Evaluating the Role of Circulating MicroRNAs in Predicting Long-Term Survival Outcomes in Breast Cancer: A Prospective, Multicenter Clinical Trial

Matthew G Davey, MRCSI, PhD, Andrew McGuire, MRCSI, PhD, Maire Caitlin Casey, MRCSI, PhD, Ronan M Waldron, MB, BCh, Maxwell Paganga, MSci, Emma Holian, PhD, John Newell, PhD, Helen M Heneghan, FRCSI, PhD, Ailbhe M McDermott, PhD, FFR, Maccon M Keane, MD, Aoife J Lowery, PhD, FRCSI, Nicola Miller, PhD, Michael J, Kerin MCh, FRCS, FRCSI, FRCSEd

BACKGROUND: While long-term outcomes have improved for patients with breast cancer, 20% to 30% will still develop recurrence, and identifying these patients remains a challenge. MicroRNAs (miRNAs) are small, noncoding molecules that modulate genetic expression and affect oncogenesis.

STUDY DESIGN: This prospective, multicenter trial (ICORG10/11-NCT01722851) recruited patients undergoing neoadjuvant chemotherapy across 8 Irish centers. Predetermined miRNAs were quantified from patient whole blood using quantitative reverse transcriptase polymerase chain reaction. Venous sampling was performed at diagnosis (timepoint 1) and midway during neoadjuvant chemotherapy (timepoint 2 [T2]). miRNA expression profiles were correlated with recurrence-free survival (RFS), disease-free survival (DFS), and overall survival. Data analysis was performed using R v3.2.3.

RESULTS: A total of 124 patients were recruited with a median age of 55.0 years. The median follow-up was 103.1 months. Increased miR-145 expression at T2 was associated with improved RFS (hazard ratio 0.00; 95% confidence interval [CI] 0.00 to 0.99; p = 0.050). Using survival regression tree analysis, a relative cutoff of increased miR-145 expression greater than 0.222 was associated with improved RFS (p = 0.041). Increased miR-145 expression at T2 trended towards significance in predicting improved DFS (hazard ratio 0.00; 95% CI 0.00 to 1.42; p = 0.067). Using survival regression tree analysis, a relative cutoff of increased miR-145 expression greater than 0.222 was associated with improved DFS (p = 0.012). No miRNAs correlated with overall survival.

CONCLUSIONS: ICORG10/11 is the first Irish multicenter, translational research trial evaluating circulatory miRNAs as biomarkers predictive of long-term survival and correlated increased miR-145 expression with enhanced outcomes in early-stage breast cancer. Validation of these findings is required in the next generation of translational research trials. (J Am Coll Surg 2023;236:317–327.

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From the Discipline of Surgery, Lambe Institute for Translational Research, National University of Ireland Galway, Galway, Ireland (Davey, McGuire, Casey, Waldron, Heneghan, McDermott, Lowery, Miller, Kerin); the School of Mathematics, Statistics and Applied Mathematics, National University of Ireland Galway, Galway, Ireland (Paganga, Holian, Newell); the Department of Medical Oncology, Galway University Hospital, Galway, Ireland (Keane); and the Cancer Trials Ireland, Innovation House, Dublin, Ireland (Kerin).

Correspondence address: Matthew G Davey, MRCSI, PhD, Department of Surgery, Galway University Hospitals, Galway H91YR71, Ireland. email: m.davey7@nuigalway.ie

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Abbreviations and Acronyms

DFS	=	disease-free survival
ER	=	estrogen receptor
HR	=	hazard ratio
HER2	=	human epidermal growth factor receptor-2
miRNA	=	microRNA
NAC	=	neoadjuvant chemotherapy
OS	=	overall survival
PCR	=	polymerase chain reaction
PgR	=	progesterone receptor
RFS	=	recurrence-free survival
T1	=	timepoint 1
T2	=	timepoint 2

Breast cancer is the most common malignancy diagnosed in women, with estimations indicating that 1.67 million new female patients are diagnosed with the disease each year.[1] Significant advancements in our understanding of the biological properties of breast cancer have facilitated the pragmatic substratification of the disease into five distinct molecular subtypes, each with varying therapeutic strategies and varying prognoses.[2] While this subclassification of the disease has translated into improved oncological outcomes for the majority,[3,4] unfortunately 20% to 30% of those diagnosed with the disease will develop disease recurrence.[5-7] Establishing disease control in the setting of breast cancer recurrence proves extremely challenging to the oncologist, leading to modest anticipated survival outcomes for the majority of patients.[8] Thus, translational research efforts have focused on discovering novel prognostic and predictive biomarkers that may identify patient subgroups who are at an increased risk of breast cancer relapse.

MicroRNAs (miRNAs) are a contemporary class of small (19 to 25 nucleotides in length), noncoding endogenous ribonucleic acids that are known to play key modulatory roles in many cellular processes, including genetic expression.[9-11] It is now established that miRNAs are responsible for regulating up to 30% of the human genome[12] and aberrant expression of miRNAs implicated in several oncogenic processes, including cancer development, progression, and metastases.[13-15] Additionally, miRNAs maintain their stability in an array of biological tissues (including tumor tissue, "normal" epithelium, and human circulation in the form of "liquid biopsies"). Moreover, miRNA expression profiles may be quantified relatively simply and inexpensively using real-time quantitative reverse transcriptase polymerase chain reaction (PCR).[16-18] Such properties are important in supporting their clinical suitability for use as prognostic biomarkers in breast cancer management.

