Generating evidence for precision medicine: considerations made by the Ubiquitous Pharmacogenomics Consortium when designing and operationalizing the PREPARE study

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Objectives **Pharmacogenetic panel-based testing represents a new model for precision medicine. A sufficiently powered prospective study assessing the (cost-)effectiveness of a panel-based pharmacogenomics approach to guide pharmacotherapy is lacking. Therefore, the Ubiquitous Pharmacogenomics Consortium initiated the PREemptive Pharmacogenomic testing for prevention of Adverse drug Reactions (PREPARE) study. Here, we provide an overview of considerations made to mitigate multiple methodological challenges that emerged during the design.**

Methods **An evaluation of considerations made when designing the PREPARE study across six domains: study aims and design, primary endpoint definition and collection of adverse drug events, inclusion and exclusion criteria, target population, pharmacogenomics intervention strategy, and statistical analyses.**

Results **Challenges and respective solutions included: (1) defining and operationalizing a composite primary endpoint enabling measurement of the anticipated effect, by including only severe, causal, and drug genotype-associated adverse drug reactions; (2) avoiding overrepresentation of frequently prescribed drugs within the patient sample while maintaining external validity, by capping drugs of enrolment; (3) designing the pharmacogenomics intervention strategy to be applicable across ethnicities and healthcare settings; and (4) designing a statistical analysis plan to avoid dilution of effect by initially excluding patients without a gene–drug interaction in a gatekeeping analysis.**

Conclusion **Our design considerations will enable quantification of the collective clinical utility of a panel of pharmacogenomics-markers within one trial as a proof-of-concept for pharmacogenomics-guided pharmacotherapy across multiple actionable gene–drug interactions. These considerations may prove useful to other investigators aiming to generate evidence for precision medicine.**
Pharmacogenetics and Genomics **30: 131–144 Copyright © 2020 The Author(s). Published by Wolters Kluwer Health, Inc.**

Pharmacogenetics and Genomics 2020, **30**:131–144

Keywords: adverse drug reactions, clinical implementation, genotyping, next-generation sequencing, pharmacogenomics, pharmacogenetics, pre-emptive, randomized controlled trial

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Received 15 November 2019 **Accepted** 2 March 2020

1744-6872 Copyright © 2020 The Author(s). Published by Wolters Kluwer Health, Inc. DOI: 10.1097/FPC.0000000000000405

Introduction

One of the first applications of genetics in precision medicine is pharmacogenomics informed pharmacotherapy. It promises to personalize medicine by using an individual's germline genetic makeup, to guide optimal drug and dose selection [1,2]. This removes the traditional 'trial and error' approach of drug prescribing, thereby promising safer, more (cost-)effective drug treatment [3,4]. Since 2005, the Dutch Pharmacogenetics Working Group (DPWG) has systematically reviewed 102 potential gene–drug interactions resulting in 56 guidelines providing therapeutic recommendations [5,6]. In parallel, the Clinical Pharmacogenetics Implementation Consortium has reviewed over 360 potential gene–drug interactions resulting in 60 recommendations [7]. A promising model for delivering pharmacogenomics-guided pharmacotherapy is through pharmacogenomics panel-based testing. At least 95% of the population carries at least one genetic variant that would result in a pharmacotherapy adjustment according to the available guidelines. Furthermore, patients may over their lifetime be started on multiple drugs for which pharmacogenomics may be relevant [8,9]. In a panel-based model, multiple variants that affect drug response are tested simultaneously and stored in the electronic medical record (EMR) for future use. When a relevant drug is prescribed, the corresponding pharmacogenomics guideline can be deployed by the clinical decision support system (CDSS) at the point of care, thereby enabling healthcare professionals to use clinically actionable pharmacogenomics information during drug prescribing [10]. However, despite the demonstrated clinical utility of pre-emptive pharmacogenomics-testing for several individual gene–drug interactions, significant implementation barriers remain [11–13].

A prominent barrier preventing widespread adoption of pharmacogenomics panel-based testing is the lack of evidence supporting this approach. Although a number of small, randomized and observational studies have indicated the potential benefits of pharmacogenomics panel-based testing [14–17], a sufficiently powered prospective study assessing the clinical and cost-effectiveness of pre-emptive pharmacogenomics-testing is yet to be executed [18]. Therefore, the Ubiquitous Pharmacogenomics Consortium initiated the PREemptive Pharmacogenomic testing for Preventing Adverse drug Reactions (PREPARE) study, (ClinicalTrials.gov: NCT03093818). The PREPARE study aims to quantify the collective clinical utility and cost-effectiveness of a panel of pharmacogenomics-markers to guide dose and drug selection in reducing the risk of clinically relevant adverse drug reactions (ADRs) [19,20], and thereby providing clinical outcome data which has been cited as a contributing factor preventing uptake of pharmacogenomics [21]. The PREPARE study is also an implementation study aiming to assess the process indicators for implementation.

This paper outlines the multiple methodological and logistical challenges that were encountered while designing and operationalizing the PREPARE study, and the solutions developed to overcome these challenges. Similar difficulties are likely to confront other investigators aiming to generate evidence to show the utility of gene panel tests affecting multiple drugs in different therapeutic areas.

Methods

An evaluation of the challenges was undertaken across the following domains: study aims and design, primary endpoint definition and collection of adverse drug events, inclusion and exclusion criteria, target population, pharmacogenomics intervention strategy, and statistical analyses. The final design has previously been published elsewhere [19] (see Box 1 for a brief overview).

Results

The following sections discuss challenges and respective solutions considered by the Ubiquitous Pharmacogenomics Consortium when designing the PREPARE study. Figure 1 provides an overview of these challenges and the respective solutions.

Study aim and design

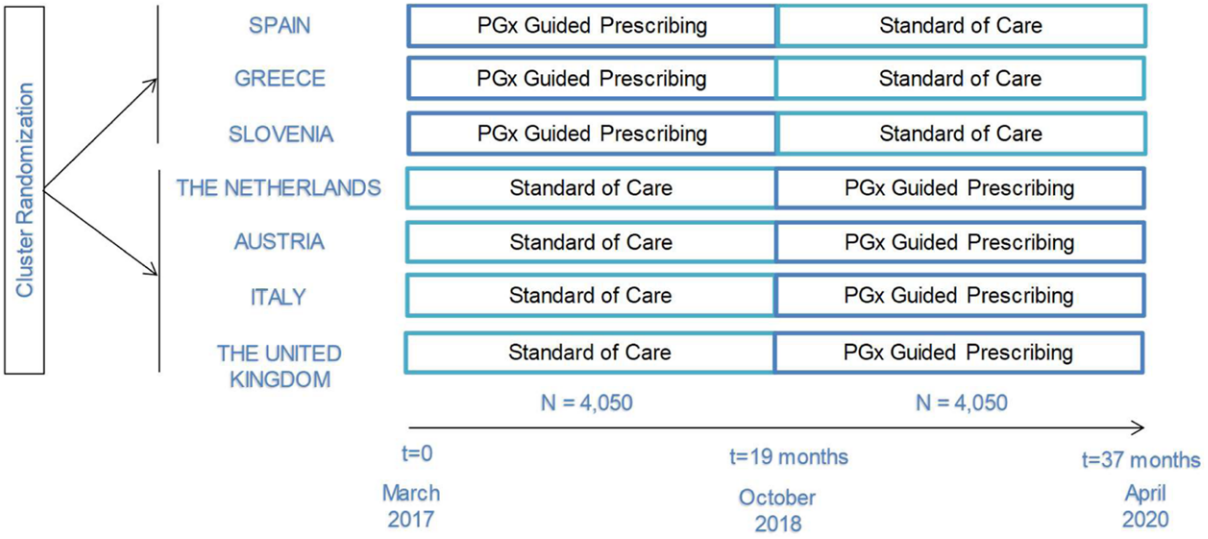
In the context of precision medicine, several fundamental options for generating evidence have been suggested such as observational research designed to identify modifiers of the effectiveness of interventions; subgroup analyses and interaction testing in standard randomized controlled trials (RCTs) of intervention effectiveness; or dedicated precision medicine RCTs that directly compare targeted vs. untargeted intervention approaches [22]. When generating evidence for pharmacogenomics effectiveness, we may envision an observational study wherein the available guidelines are implemented prospectively and compare a defined outcome with a historical control group, as recently performed for the dihydropyrimidine dehydrogenase gene (*DPYD*)–fluoropyrimidine interaction [23]. However, historical controls may likely only be feasible for patient populations who are closely monitored, such as those on high-risk drugs like fluoropyrimidines. Additionally, it is not considered ethical to prospectively recruit a control group for gene–drug interactions where there is sufficient evidence for clinical implementation as for example abacavir hypersensitivity. On the other hand, many drugs included in the DPWG guidelines are low-risk primary care drugs for which close monitoring is not routinely performed. Therefore, these studies are prone to certain forms of bias, such as information bias or selection bias.

