

Advancing Multi-Cancer Early Detection: High-performance cell-free RNA profiling with the Flomics liquid biopsy platform

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Introduction

Liquid biopsies are increasingly important diagnostic tools for cancer early detection due to their high sensitivity and minimal invasiveness.

Cell-free RNA (cfRNA) is a rich source of potential biomarkers in liquid biopsies as it is actively secreted into biofluids, including blood, by both healthy and diseased cells.

cfRNA analysis of a liquid biopsy therefore provides insight into the health of an individual, and has great potential for early cancer detection and beyond.

However, as an emerging field rife with technical challenges, cfRNA profiling for diagnostic purposes lacks gold standards for both laboratory and bioinformatic protocols.

At Flomics Biotech, we have overcome these challenges by developing **Supernal**, our proprietary high performance cfRNA-Seq platform that profiles human plasma cfRNA in a robust and reproducible manner (Figure 1).

Supernal combines customised laboratory protocols for Next-generation Sequencing of plasma cfRNA with custom-built state-of-the-art bioinformatics and machine learning classification methods to identify cfRNA biomarker signatures. Stringent quality control checkpoints are implemented at various stages in the workflow to ensure that the data that is generated is of the highest quality.

Here we applied Supernal in the LiquiDx pre-clinical study with the aim of developing a **cfRNA-based multi-cancer early detection test**.

Methods

Using SupernalTM (summarised in Figure 1), we profiled the cfRNA in plasma samples from over 1,000 individuals. The cohort includes healthy individuals and patients with early or late stage colorectal, lung, breast, pancreatic or prostate cancer.

We isolated cfRNA from 1 ml of plasma and sequenced in-house at an average depth of 36M paired-end reads per sample using the Illumina NextSeq 2000 system. A machine learning classifier was trained using the cfRNA-seq data to predict patient status and tumor tissue-of-origin.

Conclusions

- Flomics has developed Supernal, a world class cfRNA-Seq platform that will pioneer the use of cfRNA in clinical practice.
- Supernal maintains high performance across all cancer stages including stage I, illustrating the benefits of using cfRNA for cancer early detection.
- With a diverse range of applications, Supernal will have a significant impact on global health.