The Cancer Trials Ireland – Irish Clinical Oncology Research Group 10/11 (CTRIAL ICORG10/11-NCT01722851) is a prospective, multicenter trial that recruited 124 patients who were treated with standard-of-care neoadjuvant chemotherapy (NAC) for localized breast cancer in 8 independent treatment sites across the Republic of Ireland. A predetermined miRNA panel (consisting of Let-7a, miR-21, miR-145, miR-155, and miR-195) was quantified from patient whole blood using quantitative reverse transcriptase PCR across predetermined timepoints during the patient's treatment with neoadjuvant systemic therapies and during their postoperative follow-up. Previous work from our group has determined that miRNA profiling may successfully decipher patient response to NAC[16,19,20] and predict oncological outcomes in malignancy.[21] Notwithstanding, using miRNAs to predict long-term outcomes in those previously treated for breast cancer has not been established in our center, particularly in the clinical trial setting. Therefore, the primary endpoint of this clinical and translational research trial was to determine whether circulating miRNAs were capable of successfully predicting patients who were likely to develop breast cancer recurrence and mortality. miRNA expression profiles were measured from liquid biopsies taken at diagnosis (timepoint 1 [T1]) and at the halfway point during NAC (timepoint 2 [T2]) and were then correlated with recurrence-free (RFS), disease-free (DFS), and overall survival (OS) outcomes.

METHODS

Study design

The CTRIAL ICORG10/11 is a prospective, multicenter trial that recruited patients from 8 treatment sites in the Republic of Ireland (NCT01722851). Ethical approval was granted from the Galway University Hospitals (C.A.151, February 2008) and the National University of Ireland, Galway Clinical Research Institutional boards (C.A.1012, January 2014). Additionally, local hospital ethical approval was also obtained from the other participating centers. Thereafter, 124 patients who were diagnosed with breast cancer and were indicated to undergo standard-of-care NAC provided informed consent for and were recruited to the CTRIAL ICORG10/11 study. Decisions regarding the chemotherapy regimens prescribed were decided based on the professional judgement of the multidisciplinary team in each local tertiary referral center for breast cancer treatment. Consequently, a variety of treatment regimens were used.

miRNA expression profiles were measured from liquid biopsies taken at diagnosis (T1) and halfway during NAC (T2; Fig. 1). Thereafter, miRNA expression levels at each

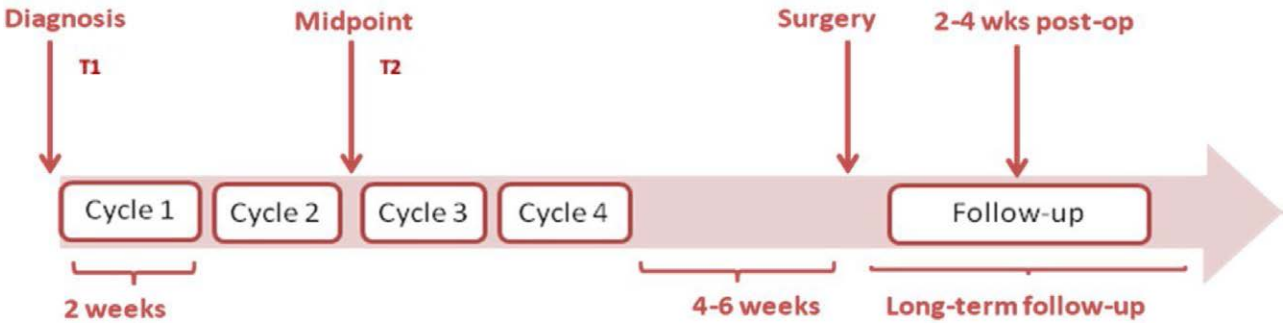

Figure 1. Schema of timepoints at which venous sampling occurred during this study. In this example, timepoint 1 (T1) involved venous sampling at breast cancer diagnosis (and prior to treatment with standard of care neoadjuvant chemotherapy), and timepoint 2 (T2) is at the halfway point (after cycle 2 of a total of 4 cycles) during neoadjuvant chemotherapy.

timepoint were evaluated to establish their roles in predicting RFS, DFS, and OS outcomes.

Inclusion and exclusion criteria

Consecutive female patients aged 18 years or older diagnosed with and treated for local breast carcinoma who were indicated to undergo standard-of-care NAC were considered for inclusion in ICORG 10/11. The breast cancer multidisciplinary team discussed each patient at length at before they were indicated to undergo NAC. Thereafter, patients were considered eligible for inclusion in this study. Patients were considered for inclusion in this study if they were: (1) female patients diagnosed with localized breast carcinoma; (2) aged 18 years or older at the time of diagnosis; (3) indicated to undergo standard-of-care NAC in accordance with best practice guidelines and recommendations; and (4) were capable of providing informed written consent. Patients were excluded from this study if they: (1) failed to meet the above inclusion criteria; (2) were diagnosed with advanced (ie, stage IV breast cancer) at diagnosis; (3) were involved in another clinical trial; or (4) were unwilling to be recruited to ICORG 10/11.