An RCT can also, of course, be used to generate evidence. Indeed, several RCTs have provided gold-standard evidence showing the clinical utility of individual gene–drug interactions to guide dosing [3,24–26] and

Box. 1

Aim: In brief, PREPARE aims to quantify the collective clinical utility of a pre-emptive panel of PGx-markers on the occurrence of clinically relevant drug-genotype associated ADRs.

Design: PREPARE is a multi-center, open-label, cluster-randomized, cross-over implementation study, with partially blinded outcome assessment, conducted in seven countries across Europe (n=8,100). Countries are randomized to start with either PGx-guided prescribing (study arm) or standard of care (control arm) for 18 months. After this period, a new set of patients will be recruited and the opposite strategy implemented for 18 months.



Inclusion criteria: Adults of any ethnicity who receive a first prescription for one of 42 drugs for which a Dutch Pharmacogenomics Working Group (DPWG) guideline is available (1-3), are eligible for participation.

Intervention: All patients will donate a DNA sample that will be genotyped for a panel of 50 genetic variants in 13 pharmacogenes (4). For patients within the study arm, their results will be: 1) recorded in the (electronic) medical record to be utilized in future prescriptions and 2) provided to the patient in the form of plastic card, akin to a credit card (5). Pharmacotherapy initiation is not delayed by the turn-around time of the PGx panel test. PGx results and the relevant DPWG guideline can used by physicians and pharmacists to guide the dose and drug selection for the initial drug of enrolment, and for the prescription of any subsequent drugs for which a DPWG guideline is available. Physicians and pharmacists are given PGx test results within a seven day turn-around time but are not mandated to adhere to the DPWG guidelines.

Follow-up: Patients are followed-up through two methods; through a web-based survey at 2 and 8 weeks, and through live or telephone interviews at 4 weeks, 12 weeks and cross-sectionally at the end of each respective time-block.

Current status: PREPARE has been recruiting since March 2017 and has currently enrolled over 6,000 patients. Sites have crossed-over to the opposite strategy and recruit a new set of patients as of October 1st 2018. PREPARE enrollment is anticipated to be completed in April 2020.

Changes to the initial protocol: Since study initiation both the drugs of enrolment and PGx panel have been adjusted as per the study protocol. The panel currently tests 48 genetic variants in 13 pharmacogenes (where variants in *HLA-A* have been removed and variants in *NUDT15* have been added) and drugs of enrolment now consist of 39 drugs (where oxycodone, clozapine and carbamazepine have been removed). The first 18 month block was also prolonged with an additional month due to start up delays.

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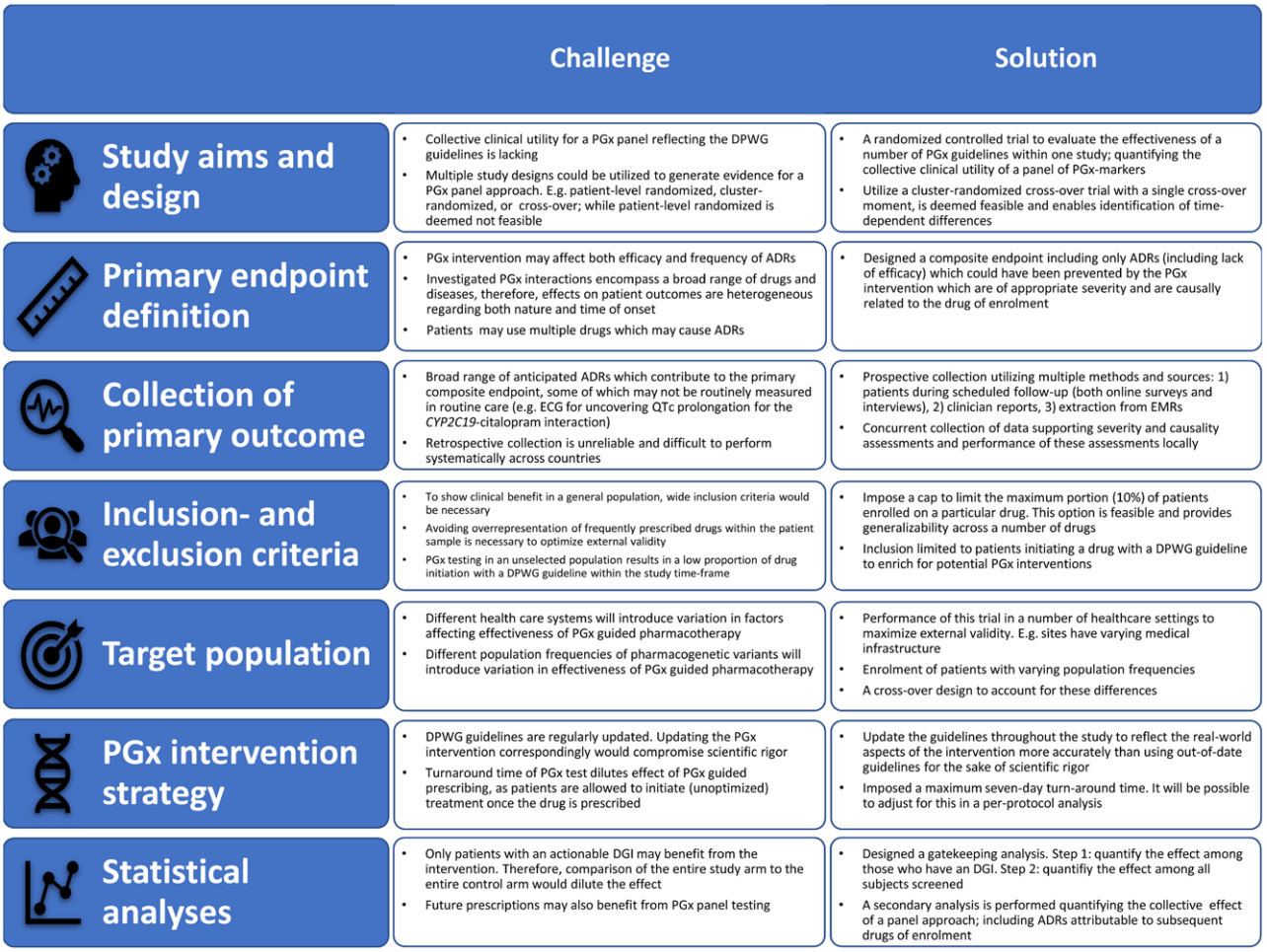
PREPARE study design in brief. PREPARE, PREemptive Pharmacogenomic testing for prevention of Adverse drug Reactions.

drug selection [27,28]. However, the DPWG has recommendations for 51 gene–drug interactions, most of which have been developed in the absence of RCTs. Some argue that gold-standard RCT evidence is required for each gene–drug interaction before undertaking clinical implementation [18]. By contract, others have argued that a mandatory requirement for prospective evidence to support the clinical validity for pharmacogenomics is

Fig. 1



An overview of challenges encountered by the Ubiquitous Pharmacogenomics Consortium and respective solutions utilized.

