

Review

Cite this article: Schork NJ, Beaulieu-Jones B, Liang WS, Smalley S and Goetz LH (2023). Exploring human biology with N-of-1 clinical trials. *Cambridge Prisms: Precision Medicine*, 1, e12, 1–9
<https://doi.org/10.1017/pcm.2022.15>

Received: 26 September 2022

Revised: 12 December 2022

Accepted: 26 December 2022

Keywords:


clinical trials; interventions; intervention response; longitudinal; precision medicine

Author for correspondence:

N. J. Schork,

Email: nschork@tgen.org

Exploring human biology with N-of-1 clinical trials

N. J. Schork^{1,2} , B. Beaulieu-Jones^{2,3}, W. S. Liang², S. Smalley^{2,4} and L. H. Goetz^{1,2} 

¹Department of Quantitative Medicine, The Translational Genomics Research Institute (TGen), Phoenix, AZ, USA; ²Net. bio Inc., Los Angeles, CA, USA; ³University of Chicago, Chicago, IL, USA and ⁴The University of California Los Angeles, Los Angeles, CA, USA

Abstract

Studies on humans that exploit contemporary data-intensive, high-throughput ‘omic’ assay technologies, such as genomics, transcriptomics, proteomics and metabolomics, have unequivocally revealed that humans differ greatly at the molecular level. These differences, which are compounded by each individual’s distinct behavioral and environmental exposures, impact individual responses to health interventions such as diet and drugs. Questions about the best way to tailor health interventions to individuals based on their nuanced genomic, physiologic, behavioral, etc. profiles have motivated the current emphasis on ‘precision’ medicine. This review’s purpose is to describe how the design and execution of N-of-1 (or personalized) multivariate clinical trials can advance the field. Such trials focus on individual responses to health interventions from a whole-person perspective, leverage emerging health monitoring technologies, and can be used to address the most relevant questions in the precision medicine era. This includes how to validate biomarkers that may indicate appropriate activity of an intervention as well as how to identify likely beneficial interventions for an individual. We also argue that multivariate N-of-1 and aggregated N-of-1 trials are ideal vehicles for advancing biomedical and translational science in the precision medicine era since the insights gained from them can not only shed light on how to treat or prevent diseases generally, but also provide insight into how to provide real-time care to the very individuals who are seeking attention for their health concerns in the first place.

Impact statement

Individuals do not respond to health interventions in the same way. This creates a need for identifying what it is (e.g., a behavior, a gene, a biomarker, or their combinations) that may indicate which interventions should be provided to different individuals. In fact, a great deal of modern biomedical science has focused on the identification of the mechanisms that contribute to disease, and relevant research has revealed that most disease processes are indeed multifactorial and can differ substantially between individuals. However, only now are studies being pursued in earnest that seek to identify links between measurable factors and likely response to health interventions. In this light, studies designed to identify unequivocal individual responders and non-responders to health interventions are needed. Current approaches, specifically those involving large cohort-based clinical trials with single endpoints and a focus on average effects of an intervention, are not necessarily designed for this. Rather, emerging N-of-1 trial designs that focus on individual responses to an intervention by collecting enough data on a participant to statistically determine and quantify their responses are better suited for this. We provide the basic motivation and techniques used in N-of-1 studies, contrasting them with standard population-based clinical trials, and focus on directions in which the research community is going that could accelerate the use of strategies for providing health interventions to the individuals most likely to benefit from them. One key area where clinical studies of health interventions have fallen short is in limiting their focus on one health outcome or measure. It is underappreciated that what individuals put in their bodies may impact them in a wide variety of ways – both good and bad – and N-of-1 studies have the potential to help overcome this and thereby push the understanding of human biology in unprecedented ways.

Introduction

The rapid development of high-throughput, cost-efficient and data-intensive assays for use in molecular biology and the biomedical sciences (e.g., DNA sequencing, proteomics, metabolomics, etc.) is revolutionizing the manner in which studies are pursued by seeking a deeper

© The Author(s), 2023. Published by Cambridge University Press. This is an Open Access article, distributed under the terms of the Creative Commons Attribution licence (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted re-use, distribution and reproduction, provided the original article is properly cited.

understanding of the pathological processes underlying diseases of all sorts. The application of such technologies to, for example, explorations of the differences between diseased and non-diseased human tissue specimens or genome-wide association studies (GWAS) interrogating DNA collected on tens of thousands of individuals with and without a particular condition, has led to many very useful insights into how to combat diseases (Karczewski and Snyder, 2018). However, such investigations have also exposed one very complicated set of issues: most pathological processes underlying diseases are heterogeneous and nuanced, to the point where mechanisms contributing to disease in one individual may be different from those in another individual. Given this, it has also been shown that available treatments or preventive interventions for different diseases tend not to work in everyone with the same general diagnosis. These two facts have led to concerted efforts to promote ‘precision’ or ‘personalized’ medicine and nutrition whereby health interventions are tailored to the unique genomic, physiologic, clinical, behavioral and exposure profiles of individuals who could benefit from them (Ginsburg and Willard, 2016; Karczewski and Snyder, 2018; Zeggini *et al.*, 2019).

The two largest impediments to enabling and deploying precision medicine at scale are (1) simply not having a more complete understanding of human *in vivo* biology and (2) not having insight into whether the differences exhibited by individuals at the molecular level – that have largely been identified from *in vitro* or *ex vivo* studies of human tissues – are truly clinically meaningful. Comprehensive longitudinal evaluations of humans using state-of-the-field assays have been pursued, but they have focused on identifying patterns among individuals in their natural environments without any controlled perturbation or design to relevant data collections (Chen *et al.*, 2012; Li-Pook-Than and Snyder, 2013; Price *et al.*, 2017; Earls *et al.*, 2019; Schussler-Fiorenza Rose *et al.*, 2019; Levy *et al.*, 2020; Sailani *et al.*, 2020; Zimmer *et al.*, 2021; Metwally *et al.*, 2022). Such studies are essential to explore human intra- and inter-individual variation but leave open the question of how different factors might contribute to different responses to health interventions (Atkinson and Batterham, 2015; Atkinson *et al.*, 2019; McInnes *et al.*, 2021). We note that there are examples of specific therapeutic modalities whose development is consistent with and motivated by a precision medicine orientation in the discussion section.

The purpose of this review is to provide an argument that clinical trials can be pursued that will allow researchers to probe human physiology in ethically-sound ways with unprecedented sophistication. Relevant trials should be rooted in N-of-1 and aggregated N-of-1 designs (Schork, 2015; Nikles *et al.*, 2021) and focus on exploring multiple phenotypes simultaneously and identifying causal relationships between phenotypes by leveraging emerging, largely non-invasive, health monitoring devices and assays (Izmailova *et al.*, 2018; Bentley *et al.*, 2019; Tehrani *et al.*, 2022). We do not provide an exhaustive review of N-of-1 trials, as there are many excellent resources and introductions to the basic motivation and methodologies (Lillie *et al.*, 2011; Nikles *et al.*, 2021; Davidson *et al.*, 2022), including comprehensive reviews of the applications of N-of-1 trials (Gabler *et al.*, 2011; Li *et al.*, 2016; Mirza *et al.*, 2017) as well as practical guides as to how to conduct N-of-1 trials (Guyatt *et al.*, 1988; Kravitz *et al.*, 2014; Nikles *et al.*, 2021; Duan *et al.*, 2022). In fact, N-of-1 trials are now receiving attention as strategies for improving health care generally (Keller *et al.*, 1988; Senn, 1998; Derby *et al.*, 2021; McDonald and Nikles, 2021; Selker *et al.*, 2022). Rather, we focus on N-of-1 trials that can

address issues plaguing precision medicine and can provide a better understanding of human biology for at least four reasons: (1) They can provide unprecedented insights into human biology, including intra-individual causal claims about interventions and health measures. (2) They provide very comprehensive ways of vetting interventions to see if they work and for whom they work. (3) Their results provide insight into an individual’s health that may benefit them almost immediately, as opposed to much later after all relevant data have been collected and analyzed as part of a larger study. (4) Their results can be aggregated to explore patterns among individuals who exhibit robust responses to interventions. The organization of the review is as follows. We first provide greater insight into why legacy population-wide effect-focused randomized clinical trials (RCTs) are inadequate to address fundamental questions about human biology. We then consider different aspects of, and settings for, the proposed multivariate N-of-1 clinical trials, including the need for better markers of drug activity and availability. We end with a brief discussion of a few emerging therapeutic areas that could benefit from the proposed trials as well as suggestions for future research.