Histopathology and molecular subtyping

Breast cancer molecular subtypes were classified in accordance with the 11th St. Gallen Expert Consensus panel,[22] based on the previous seminal work of Perou and coworkers.[23] Tumor specimens were analyzed in accordance with the 2010 American Society of Clinical Oncology/College of American Pathologists histopathological consensus guidelines for estrogen receptor (ER) and progesterone receptor (PgR) status using immunohistochemistry, which were then reported in accordance with the Allred scoring system. As per American Society of Clinical Oncology guidelines (Allred score >2, or more than 1% stain positive), the ER status and PR status were determined independently by clinical pathologists, as per standard clinical breast pathology guidelines.[24,25] Human epidermal growth factor receptor-2 (HER2) receptor status was identified by Herceptest (DAKO Agilent Pathology Solutions, Santa Clara, CA), with a score of 3+ considered to be positive. Any 2+ inconclusive results were confirmed using fluorescent in situ hybridization testing, as per American Society of Clinical Oncology guidelines, with a HER2/CEP17 > 2.0 considered amplified.[26,27] Appraisal of Ki-67 was performed using MIB1 antibody testing,[28,29] although this was not performed as routine and therefore was not used to establish breast cancer molecular subtype. In brief, luminal A disease was classified as possessing ER and PgR positivity with HER2 negativity (ER+/PgR+/HER2-), luminal B disease (LBBC-HER2+) was classified as possessing ER+ and HER2 positivity with variable PgR expression (ER+/HER2+), HER2 disease (HER2+) was classified as possessing ER and PgR negativity with HER2 positivity (ER-/PgR-/HER2+), and triple negative disease was classified as possessing ER-, PgR-, and HER-negative disease (ER-/PgR-/HER2-). Tumor staging was performed in accordance with the American Joint Committee on Cancer version 8 guidelines.[30] Treatment response to NAC measured using histopathology was performed using the Miller–Payne classification system, as outlined initially by Ogston and colleagues.[31] This involved patients substratification into those who achieved a pathological complete response (Miller–Payne grade 5) vs those who did not (Miller–Payne grades 1, 2, 3, or 4).

Venous blood sampling

Venous blood samples from the 124 included patients were collected from 8 centers across Republic of Ireland over a 3-year period (May 2011 to April 2014).

Whole blood liquid biopsies were collected at two independent timepoints. As described, these included at T1, which was at breast cancer diagnosis and prior to treatment with NAC, and at T2 at the halfway point during treatment with NAC (Fig. 1). Venous blood samples were collected in ethylenediaminetetraacetic acid tubes and stored at the Department of Surgery Cancer Biobank at the National University of Ireland, Galway, on Ireland's west coast.

miRNA expression panel

Based on their previous reported relevance to breast cancer, a panel of five miRNAs were selected for evaluation (Let-7a, miR-21, iR-145, miR-155, and miR-195).[32-34] Two additional miRNAs were selected and used as validated endogenous controls, which was based on previous work from our laboratory (miR-16 and miR-425). These endogenous controls were used to standardize miRNA expression due to their stability in the blood of breast cancer patients.[35] The relevance of the miRNA selected for inclusion in the panel for this study is outlined in Table 1.

RNA isolation and storage

Total RNA was extracted from whole blood (1 mL) using TRIzol (as per the manufacturer's instructions). RNA concentrations were determined using spectrophotometry (NanoDrop ND-1000 Technologies Inc., Wilmington, DE, USA), as previously described in the work of Heneghan and colleagues.[32,33] RNA was then transferred to storage tubes, labeled, and stored at -70°C in the Cancer Biobank at the Department of Surgery at National University of Ireland, Galway.

Analysis of miRNA expression levels

For each venous sample, miRNAs were relative quantified using PCR. TaqMan assays were used, in accordance to the manufacturer's instructions, for the values that were relative quantified using PCR of the indicated target miRNA (miRNA: TaqMan assay ID; miR-195: 000494; miR-155: 002623; miR-145: 002278; miR-21: 000397; Let-7a: 000377; miR-10b: 002218) and the endogenous control (miR-16: 000391; miR-425: 001104), as previously described (TaqMan Fast Universal Master Mix [2×], no AmpErase UNG: Applied Biosystems, Foster City, CA, USA, catalog no. 4367846).[10,35] Assays were performed using an AB7900HT fast real-time PCR system (Applied Biosystems), using standard conditions in accordance with the manufacturer's instructions. The reactions were initiated with a 10-minute incubation at 95°C followed by 40 cycles at 95°C for 15 seconds and 60°C for 60 seconds. We used miR-26b as an interassay control derived from a breast cancer cell line included on each plate. All reactions were performed in triplicate (with each individual assay performed using technical triplicates). The threshold SD for intra-assay and interassay replicates was 0.3. The percentage PCR amplification efficiencies (E) for each assay were calculated using the slope of the semilog regression plot of cycle threshold vs log input of cDNA (10-fold dilution series of 5 points), with the following equation, and a threshold of 10% above or below 100% efficiency was applied: $E = (10^{-1/\text{slope}} - 1) \times 100$. Moreover, miRNA expression levels were calibrated and normalized using endogenous controls. Thereafter, miRNA expression levels were calculated using QbasePlus software (Biogazelle, Gent, Belgium) using the geNorm method to ensure that results were calibrated and normalized before being relatively quantified compared to the endogenous controls (miR-16 and miR-425). miRNA analysis was performed blinded to clinicopathological data.