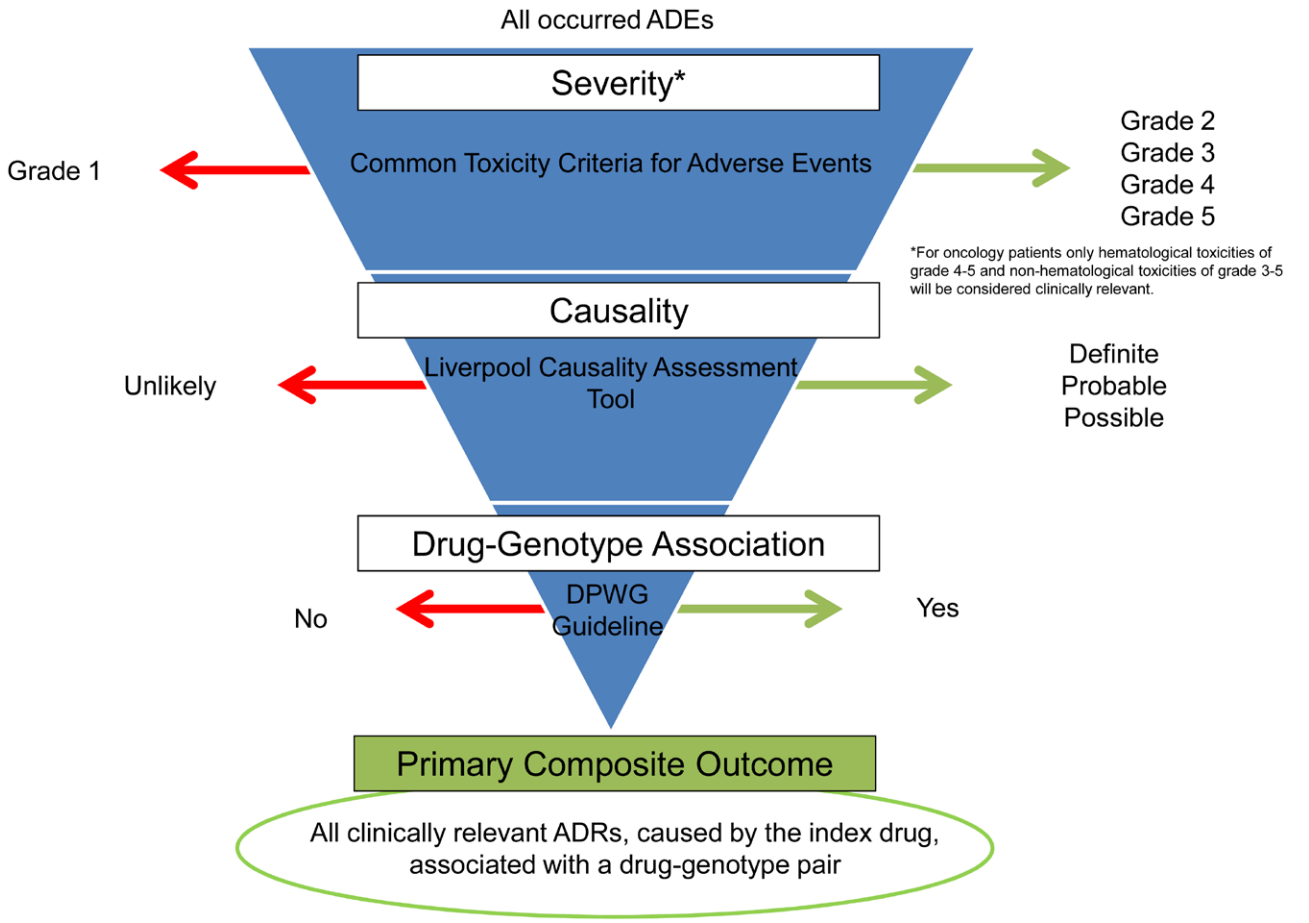
incongruous and excessive [29–32]. We support the latter view because generating gold-standard evidence for each of the 51 individual gene–drug interactions separately would require large amounts of funding which may be unrealistic. In addition, it may not be feasible to conduct RCTs for specific gene–drug interactions where the anticipated efficacy is only observed after a long follow-up. For example, the improved efficacy of tamoxifen by guiding dose on *CYP2D6* genotype may only be observed after an estimated 10-year follow-up [33]. It is important to note that nonpharmacogenomics interventions, such as dose adjustment of renally excreted drug in response to impaired kidney function, has been widely implemented in the absence of RCTs validating its effectiveness for each individual drug. Genetic exceptionalism has been held responsible for the double standard [34].

Regardless of the inconvenience, there is still a need for evidence showing patient benefit and cost-effectiveness to enable implementation of pharmacogenomics-guided drug dose and drug choice. Therefore, the Ubiquitous Pharmacogenomics Consortium decided on a novel way to evaluate the (cost-)effectiveness of pharmacogenomics-guided pharmacotherapy. Instead of conducting 51 separate RCTs (one for each DPWG guideline), the consortium set out to quantify the collective clinical utility of a panel of pharmacogenomics-markers (50 variants in 13 pharmacogenes) within one trial (the PREPARE study) for proof-of-concept across multiple potentially clinically relevant gene–drug interactions.

Primary endpoint definition

The final defined primary endpoint is described in Fig. 2. Since the DPWG guidelines encompass a broad range of gene–drug interactions, the resulting effects on patient outcomes are heterogeneous and therefore difficult to capture within one endpoint. Possible universal endpoints measuring these heterogeneous effects could have been quality of life (QoL) or overall survival (OS). On the other hand, anticipated asymptomatic effects are not

Fig. 2



The composite primary outcome is the occurrence of causal (definite, probable or possible), clinically relevant (classified as CTCAE grades 2, 3, 4, or 5), drug–genotype associated ADR, attributable to the index drug, within 12 weeks of index drug initiation. For oncology patients receiving fluorouracil, capecitabine, tegafur, or irinotecan, only hematological toxicities of CTCAE grades 4–5 and nonhematological toxicities of CTCAE grades 3–5 will be considered clinically relevant. CTCAE, Common Terminology Criteria for Adverse Events.

captured with QoL, and the follow-up time to capture effects on OS require long follow-up times. Therefore, we decided to define the primary endpoint as the prevention of clinically relevant ADRs as 90% of the DPWG guidelines aim to decrease the risk of ADRs (Table 1). Clearly, gene–drug interactions that lead to improved efficacy, such as that associated with *CYP2C19* polymorphism and omeprazole efficacy to increase *Helicobacter pylori* eradication, would not contribute to this defined endpoint. Therefore, these gene–drug interactions were excluded from the study.

