PERSPECTIVE

N-of-1 Trials in Cancer Drug Development

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Summary: The current approaches for cancer drug development lag behind an accelerated need in the field for a fast and efficient method for evaluating drugs in the personalized medicine era. In that regard, N-of-1 studies emerge as a potential addition to the drug development arsenal, although there are several considerations before its broad application becomes feasible. In essence, N-of-1 trials are a departure from the traditional "drug-centric" model to a "patient-centric" model. Herein, we review the concept of N-of-1 trials and provide real-world examples of their use in the developmental therapeutics field. N-of-1 trials offer an exceptional opportunity for fast-tracking of cancer drug development in the precision oncology era.

INTRODUCTION

Precision oncology—that is, specifically targeting a tumor's genetic liabilities—offers the promise of durable clinical benefit for the treatment of cancer. As such, tailoring therapeutic strategies by leveraging an ever-expanding array of high-throughput methods such as next-generation sequencing (NGS) has heralded a new age in solid tumor oncology (1). The notion that specific drugs, only targeting cells carrying a molecular liability such as a mutation, fusion protein, or aberrantly expressed epitopes, would yield significantly better outcomes than chemotherapeutic cytoreduction is the main driver of this Copernican revolution.

Thus, the era of precision oncology relies on the effective linking of identified genomic and/or immunologic vulnerabilities with the discovery of drugs targeting them within a bench-to-bedside framework. At the same time, this evolution is accelerated by both the increasing approval rate of novel therapies and the constant decline in the cost of NGS (2, 3). Moreover, modern approaches in medicinal chemistry are increasingly exploring previously undruggable cancer targets with accumulating success (4, 5). Additionally, tumor-agnostic approvals for biomarker-driven histology-independent therapeutics complement current histology-driven efforts and offer an additional pathway for drug development and access (6–9).

Although this progress is substantially promising, it raises a concern about whether current tools evaluating the therapeutic efficacy of novel cancer drugs meet the intended clinical expectations (10, 11). The conventional framework of drug development strategies bears a substantial lag time (12). The advent of targeted therapies, immunotherapies, and now CRISPR–Cas9 technologies has advanced the field, but novel trial designs to fast-track drug development are warranted (13). Herein, N-of-1 trials emerge as an exciting tool that provides a glimpse of safety and activity before large-scale trials are executed (Fig. 1A). In this article, we discuss the potential role of N-of-1 trials in cancer drug development in the era of personalized cancer medicine. We also elaborate on a suggested modified design for N-of-1 trials able to overcome limitations and make this concept more applicable to clinical oncology.

N-OF-1 TRIALS

N-of-1 studies are trial study designs in which a single patient is the sole unit of analysis and acts as his or her own control (14). The concept was initially used in humanitarian studies, but its introduction to modern medicine occurred several decades ago (15–18). Still, this design's success has been primarily observed in studies focusing on chronic diseases with a subjective patient-reported outcome (19). Traditionally, in N-of-1 designs, patients usually receive different treatment options or a placebo in sequential order with a possible washout period in between (Fig. 1B). This allows for intraperson comparison between different comparators and overcomes the possibility of interpatient heterogeneities observed in classic clinical trial designs. Controlled N-of-1 trials can be randomized or blinded just as in classic trial methodologies but with the added benefit of eliminating treatment-unrelated differences between study participants (14).

HISTORICAL PERSPECTIVE

A classic example of an N-of-1 trial can be understood using the example of a study by Molloy and colleagues, who performed a series of N-of-1 studies in patients with Alzheimer disease (20). Individual patients received 3 weeks of tetrahydroaminoacridine and 3 weeks of placebo for three treatment periods in a randomized, double-blind design (20). Similarly, a more recent application of the classic N-of-1 trial design was used for the assessment of the effectiveness of traditional

