

The ProBio Trial: Molecular Biomarkers for Advancing Personalized Treatment Decision in Patients with Metastatic Castration-Resistant Prostate Cancer

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Abstract

Background: Multiple therapies exist for patients with metastatic castration-resistant prostate cancer (mCRPC). However, their improvement on progression free survival (PFS) remains modest, potentially explained by tumour molecular heterogeneity. Several prognostic molecular biomarkers have been identified for mCRPC that may have predictive potential to guide treatment decision and prolong PFS. We designed a platform trial to test this hypothesis.

Methods: The Prostate-Biomarker (ProBio) study is a multi-center, outcome-adaptive, multi-arm, biomarker-driven platform trial for tailoring treatment decisions for men with mCRPC. Treatment decisions in the experimental arms are based on biomarker signatures defined as mutations in certain genes/pathways suggested in the scientific literature to be important for treatment response in mCRPC. The biomarker signatures are determined by targeted sequencing of circulating tumor and germline DNA using a panel specifically designed for mCRPC.

Discussion: Patients are stratified based on the sequencing results and randomized to either current clinical practice (control), where the treating physician decides treatment, or to molecularly driven treatment selection based on the biomarker profile. Outcome-adaptive randomization is implemented to early identify promising treatments for a biomarker signature. Biomarker signature-treatment combinations graduate from the platform when they demonstrate 85% probability of improving PFS compared to the control arm. Graduated combinations are further evaluated in a seamless confirmatory trial with fixed randomization. The platform design allows for new drugs and biomarkers to be introduced in the study.

Conclusions: The ProBio design allows promising treatment-biomarker combinations to quickly graduate from the platform and be confirmed for rapid implementation in clinical care.

Introduction

Despite multiple therapeutic avenues for metastatic castration-resistant prostate (mCRPC), their impact on prolonging survival remains modest (1). The wide range of clinical outcomes and the plethora of resistance mechanisms for each treatment suggest that an ideal therapeutic approach requires accurate patient selection taking tumor biology into account (2,3). At present, no clear guidelines exist on how to choose the right treatment for the right patient at the right time. With multiple alternative therapies and a number of new therapies expected to be approved for patients with mCRPC, it is imperative to optimize the treatment selection and identify the optimal sequencing of available therapies (1).

Here, we describe the design of the ProBio study, a prospective multi-center platform trial for tailoring treatment decision-making for men with mCRPC. ProBio is an outcome adaptive, multi-arm, biomarker driven platform trial with the aim of prospectively identifying and validating predictive molecular biomarkers. Specifically, we will test whether somatic and germline alterations can predict if a patient is more likely to benefit from receiving a particular therapy (4,5). The adaptive design is particularly suitable

for addressing multiple questions at once and allows for promising treatment-biomarker signatures to quickly graduate from the trial and to be faster implemented in routine clinical care (6,7). Treatment-biomarker combinations may also exit the trial if there is accumulated evidence of them being ineffective. The platform design allows for new drugs and new biomarkers to be introduced in ProBio, to enrich the options for guiding treatment selection for men with mCRPC.

Background And Rationale

Prostate cancer is the most common cancer and the second leading cause of cancer-related death among men in the Western world (8). mCRPC is a lethal form of advanced or metastatic prostate cancer, characterized by progressive disease under androgen deprivation (9). Currently, the most common systemic standard-of-care (SOC) therapies for these patients are second-generation hormonal therapy (Abiraterone acetate and Enzalutamide), chemotherapy (Docetaxel, Cabazitaxel), and radionuclide therapy (Radium-223) (10). Novel targeted agents (e.g. PARP and PD-1 inhibitors) are expected to soon enrich the landscape of available treatments for mCRPC patients (1,3).