Most of the DPWG guidelines recommend a dose decrease. Thus, theoretically the risk of ADRs will decrease in the intervention arm and as a result, one might be suspicious of unintended decreased efficacy because of this dose lowering. Therefore, more generally, one may argue that by using this endpoint we are unable to conclude a reduction of ADRs in the absence of decreased efficacy unless the lack of efficacy is perceived as an ADR. In this context, an ideal control arm would be one where the doses of randomly selected controls, of which genotypes are unknown, are also decreased. This is; however, considered unethical. Nevertheless, since the DPWG dose adjustments are calculated based on pharmacokinetic studies, the anticipated drug exposure among those with variant genotypes treated with a guideline-recommended lowered dose, are expected to have similar drug exposures to those with wildtype genotypes treated with normal doses. This was confirmed in a pharmacokinetic sub-study among patients carrying *DPYD* variants who were treated with a lower fluoropyrimidine dose [23]. Still, one could argue that decreased efficacy is a significant outcome that should be measured, to place the results into context. Potentially, this could be inferred from surrogate endpoints, such as drug discontinuation, within PREPARE.

Table 1 An overview of actionable Dutch Pharmacogenetics Working Group guideline and their primary anticipated effects when used to guide dose and drug selection based on the systematic review of literature underlying the guidelines

Gene	Drug	Risk reduction of adverse effects	Improvement of efficacy
CYP2B6	Efavirenz	X	
CYP2C19	Citalopram	X	
	Clomipramine	X	X
	Clopidogrel		X
	Escitalopram	X	
	Imipramine	X	
	Lansoprazole[a]		X
	Omeprazole[a]		X
	Pantoprazole[a]		X
	Sertraline	X	
	Voriconazole	X	X
CYP2C9	Phenytoin	X	
	Warfarin	X	
CYP2D6	Amitriptyline	X	X
	Aripiprazole	X	
	Atomoxetine	X	X
	Brexpiprazole[a]	X	
	Clomipramine	X	X
	Codeine	X	X
	Doxepin	X	X
	Eliglustat	X	
	Flecainide	X	X
	Haloperidol	X	X
	Imipramine	X	
	Metoprolol	X	X
	Nortriptyline	X	X
	Paroxetine		X
	Pimozide	X	
	Propafenone	X	X
	Tamoxifen		X
	Tramadol	X	X
	Venlafaxine	X	X
	Zuclopenthixol	X	X
CYP3A5	Tacrolimus		X
DPYD	Fluorouracil/capecitabine	X	
	Tegafur	X	
F5	Estrogen contraceptive agents	X	
HLA-A	Carbamazepine[b]	X	
HLA-B	Abacavir[a]	X	
	Allopurinol[a]	X	
	Carbamazepine[b]	X	
	Carbamazepine[b]	X	
	Phenytoin	X	
	Flucloxacillin	X	
	Lamotrigine[a]	X	
	Oxcarbazepine[a]	X	
NUDT15	Azathioprine/mercaptopurine	X	
	Tioguanine	X	
SLCO1B1	Atorvastatin	X	
	Simvastatin	X	
TPMT	Azathioprine/mercaptopurine	X	
	Tioguanine	X	
UGT1A1	Irinotecan	X	
VKORC1	Acenocoumarol	X	
	Phenprocoumon	X	
	Warfarin	X	

Clozapine and oxycodone were initially drugs of enrolment for the PREPARE study but updates in the Dutch Pharmacogenetics Working Group guidelines concluded that these were no longer related to actionable drug-gene interactions. PREPARE, PREemptive Pharmacogenomic testing for prevention of Adverse drug Reactions.

[a]Not included as a drug of enrolment in PREPARE.

[b]Has been removed as a drug of enrolment during study.

Simply defining a composite of ADR occurrence as an endpoint is insufficiently sensitive to measure the effect of pharmacogenomics-guided prescribing for two reasons. First, since patients enrolled are often on multiple drugs, we need to distinguish between ADRs related and unrelated to the drug of enrollment. Secondly, measuring only the ADR incidence, in the absence of severity, is less meaningful clinically. To improve sensitivity of the composite endpoint to these two factors, we incorporated both causality and severity assessments (Fig. 2). To minimize measurement error, we selected systematic and validated methodologies for assessments and decided upon thresholds for clinical relevance.

The composite endpoint includes also the third assessment, further aiming to increase sensitivity to the endpoint to measure the intended effects of the pharmacogenomics intervention. If we were to include all reported ADRs in the composite endpoint, it would dilute the effects of the pharmacogenomics intervention, as other ADRs, which may not be preventable by the intervention, would be included. Therefore, to avoid dilution, the composite endpoint only includes drug-genotype associated ADRs where the increase in ADR incidence is known to be associated with a genotype, in keeping with the DPWG guidelines (Table 2). As an example, *TMPT* poor metabolizers or intermediate metabolizers who receive a lower dose of thiopurines have a 10-fold lower risk of severe leucopenia [26]. It is also plausible that there may be a reduction of other ADRs, which are not included in the DPWG guidelines because of lack of evidence. In this situation, limiting the composite endpoint only to published drug–genotype associated ADRs may underestimate the effect of pharmacogenomics-guided prescribing. To avoid this, all ADRs experienced by participants are being collected and assessed regarding their association with the predicted phenotype of the gene of interest thereby supporting the discovery of novel associations. This will be further reinforced by work aiming to undertake sequencing of pharmacogenes in patients with extreme phenotypes.

The time between drug initiation and the onset of ADRs was also considered when defining the time-window for the primary endpoint. A literature review showed that a 12-week window would cover the majority of drug–genotype associated ADRs. Additionally, when not dose-titrated, all drugs included will have reached steady-state at 12 weeks, making it likely that most intrinsic ADRs would have occurred. Nevertheless, the selection of the time-window was complicated by the fact that we expect both on-target (type A) and off-target (or idiosyncratic) (type B) ADRs to be prevented by the pharmacogenomics intervention. On-target ADRs are often related to the causal drug's pharmacology and therefore the time-of-onset is, in general, more predictable than idiosyncratic ADRs. As an example, on-target ADRs associated with amitriptyline such as anticholinergic effects and cardiotoxicity would be expected to be preventable within the time-frame of the primary end-point [35]. Off-target ADRs, which would potentially be preventable, include

flucloxacillin-induced liver injury, which occurs after an average of 23.4 days [36]. By contrast, statin-induced myopathy can sometimes fall outside this time window. A large observational study showed more than half of statin-induced myopathy cases were reported in the first year of drug initiation; indicating at least half of cases were reported far outside a 12-week time-window [37]. Yet, with an increasing time-window, the overall causality to the index drug decreases [38]. Therefore, settling a 12-week time-window is pragmatic and optimizes balance between the ability to determine causality and coverage of most of the ADRs expected to be prevented. Clearly, we will miss some ADRs outside this time-window, and possibly under-estimate the effect, and this should be borne in mind when placing the results of the PREPARE study into context.

Primary endpoint collection

The operationalization of data collection to support the severity and causality assessments required additional consideration because of to the broad range of anticipated ADRs. This was in context of the fact that extracting ADR data retrospectively from EMRs can be difficult. Prospective collection of data in PREPARE after assessment of the patient was therefore felt to be the most accurate method. To capture as much information as possible on the occurrence of ADRs, we use three sources and concurrently collect information to support severity and causality assessments. Information is obtained from patients during scheduled follow-up (both online surveys and interviews), clinician reports, and extraction from the EMR. Patient interviews consist of open-ended questions, designed to uncover any ADRs, which the patients may have endured. On the other hand, a limitation of this methodology is the inability to uncover ADRs which are asymptomatic, such as citalopram-induced corrected QT-interval (QTc) prolongation, as ECGs are not routinely performed, potentially underestimating the occurrence of ADRs. However, once the ADR manifests symptomatically, such as Torsade de Pointes, it will contribute to the endpoint.

