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On Enrichment Strategies for Biomarker Stratified Clinical Trials

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SUMMARY: In the era of precision medicine, drugs are increasingly developed to target subgroups of patients with certain biomarkers. In large all-comer trials using a biomarker stratified design (BSD), the cost of treating and following patients for clinical outcomes may be prohibitive. With a fixed number of randomized patients, the efficiency of testing certain treatments parameters, including the treatment effect among biomarker positive patients and the interaction between treatment and biomarker, can be improved by increasing the proportion of biomarker positives on study, especially when the prevalence rate of biomarker positives is low in the underlying patient population. When the cost of assessing the true biomarker is prohibitive, one can further improve the study efficiency by oversampling biomarker positives with a cheaper auxiliary variable or a surrogate biomarker that correlates with the true biomarker. To improve efficiency and reduce cost, we can adopt an enrichment strategy for both scenarios by concentrating on testing and treating patient subgroups that contain more information about specific treatment parameters of primary interest to the investigators. In the first scenario, an enriched biomarker stratified design (EBSD) enriches the cohort of randomized patients by directly oversampling the relevant patients with the true biomarker, while in the second scenario, an auxiliary-variable-enriched biomarker stratified design (AEBSD) enriches the randomized cohort based on an inexpensive auxiliary variable, thereby avoiding testing the true biomarker on all screened patients and reducing treatment waiting time. For both designs, we discuss how to choose the optimal enrichment proportion when testing a single hypothesis or two hypotheses simultaneously. At a requisite power, we compare the two new designs with the BSD design in term of the number of randomized patients and the cost of trial under scenarios mimicking real biomarker stratified trials. The new designs are illustrated with hypothetical examples for designing biomarker-driven cancer trials.

KEY WORDS: Auxiliary variables; Biomarker stratified design; Cost minimization; Enrichment strategies; Precision medicine; Treatment selection.

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1. Introduction

There is a large literature on study designs integrated with treatment-selection biomarkers. See Mandrekar and Sargent (2009), Freidlin et al. (2010) and Tajik et al. (2013) for recent reviews. Biomarker stratified clinical trials have been frequently used to evaluate the effect and safety of an experimental therapy relative to a control therapy as well as to evaluate the utility of using the biomarker in directing treatments. A trial with a biomarker stratified design (BSD) randomizes all patients to one of the treatment therapies with biomarker as a stratification factor. Such an all-comer trial allows hypothesis testing on treatment parameters related to treatment effects among biomarker positive patients, biomarker negative patients and the overall populations as well as the value of utilizing biomarker to direct treatments. A BSD trial is especially useful when the biomarker of interest has weak or moderate credentials in directing treatments based on pre-existing data (Korn and Freidlin, 2016).

In this paper, we investigate two improved designs based on biomarker stratified clinical trials. The standard BSD design is an all-comer design, in which all eligible patients are enrolled, tested for biomarker, and then randomized. The proportion of patients with given biomarker values is not optimized for efficiency in testing specific treatment parameters. Also, the number of enrolled patients in such trial is often limited by the prohibitive cost associated with treating patients and following them for clinical outcomes. For example, when the prevalence rate of biomarker positives is low, say less than 20%, with a given trial size, the efficiency for testing the treatment effect among biomarker positives and the interaction between treatment and biomarker can be very low, while the contribution of a relatively large number of biomarker negatives to the power of testing the two treatment parameters is small. In one of the improved designs, referred to as the enriched biomarker stratified design (EBSD), we increase (enrich) the relative proportion of biomarker positives among

the randomized patients from 20% to 50% or higher by keeping all biomarker positives and retaining only a proportion of biomarker negatives. With the same number of randomized patients, the EBSD design is able to include more patients with more information on the relevant treatment parameters than the BSD design. In another situation where the cost associated with testing the true biomarker is high and there exists some inexpensive auxiliary variables that is positively correlated to the true biomarker, we can utilize the same enrichment strategy to enrich the randomized patients with more information about specific treatment parameters by oversampling based on the auxiliary biomarker. This improved design is referred to as an auxiliary-variable-enriched biomarker stratified design (AEBSD). Unlike the EBSD design, AEBSD avoids testing the true maker status for all screened patients and can be a useful design when testing for the true biomarker is expensive or time-consuming and there exists a cheaper auxiliary variable or surrogate biomarker that correlates with the true biomarker and thus achieves greater cost-efficiency.

Both EBSD and AEBSD designs use an enrichment strategy - oversampling patients who contain more information about specific treatment parameters and undersampling those who do not - to improve the study efficiency of biomarker stratified trials. Like the biomarker stratified design, these improved designs permit inference on the biomarker negative population, overall population and the interaction effect between treatment and biomarker. But unlike the biomarker stratified design, the enrichment designs usually use a smaller sample of biomarker negative patients, resulting in a more cost-efficient design. In this paper, we will study how to determine the optimal enrichment proportions for both new designs to maximize the testing efficiency for specific treatment parameters. We will compare the relative efficiency of the two designs over BSD in term of the number of randomized patients and the cost of the trial conduct. Yang et al. (2015) investigated a variant of an enriched biomarker design and demonstrated that this design can improve testing efficiency

in treatment effect among biomarker positives with continuous outcome. Both EBSD and AEBSD represent new enrichment sampling strategies to improve trial efficiency and they should be distinguished from the commonly used term "enrichment design" for a targeted design or biomarker positive only design (e.g. Simon and Maitournam (2004)).

The rest of the paper is organized as follows. Section 2 introduces the background of a biomarker stratified design (BSD). In Section 3, we describe the enriched biomarker stratified design (EBSD) and discuss how to design a EBSD trial at the optimal enrichment proportion for testing specific treatment parameters. In Section 4, we describe the auxiliary-variable-enriched biomarker stratified design (AEBSD) and explain how to obtain the optimal probabilities for selecting patients based on auxiliary biomarkers. In Section 5, we compare the two enrichment designs with BSD in several settings mimicking real biomarker stratified trials. In Section 6, we illustrate EBSD with a hypothetical Herceptin trial in breast cancer and AEBSD with a EGFR-inhibitor trial in lung cancer. In Section 7, we conclude the paper with several remarks.

2. Biomarker Stratified Design (BSD)

A biomarker stratified design (BSD) is a commonly used all-comer design for evaluating treatment effects in various biomarker subgroups and the predictive value of the biomarker for optimal treatments. As illustrated in Figure 1a, in a BSD design all screened patients will be randomized to one of two treatments (Experimental E or Control C) with biomarker as a stratification factor. Denote κ_1 the selection probability for the biomarker positives and κ_0 the biomarker negatives. In a BSD design, both κ_1 and κ_0 are equal to one so that the expected proportion of biomarker positives in the randomized cohort is equal to π, the prevalence rate of biomarker positives in the underlying patient population.

[Figure 1 about here.]

2.1 *Notation and Assumptions*

For illustrative purpose, we focus on a biomarker stratified trial in which the effect of an experimental therapy E over a control therapy C on a binary outcome, such as tumor response (yes vs. no), on patients with positive biomarker and negative biomarker. Let $M = \{+, -\}$ or $M = \{1, 0\}$ denote the biomarker status with $P(M+) = \pi$ and $P(M-) = 1 - \pi$. Let $D = \{E, C\}$ or $D = \{1, 0\}$ denote the treatment to which a patient is assigned by random allocation and Y represent the response outcome ($Y = 1$ for response; $Y = 0$ for no response). Denote the response rates for patients with $D = \{E, C\}$ and $M = \{1, 0\}$ as $\eta_{E1} = P(Y = 1|D = 1, M = 1)$, $\eta_{E0} = P(Y = 1|D = 1, M = 0)$, $\eta_{C1} = P(Y = 1|D = 0, M = 1)$ and $\eta_{C0} = P(Y = 1|D = 0, M = 0)$. Several treatment effects can be defined based on the data arising from a BSD design. In this paper, we focus on the response rate, although other related measures, such as log odds, could also be used.

- Treatment effect in $M+$ patients: $B_1 = \eta_{E1} - \eta_{C1}$
- Treatment effect in $M-$ patients: $B_0 = \eta_{E0} - \eta_{C0}$
- Overall treatment effect: $B = \pi B_1 + (1 - \pi)B_0$, which is average treatment effect weighted by the prevalence of biomarker positivity in the population.
- Interaction between treatment and biomarker: $\delta = B_1 - B_0 = (\eta_{E1} - \eta_{C1}) - (\eta_{E0} - \eta_{C0})$
- Clinical benefit between biomarker-guided approach and a standard biomarker-unguided approach:

$$\begin{aligned}
\theta_\gamma &= \text{response rate in biomarker-guided patients} - \text{response rate in biomarker-unguided patients} \\
&= [\pi\eta_{E1} + (1 - \pi)\eta_{C0}] - [\gamma\pi\eta_{E1} + \gamma(1 - \pi)\eta_{E0} + (1 - \gamma)\pi\eta_{C1} + (1 - \gamma)(1 - \pi)\eta_{C0}] \\
&= (1 - \gamma)\pi B_1 - \gamma(1 - \pi)B_0
\end{aligned}$$

where γ is the proportion of patients treated by the experimental therapy E in the biomarker-unguided approach. θ_γ is a measure of treatment benefit difference of two strategies: a biomarker-guided strategy in which optimal treatment is determined by biomarker

and a biomarker-unguided strategy where treatment is assigned to a proportion γ of patients without considering biomarker status. Notice that θ_γ can be directly estimated from biomarker-strategy trials (e.g. Sargent et al. (2005)). When $\gamma = 0$ we have $\theta_0 = \pi B_1$, commonly used as a global measure for biomarker performance in treatment selection (Brinkley et al., 2010; Janes et al., 2011, 2014).