The number of therapeutic options for mCRPC patients is increasing, but the response rates in unselected patient populations remain moderate. This leads to missed opportunities of immediately selecting optimal therapy, unnecessary side-effects for the patient, and costs to the health care systems. Although approved for unselected mCRPC patients, these SOC agents are likely more beneficial for particular subgroups of the patient population (3). Biomarker driven clinical trials for mCRPC have been hampered by the difficulty of obtaining metastatic tissue (11). Also, profiling a single metastatic lesion is not capable of providing the full spectrum of the molecular heterogeneity that may exist within the patient (12,13). A liquid biopsy, either in the form of circulating tumour cells (CTCs) or tumour-derived cell-free DNA (circulating tumor DNA, ctDNA), is an attractive alternative (14,15). Circulating tumor DNA has been shown to be highly concordant to metastatic tissue for detecting somatic variations, and allows for longitudinal monitoring and detection of acquired resistance (16–19). The use of molecular biomarkers has been successful for patient prognostication and holds the promise to inform treatment selection as predictive biomarkers for mCRPC (20). Currently, the IND.234 trial is applying ctDNA sequencing in second- or third line mCRPC to test pre-defined biomarker - treatment hypothesis for enriched responses that may subsequently be investigated in randomised trials (21).

The multiplicity of available treatments with an evolving therapeutic landscape and the molecular heterogeneity with low prevalence of patients carrying a specific marker highlights the limitations of current clinical trials in evaluating the efficacy of comparative treatments and potentially treatment-predictive biomarkers (3). A possible remedy for addressing multiple research questions within the same clinical trial is the implementation of a platform design (22–24). The multi-arm structure of a platform trial allows to compare alternative therapies with a common control group. Given the flexibility of a platform design it is possible to add or drop experimental arms, and use the accumulated data to change the course of the trial according to prespecified criteria. The outcome-adaptive component assigns more patients to promising arms and can therefore be more efficient than an equal randomization, more

beneficial for the participants in the study (since patients on average have higher probability to be assigned to effective treatments), and can dramatically reduce costs (6).

The ProBio Trial

ProBio is the first outcome-adaptive trial for mCRPC, designed to accelerate the implementation of novel results generated by molecular epidemiology into routine clinical care. ProBio incorporates several multidisciplinary innovations including prospective liquid biopsy-based molecular profiling (Figure 1), novel features in the clinical study design (Figure 2 and Figure 3), and dedicated solutions for logistics and clinical implementation. The trial was initiated in Sweden, and will expand internationally during 2020 to hasten recruitment of a large number of patients. ProBio will create a learning environment not only to identify biomarker profiles where therapies are more effective, but to answer a multiplicity of prespecified research questions (e.g. surrogacy role of ctDNA fraction, identification of new biomarker signatures based on collected data, comparing RNA analysis from plasma and thrombocytes) and new hypotheses that will arise throughout the study.

Trial design

ProBio is an outcome-adaptive, multi-arm, biomarker driven platform trial to determine whether treatment selection based on a liquid biopsy-derived biomarker profile can prolong progression-free survival (PFS) in men with mCRPC. The trial will be analyzed within a Bayesian framework where alternative treatments will be compared in terms of their probability of superiority, using a common comparator (25).

Patients are stratified based on their ctDNA biomarker signature and randomized to either one of the experimental arms where treatment decisions (abiraterone, enzalutamide, carboplatin, docetaxel, or cabazitaxel) are based on the biomarker signatures or the control group defined by current clinical practice (Figure 2). Radium-223 is not included due to the recent EMA recommendations restricting its use only to mCRPC patients who already received two treatments. Carboplatin is included despite not being approved for mCRPC since there is some evidence that it is effective against mCRPC tumors with alterations in the DNA repair genes. The outcome-adaptive randomization is implemented to assign more patients to biomarker-treatment combinations with the highest probability of being superior to SOC, and to early identify drugs which are promising in the subpopulation of patients defined by a specific biomarker signature. As data accumulates and it begins to become evident which treatments are least effective for certain biomarker signature, fewer of those patients are randomized to poorly performing therapies. This has the obvious advantage of providing patients with a treatment more likely to work for them, rather than a less effective therapy, but also of using the finite resources (patients) in a more efficient way: assigning patients toward the latter stages of the trial only to the treatments still competing to be the best treatment for that disease type.

Biomarker-treatment combinations that exit the trial based on superiority are further evaluated in a seamless confirmatory trial nested in the ProBio platform using fixed randomization (Figure 3). Upon progressive disease, the patient will re-enter the trial and be re-randomized one additional time (with a

maximum of 2 randomizations) to another treatment based on their current biomarker profile. Patients that have undetectable ctDNA (26) and do not harbor any relevant gDNA alterations cannot be randomized and will enter an observation arm of the study where SOC is administered. Upon progression of the disease, the patients will be re-analysed with the ProBio-panel and randomized into the platform trial (Figure 2). Both the re-randomization and the observation arm will provide important insights for selecting an optimal treatment sequence for mCRPC patients.