Supernal: The platform for profiling cell-free RNA in liquid biopsies

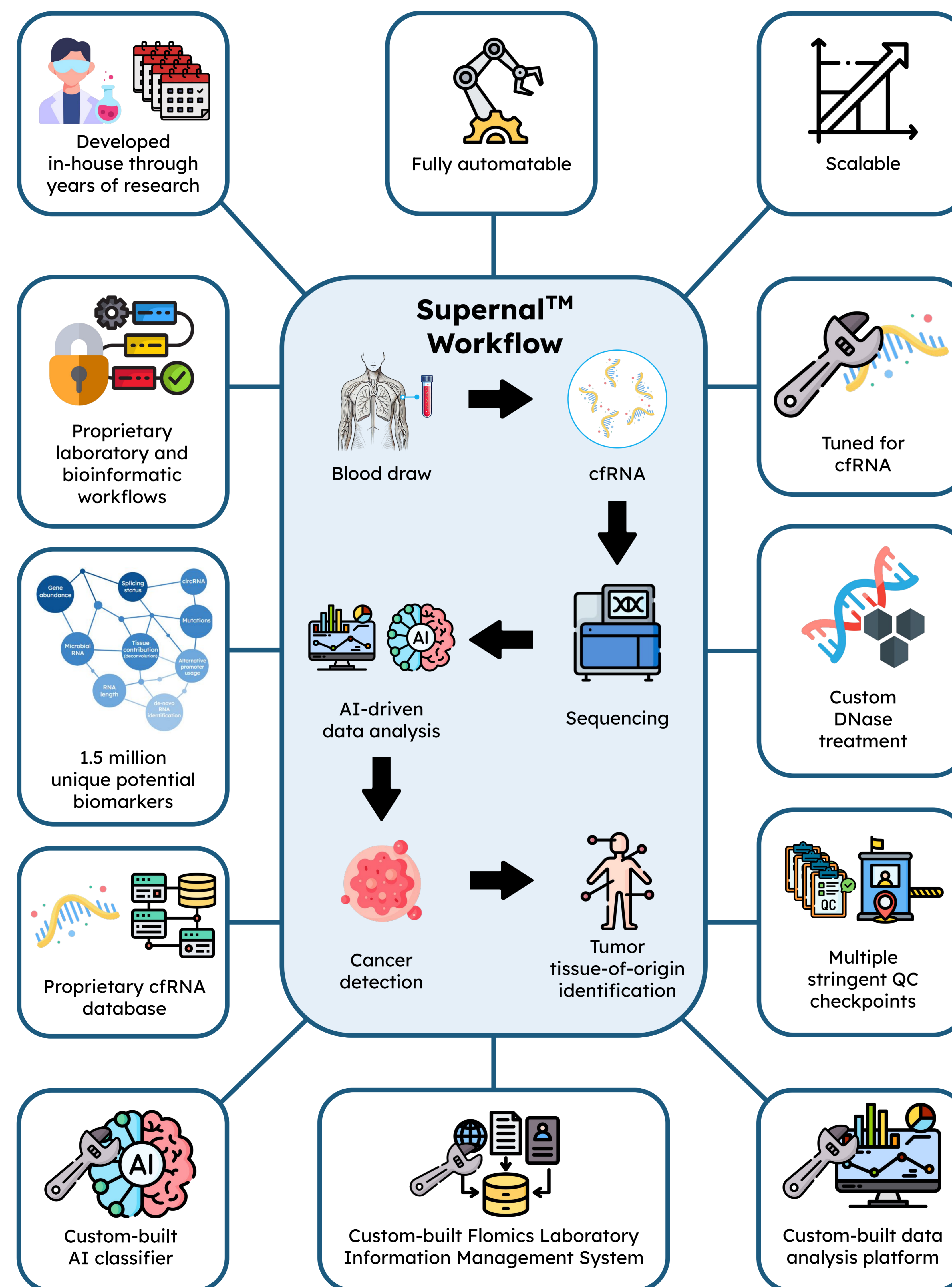


Figure 1. Overview of Supernal. **Centre:** Summary of the Supernal workflow from blood draw to cancer detection and tumor tissue-of-origin identification. **Edges:** Properties of Supernal that elevate it above other cfRNA-based methods and position Flomics as a leader in cfRNA-based diagnostics.

High-performance stage I cancer detection

We leverage differences in cfRNA expression between cancer patients and healthy individuals to build a **machine learning model** to detect cancer. Model training is ongoing, however using currently available data (Figure 2 - Left) we have developed a binary classifier that **predicts cancer with promising results** (Figure 2 - Middle). The model displays a **high performance level for the prediction of all cancer stages including stage I** (Figure 2 - Right).

