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Introduction

- Colorectal cancer (CRC) is common worldwide and is the second leading cause of cancer death
- The best characterized pathway leading to the development of CRC is the adenoma-carcinoma sequence:
 - Normal colon epithelium → Colorectal Advanced Adenomas (CAA) → Colorectal Adenocarcinoma
- microRNAs (miRNAs) are short, non-coding RNAs that play an important role in gene expression
- miRNAs have been associated with both the diagnosis and regulation of different disease processes
- They are closely associated with cell differentiation, proliferation, and apoptosis, all very important processes in tumorigenesis
- Current plasma-based assays used for monitoring response to therapy **lack sensitivity and specificity** for detecting recurrence of disease
- We believe miRNAs have a potential role in monitoring therapy following removal of a colorectal adenoma or cancer
- We have previously identified changes before and after excision of plasma miRNA expression in patients with CAA and CRC
- Our aim is to validate a panel of 11 significantly dysregulated miRNAs identified from screening of plasma samples from patients obtained before and after endoscopic or surgical removal of colorectal neoplasia

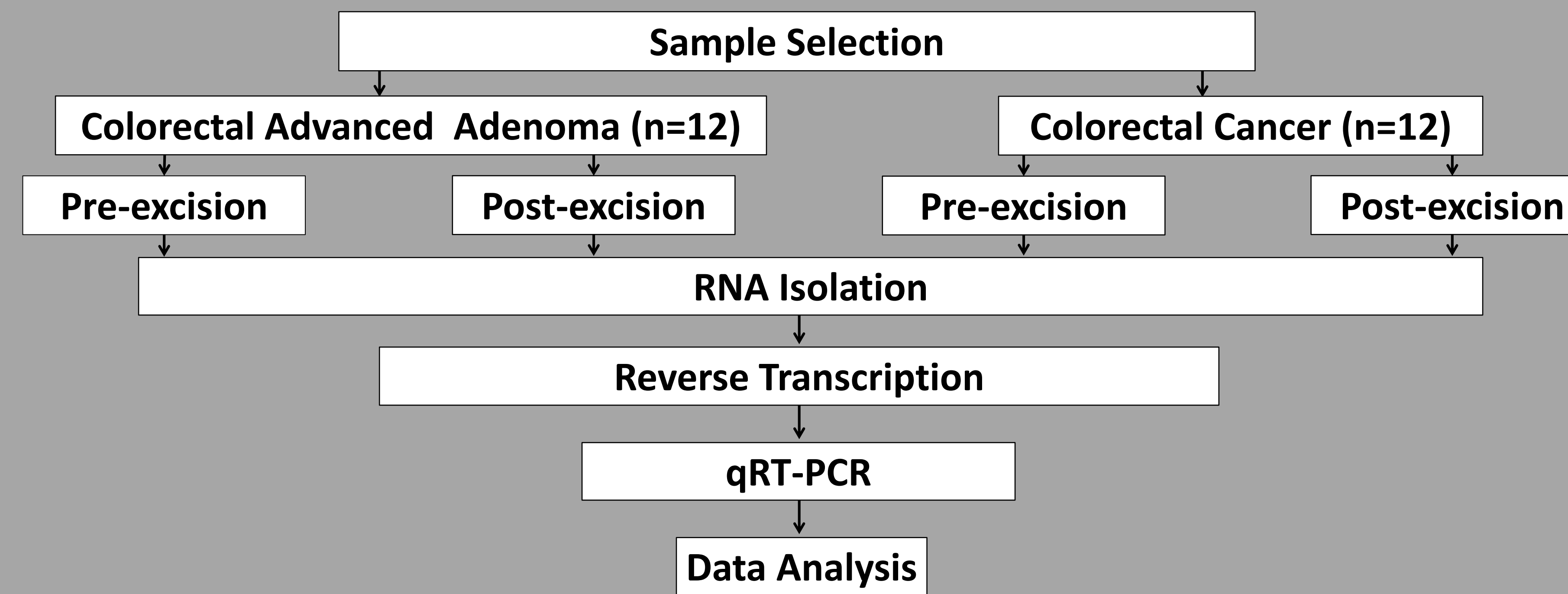
Hypothesis

- We hypothesize that miRNA expression differs between pre-treatment samples and post-removal samples of colorectal neoplasia