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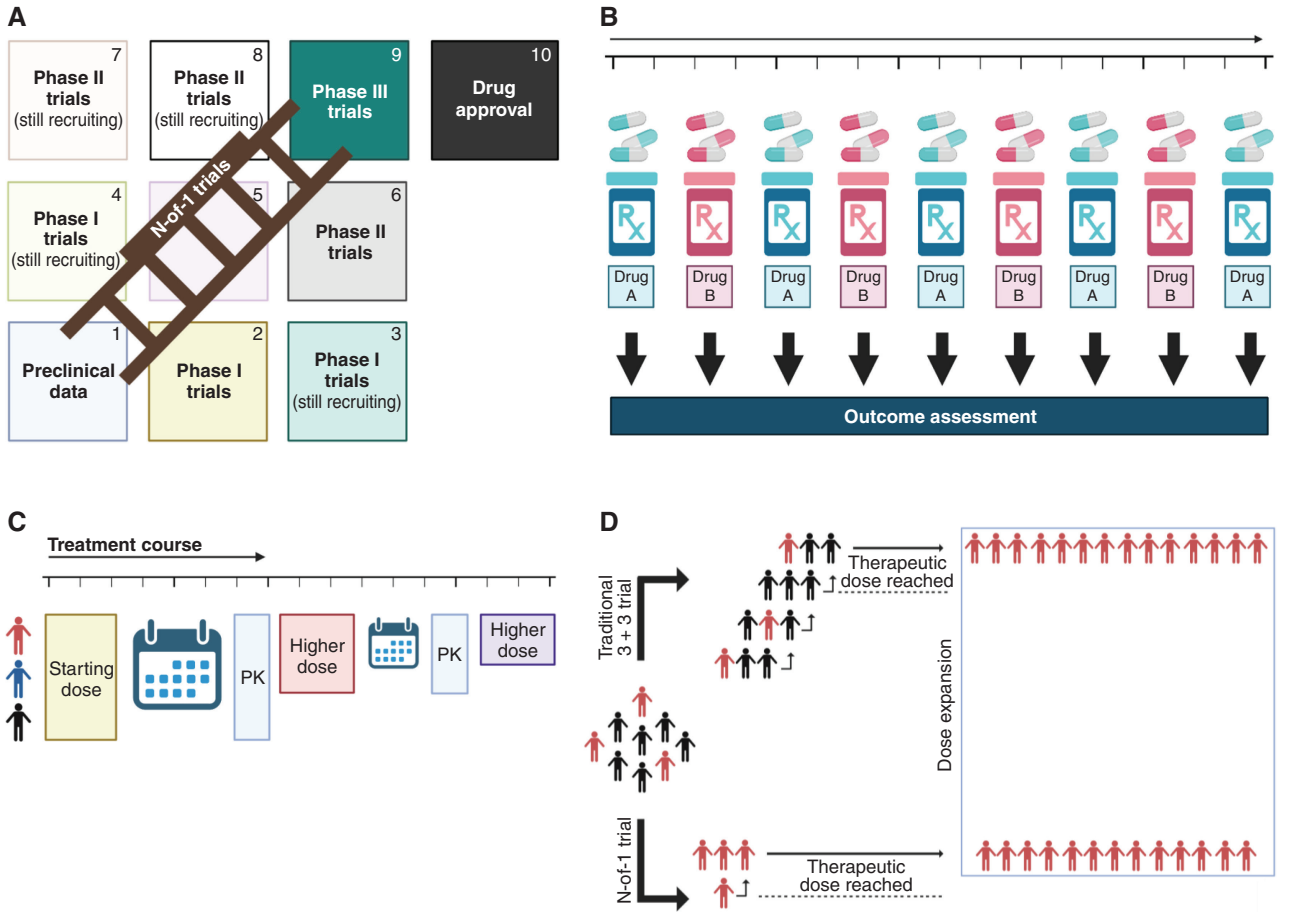


Figure 1. **A,** N-of-1 trials can enable the fast-tracking of cancer drug development. With a substantially long duration for patient recruitment in traditional early-phase trials, especially in biomarker-driven therapeutics that target rare molecular alterations, N-of-1 trials can provide a tool for speeding up drug development via intrapatient dose escalation, which also overcomes the subtherapeutic dosing in patients in the lower dose cohorts in conventional trials. **B,** Classic design of sequential N-of-1 trials. Patients receive different treatment options or a placebo in sequential order with a possible washout period, which allows for intrapatient comparisons. **C,** Model for a pharmacokinetic (PK)-guided real-time dose escalation in single-patient protocols. **D,** Such a PK-guided approach can fast-track drug development compared to conventional study designs. For example, the conventional 3 + 3 design for phase trials would allow dose escalation based on the observed toxicity profile in the current cohort. The incremental increase in dose would be tested at the next dose level and would lead to prolonged time for trial completion, as well as suboptimal dosing in some patients despite the biological plausibility of the investigated agent. Fig. 1D is adapted from Drilon and colleagues (24). Created with BioRender.com.

Chinese medicine (TCM) on chemotherapy-induced leukopenia. Patients with gastric cancer after gastrectomy received 3 weeks of standard chemotherapy and 3 days of TCM in a randomized, double-blinded, controlled N-of-1 trial design (21). Because N-of-1 trials allow for the integration of patients' input into outcome assessment, they have been commonly used to assess the usefulness of psychiatric interventions as well. For example, different N-of-1 trials were conducted to assess the efficacy of possible off-label pharmacologic interventions in treatment-resistant depression (22). Several other examples exist in different specialties of the medical field, but most were focused on neuropsychiatric disorders (23).

N-OF-1 TRIALS IN ONCOLOGY

In oncology, however, such designs can be challenging and even infeasible for patients with metastatic cancer due to disease burden and dynamic disease progression. One fundamental requirement for N-of-1 trials is their use in chronic and stable diseases with different treatments that have comparable efficacy. In addition, frequently changing an already working drug with a washout period is another ethical dilemma. Response assessment is another critical issue for N-of-1 designs in oncology, since time to response may vary between different treatment options with variable mechanisms of action. All of the above reasons represent challenges for implementing N-of-1 trial designs in oncology and thereby hamper their adoption during drug development.