We have selected the threshold values for graduation a treatment-biomarker combination or stopping for futility based on extensive simulation studies, since operating characteristics cannot be easily calculated for complex platform trials (7). In particular, we assumed multiple scenarios ranging from no differences in treatments in any of the biomarker combinations to treatments prolonging the mean PFS by 5 to 10 months. The thresholds were chosen to control the type I error rate and assure an adequate power for graduating treatment-biomarker combinations. A comprehensive description of the simulation study will be published elsewhere. A summary of the simulations' results is available at http://alessiocrippa.com/shiny/probio_dsmb/.

Inclusion/exclusion criteria

ProBio will enroll patients with mCRPC, aged 18 years and above, with an Eastern Cooperative Oncology Group (ECOG) performance status of 0-2, histologically confirmed prostate adenocarcinoma, and castrate levels (<50 ng/dl) of serum testosterone, conforming to EAU guidelines (2). The patient should have an adequate health, bone-marrow, hepatic and renal function to receive all available treatments in the trial. Distant metastatic disease needs to be documented by positive Tc-99 bone scintigraphy or by computed tomography (CT) or magnetic resonance imaging (MRI) scans. The ProBio trial will initially allow to recruit mCRPC patients starting both 1st or 2nd line systemic therapy for progressive disease, but will in the near future limit enrollment to 1st line patients to infer a better understanding on treatment sequencing. Patients are not eligible if they have received more than two of the drugs under investigation in the platform, prior to study inclusion.

Biomarker subtypes and signatures

Molecular characterization of the tumor through a ctDNA-driven liquid biopsy is a key feature of the ProBio trial. Multiple molecular perturbations (splice variants, point mutations, amplifications and genomic rearrangements) can be associated with treatment outcome and response for men with mCRPC (27–29). In men treated with enzalutamide or abiraterone, the AR-V7 splice variant (up to 60% prevalence) has been suggested as a negative response marker (30,31). However, the combination of *TP53* inactivation (occurring in 25-40% of mCRPC patients) and multiple *AR* alterations have demonstrated more promising results (32–34). Metastatic prostate cancer with DNA repair deficiency (DRD), occurring in about 20% of mCRPC cases, have been suggested to have a higher sensitivity to PARP inhibition (35) and platinum-based chemotherapy (36,37). The FDA approved the anti-PD1 immunomodulator pembrolizumab in patients with any microsatellite instable (MSI) or mismatch repair deficient (dMMR)

solid tumour (38,39). Approximately 3-4 percent of mCRPC are MSI positive (28,40), with partial- or complete responses to checkpoint inhibition being observed in up to 50% of these patients (39,41–43). Finally, The *TMPRSS2-ERG* gene-fusion, occurring in 40-50% of prostate cancer (33,51), has been suggested to predict response to docetaxel (45).

The ProBio trial will initially evaluate four classes of pre-defined genomic biomarker signatures, which have been recognized as the major candidates for guiding prognosis and treatment decision (26,28,46):

1. mutations and structural rearrangements in *AR*;
2. mutations, homozygous deletions and structural rearrangements in *TP53*;
3. DNA-repair deficiency by detection of mutations, homozygous deletions and structural rearrangements in *ATR*, *ATM*, *BARD1*, *BRCA1*, *BRCA2*, *BRIP1*, *CHEK2*, *FANCA*, *MRE11*, *NBN*, *PALB2*, *RAD50*, *RAD51*, *RAD51B*, *RAD51C* and *RAD51D*; and
4. *TMPRSS2-ERG* fusions by structural rearrangements and deleterious events.