Let n denote the total number of randomized patients in a BSD trial. Let $n_{E1}, n_{C1}, n_{E0}, n_{C0}$ denote the sample sizes in the $D = \{E, C\}$ and $M = \{1, 0\}$ groups, respectively. Let $m_{E1}, m_{C1}, m_{E0}, m_{C0}$ denote the number of responding patients in the corresponding patient groups. The unbiased estimators for these parameters and the corresponding variance estimators can be written as:

- $\hat{B}_1 = \hat{\eta}_{E1} - \hat{\eta}_{C1}$ and $\widehat{var}(\hat{B}_1) = \hat{\eta}_{E1}(1-\hat{\eta}_{E1})/n_{E1} + \hat{\eta}_{C1}(1-\hat{\eta}_{C1})/n_{C1}$, where $\hat{\eta}_{E1} = m_{E1}/n_{E1}$ and $\hat{\eta}_{C1} = m_{C1}/n_{C1}$ are the estimates for the response rates for groups $E1$ and $C1$, respectively.
- $\hat{B}_0 = \hat{\eta}_{E0} - \hat{\eta}_{C0}$ and $\widehat{var}(\hat{B}_0) = \hat{\eta}_{E0}(1-\hat{\eta}_{E0})/n_{E0} + \hat{\eta}_{C0}(1-\hat{\eta}_{C0})/n_{C0}$, where $\hat{\eta}_{E0} = m_{E0}/n_{E0}$ and $\hat{\eta}_{C0} = m_{C0}/n_{C0}$ are the estimates for the response rates for groups $E0$ and $C0$, respectively.
- $\hat{B} = \pi\hat{B}_1 + (1-\pi)\hat{B}_0$ and

$$\widehat{var}(\hat{B}) = \pi^2\widehat{var}(\hat{\eta}_{E1}) + \pi^2\widehat{var}(\hat{\eta}_{C1}) + (1-\pi)^2\widehat{var}(\hat{\eta}_{E0}) + (1-\pi)^2\widehat{var}(\hat{\eta}_{C0})$$

- $\hat{\delta} = \hat{B}_1 - \hat{B}_0$ and $\widehat{var}(\hat{\delta}) = \widehat{var}(\hat{B}_1) + \widehat{var}(\hat{B}_0)$
- $\hat{\theta}_\gamma = (1-\gamma)\pi\hat{B}_1 - \gamma(1-\pi)\hat{B}_0$ and $\widehat{var}(\hat{\theta}_\gamma) = \pi^2(1-\gamma)^2\widehat{var}(\hat{B}_1) + \gamma^2(1-\pi)^2\widehat{var}(\hat{B}_0)$.

For $\hat{B}$ and $\hat{\theta}_\gamma$ we have assumed that π is known. If π is unknown it can be estimated by n_1/n where n_1 is the total number of biomarker positives in the randomized cohort. In this case, the variance expressions are more complicated.

2.2 *Hypothesis testing on treatment parameters*

A typical BSD trial is designed to test one or more hypotheses involving the aforementioned treatment parameters and the results of these tests reveal different aspects of the effect of the experimental therapy over the control therapy conditional or unconditional on biomarker status. Several common scenarios are listed in Table 1. The primary task designing a BSD trial is to ensure that the design is adequately powered for testing the chosen hypothesis. Let $\xi = (B_1, B_0, B, \delta, \theta_\gamma)$ and $\hat{\xi} = (\hat{B}_1, \hat{B}_0, \hat{B}, \hat{\delta}, \hat{\theta}_\gamma)$. Each element of $\hat{\xi}$ is a linear combination of $(\hat{\eta}_{E1}, \hat{\eta}_{C1}, \hat{\eta}_{E0}, \hat{\eta}_{C0})$, which follows a multivariate normal distribution by the central limit theorem. As a result, each element of $\hat{\xi}$ has an asymptotic normal distribution by Slutsky's theorem. That is, when n is large, $Z_i = \frac{\hat{\xi}_i - \xi_i}{\sqrt{\widehat{var}(\hat{\xi}_i)}} \dot{\sim} \mathcal{N}(0,1)$ for $i = 1, \cdots, 5$. Standard normal distribution results can be used to derive the coverage probability for the 95% confidence interval and calculate the power for testing each treatment parameter. As an illustration, a proof that $\hat{B}$ has an asymptotic normal distribution is given in the supplementary materials.

[Table 1 about here.]

3. Enriched Biomarker Stratified Designs (EBSD)

Figure 1b shows a diagram for the EBSD design, in which biomarker positive patients will be selected into the cohort of randomized patients with probability κ_1 and the biomarker negative patients will be selected into the randomized cohort with probability κ_0, and only those patients in the randomized cohort will be treated and followed up. In this paper, our discussion is focused on equal allocation of patients to the two treatment arms. The proposed approach can be easily extended to unequal allocation between treatment arms. Indeed, the allocation ratio between treatment arms can be another design parameter subject to optimization for the power of testing specific hypotheses. For all scenarios of hypothesis testing listed in Table 1, we will search for the optimal enrichment proportion $\pi_e > 0$. The

expected proportion of positives in the trial is $\frac{\kappa_1\pi}{\kappa_1\pi+\kappa_0(1-\pi)}$. If we set the above $= \pi_e$ then $\kappa_0 = \kappa_1\frac{\pi/(1-\pi)}{\pi_e/(1-\pi_e)}$. Any pair (κ_0, κ_1) satisfying the above will work. Thus, there is no unique solution pair (κ_0, κ_1) for any given $\pi_e > 0$. However, we want to minimize the number of patients omitted from the study (i.e., maximize the number selected for randomization among screened patients), so we choose κ_0 and κ_1 to be as large as possible. This additional consideration yields the following unique values for κ_0 and κ_1:

$$\kappa_0 = \kappa_1 = 1 \text{ if } \pi_e = \pi$$

$$\kappa_0 = \frac{\pi/(1-\pi)}{\pi_e/(1-\pi_e)},\ \kappa_1 = 1,\ \text{if } \pi_e > \pi$$

$$\kappa_0 = 1,\ \kappa_1 = \frac{\pi_e/(1-\pi_e)}{\pi/(1-\pi)},\ \text{if } \pi_e < \pi$$

Thus, for any given π_e, including the optimal π_e^{opt}, the values of κ_0 and κ_1 are uniquely determined as above.

3.1 *Test on B*

The variance for the estimate of the overall treatment effect $\hat{B} = \pi\hat{B}_1 + (1-\pi)\hat{B}_0$ can be written as

$$var(\hat{B}) = \pi^2\frac{2\eta_{E1}(1-\eta_{E1})}{n\pi_e} + \pi^2\frac{2\eta_{C1}(1-\eta_{C1})}{n\pi_e} + (1-\pi)^2\frac{2\eta_{E0}(1-\eta_{E0})}{n(1-\pi_e)} + (1-\pi)^2\frac{2\eta_{C0}(1-\eta_{C0})}{n(1-\pi_e)} \tag{1}$$

For an EBSD trial with n randomized patients, the optimal enrichment proportion π_e^{opt} for biomarker positive patients can be obtained by minimizing $\widehat{var}(\hat{B})$. It is straightforward to show the optimal enrichment proportion for biomarker positives

$$\pi_e^{opt} = \frac{1}{1 + \frac{1-\pi}{\pi}\sqrt{\phi}} \tag{2}$$

where

$$\phi = \frac{\eta_{E0}(1-\eta_{E0}) + \eta_{C0}(1-\eta_{C0})}{\eta_{E1}(1-\eta_{E1}) + \eta_{C1}(1-\eta_{C1})} \tag{3}$$

Note that π_e^{opt} approaches π when ϕ approaches 1.

3.2 *Test on δ*

For an EBSD trial with n randomized patients, the optimal enrichment proportion π_e^{opt} for biomarker positive patients in testing δ can be obtained by finding the minimizer for $var(\hat{\delta})$

$$var(\hat{\delta}) = \frac{2\eta_{E1}(1-\eta_{E1})}{n\pi_e} + \frac{2\eta_{C1}(1-\eta_{C1})}{n\pi_e} + \frac{2\eta_{E0}(1-\eta_{E0})}{n(1-\pi_e)} + \frac{2\eta_{C0}(1-\eta_{C0})}{n(1-\pi_e)} \tag{4}$$

The optimal enrichment proportion in this case is given by

$$\pi_e^{opt} = \frac{1}{1+\sqrt{\phi}} \tag{5}$$

where ϕ is defined in (3). Note that π_e^{opt} approaches 0.5 when ϕ approaches 1.

3.3 *Test on θ_γ*

When testing $\theta_\gamma = (1-\gamma)\pi B_1 - \gamma(1-\pi)B_0$ with an EBSD design with $0 \leqslant \gamma \leqslant 1$, one can minimize

$$var(\hat{\theta}_\gamma) = \frac{2(1-\gamma)^2\pi^2}{n\pi_e}\cdot(\eta_{E1}(1-\eta_{E1}) + \eta_{C1}(1-\eta_{C1})) + \frac{2\gamma^2(1-\pi)^2}{n(1-\pi_e)}\cdot(\eta_{E0}(1-\eta_{E0}) + \eta_{C0}(1-\eta_{C0})) \tag{6}$$

It is straightforward to obtain the solution

$$\pi_e^{opt} = \frac{1}{1+\frac{\gamma(1-\pi)}{(1-\gamma)\pi}\sqrt{\phi}} \tag{7}$$

where ϕ is defined in (3). Note that when $\gamma = 0$ we have $\theta_0 = \pi B_1$ and $\pi_e^{opt} = 1$ and when $\gamma = 1$ we have $\theta_1 = -(1-\pi)B_0$ and $\pi_e^{opt} = 0$.

