Breast Cancer

The most global cancer incidence in women

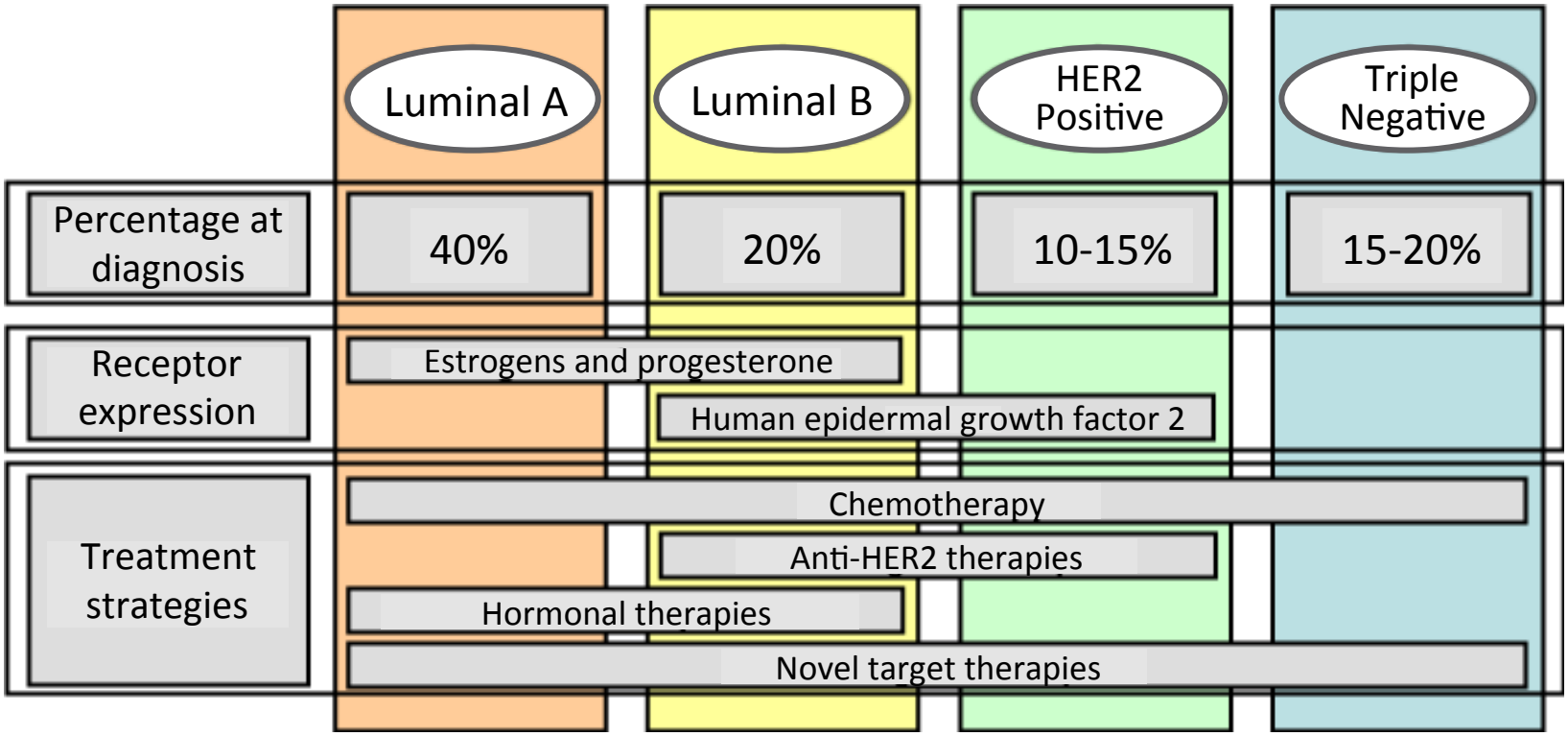
Rank	Cancer	New cases diagnosed in 2018	% of all cancers (excl. non-melanoma skin cancer)
1	Breast	2,088,849	25.4
2	Colorectal	794,958	9.7
3	Lung	725,352	8.8
4	Cervix uteri	569,847	6.9

World Cancer Research Fund

World Health Organization

- Breast cancer causes the greatest number of cancer-related deaths among women. In 2018, it is estimated that 627,000 women died from breast cancer – that is approximately 15% of all cancer deaths among women.
- While breast cancer rates are higher among women in more developed regions, rates are increasing in nearly every region globally.

Diversity of Breast Cancer: Subtypes

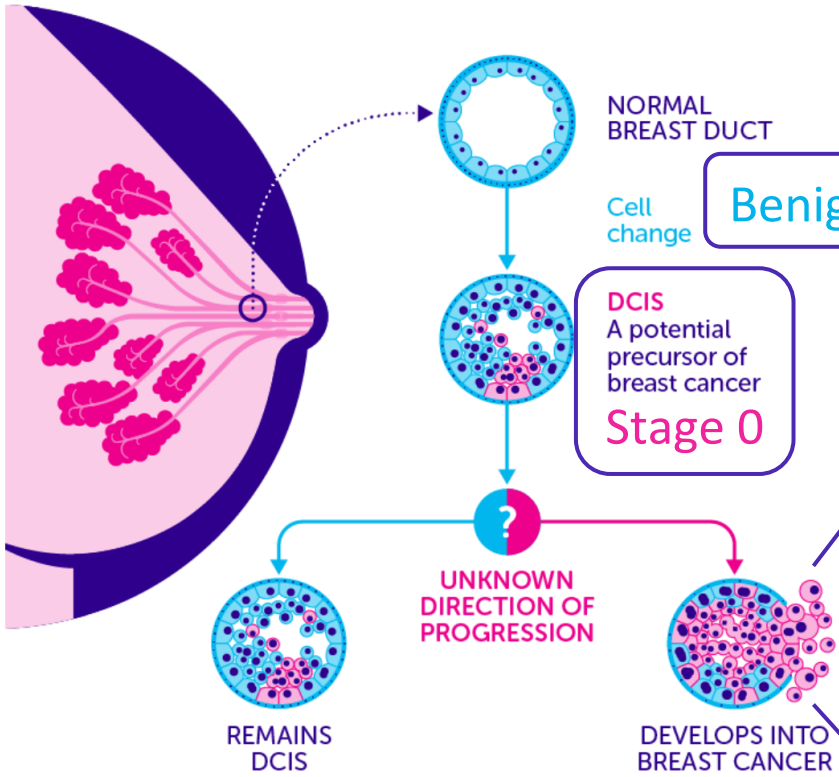


Catalanotti, V., Bertaglia, V., Tariq, N., Califano, R. (2014). Treatment of Advanced Breast Cancer (ABC): The Expanding Landscape of Targeted Therapies. *J Cancer Biol Res*, 2(1), 1036.

Clinical unmet needs in breast cancer

1. Early detection
2. New progression mechanism for targeted therapies (e.g. TNBC)
3. Heterogeneity
4. Drug Resistance
5. Companion diagnosis
6. Racial disparity

Progression & Diversity of Breast Cancer



TNM Stage	Five year survival rate
Stage I	> **95%**
Stage II	About 90%
Stage III	About **70%**
Stage IV	About **25%**

DCIS: Ductal carcinoma *in situ*

TNM Stage
T = size of primary tumor
N = the extent of spread to nearby lymph nodes
M = presence or absence of distant metastases

Diversity of Breast Cancer: Racial Disparity

Figure 6a. Trends in Female Breast Cancer Incidence Rates by Race/Ethnicity, 1975-2014, US

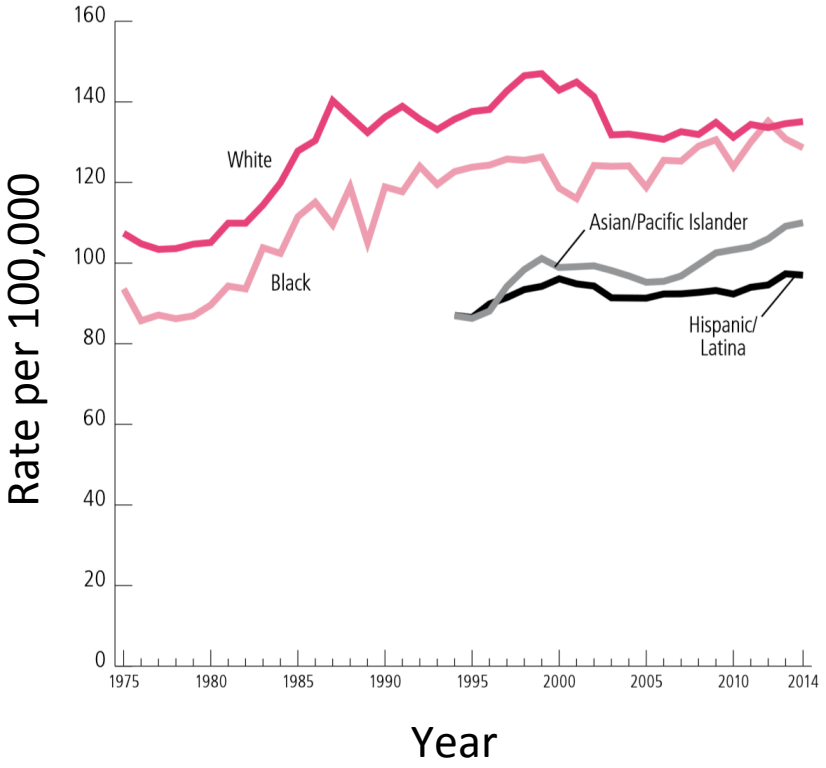
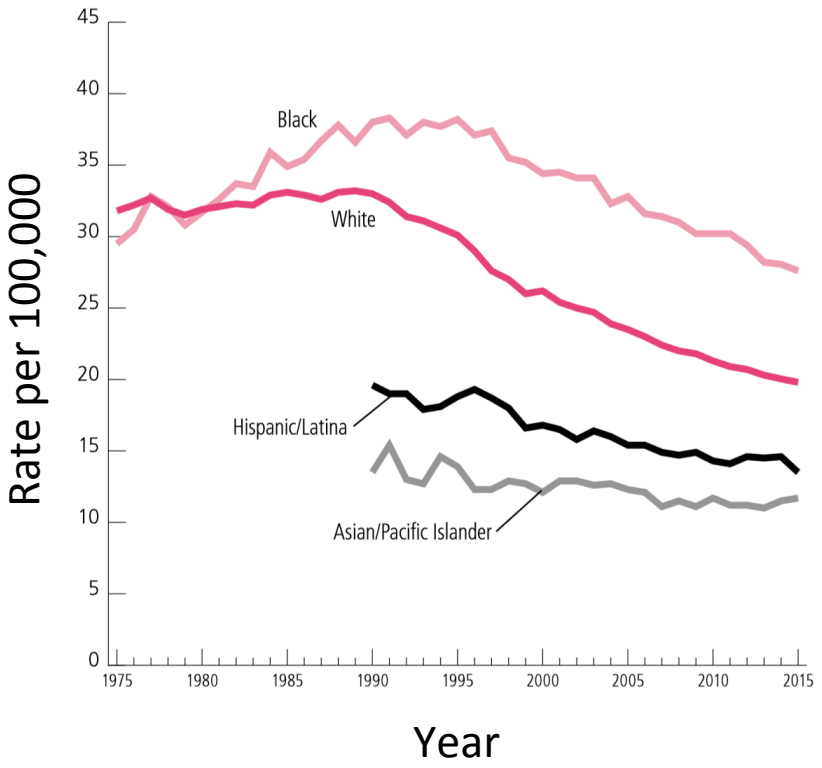