The combination of these 4 biomarkers defines the biomarker subgroup combination of a patient, i.e. the molecular characteristics of the tumor including germline DNA alterations (Figure 3). Randomization to either the control group or one of the active treatments occurs conditional on the patient's biomarker subtype combination, where a patient belongs to one and only one biomarker subtype. Initially, four binary biomarkers will be considered, which defines $2^4 = 16$ biomarker subtype combinations. The effect of a treatment within one of these 16 biomarker subtype combinations is however typically of limited interest because of the low prevalence of each combination. However, treatments may be more effective in a subpopulation defined by a group of biomarker subtypes, all harboring alterations in e.g. the target pathway that a specific drug aims to block. We refer to such groups as a *biomarker signature* (47). Initially the ProBio trial will test 5 different biomarker signatures (Table 1) (26,27). Contrary to the biomarker subtype, a patient may belong to more than one biomarker signature (Figure 3). For example, an *AR* and *TP53* wild-type patient belongs both to the signature "All patients" and "*AR*- & *TP53*". While randomization happens at a biomarker subtype level, therapies are evaluated at the higher level of biomarker signatures.

Outcome adaptive randomization

The biomarker subtype works as a stratification variable for randomizing each patient to either the control group or one of the active arms in the platform. The control group reflects current clinical practice, i.e. treatment selection according to national guidelines without the information on the tumor biology, and consists of a mix of available treatments. Each biomarker subtype has a separate control group (Figure 1).

Fixed randomization within biomarker subtypes will be implemented before accruing a minimum number of patients across the active arms ($n=50$), after which the adaptation starts to be applied. Thenceforth, experimental therapies will be randomized proportional to their Bayesian probability of prolonging PFS

compared to the control. For each biomarker subtype, we will assure that the control groups receive at least as many patients as any single drug in the experimental arm (i.e. a 1:1 randomization between the control and the current most promising biomarker signature-treatment combination). Randomization probabilities will be updated monthly based on the accumulated data throughout the trial. We chose to update randomization probabilities using the observed PFS times because time to progression in first-line and all-comer mCRPC patients may be relatively short (less than 7 months) (27,48).

Evaluation of therapies

Therapies will be evaluated in two different stages. In the first one all the therapies will be compared to the respective controls in all the biomarker signatures (screening stage). Treatments that show evidence of superiority in selected biomarker signatures will graduate from the screening stage and enter the confirmatory stage, where the therapies will be tested only for the associated graduating signature.

Screening stage. Therapies will be evaluated for effectiveness as compared to the control group separately for each biomarker signature of interest. The main outcome is PFS, where progression is defined according to Prostate Cancer Working Group 3 (49). We will use Bayesian methods for survival analysis to contrast the distributions of PFS times across the active arms and within the biomarker signatures (25). In particular, we will adopt the two-parameter Weibull distribution to model the observed PFS times. The posterior distributions of the modelled parameters will be used to evaluate the effectiveness of the active arms in the trial by computing the probabilities of superiority within the biomarker signatures, i.e. the probability that each treatment offers a longer time to progression than the control for each biomarker signature. After enrolling a minimum number of 20 patients, an active treatment may graduate and exit from the platform trial for a specific biomarker signature if its probability of superiority exceeds a predefined threshold (85%), as well as the probabilities of superiority for the biomarker subtypes that belong to the specific signature are sufficiently high (greater than 40%). On the other hand, if a treatment appears to be particularly ineffective (i.e. probability of superiority less than 15%), it will exit the trial for that biomarker signature. If none of the conditions are met and the maximum number of patients is reached ($n = 150$), randomization to the treatment under investigation will continue. Once a treatment graduates for a biomarker signature, it will exit the study and enter seamlessly in the confirmatory trial which is nested within the ProBio platform.

Confirmatory trial. The rationale for the ProBio platform study design is to learn from the data that accumulates in the trial and quickly generate solid hypotheses in a prospective way. To generate practice changing level of evidence, we will subsequently validate the promising biomarker-therapy combinations in a confirmatory trial (Figure 2). When a treatment graduates from the platform for a biomarker signature it will no longer be available in the active arms of the associated signature and will enter in a side trial nested within the ProBio platform. The control group for the biomarker subtypes belonging to the graduating signature will be divided in two halves using fixed randomization, the first receiving SOC and the other the graduating treatment. Hence, the patients in the SOC arm will at this stage act as a

comparator both for the treatment in the confirmatory trial, and for the remaining active arms in the platform study. The confirmatory trial will be analyzed in a frequentist manner.

Final analysis of the screening stage

The main and final analysis of all the active arms will be performed at the end of the screening stage of the ProBio trial. The treatment comparison, regardless if it has been validated or not, will be based on PFS within a biomarker signature. The measure of effectiveness will be the probability of superiority over the control group computed using parametric models within a Bayesian framework. Since the control group is a mix of treatments (some present also in the active arms), we will compare the efficacy of the active arms in terms of their PFS distribution against each other using an appropriate contrast matrix.