3.4 *Testing two hypotheses*

Without loss of generality, we will use an α splitting approach in the discussion of simultaneously testing two hypotheses. Other testing procedures for control of the overall type I error involving multiple hypotheses can be adopted (e.g. (Matsui et al., 2014)) but these will not be discussed in this paper. When testing two hypotheses, as in cases 12, 13, 14, 15 in Table 1, we can find the optimal enrichment proportion π_e by minimizing the maximum of the required sample sizes for the first hypothesis and the second hypothesis at given type I

errors (α_1, α_2) and type II errors (β_1, β_2). For example, for testing B_1 and δ, the sample size $n(\pi_e; H_{1a})$ for the first hypothesis is given as

$$n(\pi_e; H_{1a}) = \frac{(z_{\alpha_1/2} + z_{\beta_1})^2}{B_1^2/var^*(\hat{B}_1)} \tag{8}$$

For the second hypothesis, the sample size $n(\pi_e; H_{2a})$ is

$$n(\pi_e; H_{2a}) = \frac{(z_{\alpha_2/2} + z_{\beta_2})^2}{\delta^2/var^*(\hat{\delta})} \tag{9}$$

where $var^*(\hat{B}_1) = nvar(\hat{B}_1)$ and $var^*(\hat{\delta}) = nvar(\hat{\delta})$. The optimal π_e, i.e. π_e^{opt}, such that $n_{max} = \max(n(\pi_e; H_{1a}), n(\pi_e; H_{2a}))$ is minimized can be obtained straightforwardly by numerical method.

4. Auxiliary-variable-enriched Biomarker Stratified Design (AEBSD)

The cost of the assessment of the true status of a biomarker M for all patients is often prohibitive. However, suppose that we have an auxiliary variable or a biomarker based on another assay $\tilde{M}$ that is predictive of M and can be easily and cheaply assessed. One can enrich the study with true biomarker positive patients by selecting patients to be randomized based on the values of $\tilde{M}$. Only the patients selected for randomization will have their true biomarkers M measured. Let π and $\tilde{\pi}$ denote the prevalence rates of patients with positive true biomarker $(M = 1)$ and positive auxiliary biomarker $(\tilde{M} = 1)$ respectively in the population. The positive predictive value PPV is the probability that a patient with positive auxiliary biomarker $(\tilde{M} = 1)$ also has a positive true biomarker $(M = 1)$. That is, $PPV = Pr(M = 1|\tilde{M} = 1)$. Let $\tilde{\kappa}_1 \in [0, 1]$ and $\tilde{\kappa}_0 \in [0, 1]$ represent the probability of patients with positive and negative auxiliary variable $\tilde{M}$ being selected into the randomized cohort, respectively. The enrichment proportion for an auxiliary positive patient is $\tilde{\pi}_e = \frac{\tilde{\pi}\tilde{\kappa}_1}{\tilde{\pi}\tilde{\kappa}_1 + (1-\tilde{\pi})\tilde{\kappa}_0}$. The probability of a randomized patient with a positive true biomarker can be written as

$$\pi_e = PPV\tilde{\pi}_e + \left(\frac{\pi - \tilde{\pi}PPV}{1 - \tilde{\pi}}\right)(1 - \tilde{\pi}_e) \tag{10}$$

For statistical testing and inference concerning B or θ_γ we need a consistent estimate for π when π is unknown. We may estimate π by noting $\pi = e_{11}\tilde{\kappa}_1\tilde{\pi} + e_{01}\tilde{\kappa}_0(1-\tilde{\pi})$, where $e_{11} = P(M=1|\tilde{M}=1, R=1)$ and $e_{01} = P(M=1|\tilde{M}=0, R=1)$ and $R=1$ indicates that the patient is selected into the randomized cohort.

4.1 *Testing one hypothesis*

In designing an AEBSD trial, our goal is to find the optimal $\tilde{\pi}_e$ that minimizes the number of randomized patients for testing a specific hypothesis (or hypotheses) as in Table 1. Here we illustrate the idea for testing $H_0 : \delta = 0$ against $H_a : \delta = \delta^*$, where δ is the interaction between treatment and biomarker. To minimize the number of randomize patients we minimize $var(\hat{\delta})$, which is

$$var(\hat{\delta}) = \frac{2\eta_{E1}(1-\eta_{E1})}{n\pi_e} + \frac{2\eta_{C1}(1-\eta_{C1})}{n\pi_e} + \frac{2\eta_{E0}(1-\eta_{E0})}{n(1-\pi_e)} + \frac{2\eta_{C0}(1-\eta_{C0})}{n(1-\pi_e)} \tag{11}$$

where the denominator of each term is the expected number of patients in subgroups defined by D and M. Thus, for given n, π, $\tilde{\pi}$, PPV, $\eta_{E1}, \eta_{C1}, \eta_{E0}, \eta_{C0}$, we can find the optimal $\tilde{\pi}_e$ in $[0,1]$ that minimizes $var(\hat{\delta})$. The result is given by

$$\tilde{\pi}_e^{opt} = \frac{(1-\tilde{\pi})\pi_e^{localopt} - \pi + \tilde{\pi}PPV}{PPV - \pi} \tag{12}$$

where $\pi_e^{localopt}$ is the local optimal solution whose global optimal solution is the same as π_e^{opt} in Section 3.2 but adjusted according to π and PPV. When $\pi_e^{opt} \in [\min(\pi, PPV), \max(\pi, PPV)]$, $\pi_e^{localopt} = \pi_e^{opt}$. Otherwise $\pi_e^{localopt} = \pi$ or PPV, whichever is closer to π_e^{opt}.

4.2 *Testing two hypotheses*

When testing two hypotheses is of interest, as the cases 12, 13, 14, 15 in Table 1, we can find the optimal $\tilde{\pi}_e$ by minimizing the maximum of the required sample sizes for the first hypothesis and the second hypothesis at given $\alpha_1, \beta_1, \alpha_2, \beta_2$. For example, for case 13, the sample size $n(\tilde{\pi}_e; H_{1a})$ for the first hypothesis is given as $n(\tilde{\pi}_e; H_{1a}) = \frac{(z_{\alpha_1/2}+z_{\beta_1})^2}{B_1^2/var^*(\hat{B}_1)}$ where z_{α_1} and z_{β_1} is the standard normal distribution percentile for $\alpha_1/2$ and β_1. For the second

hypothesis, the sample size $n(\tilde{\pi}_e; H_{2a})$ is $n(\tilde{\pi}_e; H_{2a}) = \frac{(z_{\alpha_2/2}+z_{\beta_2})^2}{\delta^2/var^*(\hat{\delta})}$ where $z_{\alpha_2/2}$ and z_{β_2} is the standard normal distribution percentile for $\alpha_2/2$ and β_2. The goal is to find the optimal $\tilde{\pi}_e$ such that $n_{max} = \max(n(\tilde{\pi}_e; H_{1a}), n(\tilde{\pi}_e; H_{2a}))$ is minimized. The local optimal $\pi_e^{localopt}$ can be determined by π, PPV, π_e^{opt}, the global optimal solution in Section 4.1 and the solution for $n(\tilde{\pi}_e; H_{1a}) = n(\tilde{\pi}_e; H_{2a})$. Details are given in supplementary materials. The optimal $\tilde{\pi}_e$ in this case, $\tilde{\pi}_e^{opt}$, can also be calculated by equation (12) using $\pi_e^{localopt}$.

5. Numerical Studies

5.1 *EBSD design*

In this numerical study, we assume that the prevalence of biomarker positive patients in the population is 0.2 and that selected patients will be randomized with equal allocation to treatment $D = \{1, 0\}$. For the sake of illustration, we assume the response of each patient follows a logistic regression model $\text{logit}(Y = 1|D, M) = b_0 + b_1 D + b_2 M + b_3 TM$. We consider two types of interaction between treatment and biomarker, quantitative and qualitative (Polley et al., 2013). In the case of quantitative interaction between treatment and biomarker, we set $b_0 = -0.5, b_1 = 0.4, b_2 = -0.8, b_3 = 0.6$, as seen in Figure 2a, and the logistic model yields the response rates 0.43, 0.21, 0.48 and 0.38 for patient groups in $E1, C1, E0$ and $C0$, respectively. Figure 3 describes the relationship between statistical power for testing specific treatment parameters B, B_1, B_0, δ and θ_γ and the enrichment proportion π_e at the given number of randomized patients $n = 200, 300, 500, 1000$. These plots demonstrate that the optimal enrichment proportion π_e varies by the specific testing parameter and π_e reaches the highest power for B_1 at 1, B_0 at 0, B at 0.19, δ at 0.48 and θ_γ at 0.68. Note that the BSD design corresponds to $\pi_e = 0.2$ in these plots, demonstrating the EBSD design can achieve significant efficiency gain for a given sample size at optimal enrichment proportion π_e^{opt}.

[Figure 2 about here.]

[Figure 3 about here.]

As seen in Figure 2b, for the case of qualitative interaction between treatment and biomarker, we set $b_0 = -0.5, b_1 = -0.8, b_2 = -0.1, b_3 = 1.5$, which yields the response rates 0.21, 0.10, 0.12 and 0.11 for patient groups $E1, C1, E0$ and $C0$, respectively. Figure 4 describes the relationship between the power for the specific treatment parameters B, B_1, B_0, δ and θ_γ and the enrichment proportion π_e at the number of randomized patients $n = 200, 300, 500, 1000$. Again, these plots show that the optimal enrichment proportion π_e varies by the specific testing parameter and π_e reaches the highest power for B_1 at 1, B_0 at 0, B at 0.21, δ at 0.52 and θ_γ at 0.71.

