Enhancing Breast Cancer Treatment Response Prediction with Single-Cell RNA Sequencing and Large Language Models

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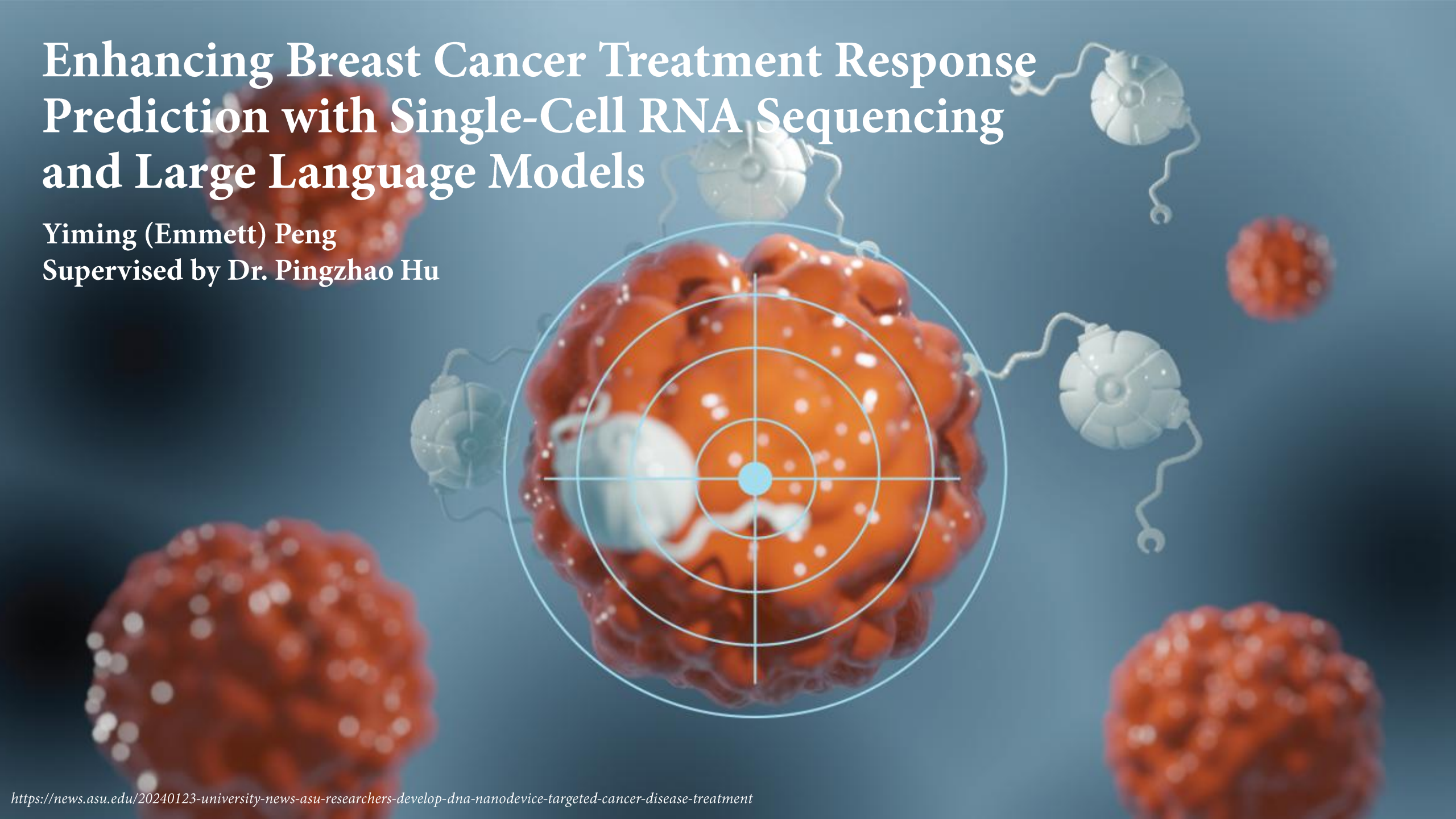
Supervised by Dr. Pingzhao Hu



https://news.asu.edu/20240123-university-news-asu-researchers-develop-dna-nanodevice-targeted-cancer-disease-treatment

Background

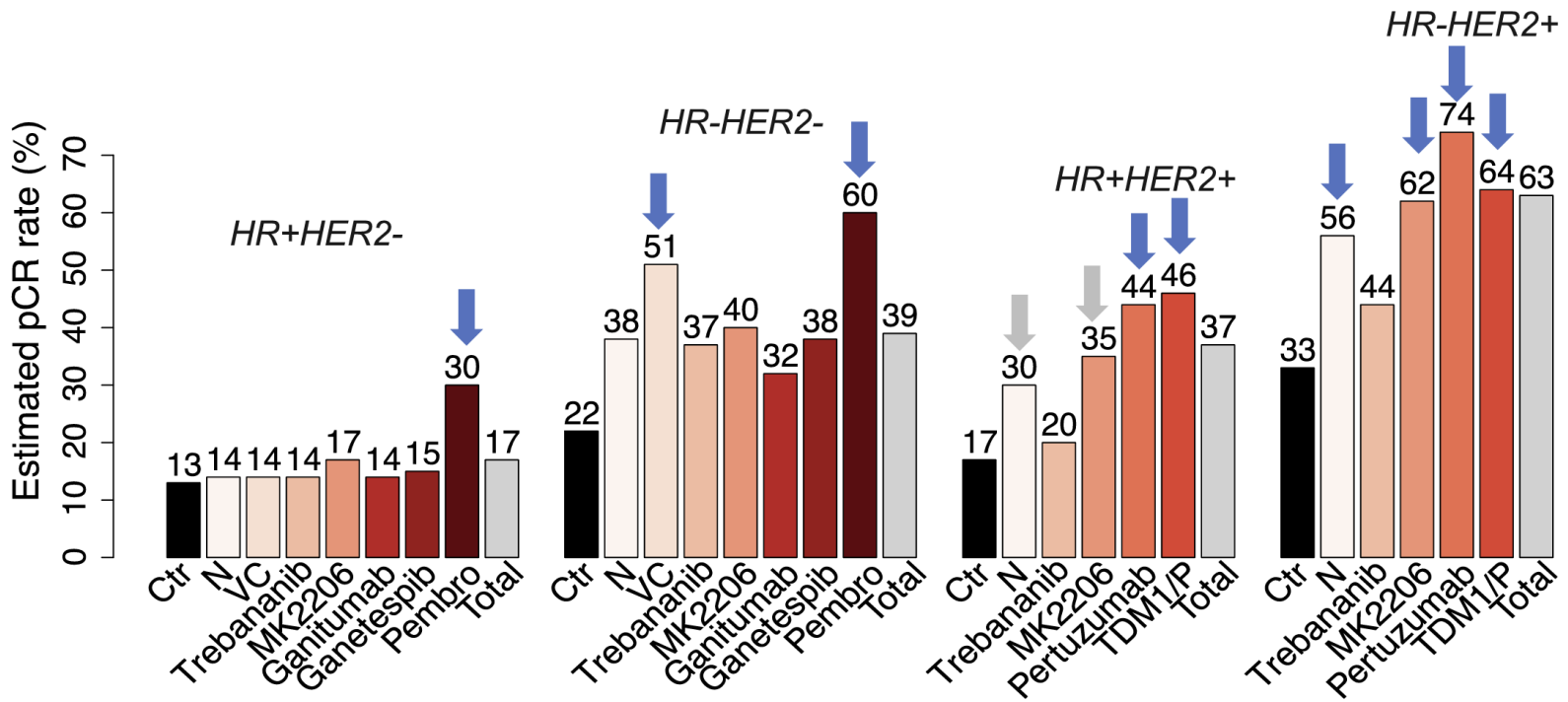
Challenges in Breast Cancer Treatment Response Prediction