Still, the N-of-1 concept stands out, fascinating oncologists versed in applying targeted therapies. Current treatment options are mostly biologically driven, and the conventional study designs for clinical trials may be deficient in their ability to promptly translate recent scientific insights. For example, the dose-escalation approach in the classic 3 + 3 design for early testing of drugs with preclinical activity requires a relatively long duration for patient recruitment, which is

especially true for biomarker-oriented drugs that target rare molecular lesions. In addition, ethical considerations emerge as some patient cohorts may receive nontherapeutic doses at low dosage levels. Importantly, molecular heterogeneity between study subjects, which most likely drives observed differences in both responses and resistance, is another issue that classic designs fail to cope with. Therefore, it is imperative that new perspectives in the drug development process evolve to accommodate the changing landscape of precision oncology drug needs.

FROM A DRUG-CENTRIC TO A PATIENT-CENTRIC MODEL FAST-TRACKING DRUG DEVELOPMENT

Current clinical trials are designed using a drug-centric model. An N-of-1 trial is a departure from this, as it proposes a patient-centric model. In this regard, a patient-centered N-of-1 trial with a modified design standardized to assess different dosages in the same patient (instead of the regular sequential N-of-1 design) may lead to enhanced and swift development of novel agents. As such, patients act as their own control, effectively eliminating any possible interpatient heterogeneity bias. Patients can also provide feedback that can be informative given the recent increasing interest in patient-reported outcomes. Aggregating data from different N-of-1 trials can then provide input that can further advance drug development based on a cumulative analysis. Below we discuss some cases of N-of-1 studies in precision oncology that could fast-track drug development.

Second-Generation NTRK Inhibitor Development in Real Time

A model of the adjusted N-of-1 trial design was proposed by Drilon and colleagues in 2017 (Fig. 1C and D; ref. 24). Drilon and colleagues used that model for treating two patients who received larotrectinib for *TRK* fusion–positive cancers. Both patients developed an acquired resistance to larotrectinib that was attributed to G595R and G623R mutations. Following promising preclinical data for LOXO-195 (now being investigated as selitrectinib)—which was demonstrated to possess activity against *NTRK* resistance mutations, including solvent-front mutations and xDFG substitutions (25)—patients started treatment with LOXO-195 using single-patient protocols. Intrapatient dose escalation was guided by real-time pharmacokinetics, and a tolerated dose of 100 mg twice daily in both patients led to a sustained clinical and radiologic response (24).

Selective RET Inhibitor Drug Development

The same study design was used in another study for treating two patients with *RET* alterations. Both patients, one with a RET^{M918T} mutation and the other with a *KIF5B–RET* fusion, received experimental LOXO-292 (now FDA approved as selpercatinib) following progression on multikinase inhibitors and with limited availability of treatment alternatives. The patient with the RET^{M918T}-mutant tumor progressed on six different multikinase therapies and developed a RET^{V804M} gatekeeper mutation. In the context of a rapidly declining Eastern Cooperative Oncology Group performance status, diarrhea of up to 30 bowel movements a day, and no approved therapy to overcome gatekeeper mutation, an N-of-1 trial was designed. Intrapatient pharmacokinetic-guided dose escalation was performed at ≥7-day intervals based on a predefined dose-escalation protocol (26).

Responses to treatment with selpercatinib were very encouraging. For example, the first patient showed substantial symptomatic relief from the pretreatment tumor-related right upper quadrant pain, ascites, and diarrhea. Tumor markers (calcitonin and carcinoembryonic antigen) decreased following treatment with selpercatinib, and a radiographic partial tumor response was observed. Also, variant allele frequency for *RET*-relevant mutations, which was assessed in cell-free DNA, decreased following treatment initiation and remained suppressed. Similar results were observed in the second patient, who also exhibited a symptomatic and radiologic response to treatment, including regression of intracranial brain metastases (26). Interestingly, the tolerated dose in this N-of-1 trial, 160 mg, was the same as that reported in the later-conducted classic phase I trial of selpercatinib. The same dose was tested in phase II studies and is the currently approved dose for selpercatinib for treating *RET* fusion–positive non–small cell lung cancer (NSCLC), *RET*-mutant medullary thyroid cancer, *RET* fusion–positive thyroid cancer, and *RET* fusion–positive solid tumors (9, 27, 28). This patient was followed with serial cell-free DNA collections and eventually developed nongatekeeper resistance mechanisms, namely, *RET* solvent-front mutations $RET^{G810S/C}$ and $RET^{Y806C/N}$ (29). These novel nongatekeeper mutations were tested preclinically and were found to be resistant against both selective RET inhibitors, selpercatinib and pralsetinib (29, 30). This was the basis for the development of second-generation RET inhibitors that cover both solvent front and gatekeeper and are currently in development. This is a prime example of how N-of-1 studies can not only address response and efficacy but also unravel possible resistance mechanisms.