Figure 6b. Trends in Female Breast Cancer Death Rates by Race/Ethnicity, 1975-2015, US

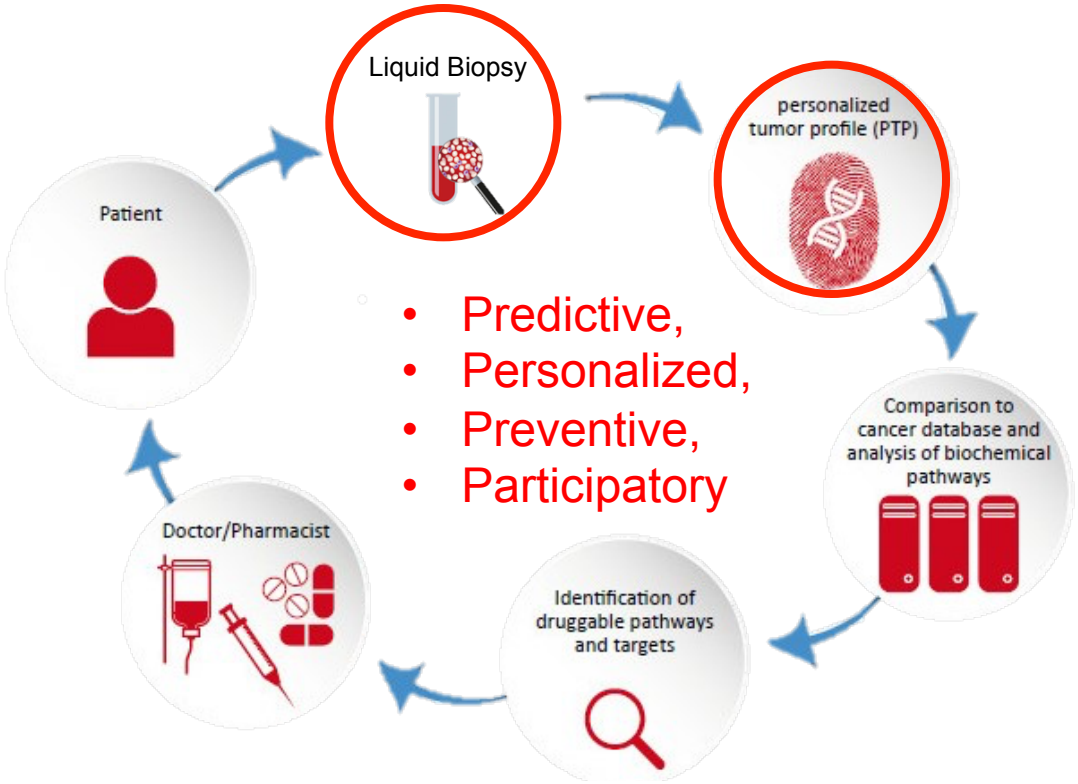


American Cancer Society®

Surveillance Research, 2017

Disease Biomarkers

From Liquid Biopsy to Precision Medicine



"Doctors have always recognized that every patient is unique, and doctors have always tried to tailor their treatments as best they can to individuals. You can match a blood transfusion to a blood type — that was an important discovery. What if matching a cancer cure to our genetic code was just as easy, just as standard? What if figuring out the right dose of medicine was as simple as taking our temperature?"

- President Obama, January 30, 2015

nature REVIEWS CLINICAL ONCOLOGY

OPINION

Predictive, personalized, preventive, participatory (P4) cancer medicine

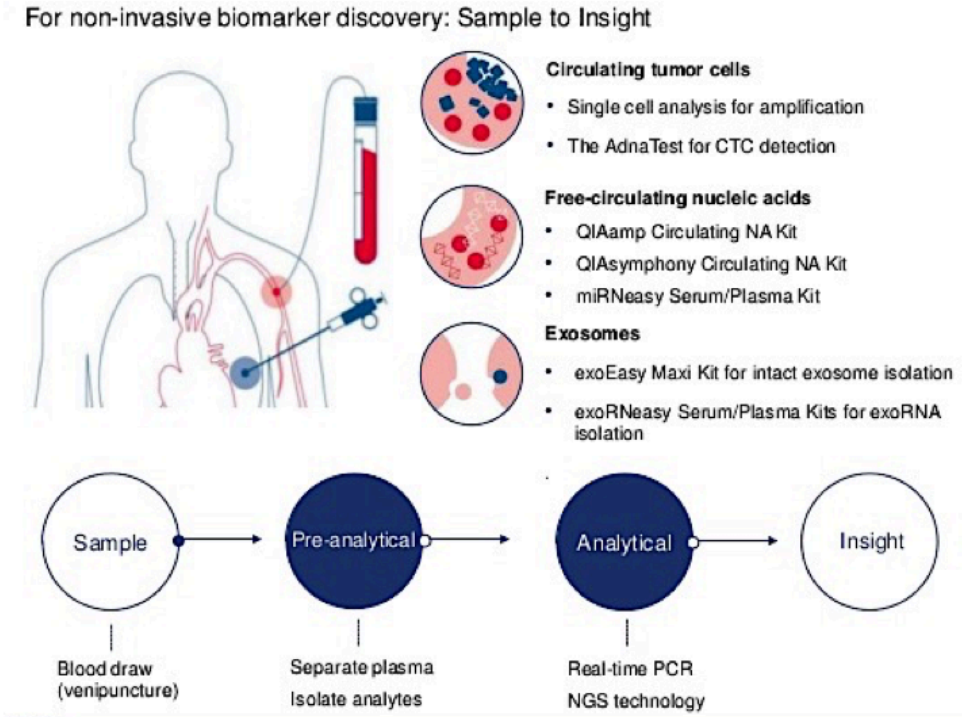
Leroy Hood and Stephen H. Friend

Published: 02 March 2011

Liquid Biopsy:

- Less invasive,
- less costly,
- less risky,
- contain more dynamic information than conventional tissue biopsies.

Trend in Cancer Precision Medicine:
Liquid Biopsy: CTC, ctDNA, Exosome



Liquid Biopsy:

Variety of biomarkers existed in bodily fluids such as blood, saliva, urine, ascites, etc

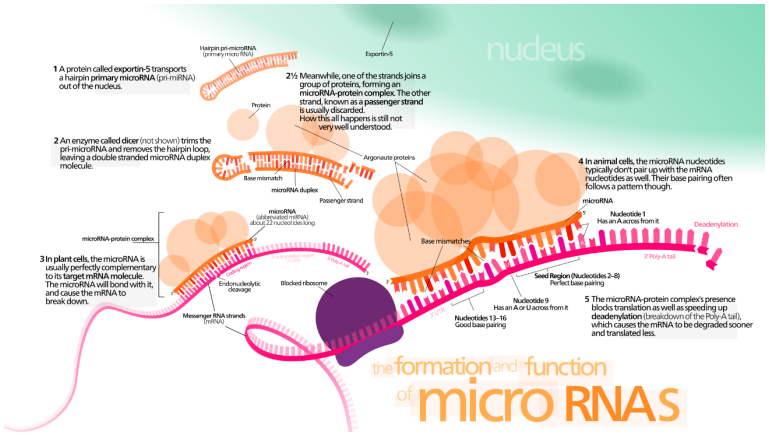
Main areas of Liquid Biopsy:

- Circulating tumor cells (CTC)
- Circulating tumor DNA (ctDNA)
- Exosomes
  - mRNA, Protein, miRNA, lncRNA

miRNA Biomarkers in Liquid Biopsy

MicroRNA(miRNA)

1. Non-coding RNA
2. 17-25 nucleotides
3. Post-transcriptional regulation: base-pair with mRNAs and silence those mRNAs
4. Appear to target about 60% of the genes of human



Evaluation of serum microRNA biomarkers for gastric cancer based on blood and tissue pools profiling: the importance of *miR-21* and *miR-331*

British Journal of Cancer (2017) 117, 266-273. doi: 10.1038/bjc.2017.190

Marek Sierzega*[,1], Marcin Kaczor[2], Piotr Kolodziejczyk[1], Jan Kulig[1], Marek Sanak[2] and Piotr Richter[1]

miR-331 and miR-21 => gastric cancer

Serum MicroRNA profile in patients with colon adenomas or cancer

BMC Medical Genomics (2017) 10:23
DOI 10.1186/s12920-017-0260-7

Yajie Zhang[1,2], Min Li[4], Yijiang Ding[3], Zhimin Fan[3], Jinchun Zhang[5], Hongying Zhang[6], Bin Jiang[3*] and Yong Zhu[3*]

8 miRNAs => colon cancer

Circulating microRNAs in breast cancer: novel diagnostic and prognostic biomarkers

Cell Death & Disease **8**, e3045 (2017)

Rimi Hamam, Dana Hamam, Khalid A Alsaleh, Moustapha Kassem, Waleed Zaher, Musaad Alfayez, Abdullah Aldahmash & Nehad M Alajez

Problems to be solved

1. Identify possible biomarkers for early detection of breast cancer from liquid biopsy (e.g. miRNAs).
2. Generate profiling on the various stages of breast cancer. Identify more insightful information regarding the critical factors that progress cancer to the next stage.

Comprehensive data (containing all variation)
+ High-throughput techniques (high sensitivity)
+ Proper data analysis (low sample number issue)
+ Artificial intelligence for optimization (predictable)

Current biomarkers for breast cancer

Breast Cancer: 3 tumor markers

- cancer antigen 15-3 (CA 15-3),
- cancer antigen 27.29 (CA 27.29), and
- carcinoembryonic antigen (CEA)

Tumor Marker	N (Total)	Sensitivity
CA 15-3	35 (145)	24.1%
CA 27.29	37 (145)	25.5%
CEA	27 (145)	18.6 %

Hou, MF, et al. (1999). Evaluation of serum CA27.29, CA15-3 and CEA in patients with breast cancer. *Kaohsiung J Med Sci.*, 23(1), pp.88-93.