- Breast cancer is the most common cancer among women worldwide, **one in three patients dies from the disease globally** (DeSantis et al., 2015)
- **Accurate treatment prediction** is crucial to ensure each patient receives the most effective therapy, maximizing the chance of achieving pCR and reducing unnecessary side effects
  - Pathologic Complete Response (pCR): absence of invasive cancer in the breast after treatment (indicator of cure)
- Breast cancer is known to have substantial **heterogeneity**, which can only be fully understood when analyzing **single-cell RNA sequence data**

Challenges in Breast Cancer Treatment Response Prediction

- Current Challenge in Breast Cancer Treatment: existing treatment strategies fail to achieve desirable pCR rates across breast cancer subtypes

Estimated pCR rate across arms by receptor subtype (adapted from Wolf et al., 2022)



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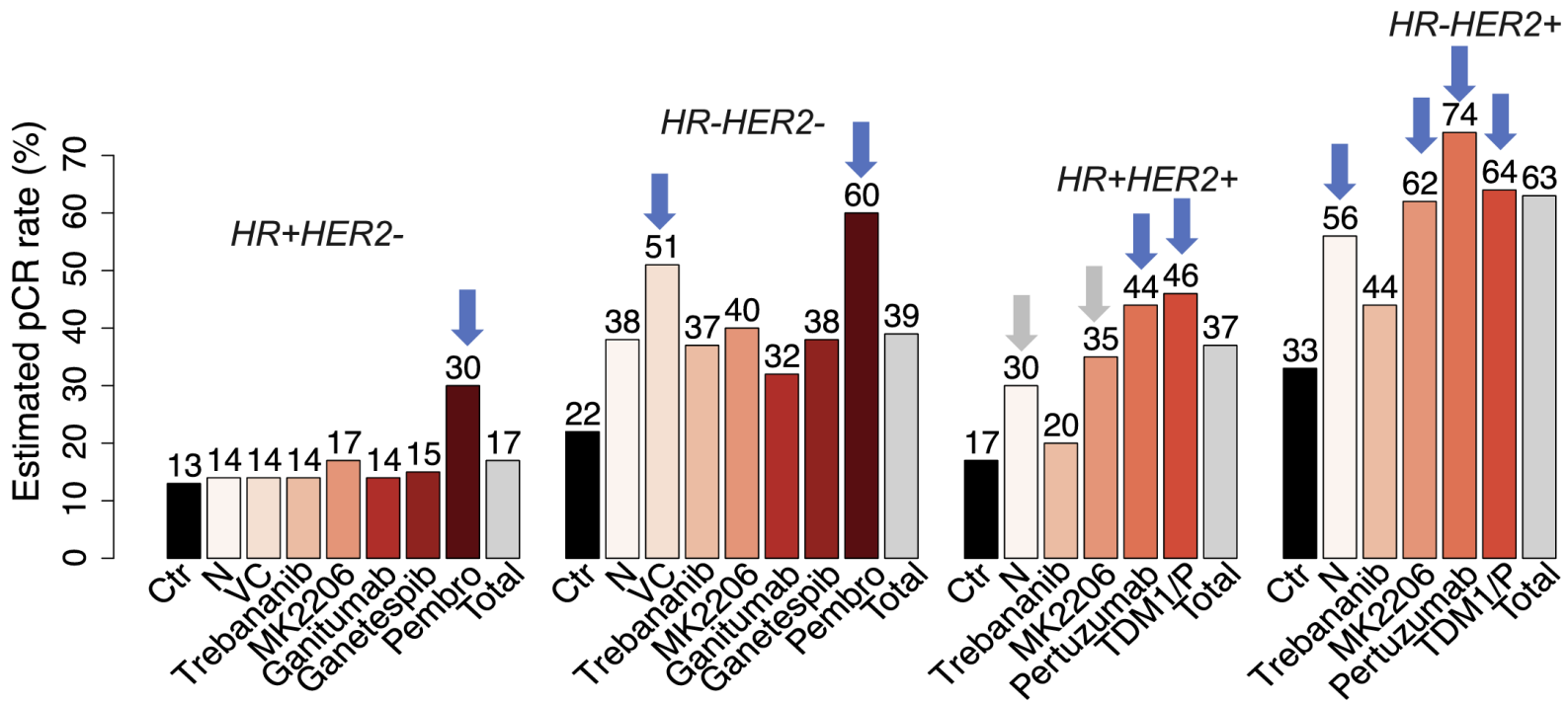