Human biology and legacy clinical trials

Strategies to understand how systems function as a whole, and which components may be dependent on other components, typically involve inducing perturbations to those systems and then determining how the systems respond (e.g., in cellular or mouse physiology studies). Studies seeking to perturb living humans systematically in this way are at worst unethical and at best logistically complicated. However, humans voluntarily subject themselves to perturbations of all sorts via pharmacologic interventions, dietary manipulations, environmental exposures, etc. In fact, clinical trials are routinely pursued to explore responses to such perturbations. Unfortunately, most clinical trials tend to focus on a singular indication (i.e., health or response measure) and the average response to the intervention in the population at large and therefore do not address broader questions about human physiology. We do not provide an in-depth review of clinical trials here (see, e.g., Friedman *et al.*, 2015), but rather highlight a few of their key aspects so they can be contrasted with the N-of-1 studies. Typically, health interventions are evaluated in stages to ensure their safety and efficacy, from small ($n = 5\text{--}20$) phase I safety trials, to moderately sized ($n = 25\text{--}200$) phase II efficacy trials, to large ($n = 250\text{--}10,000$) phase III comparative and phase IV post-marketing surveillance studies. Some phase II and virtually all phase III and IV trials are pursued as RCTs where individuals are randomized to receive or not receive the intervention in question to avoid confounding. The health measures collected on these individuals are then compared to determine what effect the intervention may have on the typical person in the population at large.

There are at least six issues in the conduct of phase I–phase IV clinical trials (Deaton and Cartwright, 2018; Schork, 2018) that motivate complementary N-of-1 trials: (1) Most standard clinical trials have inclusion and exclusion criteria to make sure the trial has been carried out in individuals likely to benefit, as well as for ensuring safety and avoiding confounding effects, which can complicate their generalizability. (2) Most, if not all, trials focus on the effect of an intervention on a single well-defined endpoint (e.g., such as blood pressure, pain, or rheumatoid arthritis symptoms). (3) Most failures of interventions in clinical trials testing occur in the phase II stage of testing; that is, despite being shown to have

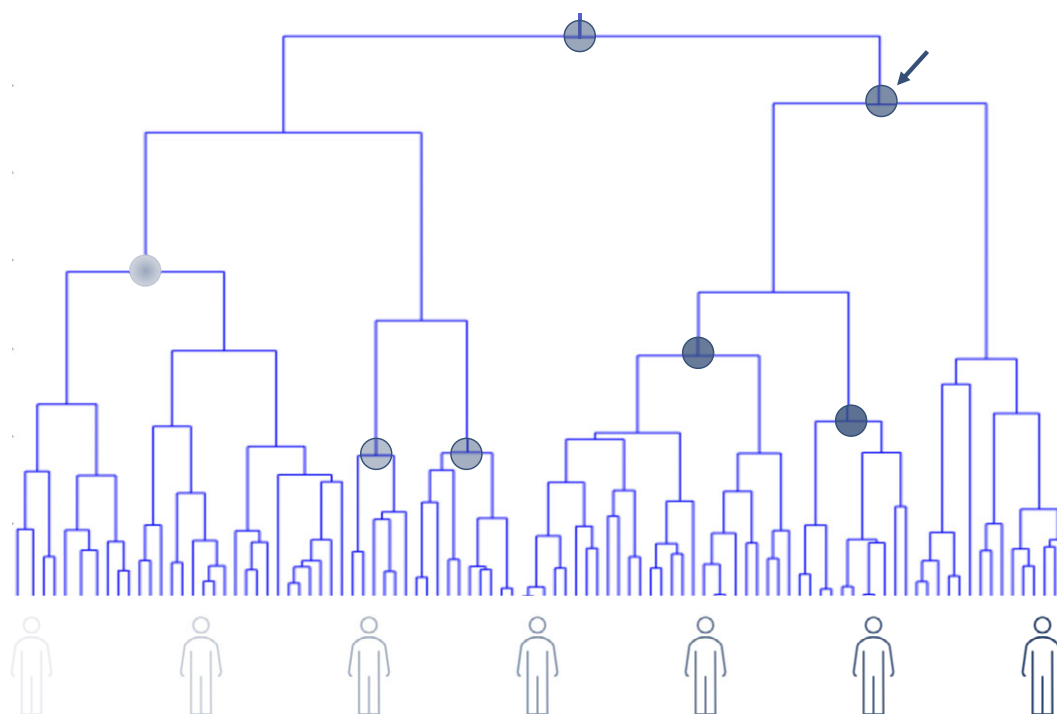


Figure 1. A tree or dendrogram reflecting how similar a number of individuals are with respect to phenotypes of relevance to drug response: the closer the bottommost branches of Figure 1 are – which represent individuals – the more similar the phenotypic profiles of those individuals are. The darkness of the shaded human figures at the bottom of the figure at different positions in the tree reflects the degree to which individuals at those positions in the tree possess a certain characteristic or profile. The circles represent interventions that can benefit different groups of individuals, such that the different locations where the shaded circles are situated represent convergence points for all individuals connected beneath that point who can benefit from the specific intervention. Thus, the topmost circle indicates that all individuals may benefit from that intervention (since all the individual tree branches converge back to that point), whereas the leftmost circle is likely to benefit the first ~25–30% of individuals. The two circles second and third from the left indicate interventions that may benefit a small number of individuals (e.g., only ~10% of individuals). The circle to which the arrow is pointing indicates an intervention that may benefit a large number of individuals but for whom other interventions (reflected by the 5th and 6th circles from the right) may benefit smaller subsets of individuals. Identifying points on trees like this that are consistent with who benefits from an intervention based on understanding of the factors responsible for mediating response is the motivation behind precision medicine and nutrition.

potential in ‘pre-clinical’ cellular and non-human experiments and to be safe in phase I trials, many interventions are shown not to modulate or affect the phenotype they were designed to impact, calling into question the pre-clinical, basic-science driven evidence suggesting that they may have benefit in humans *in vivo* (of course there are other reasons why an intervention may fail in a Phase II trial, for example, due to biased sampling, focusing on the wrong endpoint, measurement error, etc.). (4) Most late phase clinical trials, despite having inclusion and exclusion criteria, are expensive as they are conducted on very large numbers of people to ensure the trial results are generalizable and to overcome often hypothesized weak average effect sizes. (5) The results of clinical trials may identify interventions with the potential to benefit individuals, but unless it is known *a priori* how to identify individuals most likely to benefit from each intervention, it will be unclear how to optimally provide the interventions (see Figure 1). (6) Standard population-based RCTs can take a very long time to pursue and analyze, whereas more focused participant or patient-oriented alternative trial designs can be aggregated sequentially to enable population-based inferences (Schork, 2022).