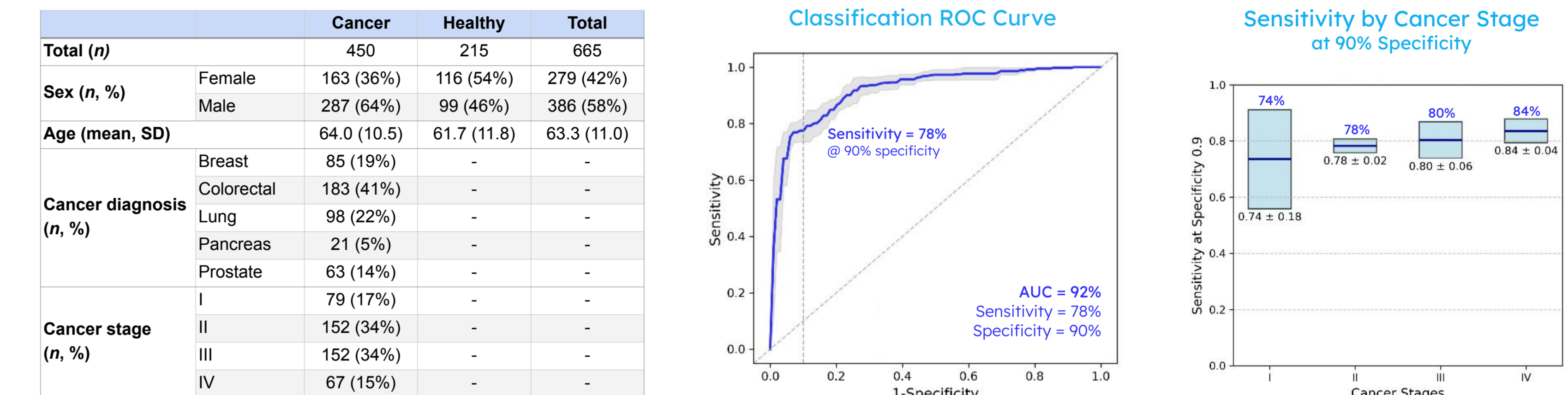


Figure 2. **Left:** Summary of the samples included in this analysis (Sentinel.trial.1.2). Note that the samples that failed QC are not included. **Middle:** Performance of our preliminary cancer vs healthy machine learning (ML) binary classifier. ROC curves (Sensitivity v.s. 1-Specificity) and Area Under the Curve (AUC) for the 4-folds cross-validation. **Right:** Performance of the ML binary classifier in predicting different stages of cancer at 90% specificity.

Tumor tissue-of-origin identification

Combining early stage cancer detection with accurate prediction of tumor tissue-of-origin (TTO) will improve patient diagnosis and prognosis. Using information from the cfRNA profiles we are developing a multi-class machine learning classifier model to predict the TTO of cancer samples. The model currently classifies the TTO in 67% of samples (Figure 3 - left). For the classified samples, TTO is determined with high accuracy, achieving 74% and 93% mean accuracy for the most probable (top-1) and the two most probable (top-2) tissues, respectively (Figure 3 - right). This approach also has great potential in identifying the tissue-of-origin in cancers of unknown primary.

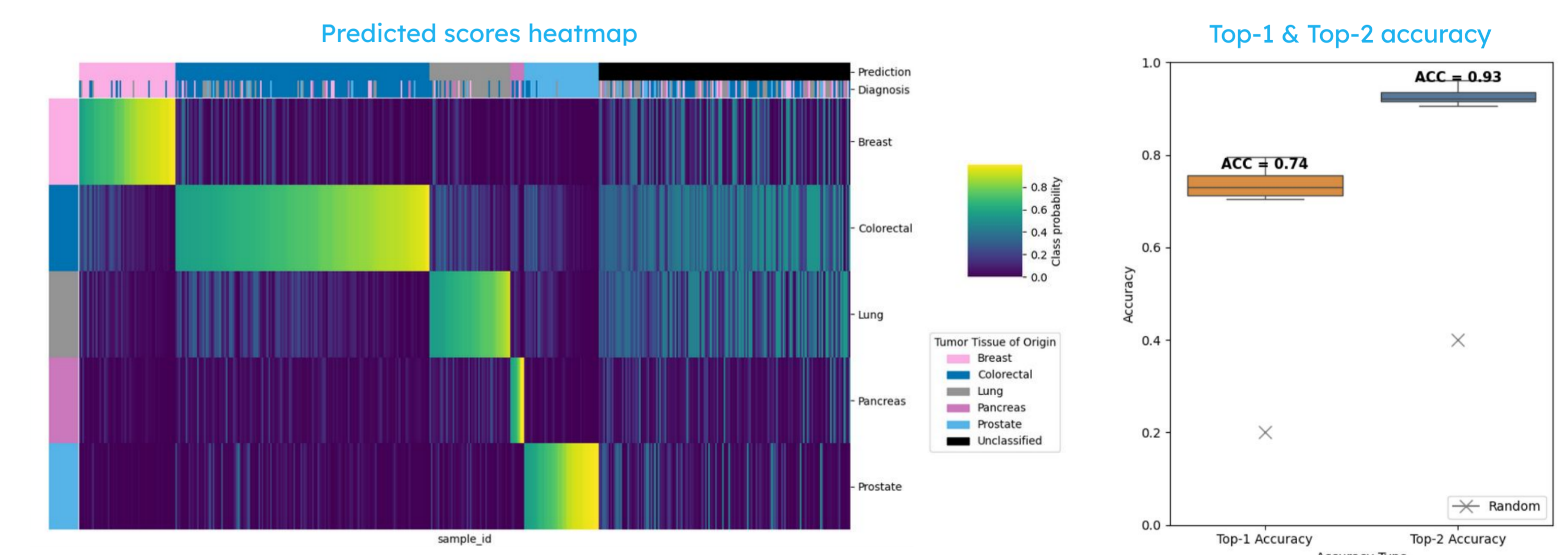


Figure 3. **Left:** Predicted scores of the model for each sample (x axis) and TTO (y axis) on the validation set using 4-folds cross-validation (CV). Predicted TTO (top row) was assigned to the tissue with highest probability. Samples with maximum probability < 0.55 for all tissues were considered as unclassified. Diagnosis (second row) indicates the actual diagnosis of the patient. **Right:** Accuracy of the TTO multi-class ML classifier model in predicting the top-1 and top-2 most probable tissues of origin of cancer samples in the validation set using 4-folds CV. Unclassified samples are excluded from this analysis. Grey crosses indicate the expected performance of the model if guessing at random.

