Circulating microRNAs in liquid biopsy for the molecular stratification of HER2+ breast cancer subtype

Vinitha Richard, PhD, Kelsey Bruce, MSc, Michael Kerin, DSc, FRCSI

Discipline of Surgery, Lambe Institute for Translational Research, University of Galway, Ireland

Ollscoil na Gaillimhe University of Galway

National Breast Cancer Research Institute

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Introduction

- 2.3 million new **breast cancer** cases in 2022 [1]
- HER2 protein and hormone receptor expression determine the molecular subtypes of Breast cancer.
- **HER2 receptor discordance** is associated with high visceral metastases but longer DFS [2]

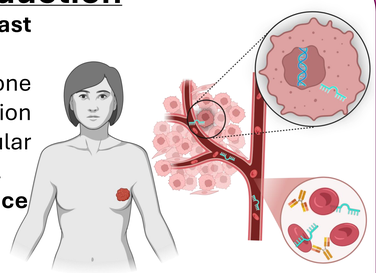


Fig 1: Circulating biomarkers for the detection of Breast cancer.

- **MicroRNAs** (miRs): short sequences of nucleic acids
  - Master regulators of gene expression
  - Reflective of disease state/subtype → "biomarker"
  - Detectable in circulation

Aim: This study seeks to identify microRNA signatures from patient-derived blood samples (plasma) for the detection and precise stratification of HER2+ breast cancer subtype.

Methods

Phase I: *In Silico* Analysis

Breast tumour-specific miRNAs identified from the SCAN-B RNA-Seq dataset [3].

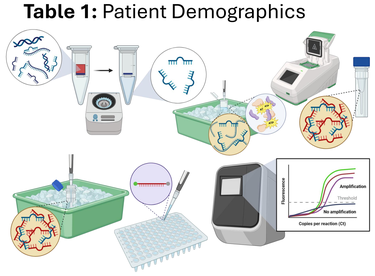
Phase II: Target Validation

- Ethics approval CREC (Galway C.A. 2073).
- MiRNA extraction via column-based kit.
- 10 ng RNA reverse transcribed to cDNA

Parameter, variable	Total	Percent/Range
Total, N	35	
Patients	30	
Age, y, median (IQR)	53	43-69
Tumor size, mm, median (IQR)	24	16.5-32.75
Nodal involvement, n (%)		
Negative	10	33.3%
Positive	20	66.7%
Tumor grade, n (%)		
Grade 1	2	6.7%
Grade 2	9	30.0%
Grade 3	18	60.0%

Molecular subtype, n	
LABC	9
LBBC	8
Basal	6
HER2+	7
IDC	
Grade 1	0
Grade 2	6
Grade 3	8
IDC noted, Unspecified	2
DCIS	
Yes	7
No	23

Table 1: Patient Demographics



- RT-PCR carried out using TaqMan Probes.
- Relative expression using $2^{-\Delta\Delta Ct}$ method [4].
- XLSTAT2023.3.1 [1416]) for data analysis.

Fig 2: Work pipeline for microRNA isolation and analysis.

Results and Discussion

Phase 1: Screening and Identification of Targets

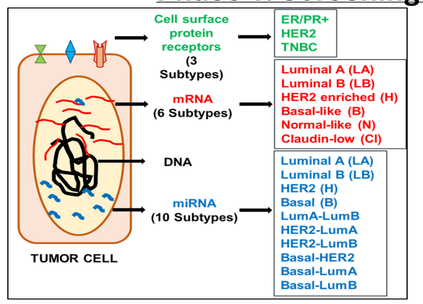


Fig 3: Molecular Classification of Breast Cancer, Richard et al, 2021 [5].

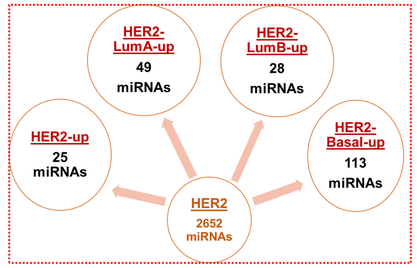


Fig 4: Significantly Dysregulated microRNAs in the SCAN-B Dataset (n=186). 33 microRNAs were selected for analysis based on: log2(FC)>1.5, p<0.05

