in more expensive [7] and formal randomized clinical trials (RCTs). Avoiding the early use of costly traditional clinical trials to identify positive drug candidates, in favor of more nimble designs, will allow the evaluation of a greater number of potential therapeutics in real world trials within a resource constrained environment. Table 1 provides an overview of the pros and cons of N-of-1 + i clinical trials and Table 2 shows some examples reported on https://clinicaltrials.gov.

Highlights

N-of-1 trials have been useful in a number of clinical conditions, most notably, cancer.

For analgesics, it may be an optimal manner to enhance drug trials to evaluate new clinical entities.

Biomarker development is critical to improved evaluation of therapeutic candidates.

Advances in neuroimaging (e.g., pharmaco-magnetic resonance imaging; brain measures of structure and function) as a potential biomarker allow for objective measures of drug effects and brain responses to be added to N-of-1 trials (N-of-1 + i).

Combining these two approaches will enhance specificity (because of repeated measures, limiting placebo response, etc.) and selectivity of choice of treatments, as well as enable multiple potential indications to be evaluated in early Phase II trials.

A better understanding of the utility of N-of-1 + i may facilitate a transformation in drug trial innovation (innovative trial design) and personalized medicine.

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Table 1. Pros and Cons of N-of-1 Trials

	Drug/intervention	Outcome	Refs
Pros			
Multiple randomized 'tests' in RCT approach allowing for trial across more than one pain profile/indication	Example 1: drug–placebo–drug–…. Example 2: drug–active comparator–drug….	Blinded. Testing of drug efficacy over more than one occasion Determination of treatment efficacy in individuals	[16] [14] [9] [27]
Early readout versus real comparator/placebo	Drug dosing	Relatively short time span depending on number of comparisons and drug PK/PD attributes and time to targeted steady state	[16] [14] [9] [27]
Potential estimate population response	Amitriptyline	Treatment effectiveness with and between patients	[76]
Within-person comparisons can substantially reduce noise variability and increase power	Without imaging	Repeated testing; subjective evaluation	[16] [14] [9] [27]
May predict population levels depending on power and effect size	Use with current best in class	Rigorous evidence-based medicine	[77] [78]
Deep clinical phenotyping in test patients	Clinical Genetic Psychological	Enhanced evaluation of effect	[79]
High probability of successful Phase II trial	Same drug Multiple data points on drug efficacy	Repeated studies Efficacy across a small cohort of well-phenotyped individuals	None
Cons			
Population-level effects	Evaluation of side effects	Difficult to establish population-level effects (e.g., percentage of responders) without large sample sizes	None
Rare adverse events	Evaluation of side effects	Little information on rare events (e.g., rare adverse effects) without large sample size	None
Carry-over effects	Drug–drug carry-over is difficult to evaluate	Need to assess carry-over effects across repeated drug and placebo administration periods	None

N-of-1 Trials and Their Relationship to Standard Clinical Trials

Standard RCTs test group average effects. RCTs report percentages of patients showing improvements in treatment and control groups, along with the statistical significance of any differences. These statistics, however, indicate simply that the difference between control and treatment is not zero on average, not whether any individual patient or percentage of patients actually improved because of drug treatment. For example, consider a difference of 10% between control and treated patients that is statistically significant at $P < 0.005$. This outcome does not mean that the drug caused improvement in 10% of the patients, just that 10% is not 0%. Indeed, the therapeutic effect could be driven by a real improvement in 5% of the patients and no improvement in the others, or by a small real improvement in all the patients, with only the upper tail of the response distribution reaching the threshold for clinical improvement. The results of multipatient, double-blind, randomized controlled trials are difficult to extrapolate to individual patients [8].

N-of-1 + i trials offer a systematic, experimental approach mimicking pharmacotherapeutic clinical practice. We recognize that N-of-1 trials cannot replace standard RCTs but see

Table 2. Examples N-of-1 Trials Reported on https://clinicaltrials.gov

	Description	NCT
Behavioral	Light therapy	NCT03167372
	Diabetes, cardiovascular disease, hyperlipidemia; drug use adherence	NCT00299169
	Paroxysmal atrial fibrillation	NCT03323099
	Fatigue in chemotherapy	NCT01983592
	Cancer-related fatigue (methylphenidate)	NCT01164956
Drug (disease/effect)	Losartan, amikodipine, hydrochlorothiazide (blood pressure)	NCT02744456
	Decoction, placebo (Chinese medicine)	NCT03147443
	Statin, placebo (statin myopathy)	NCT01259791
	Melatonin, placebo (insomnia in attention-deficit/hyperactivity disorder)	NCT02333149
	Mexiletine, placebo (nondystrophic myotonia)	NCT02045667
	Famotidine, placebo (levodopa-induced dyskinesia in pharmacodynamics)	NCT01937078
Pain (drug/indication)	Musculoskeletal pain (PREEMPT; smartphone intervention)	NCT02116621
	Osteoarthritis (diclofenac, paracetamol)	NCT00371696
	Fibromyalgia (amitriptyline and amitriptyline + fluoxetine)	NCT00000428
	Knee osteoarthritis (ibuprofen gel, topical capsaicin)	NCT03146689
	Chronic pain (palmitoylethanolamide)	NCT02699281
	Neuropathic pain (soy protein)	NCT01050244
	Ablation of epidural nerve fibers (capsaicin)	NCT01883102