Comprehensive data

台大醫院 NTUH

Wen-Hong Kuo, MD; PhD

Noncancer	Benign	10	DCIS	20

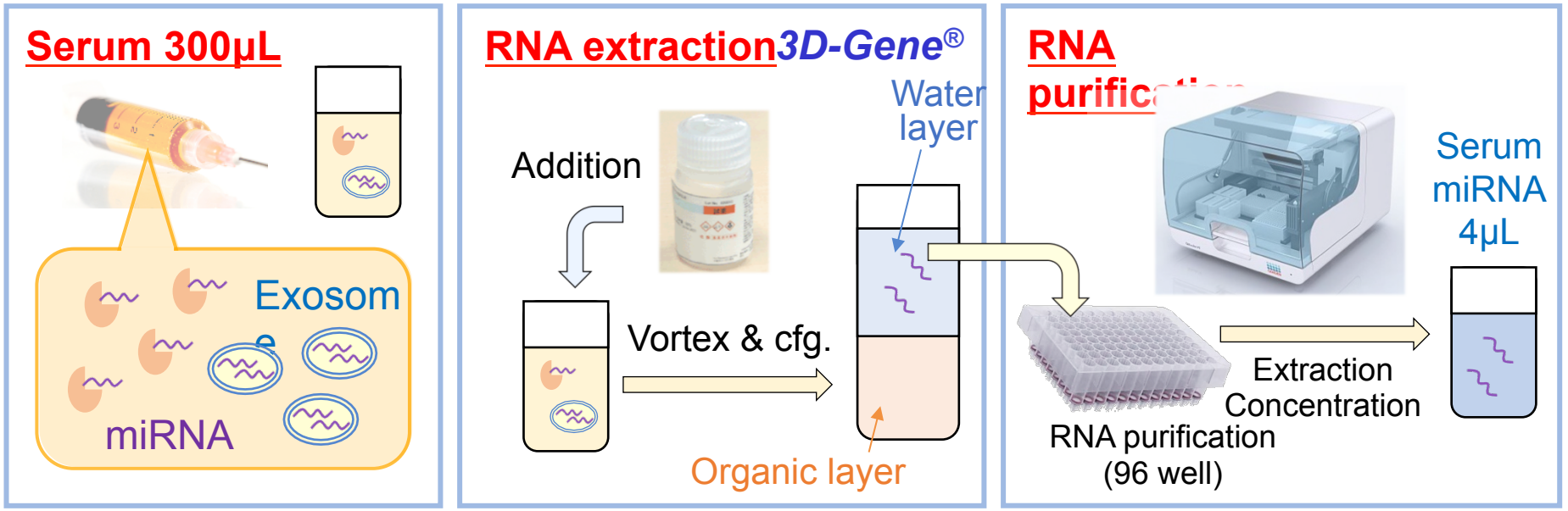
Cancer		Stage				Total
		I	II	III	IV	
Subtypes	Luminal A	8	8	12	5	33
	Luminal B	8	8	12	5	33
	HER2^{+}	8	8	12	5	33
	Triple Negative	8	8	12	4	32
Total		32	32	48	19	131

High-throughput techniques

国立研究開発法人
国立がん研究センター
National Cancer Center Japan

1. miRNA extraction from serum
2. miRNA analysis with microarray

Takahiro Ochiya, PhD



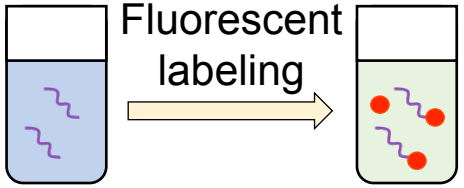
High-throughput techniques

国立研究開発法人
国立がん研究センター
National Cancer Center Japan

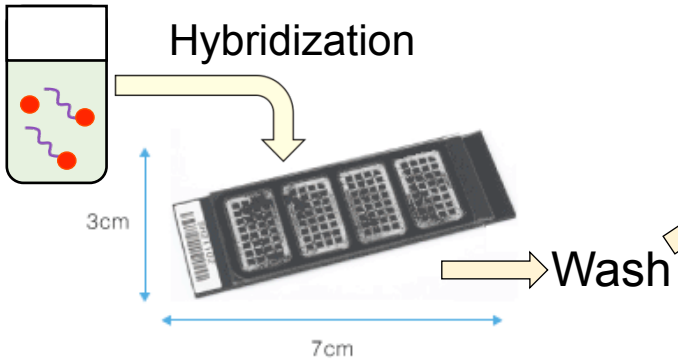
1. miRNA extraction from serum
2. miRNA analysis with microarray

RNA labeling

RNA 2μL

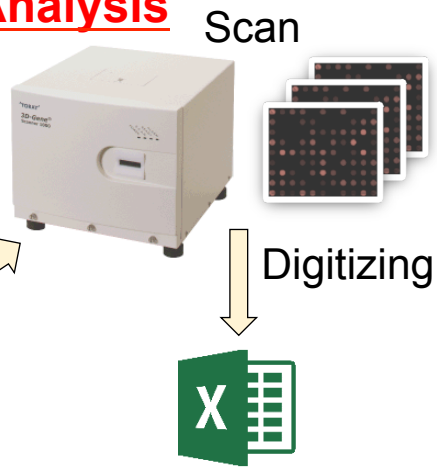


DNA chip analysis ***3D-Gene®***



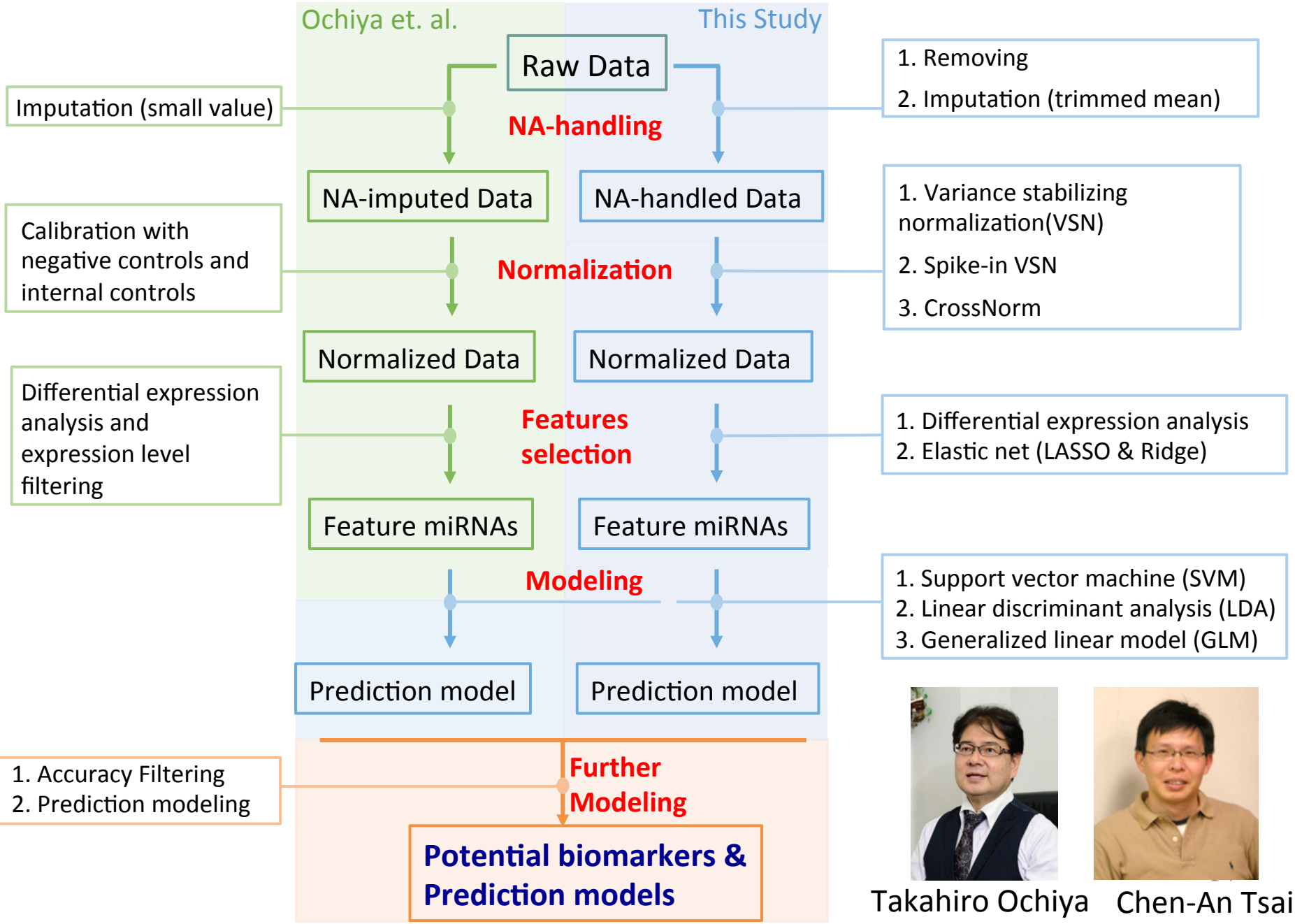
miRNA oligo chip
(2565 miRNA, miRBase ver. 21)

Analysis



miRNA expression data
(Raw data)