A new treatment outcome prediction structure is needed

Current Treatment Response Prediction Methods

- In 2022, Wolf et al. use bulk RNA-seq and protein data to refine breast cancer subtypes, incorporating biomarkers through logistic regression (**RPS-5**) to predict the treatment response
  - Bulk RNA-seq: averages gene expression across cells
  - Biomarkers: e.g., specific genes/proteins
  - Limitation: Relies on bulk data, which overlooks cellular heterogeneity present in breast cancer
- Xu et al. (2024) integrate scRNA-seq datasets to study heterogeneity and develop **InteractPrint** that computes weighted scores for cell-cell interactions for treatment response prediction
  - Limitations: **InteractPrint** is limited to known interactions

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A new cell-level prediction framework is needed for accurately predict pCR

Research Objective

Research Objective

To utilize single-cell RNA-seq data to identify cell-type-specific marker genes for predicting treatment response (pCR) in breast cancer patients, addressing cellular heterogeneity and improving upon current predictive methods

Methods

PRECISE Framework for Treatment Response Prediction

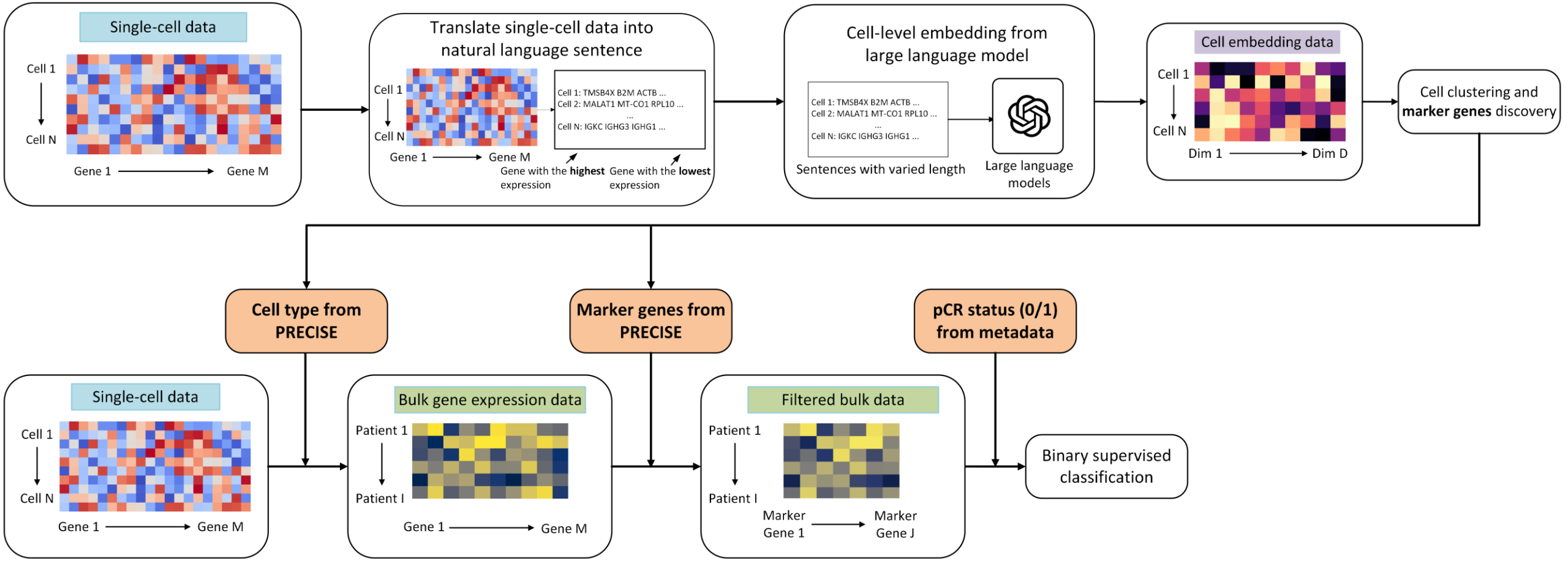
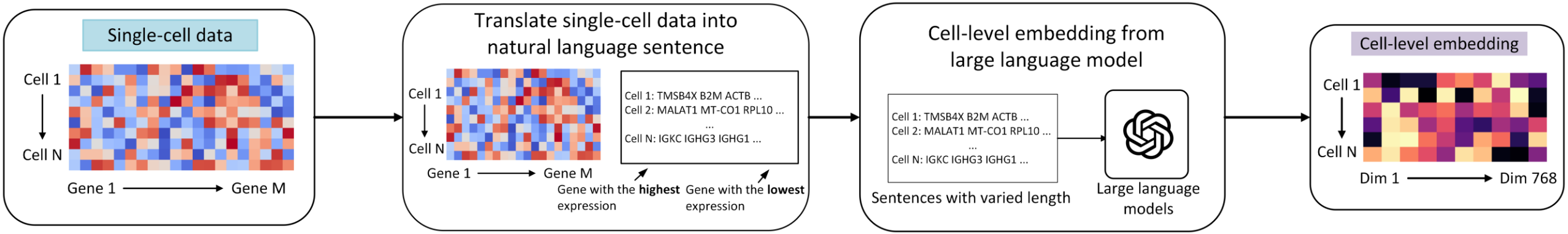


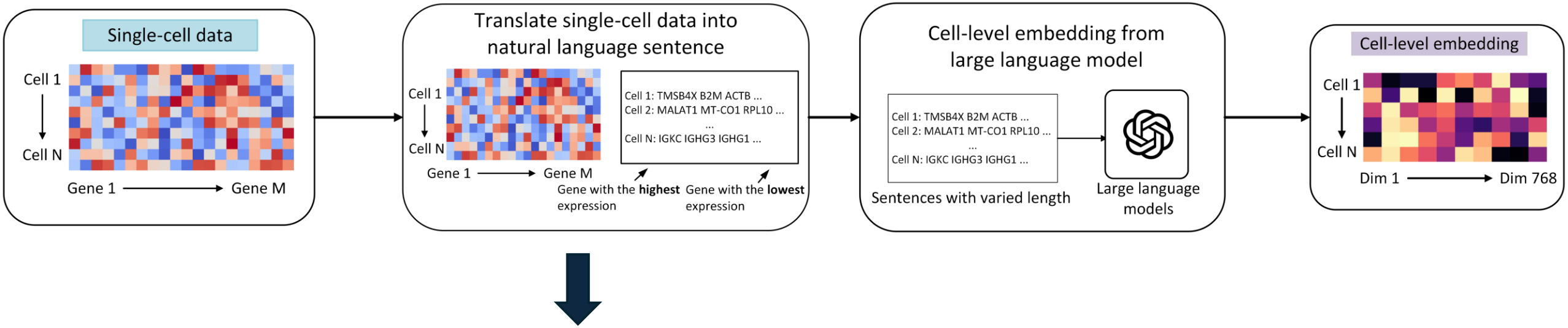
Figure 1: Overview of the PRECISE framework (**P**rediction of **RE**sponse using **C**ell-type **I**nference and **S**ingle-cell **E**mbedding), which leverages large language models and cell-type-specific markers for treatment outcome prediction