Abbreviation: NCT, National Clinical Trial Identifier.

complementary value by providing insight into comparative treatment effectiveness across a wide variety of patients [9], particularly when the disease is variable (driving large RCT trial designs) or when only small numbers of patients are available (as with rare disorders). Importantly, N-of-1 trials help us answer the question 'Does this treatment work in some fraction of individual patients?', whereas standard RCTs address the question 'Would I expect the treatment to work on average for a new individual sampled from a population of similar patients?' The use of N-of-1 trials for initial evaluation of analgesic efficacy has several unique benefits, such as: (i) establishing the best treatment for an individual phenotype [10]; (ii) insight from combining individual patient data with aggregate data from comparable patients [10]; (iii) assessment of the reliability and durability of drug effects over time with repeated administration; (iv) understanding of personalized responses (efficacy and side effects), potentially focusing future clinical development paths and reducing the costs of trials [11]; and (v) integrating patient care in clinical settings and, in turn, helping to identify optimal treatment regimens for patients in whom disease management is uncertain [11].

The design and implementation of N-of-1 trials has been reviewed elsewhere, including ethical, financial statistical, technology infrastructure, and user engagement [9,12–15]. In contrast to RCTs, N-of-1 trials are multiple within-person crossover trials using a small group of subjects, in which the dosing of drug and placebo are tested repetitively, often in a randomized sequence [14,16]. Repeated testing enables true assessment of whether an individual reliably showed improvement (or side-effects) when taking a study drug. Important factors to understand for

N-of-1 trial designs are: (i) the pharmacodynamics of the test drug, including duration of the parent and any metabolites that retain activity; (ii) any carry-over effects of the drug on subsequent processes, including responses to the next control or drug administration; and (iii) safety implications of drug withdrawal dependent on trial design. Whilst pharmacokinetic/pharmacodynamic (PK/PD) and potential carry-over effects can be easily managed with appropriate washout periods, the issue of drug provision for breakthrough pain requires serious consideration of the ethics involved, particularly in cases where no treatment (placebo) option is provided [17,18]. In summary, the intention of the N-of-1 trial is to reveal individual therapeutic profiles rather than group drug effects. We suggest that this trial design has potential to improve our probability of success in matching the right analgesic drugs with the right patients in a more personalized approach to medicine.

Issues pertaining to N-of-1 + i trials (i.e., strengths, ethical issues, financial considerations, statistical approaches, technological infrastructure, and user engagement) are actively being evaluated. While the approach may confer a step forward towards optimizing treatment strategies for individual patients or comparing treatment strategies, investigations centered around the N-of-1 + i trial need to be implemented to evaluate their overall benefits. Some of these likely benefits of the approach consist of a more focused outcome and better patient selection, amongst other (Table 3). Statistical evaluation of N-of-1 data, even without imaging, allows for flexible data analysis [19] as well as repeated measures of placebo or active treatment. However, endpoints evaluated by N-of-1 studies cannot be inferred at a population level but aggregated analyses of N-of-1 studies can be done [20].

Table 3. Pros and Cons of Adding Functional Imaging to N-of-1 Trials versus Standard N-of-1 Trials

	Description	Refs
Pros		
Objective markers	Biological measures may be: (i) less susceptible to placebo effects and decision-related biases, and (ii) more easily standardized across contexts, clinical centers, and time	[80]
Multiple disease states Can be tested and cross-evaluated if same protocols established	There are two approaches given the relatively small number in each trial: (i) multiple indications (e.g., pain or depression); (ii) multiple disease states (e.g., neuropathic pain, osteoarthritis)	[27]
Measures to have greater confidence going into Phase II or III trials	Optimizing design strategies: (i) better selection of patients for definitive studies, (ii) defining correlated end points, (iii) information that can be used to inform RTC clinical trials	[9]
Multiple comparators	Correlates of subjective, physiological, and brain measures	
Multisite	Many magnets are the same Fast data collection/collation	[81]
Increasing predictive measures of outcome/drug effects	Smaller sample size to establish efficacy Measures of placebo and treatment effects	[82] [83]
Cons		
Added costs per patient	Imaging adds to the N-of-1 trial since, for each patient, imaging would be repeated at least four times	[84]
Specialized analysis and data collection	Automated process are improving	[85] [86]
Validated process	Evolving standards for evidence and quality control Multiple measures (longitudinal)	[87] [88] [89]