CUSTOMIZED COMBINATION N-OF-1 TRIALS

Similar studies with single-patient protocols, not necessarily following the same proposed model, in different tumor types and with different early therapeutics are not infrequent. In fact, oncologists have long practiced the use of single-patient studies for testing newer antineoplastic agents or newer combinations. For example, in four patients with *RET* fusion–positive NSCLC who were previously treated with selpercatinib and progressed via *MET*-dependent resistance mechanism, adding crizotinib to selpercatinib (via four separate N-of-1 trials) led to sustained clinical activity in all patients who tolerated treatment well (31). Another study explored the value of adding BLU-667, now approved as pralsetinib, as a treatment for patients who developed resistance to the EGFR inhibitor osimertinib via off-target acquired *RET* fusions. Treatment of two patients showed rapid radiographic responses and suggested that a combination of selective RET and EGFR inhibitors can overcome secondary resistance to osimertinib (32).

Such examples provide a proof of concept on how helpful N-of-1 trials can be in treating patients with emerging resistance to targeted therapies. Because resistance mechanisms are very complex and heterogenous, a one-size-fits-all approach

might not be feasible to meet the increasing demand over the next decade. With the wide implementation of targeted therapies and the number of possible resistance mechanisms for each therapeutic agent, it is not practical or possible to use conventional designs for addressing each resistance mechanism in a classically designed clinical trial (e.g., *MET* amplification in *RET*-altered patients who received RET inhibitor therapy, as mentioned above).

Moreover, although the acquired resistance mechanism may be identified, it may be too late for the subject under investigation in many cases. In a patient with *RET* fusion–positive lung cancer treated with selpercatinib, an *NTRK3* fusion (K8;N14) was detected in the resistant tumor sample. To show proof of concept, the investigators performed preclinical experiments to show that BaF3 cells coexpressing *KIF5B–RET* and *KHDRBS1–NTRK3* were resistant to selpercatinib and cotreatment of these cells with selpercatinib and larotrectinib suppressed active *ERK1/2* and *AKT* and induced apoptosis of these cells. Unfortunately, this was identified too late, as the patient expired soon after (33). In a classic trial design, it could have taken years before this patient could enroll in a phase I trial evaluating the proposed combination. However, had an N-of-1 trial been feasible, the preclinically proven combination could likely have been fast-tracked and tested in a clinical setting that could have helped this patient and others with the same mechanism of resistance.

So, in the context of the rapidly evolving mechanisms of resistance to targeted therapies, N-of-1 trials offer a tool that can accommodate heterogeneous variations and provide evidence that can fast-track drug development and guide treatment decisions in patients with similar clinical scenarios. In an era when tissue-agnostic therapeutics are being widely explored in the precision oncology field, this is of the essence. N-of-1 trials can help provide efficacy data on some rare tumor types and also offer a chance for possible exploration of combination strategies that can overcome inherent resistant to tissue-agnostic therapies in certain sites that are not necessarily common.

N-OF-1 TRIALS IN PEDIATRIC ONCOLOGY

N-of-1 studies in the pediatric population come with their own challenges both ethically and scientifically to justify experimentation. However, we can learn from multiple single-patient protocols that have been reported. For example, Zhang and colleagues (34) used the NOTCH and γ-secretase inhibitor LY3039478 in a child patient with a metastatic glomus tumor that harbored a *CARMN–NOTCH1* fusion. With no approved therapies and only preclinical evidence, an N-of-1 trial was designed. In the first evaluation 2 months after treatment initiation, the patient had decreased FDG uptake despite having stable disease measurements. The patient continued treatment for 3 years and 4 months and achieved stable disease even after treatment discontinuation (34). In another child, a *SPECC1L–ALK* fusion was detected in high-grade glioma. In this case, the patient suffered intracranial bleeding, do-not-resuscitate was ordered, and despite the withdrawal of life support, the patient continued to breathe and show vital signs. Lorlatinib, an inhibitor of ALK, was started via a nasogastric tube, and subsequent scans showed a dramatic response with a near-complete remission of all lesions. At the time of reporting, the child was attending preschool without any appreciable neurologic deficits. The patient achieved a sustained response to lorlatinib allowing for further gross total resection not previously feasible (35). A third baby girl with a congenital spinal cord glioblastoma and *NTRK* fusion was treated with concurrent chemotherapy and larotrectinib, a TRK inhibitor, and showed a complete resolution of tumor tissue in imaging and a sustained clinical response with no evidence of disease at 17 months (36). Without single-patient protocols for using drugs with preclinical evidence on activity or evidence in another patient population, those patients would not have survived further given the lack of other treatment options and possible early-phase trials for their rare tumor types.