Basic N-of-1 trial designs

Basic designs

As emphasized, the ultimate goal of N-of-1 trials is to determine, in an appropriately powered way, if an intervention is actually

benefitting a target individual by leveraging data collections and analytical methods focused on that target individual’s response. An element common to all N-of-1 clinical trial designs is an intervention ‘crossover’ component in which measurements on a health-related phenotype (e.g., blood pressure, mood, weight, symptoms, etc.) are made while the target individual is receiving, and not receiving, an intervention. This contrast between measures while on and off the intervention can then be exploited to quantify and characterize the individual’s response to the intervention but only if enough reliable measurements are made during each of the intervention periods and data analysis methods are used to control for confounding due to, for example, placebo or unmeasured covariate effects (Lillie et al., 2011; Kravitz et al., 2014; Wang and Schork, 2019; Kravitz and Duan, 2022). Note that many of the most widely used strategies for avoiding confounding in standard RCTs can be exploited in the design and execution of N-of-1 trials, such as randomizing the order in which the interventions are provided, blinding of the received interventions to the participants and/or researchers analyzing the data, washout periods to avoid carryover effects, etc. (Lillie et al., 2011; Duan et al., 2013; Kravitz et al., 2014; Duan et al., 2022; Kravitz and Duan, 2022).

Figure 2 depicts some basic N-of-1 designs. We note that there is growing, but not complete, consensus on the definition of an N-of-1 clinical trial – which many believe requires a randomized order of interventions with, for example, blinding – as opposed to a simple ‘single case study’ which may not include randomization or blinding. We argue that both N-of-1 clinical trials and some single case

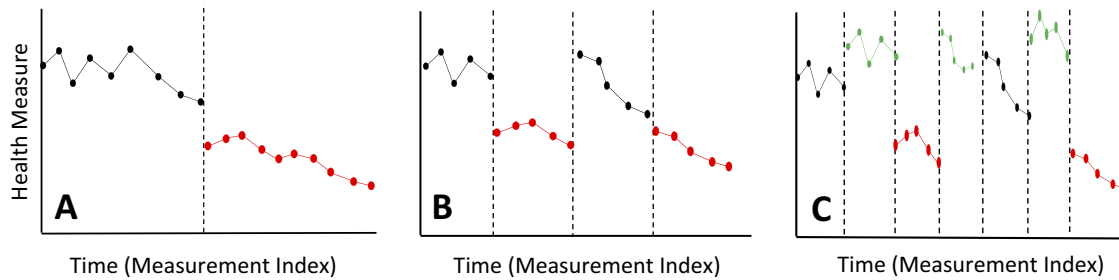


Figure 2. Different, very basic, types of N-of-1 clinical trial designs in which an intervention had a lowering effect on a health measure (like blood pressure). The black and red lines reflect hypothetical health measure trajectories (i.e., longitudinal data) while an individual is not receiving (black) or receiving (red) an intervention. The vertical dashed lines indicate when interventions were provided or changed. Panel A depicts the basic ‘interrupted time’ series design, Panel B the ‘reversal’ design and panel C a reversal design with washout periods (green lines).

studies are appropriate for advancing precision medicine (Davidson et al., 2022) and consider them both as N-of-1 clinical trials. Panel A depicts the simple and often used ‘interrupted time series single case design’ – or basic ‘AB’ design, where ‘A’ and ‘B’ correspond to interventions, one of which could be a placebo or simply no intervention (see, e.g., part V of the book by Huitema, 2011 for an excellent introduction). Panel B depicts the ‘reversal’ or ‘ABAB’ design in which the intervention periods in the interrupted time series design are repeated to ensure the initial set of observations do not reflect false positive or negative results. Panel C depicts the reversal design with washout periods (i.e., periods where no administration of an intervention, including a placebo, are provided) between each administration of an intervention to avoid confounding carryover effects (an ‘AwBwAwB’ design). Note that the number of intervention administration periods and the order of the interventions can vary depending on the sophistication of the design (e.g., ‘ABwBA’ or ‘AwAwBwAwBwAwA’).

The power of N-of-1 trials

N-of-1 trials derive their power to make inferences about the effect of an intervention on an individual from the number of measurements made on the participant while on and off an intervention (Huitema, 2011). However, serial correlations between the measurements can complicate the analysis if not appropriately accounted for, as can aforementioned covariate effects, carryover effects, missing data, non-uniform time points between measurement collections and placebo effects (Rochon, 1990; Huitema, 2011; Lillie et al., 2011; Wang and Schork, 2019; Somer et al., 2022). Many offshoots of N-of-1 trials exist to improve their efficiency and comprehensiveness; for example, sequential designs can be used to minimize the number of measurements made while preserving appropriate false positive and false negative rates (Schork and Goetz, 2017; Schork, 2022). In addition, there is no reason that N-of-1 trial methodology cannot be used in other settings, for example, assessing intervention effects in cell lines, tissue samples, mice, etc. In fact, such studies often make use of samples from a single individual or strain of mice and so, from a biological standpoint, they are, by their nature, assuming that insights from a single individual can shed light on very general biological questions. There are many recent examples of N-of-1 studies, which we will not review exhaustively here (Gabler et al., 2011; Kronish et al., 2018; Nikles et al., 2022; Samuel et al., 2022), but rather simply emphasize that they are growing in number and sophistication (Kim et al., 2019; Lamb et al., 2022; Phyland et al., 2022).

Beyond the basics

There are three important aspects of N-of-1 trials that are receiving the attention which are motivating newer approaches. First, the data and results associated with individual N-of-1 trials can be aggregated and analyzed to explore trends among the participants and their responses (Zucker et al., 2010; Araujo et al., 2016; Punja et al., 2016; Schork and Goetz, 2017; Barbosa Mendes et al., 2022). Second, with sufficient data collected over time, one could characterize *causal* relationships among the intervention and other measures (Molenaar, 2019; Izem and McCarter, 2021; Yeboah et al., 2021) (note: an entire recent issue of the journal ‘Evaluation and the Health Professions’ was devoted to causal analysis in N-of-1 trials (Miocevic et al., 2022)). Such analyses could provide unprecedented insight into human physiology. The third is that the execution of N-of-1 trials focusing on important physiologic endpoints can be greatly enhanced with emerging digital health-based monitoring devices (such as the Apple Watch and continuous glucose monitors), survey instruments made available through smartphone apps, and largely pain-free and convenient methods for obtaining blood, urine, stool and saliva samples (Enderle et al., 2016; Izmailova et al., 2018).