Current Status Of The Probio Trial

The ProBio trial is currently opening up at multiple healthcare centres across Sweden (5 sites were opened during the Spring of 2019 and an additional 6 sites will open during the Fall) with an expansion to other Scandinavian countries and Belgium planned for 2020. So far, 33 patients have been enrolled in the study, and the accrual rate is currently reaching about five patients per week. Biomarker signatures could be inferred in 28/33 enrolled patients with a median turnaround time of 15 days (from blood collection to reporting randomization results). In 5 cases the ctDNA fraction was below 2%, resulting in an incomplete assessment of the somatic biomarker signature status. These patients entered an observational standard-of-care arm of the study and hence a potential randomisation for their subsequent line of therapy.

The ProBio investigators are currently seeking to expand the described trial design towards earlier stages of the disease. This will be increasingly important in the context of the changing treatment landscape of metastatic hormone-sensitive prostate cancer, where the introduction of chemohormonal therapy (GETUG-15, CHAARTED, STAMPEDE), upfront association of abiraterone (LATITUDE, STAMPEDE), enzalutamide (ENZAMET) or apalutamide (TITAN) with standard ADT with or without local therapy of the prostate in case of de novo M1 disease (STAMPEDE), will demand a similar model for improving treatment selection for patients with advanced cancer.

Discussion

ProBio is an innovative platform design for enhancing treatment selection for mCRPC patients. Its design implements several innovations compared to standard clinical trials, including a flexible structure for adding or dropping treatments and/or biomarker signatures, adapting randomization probabilities based on the accrual data, addressing multiple hypotheses within the same study design, and validating promising therapies seamlessly to quickly change standard-of-care. This platform design has the potential to quickly corroborate hypotheses and generate new evidence which would not have been otherwise possible in a conventional randomized trial. In addition, the ProBio trial may dramatically

reduce the years and the costs associated with changing current clinical practice. Treatments that will graduate from the platform but fail in the confirmatory trial may still be valuable for refining biomarker signatures and inform treatment selection for patients outside the study. By allowing patient accrual and re-randomization throughout different lines of systemic therapy for mCRPC, ProBio will allow us to improve treatment selection that will maximize outcome and quality-of-life for the patient, and will provide essential insights into optimal treatment sequencing regimen.

Trial Status

The trial protocol, version number 3.0, was approved in April 2019 and is ongoing. The recruitment of patients was started February 1st, 2019 and will continue for at least 3 years.

Abbreviations

mCRPC: metastatic castration-resistant prostate cancer

CT: computed tomography

CTCs: circulating tumour cells

ctDNA: circulating tumour DNA

DRD: DNA repair deficiency

MRI: magnetic resonance imaging

PFS: progression free survival

SOC: standard of care

Declarations

Ethics approval and consent to participate

Men who agree to participate to the study will be asked to sign a written informed consent, according to the standard forms.

Necessary approvals of the trial protocol, the Patient Information and Informed Consent Form were obtained by the Independent Ethics Committee – Etikprövningsmyndigheten in Sweden on April 9, 2019 and by the Regulatory Authorities – Swedish Medical Products Agency on April 11, 2019. The trial is performed in accordance with the recommendations guiding physicians in biomedical research involving human patients that were adopted in 1964 by the 18th World Medical Assembly, in Helsinki, Finland, with later revisions.

Consent for publication

Not applicable.

Availability of data and materials

The questionnaires and coding sheets used in the study, as well as anonymised data, will be included in or as supplementary files to articles published, as required. Trial results will be disseminated to participants and relevant health-care providers via a stakeholder committee.

Competing interests

Henrik Grönberg has received honoraria for giving talks at Janssen, Bayer, and Astellas. All other authors declare no conflict of interest.

Authors' contributions

All the authors contributed in writing the protocol. In particular, HG is the principle investigator of the project, ME and JL are the sponsor for the statistical and genomic part of the trial, BL is the project manager. CT, EJ, GE, AU, MHE, JO, and PO are the PI for the individual study sites actively enrolling patients. Preparation of the article is primarily done by AC and BDL. AD, EEG, JTC contributed to the statistical design of ProBio. All authors critically reviewed and approved the final study protocol.