Generating Cell Embeddings with Large Language Models (LLMs)



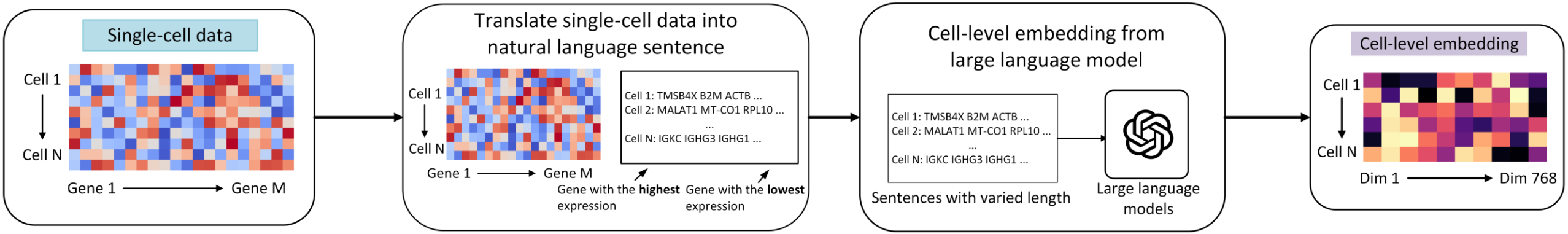
- Directly working on the sparse matrix
- Omit genes with no expression

Generating Cell Embeddings with Large Language Models (LLMs)



- Generate gene name sequences for each cell ranked by expression
- Varied length gene name sequence for each cell

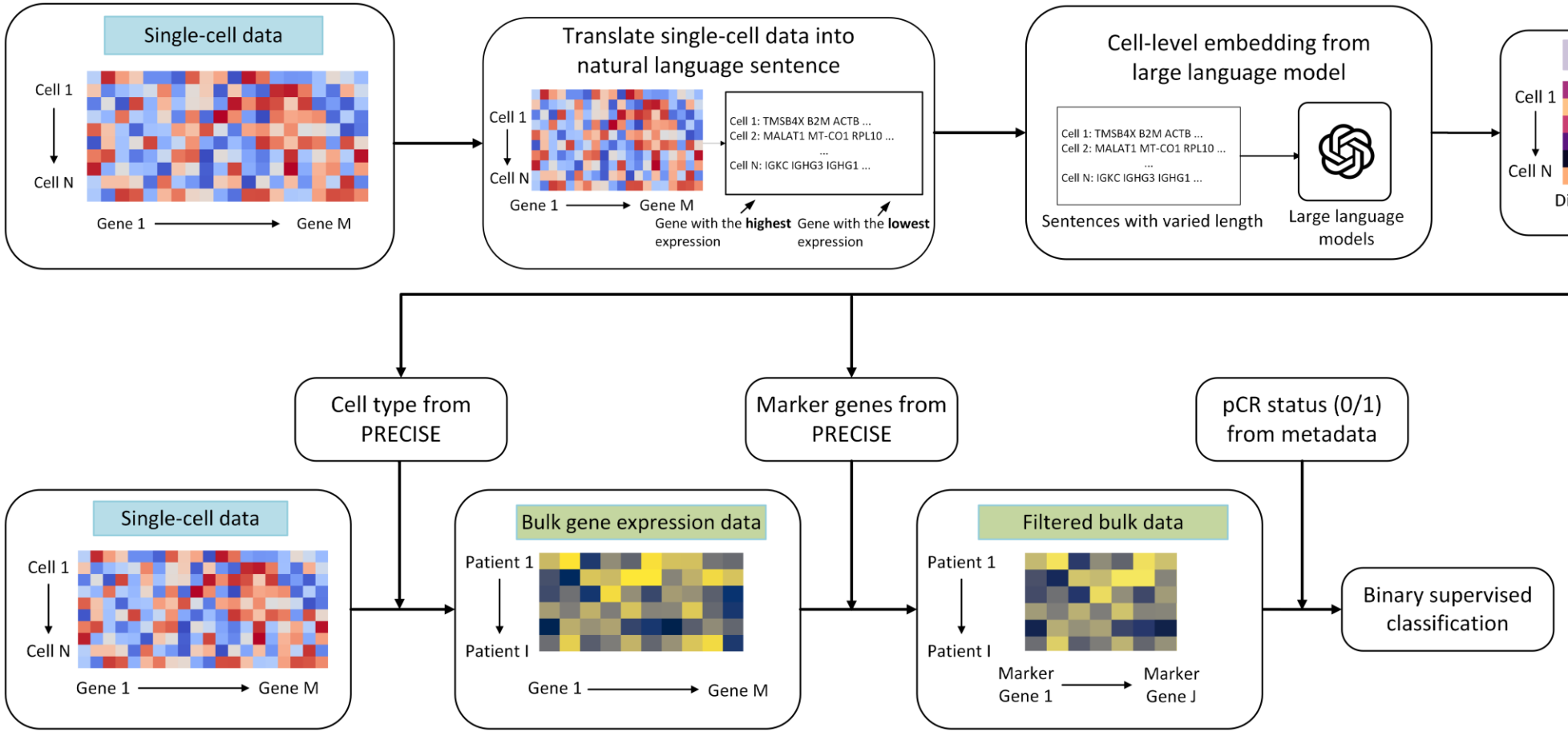
Generating Cell Embeddings with Large Language Models (LLMs)



- Model encodes each sequence into an embedding
- mxbai-embed-large-v1 (model parameter: 110M)