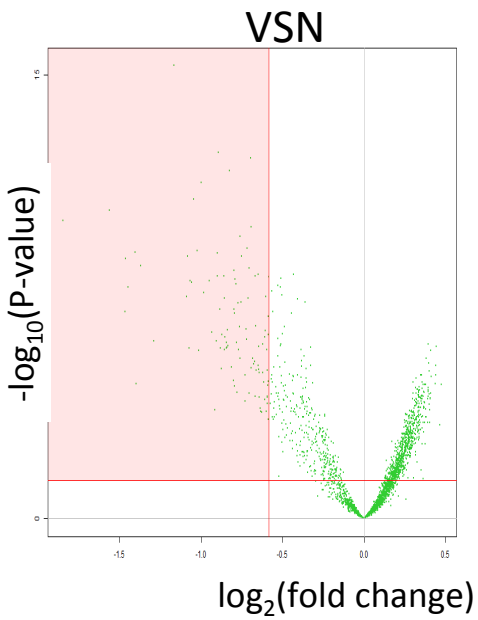
Data Analysis Pipeline



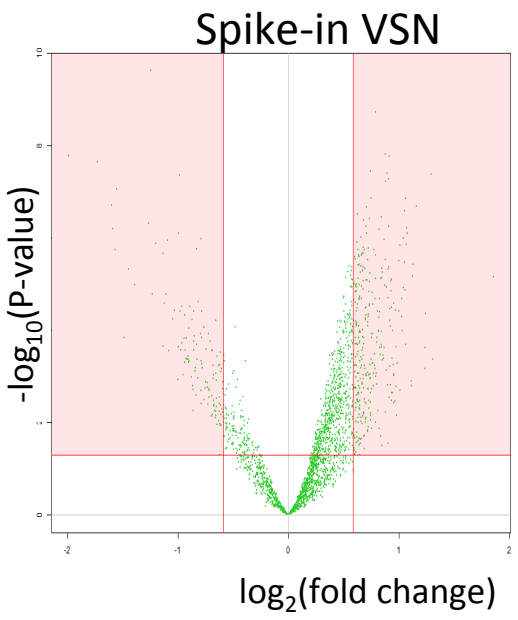
Takahiro Ochiya

Chen-An Tsai

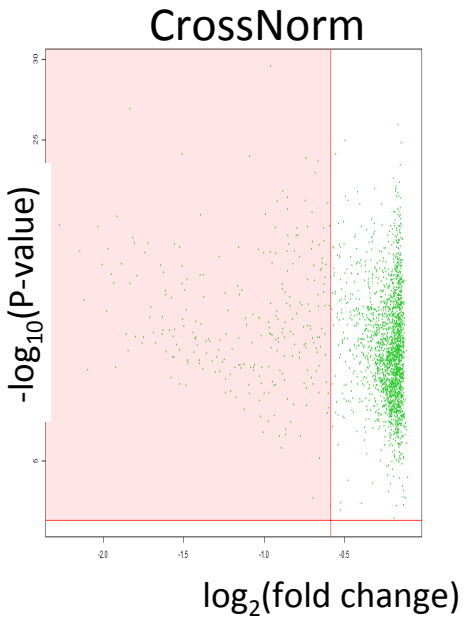
Differential Expression Analyses



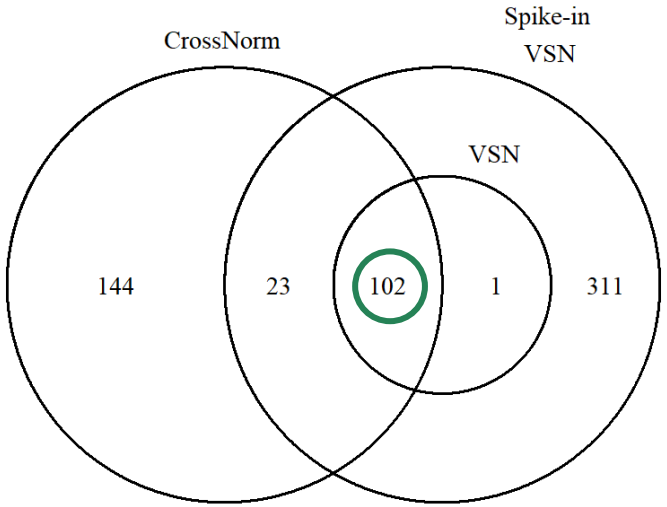
103 probes are selected



437 probes are selected



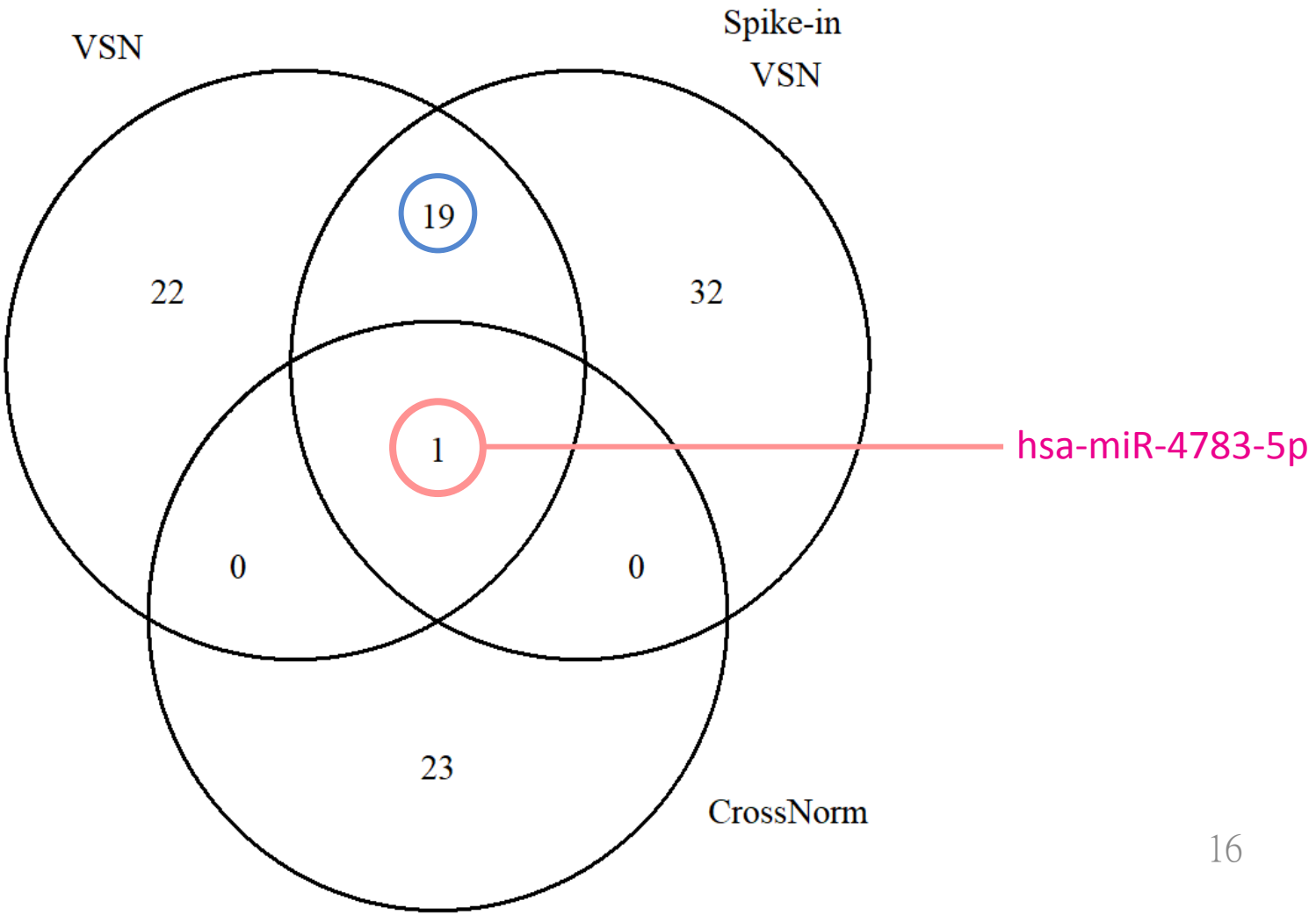
269 probes are selected



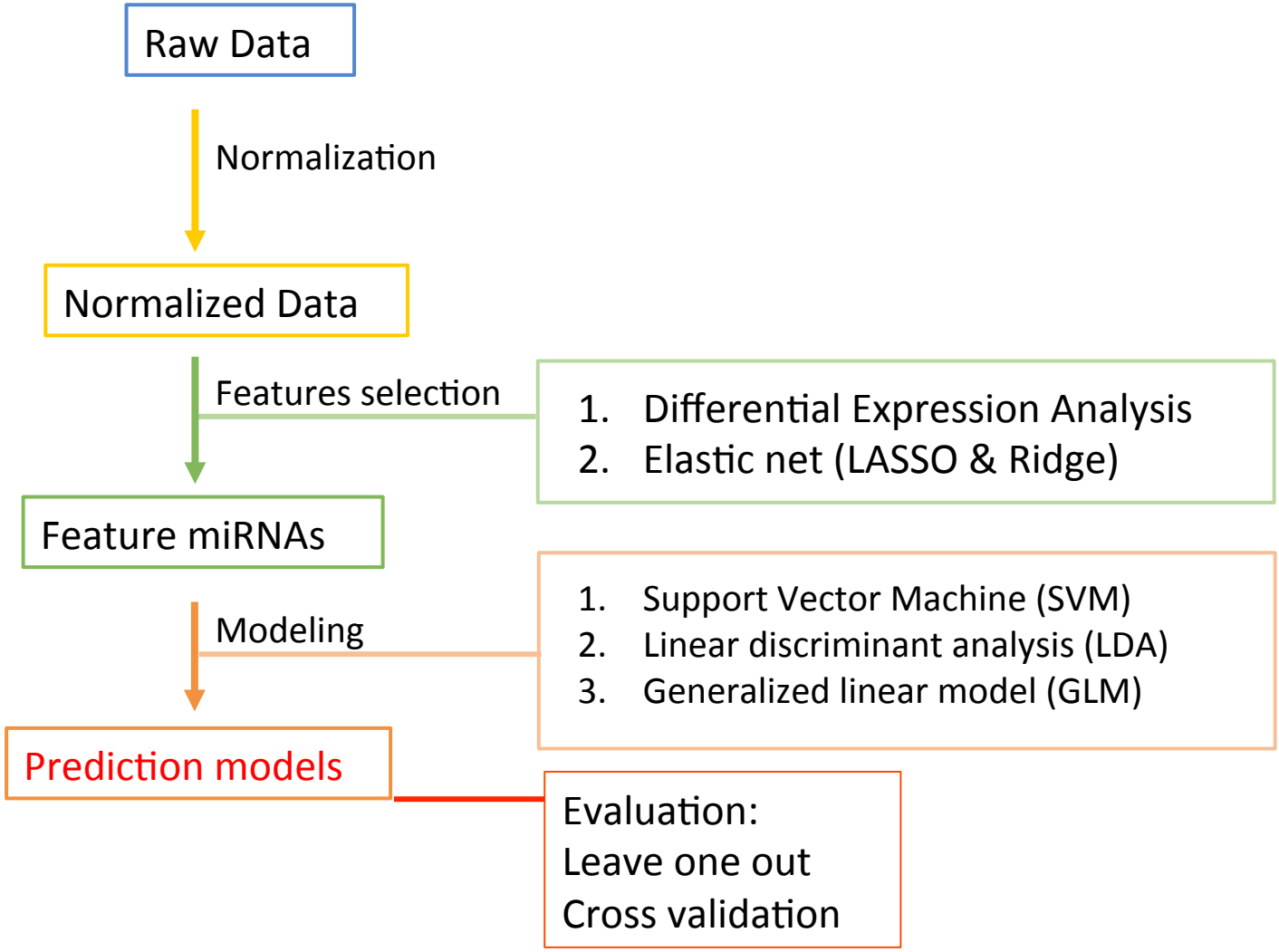
Adjusted P-values < 0.05
(Benjamini–Hochberg procedure)

