Predicting pathological Complete Response

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Project Overview

Evaluation of pam50 predictor against others

Prosigna predicted pCR using categorical pam50 predictor

We use metric to compare our classifiers against pam50 benchmark

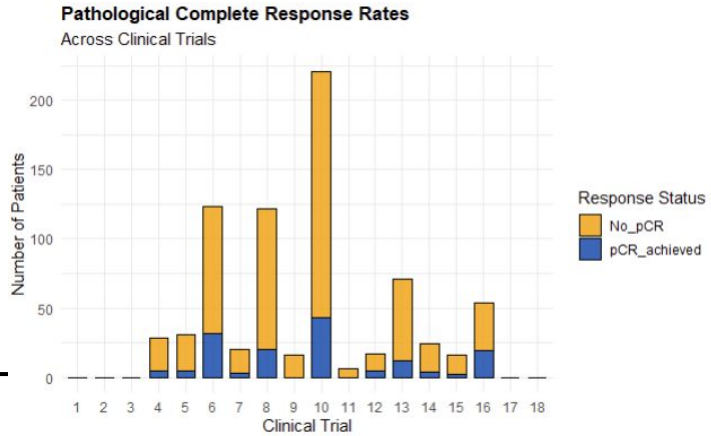
Analysis Workflow

1. **Data Exploration**: Examine distributions and relationships
2. **Feature Selection**: Identify informative biomarkers
3. **Model Training**: Train classification model using selected features
4. **Validation**: Cross-validate across multiple studies
5. **Benchmarking**: Compare performance against pam50 predictor
6. **Interpretation**: Extract biological insights from predictive feature

Exploratory Data Analysis: Structure

Overview

- **pCR Data**
  - Access study 1 responses: pCR[[1]]
  - Binary values (0 or 1)
- **Expression Data**
  - Access study 1 biomarkers: XX_pCR[[1]]
  - Matrix dimensions: 10,115 biomarkers × [variable # of patients]
  - Row names = biomarker identifiers



Objects

- **Data Source**: 18 studies with binary pCR outcomes
- **Three Main Components**:
  - pCR: List of 18 binary response vectors
  - XX_pCR: List of 18 expression matrices (biomarkers × patients)
  - pam50_recur: Benchmark prediction vectors
- **Scale**: 10,115 biomarkers consistent across all studies

Test and Train Sets:

- Study 10 selected as the **test set** (largest dataset with pam50 predictions, minimizing evaluation variance)
- Remaining studies with pam50 predictions combined as the **training set**

Cross-Validation Approach:

- Leave-one-study-out cross-validation employed during training.
- PCA to generate features for each gene

Filtering Observations

Studies containing pam50 predictions

- Studies 1, 2, 3, 17 and 18 contained only NA values for pam50
- Data from them are not included for training and testing

```
Study 1: 114 samples, 114 NAs (100.00%), Has pam50 data: FALSE
Study 2: 53 samples, 53 NAs (100.00%), Has pam50 data: FALSE
Study 3: 261 samples, 261 NAs (100.00%), Has pam50 data: FALSE
Study 4: 28 samples, 0 NAs (0.00%), Has pam50 data: TRUE
Study 5: 31 samples, 0 NAs (0.00%), Has pam50 data: TRUE
Study 6: 128 samples, 0 NAs (0.00%), Has pam50 data: TRUE
Study 7: 20 samples, 0 NAs (0.00%), Has pam50 data: TRUE
Study 8: 122 samples, 0 NAs (0.00%), Has pam50 data: TRUE
Study 9: 16 samples, 0 NAs (0.00%), Has pam50 data: TRUE
Study 10: 221 samples, 0 NAs (0.00%), Has pam50 data: TRUE
Study 11: 6 samples, 0 NAs (0.00%), Has pam50 data: TRUE
Study 12: 17 samples, 0 NAs (0.00%), Has pam50 data: TRUE
Study 13: 71 samples, 0 NAs (0.00%), Has pam50 data: TRUE
Study 14: 25 samples, 0 NAs (0.00%), Has pam50 data: TRUE
Study 15: 16 samples, 0 NAs (0.00%), Has pam50 data: TRUE
Study 16: 54 samples, 0 NAs (0.00%), Has pam50 data: TRUE
Study 17: 115 samples, 115 NAs (100.00%), Has pam50 data: FALSE
Study 18: 11 samples, 11 NAs (100.00%), Has pam50 data: FALSE

Studies without pam50 data: 1, 2, 3, 17, 18
Studies with incomplete pam50 data: None
Studies with complete pam50 data: 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16
```