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Table

Table 1: Definition of the 5 candidate biomarker signatures and estimated prevalences with included biomarker subgroup combinations, in the order: AR, DRD, TP53, and TEfus (where '+' indicates mutated, '-' wildtype/non-mutated).

signatures	---	---+	--+	---+	+-	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	prev
all	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	1
TP53- & AR-	x	x			x	x										0.5
TP53+			x	x			x	x			x	x			x	0.37
DRD+					x	x	x	x					x	x	x	0.19
TEfus+		x		x		x		x		x		x		x		0.32

Figures

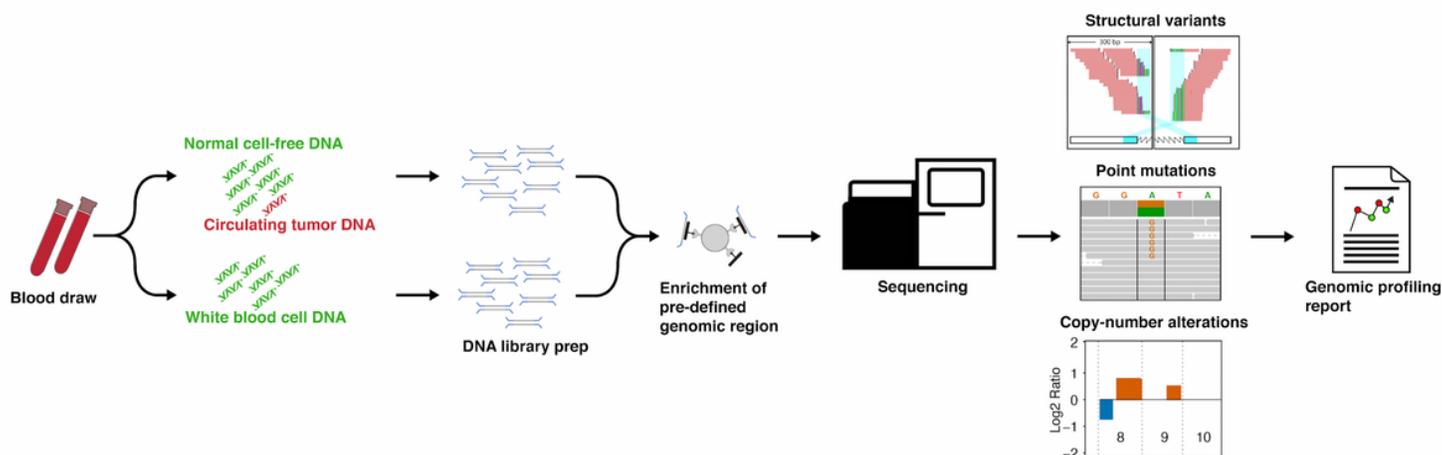


Figure 1

Genomic profiling in the ProBio platform trial. Two 10 ml tubes of blood are drawn from each study participant and plasma is enriched. Extraction is performed to obtain cell-free DNA from plasma and germline DNA from white blood cells. Targeted sequencing is applied on both cell-free and germline DNA using the ProBio-panel. The ProBio panel covers mutations in 78 genes, structural variants in 11 genes and allows for interrogation of genome wide copy-number alterations, microsatellite instability and hypermutation. Sequence data is processed using an in-house developed bioinformatics infrastructure (<https://autoseq-docs.readthedocs.io>). All variants are manually examined to remove false positive calls. This information is condensed into a report which contains the biomarker profile for subsequent randomization of the study participants. Study participants that progress are reanalyzed and re-randomized.