Significant log2 fold change = 1.5

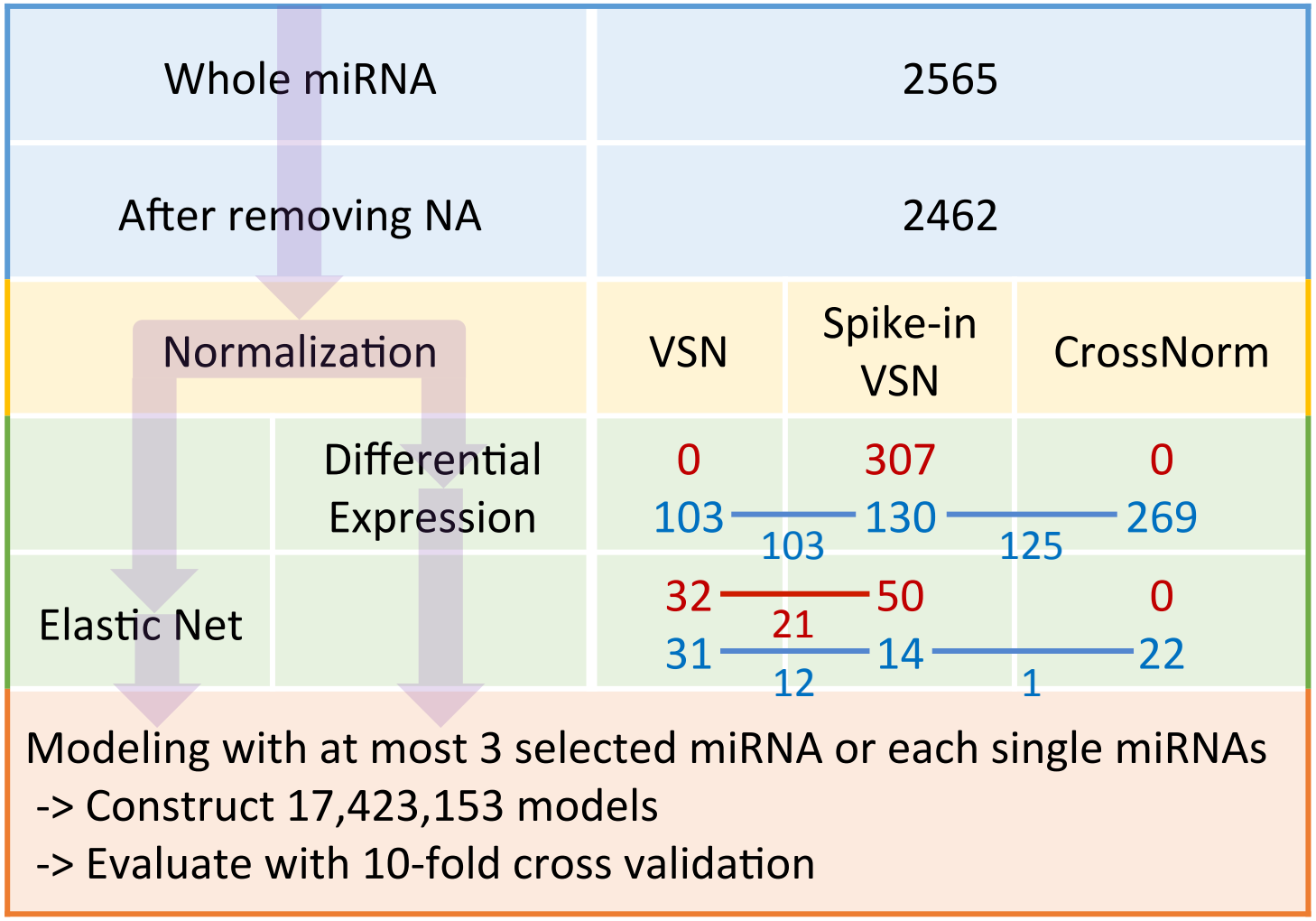
miRNAs Selected by Elastic Net Regression



miRNA Analysis Flow Chart



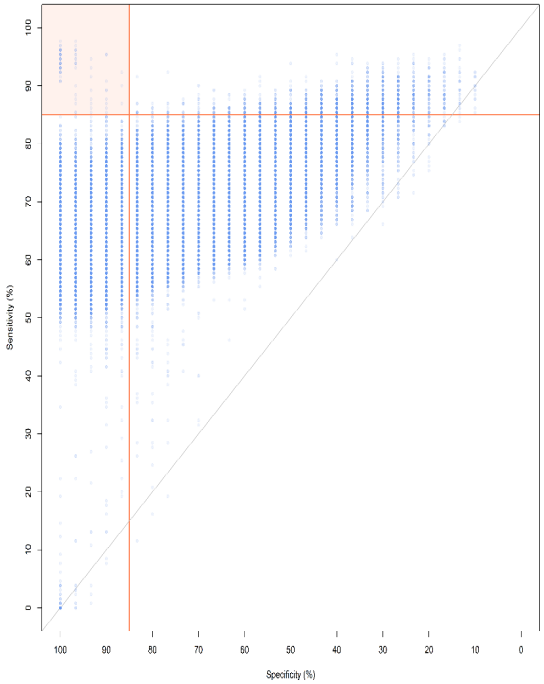
Results of miRNA through Analysis Pipeline