Definitions

- Recurrence-free survival (or RFS) was defined as freedom from detectable invasive disease recurrence,[36]

Table 1. List of 5 Target-miRNA and 2 Endogenous Controls and Their Rationale for Selection in Our Predetermined MicroRNA Panel

Target	Author	miRNA function
Let-7a	Heneghan et al.[32,33]	Increased expression in treatment naïve breast cancer patients vs controls and postresection
miR-21	Heneghan et al.[32,33]	Known as a well described oncogenic miRNA
miR-145	Heneghan et al.[32,33]	Increased expression levels in breast cancers relative to other malignancies and controls
miR-155	Heneghan et al.[32,33]	Differentiated expression levels in breast cancers relative to other malignancies and controls
miR-195	Heneghan et al.[32,33]	Increased expression in treatment naïve breast cancer patients vs controls and other cancers
miR-16	McDermott et al.[35]	Endogenous control in venous circulation
miR-425	McDermott et al.[35]	Endogenous control in venous circulation

miRNA, microRNA.

- Disease-free survival (or DFS) was defined as freedom from detectable invasive disease recurrence, a second primary cancer, or death,[37,38]
- Overall survival (or OS) was defined as mortality due to any cause.[37,38]

Statistical analysis

The data were analyzed using R statistical software version 3.2.3. Univariable and multivariable Cox regression analyses were performed on miRNA expression profiles in order to inform RFS, DFS, and OS outcomes, with the results expressed as hazard ratios (HRs) with corresponding 95% confidence intervals (CIs). Following this, regression trees were used to classify the clinically relevant cutoffs for each miRNA included in the multivariable regression analysis. Each regression tree analysis is illustrated using nodes that represent steps in the algorithm for establishing the relevance of certain parameters (eg miRNA expression levels) in predicting outcomes of interest (eg RFS, DFS, or OS). Median follow-up was calculated using the reverse Kaplan–Meier method.[39] The results were considered statistically significant when $p < 0.050$.

RESULTS

Clinicopathological and Surgical Data

In this trial, 124 patients were prospectively recruited. The median age at diagnosis was 55.0 years (interquartile range 48.0 to 63.0 years), and the median tumor size was 38.0 mm (interquartile range 28.0 to 54.0 mm). In total, 63.7% of patients had nodal involvement (79 of 124), and 46.0% had grade 3 disease (57 of 124). Overall, 49.2% of included patients had luminal A disease (61 of 124), 20.2% had triple negative disease (25 of 124), 17.8% had luminal B disease subtype HER2 (22 of 124), and 12.9% had HER2+ disease (16 of 124). Overall, 25.8% achieved a pathological complete response to NAC (32 of 124), and most were treated surgically with breast conservation surgery (55.8%, 69 of 124). Clinicopathological and surgical data for the entire cohort are outlined in Table 2.

Table 2. Clinicopathological and Surgical Data for All 124 Included Patients

Parameter, variable	Total
Total, N	124
Age, y, median (IQR)	55 (48–63)
Tumor size, mm, median (IQR)	38 (28–54)
Nodal involvement, n (%)	
Negative	45 (36.3)
Positive	79 (63.7)
Tumor grade, n (%)	
Grade 1	1 (0.8)
Grade 2	66 (53.2)
Grade 3	57 (46.0)
Estrogen receptor, n (%)	
Positive	81 (65.3)
Negative	43 (34.7)
Progesterone receptor, n (%)	
Positive	66 (53.2)
Negative	58 (46.8)
HER2 receptor, n (%)	
Positive	38 (30.6)
Negative	86 (69.4)
Molecular subtype, n (%)	
LABC	61 (49.2)
LBBC-HER2	22 (17.7)
HER2+	16 (12.9)
TNBC	25 (20.2)
Response to NAC, n (%)	
pCR	32 (25.8)
Residual disease	92 (74.2)
Surgery, n (%)	
BCS	69 (55.8)
Mastectomy	55 (44.2)
Axillary surgery, n (%)	
SLNB only	39 (31.4)
ALNB	85 (68.6)

ALND, axillary lymph node dissection; BCS, breast conservation surgery; HER2, human epidermal growth factor receptor-2; HER2+, human epidermal growth factor receptor-2-positive molecular; IQR, interquartile range; LABC, luminal A breast cancer; LBBC-HER2, luminal B breast cancer subtype; NAC, neoadjuvant chemotherapy; pCR, pathological complete response; SLNB, sentinel lymph node biopsy; TNBC, triple negative breast cancer.