[Figure 4 about here.]

To further verify the performance of the proposed treatment parameter estimators and their variance estimators under EBSD, simulation was conducted based on 1000 simulations. At a given sample size $n = 500$, Table 2 lists the estimates for $B, B_1, B_0, \delta, \theta_\gamma$ for EBSD at π_e^{opt} and BSD. Other quantities, including the standard errors based on the proposed variance estimators (*std.p*), the simulated standard error (*std.e*), and the 95%CI coverage probability based on the estimated standard error (*coverage*), are also provided. It can be seen that the proposed estimators yield consistent estimates with negligible bias and variance estimators yield standard errors close to the simulated one and a satisfying 95% nominal coverage probability. It can also be seen that the EBSD design at π_e^{opt} yields much smaller standard error than the BSD design, indicating the EBSD design is significantly more efficient that the BSD, except for testing the overall treatment effect B, where BSD at $\pi = 0.2$ is very close to its optimal $\pi_e^{pt} = 0.19$ for the quantitative interaction and 0.21 for the qualitative interaction and understandably the BSD at the setting yields similar performance as the EBSD.

[Table 2 about here.]

Table 3 summarizes the results of designing a EBSD trial to test two treatment parameters simultaneously at given powers, 90% for H_1 and 80% for H_2. The results for EBSD are obtained at π_e^{opt} with the method described in Section 3.4. The coverage probability for all treatment effect estimates achieves their corresponding nominal levels; for $\hat{B}_1$ the coverage probability is close to 99% and for the second treatment effect estimate the coverage probability is close to 96%. It can be seen that the EBSD needs significantly less randomized patients to achieve requisite powers for testing two hypotheses than BSD in all combinations of hypothesis testing. Also, the efficiency gain for testing two hypotheses is generally larger than that of testing a single hypothesis.

[Table 3 about here.]

5.2 *AEBSD design*

In this numerical study, we investigate the relationship of patient ratio and cost ratio with PPV for testing the interaction δ under AEBSD. Quantitative and qualitative interactions are both investigated. For a quantitative interaction, $\eta_{E1} = 0.43, \eta_{C1} = 0.21, \eta_{E0} = 0.48$ and $\eta_{C0} = 0.38$. For a qualitative interaction, $\eta_{E1} = 0.53, \eta_{C1} = 0.35, \eta_{E0} = 0.21$ and $\eta_{C0} = 0.38$. We assume $\alpha = 0.05, \beta = 0.1$ in the calculation. The unit cost is 500 for biomarker assay and the average unit cost is $10,000$ for treating and following each patient. Figure 5 shows decreasing trends for both patient ratio and cost ratio with an increasing PPV for both quantitative and qualitative interactions. Table 4 gives further details on the screening ratio ns_{ratio} for AEBSD over BSD. Similar results are obtained for testing two treatment parameters simultaneously. Details can be found in the supplementary materials.

[Figure 5 about here.]

[Table 4 about here.]

6. Case Studies

6.1 *Herceptin trial with EBSD*

The breast cancer chemotherapy Herceptin is a well-known success story of personalized medicine. Human epidermal growth factor receptor-2 protein (HER2) is over-expressed in approximately 20% of breast cancer patients (Korkaya and Wicha, 2013). Herceptin, a target agent on HER2, was shown to be effective in patients with HER2+ metastatic breast cancer (Baselga, 2001; Joensuu et al., 2006). Retrospective studies also suggested that HER2- patients could also benefit from Herceptin (Paik et al., 2008). For illustration, we assume that the overall response rate (ORR), a binary endpoint based on the percentage of patients whose cancer shrinks or disappears after treatment, is to be used in designing a first-line metastatic breast cancer therapy for Herceptin plus chemotherapy E versus chemotherapy C. We assume that these response rates for groups $E1$ and $C1$ are $\eta_{E1} = 45\%$ and $\eta_{C1} = 29\%$ respectively in HER2+ patients and that the response rates for groups $E0$ and $C0$ is 45% and 40%, respectively. Our goal is to illustrate how to design a EBSD trial at the optimal enrichment proportion π_e when the investigators are primarily interested in testing a single hypothesis involving a single treatment parameter from $(B_1, B_0, B, \delta, \theta_\gamma)$ with $\gamma = 0.2$. The optimal enrichment proportion π_e^{opt} is obtained by the method described in Section 3 to achieve the maximum efficiency for the specific test for given n. The top panel of Table 5 shows the required number of randomized patients for EBSD and BSD at two-sided $\alpha = 0.05$ and $\beta = 0.1$. The ratio of randomized patents n_{ratio} and the cost ratio c_{ratio} for EBSD versus BSD are also provided, where the unit cost for ascertaining true biomarker is 300 and the unit cost for treating and following patient is averaged $10,000$ for one year (Schmidt, 2011). In this case, the cost of screening and IHC testing for HER2 is significantly lower than the cost of treatment and patient follow-up.

[Table 5 about here.]

Table 5 also illustrates the case of designing an EBSD trial when testing two hypotheses. We consider the first hypothesis of interest to be the test on treatment effect among HER2+ patients B_1, which is often the primary goal in a biomarker-driven clinical trial. The second hypothesis will be chosen from $(B_0, B, \delta, \theta_\gamma)$, testing the treatment effect in biomarker negatives, the treatment effect in the overall population, the interaction between treatment and biomarker, and the clinical benefit of selecting treatment by biomarker. The response rates for the four groups of patients defined by treatment and biomarker are the same for the case of single hypothesis testing. To control the overall type error at the level of two-sided 0.05, we split the α between the first hypothesis and the second hypothesis. The number of randomized patients for testing each hypothesis for given $\alpha_1 = 0.01$, $\beta_1 = 0.10$, $\alpha_2 = 0.04$ and $\beta_2 = 0.2$ are calculated, and the maximum of the two sample sizes is chosen as the size of the trial. The optimal enrichment proportion π_e^{opt} for the EBSD design is obtained by numerical methods to achieve the smallest of the maximum number of randomized patients required by testing both hypotheses with respective power greater than $1 - \beta_1$ and $1 - \beta_2$ for the two hypotheses. In the bottom panel of Table 5, the ratio of randomized patents of the two designs n_{ratio} and the ratio of cost c_{ratio} are listed. P_{ebsd} indicates the probability of success when testing two hypotheses for EBSD. In designing trials with two primary hypotheses, one can obtain the probability of success, i.e. the probability of rejecting either null hypothesis under the alterative (Matsui et al., 2014). The probability of success is calculated using the joint distribution of two testing statistics $Z_1 = \frac{\hat{B}_1}{\sqrt{\widehat{var}(\hat{B}_1)}}$ and $Z_2 = \frac{\hat{\delta}}{\sqrt{\widehat{var}(\hat{\delta})}}$.

6.2 *EGFR-inhibitor trial using AEBSD*

In this case study, we consider designing a hypothetical AEBSD trial for comparing the 5-month progression-free-survival (5mPFS) rate of gefitinib (E) versus carboplatin and paclitaxel (C) in patients with non-small-cell lung cancer (NSCLC). The example is hypothetical, but the 5mPFS for each patient group is based on the results of an actual clinical trial

(IPASS) (Mok et al., 2009). The mutation of epidermal growth factor receptor (EGFR) is thought predictive of the effect of gefitinib in treating non-small cell lung cancer. The prevalence of EGFR mutations in approximately 50% in Asia, significantly higher than the 10% prevalence in North America (Shi et al., 2015; Kerr, 2013). As a result of the high prevalence rate of EGFR mutants, IPASS was successfully conducted in Asia and found that gefitinib significantly extended PFS among patients with EGFR mutations, but resulted in significantly shorter PFS for patients with EGFR wild types (Mok et al., 2009; Maemondo et al., 2010).

We consider a biomarker stratified trial to be conducted in North America with the goal of testing two primary hypotheses: H_1: the treatment effect among patients with EGFR mutations B_1 and H_2: the interaction between treatment and EGFR mutation δ. We set two-sided $\alpha_1 = \alpha_2 = 0.025$ and $\beta_1 = \beta_2 = 0.1$. A BSD design with the objectives of testing B_1 and δ is very inefficient, as it would enroll, treat and follow a large number of patients who are EGFR wild-types and therefore would entail a waste of limited resource. For an AEBSD design, it is known that EGFR mutations are more commonly observed in patients with adenocarcinomas and no prior history of smoking, as well as in females and those of Asian descent (Kerr, 2013). A predictive score, the auxiliary variable in this case, can be built using these easily and cheaply assessed prognostic factors. We have assumed the prevalence rate of "high-score" patients is 15% and the true EGFR mutations is at least 60% among the "high-score" patients.

Mimicking the IPASS trial, we choose the median PFS for groups $E1, C1, E0, C0$ as 9.82, 4.71, 2.00 and 5.70 months, respectively, indicating a strong qualitative interaction between treatment and biomarker. Under the exponential hazards, we assume the 5mPFS for these groups are 0.65, 0.41, 0.13 and 0.48, respectively. Under these design parameters, we find the optimal selection probability $\tilde{\kappa}_1 = 1$ for auxiliary positive patients and $\tilde{\kappa}_0 = 0$ for the

auxiliary negative patients. The number of randomized patients for the two designs are $n_{AEBSD} = 338$ and $n_{BSD} = 2023$ with $n_{ratio} = 0.167$, and the cost ratio $c_{ratio} = 0.172$. In the calculation of the trial cost, we assume that the unit cost of testing EGFR mutation is $1,000$, the average treatment cost and the average follow-up cost are $7,500$ and $2,500$ for each patient in the randomized cohort while the unit cost for determining the EGFR predictive score is 50. These cost estimates are based on the literature reflecting the experience of the United States (Horgan et al., 2011; Sauter and Butnor, 2016).