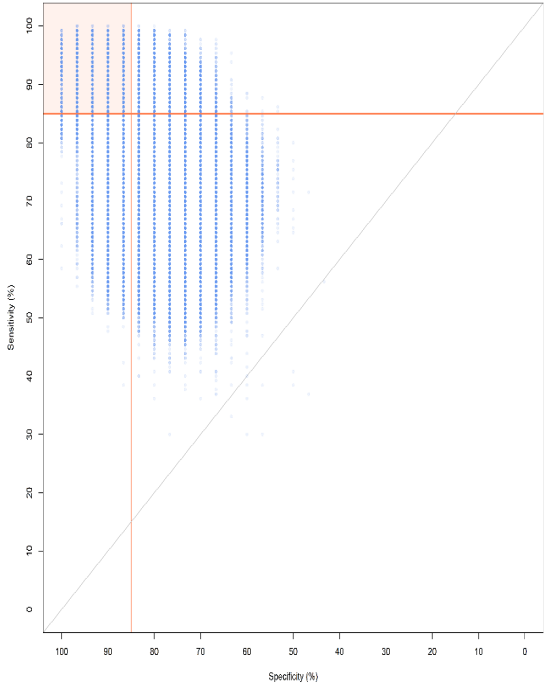
Modeling with Selected miRNAs

Selecting the miRNAs with prediction ability

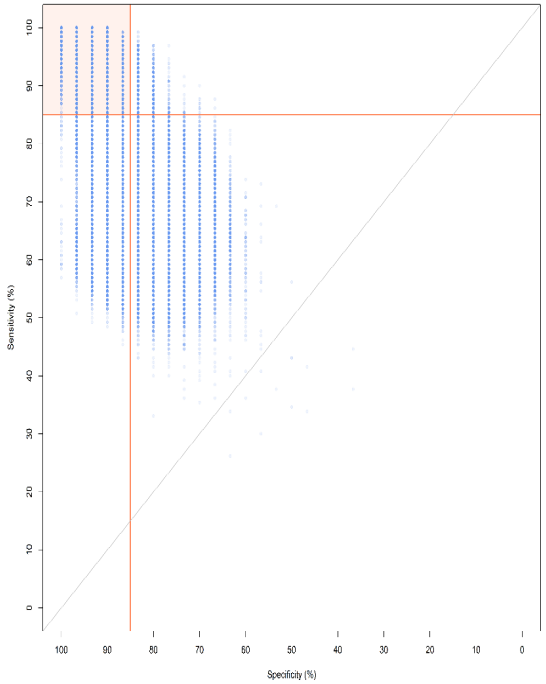
sensitivity & specificity > 85%



SVM

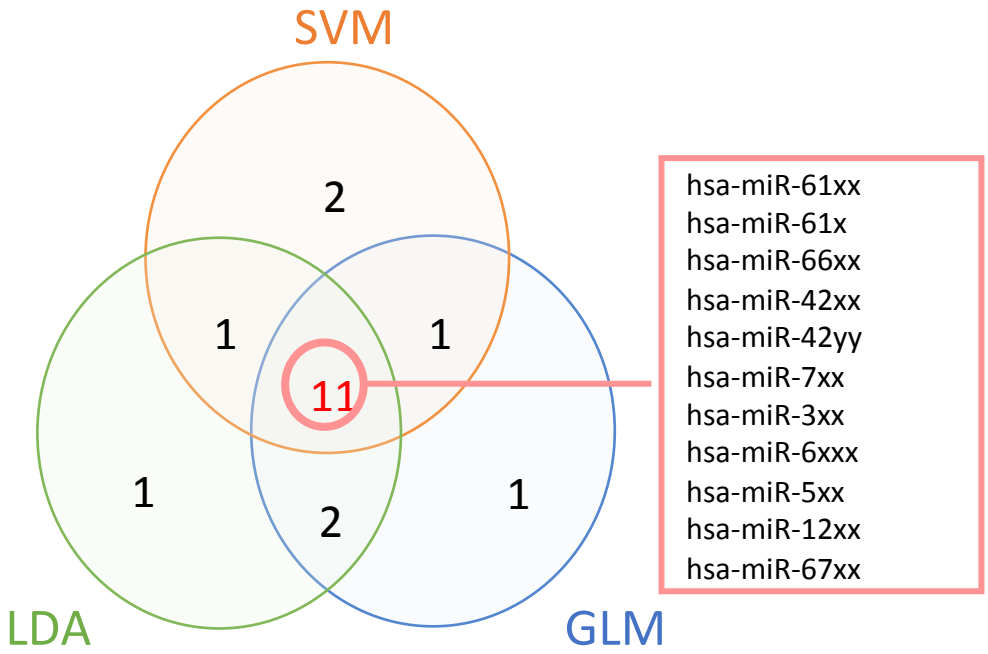


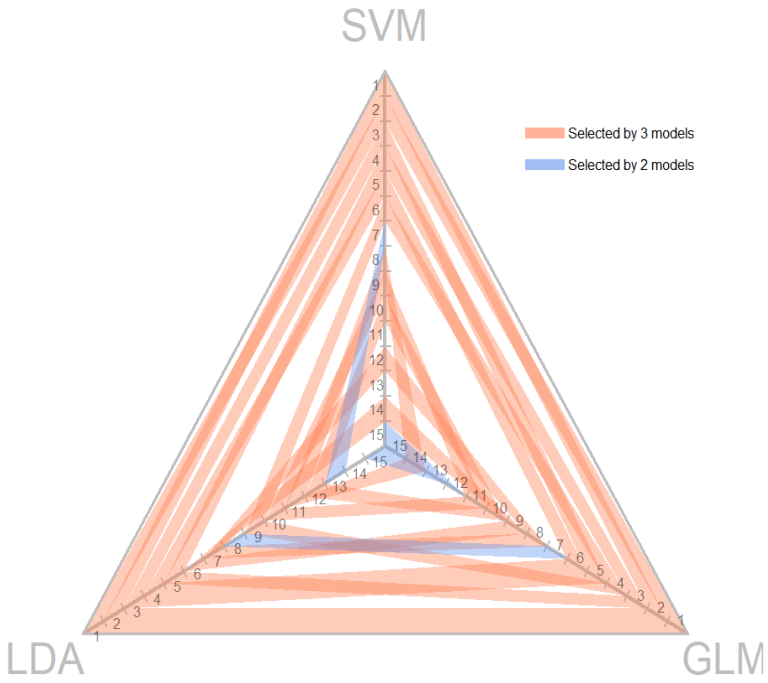
LDA



GLM

Overlap and Consistency of Each Modeling Method

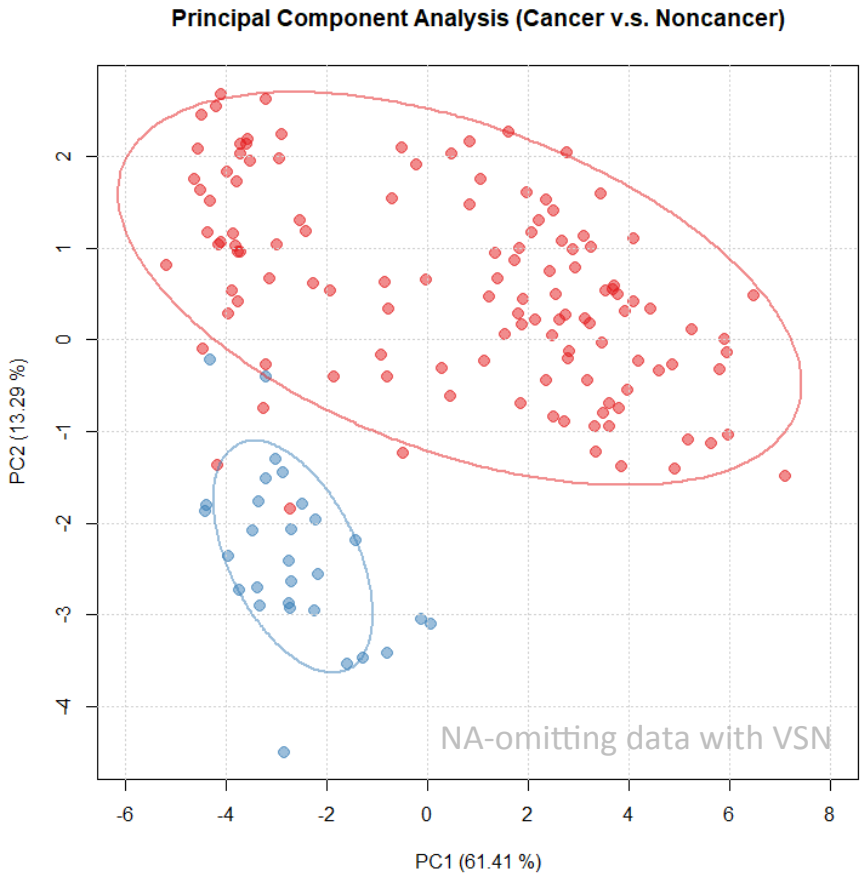


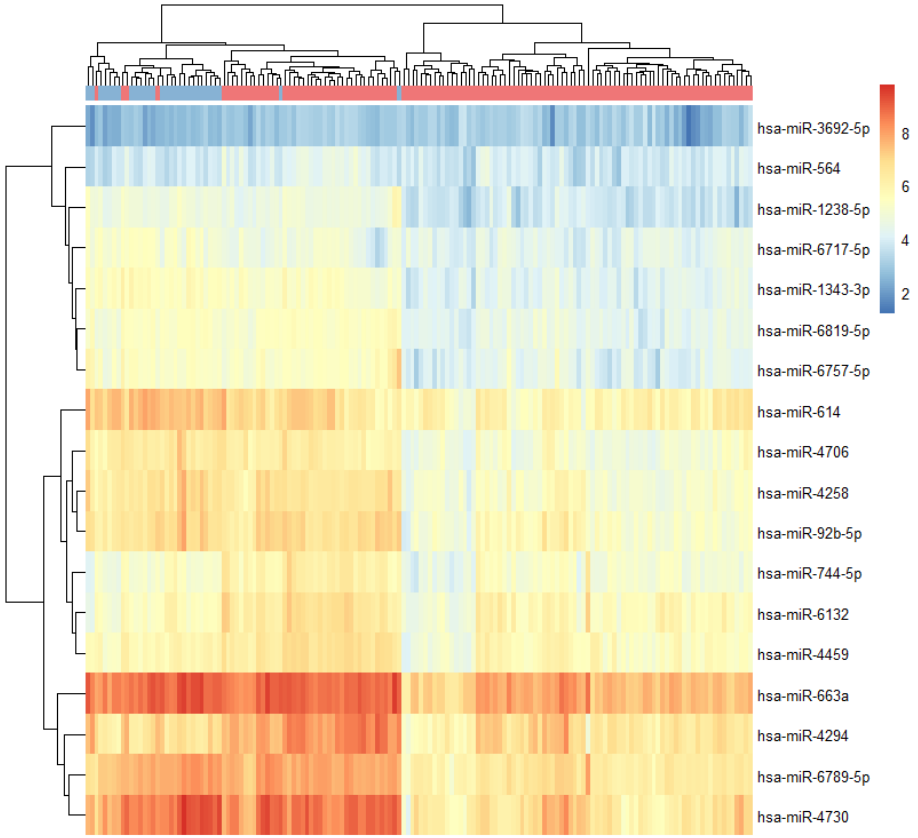


Selected miRNAs with Prediction Ability

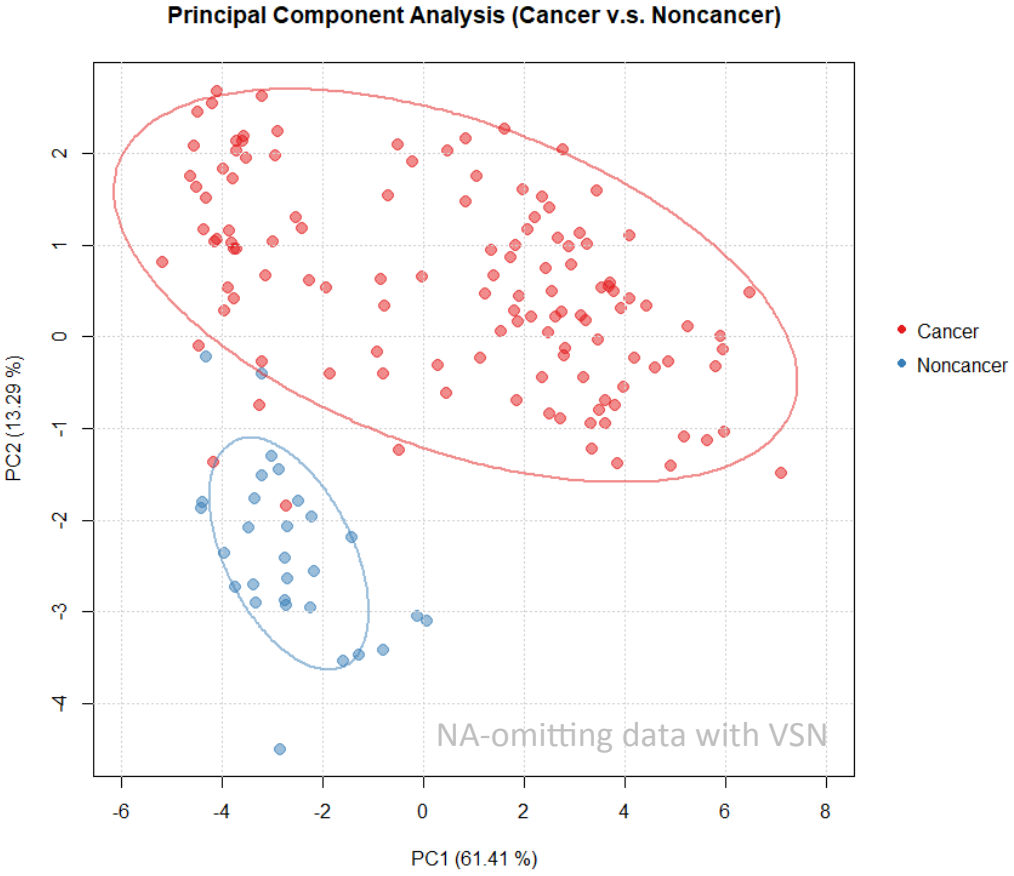
⇒18 miRNAs (overlap from 4 methods)

