Optimizing transcriptome-based synthetic lethality predictions to improve precision oncology in early-stage breast cancer: BC-SELECT

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- Figure 1: A graphical overview illustrating the adaptation of BC-SELECT pipeline.
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- Supplementary Table 1: AUC values of precision-recall curves of BC-SELECT and its two components.
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ABSTRACT

Introduction: Patients with early-stage breast cancer currently do not have approaches to predict relative benefit from targeted therapies or immunotherapies (as with Oncotype in hormone-receptor positive (HR+) patients for chemotherapy). SELECT,[1] a computational tool that leverages genetic interactions, such as synthetic lethality or rescue (SL or SR), using transcriptomic data to predict treatment response. SELECT and its commercial adaptation, ENLIGHT[2], predict patient survival response to therapy in retrospective data across cancer types. However, the breast cancer-specific therapies tested by those algorithms are largely limited to endocrine therapies or HER2-directed therapies. This does not address current treatment options, such as PARP inhibitors or immunotherapy, that may compete without clear guidance for patients. We sought to improve the application of transcriptome-based genetic interactions in patients with early-stage breast cancer by adapting the SELECT framework specifically for this population. This adaptation, BC-SELECT, seeks to optimize predictive accuracy and therapeutic guidance for patients with breast cancer.

Methods: SELECT involves two main steps: (1) a “training” step that, for a given drug target, constructs a library of clinically relevant candidate SL/SR partner genes, and (2) a “testing/validation” step that calculates patients' predicted response scores in unseen studies based on the relative expression of the candidate partner genes. We adapt this approach in BC-SELECT by using breast cancer-specific expression data and breast cancer trials in the training step, then performing testing/validation on breast cancer

clinical trials. We evaluate the prediction accuracy of BC-SELECT using multiple statistical tests.

Results: BC-SELECT predicted treatment response in 11 clinical trials in breast cancer (not used for training), with 5 out of 8 targeted therapy trials (including PARP inhibitors) showing ROC-AUCs > 0.6, and all 3 immunotherapy trials showing ROC-AUCs > 0.7. BC-SELECT showed empirical statistical significance and surpassed our 2.5% cutoff threshold compared to chance in 7/11 datasets. The algorithm outperformed the original SELECT and ENLIGHT for immunotherapy prediction in breast cancer.

Conclusion: In the early-stage setting, where pre-treatment predictors of response are limited for triple-negative and HER2-positive disease, BC-SELECT predicts patient responses better than chance and better than pre-existing algorithms for targeted therapies and immunotherapies. These findings underscore the algorithm's potential as a decision support tool for refining precision medicine, particularly prioritization of clinical treatments, for patients with early-stage breast cancer.

INTRODUCTION

Gene expression data (via RT-PCR-based panels) have transformed clinical management in early-stage breast cancer. However, this information is currently limited to patients with HR+ disease, who benefit from the opportunity to undergo this testing prior to receiving any such therapy[3–5]. Patients with HER2-positive and triple-negative breast cancer (TNBC), unfortunately, must currently undergo multiple therapies and surgery, with pathologic complete response to treatment serving as a post-facto indicator of response. Although broader transcriptomic data, which can be routinely obtained commercially, identifies not only gene expression levels, but also fusion genes that underlie FDA therapeutic approvals, this data is not currently used in early-stage breast cancer management[6–8].

Synthetic lethality (SL), a form of genetic interaction in which two genes must be concurrently inactivated to cause cell death, demonstrates clinical relevance, as shown in the example of PARP inhibitors as used to treating cancers with a *BRCA1/2* pathogenic mutation across numerous clinical trials[9,10]. Other genetic interactions, such as synthetic dosage lethality (SDL), in which one gene must be over-expressed and another under-expressed[11], or synthetic rescue (SR), in which inactivation of one gene that would cause death is ameliorated by altered activation of another gene, are being actively studied for potential clinical applications[12].

Computational approaches to identifying these interactions have been recently developed. SELECT and a commercial adaptation, ENLIGHT, use large-scale cell line and patient tumor transcriptomic data to identify clinically relevant synthetic lethal/rescue partners for a wide array of targeted therapies. Both algorithms show

efficacy in predicting treatment response in retrospective clinical trials[1,2]. Despite the breadth of these tools across numerous cancer types as published, their translational application in breast cancer was limited. The breast cancer trials that were included focused on endocrine therapy or HER2-directed therapy, where clinical subtype effectively dictates the use of these therapies (limiting the need for other tools to refine treatment selection).

In this paper, we adapt SELECT for breast cancer with the goal of predicting therapeutic response to various targeted therapy and immunotherapy drugs specific to early-stage breast cancer. We used datasets from patients with breast cancer to train our model, and we apply the algorithm to clinical trials not only of HER2-directed therapy, but also immunotherapy and PARP inhibitors in breast cancer. This adaptation, BC-SELECT, advances a translational, transcriptome-based strategy to predict treatment responders, with the eventual goal of personalizing prioritized treatment strategies for patients with breast cancer.

RESULTS

Overview of the BC-SELECT framework and adaptations for performance in early-stage breast cancer

Figure 1 provides a visual overview of the BC-SELECT pipeline. There are two components: (1) Training on large-scale cell-line and patient datasets to identify clinically relevant genetic interactions, and (2) Testing/validation by predicting response to treatments on unseen clinical trials in breast cancer. The inferred genetic interaction partner genes in step (1) are then used to generate a given patient's predicted response score using their transcriptomic data in step (2), with all patients analyzed within a given trial dataset. We tested BC-SELECT on 11 breast cancer clinical trials: 4 using trastuzumab and 1 using lapatinib in HER2+ disease, 2 using durvalumab in TNBC, 1 using pembrolizumab in TNBC and HR+ breast cancer, and 3 using PARP inhibitors (either veliparib or olaparib) in TNBC and HR+ disease. This approach allows BC-SELECT to avoid overfitting issues while achieving flexibility across datasets.

Briefly, we made the following adaptations to the previously published SELECT and ENLIGHT algorithms:

• The training step uses breast cancer-specific patient datasets, and the testing/validation step uses only breast cancer-specific trials. As all trials were in early-stage TNBC, pathologic complete response (PCR) was used as the response indicator.

• Copy number data is removed as a training step requirement to facilitate the future addition of publicly available breast cancer datasets.

• We add additional clinical covariates to the survival analyses in the Cox proportional hazards filtering step to improve clinical utility.

• We add a separate SDL score to the targeted therapy prediction component, in which a higher SDL score indicates a higher likelihood of cancer cell death and drug response.

• In the targeted therapy prediction component, we prioritize candidate partner genes based on survival analysis.

• We generate a final predicted response score (described below) that incorporates target gene expression of the tested therapy as well as a ratio of the relative expression of the partner genes for the relevant genetic interaction to the relative expression of all genes (METHODS).

BC-SELECT predicts treatment response for PARP inhibitors and trastuzumab better than chance

Table 1 reviews the ROC-AUC values and empirical p-values compared to chance for the BC-SELECT predicted response score and its two components: target gene expression and partner gene expression ratio. BC-SELECT predicted response scores showed statistical significance (one-tailed empirical p-value < 0.025) for four targeted therapy datasets (2/4 for patients receiving trastuzumab, 2/3 for patients receiving a PARP inhibitor), with AUCs ranging from 0.64-0.81. In contrast, partner gene expression ratio was statistically significant in 3 datasets, and target gene expression in 2 datasets. Figure 2 demonstrates the ROC and precision-recall curves for these datasets given the limitations of ROC-AUC alone, and AUC values of the precision-recall curves are provided in Supplementary Table 1.

BC-SELECT predicts treatment response for immunotherapy better than chance and outperforms two reference controls

Table 2 reviews the ROC-AUC values and empirical p-values compared to chance for BC-SELECT predicted response scores and the two score components: *PDCD1*/*CD274* expression and partner gene expression ratio. BC-SELECT and partner gene expression ratio showed statistical significance for all datasets; however, ROC-AUCs were consistently higher with BC-SELECT (0.74-0.76 vs. 0.65-0.72). By comparison, 2/3 datasets were statistically significant with target gene expression. Figure 3 demonstrates the ROC and precision-recall curves for these datasets, with the AUC values for the precision-recall curves shown in Supplementary Table 1.

BC-SELECT distinguishes between responders and non-responders in targeted therapy and immunotherapy

Across all datasets, responders (pathological complete response, PCR = 1) had consistently higher predicted scores from BC-SELECT compared to non-responders (PCR = 0). Using an adjusted p-value threshold of 0.1 after correction of multiple hypothesis tests, we observed statistical significance using the Wilcoxon rank sum test in 5/8 trials for targeted therapy (Figure 4) and 3/3 immunotherapy trials (Figure 5).