OTHER EXAMPLES OF N-OF-1 TRIALS IN ONCOLOGY

The list of studies that used single-patient protocols for early assessment of investigational cancer drugs is long. Again, physicians in academic centers have long used this investigational tool but without completely understanding its full potential. Moreover, without a standardized definition of design and framework, most of these studies were reported or perceived as merely case reports, which limited their potential to generate reliable evidence. But are N-of-1 just case reports? N-of-1-trials are far beyond case reports, with deeper understanding of the disease process and more comprehensive evaluation and follow-up. These test personalized and customized therapies in a single patient. It is true that there is an overlap between the ways both N-of-1 trials and case reports are reported, but the difference is usually substantial. For example, a patient with *KRAS*^{G12D}-mutant therapy-refractory pancreatic cancer was treated with neoantigen T-cell receptor gene therapy using a single-patient protocol. Using T cells that had been genetically engineered to express T-cell receptors targeting this patient's particular mutation, a specifically custom-made and designed therapy was administered to the patient, who showed a partial response upon restaging (37). Another patient with Pacak–Zhuang syndrome, a rare condition characterized by polycythemia and multiple paragangliomas, was treated with belzutifan, which is a potent HIF2α inhibitor. As Pacak–Zhuang syndrome results from mutations in the *EPAS1* gene encoding HIF2α and there were risks associated with alternative treatment options, the patient was offered investigational treatment with belzutifan, which led to a rapid response, including resolution of most symptoms (38). This is an example of an N-of-1 trial based on biology and cellular mechanisms intercepting the aberrant pathway in a rare disease. In a patient with acute myeloid leukemia and an *ETV6–NTRK2* fusion who had no other treatment options, partial remission was achieved using larotrectinib in a single-patient protocol under FDA expanded access. This followed preclinical testing in patient-derived xenograft models that showed potential sensitivity to larotrectinib (39). Hence, there are two major dissimilarities between case reports and N-of-1 trials: (i) N-of-1 trials are exploring investigational agents that have not yet been approved, and (ii) N-of-1 trials are practically first-in-human studies, which may use a more systematic way

for clinical investigation that may include intrapatient dose testing.

MOLECULAR PROFILING GUIDING CUSTOMIZED N-OF-1 DESIGN

In a trial to bridge the gap between individual study designs and clinical practice, Sicklick and colleagues (40, 41) implemented the I-PREDICT study (NCT02534675) to administer multidrug personalized treatment with combination therapies to patients with advanced cancers. The master protocol allowed patients with diverse cancer types to receive more than one molecular-matched treatment that was individualized according to their molecular profiles. The study showed successful results in treatments of both treatment-naive and previously treated patients introducing the concept of molecular matching and individualized investigation of molecularly based combination treatment options (40, 41). Because some combinations were used *de novo*, and in a trial to limit possible toxicities, the authors used 50% of the usual dose of each drug in doublet combinations and 33% in triplet combinations. They were then able to perform intrapatient dose escalation, which led to real-time determination of appropriate doses in each combination. Dosing strategies were discussed in molecular tumor boards, with input from clinical pharmacists based on prior relevant literature (40, 41).

The I-PREDICT study has in fact addressed the key challenge of individualized treatment assessment: feasibility. Although N-of-1 trial designs appear tempting, large-scale implementation has always been hindered by applicability and difficult statistical analysis. It is true that aggregation of summary statistics can generate group comparisons between mean treatment effects (42). However, such an approach was usually limited by design heterogeneity and applied therapeutic options. Therefore, without a standardized approach for performing those N-of-1 studies with the concepts of trial designs in mind, results would have remained heterogeneous, hard to combine, and useful only as preliminary clinical data. In that context, I-PREDICT was a departure of the N-of-1 norm of single-patient protocols, as it proved the feasibility of designing multiple N-of-1 customized therapies for patients in an academic center under a single umbrella protocol.

N-OF-1 AS A TOOL IN THE ERA OF LIQUID BIOPSY

With advances in liquid biopsy technologies, serial tracking of tumor behavior including molecular profiles became appealing to many clinicians. For example, using simple blood analysis, physicians can monitor the clonal evolution of their patients' cancer in real time. Such accessibility to real-time analysis was not previously possible and necessitates a new model of cancer management that can allow for dynamic changes in management based on liquid biopsy. In this setting, an N-of-1 trial might emerge as a more flexible and appropriate tool for assessing new therapeutics than conventional clinical trial designs. Evaluation models have been proposed including one by Silvestris and colleagues (43), who suggested comparing progression-free survival in the same patient between the new drug and the older regimens. Although such models do not account for heterogeneity in responses between different regimens, it may be beneficial in targeted therapies working on the same target (43).