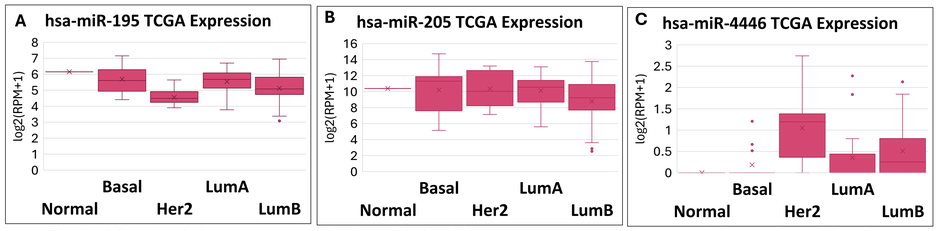
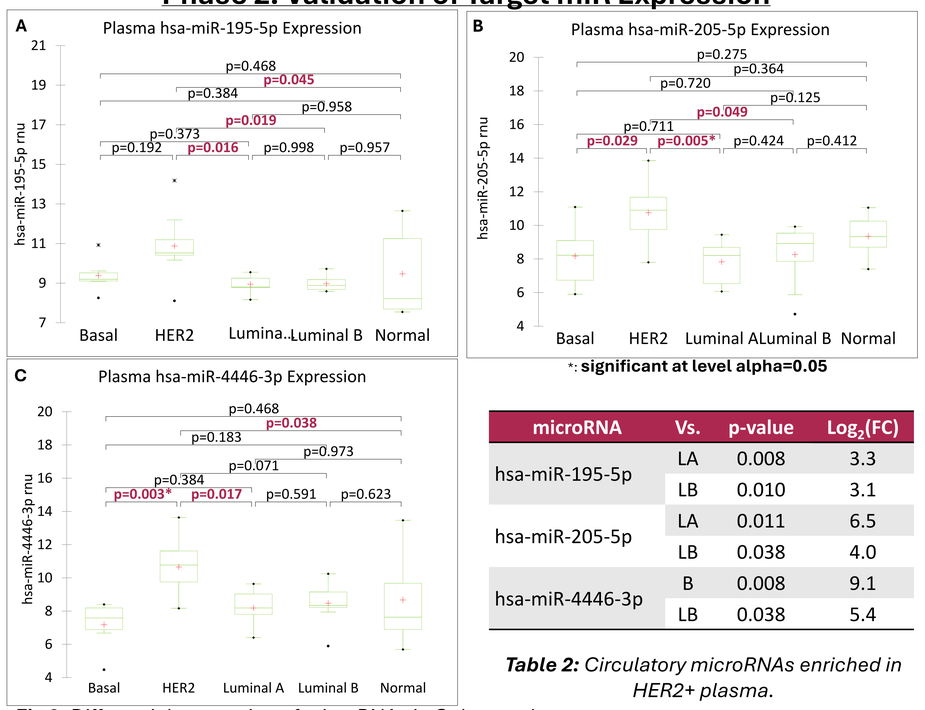


Fig 5: Differential expression of microRNAs in breast cancer and normal tissue from The Cancer Genome Atlas (TCGA) [6]. (A) hsa-miR-195-5p, (B) hsa-miR-205-5p, (C) hsa-miR-4446-3p.

Phase 2: Validation of Target miR Expression



*: **significant at level alpha=0.05**

microRNA	Vs.	p-value	Log_2(FC)
hsa-miR-195-5p	LA	0.008	3.3
	LB	0.010	3.1
hsa-miR-205-5p	LA	0.011	6.5
	LB	0.038	4.0
hsa-miR-4446-3p	B	0.008	9.1
	LB	0.038	5.4

***Table 2:** Circulatory microRNAs enriched in HER2+ plasma.*

***Fig 6:** Differential expression of microRNAs in Galway cohort*

Phase 3: Clinicopathological Correlation of 3-miR Panel

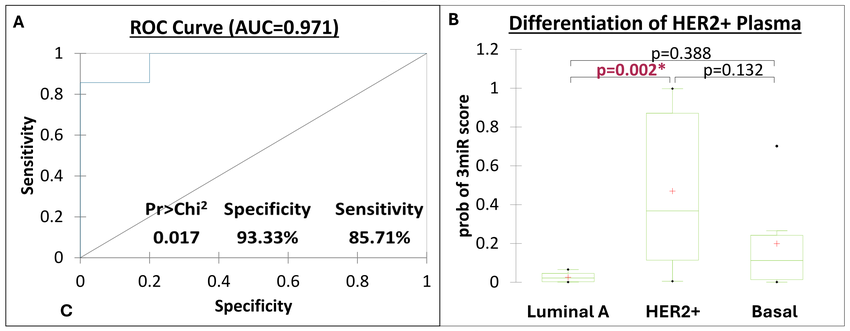


Fig 7: Binomial logistic regression model of HERCEPT Prediction. (A) ROC of Circulating microRNA Panel [AUC = 0.971]. (B) Ability of three-miR panel to differentiate between HER2+ and HER2- subtypes.

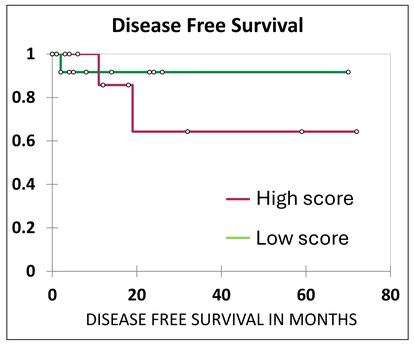


Fig 8: Kaplan Meyer Disease Free Survival Distribution of miR groups.

Mean DFS†	St Dev	Lower bound (95%)	Upper bound (95%)
43.571	12.678	18.724	68.419

***Table 3:** Median Survival Time. Predictive Potential of 3-miR Panel for DFS.* †DFS to 72 months.

While individual microRNAs are able to distinguish plasma from HER2+ patients from that of patients with other subtypes and controls, a panel comprised of the three microRNAs together has superior sensitivity and specificity.

Conclusion

MicroRNA-based liquid biopsy screening will enable stratification and potentially create a method for therapeutic screening of breast cancer subtypes, through multiple longitudinal minimally invasive samplings before the need for a core needle biopsy.

The miRNA signature outlined here provides a good diagnostic starting point for use in HER2+ cancers.

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