NA Values in remaining studies

- Remove all rows with NA values for pCR
- Assign zero to NA gene expression values

Normalization using biomarker MKI67

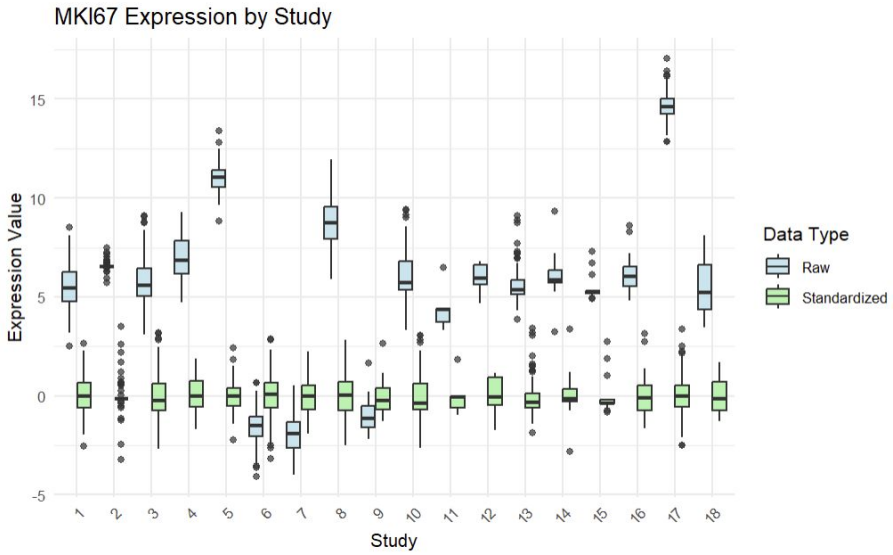
Methodology

- Identify common genes across all datasets
- Filter studies to include only shared genes for consistent analysis

Correct for Batch Effects

- Normalize gene expression data using MKI67 (proliferation marker)
  - Subtracting MKI67 expression
  - Scaling by standard deviation of MKI67 expression in each study
- Minimize differences caused by batch effects
- Preserve Data Structure
- Split data back into individual studies post-normalization
- Verify that dimensions (number of genes and samples) remain consistent