Applications beyond cancer early detection

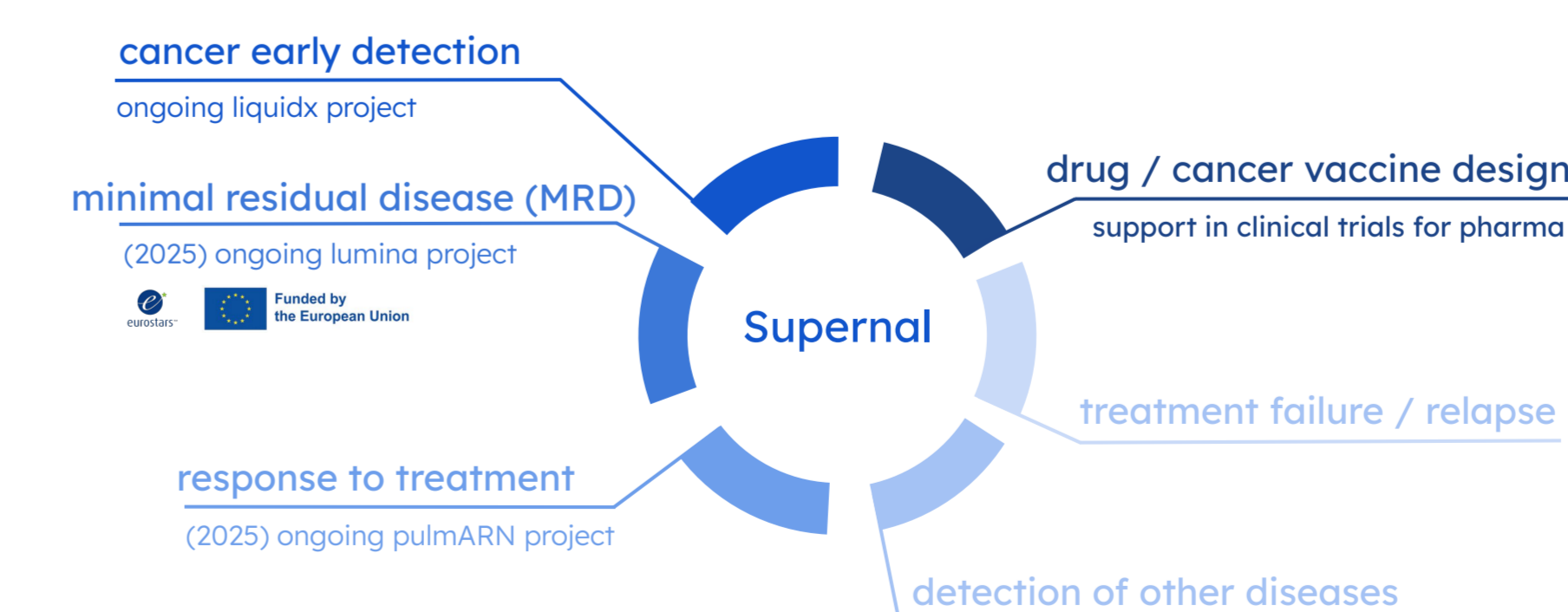


Figure 4. Applications of Supernal including ongoing projects at Flomics in these research areas.

Supernal has many potential applications in addition to early cancer detection (Figure 4), such as monitoring minimal residual disease, guiding cancer treatment selection, and assessing response to cancer therapies such as immunotherapy. Beyond cancer, the platform can be applied to many other diseases and research areas such as the detection and tracking of cardiovascular disease and neurodegenerative diseases.

Flomics is always looking for:

- Collaborations
- Opportunities to provide our technology and know-how as a service
- Clinical expertise including sample provision

Contact us at info@flomics.com