7. Discussion

In this paper, we propose two new enrichment designs for biomarker stratified clinical trials. The key idea of enrichment sampling is to oversample patients who contain more information about specific treatment parameters and undersample those who do not. We demonstrate that the new designs can significantly improve study efficiency in term of increased power and higher estimation precision with a fixed number of randomized patients and therefore reduce the cost of conducting trials. We give analytic solutions or numerical algorithms for finding the optimal probabilities for selecting patients with positive and negative biomarkers into the randomized cohort for the EBSD design and the optimal probabilities of selecting patients with positive and negative auxiliary biomarkers for the AEBSD design. We also demonstrate how to determine the sample size for EBSD and AEBSD designs when testing a single treatment parameter or two treatment parameters simultaneously. The numerical studies and the case studies demonstrate the superior performance of the new designs over the BSD.

Enrichment sampling strategies have been proposed and successfully used in observational studies to test association between disease and risk factors (Morara et al., 2007; Wang and Zhou, 2010; Strauss et al., 2010) and to estimate the accuracy of biomarkers in predicting disease condition (Wang et al., 2012, 2013). These papers demonstrate that biased sampling

with enrichment of relevant patient subgroups, those that contain more information on estimands, leads to more efficient studies that requires significantly fewer patients and study cost. The enrichment strategies can be applied to other biomarker-driven clinical trial designs, such as the biomarker strategy design. See Freidlin et al. (2010) for a review. In a biomarker strategy design, patients are randomly assigned to a biomarker-guided arm that uses the biomarker to determine whether a patient receive the experimental therapy or the control therapy or to a biomarker-unguided arm that randomly assign the patients to the experimental therapy and control therapy regardless of biomarker status.

In this paper we consider a binary endpoint such as tumor response or survival rate at a landmark time. The extension of our discussion to an unequal randomization ratio is straightforward. Indeed, the allocation ratio between treatment arms can be optimized for additional efficiency gains to test specific treatments parameters. An enrichment strategy is equally applicable to trials involving more than two treatments.

Compared to the BSD design, one limitation of the EBSD and AEBSD designs is that they may significantly prolong the time of trial completion, as the latter designs require longer time to accrue sufficient number of biomarker positive patients. In this paper, the cost introduced by prolonged trial completion time has not been considered. In practice, this issue can be addressed by verifying that the EBSD and AEBSD designs under the optimal selection on $\tilde{\kappa}_1$ and $\tilde{\kappa}_0$ will lead to an estimated time of trial completion that the investigators can accept. If not, the standard BSD design may be used.

Acknowledgements

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References

Baselga, J. (2001). Herceptin® alone or in combination with chemotherapy in the treatment of HER2-positive metastatic breast cancer: pivotal trials. *Oncology* **61,** 14–21.

Brinkley, J., Tsiatis, A., and Anstrom, K. J. (2010). A generalized estimator of the attributable benefit of an optimal treatment regime. *Biometrics* **66,** 512–522.

Freidlin, B., McShane, L. M., and Korn, E. L. (2010). Randomized clinical trials with biomarkers: design issues. *Journal of the National Cancer Institute* .

Horgan, A., Bradbury, P., Amir, E., Ng, R., Douillard, J., Kim, E., Shepherd, F., and Leighl, N. (2011). An economic analysis of the INTEREST trial, a randomized trial of docetaxel versus gefitinib as second-/third-line therapy in advanced non-small-cell lung cancer. *Annals of Oncology* **22,** 1805–1811.

Janes, H., Brown, M. D., Huang, Y., and Pepe, M. S. (2014). An approach to evaluating and comparing biomarkers for patient treatment selection. *The international journal of biostatistics* **10,** 99–121.

Janes, H., Pepe, M. S., Bossuyt, P. M., and Barlow, W. E. (2011). Measuring the performance of markers for guiding treatment decisions. *Annals of Internal Medicine* **154,** 253–259.

Joensuu, H., Kellokumpu-Lehtinen, P.-L., Bono, P., Alanko, T., Kataja, V., Asola, R., Utriainen, T., Kokko, R., Hemminki, A., Tarkkanen, M., et al. (2006). Adjuvant docetaxel or vinorelbine with or without trastuzumab for breast cancer. *New England Journal of Medicine* **354,** 809–820.

Kerr, K. M. (2013). Clinical relevance of the new IASLC/ERS/ATS adenocarcinoma classification. *Journal of Clinical Pathology* **66,** 832–838.

Korkaya, H. and Wicha, M. S. (2013). HER2 and breast cancer stem cells: more than meets the eye. *Cancer Research* **73,** 3489–3493.

Korn, E. L. and Freidlin, B. (2016). Biomarker-based clinical trials. In George, S. L., Wang,

X., and Pang, H., editors, *Cancer Clinical Trials: Current and Controversial Issues in Design and Analysis*, pages 333–364. Chapman and Hall/CRC.

Maemondo, M., Inoue, A., Kobayashi, K., Sugawara, S., Oizumi, S., Isobe, H., Gemma, A., Harada, M., Yoshizawa, H., Kinoshita, I., et al. (2010). Gefitinib or chemotherapy for non–small-cell lung cancer with mutated EGFR. *New England Journal of Medicine* **362,** 2380–2388.

Mandrekar, S. J. and Sargent, D. J. (2009). Clinical trial designs for predictive biomarker validation: theoretical considerations and practical challenges. *Journal of Clinical Oncology* **27,** 4027–4034.

Matsui, S., Choai, Y., and Nonaka, T. (2014). Comparison of statistical analysis plans in randomize-all phase III trials with a predictive biomarker. *Clinical Cancer Research* **20,** 2820–2830.

Mok, T. S., Wu, Y.-L., Thongprasert, S., Yang, C.-H., Chu, D.-T., Saijo, N., Sunpaweravong, P., Han, B., Margono, B., Ichinose, Y., et al. (2009). Gefitinib or carboplatin–paclitaxel in pulmonary adenocarcinoma. *New England Journal of Medicine* **361,** 947–957.

Morara, M., Ryan, L., Houseman, A., and Strauss, W. (2007). Optimal design for epidemiological studies subject to designed missingness. *Lifetime Data Analysis* **13,** 583–605.

Paik, S., Kim, C., and Wolmark, N. (2008). HER2 status and benefit from adjuvant trastuzumab in breast cancer. *New England Journal of Medicine* **358,** 1409–1411.

Polley, M.-Y. C., Freidlin, B., Korn, E. L., Conley, B. A., Abrams, J. S., and McShane, L. M. (2013). Statistical and practical considerations for clinical evaluation of predictive biomarkers. *Journal of the National Cancer Institute* **105,** 1677–1683.

Sargent, D. J., Conley, B. A., Allegra, C., and Collette, L. (2005). Clinical trial designs for predictive marker validation in cancer treatment trials. *Journal of Clinical Oncology* **23,** 2020–2027.

Sauter, J. and Butnor, K. (2016). Clinical and cost implications of universal versus locally advanced-stage and advanced-stage-only molecular testing for epidermal growth factor receptor mutations and anaplastic lymphoma kinase rearrangements in nonsmall cell lung carcinoma: A tertiary academic institution experience. *Archives of Pathology and Laboratory Medicine* **140,** 358–361.

Schmidt, C. (2011). How do you tell whether a breast cancer is HER2 positive? ongoing studies keep debate in high gear. *Journal of the National Cancer Institute* **103,** 87–89.

Shi, Y., Li, J., Zhang, S., Wang, M., Yang, S., Li, N., Wu, G., Liu, W., Liao, G., Cai, K., et al. (2015). Molecular epidemiology of EGFR mutations in asian patients with advanced non-small-cell lung cancer of adenocarcinoma histology–mainland china subset analysis of the PIONEER study. *PloS one* **10,** e0143515.

Simon, R. and Maitournam, A. (2004). Evaluating the efficiency of targeted designs for randomized clinical trials. *Clinical Cancer Research* **10,** 6759–6763.

Strauss, W. J., Ryan, L., Morara, M., Iroz-Elardo, N., Davis, M., Cupp, M., Nishioka, M. G., Quackenboss, J., Galke, W., Özkaynak, H., et al. (2010). Improving cost-effectiveness of epidemiological studies via designed missingness strategies. *Statistics in Medicine* **29,** 1377–1387.

Tajik, P., Zwinderman, A. H., Mol, B. W., and Bossuyt, P. M. (2013). Trial designs for personalizing cancer care: a systematic review and classification. *Clinical Cancer Research* **19,** 4578–4588.

Wang, X., Ma, J., George, S., and Zhou, H. (2012). Estimation of AUC or partial AUC under test-result-dependent sampling. *Statistics in Biopharmaceutical Research* **4,** 313–323.

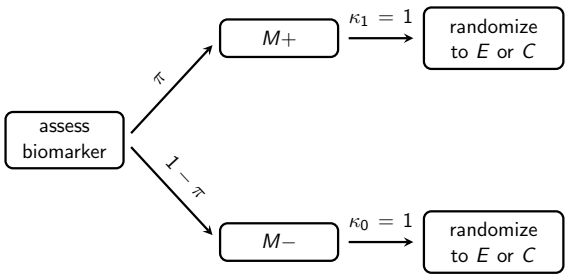
Wang, X., Ma, J., and George, S. L. (2013). ROC curve estimation under test-result-dependent sampling. *Biostatistics* **14,** 160–172.