For the N-of-1 + i, an imaging infrastructure is required: scanners, technical analysis systems/people/imaging experts, and a process for reasonable throughput if done at one center; multiple centers obviously can manage larger patient volume and cross-center validation is now well managed with good quality assurance protocols [21–23]. The issue of the value-added proposition of including imaging allows for these measures to be obtained together with clinical endpoints with likely greater statistical sensitivity, since variability within subjects is evaluated with repeated measures; the more measurements, the better the study. Smaller sample numbers can also afford improved phenotypic evaluation of those recruited to these studies, with an obvious requirement of being able to complete functional magnetic resonance imaging (fMRI) studies (i.e., not claustrophobic, do not have not-easily removed metallic objects, do not suffer undue discomfort, etc.).

The approach (N-of-1) is new in the chronic pain field and still requires pressure testing and validation [see for point [24,25] and counterpoint [26] regarding one chronic pain trial (albeit not pharmacological)]. It is noted that brain imaging methods were not integrated into the N-of-1 investigation. Future N-of-1 trials will afford insight into many of the issues raised above. As Duan and colleagues note: 'Single patient trials may be important in comparative effectiveness research…. Can enhance therapeutic precision, improve patient outcomes and reduce costs' [27].

Past Experience with N-of-1 Trials in Pain Conditions

Fibromyalgia

N-of-1 trials have been reported in fibromyalgia [28,29]. In one trial, 58 patients were enrolled in randomized, double-blind, multi-crossover trials comparing amitriptyline and the combination of amitriptyline and fluoxetine [29]. Outcome measures included quality of life, Fibromyalgia Impact Questionnaire, global well-being scales, and tender point examination. The pooled data indicated that combination treatment was significantly better than monotherapy. Several features distinguish this trial from a standard Phase II trial in advantageous ways. First, relatively few patients were recruited, reducing costs; in this case, even with the small sample size, the results were consistent with larger traditional trials [30]. Secondly, the trials could be conducted in a practice-based clinical environment, dramatically reducing overhead costs and increasing the feasibility of recruitment. Third, the trials used crossover, repeated-measures designs (monotherapy versus combination therapy each administered three times), enabling repeated assessments of PK/PD properties. Jaeschke and colleagues reported on 23 N-of-1 trials evaluating the effectiveness of amitriptyline in fibromyalgia patients [28]. By implementing the N-of-1 approach, use of amitriptyline was discontinued in 30% of cases due to lack of effect, saving patients from further exposure to an ineffective drug. With the implementation of neuroimaging, there can be confirmation that the pharmacological approach yielded no or minimal neurobiological effect(s) indicative of analgesia [31,32].

Neuropathic Pain

Gabapentin

An N-of-1 study evaluated the efficacy of gabapentin along with placebo for neuropathic pain [33]. The approach was to compare patients already on gabapentin in a randomized, crossover design; each trial lasted 12 weeks and consisted of three cycles of gabapentin and placebo treatment pairs. The aggregate response to gabapentin was better than placebo in 16 patients (29%), 15 of whom continued gabapentin post-trial [33]. All responders continued on the drug. No difference was shown in 38 individuals (69%) and 1 (2%) showed a better response to placebo. The response rate and mean reduction in symptoms with gabapentin were small. This is an important issue in treatment, since many patients may remain on medications without a measurable

outcome. Furthermore, this study exemplifies the ability of the N-of-1 approach to essentially identify responders, weak responders, and nonresponders.

Ketamine

We are aware of one N-of-1 study of ketamine for neuropathic pain. The trial involved increasing doses of oral ketamine until analgesia, adverse effects, or a maximum dose of 100 mg was reached [34]. Responders were then randomized into an N-of-1 trial of three treatment or placebo pairs, each a week in duration. Of 21 patients, 9 completed the trial. No difference between ketamine and placebo was observed. Ketamine is a drug that has shown some interesting effects in nonrandomized controlled trials for conditions such as complex regional pain syndrome (CRPS) [35] and in randomized studies [36].

Deep Brain Stimulation

Seven patients had deep brain stimulation (DBS) for neuropathic pain in the thalamus; pain assessed prior to and at 6 months after stimulating electrodes were placed into the periaqueductal gray or ventral posterolateral thalamus [37]. Patients were entered into an N-of-1 trial with the DBS switched on or off, with patients being blinded to the condition; three of seven patients accurately defined that the DBS was active. While not a pharmacological assay, this study raises the issues of how best to evaluate measures in expensive, procedural-based therapies.