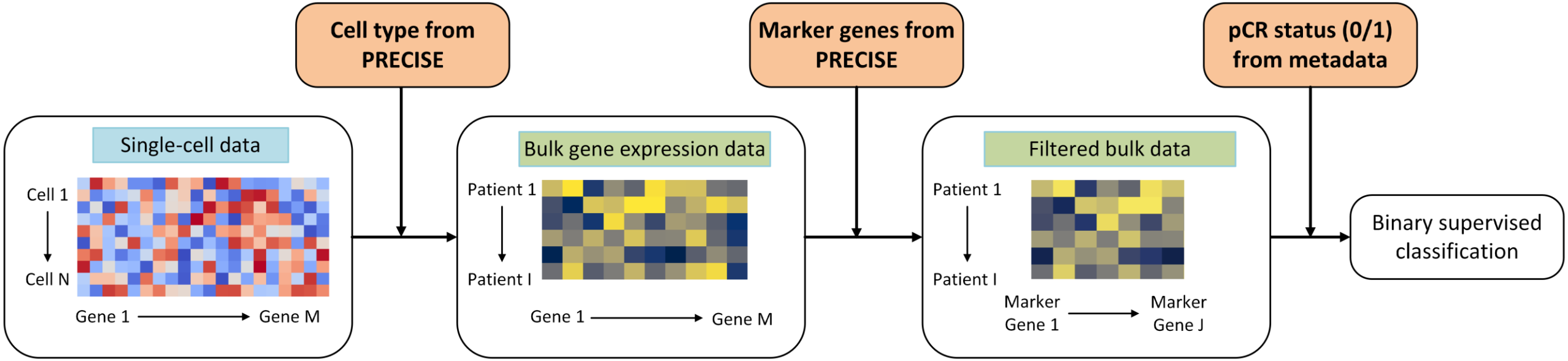
- Embedding dim: 768

Identifying Cell-Type-Specific Marker Genes

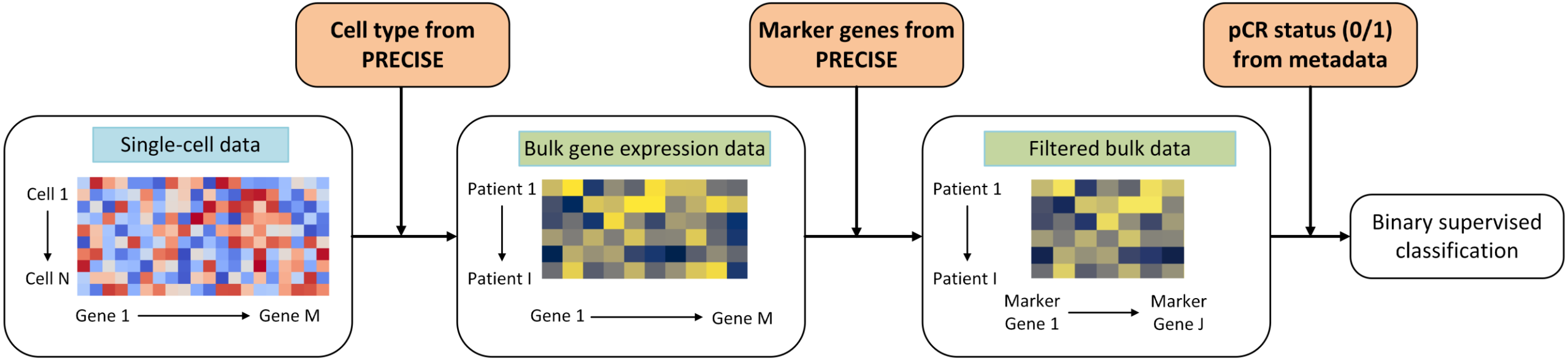


- **Cell clustering** was performed using the Louvain algorithm
- **Marker genes** for each cluster were identified using Seurat
- **Cell-type annotations** were assigned using *SingleR*