To ensure systematic assessment of the defined composite endpoint, we utilized standardized methods for assessment of severity and causality; the Common Terminology Criteria for Adverse Events (CTCAE) and Liverpool Causality Assessment Tool (LCAT), respectively.

In order to undertake severity assessment, using CTCAE, we require different types of data depending on the ADR reported. For example, to assess severity of a drug-induced dry mouth, we require data on the intervention required (grade 1: symptomatic without dietary alteration; grade 2: oral intake alterations; grade 3: tube feeding), whereas when assessing the severity of neutropenia, we require data on neutrophil counts (grade 3: ANC<1000/mm^3; grade 4: life-threatening consequences; grade 5: death). This content is collected both directly from patients during follow-up (e.g. How much did the side effect interfere with your usual daily activities? Did it limit your self-care activities?) and from the EMR. To minimize the impact of recall bias, patients are contacted at $t=4$ and $t=12$ weeks after index drug initiation. We note that a previous study indicated that a recall period of 1 week corresponded best to daily reporting, but it concluded that a 4-week recall was also acceptable [39] in order to reduce the burden of patient follow-up on each individual site (staff and patients). The initial $t=4$-week follow-up is within the acceptable range, and we expect most ADRs to be reported within this time.

Causality assessment was formalized with the LCAT, which requires information on the start- and end-date of both the index drug and the ADR, the severity of the ADR and the outcome of the re-challenge, when applicable. As with the severity assessment, data to support the causality assessment is collected from direct questioning of patients after the report the adverse event during scheduled follow-up (e.g. Did you lower the dose or temporarily stop taking the index drug after having the side effect? Did this reduce the side effect?).

In contrast to severity and causality assessment, there is no standardized method available for assessment of drug-genotype association. Therefore, to ensure unbiased assessment, an algorithm will be used to perform the analysis at study completion, to ensure consistent pharmacogenomics literature across assessments. This algorithm is being created by an expert panel, blinded to patient allocation. It is based on ADRs associated with certain genotypes in the literature underlying the DPWG (Table 1) and the ADRs reported in a drug's Summary of Product Characteristics.

Study design and blinding

To account for center-heterogeneity and time-dependent differences, PREPARE is a cluster-randomized cross-over trial comparing pharmacogenomics-guided strategy with standard care using a single cross-over moment. Clusters are formed by the countries participating in the study. The choice of a single cross-over time-point, instead of many, is dictated by the substantial logistic effort to switch between strategies. Without randomization, all countries could have started with standard of care followed by pharmacogenomics-guided prescribing synchronously. This could potentially introduce time-dependent differences, for example, healthcare professional (HCP) awareness and knowledge of pharmacogenomics may increase over time. Risk of protocol violation of the crossover approach results from centers randomized to begin with the pharmacogenomics-strategy, being unable to switch back to standard of care, resulting in an absence of a site-specific control arm. In our project, this has not occurred. A statistically more powerful alternative, for cluster randomization, would be individual

Table 2 An overview of actionable Dutch Pharmacogenetics Working Group guideline and their primary anticipated effect based on the literature underlying the Dutch Pharmacogenetics Working Group guidelines

DPWG Clinical relevance score	CTCAE score	Adverse drug event
AA#:		Positive clinical effect.
AA: No kinetic effect:		No change or nonsignificant change of kinetic parameters. No clinical effect: no change or nonsignificant change of clinical parameters.
A: Kinetic effect:	0	Significant change of kinetic parameters. Clinically insignificant effect: increase in QTc interval not higher than 470 ms for women or 450 ms for men or an absolute increase in QTc time of no more than 60 ms; increase INR to 4.5; expected asymptomatic bradycardia.
B: Clinical effect, short-term discomfort (<48 h) without residual symptoms:	1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.	Reduced decrease in resting heart rate; decrease in exercise tachycardia; short-term symptomatic bradycardia; insufficient pain relief with oxycodone; side effects due to increased bioavailability of atomoxetine (decreased appetite, insomnia, sleep problems, depressed mood, early awakening); neutropenia> 1.5×10^9/L; leukopenia> 3.0×10^9/L; thrombocytopenia> 75×10^9/L; moderate diarrhea without affecting daily activities; reduced rise in glucose levels in glucose tolerance test; muscle complaints with creatine kinase <3 times the upper limit of normal.
C: Clinical effect, long-term discomfort (48–168 h) without residual symptoms:	2: Moderate; minimal, local or Noninvasive intervention indicated; limiting age-appropriate instrumental ADL.	Failure therapy for a nonserious condition: tricyclic antidepressants, atypical antipsychotics; extrapyramidal side effects, parkinsonism (shaky/shaky); side effects due to increased plasma concentration of tricyclic antidepressants, metoprolol, propafenone (central side effects such as dizziness); long-term symptomatic bradycardia; increase INR to 4.5–6.0; neutropenia 1.0–1.5×10^9/L; leukopenia 2.0-3.0×10^9/L; thrombocytopenia 50–75×10^9/L; muscle complaints with creatine kinase 3–10 times the upper limit of normal.
D: Clinical effect, long-term discomfort (>168 h) or residual symptoms or disability:	3: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL	Failure of prophylaxis in atrial fibrillation; venous thromboembolism; reduction of inhibition of platelet aggregation by clopidogrel; side effects due to increased plasma concentration of phenytoin; increase INR>6.0; neutropenia 0.5–1.0×10^9/L; leukopenia 1.0–2.0×10^9/L; thrombocytopenia 25–50×10^9/L; severe diarrhea; myopathy (muscle complaints with creatine kinase ≥10 times the upper limit of normal); hospitalization due to bradycardia.
E: Failure of (in the short- or long-term) life-saving therapy:	4: Life-threatening consequences; urgent intervention indicated	Prevention of breast cancer recurrence; arrhythmias; expected bone marrow depression; neutropenia <0.5×10^9/L; leukopenia <1.0×10^9/L; thrombocytopenia <25×10^9/L; life-threatening effects of diarrhea; life-threatening side effects (such as SJS, TEN or DRESS); rhabdomyolysis.
F: Death:	5: Death related to adverse event	Arrhythmias; unexpected bone marrow depression

ADL, activities of daily living; CTCAE, Common Terminology Criteria for Adverse Events; DPWG, Dutch Pharmacogenetics Working Group; DRESS, drug rash with eosinophilia and systemic symptoms; SJS, Stevens-Johnson Syndrome; TEN, toxic epidermal necrolysis, QTc, corrected QT-interval.

randomization at the patient level. This would likely be logistically challenging and error-prone for the participating clinicians, who would need to follow different strategies for different patients simultaneously and the design was therefore excluded in the design phase of this study.

Certainly, blinding both patients and clinicians from the pharmacogenomics-test results would optimize scientific rigor and prevent potential information bias [40]. Nevertheless, this was not deemed feasible and therefore an open-label approach was chosen. This may potentially affect results as pharmacogenomics-testing could provide false reassurance to wild-type patients enrolled in the intervention arm that a given drug will be effective and cause minimal side effects; and conversely, may also motivate patients with variant genotypes to report side effects. By contrast, patients enrolled in the intervention arm may also be susceptible to the nocebo effect [41]. This may be the case when patients are told they have an actionable gene-drug interaction, requiring immediate pharmacotherapy adjustment, although they have already been using an untailored regimen for a number of days. In this case, the patient may be more prone to perceiving ADRs, which otherwise may not have been perceived. This notion is supported by a recent study that showed that learning one's genetic risk may evoke physiological changes consistent with the expected risk profile [42]. This underlines the importance of both the clinician's role in communicating the pharmacogenomics results and their effects on the drug regimen and the ability of patients to understand the probabilistic nature of the test result. Despite these issues, an advantage of performing an open-label study is that it closely mimics real-world application of pharmacogenomics, where both patients and clinicians are aware of the pharmacogenomics result. It is also an opportunity to study the interactions between patients and clinicians when implementing pharmacogenomics. Finally, in an effort to minimize differences in participant experience between intervention and control arms, we are providing control patients with a mock safety-code card, given that intervention patients also receive their pharmacogenomics recommendations on a safety-code card.