MODERN N-OF-1 TRIAL DESIGN BEYOND ONCOLOGY

Beyond oncology, the same design can still be applicable, and hence collaborative work between different fields might help in the conceptualization of a standardized way for performing N-of-1 trials. For example, an approach for customized design of oligonucleotide therapy was used by Kim and colleagues (44) to treat a young patient with the rare genetic neurodegenerative condition of neuronal ceroid lipofuscinosis 7 (CLN7). Investigators first identified the genetic mutation of concern in their 6-year-old patient, whom they assumed would benefit from a patient-customized oligonucleotide therapy. Next, they used the investigational agent in rats to study potential toxicity before starting the testing in the patient. They then performed intrapatient dose escalation until reaching the maintenance dose that established clinical efficacy with a tolerable toxicity profile (44). This application and others are proof of the feasibility of using such designs in developing drugs with varying mechanisms of action and in different clinical scenarios, including those related to cancer-related conditions and different cancer types.

REGULATORY CHALLENGES FOR N-OF-1 STUDIES

One important aspect that needs to be addressed with a proposal for the wide-scale adoption of N-of-1 studies is drug approval regulation. For example, there are certainly challenges in identifying cutoffs for preclinical data that are considered safe for human subject testing (45). However, current approaches used to establish enough preclinical evidence on biological plausibility before phase I trial initiation can be hypothetically used for N-of-1 initiation. It is possible that a future implementation of artificial intelligence in the drug development industry might be helpful in identifying agents with potential efficacy (46). Also, how single-patient trials can be pooled to inform evidence generation and how drug approval regulations can be adapted to the proposed models is another important aspect (45). Such issues will necessitate thorough discussions that include experts in drug development, early-phase trials, statistics, regulatory approvals, and public health. However, as we have more trials underway, we might be able to gain insight into the best approach that can ultimately be used for creating new regulations.

Another issue related to regulatory challenges is how patients will access investigational drugs before approval. Right now, there are two pathways that permit the use of non-approved agents in an N-of-1 setting. These are expanded access use and right to try use (47). Although these pathways were primarily designed to allow access to drugs for patients who are trial ineligible, their possible use in patients who might have other options should still be considered as we start adopting the N-of-1 approach, because this can allow individualized drug development for each patient.

EVIDENCE GENERATION FROM N-OF-1 STUDIES

Accumulating data from different N-of-1 trials can be practically summated to establish important endpoints similar to those in phase I/II designs. For example, data from multiple N-of-1 trials can be used to establish a recommended phase II dose and even preliminary efficacy. Toxicity profiles from different patients in different studies can also be used to understand possible side effects and approaches to mitigate. With more standardized conduct of N-of-1 trials, data generated should be of high quality to inform clinical evidence (Fig. 2A).

Response and anticipated duration of response can, however, be variable based on tumor type and patient-to-patient differences within the same tumor type. This can establish the rationale for needing control arms in studies addressing clinical efficacy of newer agents, which is not present in N-of-1 trials. However, patients can still act as their own control, and an exploratory comparison can be made with patients' last and previous therapies. In this case, a progression-free survival on N-of-1 studies can be compared with patients' progression-free survival while on prior therapies. This calculation can be similar to growth modulation index (GMI; refs. 9, 48). GMI is the ratio of time spent on an N-of-1 trial to time spent on last previous therapy. A GMI of >1.33 is generally used as an indicator of the clinical activity/efficacy of a drug. With genomically targeted agents, additional measures could be collected, such as time to emergence of a specific resistance mechanism in liquid biopsy and how that compares with RECIST-based imaging resistance/progression.

One issue would be how to place evidence generated from N-of-1 trials into current evidence pyramids. Because the proposed model offers a prospective controlled study design in which patients act as their own controls, it would be plausible to place such evidence higher in the evidence pyramid than case reports, case series, and case–control studies (Fig. 2B). Most of these studies either are not systematically designed or use a different control cohort that might vary in its inherent characteristics from the patient cohort because each patient is genomically unique. A higher position in the evidence pyramid might be possible for aggregated data from N-of-1 trials, but this will remain controversial, as the design evolves to accommodate the growing needs in oncology (46).

A key challenge related to evidence generation from N-of-1 trials would be the possible underreporting of negative studies. This is not purely a design limitation but rather a publication bias that might be currently affecting other study designs, although it can certainly be worse with a negative study reporting on data from only one patient (49). Access to FDA data from different N-of-1 trials might be another relevant issue that currently limits pooled analysis but will hopefully improve with further discussions and more wide-scale acceptance of the notion of open data sharing (50). Whether a central registry for recording results of individual N-of-1 trials can be adapted on a wide scale remains a challenging but feasible solution.