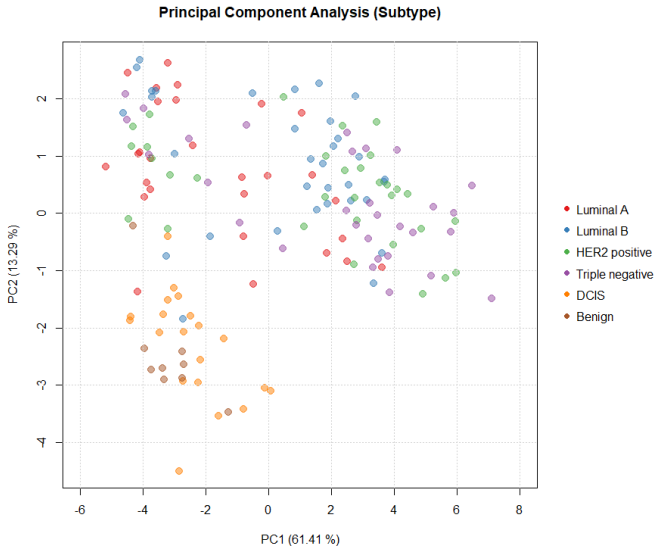
PCA and heatmap with Selected 18 miRNAs

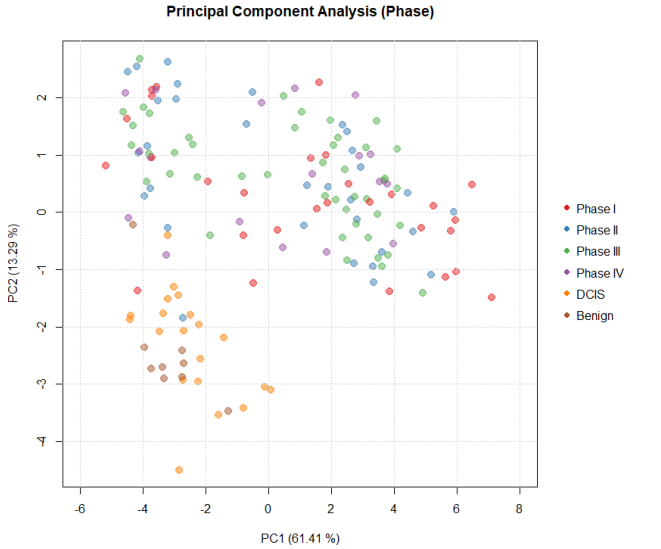




PCA plots show selected 18 miRNAs fit-in early detection







Further modeling

After previous prediction modeling, we used the union of selection from each pipeline to build more prediction models. Fisher's linear discriminant analysis (LDA) was performed with each of these miRNA marker or a combination of at most six miRNA markers.

To evaluation the prediction performance, 10-fold cross validation were applied to each model. We separated the data into 10 groups, built the model with 9 groups and used the residual group as testing cohort to calculate the prediction accuracy, sensitivity and specificity. After repeating the estimation process with different testing group 10 times, the average values of each test result were calculated for model evaluation.

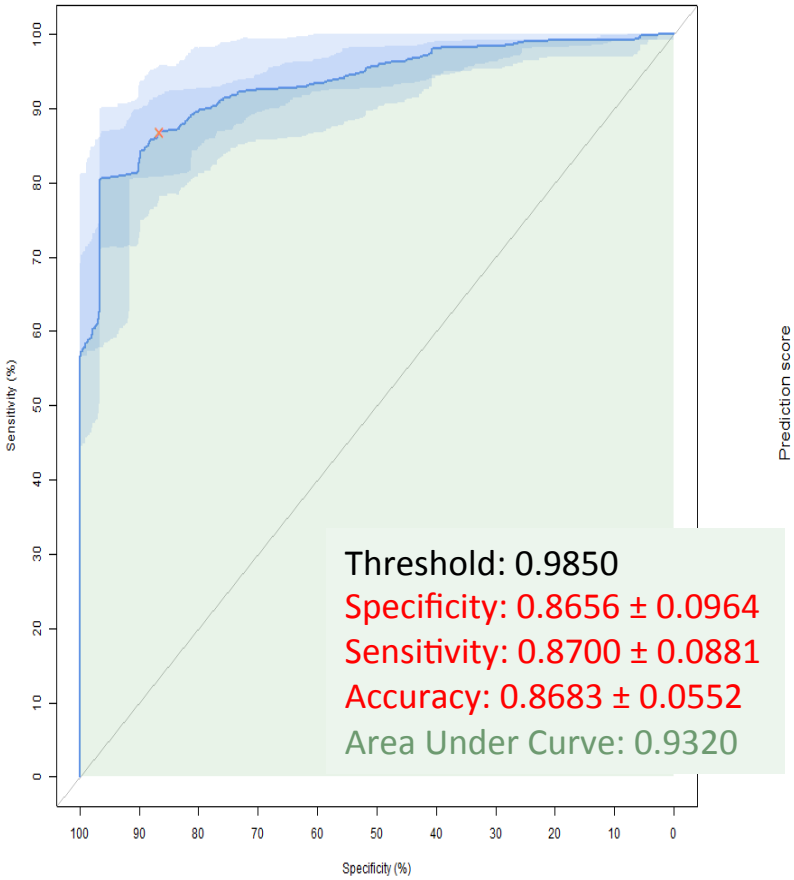
The resulting values of the discriminant functions were used to prepare the diagnostic index.

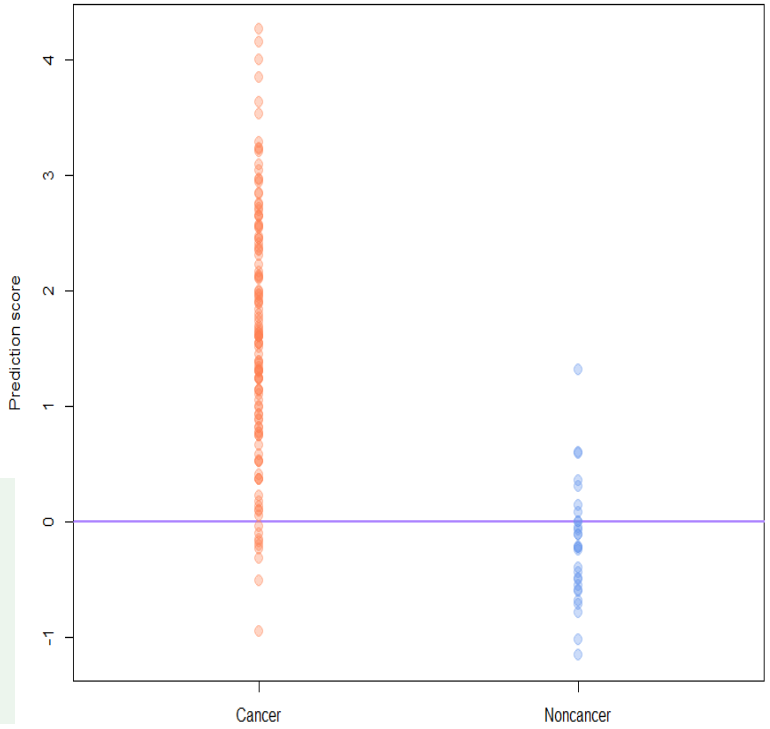
Index score ≥ 0: breast cancer

Index score < 0: non-breast cancer or other clinical conditions

Further Modeling (with 1 miRNA)

Modeling with 1 miRNA (hsa-miR-614)

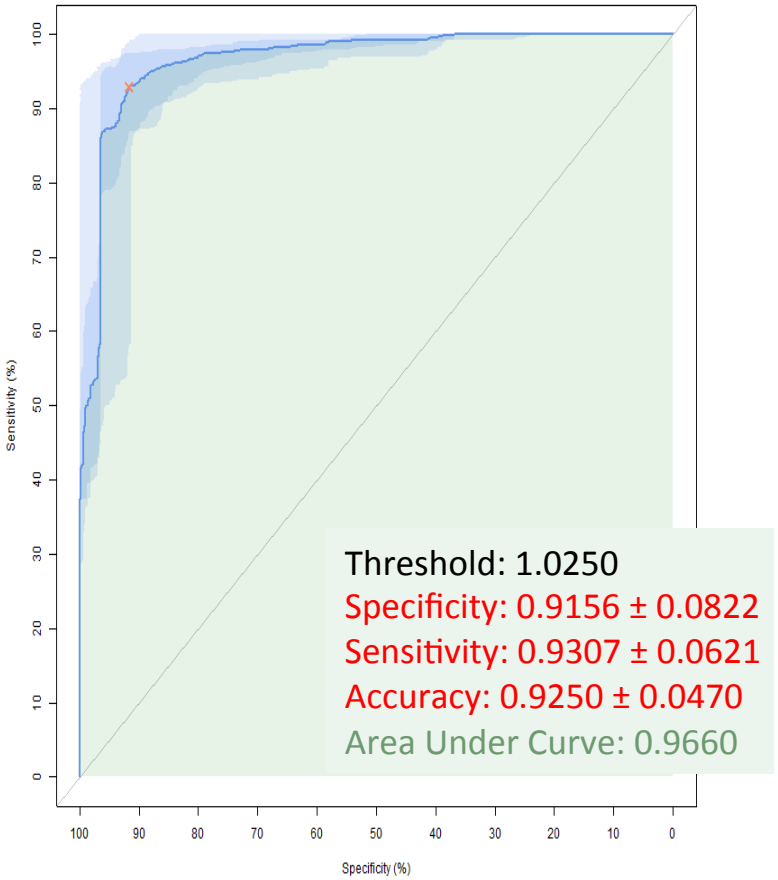


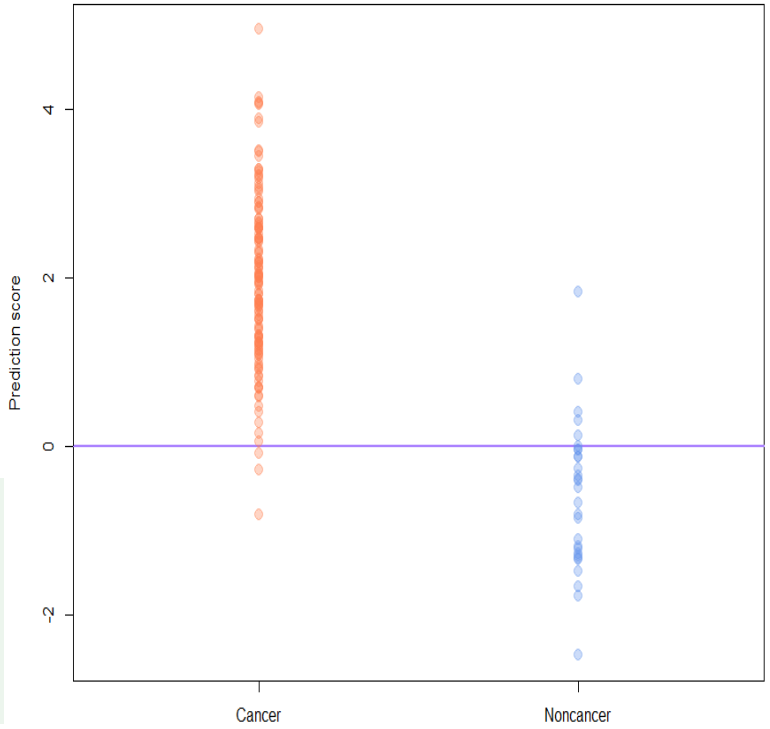


Prediction score = 11.3262 - 1.5682 x hsa-miR-614

Further Modeling (with 3 miRNAs)