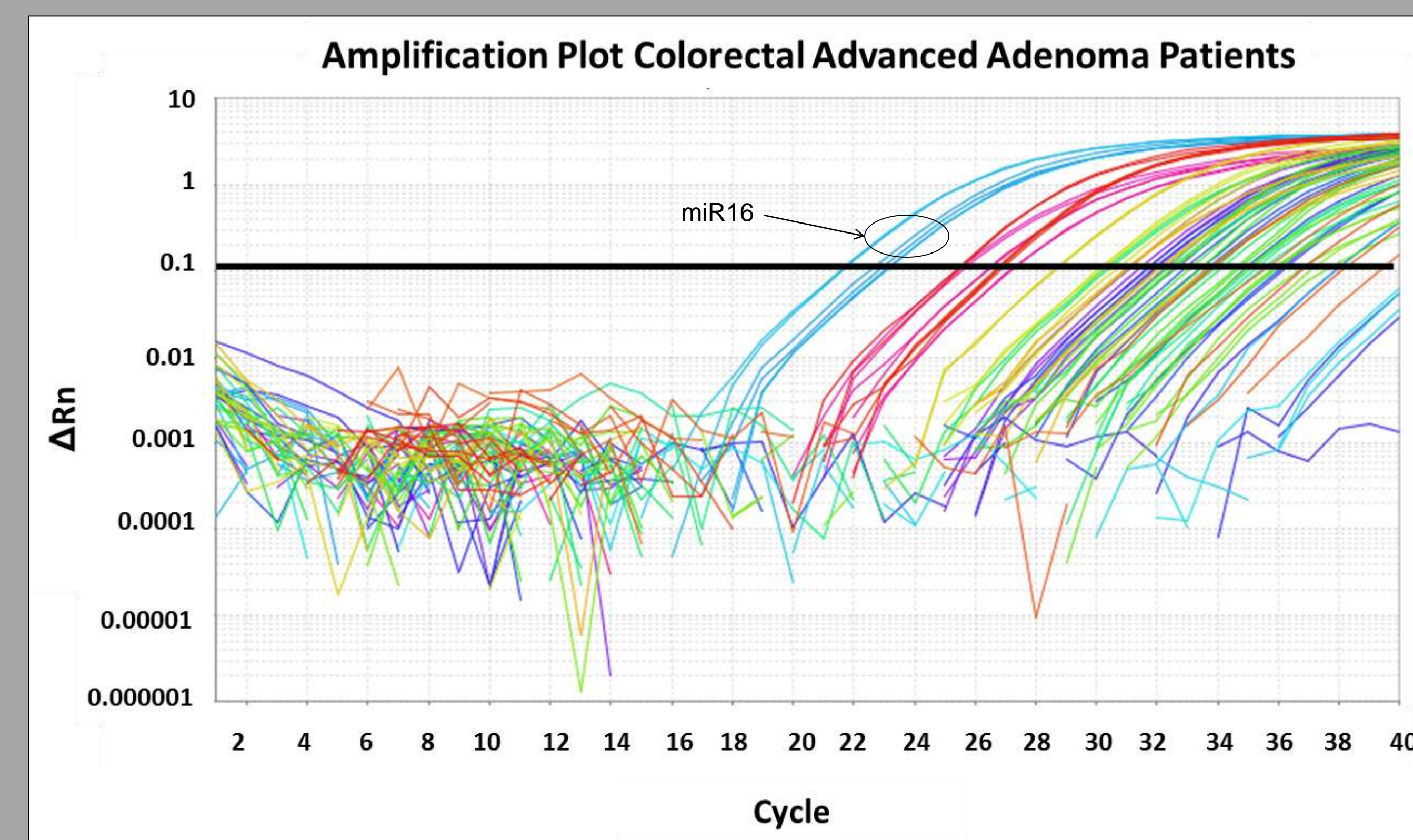