Multivariate n-of-1 trials

N-of-1 clinical trials can be pursued to characterize the effect of an intervention on a specific phenotype (blood pressure) for a target individual and as such complement population-based RCTs, especially when it is unclear if an individual is likely to benefit from the intervention. However, many diseases are not associated with singular phenotypes and, in fact, most individuals who suffer from them do not only have one major symptom or problem (Ong et al., 2020). This is especially the case for older individuals with many comorbidities (Pearson-Stuttard et al., 2019; Onder et al., 2020; Skou et al., 2022). As a result, it makes sense to pursue appropriately powered N-of-1 trials that explore the impact of an intervention on more than one outcome (i.e., multivariate N-of-1 trials). Although multivariate trials have been proposed in the context of standard RCTs, there are few, if any, precedents in N-of-1 study contexts (Zhao et al., 2009). Few published precision medicine studies have measured more than one clinically relevant health measure despite the availability of newer health monitoring technologies (Viana et al., 2021). Although we will not go into the mathematical or statistical details here for how such trials can achieve sufficient power, it is arguable that if health is defined broadly (e.g., normal blood pressure, quality sleep, good blood biochemistry profile, etc.)

then a good health intervention should at a minimum not negatively affect any of them and at best positively affect them all. In this light, testing multiple measures for intervention effects simultaneously using an omnibus statistical test of the hypothesis that an intervention positively affects them all could lead to an increase in power (Huitema, 2011; Tabachnick and Fidell, 2012), but only if the number of measures is large (Leroy et al., 2022). Reaching appropriate numbers of observations could be achieved, for example, through the use of the aforementioned continuous wireless devices or microsampling techniques which involve collecting minute amounts of blood or urine for analyses to avoid a standard blood draw or logistically challenging biospecimen collections (Enderle et al., 2016; Bentley et al., 2019; Anderson et al., 2020).

There are many settings beyond multimorbidity issues that justify an evaluation of multiple health measures in N-of-1 clinical trials. For example, depression is known to impact virtually all aspects of a person's health due to the various behaviors adopted by depressed individuals (Triolo et al., 2020; Aprahamian et al., 2022). Testing the effect of an antidepressant on mood and depressive symptoms in addition to, perhaps, weight, blood pressure, sleep quality, etc. makes sense. Another example involves geroprotectors, or interventions meant to slow the aging rate and thereby influence susceptibility to, or processes associated with, many different age-related diseases (Mahmoudi et al., 2019; Kritchevsky and Justice, 2020; Triolo et al., 2020; Aprahamian et al., 2022; Moskalev et al., 2022). Thus, by definition, a geroprotector should affect multiple systems and hence could be tested for this. In fact, if only one or some subset of health measures among many different measures is in fact affected by a purported geroprotector, then the intervention is probably not a geroprotector (Schork et al., 2022).

In addition to testing for the effect of an intervention on multiple health measures, N-of-1 and aggregated N-of-1 studies can be pursued to exploit interventions as ways of perturbing or probing human physiology – the goal being to identify relationships among different health measures or processes. Thus, if enough measures are collected over the time an individual is both receiving and not receiving an intervention, then temporal relationships between the measures can reveal likely causal relationships among them based on, for example, time series analysis, Granger regression and other techniques (McCracken, 2016; Molenaar, 2019). Such analyses would again be significantly enhanced if the relevant health measures were collected continuously (Enderle et al., 2016; Bentley et al., 2019; Anderson et al., 2020). In addition, by assessing the effect of the intervention on health measures beyond a primary measure in relevant trials, potential intervention ‘repurposing’ opportunities could arise (Pushpakom et al., 2019; Krishnamurthy et al., 2022; Mucke, 2022). In this way, N-of-1 trials can be pursued as proof-of-concept studies for identifying multiple indications, or at least one on solid footing, for an intervention (Pushpakom et al., 2019; Mucke, 2022). In addition, by collecting multiple health measures on an individual N-of-1 trial participant, possibly continuously and in real time, insights into that participant's health and health trajectory can be obtained even if an intervention being tested is shown not to benefit the participant.

Whole body, biomarker validation and therapeutic drug monitoring studies

There are some very specific areas where multivariate N-of-1 trials can be pursued that will enhance the assessment of individual

intervention response and enable deeper insight into human physiology, as emphasized throughout this review. We briefly describe four such areas below.

General assessment of inter-individual variation in intervention response

As noted, given that N-of-1 trials focus on individuals' responses, they can be used to more precisely identify responders to particular interventions. In addition, if relevant studies collected sufficient data on more than one health measure then they can be used to identify potential side effects, alternative uses for the intervention and different mechanisms of action or physiological processes modulated by the intervention. In fact, it might make sense for all interventions to be evaluated for their whole-body effects in a small number of individuals as they are being developed. If done along the lines outlined in the review, such trials could shed enormous light on how substances put into the human body affect it systemically (see Figure 3).

Biomarker and surrogate endpoint validation

There is great interest in identifying better biomarkers of an intervention's activity so that these biomarkers can be correlated with other health measures of interest (see, e.g., ‘Therapeutic Drug Monitoring Studies’ section below) (Hendrickson et al., 2020). In addition, there is also interest in identifying ‘surrogate endpoints’ for clinical trials that initially focus on expensive, lengthy and logistically challenging health outcome measures, and N-of-1 trials are excellent vehicles for validating biomarkers and surrogate endpoints (Burzykowski et al., 2005). As an example, consider the development and use of epigenetic clocks as surrogate endpoints in trials of geroprotectors (Schork et al., 2022). The belief is that if an intervention modulates or changes an epigenetic clock among participants in a trial in positive ways – thereby indicating that the intervention in question is slowing the aging rate of the individuals – then those individuals do not necessarily have to be tracked longitudinally until they develop (or do not develop) age-related diseases that the candidate geroprotector is hypothesized to prevent or treat (Mahmoudi et al., 2019; Kritchevsky and Justice, 2020; Schork et al., 2022). Thus, the epigenetic clocks would act as a surrogate endpoint for the processes that are associated with the disease endpoints of real interest, which are modulated by the intervention. Although epigenetic clocks have been shown to be correlated with disease endpoints, they have been done so via large epidemiological studies and not in focused clinical trials measuring appropriate health measures. Therefore, it is arguable that by measuring epigenetic clocks along with health measures that underlie many common chronic age-related diseases and conditions, such as blood pressure, cholesterol level, sleep quality, etc. in appropriately powered N-of-1 trials, one might not only show that the geroprotector influences these health measures in positive ways, but also that an epigenetic clock is correlated with them as well. This would in effect validate surrogacy of the epigenetic clock at the ‘level of the individuals and the trial’ (Burzykowski et al., 2005; Buyse et al., 2022).

Therapeutic drug monitoring studies

Therapeutic drug monitoring (TDM) studies consider the measurement of a drug's concentration in an individual's bloodstream in