Oncological and survival outcomes

At median follow-up of 103.1 months, 23.4% of included patients had experienced disease recurrence (29 of 124). Furthermore, 25.0% of patients experienced disease recurrence, a new primary cancer, or death (31 of 124). Overall, 17.7% of patients had died during this study (22 of 124). Of note, just 1 patient suffered cardiotoxicity to systemic chemotherapy in this trial (0.8%), leading to no formal analysis being performed to correlate miRNA expression profiles with cardiotoxicity.

MicroRNA predicting recurrence-free survival

At univariable analysis, increased miR-145 expression at T2 was associated with improved RFS in this study (HR 0.59; 95% CI 0.35 to 1.01; $p = 0.054$). With multivariable

analysis, increased miR-145 expression at T2 was associated with improved RFS (HR 0.00; 95% CI 0.00 to 0.99; p = 0.050). Using survival regression tree analysis, a relative cutoff of increased miR-145 expression greater than 0.222 was associated with improved RFS (p = 0.041; Fig. 2). miRNA expression profiles that failed to predict RFS in this analysis are illustrated in Table 3.

MicroRNA predicting disease-free and overall survival

At univariable analysis, increased miR-145 expression at T2 was associated with improved DFS (HR 0.57; 95% CI 0.34 to 0.97; p = 0.037). At multivariable analysis, increased miR-145 expression at T2 trended towards having improved DFS (HR 0.00; 95% CI 0.00 to 1.42; p = 0.067). Using survival regression classification tree analysis, a relative cutoff of increased miR-145 expression greater than 0.222 was associated with improved DFS (p = 0.012; Fig. 3). miRNA expression profiles that failed to predict DFS and OS in this analysis are illustrated in Table 4.

DISCUSSION

This study correlated circulatory miRNAs with long-term oncological and survival outcomes in a prospective, multicenter translational clinical research trial that recruited 124 patients being treated with standard-of-care neoadjuvant therapies for localized breast cancer. The most important finding in this phase of the ICORG10/11 trial is the data supporting miR-145 measurement taken from liquid biopsies as a sensitive biomarker of predicting RFS and DFS outcomes. While breast cancer translational research efforts have been heavily focused upon identifying novel biomarkers with clinical utility in predicting disease recurrence, prognoses, and response to conventional therapeutic strategies, there remains a paucity of clinical trial data available assessing the value of miRNAs to inform such outcomes. Therefore, the ICORG10/11 trial provides novel, valuable information that, if further validated, has the potential to be translated into clinical practice and indirectly affect oncological outcomes for prospective breast cancer patients.

In this study, increased miR-145 expression correlated with improved RFS and DFS outcomes at almost 9 years