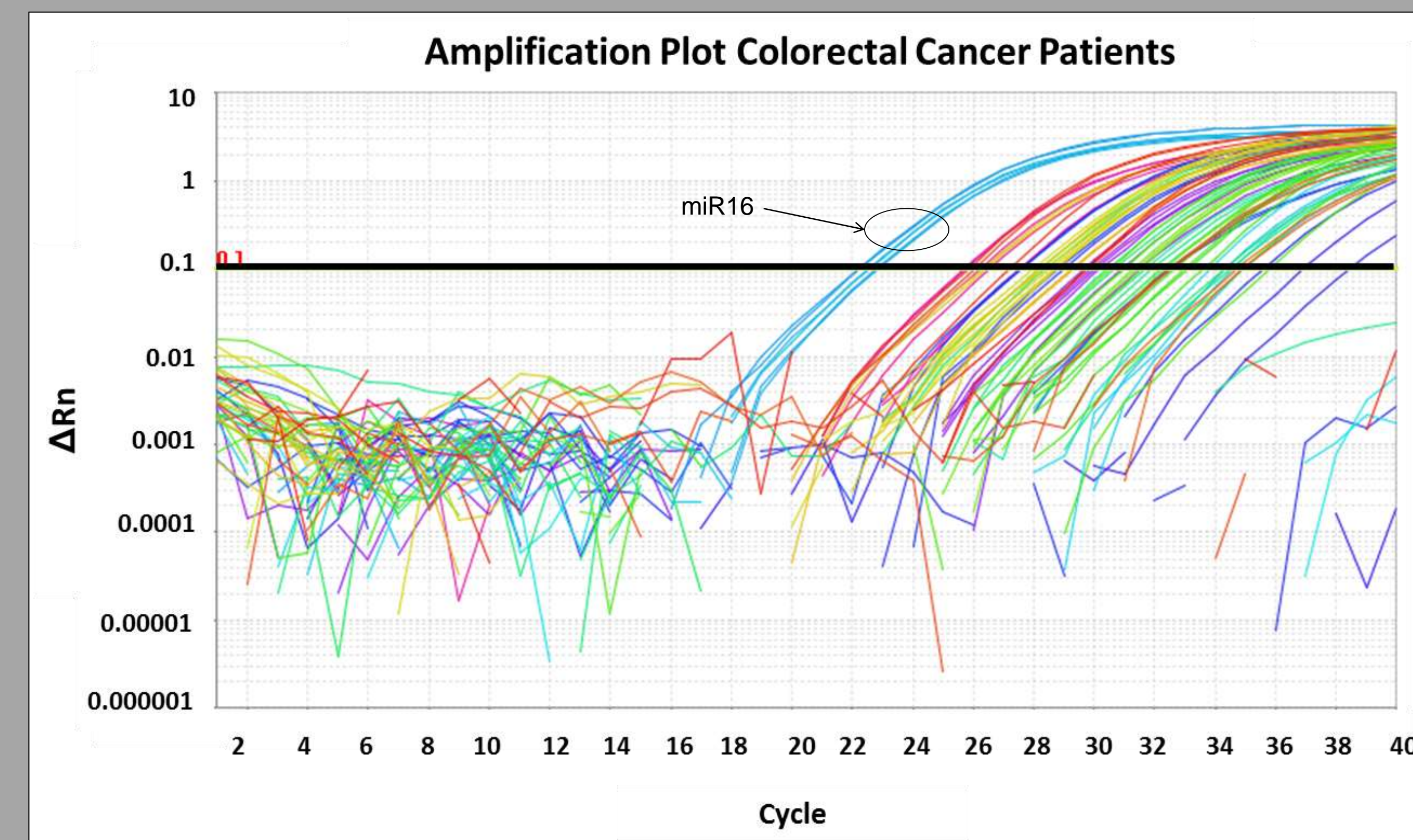
Methods