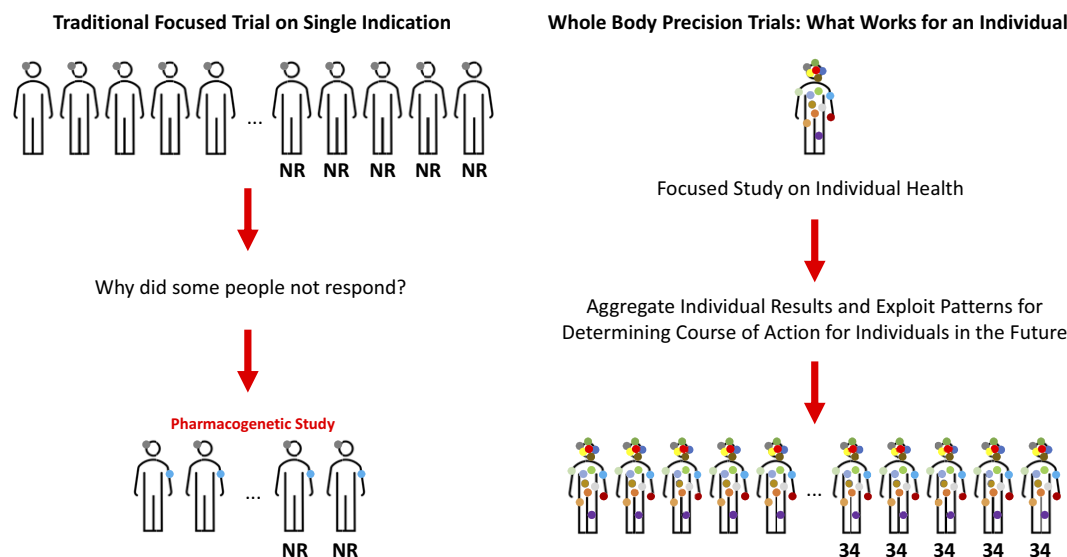


Figure 3. Contrasting clinical trial designs. The design depicted on the left is consistent with standard RCTs focusing on a singular health measure or indication (the gray colored dot on the left side of the head of the human figures indicating a single phenotype of interest; for example, depression symptoms). If individuals are found not to respond (NR = Non-Responders) then a future study seeking to identify biomarkers of response could be pursued, whereby a new biomarker phenotype is associated with the response/non-response phenotype (e.g., genomic profile). The design depicted on the right provides the motivation for complementary trials to traditional RCTs whereby the effect of an intervention is evaluated on an individual from a whole-body perspective. The results of this trial are aggregated with trials on other individuals and patterns that could identify responders and non-responders are explored that may also reveal intervention effects on different phenotypes and how those phenotypes interact.

order to correlate the levels of the drug with the phenotype that the drug is hypothesized to modulate (Dasgupta, 2012; Clarke and Dasgupta, 2019). Most drugs do not undergo such evaluation and testing, which is unfortunate since such studies could in theory better characterize mechanisms of action of the drug and its effects on different phenotypic endpoints. Of course, TDM studies are predicated on the assumption that there is a definable relationship between drug dose and plasma or blood drug concentration, and between concentrations and therapeutic effects. In addition, TDM studies require ways of measuring blood levels of a drug which may not be trivial. However, by more precisely measuring drug bioavailability and activity in N-of-1 trials, especially in trials for which participants are monitored for multiple health measures, one could explore temporal relationships between drug bioavailability and activity and not just, for example, pill count-based dosing and outcomes (Dasgupta, 2012; Clarke and Dasgupta, 2019; Irving and Gecse, 2022; Ordutowski *et al.*, 2022).

Matching based on data aggregation

As noted previously, if enough N-of-1 trials are pursued using the same interventions, and baseline health assessments with common measures have been collected on each participant, then the data and results can be aggregated and analyzed. The common baseline health examination profiles of the individuals could then be explored for patterns and correlations with intervention responses. This can enable matching a future target individual's baseline health profile with others' profiles who previously went through N-of-1 trials. If good matches (however defined) are found, then the interventions to which those individuals matching the target individual responded, would be reasonable first-choice interventions for the target individual (Wicks *et al.*, 2011; Schork and Goetz, 2017; Schork *et al.*, 2020; Davidson *et al.*, 2022). Different strategies for identifying the matches could be pursued based on, for example, propensity scores and related techniques (Guo and Fraser, 2014; Liu and Meng, 2016).

Conclusions and future directions

There are few health interventions whose effectiveness is ubiquitous. This can be attributed to the great genetic, physiologic, clinical, behavioral and exposure profile variation exhibited by individuals susceptible to or suffering from diseases (Schork, 2015). Identifying interventions that benefit individuals on the basis of their nuanced and possibly unique profiles is the goal of precision or personalized medicine. However, tailoring or matching interventions to individuals will require greater understanding of intra- and inter-individual variation and intervention response and, as argued throughout, can be enabled or enhanced through the use of whole-body N-of-1 clinical trials (Figures 1 and 3).

In this light, many emerging interventions, such as cytotoxic T-cell therapies (Kiyotani *et al.*, 2021; Roesler and Anderson, 2022), brain anatomy-guided Transcranial Magnetic Stimulation (TMS) therapies (Siddiqi *et al.*, 2021; Williams *et al.*, 2021) and sequence-based antisense oligonucleotide therapies (Kim *et al.*, 2019; Helm *et al.*, 2022), are designed to only work on specific individuals given that the targets they exploit and constructs they use are based on the unique features underlying the pathologies of the individuals for whom they are designed. Testing the effectiveness of these interventions, given that no two individuals with the same condition will likely get exactly the same intervention, could make use of the proposed N-of-1 strategies. Of course, one could address very broad questions about the utility of such interventions using standard RCTs, such as whether individuals who receive the personalized interventions fare better than individuals who receive a more 'one-size-fits-all' intervention (Schork *et al.*, 2020).

Ultimately, the current emphasis on precision medicine, the emergence of sophisticated health monitoring technologies, and the desire of individuals to optimize their health and not simply contribute to studies that may only benefit future generations, demand better approaches to biomedical and translational science. We recognize that there might be impediments to the implementation of multivariate N-of-1 trials of the type described. For

example, a greater patient burden for data collection, logistical complications in collecting different data types, and the costs of conducting and monitoring the individual participants may create barriers to the adoption and use of multivariate N-of-1 trials. However, efficient, cost-effective and participant-friendly N-of-1 clinical trials – to the degree that they can be pursued – are very likely to be an appropriate addition to biomedical and translational studies in the future given that they have at least 4 very overt advantages, including: (1) the ability to shed light on fundamental questions about human biology; (2) determine which interventions work and on whom; (3) benefit the participants in the trials directly and almost immediately by collecting vast amounts of health data on them possibly continuously and with real-time interpretive ability; and (4) pave the way for their aggregation and analysis to identify patterns that may inform their use and execution in the future.

Open peer review. To view the open peer review materials for this article, please visit <http://doi.org/10.1017/pcm.2022.15>.

Acknowledgements. The authors would like to thank Drs. Mark Adler and Stephanie Venn Watson for commenting on earlier versions of this manuscript.

Author contributions. N.J.S. conceived of the orientation and format for the review, and N.J.S., B.B.-J., W.S.L., S.S. and L.H.G. pursued relevant literature reviews. N.J.S. wrote the initial draft, L.H.G. edited the initial draft and B.B.-J., W.S.L. and S.S. edited the subsequent drafts.

Financial support. N.J.S. is supported in part by the following grant support from the National Institutes of Health: 1 U19 AG056169-01A1; U2C CA252973; UH3 AG064706; UH2 AG06470602S1 and U19 AG023122.

Competing interest. N.J.S., S.S. and L.H.G. are founders of net. Bio, a company focusing on pursuing novel clinical protocols to ensure that individuals benefit from health interventions of all sorts. B.B.-J. and W.S.L. are paid consultants for net. Bio.