Predicting Treatment Outcomes from Bulk Gene Expression

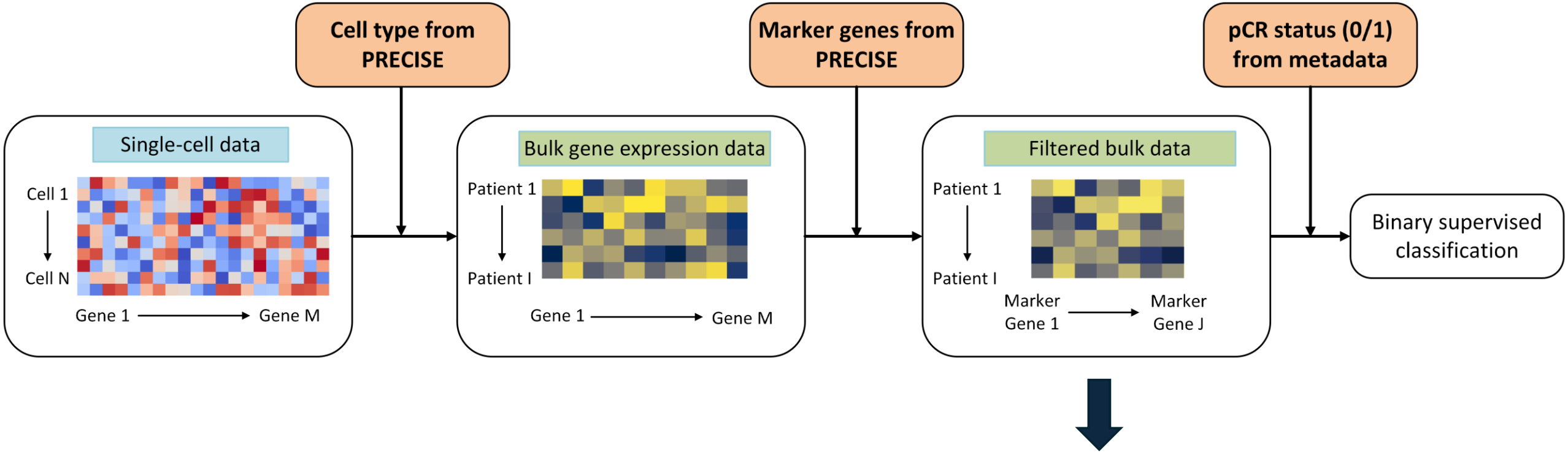


Predicting Treatment Outcomes from Bulk Gene Expression



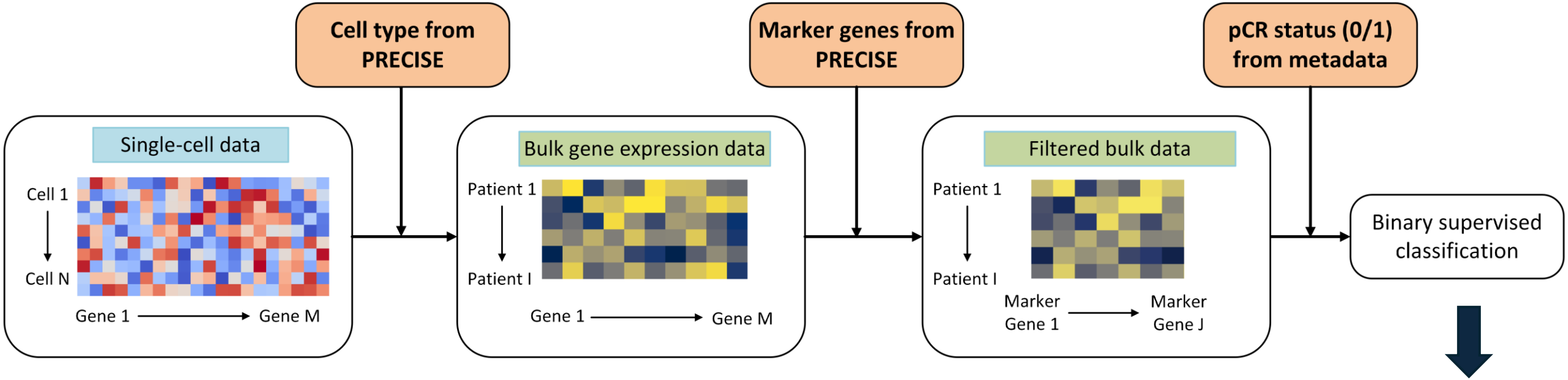
- Group cells by type for each patient
- Average gene expression within each group

Predicting Treatment Outcomes from Bulk Gene Expression



- Filter bulk expression matrix to keep only marker genes for prediction

Predicting Treatment Outcomes from Bulk Gene Expression



- Train classifiers using LASSO
- Evaluate performance using 3-fold stratified cross-validation

Results and Future Work

Discovery Dataset - Bassez et al. Cohort (2021)

- Study design:
  - Paired scRNA-seq data were collected before and after anti-PD1 treatment
  - scRNA-seq dimensions: 157,760 cells × 25,291 genes
- Motivation for our project:
  - Better understand the mechanisms underlying breast cancer by using **single-cell gene expression data**
  - Improve treatment outcome prediction for **anti-PD1 treatment**
  - Identify novel **marker genes** predictive of treatment response

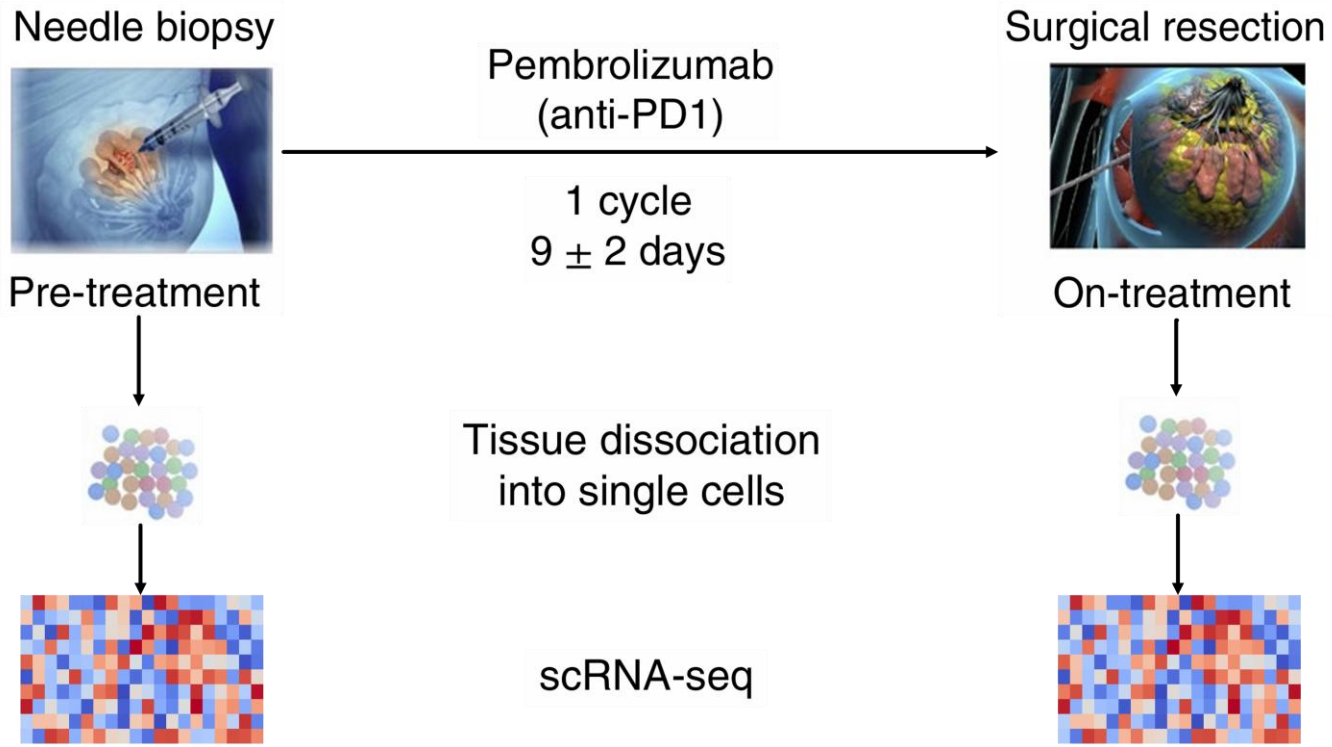


Figure 2: Sampling process of the discovery dataset (modified from Bassez et al., 2021)