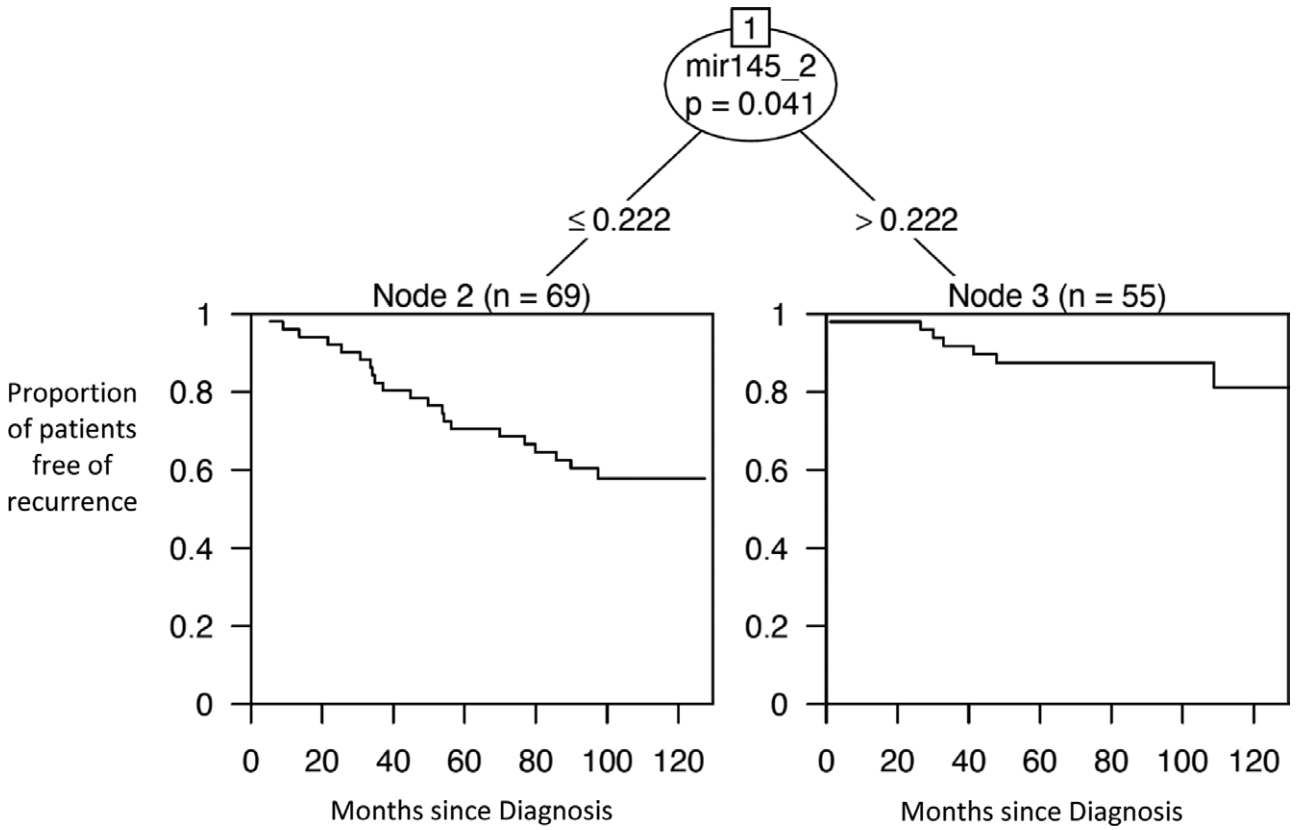

Figure 2. Survival regression classification tree analysis used to determine a relative cutoff at timepoint 2 for miR-145 measurement to predict recurrence-free survival.

Table 3. Cox Regression Analyses for MicroRNA Predicting Recurrence-Free Survival

Parameter	Univariable, HR (95% CI)	p Value	Multivariable, HR (95% CI)	p Value
Timepoint 1				
Let-7a	1.16 (0.67–2.00)	0.597	0.00 (0.00–146.90)	0.254
miR-10b	1.02 (0.33–3.16)	0.970	13.06 (0.03–5,644.35)	0.407
miR-21	1.35 (0.71–2.57)	0.358	186.07 (0.00–1,145.00)	0.146
miR-145	0.65 (0.36–1.30)	0.171	45.01 (0.09–23,175.37)	0.232
miR-155	0.97 (0.63–1.51)	0.898	0.00 (0.00–3.87)	0.103
miR-195	0.86 (0.43–1.71)	0.663	0.16 (0.00–7.26)	0.346
Timepoint 2				
Let-7a	0.87 (0.47–1.61)	0.660	2.16 (0.01–702.70)	0.794
miR-10b	0.96 (0.40–2.29)	0.918	5.19 (0.00–13,664.06)	0.682
miR-21	1.03 (0.66–1.60)	0.904	12.95 (0.08–2,228.53)	0.330
miR-145	0.59 (0.35–1.01)	0.054	0.00 (0.00–0.99)	0.050*
miR-155	0.77 (0.40–1.48)	0.429	1.10 (0.00–565.01)	0.975
miR-195	0.85 (0.55– 1.30)	0.447	0.53 (0.01–40.03)	0.772

*Statistically significant.
CI, confidence interval; HR, hazard ratio.

follow-up: Increased miR-145 independently predicted improved RFS (HR 0.00; 95% CI 0.00 to 0.99; p = 0.050) and trended towards improved DFS (HR 0.00; 95% CI 0.00 to 1.42; p = 0.067) when Cox regression analyses were performed. This illustrates the biomarker's clinical value in providing prognostication for those undergoing NAC for breast cancer, while identifying those at an increased risk of systemic metastases. Accordingly, miR-145 could potentially have utility in the selection of "high-risk" patients who may derive benefit from a tailored treatment strategy in the postoperative phase of treatment or perhaps closer breast cancer surveillance. Interestingly, increased miR-145 expression levels failed to predict OS. This illustrates the true potential of the biomarker as key modulator in the metastatic dissemination of breast cancer during disease recurrence. In tandem, these are interesting findings that may somewhat unsurprising: miR-145 is located on chromosome 5 (5q32-33) and is a known tumor suppressor miRNA, indicating overexpression of the biomarker has inhibitory effects on several cancers, including breast carcinoma.[40] Wang and colleagues[41] previously illustrated that miR-145 overexpression had an inhibitory impact upon breast cancer cell growth and development. Furthermore, Ding and colleagues[42] subsequently highlighted the role of miR-145 expression as a key modulator of proliferation and migration within breast cancer cells in their preclinical study. Given the well established repressive role of miR-145 on cancer progression in the preclinical setting, the data supporting miR-145 as a sensitive predictor of disease metastases and recurrence in the current analysis prove extremely promising to the oncologist. Therefore, the current analysis supports the potential utilization of circulatory miR-145 as a prognostic biomarker within breast cancer patient management, although further experimentation and validation is required before translation in the clinical setting.

At present, prospective, multicenter translational research trials have focused their efforts into the application of miRNA profiling to aid breast cancer diagnoses,[43] to develop novel miRNA-based therapeutics,[44] and to inform therapeutic response to neoadjuvant therapies.[19,45,46] There have been but few studies similar to ICORG 10/11 in correlating miRNA expression patterns with long-term oncological and survival outcomes: Evidence from the Women's Healthy Eating and Living (or WHEL) study illustrated the prognostic significance of miR-29c and miR-210 in differentiating 10-year OS among 1,253 patients treated for breast carcinoma.[47] Furthermore, in the translational research arm of the prospective, multicenter randomized NeoALTTO trial, Di Cosimo and colleagues[48] reported the value of circulatory miR-140-5p as a sensitive biomarker of event-free survival (HR 0.48; 95% CI 0.22 to 0.84). Interestingly, these authors reported prognostic significance surrounding the measurement of miR-140-5p taken 2 weeks into treatment with trastuzumab, similar to the delayed venous sampling that occurred during neoadjuvant treatment (at T2) in the current study. These seminal findings illustrate the importance of serial venous sampling methodology in gaining additional predictive and prognostic data, which should be noted by the principal investigators of the next generation of translational research breast cancer trials.