N-OF-1 TRIALS AND THE MOLECULAR TUMOR BOARD: A SYMBIOTIC RELATIONSHIP

As the vehicle driving precision oncology approaches in the day-by-day clinical routine, molecular tumor boards (Fig. 2C) became an integral part of comprehensive cancer centers across the globe (51, 52). Exploiting state-of-the-art high-throughput sequencing methods, they drastically expand the data complexity within individual patient's cases, enabling oncologists to apply therapies based on more detailed information than ever before. Even though many riddles remain unsolved and most of the generated data have not immediately translated into therapy yet, the molecular tumor board offers the most granular library of tumor characteristics, allowing for subtype differentiation beyond the expression of surface markers or singular mutations. The exponential increase in resolution requires an exponential growth of clinical trials to test treatments in the newly defined subgroups. Although the conventional pipeline of drug testing from phase I to III offers robust results, it is not suitable to test an increasing number of therapeutic approaches quickly. N-of-1 trials may function as a feasible methodology to test molecularly informed hypotheses in an agile manner, enabling the molecular tumor board to reach more of its potential. By default, any case treated based on a molecular tumor board recommendation can be considered an N-of-1 trial if monitored and evaluated accordingly.

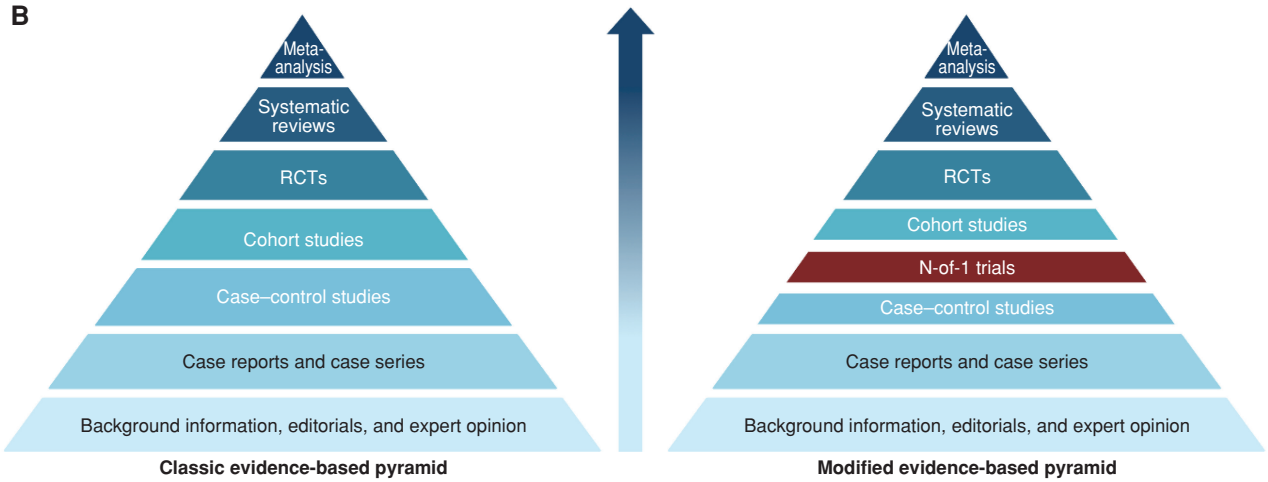
DIGITAL TWINS

Both the increasing availability of targeted molecular therapies, by virtue of exclusively targeting a genetic lesion without affecting healthy cells, and the increased availability of genomics portraying a tumor's genetic makeup shatter the tree of classic trial landscapes. As such, novel concepts emerge that try to encompass the benefits of precision medicine and apply these to patients affected by cancer. One of the trial concepts is the N-of-1 trial, whose benefits are discussed herein. However, unlike the ease of comparing clinical response across large patient collectives enrolled in phase III trials, interpatient response rates are, by definition, difficult to compare. The inherent heterogeneity, which bears a benefit when looking at effectiveness in one individual patient, becomes an Achilles' heel in defining evidence applicable to genetically distinct patients. Thus, sharing experiences from these N-of-1 trials will be instrumental to achieving clinical evidence, which can then be leveraged in subsequent cases. One proposed way to solve this asymmetry could be successful through the implementation of the use of so-called "digital twins." Digital twin refers to a virtual representation of