Wang, X. and Zhou, H. (2010). Design and inference for cancer biomarker study with an

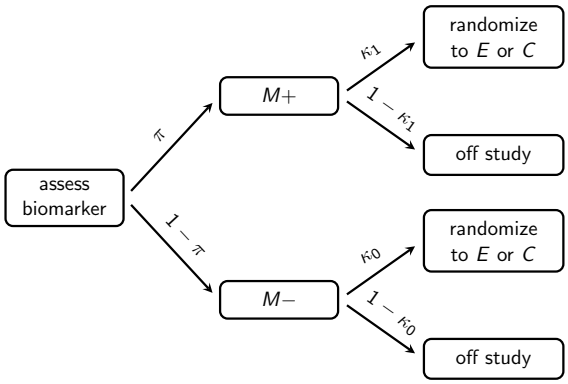
outcome and auxiliary-dependent subsampling. *Biometrics* **66,** 502–511.

Yang, B., Zhou, Y., Zhang, L., and Cui, L. (2015). Enrichment design with patient population augmentation. *Contemporary Clinical Trials* **42,** 60–67.

(a) Biomarker Stratified Design (BSD)



(b) Enriched Biomarker Stratified Design (EBSD)



(c) Auxiliary-variable-enriched Biomarker Stratified Design (AEBSD)

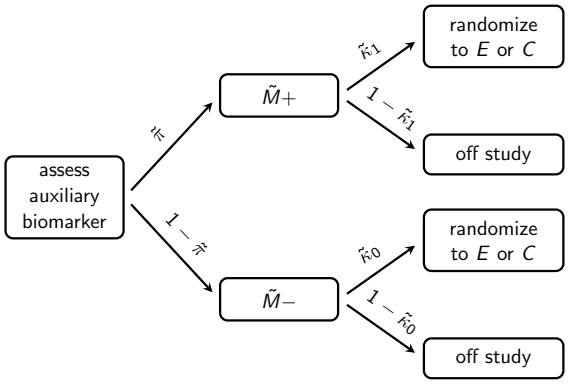


Figure 1: Diagram for (a) Biomarker stratified design (BSD), (b) Enriched biomarker stratified design (EBSD) and (c) Auxiliary-variable-enriched biomarker stratified design (AEBSD). For BSD and EBSD, π is the prevalence of biomarker positives in the population; κ_1 and κ_0 are the selection probability for biomarker positives and biomarker negatives into the randomized cohort, respectively. For AEBSD, $\tilde{\pi}$ is the prevalence of auxiliary positives in the population; $\tilde{\kappa}_1$ and $\tilde{\kappa}_0$ are the selection probability for auxiliary positives and auxiliary negatives into the randomized cohort, respectively.

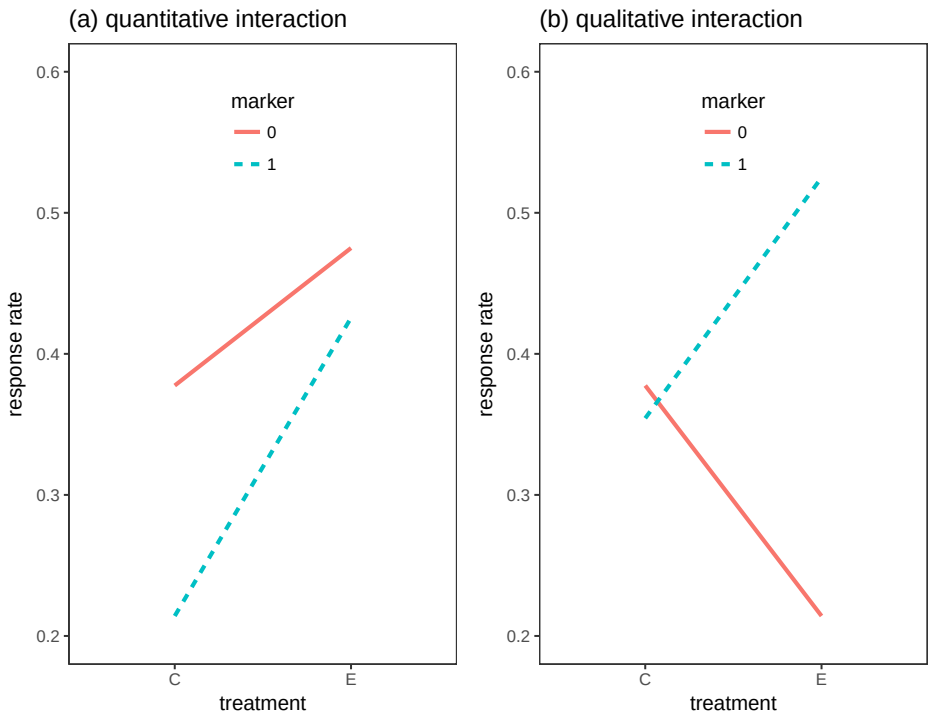


Figure 2: Illustration for (a) quantitative interaction with response rates $\eta_{E1} = 0.43$, $\eta_{C1} = 0.21$, $\eta_{E0} = 0.48$ and $\eta_{C0} = 0.38$ and (b) qualitative interaction with response rates $\eta_{E1} = 0.21$, $\eta_{C1} = 0.10$, $\eta_{E0} = 0.12$ and $\eta_{C0} = 0.11$

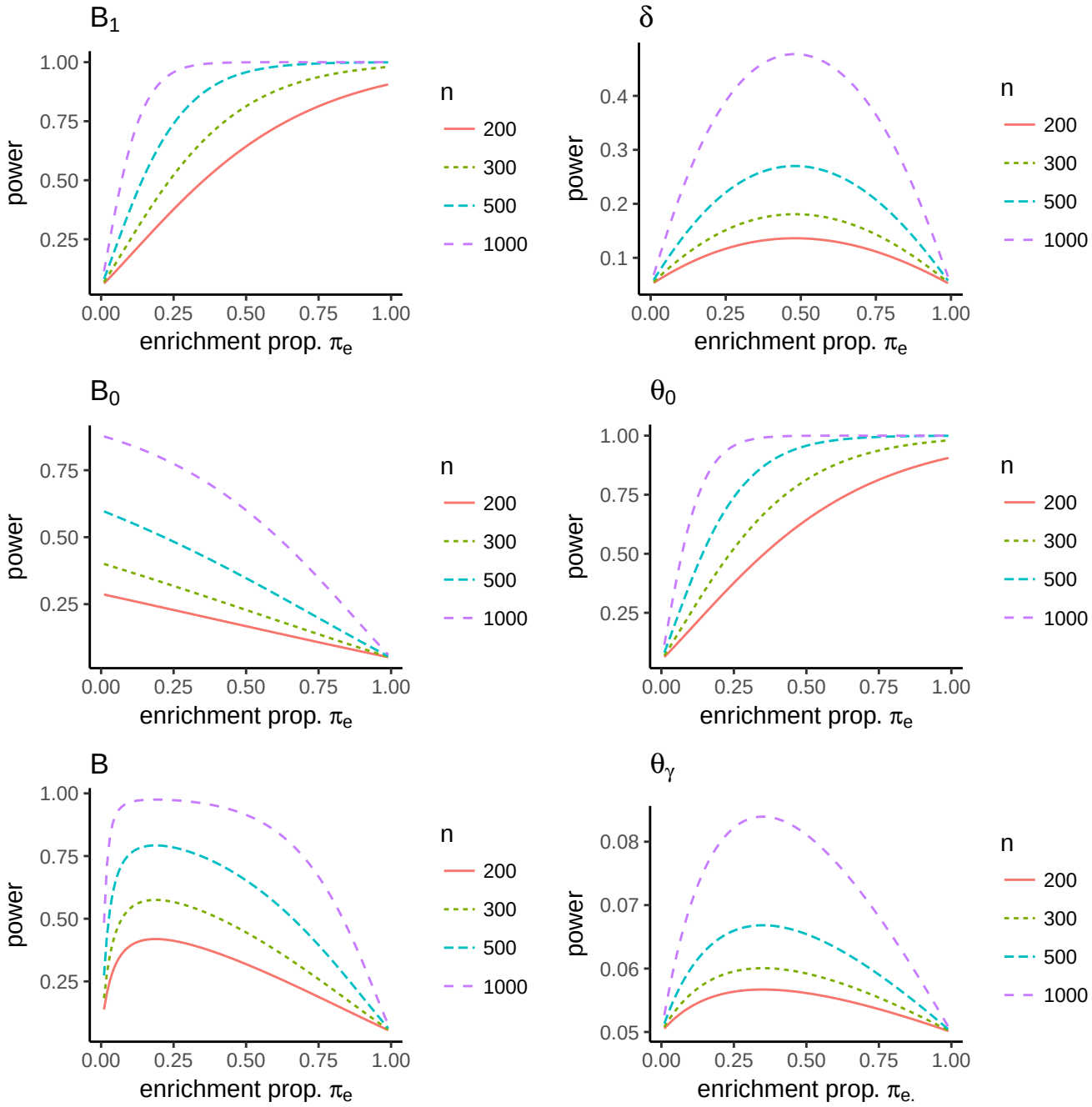


Figure 3: The power for testing a specific treatment parameter at different enrichment proportions π_e for EBSD for quantitative interaction