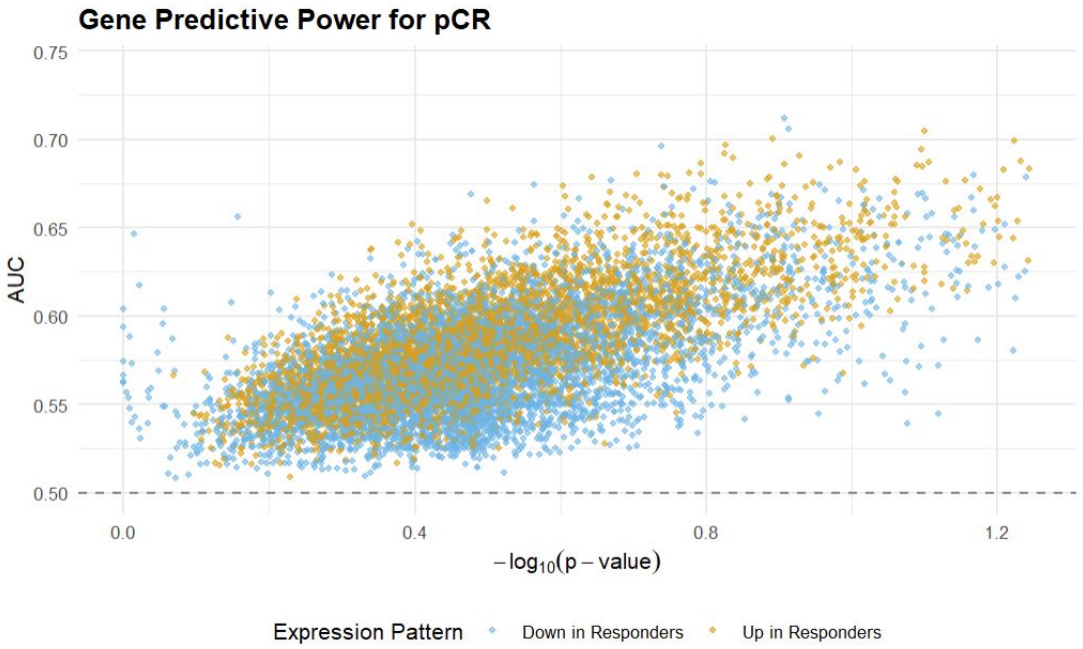
Comparison



Source: Ki-67 labeling index is a predictive marker for a pathological complete response to neoadjuvant chemotherapy in breast cancer

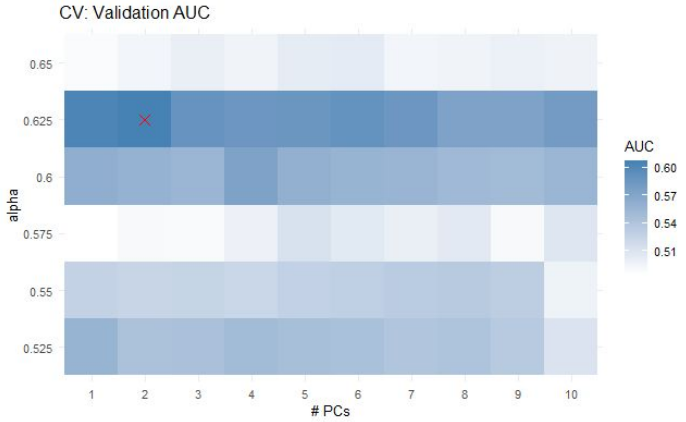
Feature selection

AUC threshold



Using cross-validation to select alpha

```
set.seed(117)
alphas <- seq(0.525, 0.650, 0.025)
kmax <- 10
cat("Searching α ∈ [", min(alphas), "-", max(alphas), "] and k = 1…", kmax, "\n")
cv <- CV_pca(XX_pCR, pCR, alphas, kmax, leave_one = TRUE)

print(cv$heatmap)
cat("→ Best α =", cv$alpha_best, "  k =", cv$k_best, "\n")
```



Feature selection

Clustering based on corr threshold of 0.8

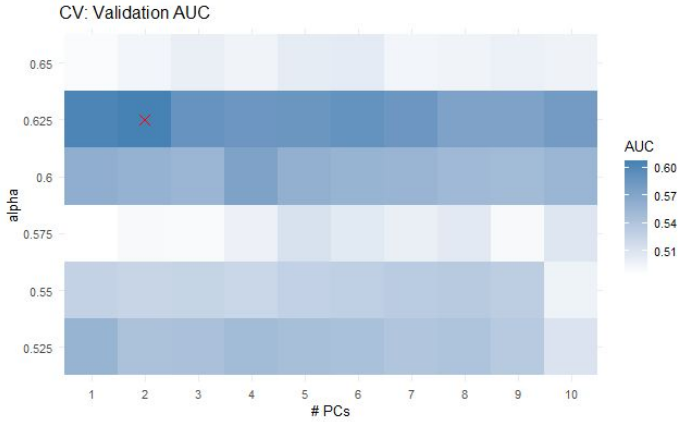
- Assign +1 or -1 weight based on whether AUC is above or below 0.5
- For each cluster, compute the weighted sum of all genes in that cluster
- Using PCA, obtain k features, built from genes that both correlate positively and negatively with pCR

```r
# Function to cluster genes based on correlation
corr_cluster <- function(expr_mat, corr_thr = 0.8) {
  # Calculate the pairwise correlation matrix
  cor_mat <- cor(t(expr_mat), method = "pearson", use = "pairwise.complete.obs")

  # Take absolute values of correlations for clustering purposes
  abs_cor_mat <- abs(cor_mat)

  # Create a distance matrix where distance = 1 - abs(correlation)
  dist_mat <- as.dist(1 - abs_cor_mat)

  # Perform hierarchical clustering using average linkage
  hc <- hclust(dist_mat, method = "average")

  # Cut the dendrogram at a height corresponding to the threshold
  cluster_assignment <- cutree(hc, h = 1 - corr_thr)

  # Organize genes into groups
  groups <- split(names(cluster_assignment), cluster_assignment)

  # Sort groups by size (largest first)
  group_sizes <- sapply(groups, length)
  groups_sorted <- groups[order(group_sizes, decreasing = TRUE)]

  return(groups_sorted)
}
```