References

- Anderson L, Razavi M, Pope ME, Yip R, Cameron LC, Bassini-Cameron A and Pearson TW (2020) Precision multiparameter tracking of inflammation on timescales of hours to years using serial dried blood spots. *Bioanalysis* **12**, 937–955.
- Aprahamian I, Borges MK, Hanssen DJC, Jeuring HW and Oude Voshaar RC (2022) The frail depressed patient: A narrative review on treatment challenges *Clinical Interventions in Aging* **17**, 979–990.
- Araujo A, Julious S and Senn S (2016) Understanding variation in sets of N-of-1 trials. *PLoS One* **11**, e0167167.
- Atkinson G and Batterham AM (2015) True and false interindividual differences in the physiological response to an intervention. *Experimental Physiology* **100**, 577–588.
- Atkinson G, Williamson P and Batterham AM (2019) Issues in the determination of ‘responders’ and ‘non-responders’ in physiological research. *Experimental Physiology* **104**, 1215–1225.
- Barbosa Mendes A, Jamshidi L, Van den Noortgate W and Fernandez-Castilla B (2022) Network meta-analysis for single-case design studies: An illustration. *Evaluation and the Health Professions* **45**, 66–75.
- Bentley KH, Kleiman EM, Elliott G, Huffman JC and Nock MK (2019) Real-time monitoring technology in single-case experimental design research: Opportunities and challenges. *Behavior Research and Therapy* **117**, 87–96.
- Burzykowski T, Molenberghs G and Buyse M (eds.) (2005) *The Evaluation of Surrogate Endpoints*. New York: Springer.
- Buyse M, Saad ED, Burzykowski T, Regan MM and Sweeney CS (2022) Surrogacy beyond prognosis: The importance of “trial-level” surrogacy. *The Oncologist* **27**, 266–271.
- Chen R, Mias GI, Li-Pook-Than J, Jiang L, Lam HY, Chen R, Miriami E, Karczewski KJ, Hariharan M, Dewey FE, Cheng Y, Clark MJ, Im H, Habegger L, Balasubramanian S, O’Huallachain M, Dudley JT, Hillenmeyer S, Haraksingh R, Sharon D, Euskirchen G, Lacroute P, Bettinger K, Boyle AP, Kasowski M, Grubert F, Seki S, Garcia M, Whirl-Carrillo M, Gallardo M, Blasco MA, Greenberg PL, Snyder P, Klein TE, Altman RB, Butte AJ, Ashley EA, Gerstein M, Nadeau KC, Tang H and Snyder M (2012) Personal omics profiling reveals dynamic molecular and medical phenotypes. *Cell* **148**, 1293–1307.
- Clarke W and Dasgupta A (2019) *Clinical Challenges in Therapeutic Drug Monitoring: Special Populations, Physiological Conditions and Pharmacogenomics*. Amsterdam: Elsevier.
- Dasgupta A (2012) *Therapeutic Drug Monitoring: Newer Drugs and Biomarkers*. Cambridge, MA: Academic Press.
- Davidson K, Cheung K, Friel C and Suls J (2022) Introducing data sciences to N-of-1 designs, statistics, use-cases, the future, and the moniker ‘N-of-1’ Trial. *Harvard Data Science Review*, (Special Issue 3). <https://doi.org/10.1162/99608f92.116c43fe>
- Deaton A and Cartwright N (2018) Understanding and misunderstanding randomized controlled trials. *Social Science and Medicine* **210**, 2–21.
- Derby L, Kronish IM, Wood D, Cheung YKK, Cohn E, Duan N, St Onge T, Duer-Hefe J, Davidson KW and Moise N (2021). Using a multistakeholder collaborative and patient surveys to inform the conduct of personalized (N-of-1) trials. *Health Psychology* **40**, 230–241.
- Duan N, Kravitz RL and Schmid CH (2013) Single-patient (n-of-1) trials: A pragmatic clinical decision methodology for patient-centered comparative effectiveness research. *Journal of Clinical Epidemiology* **66**, S21–S28.
- Duan N, Norman D, Schmid C, Sim I and Kravitz RL (2022) Personalized data science and personalized (N-of-1) trials: Promising paradigms for individualized health care. *Harvard Data Science Review*, (Special Issue 3). <https://doi.org/10.1162/99608f92.8439a336>
- Earls JC, Rappaport N, Heath L, Wilmanski T, Magis AT, Schork NJ, Omenn GS, Lovejoy J, Hood L and Price ND (2019) Multi-omic biological age estimation and its correlation with wellness and disease phenotypes: A longitudinal study of 3,558 individuals. *Journals of Gerontology Series A: Biological Sciences and Medical Sciences* **74**, S52–S60.
- Enderle Y, Foerster K and Burhenne J (2016) Clinical feasibility of dried blood spots: Analytics, validation, and applications. *Journal of Pharmaceutical and Biomedical Analysis* **130**, 231–243.
- Friedman LM, Furburg CD, DeMets DL, Reboussin DR and Granger CB (2015) *Fundamentals of Clinical Trials*. New York: Springer.
- Gabler NB, Duan N, Vohra S and Kravitz RL (2011) N-of-1 trials in the medical literature: A systematic review. *Medical Care* **49**(8), 761.
- Ginsburg GS and Willard HF (ed.) (2016) *Genomic and Precision Medicine: Foundations, Translation, and Implementation*. London: Academic Press.
- Guo S and Fraser MW (2014) *Propensity Score Analysis: Statistical Methods and Applications*. Thousand Oaks, CA: SAGE Publications.
- Guyatt G, Sackett D, Adachi J, Roberts R, Chong J, Rosenbloom D and Keller J (1988) A clinician’s guide for conducting randomized trials in individual patients. *Canadian Medical Association Journal* **139**, 497–503.
- Helm J, Schols L and Hauser S (2022) Towards personalized allele-specific antisense oligonucleotide therapies for toxic gain-of-function neurodegenerative diseases. *Pharmaceutics* **14**, 1708.
- Hendrickson RC, Thomas RG, Schork NJ and Raskind MA (2020) Optimizing aggregated N-Of-1 trial designs for predictive biomarker validation: Statistical methods and theoretical findings. *Frontiers in Digital Health* **2**, 13.
- Huitema BE (2011) *The Analysis of Covariance and Alternatives: Statistical Methods for Experiments, Quasi-Experiments, and Single-Case Studies*. New Jersey: Wiley.
- Irving PM and Gecse KB (2022) Optimizing therapies using therapeutic drug monitoring: Current strategies and future perspectives. *Gastroenterology* **162**, 1512–1524.
- Izem R and McCarter R (2021) Randomized and non-randomized designs for causal inference with longitudinal data in rare disorders. *Orphanet Journal of Rare Diseases* **16**, 491.
- Izmailova ES, Wagner JA and Perakslis ED (2018) Wearable devices in clinical trials: Hype and hypothesis. *Clinical Pharmacology and Therapeutics* **104**, 42–52.
- Karczewski KJ and Snyder MP (2018) Integrative omics for health and disease. *Nature Reviews Genetics* **19**, 299–310.