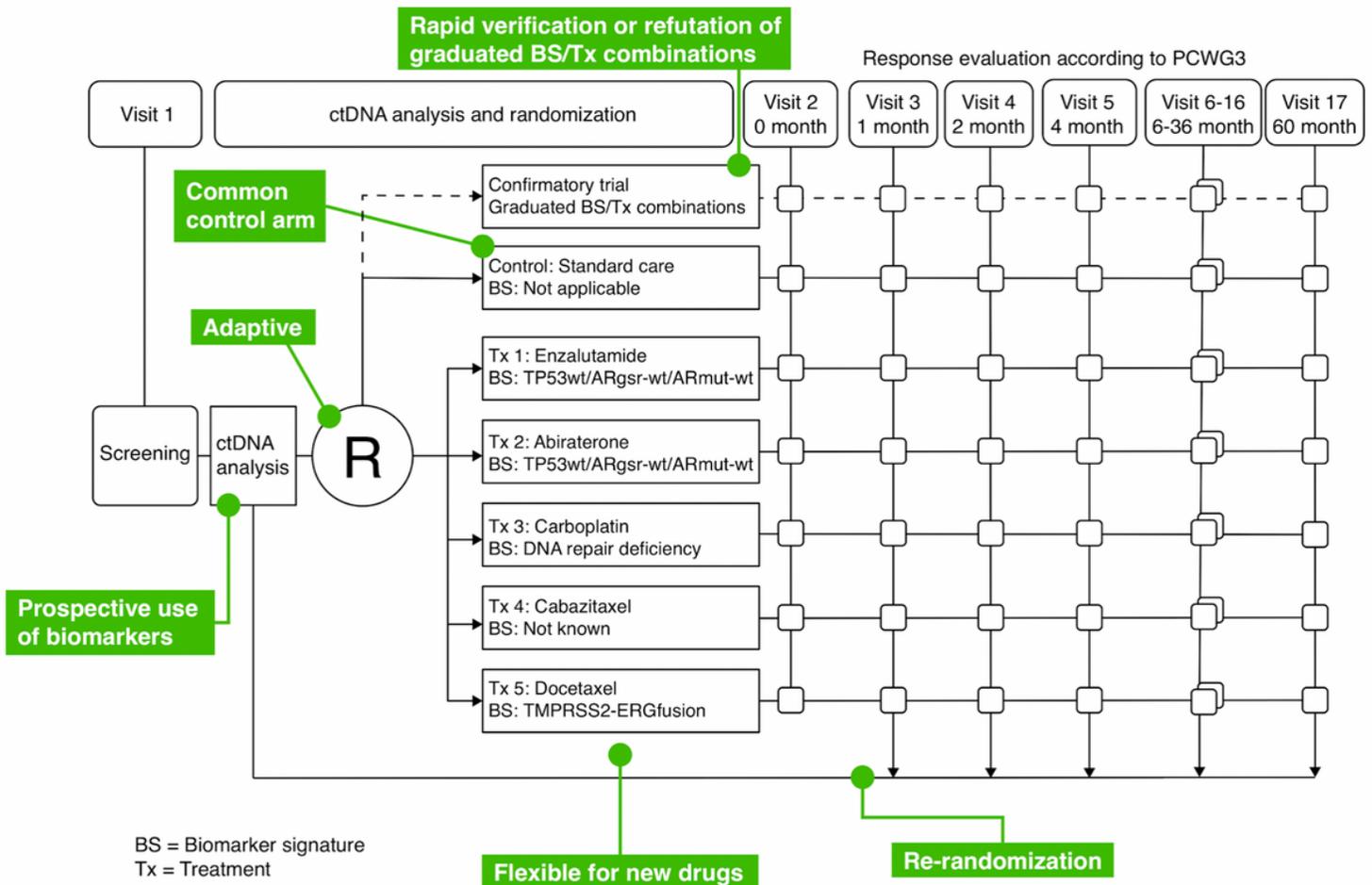


Figure 2

Study design of the ProBio platform trial. Participants who meet the inclusion criteria and agreed to participate in the study are genotyped and their biomarker profile is derived. Based on their biomarker subgroup combination they are randomized to either the control group (standard-of-care) or one of the active arms. Patients are regularly followed through the study. Their outcome data is used to adapt the randomization probabilities, assigning more patients to more beneficial therapies within a biomarker signature. Upon the first progression in the study, patients will be re-genotyped and re-randomized to an alternative arm based on their updated biomarker profile.