Bulk Gene Expression Dataset for Treatment Response Prediction

- Discovery dataset: *Bassez et al. Cohort* (2021)
  - Treatment: anti-PD1 treatment (pembrolizumab + paclitaxel)
  - Sample size: **29 patients** (9 achieve pCR, 20 did not)
  - Bulk gene expression data: **aggregated from the cell-by-gene matrix using the PRECISE model**, 29 patients × 25,291 genes
- Validation dataset: *I-SPY2 trial cohort treated with anti-PD1 treatment*
  - Sample size: **69 patients** (31 achieve pCR, 38 did not)
  - Bulk gene expression data dimensions: 69 patients × 19,134 genes

Treatment Outcome Prediction: PRECISE vs. Seurat

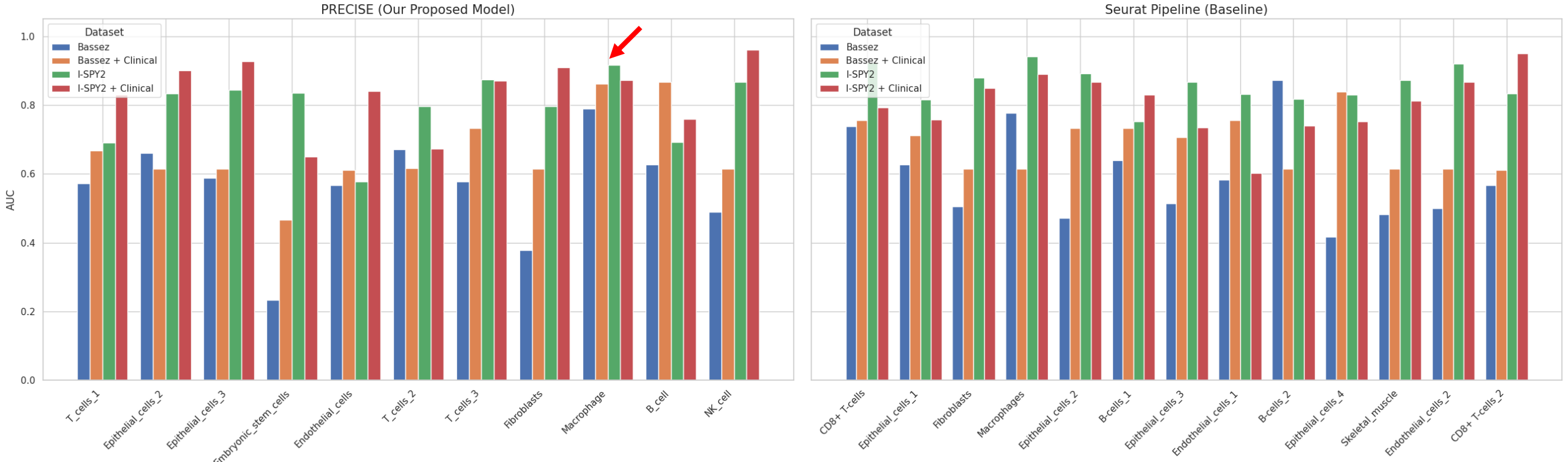


Figure 3: Cell type-specific treatment response prediction performance across models and datasets (LASSO)

PRECISE-identified **Macrophage** marker genes showed **consistently high AUC** across all datasets

- Strong performance on both **Bassez** and **I-SPY2** and Maintained performance even after incorporating **clinical covariates**
- Outperformed the baseline **Seurat pipeline** in nearly all conditions for macrophages
- Fine-tuned **XGBoost** and **SVM** models underperformed compared to **LASSO**, yielding lower AUC scores

Treatment Outcome Prediction: PRECISE vs InteractPrint

PRECISE-identified **Macrophage** marker genes **Outperforms Published Models for Treatment Outcome Prediction**

- PRECISE achieved **AUC = 0.861** on the Bassez et al. dataset (top left) and **AUC = 0.917** on the I-SPY2 trial (bottom left)
- Outperforms **PD-L1 expression** and **T Cell InteractPrint** baselines from Xu et al., 2024 (right panel)