Using cross-validation to select k

-

```r
set.seed(117)
alphas <- seq(0.525, 0.650, 0.025)
kmax <- 10
cat("Searching α ∈ [", min(alphas), "-", max(alphas), "] and k = 1…", kmax, "\n")
cv <- CV_pca(XX_pCR, pCR, alphas, kmax, leave_one = TRUE)

print(cv$heatmap)
cat("→ Best α =", cv$alpha_best, "  k =", cv$k_best, "\n")
```



Source: WGCNA: Zhang & Horvath (2005) - "A General Framework for Weighted Gene Co-Expression Network Analysis"

Training and testing data sets

Method

- **Methodology:** Leave-one-study-out
- Chose study 10 because it had the most samples -> model eval of least variance
- Through studies 4 to 16

Benchmarking

- **Goal**: Develop a classifier to predict pCR status
- **Approach**:
  - Train using biomarker expression data (XX_pCR)
  - Predict binary pCR response
  - Compare performance against pam50 benchmark
- **Evaluation**: Use metrics like AUC to assess model performance

Source: WGCNA: Zhang & Horvath (2005) - "A General Framework for Weighted Gene Co-Expression Network Analysis"

Baseline Model: Logistic Regression

Merge all studies together to generate one big XX_pCR dataset including outcome, study ID

```
j <- 10
k_best <- 2

# 1) split indices
idx_tr <- which(YY$study != j)
idx_te <- which(YY$study == j)

# 2) fit logistic model on training subset only
lr_mod <- fit_model(
  predictors = P,
  YY_train   = YY$response[idx_tr],
  indexes    = idx_tr,
  k          = k_best,
  lr         = TRUE
)

# 3) compute AUCs
df_tr <- as.data.frame(P[idx_tr,]); colnames(df_tr) <- paste0("P",1:k_best)
df_te <- as.data.frame(P[idx_te,]); colnames(df_te) <- paste0("P",1:k_best)

lr_train_auc <- auc_safe(
  predict(lr_mod, newdata = df_tr, type="response"),
  YY$response[idx_tr]
)
lr_test_auc <- auc_safe(
  predict(lr_mod, newdata = df_te, type="response"),
  YY$response[idx_te]
)
```

- **Only provides appropriate fits when the true response is a logistic function of the features.**
- train two flexible models (namely, a random forest classifier and an gradient boosting classifier) on top of a baseline logistic regression model
- Flexible because they can (essentially) fit arbitrarily well to a prescribed training set
- Improvement:
  - Consider applying regularization (e.g., L1 or L2) to address multicollinearity among predictors.
  - Validate the model across multiple folds or studies for further robustness.

Old but gold!