We also derived the odds ratios with 95% confidence intervals of the predicted response scores between responders and non-responders. We chose a classification threshold of 0.5 given the binary response metric. Among the 11 datasets, 7 (all 3

PARP inhibitors, all 3 immunotherapy, and 1 of 4 trastuzumab) demonstrate statistically significant odds ratios, further supporting BC-SELECT scores being consistently higher among responders. Detailed 95% confidence intervals of odds ratios and multiplicity-corrected p-values from the Fisher exact tests for significance are provided in Supplementary Table 2.

BC-SELECT performs better than SELECT and ENLIGHT in early-stage breast cancer immunotherapy datasets

As shown in Figure 7, BC-SELECT consistently achieved higher ROC-AUCs than SELECT where original SELECT results could be estimated (with immunotherapy). We were also able to compare to previously included four breast cancer datasets in ENLIGHT (Figure 8). In the context of ROC-AUC values, BC-SELECT also performed better than ENLIGHT in the one immunotherapy dataset. However, BC-SELECT's performance was mixed relative to ENLIGHT for trastuzumab, with superior performance in only 1/3 datasets.

DISCUSSION

Our computational algorithm, BC-SELECT, effectively predicts responders to PARP inhibitors and immunotherapy in early-stage breast cancer, demonstrating translational application feasibility. We adapted the SELECT algorithm to use breast cancer-specific datasets for training and breast cancer-specific trials for testing/validation, added additional clinical covariates as part of the filtering of genetic interaction pairs, and sought to demonstrate with statistical rigor how well BC-SELECT predicts treatment response relative to chance. Given that the eventual goal is to support and refine current clinical treatment strategies with clinical utility, we intentionally applied multiple measures with stringent thresholds to test performance. These statistical measures included not only ROC-AUCs, but also PR-AUCs, odds ratios, and Monte-Carlo simulations (to develop an estimate of “chance”) to better estimate how well BC-SELECT performs. BC-SELECT showed ROC-AUCs above 0.6 in 8/11 datasets and showed empirical statistical significance with a 2.5% cutoff threshold compared to chance in 7/11 datasets. Seven datasets, including all of the PARP inhibitors and immunotherapy trials, also demonstrated statistically significant odds ratios in distinguishing responders from non-responders.

In addition to developing this tool, we observed patterns that may be notable in the context of future translational research work. For example, in the targeted therapy module, we handled SL and SDL separately and found that, across trials, different therapeutic targets would show better performance with one interaction type over another. PARP inhibitors demonstrated better performance with SL partners, consistent with their known mechanism of interaction. However, HER2-targeted therapies showed

better performance with SDL partners. HER2 has only been studied as a participant in synthetic lethal interactions to a minimal extent to date[13,14]. Our result encourages further investigation into HER2-targeted agents potentially applied through this mechanism.

We also observed that target gene expression, specifically, the product of *PDCD1* and *CD274*, performed well in predicting response to immunotherapy. *CD274* / *PD-L1* is an existing therapeutic biomarker via protein expression, with part of the challenge in its current predictive use being multiple assays and incomplete ascertainment of potential treatment responders[15]. RNA-based expression of these genes is known to correlate with immunotherapy responders[16–18], but these genes have not demonstrated pure predictive performance in themselves. In this paper, we still observed that BC-SELECT was improved upon target gene expression performance in immunotherapy, reinforcing that consideration of both the synthetic genetic interaction mechanism (where it applies) as well as the mechanism of the drug can support prediction.

Potential future applications of BC-SELECT encompass multiple clinical needs. One application already alluded to is the ability to predict responders to therapy in HER2+ and TNBC breast cancers in advance of treatment. While HER2+ subtyping with immunohistochemistry and fluorescence in situ hybridization has been long established, research remains ongoing into methods that can improve on these subtyping strategies specifically for treatment response prediction[19]. Another potential area of need is the tool's ability to identify potential responders to therapy that would be outside of current guidelines. For example, patients with metastatic TNBC are currently only candidates

for immunotherapy if they have a combined PD-L1 positive expression score above 10, with an active question being how to identify potential responders that do not meet this specific threshold[20]. The structure of BC-SELECT facilitates the addition of metastatic breast cancer datasets in future.

This tool can also prove valuable in the context of patients who have multiple options potentially available, supporting clinicians in prioritizing given options. Patients with pathogenic germline *BRCA1/2* variants and breast cancer, for example, can often be eligible for both PARP inhibitors and immunotherapy, with guidelines having limited data to support current recommendations for prioritization. Multiple clinical trials have also not demonstrated any outcome benefit to combining these therapies, and a downside of additional side effects[21,22]. This approach could potentially optimize decision making in such scenarios.

BC-SELECT does have limitations consistent with the other computational algorithms in this family. It requires specific drug targets. Many chemotherapies, unfortunately, have numerous target effects by mechanism, limiting the ability to predict with this tool. Additionally, if a drug has multiple targets, as of now, we consider the average gene expression of these targets, weighting the targets equally. We observe the issue with this in dataset GSE66399[23] (lapatinib), a drug that is designated as having both *ERBB2* (HER2) and *EGFR* as targets in DrugBank. If we prioritize *ERBB2* (HER2) instead of equally weighting these targets, BC-SELECT achieved an ROC-AUC of 0.67 (as opposed to 0.51), suggesting that future research on assigning informed weights to the targets can enhance BC-SELECT's prediction performance. We also observed that datasets with fewer than 25 patients showed reduced prediction

accuracy. As more clinical datasets in breast cancer become available for training and testing/validation, we will reinforce BC-SELECT's vulnerability to "noise."

In summary, we have developed BC-SELECT, a computational tool designed to predict drug response to targeted and immunotherapy in patients with breast cancer using gene expression and synthetic genetic interactions. This tool maximizes roles for publicly available datasets with human patient data and focuses on clear, patient-centered outcomes. Given the increased success rates of SL oncology trials even outside of PARP inhibitor trials[24], it is worth exploring this mechanism and its application across other therapeutic options. This tool demonstrates predictive performance across targeted therapies and immunotherapies, enabling the differentiation of responders and non-responders for future translational purposes and eventual clinical decision support.

METHODS

Datasets used for training

To train BC-SELECT, we use large, publicly available breast cancer-specific datasets that included gene expression data (microarray or normalized RNA-seq), clinical measures (age, stage), and overall survival. This includes all patients with breast cancer in TCGA (n=1075)[25] as well as METABRIC (n=1989)[26]. The cell-line data is identical to the original SELECT.

Drug targets

We use DrugBank as our reference for the drug targets for targeted therapy[27]. For immunotherapy, the "drug targets" were the simple products of the expression of *PDCD1* and *CD274.*

BC-SELECT training framework

Our model adapts the original SELECT training framework[1]. Screening for genetic interactions is performed in two independent modules, one that employs SL/SDL interactions for targeted therapies, and one that employs SR interactions for immunotherapy.

There are three general filtering steps in the training framework to identify gene pairs associated with clinical relevance:

(1) Either a large-scale data screen using tumor cell line data[28–33] and pharmacological data[34–36] with a Wilcoxon rank sum test to identify significant

associations (targeted therapy) or a hypergeometric test (described in ISLE[37] and SELECT[1]) in the training datasets above to identify pairs of genes that were underexpressed or inactivated together more frequently than expected by chance in a hypergeometric distribution.

(2) Cox proportional hazards models using the genetic interaction partners' gene expressions from patients with breast cancer (in the training datasets above) to identify genetic interactions associated with patient survival.

(3) Evolutionary analysis of preserved gene expression relationships using the Euclidean distance of phylogenetic similarity scores[38] and feature weight[39].

Gene pairs advance through filters if they meet the pre-specified FDR threshold of 0.2 for the Wilcoxon rank sum test / hypergeometric test in step (1) and survival analysis in (2). This threshold is used to avoid overly conservative exclusion of potential genetic interactions, and is the same uniform threshold used in ISLE[37]. For the phylogenetic analysis in step (3), we identify phylogenetically linked candidate partner genes by analyzing phylogenetic profiles.