Orofacial Pain

Sympathetic Blockade

Orofacial pain is a condition affecting the face, mouth and jaw) and includes a number of causes and is a difficult to treat. Nixdorf *et al*. [38] utilized an N-of-1 approach to treat a patient with presumed CRPS based on clinical signs and symptoms, implementing a double-blind crossover design with three periods of injections of either placebo or lidocaine without epinephrine. The trial failed to show a drug versus placebo difference but provided an evidence-based process for the patient to receive no further treatment.

Migraine

Dextroamphetamine

Migraine and tension-type headaches are common clinical problems. An N-of-1 trial [39] evaluated two subpopulations within each of the migraine and tension-type headache samples: patients already on dextroamphetamine and medication-naïve patients. Both groups received dextroamphetamine or caffeine (a control intended to match for effects on arousal) during four alternating 20-day periods. Both the tension-type and migraine groups had lower average daily headache grades in the amphetamine arm compared with the caffeine treatment. Significant differences were observed for the migraine but not the tension headache groups. Segregation of effects for the drugs and number of responders were defined. The conclusions indicated that the drug had preventive effects in a subgroup of each headache subtype.

Arthritis

Of all the pain groups, the use of N-of-1 trials has been most frequently studied in patients with osteoarthritis (OA) [40–43]. In an OA study [43] celecoxib was compared with sustained release paracetamol in 2-week treatment periods, randomized in 59 patients, and it was concluded that this approach may be an effective way to choose drugs for individuals. As noted above, in many cases this approach, although not formalized in this way, is what clinicians do. Additionally, there has been an N-of-1 trial in juvenile idiopathic arthritis (JIA) [44]. In the JIA study, the effects of

amitriptyline in an N-of-1 trial in six children were evaluated and the authors concluded that 'Bayesian techniques were used successfully to obtain estimates of population effect, despite the small number of participants'.

The Future: Systematic Use of N-of-1 Clinical Trials in Chronic Pain

Chronic pain is an ideal condition in which to use N-of-1 + i trials given the availability of interventions across multiple chronic pain conditions. The N-of-1 + i trial design in chronic pain accommodates differences between individuals with respect to phenotype (e.g., gender, duration of disease, peripheral and central pathophysiology, psychological factors, or disease evolution), as well as specific condition (e.g., OA and chronic back pain). For some pain indications there is the added benefit that patients are otherwise medically stable and are not on multiple therapeutics and, in turn, there is a limit to confounds emanating from drug–drug interactions. Though pharmacological and nonpharmacological treatments have long been available for pain treatment, what makes certain patients prone or resistant to therapy, or relatedly, prone or resistant to disease progression, is unknown. With N-of-1 + i trials, it may be possible to select a group of patients with a similar etiology and pathophysiology, which could reduce between-person heterogeneity. An example for the use of the N-of-1 trial design in chronic pain can be found on https://clinicaltrials.gov [N-of-1 trials using mHealth in chronic pain (PREEMPT); NCT0211662145] in the PREEMPT mental health in chronic pain study [45].

Adding an Objective Assay to N-of-1 Trials: Is There a Role for Functional Imaging?

Traditional analyses of clinical trials involve estimating a parameter known as the number needed to treat (NNT). This statistic represents the number of patients who need to be treated for one of them to benefit compared with a control in a clinical trial. The NNT is an index of the impact of a medicine or therapy. The NNT helps us to understand the benefit risk ratio of an intervention. In small trials such as the N-of-1 clinical studies, if the NNT is substantially less than the number of participants in the trial then the chances are high that the drug is having the desired effect and may show benefits across larger patient populations. The advent of NMR techniques for measures of brain function or disease state have allowed for new insights in understanding and evaluating chronic pain and analgesic responses [46]. Most studies have utilized groups of patients to measure these changes. However, the ability to define changes in the individual are now promising and the integration of neuroimaging into the N-of-1 paradigm may permit the objective measurement of changes in brain pathophysiology alongside subjective reporting during treatment for chronic pain. Inclusion of functional imaging methods could increase the sensitivity of detecting a pharmacodynamic effect and potentially increase the precision with which we could define the NNT. While most useful in novel drug evaluation, imaging may also be used in the future to fingerprint novel drug effects for comparison with more extensive datasets that are currently being collected in large-scale imaging studies [21].

In Box 1 we have summarized functional imaging readouts that could be included in N-of-1 + i clinical trials of treatments for chronic pain. Table 3 provides an overview of the pros and cons of adding functional imaging to N-of-1 clinical trials for the treatment of pain.

fMRI Studies of Single Subjects in Pain and Analgesia

Multimodal functional imaging is often used to assess the responses of the brain to interventions, but the reported outcomes are often grouped across individuals. There are, however, a number of examples where functional imaging has been used to evaluate single patients in a number of medical conditions [9,47], including pain (Table 1). We discuss these in terms of drug evaluation, clinical state assessment, and the nature of information garnered from each study.