- Following informed consent, blood samples were taken from patients prior to and after removal of colorectal neoplasia
 - 12 patients with advanced CAA (>0.6 cm diameter +/- villous component)
 - 12 patients with stage II or III CRC
- Plasma was isolated from each patient sample (n=48)
- Total RNA was extracted from plasma (Qiagen® miRNeasy) and quality and quantity were assessed
- Reverse Transcription followed by quantitative real-time polymerase chain reaction (qRT-PCR) was performed using specific primers and probes to the 11 miRNAs of interest
- Data analyzed using paired t-tests after normalizing raw cycle threshold data to endogenous miR-16 and RNU6

Study Design



Results



Results (cont.)

Colorectal Cancer						
Target Name	ΔCT Pre	ΔCT Post	ΔΔCT	Fold Change	Fold Regulation	p-value
let7b	0.813	-1.051	1.864	1.039	1.039	0.061
miR19b	-2.097	-3.447	1.350	3.494	3.494	0.273
miR324-5p	4.615	2.594	2.022	0.670	-1.492	0.019
miR454	6.633	4.830	1.803	0.787	-1.270	0.043
miR29c	3.467	1.143	2.324	0.510	-1.962	0.027
miR122	-1.927	-3.905	1.979	0.845	-1.183	0.069
miR192	0.605	0.081	0.524	1.347	1.347	0.408
miR21	-0.553	-2.009	1.456	2.539	2.539	0.245
miR346	5.527	3.978	1.550	0.994	-1.006	0.058
miR372	5.210	3.372	1.838	0.858	-1.165	0.039
miR374a	3.809	1.655	2.154	0.726	-1.378	0.036
u6	Housekeeper					
miR16	Housekeeper					

Colorectal Advanced Adenoma						
Target Name	ΔCT Pre	ΔCT Post	ΔΔCT	Fold Change	Fold Regulation	p-value
let7b	2.587	1.340	1.247	0.740	-1.351	0.079
miR19b	-1.889	-2.358	0.470	0.992	-1.008	0.362
miR324-5p	7.794	7.797	-0.003	2.335	2.335	0.997
miR454	5.941	5.051	0.891	0.936	-1.068	0.113
miR29c	4.037	3.799	0.239	1.288	1.288	0.604
miR122	5.356	3.917	1.439	1.359	1.359	0.075
miR192	3.701	3.355	0.346	1.487	1.487	0.594
miR21	-2.162	-2.566	0.405	0.941	-1.063	0.240
miR346	8.429	8.980	-0.551	4.274	4.274	0.460
miR372	9.791	9.778	0.013	4.794	4.794	0.987
miR374a	5.605	4.585	1.020	0.665	-1.505	0.016
u6	Housekeeper					
miR16	Housekeeper					

Conclusions and Future Directions

- We validated expression levels of 5 miRNAs (miR-324-5p, miR-454, miR-29c, miR-372 & miR-374a) to be different in pre-treatment compared to post-removal plasma samples in patients with CRC and CAA
- miR-374a was significantly downregulated in both CRC and CAA in pre-treatment samples compared to post-treatment samples
- These findings may help provide for a relatively non-invasive method of monitoring therapy or assessing response to treatment and can be used as an innovative tool in diagnostics
- Future considerations should include more specific miRNAs that play a role in initiation, progression, and outcomes in colorectal cancer and the use of miRNAs as potential therapeutic targets

Acknowledgments

National Cancer Institute grant R25-CA134283, John Williamson and Barbara Thruston Atwood Price Trust