Each module includes tailored adaptations. In the targeted therapy module, step (1) is the large-scale data screen using tumor cell line and pharmacological data as described. For step (2), we conduct a total of four separate Cox proportional hazard analyses: one for SDL and one for SL, each performed separately in the TCGA[25] and METABRIC[26] datasets to avoid batch effect confounding. Clinical covariates include age, stage, sex (for TCGA) and race (for TCGA). Gene expression values are included

as covariates in addition to clinical variables to determine if expression of those genes is significantly associated with patient survival. Gene expression is assessed only within datasets (not across datasets, again to limit batch effect confounding) and split into tertiles (where upregulated is the top tertile, and downregulated is the bottom tertile based on relative expression levels across patients. Significance is determined by using the multiplicity-corrected marginal p-values of covariates for gene expression and the p-values from the associated log-likelihood ratio tests.

After the partner genes are identified, the training framework selects the top 50 SL or SDL partner genes, prioritized by their adjusted significance in the Cox proportional hazards testing. These genes are used to calculate the patient's partner gene expression ratios (Supplementary Table 3).

For the immunotherapy module, for step (1), the cell-line and drug screen filter did not identify enough immune-related candidate partner genes. As such, we substitute a hypergeometric test only for this module, as performed in ISLE[37] and SELECT[1], using the breast TCGA and METABRIC datasets. This test identifies pairs of genes that are underexpressed or inactivated together more frequently than expected by chance in a hypergeometric distribution (suggesting that this would support cancer cell survival). For step (2), two survival analyses were performed using the same TCGA and METABRIC datasets. We focused on anti-PD1/PDL1 agents, as other classes of immunotherapy are not yet approved in breast cancer and did not have corresponding clinical trial data available. For step (3), we compute the phylogenetic similarity score between each

candidate partner gene and both *PDCD1* and *CD274*. The final phylogenetic score for the candidate gene is determined as the smaller value between the two similarity scores. We identify phylogenetically linked candidate partner genes using a threshold (acceptable maximum Euclidean distance between two linked genes) of 10.5 for targeted therapy[37] and 23.15 for immunotherapy[1].

After the partner genes are identified, the training framework selects the top 10 SR partner genes, prioritized by phylogenetic similarity scores. These genes are used to calculate the patient's partner gene expression ratios (Supplementary Table 3).

BC-SELECT predicted response scores

The partner gene expression ratio for SL in the targeted therapy module for each patient is calculated as the ratio of down-regulated partner genes (as defined by tertiles) over all 50 SL genes identified. The partner gene expression ratio for SDL in the targeted therapy module for each patient is calculated as the ratio of up-regulated partner genes (as defined by tertiles) over all 50 SDL genes identified. The partner gene expression ratio for SR in the immunotherapy module is calculated as 1 minus the ratio of down-regulated partner genes over all 10 SR genes identified (as we are measuring the likelihood of drug response).

These gene expression ratios are then revised to include only those genes in both the numerator and denominator that are in curated gene lists of 776 genes (breast cancer-

specific) and 1154 genes (immune-specific) derived from Nanostring[40,41] (Supplementary Table 4) for normalization and noise reduction.

The final predicted response scores were calculated by multiplying the partner gene expression score by target gene expression. Where a drug has multiple targets, the score uses the mean expression value of all targets for each patient. These predicted response scores are what are reported as the predicted response to targeted therapy or immunotherapy.

BC-SELECT testing and validation framework

Clinical trials used for testing and validation

To test and validate BC-SELECT, we sought publicly available clinical trial datasets that included at least 25 patients with early-stage breast cancer, individual level gene expression data, and treatment response. Pathologic complete response (PCR) was the most commonly studied trial endpoint available. This was consistently reported across studies as a binary measure with either 0 (residual disease) or 1 (complete response). Three datasets (two for targeted therapy and one for immunotherapy) are used as tuning cohorts to determine the hyperparameters. These fixed parameters are then used for drug response predictions in independent targeted and immunotherapy datasets to avoid overfitting. We included a total of 8 clinical trials across 4 therapies for targeted therapy and 3 clinical trials for 2 therapies for immunotherapy. Trials used in this paper are described in Supplementary Table 5.

Performance evaluation

To test the performance of BC-SELECT, we compared the final prediction scores of BC-SELECT in response to a given therapy to the actual drug responses within each clinical trial. We calculated ROC-AUC, precision-recall curves with PR-AUCs, and odds ratios to assess prediction performance. The final reported ROC-AUC values for targeted therapy represent either all SL or all SDL gene pairs – ROC-AUCs were independently generated and compared within the module.

We estimated the statistical significance of BC-SELECT's predictions compared to chance by developing a prediction "null distribution" using Monte-Carlo simulations (Supplementary Figure 1).

For the Monte-Carlo simulations, we randomly selected a subset of genes with the same number of partners as our candidates (50 for targeted therapy and 10 for immunotherapy). Using these genes, we calculated ROC-AUC values and iterated this process 5000 times to yield a distribution of "null AUC values". We then could compare the predictive efficacy of the BC-SELECT final prediction score via percentile of the ROC-AUC to that achieved by chance. We used a similar approach to generate a separate "null" distribution in order to compare the predictive performance of the gene expression ratios to chance. For target gene expression, we used the same number of randomly selected genes as number of targets for the drug of interest to generate a separate "null" distribution. We also report our findings using an empirical p-value ($p=0.025$), the probability of the null AUC distribution exceeding the obtained ROC-AUC

of BC-SELECT. Based on empirical rules, the top 2.5% corresponds to the single tail with prediction better than two standard deviations from the mean in the null distribution.

Statistical Analysis

Statistical tests used to prioritize partner genes were described in "**BC-SELECT training framework."** Cox proportional hazards regression models were fitted using the R library survival. Wilcoxon rank sum tests were performed using R functions wilcox.test. ROC-AUC values are calculated using the R library ROCR and pROC. Precision-recall curves were generated using the R libraries pROC and ggplot2. The Fisher exact test was performed using the fisher.test function in R. To control the false discovery rate (FDR), we apply the Benjamini–Hochberg procedure (BH step-up procedure) as a multiplicity correction using p.adjust function in R. All analyses were conducted using version 4.2 of the R software.

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Conflicts of Interest:

Yewon Kim: None
Matthew Nagy: None
Becca Pollard: None
Padma Sheila Rajagopal: None

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Data availability:

All available data and the source codes are accessible on GitHub: https://github.com/yewon-kim76/BC-SELECT, and the list of references can be found in Zotero: https://www.zotero.org/yewon76/collections/G396YYNB.