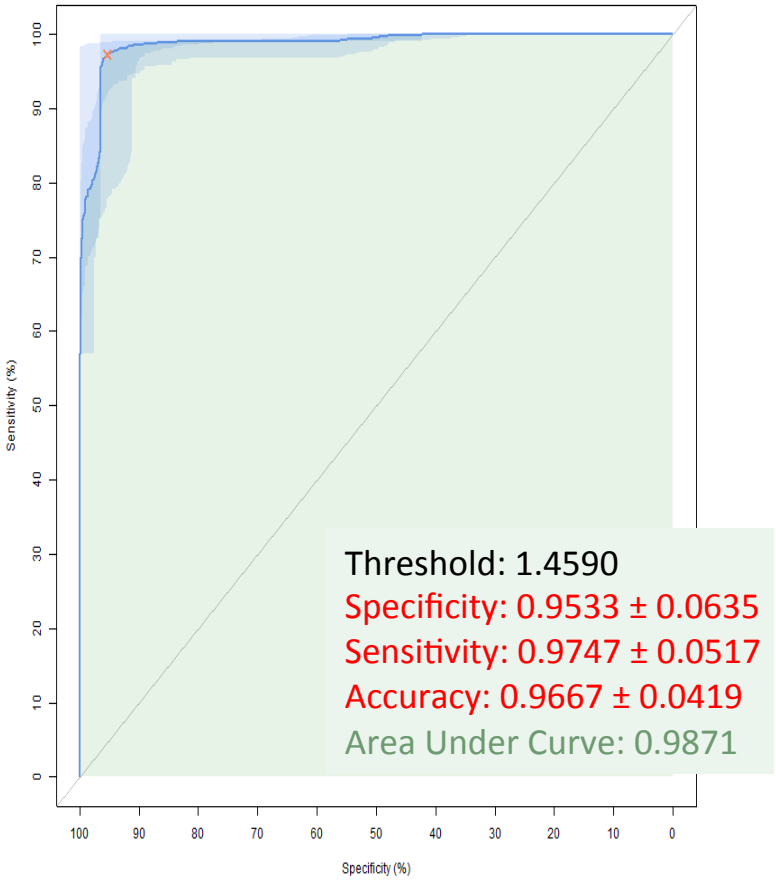
Prediction score = 5.4049 - 1.7271 x hsa-miR-614 + 0.0937 x hsa-miR-42XX + 1.1171 x hsa-miR-61XX

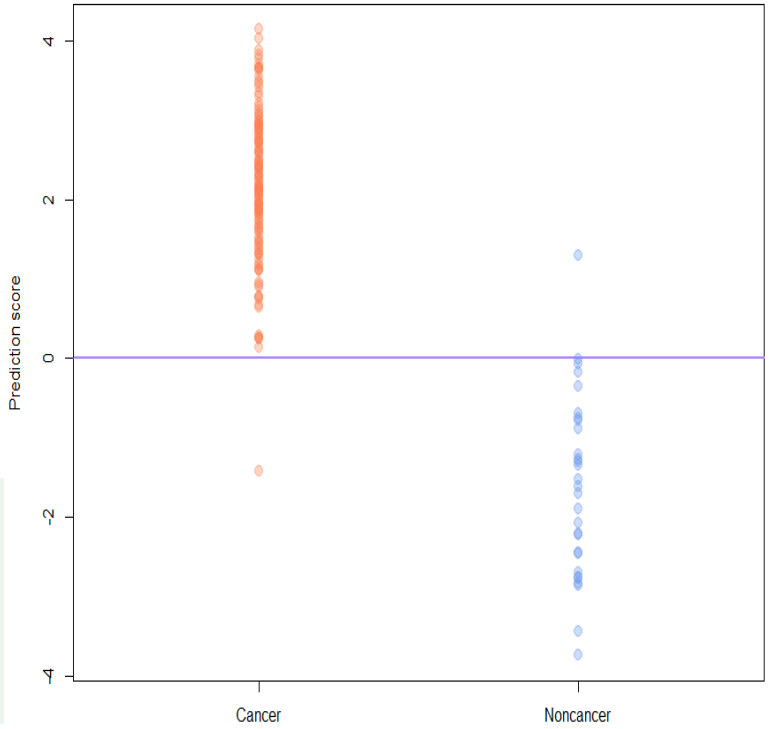




Further Modeling (with 4 miRNAs)

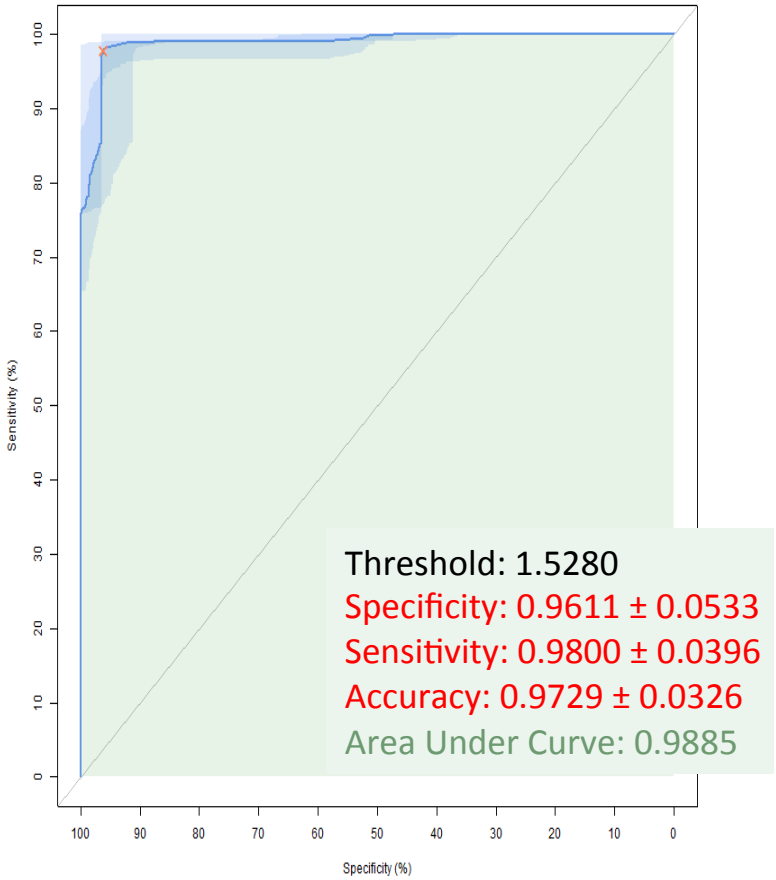
Prediction score = 9.225 - 0.9554 x hsa-miR-614 + 0.8076 x hsa-miR-42xx + 1.4167 x hsa-miR-61xx - 1.9153 x hsa-miR-66XX

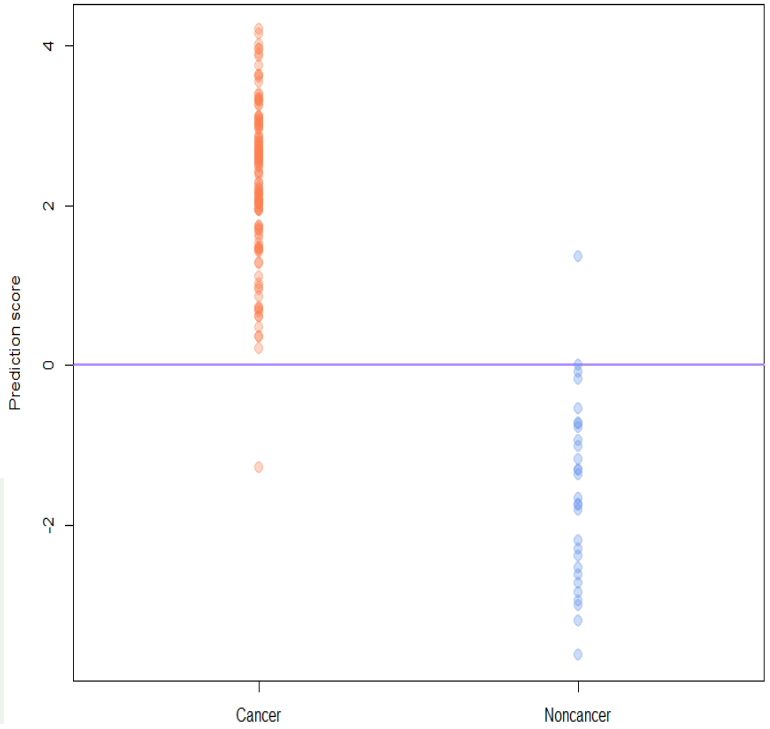




Further Modeling (with 5 miRNAs)

Prediction score = 8.763 - 0.665 x miR-614 + 0.865 x miR-42xx + 1.413 x miR-61xx - 1.697 x miR-66xx - 0.716 x miR-1xxx





Validation

- Patient Serum Collection: Healthy, Benign, pre-Cancer & Cancer
- Workflow:
    1. Isolate RNA
    2. Optimize primer
    3. Reverse transcription
    4. Amplify cDNA
    5. Run qPCR / nanostring/(liquid) chip
    6. Analyze

Conclusions

1. While applying different analysis pipeline would get quiet different outcomes, there are some overlaps, which show the consistency of these methods.

2. Several serum miRNAs are enough to identify the group (cancer or noncancer) of a patient at a high accuracy level. Thus, these selected miRNAs could be viewed as potential biomarkers for implementing early detection of breast cancer.

Perspectives

1. The models seem to be precise enough to fit early detection, more validations are still required to establish robust criteria.
2. The established useful analysis pipeline enables applying for other different expression data derived from other diseases.
3. The mechanisms of these selected miRNAs related are unknown. It is much more meaningful and critical for the understanding of these identified biomarkers. By comprehending the molecular mechanisms underlying these biomarkers, the developing effective treatments and translational research would be promoted.

Acknowledgements

Lab members

Bao-Hong Lee, PhD

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Ru-Ying Fang (Master)

Yi-Ting Chen (Master)

Dan-Jung Chuang (Master)

Shi-Chen Wang (Master)

Mitch Lin (Master)

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David C. Lyden, MD, PhD

Ayuko Hoshino, PhD

Héctor Peinado, PhD (CNIO, Spain)

Japan

Takahiro Ochiya, PhD (NCC)

Hidetoshi Tahara, PhD (U Hiroshima)

Koji Ueda, PhD (Cancer Found.)

Others

Arthur Lander, MD,PhD (UC Irvine)

Kuo-Kan Liang (AS)

Ali Mortazabi, PhD (UC Irvine)

Zhongmin Zhao, PhD (UT Health)