Inclusion and exclusion criteria

Some drugs are more frequently prescribed than others in routine care. To avoid overrepresentation of those drugs, which are frequently prescribed within the patient sample, a number of potential solutions were considered. First, imposing a cap to limit the maximum portion (10%) of patients enrolled in a particular drug. This option is feasible and would have a positive effect on clinical relevance since it would provide the opportunity for a minimum of 10 eligible drugs to be enrolled in the study. Second, reflecting the enrolment of drugs in the study to prescription frequencies in the European population. This option would be feasible, but drugs, which are uncommonly prescribed, will not be equally represented as commonly

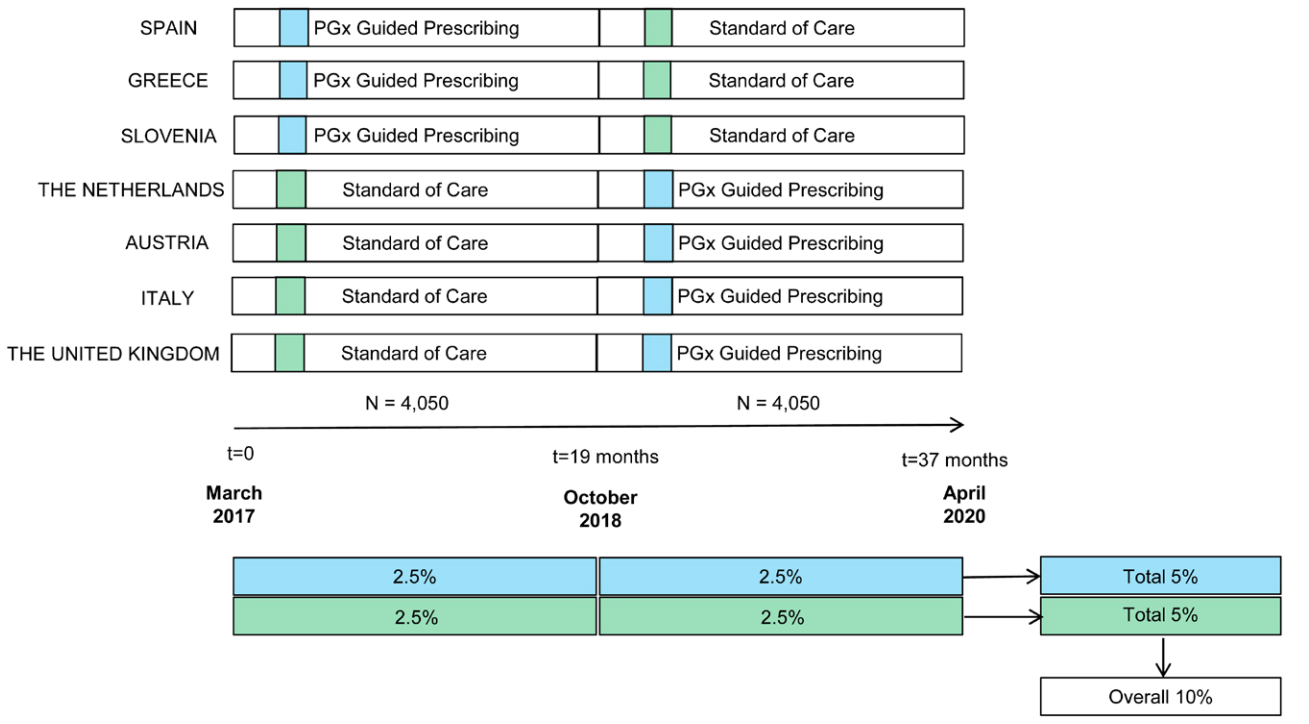
prescribed drugs thereby decreasing the generalizability of the results. Third, to only select gene–drug interactions that we anticipate having the greatest effect size and to maximize the proportion of patients enrolled on the corresponding drug. Selecting this option; however, would synthetically increase the effect size of the intervention, thereby leading to a biased outcome with low generalizability. After consideration, the first option was chosen and implemented since it was most feasible and is likely to have a positive effect on the generalizability of results across all 41-index drugs (Fig. 3). In order to avoid ethical problems and maintain equipoise, countries already implementing specific gene–drug interactions as part of routine care are exempt from enrolling patients on the corresponding drugs. For example, pre-emptive routine *DPYD* testing is standard of care in the Netherlands and therefore patients initiating fluoropyrimidines are not being recruited in the Netherlands, but are being recruited in other countries where *DPYD* testing is not standard of care.

Target setting and population

Whether pharmacogenomics-guided pharmacotherapy is effective in reducing ADRs is partially dependent on the health-care system in which it is applied, as the following vary across healthcare systems beliefs and knowledge about pharmacogenomics amongst HCPs [43], adherence to pharmacogenomics guidelines, patient baseline health, cultural aspects, the usability of the CDSS, and the infrastructure of the healthcare systems in which it is implemented. This underlines the importance of performing this experiment in a number of healthcare settings, where these variables may vary, to maximize external validity. Each of the seven countries has its own healthcare system ranging from highly automated electronic CDSS (such as in The Netherlands) to paper-based systems (such as in Slovenia and Greece). The considerations made in developing local solutions for CDSS have been published previously [44]. An added advantage is the ability to assess implementation process metrics across diverse healthcare systems, which may be helpful for future implementation efforts outside the scope of this trial. However, current sites are considered early adopters of pharmacogenomics, potentially limiting the external validity.

Whether pharmacogenomics-guided pharmacotherapy is effective in reducing ADRs is also dependent on the intersection of variants included in the panel and the ethnicity of the target population. Allele frequencies in pharmacogenes vary significantly between different ethnic groups

Fig. 3



Index drugs are capped at 10% of the total sample, 5% for each arm. This 5% is in turn equally divided over two groups of sites (2.5% in each), equally providing all sites with the opportunity to enroll patients on certain index drugs; the first group being those starting with the intervention arm and the second group being those staring with the control arm. Index drug enrollment is monitored centrally in real-time to ensure cessation of enrolment of index drugs once their cap is reached.

[45]. As a result, the incidence of patients carrying an actionable gene-drug interaction may also vary. Additionally, certain gene-drug interactions may only be relevant in some ethnic groups [46]. In an effort to optimize the applicability of the PREPARE results, the variants selected for ubiquitous pharmacogenomics panel should have at least an overall minor allele frequency (MAF) ≥1%, or the MAF in selected populations must be (European/Asian/African) ≥1%. The considerations made in developing the ubiquitous pharmacogenomics panel (pharmacogenomics passport) have been published previously [47]. Tagging single neucleotide polymorphisms (SNPs) were included in the initial panel selection to assess *HLA-B**15:02 and *HLA-A**31:01 status. However, the tagging SNPs only appeared suitable in subjects of Asian origin and not in a cohort of subjects of predominantly Caucasian origin. As a result, these tagging SNPs were removed from the panel and carbamazepine was removed as a drug of enrolment.