Tables and Figures

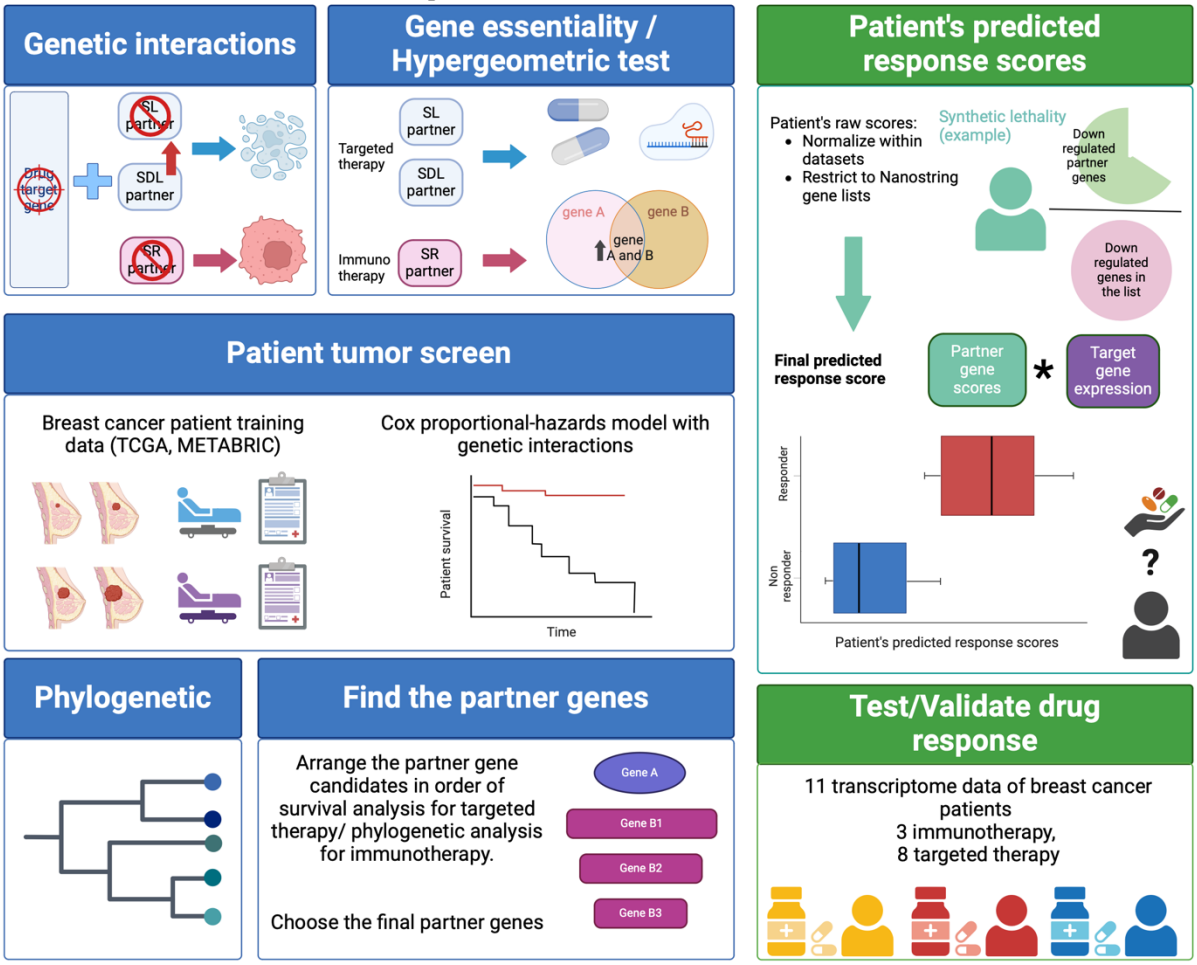


Figure 1: A graphical overview illustrating the adaptation of BC-SELECT pipeline. The training component is depicted in the blue box, while the testing/validation component is illustrated in the green box.

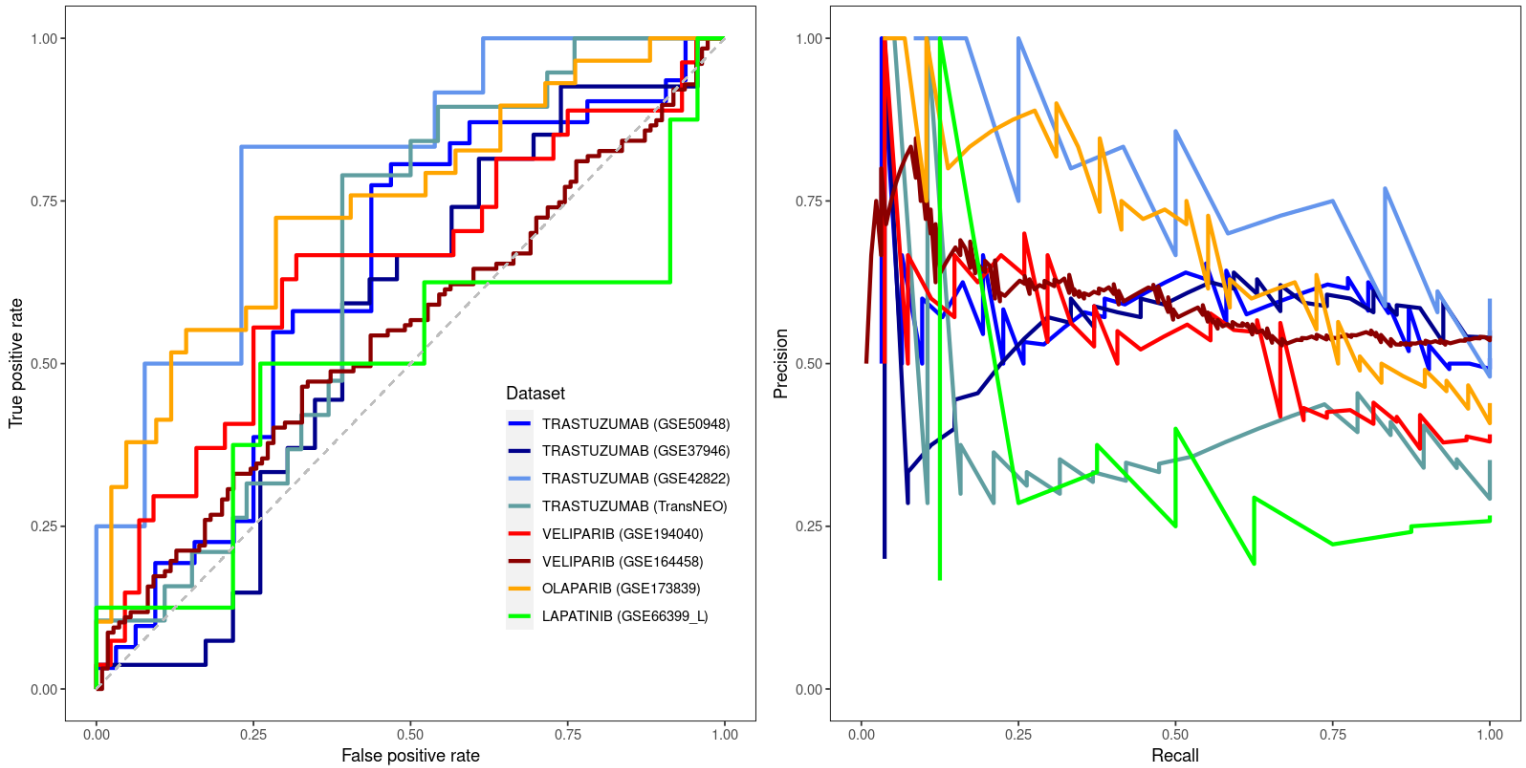


Figure 2: ROC and precision-recall curves for BC-SELECT in targeted therapy datasets.
The left panel shows the false positive rate plotted against the true positive rate for the ROC curve. The right panel shows the precision recall curve. HER2-directed trials are in cool colors (trastuzumab-based trials in blue and lapatinib in green), while PARP inhibitors are in warm colors (veliparib-based trials in red and olaparib in orange). Precision-recall values are presented in Supplementary Table 1.