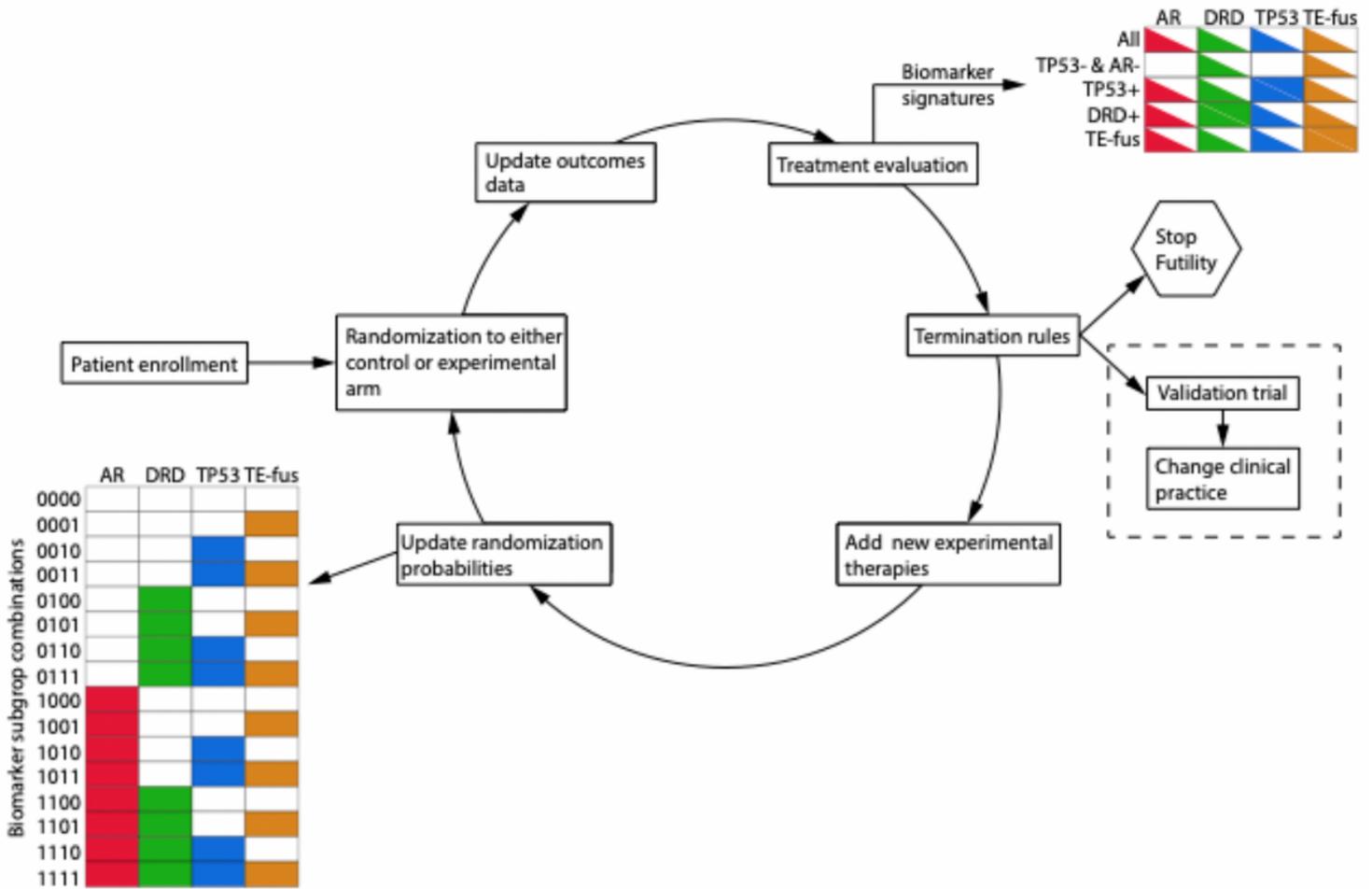


Figure 3

Life cycle of the ProBio platform trial. After written informed consent, the biomarker subgroup combination of the patient is determined and used for randomization to either the control group (standard of care) or one of the experimental arms. Outcome data are updated monthly throughout the trial and will be used to calculate the probabilities of superiority for the active arms over the control group for each biomarker signature of interest. Based on selected threshold, a decision to continue enrollment or to terminate (for futility or superiority) each treatment-biomarker signature will be made. As treatments-biomarker signatures leaves the platform, new treatments can possibly entry in the study. The outcome data is also used to update the randomization probabilities within the biomarker subgroup combinations. Graduating treatments-biomarker signatures will enter a confirmatory trial to validate the hypotheses generated from the platform.