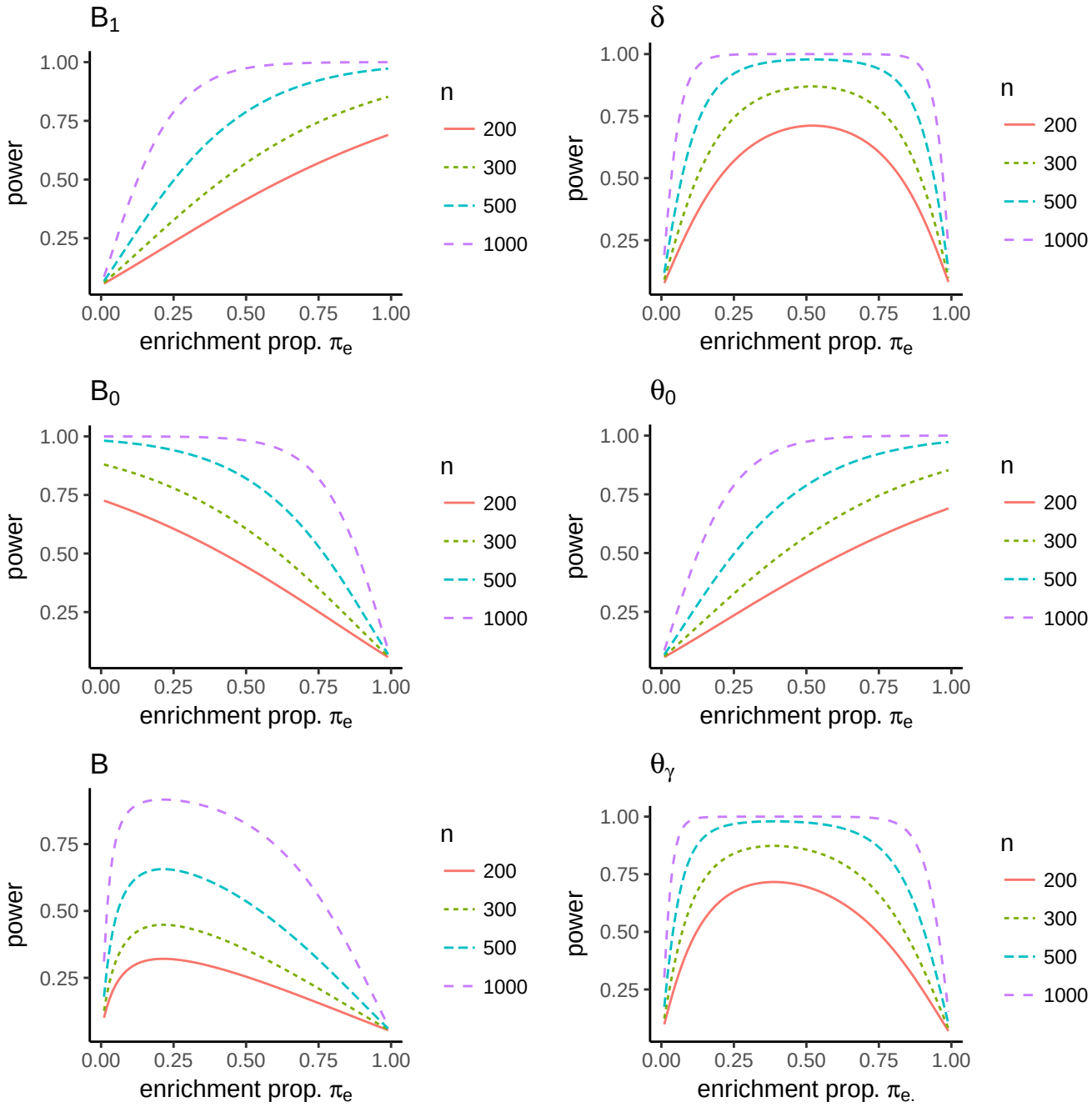


Figure 4: The power for testing a specific treatment parameter at different enrichment proportions π_e for EBSD for qualitative interaction

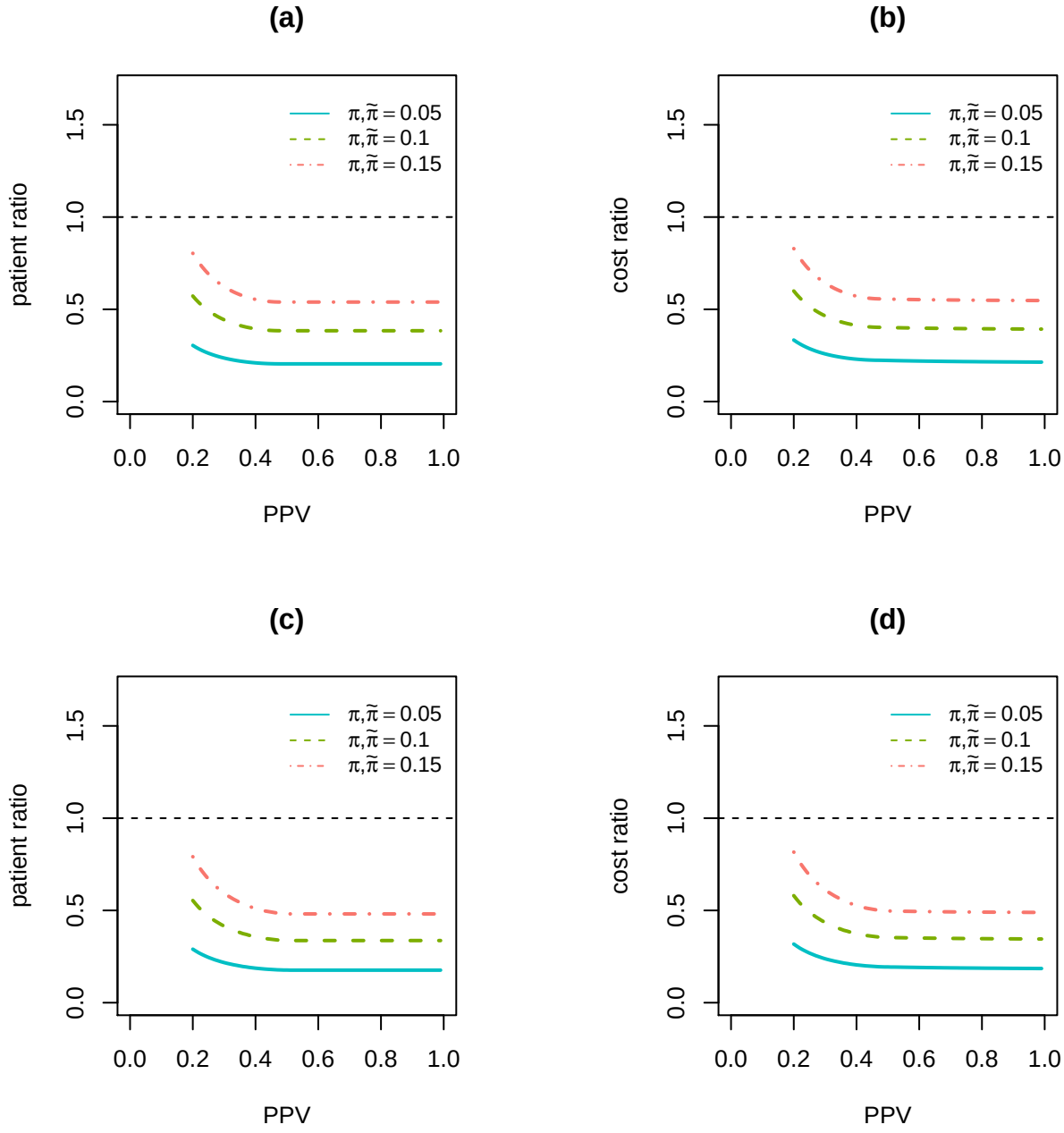


Figure 5: Relationship of patient and cost ratio with PPV for testing the interaction between treatment and biomarker δ under AEBSD. (a) Patient ratio with quantitative interaction; (b) Cost ratio with quantitative interaction; (c) Patient ratio with qualitative interaction; (d) Cost ratio with qualitative interaction.

Table 1: Tests on different treatment parameters and their combinations

Case	Hypothesis	Interpretation
1	H_0: $B_1 = 0$ vs. H_a: $B_1 \neq 0$	Test on B_1
2	H_0: $B_0 = 0$ vs. H_a: $B_0 \neq 0$	Test on B_0
3	H_0: $B = 0$ vs. H_a: $B \neq 0$	Test on B
4	H_0: $\delta = 0$ vs. H_a: $\delta \neq 0$	Test on δ
5	H_0: $\theta_\gamma = 0$ vs. H_a: $\theta_\gamma \neq 0$	Test on θ_γ
12	H_{10}: $B_1 = 0$ vs. H_{1a}: $B_1 \neq 0$ H_{20}: $B_0 = 0$ vs. H_{2a}: $B_0 \neq 0$	Test on B_1 and B_0
13	H_{10}: $B_1 = 0$ vs. H_{1a}: $B_1 \neq 0$ H_{20}: $B = 0$ vs. H_{2a}: $B \neq 0$	Test on B_1 and B
14	H_{10}: $B_1 = 0$ vs. H_{1a}: $B_1 \neq 0$ H_{20}: $\delta = 0$ vs. H_{2a}: $\delta \neq 0$	Test on B_1 and δ
15	H_{10}: $B_1 = 0$ vs. H_{1a}: $B_1 \neq 0$ H_{20}: $\theta_\gamma = 0$ vs. H_{2a}: $\theta_\gamma \neq 0$	Test on B_1 and θ_γ