Box 1. Potential Contributions of Functional, Structural, and Anatomical Imaging to N-of-1 Trials

General

Adding imaging may provide a measure of: (i) alterations in brain systems that may precede clinical changes; (ii) activity in regions that may predict side effects; (iii) a change compared with a standard in field; (iv) metrics that may be compared with larger populations; and (v) increase the power of a study, thus limiting exposure of a drug to volunteers. Depending on the number of individuals recruited to the study, it may be possible to delineate insights into responsivity (responders versus nonresponders). (See Pros and Cons of Adding Neuroimaging to N-of-1 trials Table 3.)

Specific

Resting State Networks (RSNs)

RSNs have the advantage of being: (i) quickly acquired, (ii) stable over time, and (iii) differentiating between drug versus nondrug or comparative drug. If an N-of-1 trial is conducted with a repeat of the test medication, a within-trial comparison may be evaluated for further objective evaluation of the drug in question.

Morphological Changes

Two morphological changes may be evaluated: (i) gray matter volume, and (ii) white matter integrity. Drug-induced changes may, for example, correlate with reversal of gray matter changes induced by pain. White matter changes may indicate a number of alterations, including potential neuroinflammation.

Neurochemistry

With the use of magnetic resonance spectroscopy, a number of measures may be acquired, including: (i) neuronal integrity; and (ii) chemical changes, particularly with inhibitory (e.g., GABA) or excitatory (glutamate) neurotransmitters that may predict responsivity.

Drug Evaluation

The value of imaging drug effects is that there should be a correlation with clinical outcomes (in this case pain), such that the underlying brain response confers an objective measure of the pharmacological intervention. Ketamine has been used in the treatment of CRPS, through mechanisms that may relate to 'resynchronizing' networks, as we have reported in pediatric CRPS [48]. For example, a patient with CRPS underwent imaging prior to and following intravenous ketamine treatment to evaluate resting state changes while not performing any tasks or responding to any stimuli during the fMRI resting state networks (RSN) acquisition [49]. The default RSN following treatment was reversed by the treatment, showing a pattern that paralleled the default mode network (DMN) in healthy volunteers. While this was not a comparative drug study, the single patient study can be designed to capture specific changes of nonsubjective markers.

In the second example using fMRI in a single subject to evaluate drugs, brain activity in psoriatic arthritis was evaluated using a cyclooxygenase-2 (COX2) inhibitor [50]. These investigators reported that the clinical condition was 'delineated' by brain activity and modulated by a COX-2 inhibitor. Interestingly, the authors compared the outcome in patients to similar responses to thermal stimuli in healthy subjects as control, on the assumption that nociceptive responses were also predominantly activated by the stimuli in psoriasis patients

Clinical State

One of the best single subject reports utilizing fMRI (arterial spin labeling) as a functional outcome measure is in erythromelalgia patients. Erythromelalgia is a genetically driven episodic or continuous painful condition affecting the extremities, with clinical manifestations of burning pain, redness, and increased skin temperature [51]. Here, the fMRI showed the central nervous system (CNS) correlates of 'pain, pain affect and reward-related areas' [52] to pain relief (cooling) in a

patient whose genotype was consistent with *SCN9A (Nav1.7)* mutation. Such approaches offer the opportunity to evaluate specific conditions in rare diseases (see [53]).

In two studies relating to the eye, single-subject imaging produced novel information. In the first on corneal pain [54], activation of the trigeminal nociceptive pathway to bright light was reported; this is consistent with our knowledge of the pathway [55]. In the second on photophobia induced by a corneal abrasion, the authors used a design to capture change with pain (and blinking) and without pain (and blinking) and show for the first time the location of the cornea in the somatosensory cortex [56].

Design of N-of-1 + i Trials

Functional imaging can be included in N-of-1 trial designs using a set drug–control sequence design or using random allocation of interventions (Figure 1). It is important to integrate repeated subjective (sensory, emotional, physiological) reporting in a matrix with objective functional imaging measures. The richness and return from adding imaging to the N-of-1 designs can be further enhanced by conducting several different functional imaging sequences/acquisitions in each patient to maximize the opportunity to study both drug and condition (Figure 1B). Finally, since there is crosstalk between pharmacology and chronic pain conditions, it is important to consider running several small N-of-1 trials across several indications using multiple sequences for each condition (Figure 2). Composite orthogonal readouts from multiple imaging protocols [e.g., RSN, grey matter, and neurochemical spectroscopy (magnetic resonance spectroscopy)] that can be conducted rapidly in a patient-friendly manner (<1 h imaging time) may provide the best discrimination between active treatment and control conditions.