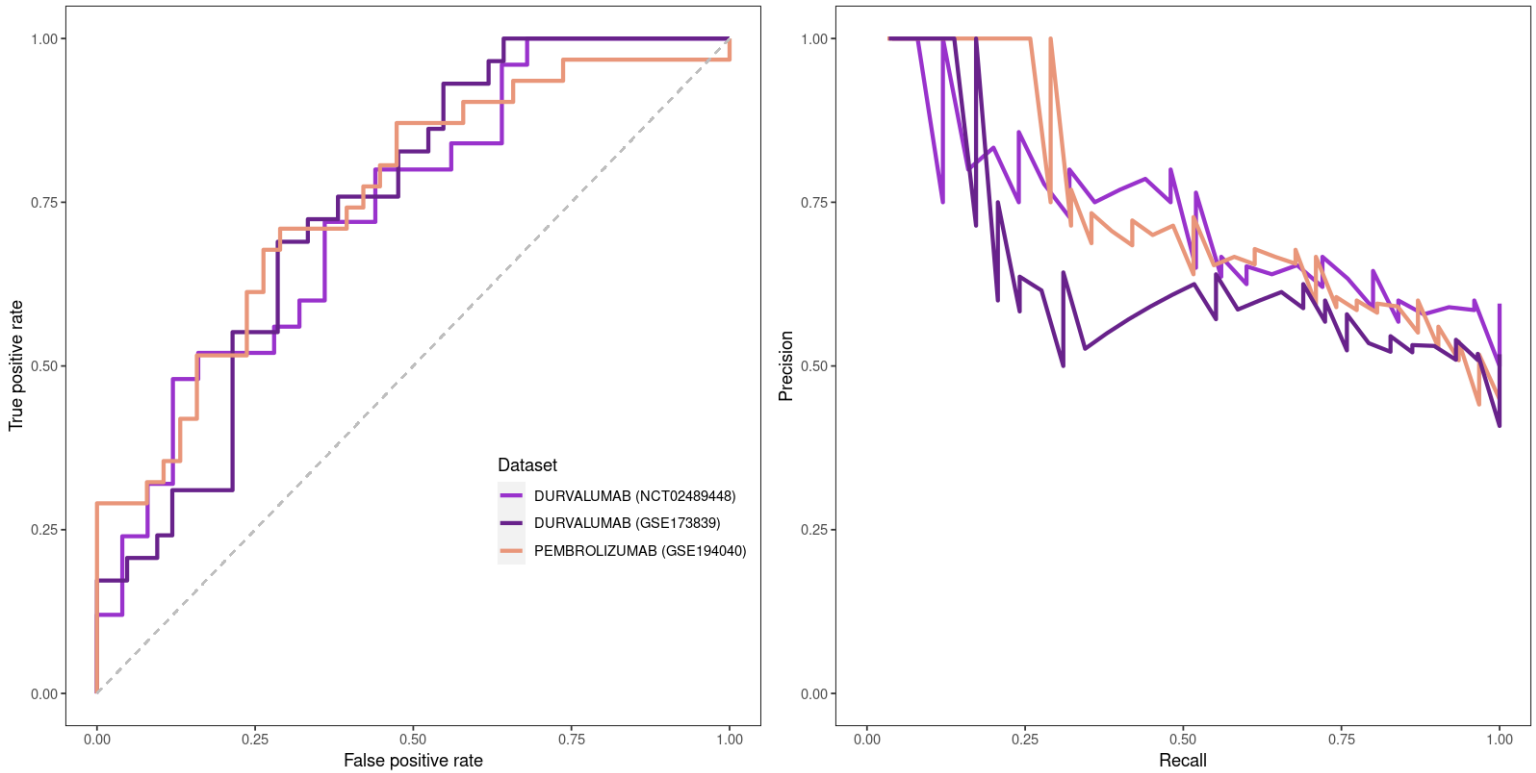


Figure 3: ROC and precision-recall curves for BC-SELECT in immunotherapy datasets. The left panel shows the false positive rate plotted against the true positive rate for the ROC curve. The right panel shows the precision recall curve. Immunotherapy trials are shown in pinks and purples (Durvalumab is represented in purple, and pembrolizumab is shown in dark-salmon). Precision-recall AUC values are shown in Supplementary Table 1.

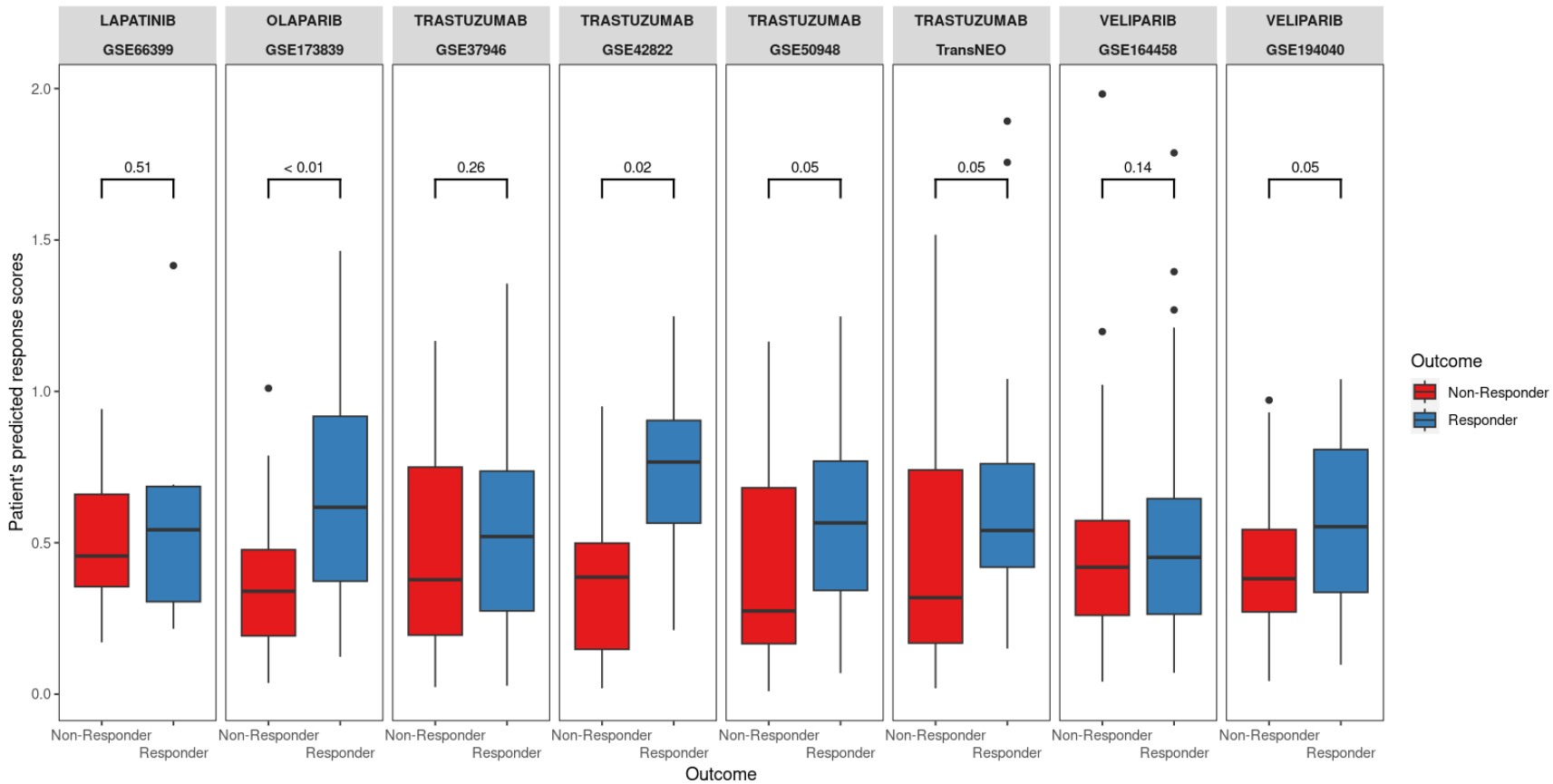


Figure 4: Patient's predicted response scores of BC-SELECT among responders and non-responders for targeted therapies. BC-SELECT scores are significantly higher in responders compared to non-responders in 5 out of 8 datasets, as determined by the Wilcoxon rank sum test with correction for multiple hypotheses. Adjusted p-values for the test results are reported in each box plot.