- Keller JL, Guyatt GH, Roberts RS, Adachi JD and Rosenbloom D (1988) An N of 1 service: Applying the scientific method in clinical practice. *Scandinavian Journal of Gastroenterology Supplement* **147**, 22–29.
- Kim J, Hu C, El Achkar CM, Black LE, Douville J, Larson A, Pendergast MK, Goldkind SF, Lee EA, Kuniholm A, Soucy A, Vaze J, Belur NR, Fredriksen K, Stojkowska I, Tsytyskova A, Armant M, DiDonato RL, Choi J, Cornelissen L, Pereira LM, Augustine EF, Genetti CA, Dies K, Barton B, Williams L, Goodlett BD, Riley BL, Pasternak A, Berry ER, Pflöck KA, Chu S, Reed C, Tyndall K, Agrawal PB, Beggs AH, Grant PE, Urion DK, Snyder RO, Waisbren SE, Poduri A, Park PJ, Patterson A, Biffi A, Mazzulli JR, Bodamer O, Berde CB and Yu TW (2019) Patient-customized oligonucleotide therapy for a rare genetic disease. *New England Journal of Medicine* **381**, 1644–1652.
- Kiyotani K, Toyoshima Y and Nakamura Y (2021) Personalized immunotherapy in cancer precision medicine. *Cancer Biology and Medicine* **18**, 955–965.
- Kravitz RL and Duan N (2022) Conduct and implementation of personalized trials in research and practice. *Harvard Data Science Review*, (Special Issue 3). <https://doi.org/10.1162/99608f92.901255e7>
- Kravitz RL, Duan N and DECIDE Methods Center N-of-1 Guidance Panel (2014) Design and implementation of N-of-1 trials: A user's guide. In *AHRQ Publication No. 13(14)-EHC122-EF*. Rockville, MD: Agency for Healthcare Research and Quality.
- Krishnamurthy N, Grimshaw AA, Axson SA, Choe SH and Miller JE (2022) Drug repurposing: A systematic review on root causes, barriers and facilitators. *BMC Health Services Research* **22**, 970.
- Kritchevsky SB and Justice JN (2020) Testing the geroscience hypothesis: Early days. *Journals of Gerontology. Series A: Biological Science and Medical Science* **75**, 99–101.
- Kronish IM, Hampsey M, Falzon L, Konrad B and Davidson KW (2018) Personalized (N-of-1) trials for depression: A systematic review. *Journal of Clinical Psychopharmacology* **38**, 218–225.
- Lamb JJ, Stone M, D'Adamo CR, Volkov A, Metti D, Aronica L, Minich D, Leary M, Class M, Carullo M, Ryan JJ, Larson IA, Lundquist E, Contractor N, Eck B, Ordovas JM and Bland JS (2022) Personalized lifestyle intervention and functional evaluation health outcomes SurvEy: Presentation of the LIFEHOUSE study using N-of-one tent-umbrella-bucket design. *Journal of Personalized Medicine* **12**, 115.
- Leroy JL, Frongillo EA, Kase BE, Alonso S, Chen M, Dohoo I, Huybregts L, Kadiyala S and Saville NM (2022) Strengthening causal inference from randomised controlled trials of complex interventions. *British Medical Journal Global Health* **7**(6):e008597. doi: [10.1136/bmjgh-2022-008597](https://doi.org/10.1136/bmjgh-2022-008597). PMID: 35688484; PMCID: PMC9189821.
- Levy R, Magis AT, Earls JC, Manor O, Wilmanski T, Lovejoy J, Gibbons SM, Omenn GS, Hood L and Price ND (2020) Longitudinal analysis reveals transition barriers between dominant ecological states in the gut microbiome. *Proceedings of the National Academy of Sciences: U S A* **117**, 13839–13845.
- Li J, Gao W, Punja S, Ma B, Vohra S, Duan N, Gabler N, Yang K and Kravitz RL (2016) Reporting quality of N-of-1 trials published between 1985 and 2013: A systematic review. *Journal of Clinical Epidemiology* **76**, 57–64.
- Lillie EO, Patay B, Diamant J, Issell B, Topol EJ and Schork NJ (2011) The n-of-1 clinical trial: The ultimate strategy for individualizing medicine? *Personalized Medicine* **8**, 161–173.
- Li-Pook-Than J and Snyder M (2013) iPOP goes the world: Integrated personalized omics profiling and the road toward improved health care. *Chemistry and Biology* **20**, 660–666.
- Liu K and Meng XL (2016) There is individualized treatment. Why not individualized inference? *Annual Review of Statistics and Its Application* **3**, 79–111.
- Mahmoudi S, Xu L and Brunet A (2019) Turning back time with emerging rejuvenation strategies. *Nature Cell Biology* **21**, 32–43.
- McCracken JM (2016) *Exploratory Causal Analysis with Time Series Data*. San Rafael, CA: Morgan and Claypool.
- McDonald S and Nikles J (2021) N-of-1 trials in healthcare. *Healthcare (Basel)* **9**, 330.
- McInnes G, Yee SW, Pershad Y and Altman RB (2021) Genomewide association studies in pharmacogenomics. *Clinical Pharmacology and Therapeutics* **110**, 637–648.
- Metwally AA, Zhang T, Wu S, Kellogg R, Zhou W, Contrepois K, Tang H and Snyder M (2022) Robust identification of temporal biomarkers in longitudinal omics studies. *Bioinformatics* **38**, 3802–3811.
- Miocevic M, Moeyaert M, Mayer A and Montoya AK (2022) Causal mediation analysis in single case experimental designs: Introduction to the special issue. *Evaluation and the Health Professions* **45**, 3–7.
- Mirza RD, Punja S, Vohra S and Guyatt G (2017) The history and development of N-of-1 trials. *Journal of the Royal Society of Medicine*, **110**, 330–340.
- Molenaar PCM (2019) Granger causality testing with intensive longitudinal data. *Prevention Science* **20**, 442–451.
- Moskalev A, Guvatova Z, Lopes IA, Beckett CW, Kennedy BK, De Magalhaes JP and Makarov AA (2022) Targeting aging mechanisms: Pharmacological perspectives. *Trends in Endocrinology and Metabolism* **33**, 266–280.
- Mucke HAM (2022) Drug repurposing patent applications March-June 2022. *Assay and Drug Development Technologies* **20**, 286–293.
- Nikles J, Evans K, Hams A and Sterling M (2022) A systematic review of N-of-1 trials and single case experimental designs in physiotherapy for musculoskeletal conditions. *Musculoskeletal Science and Practice* **62**, 102639.
- Nikles J, Onghena P, Vlaeyen JWS, Wicksell RK, Simones LE, McGree JM and McDonald S (2021) Establishment of an international collaborative network for N-of-1 trials and single-case designs. *Contemporary Clinical Trials Communications* **23**, 100826.
- Onder G, Bernabei R, Vetrano DL, Palmer K and Marengoni A (2020) Facing multimorbidity in the precision medicine era. *Mechanisms of Ageing and Development* **190**, 111287.
- Ong KY, Lee PSS and Lee ES (2020) Patient-centred and not disease-focused: A review of guidelines and multimorbidity. *Singapore Medical Journal* **61**, 584–590.
- Ordutowski H, Dal Dosso F, De Wispelaere W, Van Tricht C, Vermeire S, Geukens N, Gils A, Spasic D and Lammertyn J (2022) Next generation point-of-care test for therapeutic drug monitoring of adalimumab in patients diagnosed with autoimmune diseases. *Biosensors & Bioelectronics* **208**, 114189.
- Pearson-Stuttard J, Ezzati M and Gregg EW (2019) Multimorbidity—a defining challenge for health systems. *The Lancet Public Health* **4**, e599–e600.
- Phyland RK, McKay A, Olver J, Walterfang M, Hopwood M, Ponsford M and Ponsford JL (2022) Use of olanzapine to treat agitation in traumatic brain injury: A series of N-of-one trials. *Journal of Neurotrauma* **40**, 33–51.
- Price ND, Magis AT, Earls JC, Glusman G, Levy R, Lausted C, McDonald DT, Kusebauch U, Moss CL, Zhou Y, Qin S, Moritz RL, Brogaard K, Omenn GS, Lovejoy JC and Hood L (2017) A wellness study of 108 individuals using personal, dense, dynamic data clouds. *Nature Biotechnology* **35**, 747–756.
- Punja S, Xu D, Schmid CH, Hartling L, Urchuk L, Nikles CJ and Vohra S (2016) N-of-1 trials can be aggregated to generate group mean treatment effects: A systematic review and meta-analysis. *Journal of Clinical Epidemiology* **76**, 65–75.
- Pushpakom S, Iorio F, Eyers PA, Escott KJ, Hopper S, Wells A, Doig A, Guillemins T, Latimer J, McNamee C, Norris A, Sanseau P, Cavalla D and Pirmohamed M (2019) Drug repurposing: Progress, challenges and recommendations. *Nature Reviews Drug Discovery* **18**, 41–58.
- Rochon J (1990) A statistical model for the “N-of-1” study. *Journal of Clinical Epidemiology* **43**, 499–508.
- Roesler AS and Anderson KS (2022) Beyond sequencing: Prioritizing and delivering neoantigens for cancer vaccines. *Methods in Molecular Biology* **2410**, 649–670.
- Sailani MR, Metwally AA, Zhou W, Rose SMS, Ahadi S, Contrepois K, Mishra T, Zhang MJ, Kidzinski L, Chu TJ and Snyder MP (2020) Deep longitudinal multiomics profiling reveals two biological seasonal patterns in California. *Nature Communications* **11**, 4933.
- Samuel JP, Wootton SH, Holder T and Molony D (2022) A scoping review of randomized trials assessing the impact of n-of-1 trials on clinical outcomes. *PLoS One* **17**, e0269387.
- Schork NJ (2015) Personalized medicine: Time for one-person trials. *Nature* **520**, 609–611.
- Schork NJ (2018) Randomized clinical trials and personalized medicine: A commentary on deaton and cartwright. *Social Science and Medicine* **210**, 71–73.