Concept of Change in Point Analysis (CPA) or Detection

Often, in N-of-1 trials, it may be desirable to monitor a patient over a period of time for a change in symptoms. Researchers may hypothesize that a drug will have an effect but be unsure about when the drug effect will begin to affect outcomes for a given individual patient. Incorrect assumptions about when an effect should occur can result in missed treatment effects. CPA provides a solution (e.g., [57]). It involves measuring symptoms for a baseline time period (e.g., several weeks) and developing a statistical model of natural symptom fluctuation. After this, change-point statistics are calculated on a moving average over future observations and compared with the baseline model. When a statistically significant deviation from baseline symptoms occurs, the researcher can use statistical methods to track back and estimate when the change began. One natural use is in an A-B or A-B-A design, when baseline observation (A) is followed by a treatment (B), and symptom fluctuation at baseline is used to estimate when changes after the start of treatment occur. It can also be used with multiple crossovers. Models for group data have been developed that can statistically model a deviation from baseline symptoms in a group of individuals and that model distributions across change points in a collection of individual people [58]. This can increase the accuracy of inferences about the onset of changes in individuals by providing an informed prior distribution for Bayesian analysis.

CPA can also be applied to fMRI measures [59–61]. In fMRI, activity levels are stable only over short time intervals (i.e., up to a few minutes). Thus, while activity levels have been found to be reproducibly affected by acute drug administration in pharmacological fMRI studies, longer-term monitoring of treatment response and estimation of change points across repeated scans (e.g., weeks apart) has been challenging. However, fMRI functional connectivity can be relatively stable across long periods of time [62]. Connectivity within and between large-scale functional networks, or functional brain network analysis (FBNA) and functional frequency analysis (FFA),