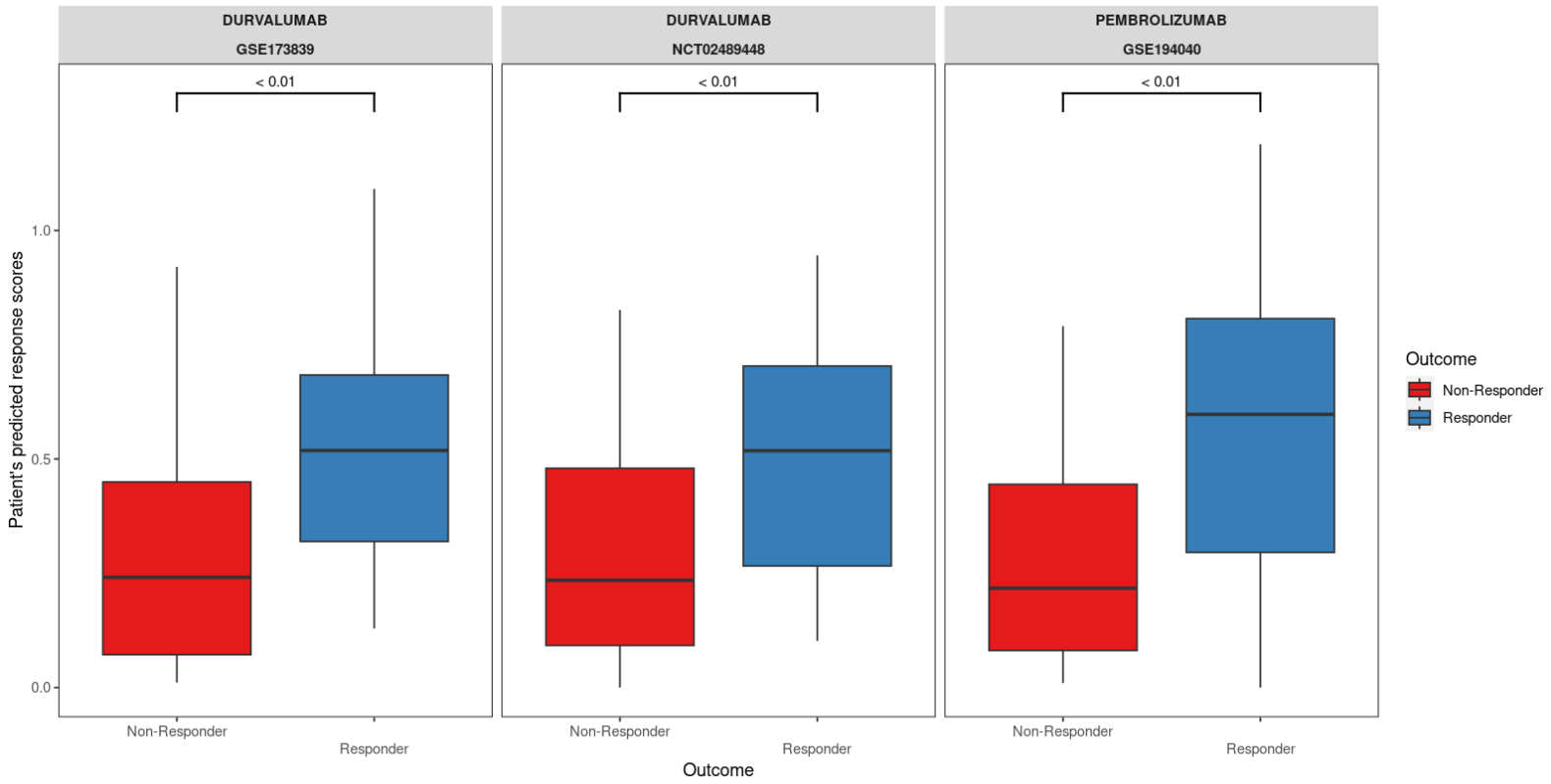


Figure 5: Patient's predicted response scores of BC-SELECT among responders and non-responders for immunotherapies. BC-SELECT scores are significantly higher in responders compared to non-responders in 5 out of 8 datasets, as determined by the Wilcoxon rank sum test with correction for multiple hypotheses. Adjusted p-values for the test results are reported in each box plot.

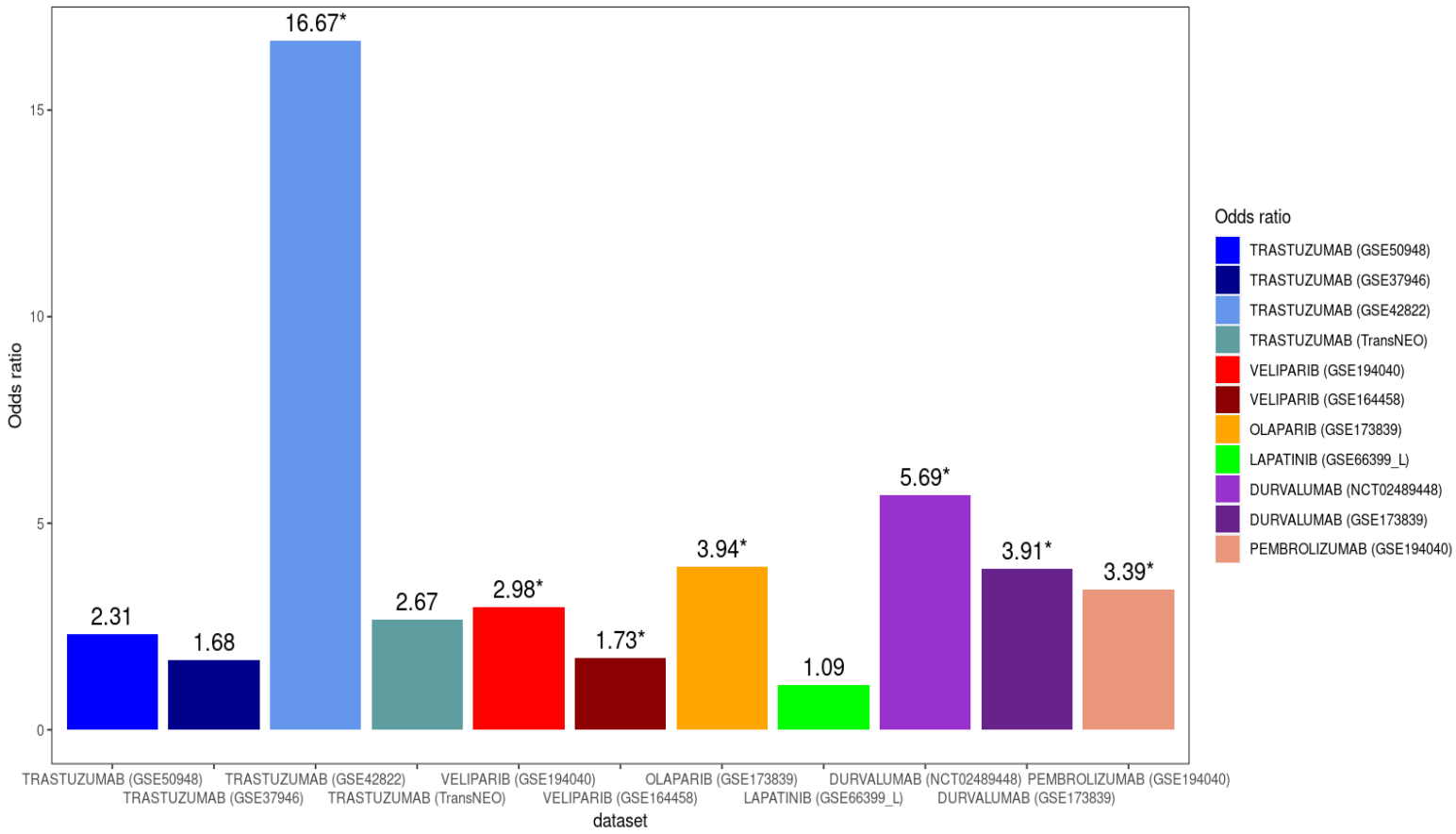


Figure 6: Odds ratios between responders and non-responders using patients' predicted response scores from BC-SELECT. Using a threshold of 0.5, we calculated the odds ratios between responders and non-responders across 11 breast cancer datasets. All odds ratios were greater than 1, and 7 datasets showed statistical significance (marked with *), with the 95% confidence interval not containing not containing 1. Detailed 95% confidence intervals of odds ratios and multiplicity-corrected p-values from Fisher exact tests are provided in Supplementary Table 2.