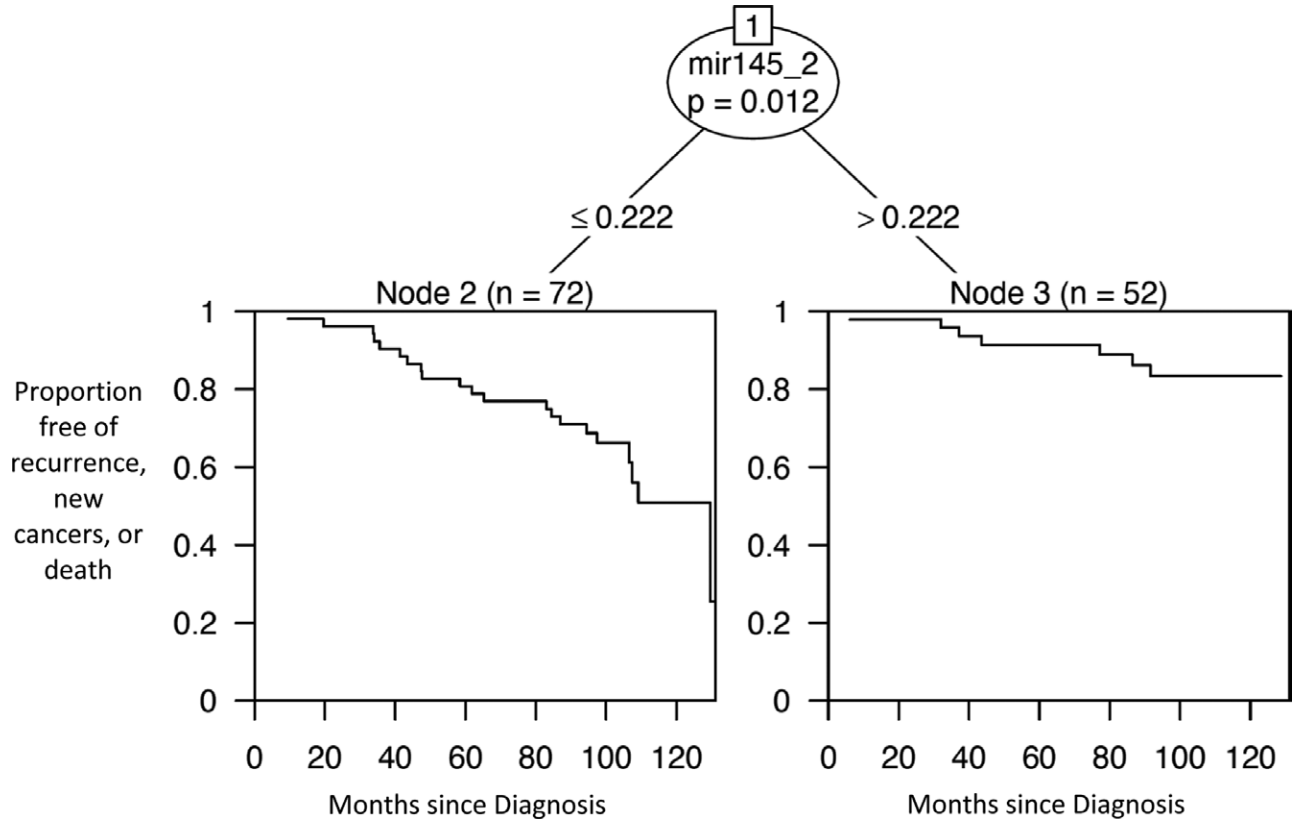

Figure 3. Survival regression classification tree analysis used to determine a relative cutoff at timepoint 2 for miR-145 measurement to predict disease-free survival.

While this study has several strengths, we acknowledge that it is subject to certain unavoidable limitations. Firstly, the miRNA panel evaluated in this study were predetermined by the principal investigators to include the targets of most relevance and interest at that time of trial design. During the time elapsed between study initiation and completion, several new miRNA targets have been discovered, which may bring into question the clinical relevance of the predetermined panel evaluated in this trial. Secondly, while miR-145 measurement at T2 successfully correlated with RFS and DFS, miR-145 expression levels in the treatment-naïve liquid biopsy at T1 failed to inform patient outcomes. Therefore, this may limit the validity and transferability of these results into settings where NAC is not been prescribed, leading to scrutiny of our results from T2. Thirdly, the patients recruited to this study were all habitants in a unique cultural region off the coast of mainland Europe. This inevitably leads to an unavoidable selection bias from a relatively limited genetic pool, which may be considered an inaccurate representation of patients in other ethnic and cultural regions, ultimately limiting the robustness of the translation of results and conclusions to a continental and global level. Importantly, there is a lack of uniformity in the NAC regimens prescribed that may further affect the translation of results into clinical practice. Furthermore, ICORG10/11 was not designed to be powered to determine the prognostic significance among breast cancer molecular subtypes. Contemporary breast cancer management relies heavily upon such substratification, thereby limiting the importance of the results of this study. Finally, the current study uses relative quantification methodology in measuring the expression profiles of the miRNA evaluated in this trial. Ultimately, until the absolute quantification of miRNAs is established, the relevance of translating the results from current study in a clinical setting may be challenged.