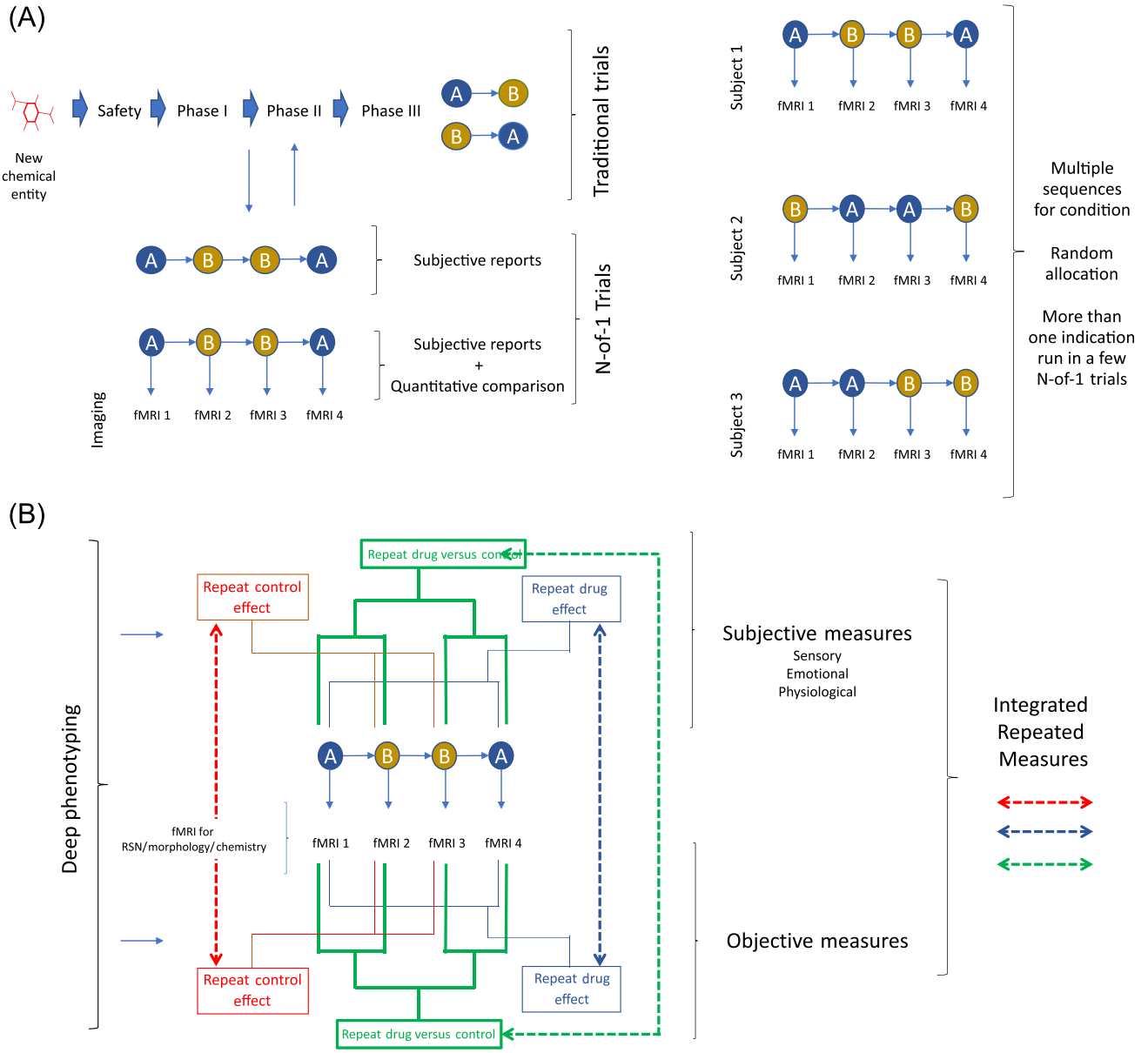

Figure 1. Integrating N-of-1 Trials with Imaging. (A) Top: standard N-of-1 approach and N-of-1 + imaging (N-of-1 + i) approach: the figure shows the adaptive use of functional magnetic resonance imaging (fMRI) to the N-of-1 imaging approach for evaluating drugs at an early stage to enhance choice of disease state or drug choice compared with traditional approaches. Bottom: N-of-1 randomization: details of a typical approach for implementing N-of-1 + i trial across subjects. The strategy facilitates multiple evaluations of a drug (multiple sequences of drug or placebo), randomization to patients, and the possibility of using small numbers to evaluate a drug for more than one indication. (B) Integrated approach implementing N-of-1 + i: the integrated approach allows for drug, placebo, or comparative treatment to be evaluated utilizing drug–nondrug (placebo or comparative drug or both). The use of deep phenotyping in smaller trials can be applied to enhance choice of the appropriate disease state to therapeutic target. Subjective or objective measures can be deployed in repeated trials to enhance definition of specificity and sensitivity. In this way, integrated repeated measures of subjective and objective changes for a drug candidate (or other treatment) can be evaluated. Abbreviation: RSN, resting state network.

can thus serve as useful measures for evaluating normal connectivity patterns and how those patterns are altered by drugs. In the FBNA approach, connectivity changes may define the underlying disease state [63–65], including pain [66,67], and how the disease state may be modulated by