Model 2: Random Forest

Limited Depth of Tree & Number of Samples Per Leaf

```
# 1) train/test indices
idx_tr <- which(YY$study != j)
idx_te <- which(YY$study == j)

# 2) pull out and rename your predictors to P1…Pk
df_tr <- as.data.frame(P[idx_tr, , drop = FALSE])
df_te <- as.data.frame(P[idx_te, , drop = FALSE])
colnames(df_tr) <- colnames(df_te) <- paste0("P", seq_len(k_best))

# 3) fit the RF on the training data frame
rf_mod <- randomForest(
  x        = df_tr,
  y        = as.factor(YY$response[idx_tr]),
  ntree    = 500,
  mtry     = floor(sqrt(k_best)),
  maxnodes = 10,          # cap max tree depth
  nodesize = 5            # minimum number of samples per leaf
)

# 4) get predicted probabilities
rf_train_probs <- predict(rf_mod, newdata = df_tr, type = "prob")[,2]
rf_test_probs  <- predict(rf_mod, newdata = df_te, type = "prob")[,2]

# 5) compute AUCs
rf_train_auc <- auc_safe(rf_train_probs, YY$response[idx_tr])
rf_test_auc  <- auc_safe(rf_test_probs,  YY$response[idx_te])
```

- Considered refining feature selection, adjusting hyperparameters
- Balanced Hyperparameters:
    - The choice of mtry, maxnodes, and nodesize is aimed at balancing model complexity and generalization.
- Advantages of Random Forest:
    - Handles high-dimensional data effectively, which is common in gene expression datasets.
- Robust against overfitting, particularly with the use of default bootstrapping and ensemble averaging.
- Suggestions for Further Enhancements:

Model 3: Gradient Boosting Machine

MODERNIZED & EFFECTIVE Classifer

```r
# 1. Extract predictors and response
X_tr <- as.data.frame(P[idx_tr, , drop = FALSE])
X_te <- as.data.frame(P[idx_te, , drop = FALSE])
y_tr <- YY$response[idx_tr]
y_te <- YY$response[idx_te]

# 2. Fit GBM model
gbm_mod <- gbm::gbm(
  formula = y_tr ~ .,
  data = data.frame(y_tr = y_tr, X_tr),
  distribution = "bernoulli",
  n.trees = 100,
  interaction.depth = 3,
  shrinkage = 0.05,
  n.minobsinnode = 5,
  verbose = FALSE
)

# 3. Find best number of trees via internal cross-validation (if using cv.folds)
best_iter <- gbm::gbm.perf(gbm_mod, method = "OOB", plot.it = FALSE)

# 4. Predict
gbm_train_probs <- predict(gbm_mod, newdata = X_tr, n.trees = best_iter, type = "response")
gbm_test_probs  <- predict(gbm_mod, newdata = X_te, n.trees = best_iter, type = "response")
```

- Iteratively combine weak decision trees by sequentially fixing error
- Excels at capturing nonlinearities and high-order interactions without manual feature construction.
- Offers fine-grained control via multiple regularization knobs (learning rate, tree depth, early stopping)
- In practice often outperforms single models like random forests—at the cost of more tuning
- See results…

Interpretation

Overview

- **AUC measurements**
  - Successful strategy: rigorous feature engineering with flexible models.
  - Simple logistic regression on our features > Prosigna's pam50 features.
  - More flexible models improved performance further (GBM)
- GBM showed good ability to generalize
  - Test AUC slightly higher than train AUC

Suggestions

- Suggests need for stronger dimensionality reduction/feature selection with Model III.
- Random forest model achieved highest test AUC of all models but also most overfitting
- Need to benchmark against pam50

Model	Train AUC	Test AUC	Gap
Logistic Regression	0.674	0.754	-0.079
Random Forest	0.830	0.705	0.125
GBM	0.719	0.755	-0.037

Other metrics

AUC

- Everyone uses this
- Compared with concordance index, better visualizations

Brier score

- The Brier score measures the accuracy of probabilistic predictions. It is a proper scoring rule that evaluates both calibration and discrimination, providing a comprehensive measure of model performance
- Possibly dominated by large majority class

Log loss

- Penalizes incorrect predictions and is particularly useful for evaluating models that output probabilities. It provides a measure of the model's confidence in its predictions.
- Possibly use weighting to compare models given imbalanced data

Future Steps

Overview

- **Three Main Extensions**:
  - Use feature importance scores from the GBM model to analyze which predictors most strongly contribute to pCR prediction
  - Consider applying regularization (e.g., L1 or L2) to address multicollinearity among predictors
  - Account for non-linear relationships - for PCA (kernel) and for Pearson correlation