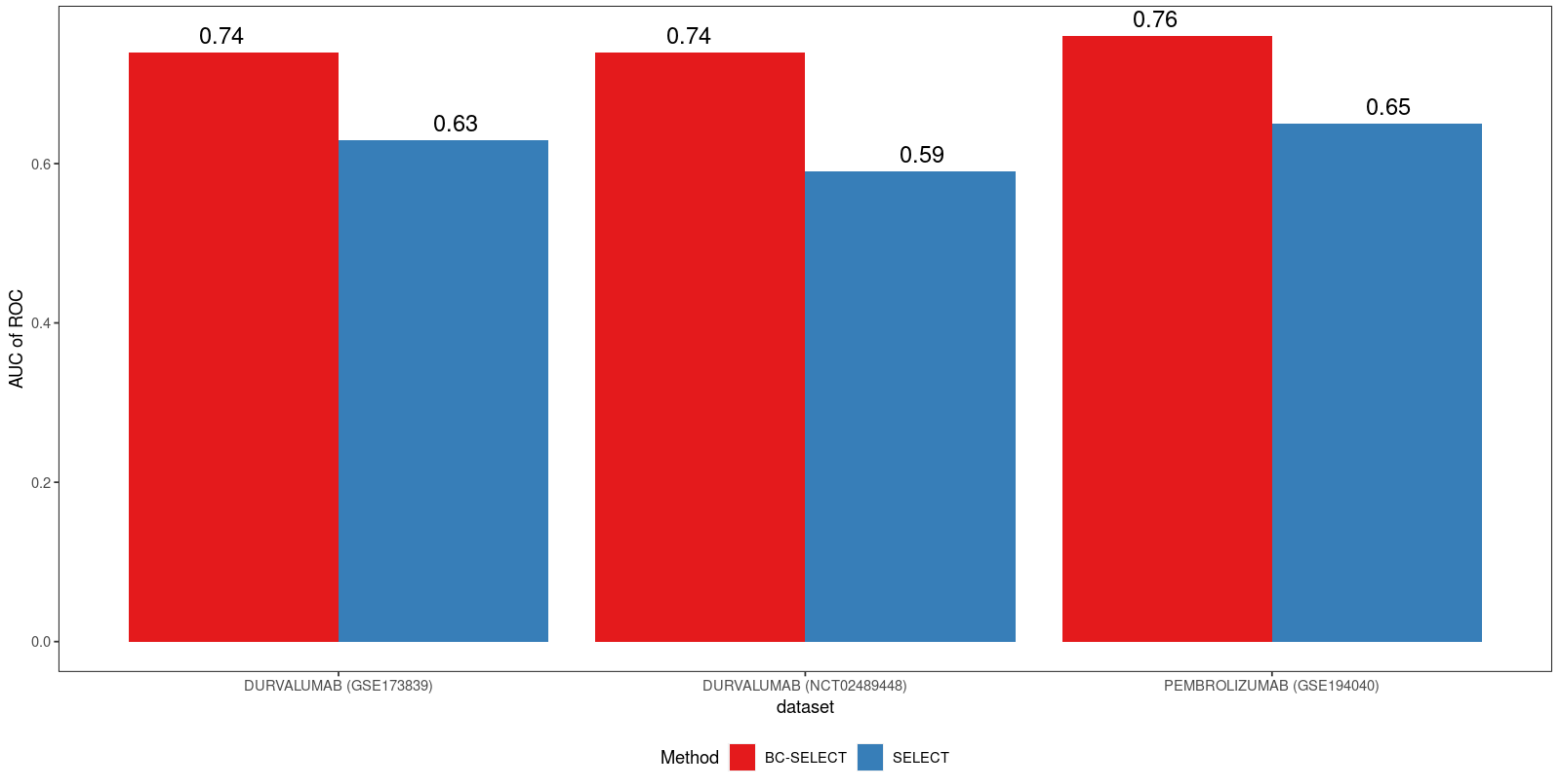


Figure 7: Comparison of ROC-AUC values between BC-SELECT and SELECT. The evaluation includes one for pembrolizumab and two durvalumab. BC-SELECT demonstrates superior prediction performance compared to the SELECT.

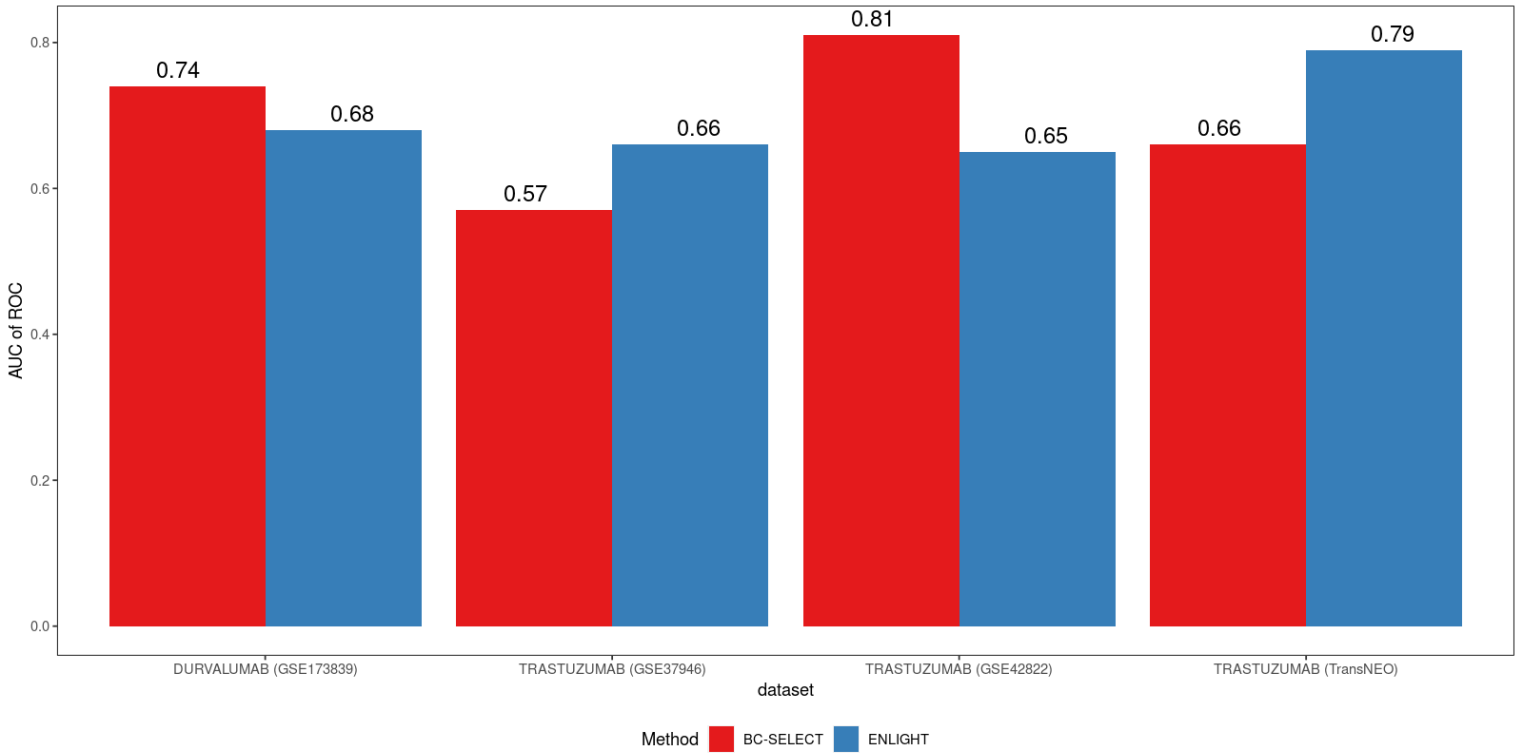


Figure 7: Comparison of ROC-AUC values between BC-SELECT and ENLIGHT for one durvalumab (immunotherapy) and three trastuzumab (targeted therapy). BC-SELECT demonstrates superior prediction performance in the one immunotherapy study, but mixed performance across the trastuzumab studies.

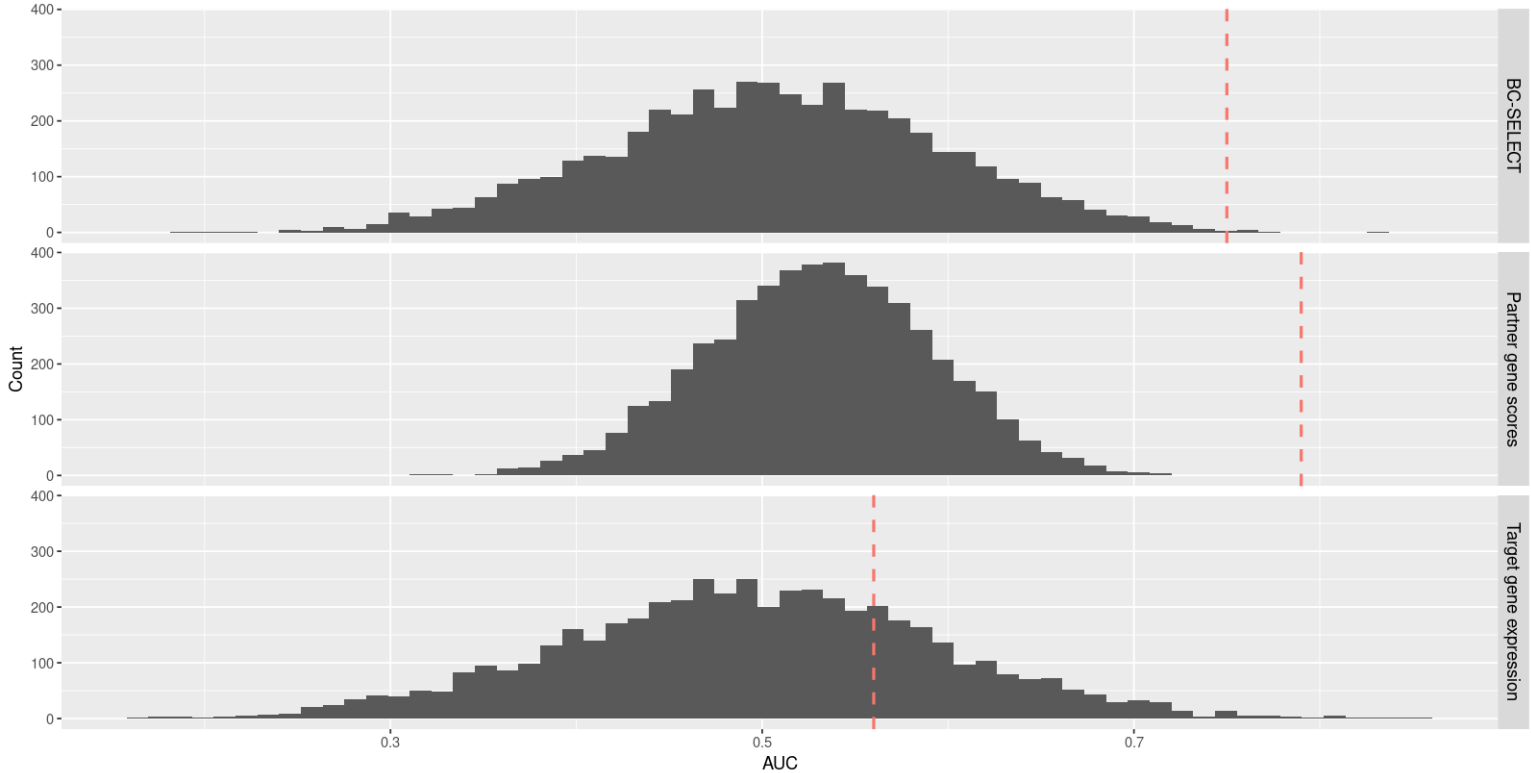
DRUG	TARGET	DATASET	GENETIC INTERACTION	BC-SELECT ROC-AUC (Empirical P-value)	Partner gene expression ratio ROC-AUC (Empirical P-value)	Target gene expression ROC-AUC (Empirical P-value)
TRASTUZUMAB	ERBB2 (HER2)	GSE50948[42] (R:31, NR:32)	SDL	**0.64** (0.01)	**0.63** (0.02)	0.6 (0.06)
		GSE37946[43] (R:27, NR:23)	SDL	0.57 (0.13)	0.44 (0.48)	0.6 (0.1)
		GSE42822[44] (R:12, NR:13)	SDL	**0.81** (0.01)	0.68 (0.23)	**0.78** (0.01)
		TransNEO[45] (R:19, NR:46)	SL	0.66 (0.14)	0.54 (0.64)	0.69 (0.04)
VELIPARIB	PARP1, PARP2	GSE194040[46] (R: 27, NR: 44)	SDL	**0.65** (0.02)	**0.59** (0.02)	0.63 (0.1)
		GSE164458[47] (R:127, NR:110)	SL	0.55 (0.39)	0.44 (0.99)	**0.61** (0.02)
OLAPARIB	PARP1, PARP2, PARP3	GSE173839[48] (R:29, NR:42)	SL	**0.75** (<0.01)	**0.79** (<0.01)	0.56 (0.26)
LAPATINIB	EGFR, ERBB2 (HER2)	GSE66399[23] (R:8, NR:23)	SDL	0.51 (0.53)	0.64 (0.11)	0.43 (0.74)