- Schork NJ (2022) Accommodating serial correlation and sequential design elements in personalized studies and aggregated personalized studies. *Harvard Data Science Review*, (Special Issue 3). <https://doi.org/10.1162/99608f92.f1eef6f4>.
- Schork NJ, Beaulieu-Jones B, Liang W, Smalley S and Goetz LH (2022) Does modulation of an epigenetic clock define a geroprotector? *Advances in Geriatric Medicine and Research* 4, e220002.
- Schork NJ and Goetz LH (2017) Single-subject studies in translational nutrition research. *Annual Review of Nutrition* 37, 395–422.
- Schork NJ, Goetz LH, Lowey J and Trent J (2020) Strategies for testing intervention matching schemes in cancer. *Clinical Pharmacology and Therapeutics* 108, 542–552.
- Schussler-Fiorenza Rose SM, Contrepois K, Moneghetti KJ, Zhou W, Mishra T, Mataraso S, Dagan-Rosenfeld O, Ganz AB, Dunn J, Hornburg D, Rego S, Perelman D, Ahadi S, Sailani MR, Zhou Y, Leopold SR, Chen J, Ashland M, Christle JW, Avina M, Limcaoco P, Ruiz C, Tan M, Butte AJ, Weinstock GM, Slavich GM, Sodergren E, McLaughlin TL, Haddad F and Snyder MP (2019) A longitudinal big data approach for precision health. *Nature Medicine* 25, 792–804.
- Selker HP, Cohen T, D'Agostino RB, Dere WH, Ghaemi SN, Honig PK, Kaitin KI, Kaplan HC, Kravitz RL, Larholt K, McElwee NE, Oye KA, Palm ME, Peretto E, Ramanathan C, Schmid CH, Seyfert-Margolis V, Trusheim M and Eichler HG (2022) A useful and sustainable role for N-of-1 trials in the healthcare ecosystem. *Clinical Pharmacology and Therapeutics* 112, 224–232.
- Senn S (1998) Applying results of randomised trials to patients. N of 1 trials are needed. *British Medical Journal* 317, 537–538.
- Siddiqi SH, Weigand A, Pascual-Leone A and Fox MD (2021) Identification of personalized transcranial magnetic stimulation targets based on subgenual cingulate connectivity: An independent replication. *Biological Psychiatry* 90, e55–e56.
- Skou ST, Mair FS, Fortin M, Guthrie B, Nunes BP, Miranda JJ, Boyd CM, Pati S, Mtenga S and Smith SM (2022) Multimorbidity. *Nature Reviews Disease Primers* 8, 48.
- Somer E, Gische C and Miocevic M (2022) Methods for modeling autocorrelation and handling missing data in mediation analysis in single case experimental designs (SCEDs). *Evaluation and the Health Professions* 45, 36–53.
- Tabachnick GB and Fidell LS (2012) *Using Multivariate Statistics*. New York: Pearson.
- Tehrani F, Teymourian H, Wuerstle B, Kavner J, Patel R, Furmidge A, Aghavali R, Hosseini-Toudeshki H, Brown C, Zhang F, Mahato K, Li Z, Barfidokht A, Yin L, Warren P, Huang N, Patel Z, Mercier PP and Wang J (2022) An integrated wearable microneedle array for the continuous monitoring of multiple biomarkers in interstitial fluid. *Nature Biomedical Engineering* 6, 1214–1224.
- Triolo F, Harber-Aschan L, Murri MB, Calderon-Larranaga A, Vetrano DL, Sjoberg L, Marengoni A and Dekhtyar S (2020) The complex interplay between depression and multimorbidity in late life: Risks and pathways. *Mechanisms of Ageing and Development* 192, 111383.
- Viana JN, Edney S, Gondalia S, Mauch C, Sellak H, O'Callaghan N and Ryan JC (2021) Trends and gaps in precision health research: A scoping review. *British Medical Journal Open* 11, e056938.
- Wang Y and Schork NJ (2019) Power and design issues in crossover-based N-of-1 clinical trials with fixed data collection periods. *Healthcare (Basel)* 7, E84.
- Wicks P, Vaughan TE, Massagli MP and Heywood J (2011) Accelerated clinical discovery using self-reported patient data collected online and a patient-matching algorithm. *Nature Biotechnology* 29, 411–414.
- Williams LM, Coman JT, Stetz PC, Walker NC, Kozel FA, George MS, Yoon J, Hack LM, Madore MR, Lim KO, Philip NS and Holtzheimer PE (2021) Identifying response and predictive biomarkers for transcranial magnetic stimulation outcomes: Protocol and rationale for a mechanistic study of functional neuroimaging and behavioral biomarkers in veterans with pharmacoresistant depression. *BMC Psychiatry* 21, 35.
- Yeboah E, Mauer NS, Hufstедler H, Carr S, Matthay EC, Maxwell L, Rahman S, Debray T, de Jong VMT, Campbell H, Gustafson P, Janisch T and Barnighausen T (2021) Current trends in the application of causal inference methods to pooled longitudinal non-randomised data: A protocol for a methodological systematic review. *British Medical Journal Open* 11, e052969.
- Zeggini E, Gloyn AL, Barton AC and Wain LV (2019) Translational genomics and precision medicine: Moving from the lab to the clinic. *Science* 365, 1409–1413.
- Zhao L, Hu XJ and Lagakos SW (2009) Statistical monitoring of clinical trials with multivariate response and/or multiple arms: A flexible approach. *Biostatistics* 10, 310–323.
- Zimmer A, Korem Y, Rappaport N, Wilmanski T, Baloni P, Jade K, Robinson M, Magis AT, Lovejoy J, Gibbons SM, Hood L and Price ND (2021) The geometry of clinical labs and wellness states from deeply phenotyped humans. *Nature Communications* 12, 3578.
- Zucker DR, Ruthazer R and Schmid CH (2010) Individual (N-of-1) trials can be combined to give population comparative treatment effect estimates: Methodologic considerations. *Journal of Clinical Epidemiology* 63, 1312–1323.