Serum-Based miRNAs in the

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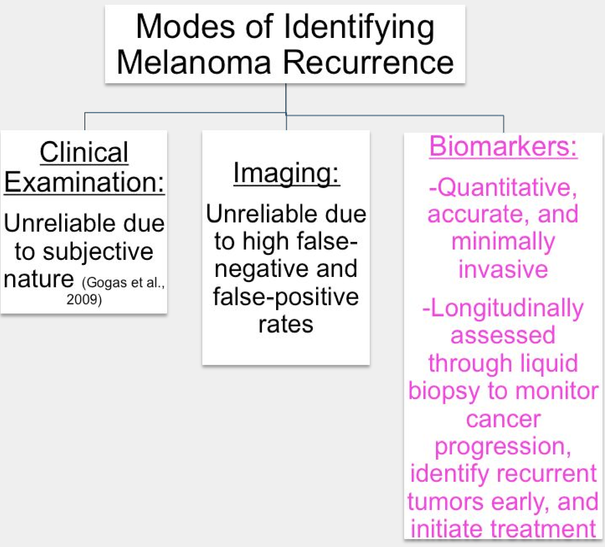
BACKGROUND

Melanoma

- On average, 1 American dies from melanoma, the most commonly diagnosed cancer, **every hour** (Facts About Skin Cancer, 2014, p. 2)

Current Prediction and Detection of Melanoma Recurrence



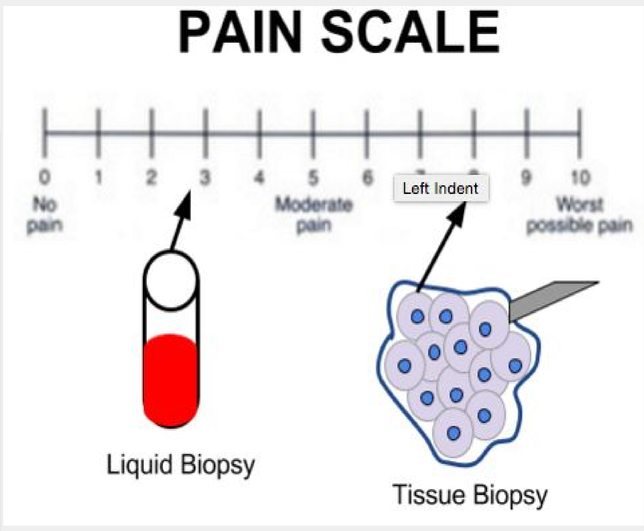


Figure 1. Advantages of Biomarkers. Biomarkers are more reliable than other modes of identifying melanoma recurrence (i.e., clinical examination and imaging) and are measured by minimally invasive liquid biopsy, causing less patient discomfort.

microRNAs as Biomarkers

- miRNAs regulate gene expression, are remarkably stable in blood, and exhibit unique expression in different diseases (Mar-Aguilar et al., 2013, p. 163)
- **miRNAs in melanoma have only been used as markers to detect recurrence, not prognostication** (Alegre et al., 2014)

PURPOSE

Determine a serum-based miRNA signature capable of both prediction and detection of melanoma recurrence by:

1. Identifying a **serum-based miRNA signature** in primary melanoma patients
2. Analyzing **prognostic value** of the miRNAs by assessing their ability to:
   a. Stratify patients by risk of recurrence
   b. Predict patient survival according to risk stratification
3. Assessing ability of the miRNAs to serve as **follow-up markers** for detecting recurrence
4. Establishing role of the serum-based miRNA signature in **melanoma biology**

METHODOLOGY

1. **Patient and Sample Collection**
   - Melanoma patients (n=283) split into Training (n=201) and Validation (n=82) cohorts

2. Identification of 4-miRNA Signature

- Logistic regression analysis and Akaike's information criterion (AIC) selected a 4-miRNA signature (miR-150, miR-15b, miR-425, miR-30d) associated with recurrence (Friedman et al., 2012)

3. Recurrence Prediction of 4-miRNA Signature

- miRNA-based predictive model stratified patients by predicting risk of recurrence
  - Sensitivity and specificity evaluated by Receiver Operating Characteristic (ROC) curve