Table 2: Simulation results for EBSD and BSD for testing a single hypothesis ($n = 500$)

Test	B_1		B_0		B		δ		θ_γ	
	BSD	EBSD	BSD	EBSD	BSD	EBSD	BSD	EBSD	BSD	EBSD
					quantitative interaction					
π, π_e^{opt}	0.2	1	0.2	0	0.2	0.188	0.2	0.480	0.2	0.675
true	0.211	0.211	0.097	0.097	0.120	0.120	0.114	0.114	0.030	0.030
estimate	0.211	0.212	0.097	0.098	0.120	0.118	0.110	0.113	0.030	0.030
std.p	0.090	0.041	0.049	0.044	0.043	0.043	0.103	0.084	0.017	0.011
std.e	0.091	0.040	0.049	0.044	0.044	0.043	0.103	0.085	0.017	0.011
coverage	0.943	0.952	0.949	0.945	0.951	0.949	0.942	0.946	0.944	0.947
					qualitative interaction					
π, π_e^{opt}	0.2	1	0.2	0	0.2	0.214	0.2	0.521	0.2	0.710
true	0.171	0.171	-0.163	-0.163	-0.097	-0.097	0.334	0.334	0.044	0.044
estimate	0.171	0.171	-0.162	-0.164	-0.097	-0.096	0.335	0.333	0.044	0.044
std.p	0.097	0.044	0.045	0.040	0.042	0.041	0.107	0.084	0.018	0.011
std.e	0.100	0.044	0.045	0.041	0.042	0.041	0.108	0.085	0.018	0.011
coverage	0.944	0.948	0.951	0.941	0.949	0.945	0.947	0.948	0.941	0.951

$\gamma = 0.1$ is assumed for θ_γ. We also set $\pi = 20\%, \alpha = 0.05, n_{sim} = 1000$

Table 3: Simulation results for EBSD and BSD for testing two hypotheses at targeted powers

Test	B_1 & B_0		B_1 & B		B_1 & δ		B_1 & θ_γ	
	BSD	EBSD	BSD	EBSD	BSD	EBSD	BSD	EBSD
				quantitative interaction				
π, π_e^{opt}	0.2	0.244	0.2	0.416	0.2	0.480	0.2	0.675
n	1374	1130	1374	661	3449	2315	1374	538
	B_1		B_1		B_1		B_1	
true	0.211	0.211	0.211	0.211	0.211	0.211	0.211	0.211
estimate	0.211	0.211	0.211	0.212	0.211	0.212	0.211	0.211
std.p	0.055	0.054	0.055	0.055	0.035	0.027	0.055	0.047
std.e	0.055	0.055	0.055	0.054	0.034	0.027	0.054	0.048
coverage	0.987	0.988	0.989	0.987	0.991	0.991	0.987	0.990
	B_0		B		δ		θ_γ	
true	0.097	0.097	0.120	0.120	0.114	0.114	0.030	0.030
estimate	0.097	0.097	0.120	0.120	0.113	0.114	0.030	0.030
std.p	0.030	0.034	0.026	0.041	0.039	0.039	0.010	0.010
std.e	0.030	0.033	0.027	0.041	0.039	0.039	0.010	0.011
coverage	0.963	0.961	0.958	0.962	0.961	0.960	0.955	0.951
				qualitative interaction				
π, π_e^{opt}	0.2	0.658	0.2	0.495	0.2	0.873	0.2	0.939
n	2444	742	2444	988	2444	560	2444	520
	B_1		B_1		B_1		B_1	
true	0.171	0.171	0.171	0.171	0.171	0.171	0.171	0.171
estimate	0.170	0.170	0.171	0.172	0.171	0.171	0.171	0.171
std.p	0.044	0.044	0.044	0.044	0.044	0.044	0.044	0.044
std.e	0.045	0.044	0.046	0.044	0.044	0.045	0.045	0.045
coverage	0.988	0.990	0.987	0.990	0.989	0.990	0.990	0.989
	B_0		B		δ		θ_γ	
true	-0.163	-0.163	-0.097	-0.097	0.334	0.334	0.044	0.044
estimate	-0.163	-0.164	-0.096	-0.096	0.335	0.335	0.044	0.044
std.p	0.020	0.056	0.019	0.033	0.049	0.114	0.008	0.015
std.e	0.021	0.057	0.019	0.034	0.049	0.116	0.008	0.015
coverage	0.958	0.958	0.954	0.956	0.960	0.954	0.957	0.941

$\gamma = 0.1$ is assumed for θ_γ. We also set $\pi = 20\%, \alpha_1 = 0.01, \alpha_2 = 0.04, \beta_1 = 0.1, \beta_2 = 0.2, n_{sim} = 1000$

Table 4: Numerical results for AEBSD design for testing δ

π	$\tilde{\pi}$	PPV	$\tilde{\pi}_e^{opt}$	n_{aebsd}	ns_{aebsd}	n_{bsd}	n_{ratio}	c_{ratio}	ns_{ratio}
quantitative interaction									
0.05	0.05	0.2	1.000	4325	86500	14199	0.305	0.334	6.092
		0.5	0.958	2902	55592	14199	0.204	0.223	3.915
		0.8	0.595	2902	34516	14199	0.204	0.216	2.431
0.1	0.1	0.2	1.000	4325	43251	7559	0.572	0.599	5.722
		0.5	0.955	2902	27716	7559	0.384	0.401	3.667
		0.8	0.589	2902	17082	7559	0.384	0.395	2.260
0.15	0.15	0.2	1.000	4325	28834	5381	0.804	0.829	5.358
		0.5	0.951	2902	18408	5381	0.539	0.556	3.421
		0.8	0.582	2902	11252	5381	0.539	0.549	2.091
qualitative interaction									
0.05	0.05	0.2	1.000	546	10920	1882	0.290	0.318	5.802
		0.5	1.000	333	6660	1882	0.177	0.194	3.539
		0.8	0.647	332	4296	1882	0.176	0.187	2.283
0.1	0.1	0.2	1.000	546	5461	986	0.554	0.580	5.539
		0.5	1.000	333	3330	986	0.338	0.354	3.377
		0.8	0.642	332	2131	986	0.337	0.347	2.161
0.15	0.15	0.2	1.000	546	3640	690	0.791	0.816	5.275
		0.5	1.000	333	2220	690	0.483	0.498	3.217
		0.8	0.635	332	1407	690	0.481	0.491	2.039

$\tilde{\pi}_e^{opt}$ is optimal enrichment proportion for auxiliary positive patient; n_{aebsd} is the number of randomized patients for AEBSD; n_{bsd} is the number of randomized patients for BSD; n_{bsd} is the number of randomized patients for BSD; n_{ratio} is the ratio of n_{EBSD} and n_{BSD}; ns_{ratio} is the ratio of the number of screened patients for AEBSD versus BSD; c_{ratio} is the cost ratio for conducting AEBSD and BSD. $\alpha = 0.05, \beta = 0.1$. The unit cost is 500 for ascertaining true biomarker, the average unit cost is $10,000$ for treatment and follow-up, and the unit cost is 50 for ascertaining auxiliary variable.

Table 5: Herceptin trial: Testing one or two hypotheses with EBSD at optimal enrichment proportion π_e^{opt} compared to BSD

Test	true	π_e^{opt}	n_{ebsd}	P_{ebsd}	n_{bsd}	P_{bsd}	n_{ratio}	ns_{ratio}	c_{ratio}
Testing a single hypothesis									
B_1	0.160	1.000	372	0.900	1861	0.900	0.200	1.000	0.216
B_0	0.050	0.000	4098	0.900	5122	0.900	0.800	1.000	0.804
B	0.072	0.194	1948	0.900	1949	0.900	1.000	1.007	1.000
δ	0.110	0.491	3267	0.900	4996	0.900	0.654	1.605	0.673
θ_0	0.032	1.000	372	0.900	1861	0.900	0.200	1.000	0.216
θ_γ	0.025	0.685	1071	0.900	2643	0.900	0.405	1.387	0.425
Testing two hypotheses									
B_1 & B_0	0.160, 0.050	0.139	3797	0.980	4087	0.997	0.929	1.000	0.930
B_1 & B	0.160, 0.072	0.318	1663	0.961	2635	0.985	0.631	1.002	0.638
B_1 & δ	0.160, 0.110	0.491	2607	1.000	3986	0.985	0.654	1.606	0.673
B_1 & θ_0	0.160, 0.032	0.999	528	0.965	2635	0.964	0.200	1.001	0.216
B_1 & θ_γ	0.160, 0.025	0.685	855	0.940	2635	0.909	0.325	1.111	0.340

π_e^{opt} is optimal enrichment proportion for biomarker positives; n_{ebsd} is the number of randomized patients for EBSD; P_{ebsd} is the power of testing a single hypothesis and the probability of success of testing two hypotheses for EBSD; n_{bsd} is the number of randomized patients for BSD; P_{bsd} is the power of testing a single hypothesis and the probability of success of testing two hypotheses for BSD; n_{ratio} is the ratio of n_{EBSD} and n_{BSD}; ns_{ratio} is the ratio of the number of screened patients for EBSD versus BSD; c_{ratio} is the cost ratio for conducting EBSD and BSD. $\gamma = 0.2$ is assumed for θ_γ. We also assume $\pi = 0.2$, the unit cost is 300 for ascertaining true biomarker and the average unit cost is $10,000$ for treatment and follow-up. For testing on a single hypothesis, $\alpha = 0.05, \beta = 0.1$. For testing two hypotheses, $\alpha_1 = 0.01, \beta_1 = 0.1$, $\alpha_2 = 0.04, \beta_2 = 0.2$.