Pharmacogenomics intervention strategy

As literature underlying pharmacogenomics accumulates over time, the DPWG guidelines are regularly updated. In the PREPARE study, these updates are also implemented regularly, as we felt it unethical not to treat patients with the most up-to-date knowledge. However, one may argue that this imposes limitations on the scientific rigor of determining the effect of the intervention since it is not constant over time. However, it is inevitable that the guidelines will be updated regularly even after completion of the PREPARE study. Therefore, updating the guidelines within the study reflects the real-world aspects of the intervention more accurately than using out-of-date guidelines for the sake of scientific rigor. Examples of this during the study are the removals of oxycodone and clozapine as drugs of enrolment. At study initiation, the *CYP2D6*–oxycodone and *CYP1A2*–clozapine interactions were classified as actionable interactions requiring pharmacotherapy adjustment for at least one phenotype category. However, after preplanned updates of these guidelines by the DPWG, both were no longer considered actionable. Therefore, these drugs cannot result in a pharmacotherapeutic recommendation and were removed as drugs of enrolment. Additionally, *CYP1A2* was removed from the panel since there remained no actionable drug-gene interactions related to this gene. In addition to updates of DPWG guidelines, new guideline for novel gene–drug interactions are also developed. For example, for *CYP2D6*–brexpiprazole, *HLA-B*–allopurinol, *HLA-B*–lamotrigine, and *HLA-B*–oxcarbazepine. However, to avoid unequal number of patients on these drugs in study and control arms, these drugs were not added to the list of drugs of enrolment throughout the study. Indeed, removal of oxycodone and clozapine also resulted in an unequal number of patients on these drugs in study and control arms. However, the resulting nonactionability of the DPWG updates could not have been foreseen and therefore is substantiated.

The timing and methodology of delivering the relevant actionable DPWG guideline to treating clinicians may also have an effect on outcomes. To quantify the effectiveness of pharmacogenomics to prevent ADRs, one would prefer to initiate the 'correct' drug and dose, tailored to the patient's pharmacogenomics profile. However, this would require patients to delay the initiation of their pharmacotherapy while awaiting the turn-around of their pharmacogenomics test result. We felt this would be unethical, especially for analgesic therapies. For this reason, we have imposed a maximum 7-day turn-around time in which the gene important to the drug of enrollment and the corresponding DPWG guideline should be reported to the treating physician. However, a limitation of this delay is the potential underestimation of the true effect of pharmacogenomics-guided pharmacotherapy, since patients start the index drug on an unoptimized dose or drug to bridge the turn-around time and the risk of ADRs may increase with an increasing number of days using the un-optimized dose. It will be possible to adjust for this in a per-protocol analysis.

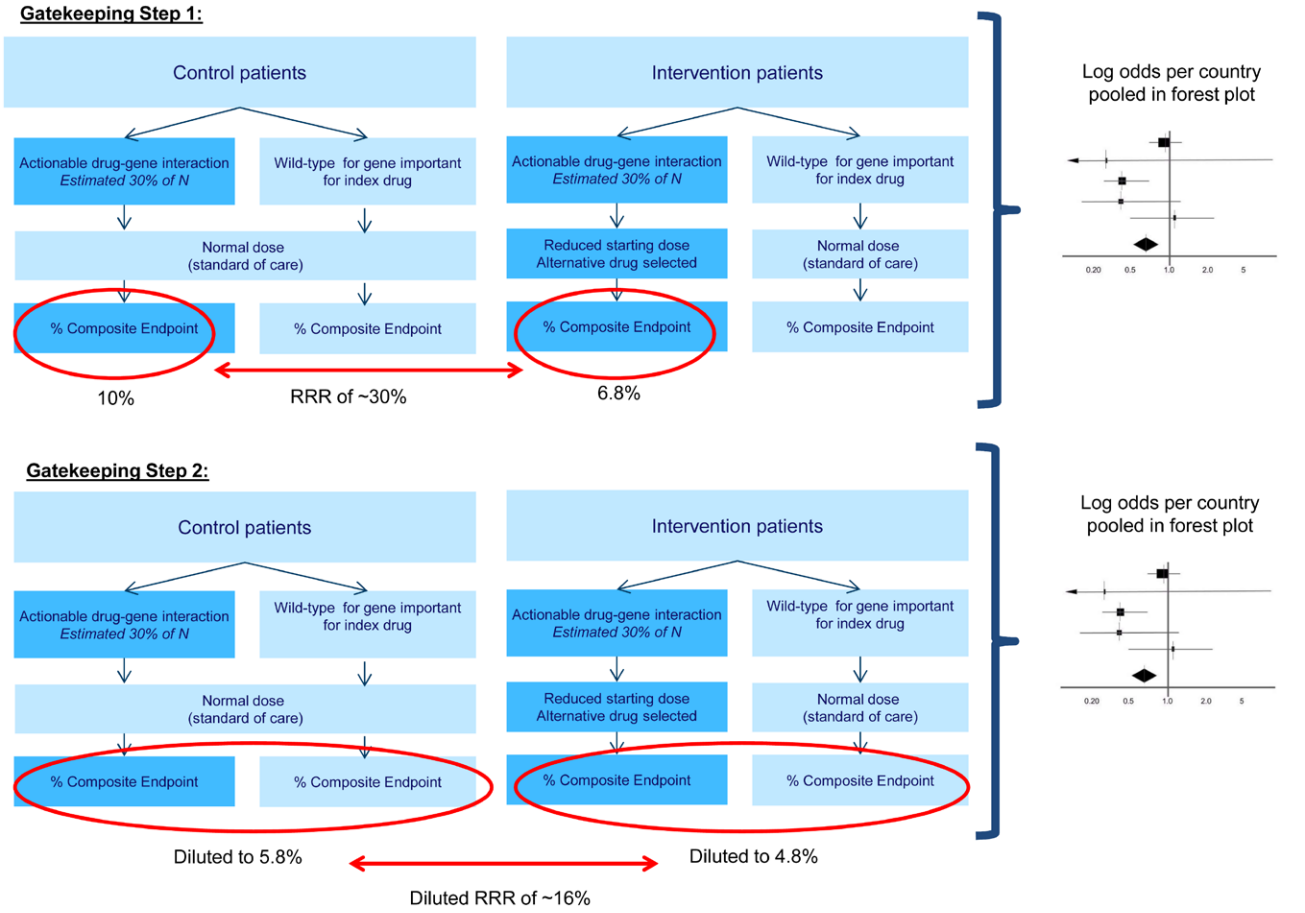
Statistical analysis

The added value of testing a panel of pharmacogenomics markers as opposed to multiple single genes is the ability to reuse the pharmacogenomics-panel results in the future without the logistical hassle of turn-around time. However, this added value is not captured by the primary statistical analysis since it is limited to ADRs attributed to the index drug and excludes ADRs attributed to subsequent drugs. Our reasoning for this is to avoid selection bias since patients initiating subsequent drugs use more concomitant drugs and are therefore at higher risk of experiencing more ADRs. Nevertheless, there is a need to quantify the effectiveness of ADR prevention through pre-emptive panel testing. Since PREPARE enables data collection not only for the index drug but also for (multiple) subsequent drugs, we are able to address this need by re-running the primary analysis using ADRs attributed to subsequent drugs in a secondary analysis.