4. Prognostic Value of 4-miRNA Signature

- Kaplan-Meier survival curves and log-rank tests compared recurrence-free and overall survival of low- and high-risk groups

5. Monitoring Melanoma Recurrence with 4-miRNA Signature

- Separate linear mixed effect models longitudinally assessed miR-15b serum levels in melanoma samples

6. Role of 4-miRNA Signature in Melanoma Biology

- Gene set enrichment analysis software (GSEA) identified functions of mRNA targets significantly associated ($p<0.01$) with miRNAs

Prediction and Detection of

RESULTS & DISCUSSION

Patient and Sample Collection

- Training (n=201) and Validation (n=82) cohorts are **well-matched** in all categories, e.g., recurrence and survival status (Table 1)

Characteristic n (%)	Training Cohort (n=201)	Independent Validation Cohort (n=82)	Total (n=283)
Gender			
Male	112 (56)	52 (63)	164 (58)
Female	89 (44)	30 (37)	119 (42)
Age, median (range)	61 (21–96)	59.5 (25–88)	
Thickness, median (range)	1.5 (0.16–30)	1.3 (0.27–28)	
Ulceration			
Present	65 (32)	24 (29)	89 (31)
Absent	136 (68)	58 (71)	194 (69)
Mitoses			
Present	141 (70)	60 (73)	201 (71)
Absent	58 (29)	22 (27)	80 (28)
Unclassified	2 (1)	0 (0)	2 (1)
Initial Stage & Recurrence Status			
I	89 (44)	41 (50)	130 (46)
Recurrent	*14 (16)*	*6 (15)*	*20 (15)*
Non-Recurrent	*75 (84)*	*35 (85)*	*110 (85)*
II	52 (26)	20 (24)	72 (25)
Recurrent	*15 (29)*	*7 (35)*	*22 (31)*
Non-Recurrent	*37 (71)*	*13 (65)*	*50 (69)*
III	60 (30)	21 (26)	81 (29)
Recurrent	*33 (55)*	*13 (62)*	*46 (57)*
Non-Recurrent	*27 (45)*	*8 (38)*	*35 (43)*
Follow-up time in months, median (range)	90.9 (17.5–128.9)	39.0 (0.8–95.0)	68.8 (0.8, 128.9)
Status as last follow-up			
Alive	157 (78)	62 (76)	219 (77)
Died, of Melanoma	39 (19)	18 (22)	57 (20)
Died, Other Causes	5 (2)	2 (2)	7 (2)

Table 1. Baseline demographic and primary tumor characteristics of the well-matched Training (n=201) and Validation (n=82) cohorts.

Recurrence Prediction of 4-miRNA Signature

- **Clinical staging with miRNA signature** achieved an area under the ROC curve of **AUC=0.760 and AUC=0.790** in the Training and Validation cohorts, respectively (Fig. 2)
- Training cohort: sensitivity=80.9%, specificity=60.1% (Fig. 3)
  - Validation cohort: sensitivity=84.6%, specificity=66.1% (Fig. 3)
- The serum-based miRNA signature **enhances** recurrence prediction done by clinical staging, with **sensitivity and specificity** (Zhang et al., 2015)

	Training Cohort			Independent Validation Cohort		
	Stage I	Stage II	Stage III	Stage I	Stage II	Stage III
Number of patients	89	52	60	40	20	22
Stage alone	0.5	0.5	0.5	0.5	0.5	0.5
Stage + miRNA classifier	0.544	0.785	0.640	0.663	0.637	0.738

Figure 2. Recurrence Prediction. Clinical stage with miRNA signature achieved AUC=0.760 and AUC=0.790 in the Training and Validation cohorts, respectively.