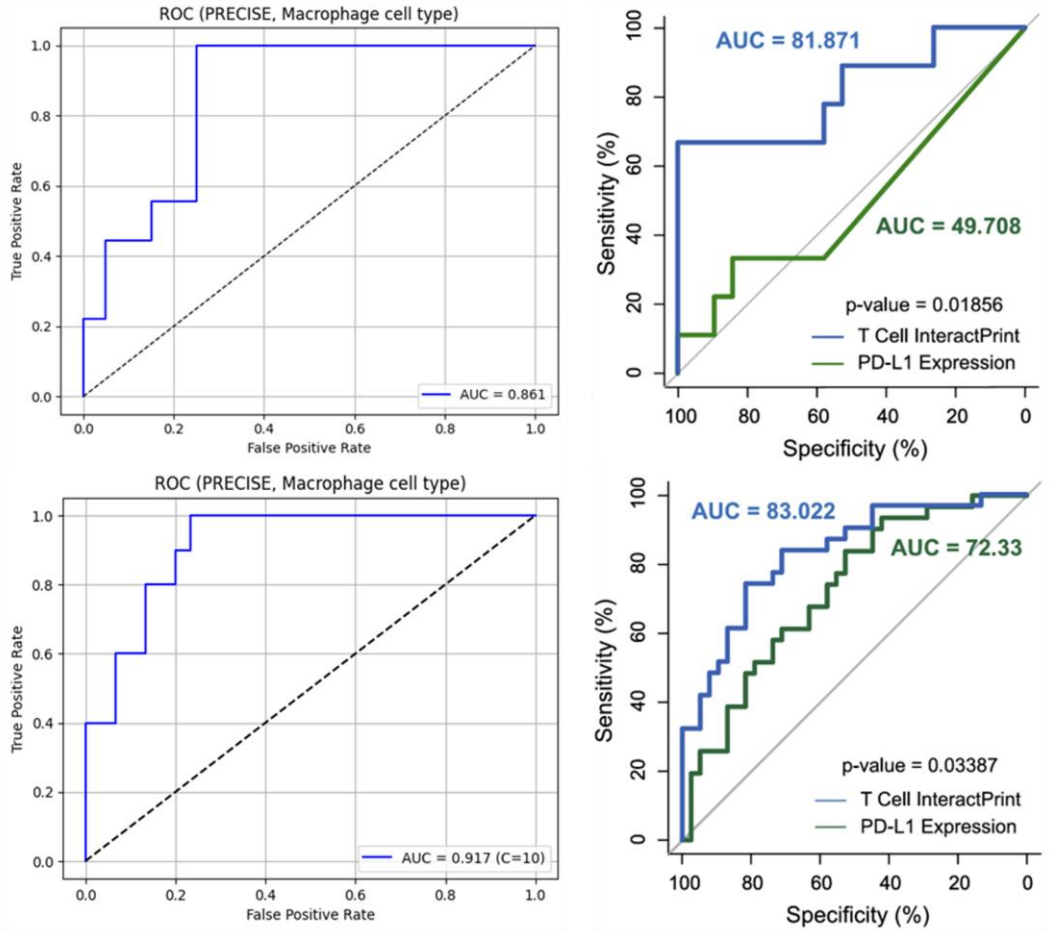


Figure 4: Comparison of PRECISE Macrophage-Based Prediction with Existing Published Models (**Left**: ROC curves from PRECISE using macrophage marker genes on the Bassez et al. dataset (top) and the I-SPY2 trial dataset (bottom). **Right**: ROC curves adapted from Xu et al., Cell Reports Medicine (2024) [https://doi.org/10.1016/j.xcrm.2024.101511]

Uncertainty Quantification of Treatment Response Prediction

Conformal prediction:

- **Motivation**: Beyond point prediction, we aim to quantify **uncertainty** in model outputs

Conformal histogram (right, e.g.):

- Majority of predictions are **high-confidence**
- **Only a few uncertain samples** fall near the decision threshold

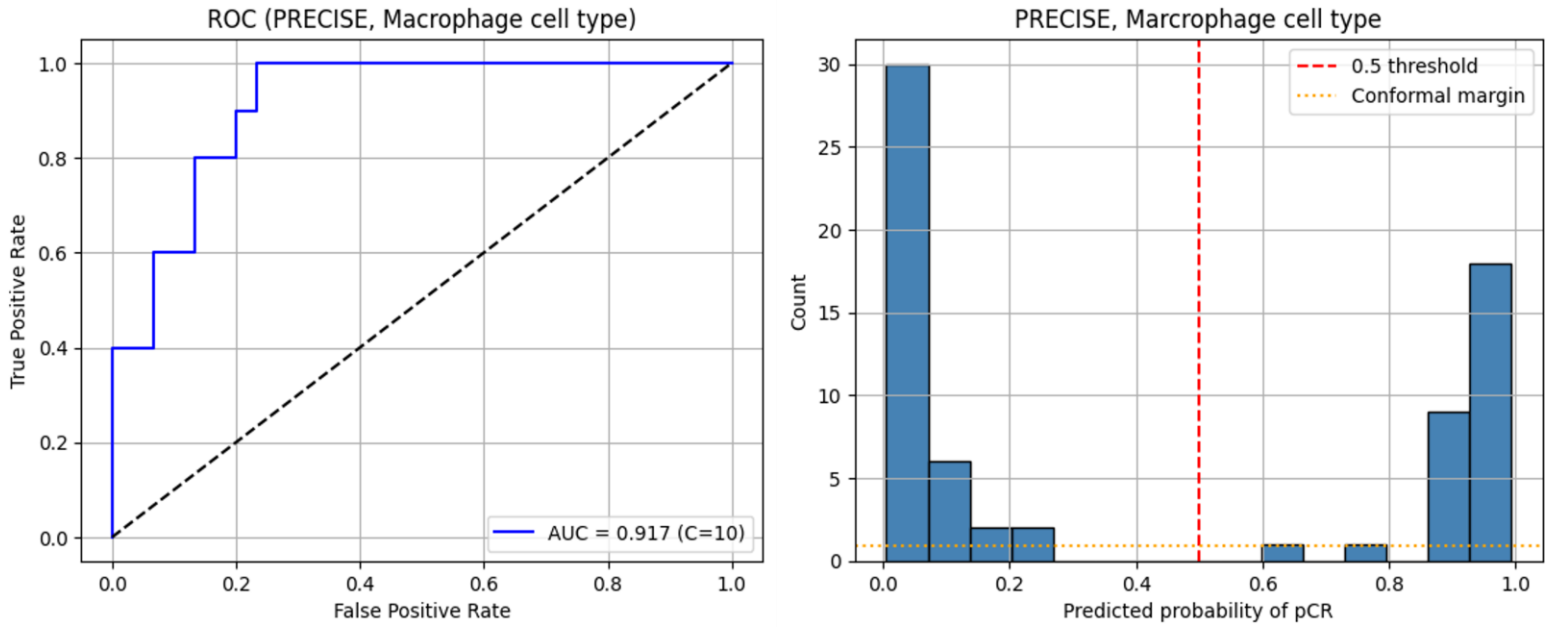


Figure 5: Quantifying prediction uncertainty with conformal prediction: macrophage markers in I-SPY2 trial
Left: *ROC curve (AUC = 0.917) using PRECISE-identified macrophage markers*
Right: *Conformal prediction at 90% confidence (Actual coverage: 0.97, Confident predictions: 67 / 69) illustrating model uncertainty*

Conclusion

1. **PRECISE's framework outperforms existing models**
   - Achieved higher AUCs than published and clinically used approaches across datasets
2. **Consistently better performance than Seurat pipeline**
   - Embedding-based features from foundation models generalize well in both discovery and validation cohorts
3. **Explored uncertainty quantification via conformal prediction**
   - Initial results demonstrate promise in flagging low-confidence predictions

Future Work

1. **Advance uncertainty quantification with conformal prediction**
   - Apply more rigorous conformal methods and validate confidence intervals across datasets
2. **Implement and benchmark published baselines**
   - Reproduce PD-L1 Expression and InteractPrint pipelines for formal statistical comparison (e.g., DeLong's test)
3. **Investigate clinical interpretability of key features**
   - Analyze top marker genes driving predictions for biological and clinical relevance

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Any Questions?