CONCLUSIONS

The ICORG 10/11 study is the first prospective, multicenter, neoadjuvant translational research trial conducted to evaluate the role of circulatory miRNAs in predicting oncological and survival outcomes in an Irish population. This study successfully illustrates the value of measuring circulatory miRNA expression profiles as potential biomarkers to aid patient prognostication for patients being treated with curative intent for early-stage breast cancer.

Table 4. Cox Regression Analyses for MicroRNA Predicting Disease-Free and Overall Survival

Parameter	Univariable, HR (95% CI)	p Value	Multivariable, HR (95% CI)	p Value
Disease-free survival				
Timepoint 1				
Let-7a	1.24 (0.69–2.22)	0.476	0.00 (0.00–90.26)	0.245
miR-10b	1.38 (0.42–4.56)	0.595	24.27 (0.06–10,030.34)	0.299
miR-21	1.25 (0.65–2.38)	0.502	696.07 (0.07–6,846.00)	0.087
miR-145	0.65 (0.34–1.25)	0.194	9.91 (0.06–1,535.39)	0.373
miR-155	1.24 (0.73–2.11)	0.433	0.00 (0.00–2.60)	0.091
miR-195	0.71 (0.36–1.42)	0.339	0.06 (0.00–2.90)	0.153
Timepoint 2				
Let-7a	0.91 (0.49–1.71)	0.770	0.41 (0.00–101.82)	0.752
miR-10b	0.91 (0.36–2.33)	0.850	1.38 (0.00–655.53)	0.919
miR-21	1.02 (0.65–1.58)	0.943	2.31 (0.03–198.36)	0.712
miR-145	0.57 (0.34–0.97)	0.037*	0.00 (0.00–1.42)	0.063
miR-155	0.92 (0.46–1.83)	0.813	0.18 (0.00–227.89)	0.641
miR-195	0.78 (0.52–1.17)	0.230	7.66 (0.06–939.62)	0.407
Overall survival				
Timepoint 1				
Let-7a	1.11 (0.60–2.06)	0.737	0.00 (0.00–95.77)	0.216
miR-10b	1.46 (0.37–5.76)	0.585	3.69 (0.01–1,943.42)	0.683
miR-21	1.04 (0.54–2.01)	0.911	198.07 (0.00–14,746.00)	0.125
miR-145	0.58 (0.30–1.14)	0.116	1.91 (0.00–933.94)	0.837
miR-155	1.14 (0.64–2.01)	0.659	0.01 (0.00–4,631.03)	0.456
miR-195	0.73 (0.35–1.53)	0.406	0.45 (0.01–24.38)	0.696
Timepoint 2				
Let-7a	0.90 (0.44–1.83)	0.771	0.17 (0.00–151.38)	0.607
miR-10b	0.91 (0.33–2.52)	0.862	3.09 (0.00–5,034.71)	0.765
miR-21	0.99 (0.60–1.63)	0.968	17.40 (0.02–15,242.77)	0.409
miR-145	0.60 (0.32–1.10)	0.098	0.00 (0.00–4.19)	0.111
miR-155	1.10 (0.48–2.54)	0.817	0.01 (0.00–4,361.03)	0.456
miR-195	0.77 (0.49–1.20)	0.245	0.71 (0.00–116.57)	0.895

*Statistically significant.
CI, confidence interval; HR, hazard ratio.

In this analysis, aberrant expression of miR-145 correlated with oncological outcomes, and validation of these results is required in the next generation of prospective, translational research trials before definitive conclusions may definitively be drawn regarding its utility. This study supports the ideology that translational research trials should focus on comprehensively evaluating miRNA as potential biomarkers to inform oncological and survival outcomes in the modern breast cancer management paradigm.

Author Contributions

Conceptualization: Davey, Casey, Heneghan, McDermott, Keane, Millern, Kerin
Funding acquisition: Keane
Data curation: Davey, McGuire, Casey, Kerin
Formal analysis: Davey, Waldron, Paganga, Holian, Newell, Miller
Investigation: Davey, Casey, Miller, Kerin
Methodology: McGuire, Casey, Waldron, Kerin
Project administration: Waldron, Miller
Supervision: Lowery, Miller, Kerin
Resources: Waldron, Keane
Validation: Davey, Paganga, Holian, Newell, Miller, Kerin
Visualization: Davey, McGuire, Waldron, Heneghan, McDermott, Keane, Lowery, Miller, Kerin
Writing – original draft: Davey, Casey, Paganga, Holian, Newell, Heneghan, Keane, Lowery, Miller, Kerin
Writing – review & editing: Davey, McGuire, Waldron, Holian, McDermott, Miller, Kerin

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