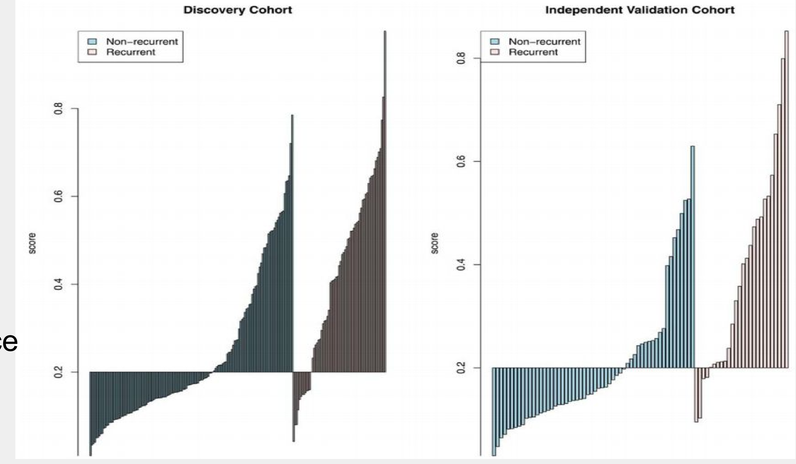


Figure 3. Sensitivity and Specificity of Recurrence Prediction. A. Training cohort: sensitivity=80.9%, specificity=60.1%. B. Validation cohort: sensitivity=84.6%, specificity=66.1%.

Prognostic Value of 4-miRNA Signature

- **Significant** distinction in recurrence free (p<0.001) and overall (p<0.001) survival between low- and high-risk groups in both cohorts (Fig. 4)
- miRNA-based risk stratification **accurately predicts melanoma patient survival** (Zhang et al., 2015)

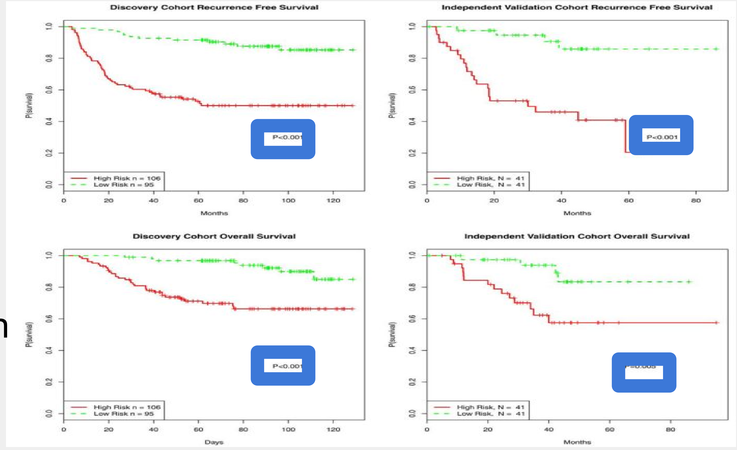


Figure 4. Prognostic Value of 4-miRNA Signature. Significant difference between low- and high-risk groups in recurrence-free (p<0.001) and overall (p<0.001) survival.

Recurrence in Melanoma Patients

Monitoring Melanoma Recurrence with 4-miRNA Signature

- **miR-15b serum levels increased in recurrent patients (p<0.001)**, no significant change in nonrecurrent patients (p=0.17; Fig. 5)
- miR-15b is an early marker of melanoma recurrence (Satzger et al., 2010)