Gatekeeping analysis

Traditional RCTs use strict eligibility criteria to select only patients in whom a benefit is most likely to be observed from the assessed intervention. However, with pharmacogenomics testing, we are unable to perform such a selection because the genotype is not known prior to recruitment. Only those patients with an actionable gene–drug interaction for the index drug may benefit from the pharmacogenomics intervention for the drug of enrolment. Therefore, comparison of the entire study arm to the entire control arm would underestimate the effectiveness for the subgroup of patients who may benefit from the intervention. For example, a study by Coenen *et al.* [26] found screening for variants in *TPMT* did not reduce the overall proportions of patients with hematologic ADRs during thiopurine treatment. However, within the subgroup carrying an actionable gene-drug

Fig. 4



Primary statistical analysis: a two-step gatekeeping analysis. First, a logistic regression analysis will be performed among patients who had an actionable drug-genotype combination. This analysis is first performed per country, and the log-odds are pooled in a forest plot. Only if this is statistically significant a second analysis will be performed: a logistic regression analysis among all patients included in the study. Again, this is first performed per country, and log-odds are pooled in a forest-plot.

interaction, there was a 10-fold reduction in hematologic ADRs. To minimize the risk of an unjust nonsignificant outcome when comparing arms, we decided upon a gatekeeping analysis (Fig. 4), where the first analysis quantifies the effect among those who have an actionable gene-drug interaction. However, when applied in a clinical setting, an entire population will need to be genotyped to be able to identify those who carry a variant genotype relevant for the drug to be used in the patient. This is addressed in the second analysis, where the effect of pharmacogenomics-guided pharmacotherapy among the entire screened population will be quantified.

Statistical model and estimand

To account for the clustered nature of the data, the analysis will be stratified by country, correcting for study center and other relevant covariates on the patient level. Analyses will be pooled using a meta-analysis. The composite endpoint is binary representing – in general – ADE severity 0/1 in one group and ADE severities 2/3/4/5 in the other. We use logistic regression to assess the influence of the pharmacogenomics-intervention on the probability of transitions between these two groups. Transitions within the groups, for example between grades 3 and 4, are not considered by this analysis as not aligning with the implementation goal. The estimand of the primary analysis is, therefore, the odds ratio for transitioning between these two groups based on the intervention.

An ordered logistic regression (ordinal regression) is more powerful when the goal is to detect the influence of the intervention on lowering severity in general by choosing as an outcome the ordinal ADEs (not grouping ADE grade). As such an approach can give additional insight into mechanisms underlying the effect of pharmacogenomics-intervention, ordinal regression will be performed as a secondary analysis.

Extreme phenotypes

In addition to the aim of quantifying the clinical utility of a pharmacogenomics-panel, the PREPARE study also aims to expand our understanding of genetic variation on drug response. The PREPARE study is a unique opportunity to identify and further investigate patients who express unexpected drug responses, known as extreme phenotypes. Three criteria for defining extreme phenotypes were devised to identify patients within PREPARE: (1) the physician considers the ADR important, (2) ADR is of severity grade 3 or above, or in the case of hematological toxicity grade 4 or above, and (3) patient experienced (re)hospitalization as a result of the ADR. A major problem as a result of these criteria is that they are applied differently across study centers. In some cases, it is difficult to stratify a true ADR from disease-induced adverse events. To overcome this issue, interesting cases are identified from the existing list of extreme phenotypes. These are further analyzed for possible novel genetic variants or combination of variants by an initial genome-wide association study encompassing all relevant pharmacogenes and followed in special cases by whole genome sequencing. Based on the multitude of rare genetic variants uniquely present in specific individuals, we believe this is an important step toward higher future predictability of genetically related ADRs.

Discussion

Future perspective of pharmacogenomics testing to optimize pharmacotherapy

The potential effectiveness of pharmacogenomics testing to reduce on-target ADRs is determined by a number of factors including the ability of the tested variants to accurately predict the patient's phenotype, the extent to which genetic variation affects target protein functionality, the extent to which the target protein determines drug exposure and finally, the causal relationship between the drug of exposure and ADR risk. In the future, we expect a shift in the field regarding the first two determinants; phenotype prediction will become more accurate due to accumulating knowledge of the effects of individual variants on enzyme and transporter functionality. Currently, phenotypes are predicted using a categorical approach. However, enzyme activity is usually normally distributed within a population and therefore is better described by a continuous phenotype scale. We envision a future where phenotypes can be predicted more precisely by using all of an individual's genetic variation (common and rare variants), as opposed to limiting the assessment to only those variants included in a tested panel. Although we expect the variants in the ubiquitous pharmacogenomics panel to correctly predict most phenotypes, it may not be able to do so correctly for all patients, since rare, untested variants may also affect phenotype. As our understanding of the effects of individual variants to inform phenotype prediction improves, we imagine that phenotype prediction will ultimately become substrate-specific as opposed to simply gene-specific. Thus, as our knowledge improves and increases resolution, we expect further improvements in the effectiveness of pharmacogenomics testing to reduce ADRs. Looking even further forward into the future pharmacogenomics profiles may be combined with other -omic profiles including the epigenome [48], microbiome [49], and metabolome [50], to further inform truly personalized, as opposed to stratified, prescribing.

Generating evidence for other precision medicine approaches

In this article, we have provided an overview of the challenges and respective solutions while designing the PREPARE study. We expect similar challenges to confront fellow investigators aiming to generate evidence for precision medicine approaches other than pharmacogenomics. Conventionally, evidence supporting novel interventions is generated by prospective trials. However, in an era where digitalization is driving data accumulation and a concomitant increase in stratification of patient groups, we are moving towards utilization of real-world data to support precision medicine. Several authors have pointed out that precision medicine, and genomic medicine, in particular, would benefit from a convergence of implementation science and a learning health system approach to measure outcomes and generate evidence simultaneously [51,52]. However, this requires standardization of outcomes in EMRs to enable aggregation of phenotype data across large populations for both discovery and outcomes assessment as part of implementation [21,53]. Many nationwide, large-scale initiatives are generating prospective longitudinal evidence supporting precision medicine approaches [54–57].

Generating evidence for pharmacogenomics: the PREemptive Pharmacogenomic testing for prevention of Adverse drug Reactions study

In conclusion, we have undertaken detailed considerations to enable quantification of the collective clinical utility of a panel of pharmacogenomics-markers within one trial as a proof-of-concept for pharmacogenomics-guided pharmacotherapy across multiple actionable gene–drug interactions. Although PREPARE will enable quantification of the clinical utility of a pharmacogenomics panel, it may also potentially underestimate the effects due to delayed initiation of a pharmacogenomics-guided dose or drug choice because of our turn-around-time (<7 days), and thereby limit the primary endpoint which is the reduction of ADRs caused by the initiated drug. Further research is therefore needed to quantify the effectiveness of panel-based pharmacogenomics-guided pharmacotherapy in patients encountering multiple gene–drug interactions over a longer time-horizon. This can be achieved within a clinical trial, or a more practical

approach may be to use real-world data to estimate long-term (cost-)effectiveness. PREPARE provides the framework not only for future efforts in this area but also in providing the evidence base for ubiquitous adoption of pharmacogenomics-guided pharmacotherapy [58].

Acknowledgements

We would like to thank the Scientific Advisory Board (Prof. Russ Altman, Prof. Michel Eichelbaum, David Haerry, Prof. Mark Ratain, Prof. Mary Relling, and Prof. Dan Roden) for their insights in the development of the study protocol and statistical analysis plan.

The research leading to these results has received funding from the European Community's Horizon 2020 Programme under grant agreement No. 668353 (Ubiquitous Pharmacogenomics). M.S. is in part supported by the Robert Bosch Stiftung, Stuttgart, Germany.

Conflicts of interest

G.P.P. is a full member and National Representative of the European Medicines Agency, Committee for Human Medicinal Products – Pharmacogenomics Working Party (Amsterdam, the Netherlands). There are no conflicts of interest for the remaining authors.

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