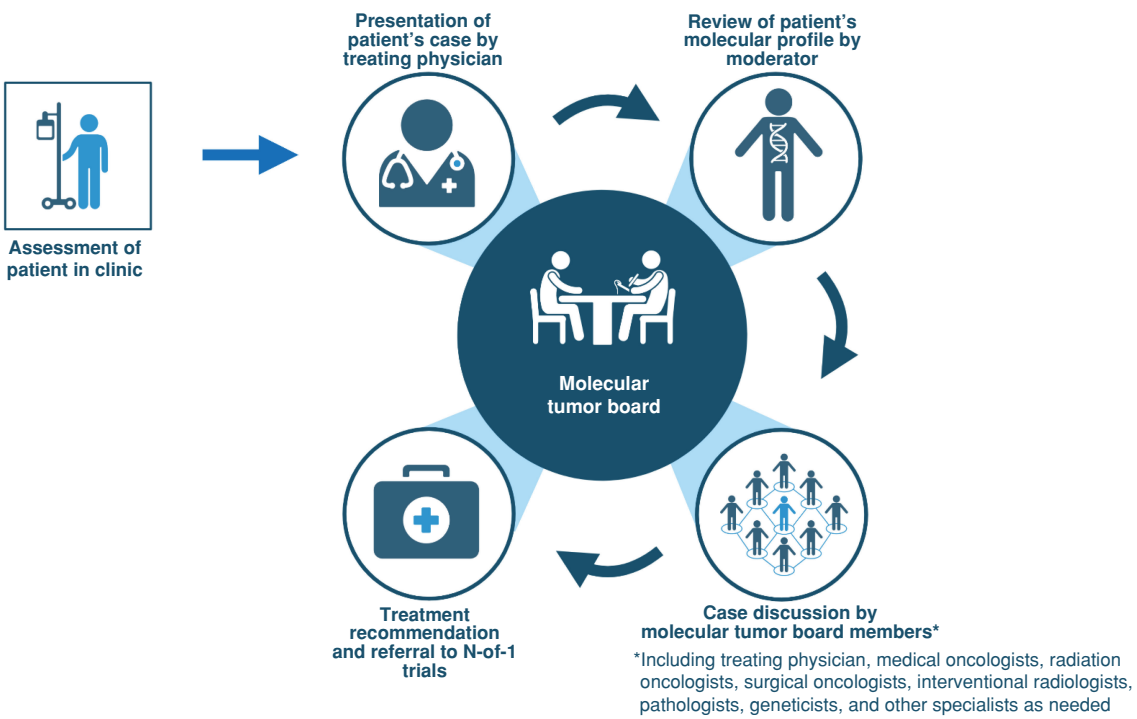
Figure 2. **A,** N-of-1 trials can act as the building blocks for evidence synthesis. **B,** Evidence-based medicine (EBM) pyramid and possible position of evidence generated from N-of-1 studies. In the classic EBM pyramid, N-of-1 trials can provide evidence higher in quality than case–control trials given that patients are used as their own controls. With more advances in designs of N-of-1 trials, this can even be moved up in the evidence hierarchy. RCT, randomized controlled trial. **C,** Molecular tumor boards as a tool for precision oncology in the molecular medicine era. The comprehensive discussions between different specialists in the molecular tumor board, including medical oncologists, radiation oncologists, surgeons, radiologists, pathologists, geneticists, and other specialists, can enable optimum drug selection for each individual patient based on the molecular profile. **A** was created with DrawImpacts, and **B** and **C** were created with BioRender.com.

A

B


Meta-analysis
Systematic reviews
RCTs
Cohort studies
Case–control studies
Case reports and case series
Background information, editorials, and expert opinion
Classic evidence-based pyramid
Meta-analysis
Systematic reviews
RCTs
Cohort studies
N-of-1 trials
Case–control studies
Case reports and case series
Background information, editorials, and expert opinion
Modified evidence-based pyramid

C


Assessment of patient in clinic
Presentation of patient's case by treating physician
Review of patient's molecular profile by moderator
Molecular tumor board
Treatment recommendation and referral to N-of-1 trials
Case discussion by molecular tumor board members*
*Including treating physician, medical oncologists, radiation oncologists, surgical oncologists, interventional radiologists, pathologists, geneticists, and other specialists as needed

a real-world system that serves as an indistinguishable counterpart (53, 54). Akin to snowflakes, each tumor is different at the molecular level. Each patient, even with an *NTRK* or a *RET* fusion, has different co-occurring alterations and are heterogeneous "malignant snowflakes" at the molecular level (1). The creation of a system for "patients like me" as a digital twin based on N-of-1 trials has the potential to further personalize precision medicine. Sharing of matched genomic and clinical data from N-of-1 trials will potentially yield the possibility of finding near-exact data of patients having received a particular therapy in the past, therefore serving as a blueprint for therapy-naive cases. The prerequisite for this will be 2-fold: (i) the readiness of investigators to openly share these data and (ii) defining which technological standards and encompassing bioinformatic algorithms should be used for matching. By doing so, clinical oncologists can leverage experience from N-of-1 trials in patients with similar genetic lesions, thus likely responding more predictably than usually expected from recommendations originating from the molecular tumor board or instigated by the N-of-1 trial itself. Various attempts have been made in the past to define the frameworks as well as specific thresholds that should be given importance. Still, most importantly these efforts will only collaboratively bear fruits, arguing for the importance of raising this concept within this discussion.

CONCLUDING REMARKS

In summary, N-of-1 trials offer an exceptional opportunity for advancing the field of cancer drug development through dynamic and speedy testing with patients used as their own controls. In the precision oncology era, they may aid in fast-tracking drug development. The traditional N-of-1 design may not be suitable given the aggressive nature of cancer and its management. However, a modified, standardized N-of-1 design, potentially reached through consensus discussions between drug development experts with a focus on intrapatient investigational possibilities (e.g., dose escalation), can be extremely useful in the better evaluation of newer antineoplastic agents.

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