Table 1: Prediction performance of BC-SELECT and its two score components (used as references) compared to chance for targeted therapies. We report ROC-AUCs and describe the performance in the Monte Carlo "null distribution" with empirical p-values. ROC-AUC values are highlighted in bold and red if the ROC-AUC values fall within the top 2.5% of null AUC distributions. The reported ROC-AUC value is the higher of the analysis results between SL and SDL.

DRUG	TARGET	DATASET	BC-SELECT ROC-AUC (Empirical P-value)	Partner gene scores ROC-AUC (Empirical P-value)	Target Gene Exp ROC-AUC (Empirical P-value)
DURVALUMAB	PDCD1, CD274	NCT02489448[49] (R:25, NR:25)	**0.74** (<0.01)	**0.65** (<0.01)	**0.71** (0.01)
		GSE173839[48] (R:29, NR:42)	**0.74** (<0.01)	**0.72** (<0.01)	0.70 (0.03)
PEMBROLIZUMAB		GSE194040[46] (R:31, NR:38)	**0.76** (<0.01)	**0.67** (<0.01)	**0.75** (<0.01)

Table 2: Prediction performance of BC-SELECT and its two score components (used as references) compared to chance for immunotherapy. We report ROC-AUCs and describe the performance in the Monte Carlo "null distribution" with empirical p-values. ROC-AUC values are highlighted in bold and red if the ROC-AUC values fall within the top 2.5% of null AUC distributions.

Supplementary Figures



Supplementary Figure 1: Example of Monte Carlo-based "null distributions" for assessment of BC-SELECT performance of olaparib (GSE173839[48]). The X-axis depicts the distributions of 5000 ROC-AUCs calculated based on genes randomly selected to use for prediction. The null AUC distributions are generally centered around 0.5, indicative of random chance classification, while their varying right-tail thickness and the central tendency reflect differences reflective of the efficacy of prediction using gene expression. The three red dashed lines indicate the ROC-AUC values obtained by BC-SELECT, partner gene scores, and target gene expression. Empirical p-values are calculated based on the proportion of the right tail area of the red dashed line and the histogram results of random selections. In this example, both BC-SELECT and partner gene scores exhibit smaller empirical p-values, indicating superior performance of SL partner genes in drug response prediction compared to random guessing.

DRUG	TARGET	DATASET	GENETIC INTERACTION	BC-SELECT PR-AUC	Partner gene expression ratio PR-AUC	Target gene expression PR-AUC
TRASTUZUMAB	ERBB2 (HER2)	GSE50948[42] (R:31, NR:32)	SDL	0.56	**0.61**	0.54
		GSE37946[43] (R:27, NR:23)	SDL	0.53	0.54	**0.59**
		GSE42822[44] (R:12, NR:13)	SDL	**0.72**	0.59	0.66
		TransNEO[45] (R:19, NR:46)	SL	0.38	0.31	**0.47**
VELIPARIB	PARP1, PARP2	GSE194040[46] (R: 27, NR: 44)	SDL	**0.51**	0.43	0.47
		GSE164458[47] (R:127, NR:110)	SL	**0.6**	0.52	**0.6**
OLAPARIB	PARP1, PARP2, PARP3	GSE173839[48] (R:29, NR:42)	SL	0.67	**0.71**	0.41
LAPATINIB	EGFR, ERBB2 (HER2)	GSE66399[23] (R:8, NR:23)	SDL	0.24	**0.4**	0.23

DRUG	TARGET	DATASET	BC-SELECT PR-AUC	Partner gene expression ratio PR-AUC	Target gene expression PR-AUC
DURVALUMAB	PDCD1, CD274	NCT02489448[49] (R:25, NR:25)	**0.7**	0.61	0.65
		GSE173839[48] (R:29, NR:42)	**0.62**	0.57	**0.62**
PEMBROLIZUMAB		GSE194040[46] (R:31, NR:38)	**0.71**	0.59	0.65

Supplementary Table 1: Prediction performance of BC-SELECT and its two score components (used as references) compared to chance. AUC values of precision-recall curves (PR-AUCs) the predicted response score and score components, with the highest value highlighted in bold for each dataset.

DRUG	TARGET	DATASET	GENETIC INTERACTION	Odds ratio	95% Confidence Interval	Fisher exact test adjusted P-value
TRASTUZUMAB	ERBB2 (HER2)	GSE50948[42] (R:31, NR:32)	SDL	2.31	(0.84, 6.34)	0.18
		GSE37946[43] (R:27, NR:23)	SDL	1.68	(0.54, 5.17)	0.47
		GSE42822[44] (R:12, NR:13)	SDL	**16.67**	**(2.27, 122.22)**	**0.04**
		TransNEO[45] (R:19, NR:46)	SL	2.67	(0.88, 8.05)	0.16
VELIPARIB	PARP1, PARP2	GSE194040[46] (R: 27, NR: 44)	SDL	**2.98**	**(1.10, 8.09)**	**0.09**
		GSE164458[47] (R:127, NR:110)	SL	**1.73**	**(1.02, 2.93)**	**0.09**
OLAPARIB	PARP1, PARP2, PARP3	GSE173839[48] (R:29, NR:42)	SL	**3.94**	**(1.42, 10.92)**	**0.05**
LAPATINIB	EGFR, ERBB2 (HER2)	GSE66399[23] (R:8, NR:23)	SDL	1.09	(0.22, 5.45)	1

DRUG	TARGET	DATASET	Odds ratio	95% Confidence Interval	Fisher exact test adjusted P-value
DURVALUMAB	PDCD1, CD274	NCT02489448[49] (R:25, NR:25)	**5.69**	**(1.51, 21.42)**	**0.02**
		GSE173839[48] (R:29, NR:42)	**3.91**	**(1.40, 10.95)**	**0.02**
PEMBROLIZUMAB		GSE194040[46] (R:31, NR:38)	**3.93**	**(1.39, 11.07)**	**0.02**

Supplementary Table 2: Odds ratios between responders and non-responders using patients' predicted response scores from BC-SELECT. Using a threshold of 0.5 of BC-SELECT final predictive scores, we calculated the odds ratios between responders and non-responders across 11 breast cancer datasets. All odds ratios were greater than 1, and 7 datasets showed statistical significance, with the 95% confidence interval not containing 1 (in bold). The last column presents the p-values of the Fisher exact test after adjusting for multiple hypotheses correction.