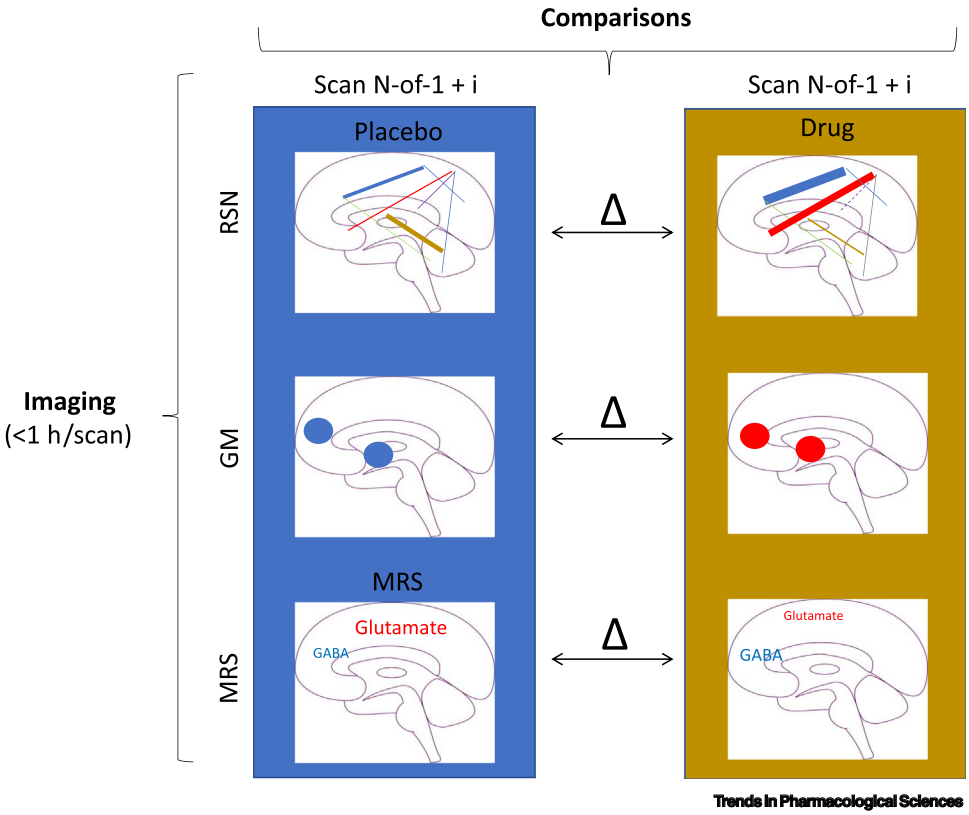

Figure 2. Utility of Imaging. Multiple imaging data sets may be acquired in individual patients, including resting state network (RSN), gray matter changes (GM) in cortical and subcortical regions, and chemical changes [magnetic resonance spectroscopy (MRS)]. Other techniques (e.g., functional brain network analysis, functional frequency analysis; see text) may also have great utility in evaluating comparative changes for treatments in specific disease conditions using N-of-1 + imaging (N-of-1 + i).

drugs [68]. For FFA, recent reports have described the use of evaluating blood oxygen level dependent spectral power characteristics [69,70] in order to differentiate conditions. CPA can also be applied to connectivity patterns, or graphs, within functional networks [58].

Discordance between Subjective versus Objective Measures

The issue of discordance between subjective and objective measures has been a problem in clinical studies, posing downstream challenges towards determining meaningful biological change [71] or, specifically, clinically relevant therapeutic effects [72,73]. Yet, a chosen central imaging-based endpoint (e.g., fMRI response in CNS network X) would have some known level of validation and association with subjective measures of pain; a highly reasonable goal considering what has been shown to date across a large body of pain neuroimaging literature. Considering that the N-of-1 + i (Figure 1) approach facilitates multiple assessments of the drug, drug dose, placebo, or positive control, an effect observed on both subjective and objective measures would give confidence in meaningful therapeutic effects taking place at the individual patient level. Moreover, in the proposed intrapatient paradigm, some therapies, particularly small molecules, may have pharmacokinetic properties that are more amendable to a 'rapid' on (active treatment) and off (placebo) switching and, therefore, yielding a higher number of opportunities towards observing a hypothesized effect on both subjective and objective measures at the

individual patient level. The approach furthermore begins to address the issues of: (i) individual differences in response and potential optimization of drug exposure in individuals balancing safety and clinical efficacy [74]; (ii) potential identification of alternate indications, again because small numbers may be evaluated for a disease state [75]; and (iii) confirmation that the observed clinical and neurobiological effects are reproducible in a single patient (Figure 1B).

How discrepancies are handled also depends on the goals of a study and the cost/benefit ratio for making errors of omission (missed true treatment response) versus errors of commission (false positive identification of treatment response). This tradeoff, and the relative importance of subjective and objective measures, is different for clinical practice and clinical trials. In clinical practice, subjective improvement is considered primary, and errors of omission are more important. One would not want to discontinue an effective treatment that is producing subjective clinical benefits. In clinical trials, particularly in drug development, objective measures of pathophysiological changes consistent with proposed drug mechanisms are important. In addition, errors of omission are more important, as a decision to go forward with drug development entails increasingly large and costly studies. Thus, clinical trials may: (i) elect to proceed forward only if subjective and objective measures agree, or (ii) identify a sample of individuals who respond both on subjective and objective measures in N-of-1 trials and identify the common characteristics underlying successful treatment response.

Concluding Remarks and Future Perspectives

'Although obvious and already pursued to some degree, well-designed and controlled N-of-1 trials can be used in early-phase trials evaluating the tolerability, dosing and potential utility of an experimental compound' [9]. An N-of-1 approach enhanced by the use of neuroimaging (N-of-1 + i) could provide a robust system to evaluate analgesic drugs in small numbers of patients. The addition of functional neuroimaging techniques and analysis approaches to subjective endpoints in N-of-1 analgesic treatment trials provide an objective measure encompassing within-subject controls in trials across treatment conditions (e.g., active drug versus placebo). We therefore propose that the use of a robust N-of-1 + i trial design could reduce the cost and accelerate decision making on whether to progress potential novel analgesic candidates to larger scale clinical trials. This approach may offer early insights as to which patient subgroups most likely will respond to treatment. A new paradigm for analgesic drug discovery and development is badly needed, perhaps this one deserves attention (see Outstanding Questions).

Outstanding Questions

In the real world, would the N-of-1 + i contribute to better evaluation of analgesic candidates and allow for more rational drug development decisions?

For initial testing of new clinical entity by smaller companies, would this approach allow for more useful data to be evaluated?

Will the added expense of imaging provide benefits such as predicting side effects, observing early changes that may predict the outcome (symptoms or disease modification), when compared with standard approaches in Phase II trials?

Can these approaches be leveraged to large trials of N-of-1?

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