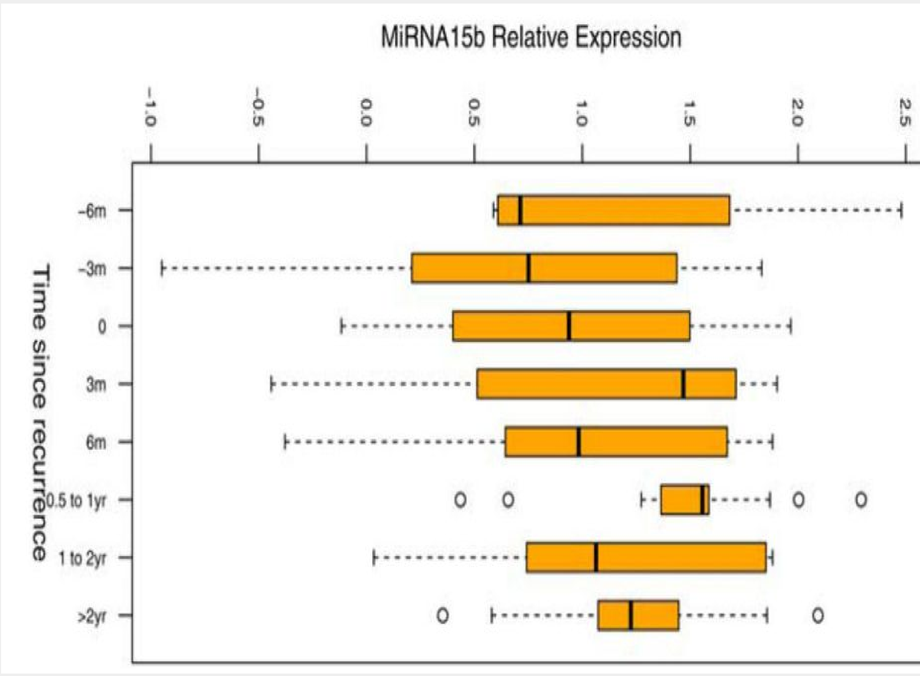
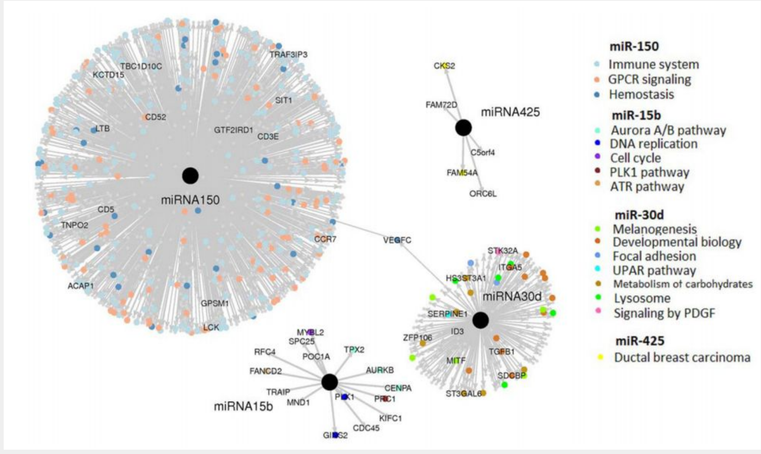


Figure 5. 4-miRNA Signature Indicates Recurrence. miR-15b serum levels increased leading up to recurrence (p<0.001).

Role of 4-miRNA Signature in Melanoma Biology

- ITGA5, SERPINE1, and ADAM19 are mRNA targets for miR-30d involved in **immune signaling, melanogenesis, and mitotic regulation** (Fig. 6)
- The signature miRNAs play key roles in processes critical to melanoma biology (Gaziel-Sovran et al., 2011)



Summary of major functional pathways associated with signature miRNAs

microRNA	Gene Set Description	# of Genes	Key Genes	P-value	FDR Q-Value
miR-150	Immune System	107	VAV1, RASGRP1/2	<0.001	<0.001
	Cytokine Signaling in Immune System	27	PTAFR	<0.001	<0.001
	Signaling by GPCR	53	CCXR3, CCL21	<0.001	<0.001
miR-30d	Melanogenesis	5	MITF, GNAI2, TYR	<0.001	0.005
	Developmental Biology	11	TGFB1, ITGA5	<0.001	<0.001
miR-15b	Cell Cycle	6	AURKB, CENPA	<0.001	<0.001
	Cell Cycle (mitotic)	6	AURKB, CENPA	<0.001	<0.001
	DNA Replication	5	AURKB, CENPA	<0.001	<0.001
miR-425	Up-regulated in ductal carcinoma tumor cells	2	CKS2, FAM54A	<0.001	0.005

Figure 6. Role of miRNA in Melanoma Biology. mRNA targets (colored dots) of the key miR-30d- ITGA5, SERPINE1, and ADAM19- are involved in immune signaling, melanogenesis, and mitotic regulation.

CONCLUSION/FUTURE INVESTIGATIONS

- The 4-miRNA signature is **useful for melanoma prognosis** via risk stratification
- miR-15b levels can be monitored to **detect melanoma recurrence** (Satzger et al., 2010)
- A blood-based miRNA assay can **inexpensively and noninvasively** measure miRNA levels to improve health care providers' follow-up practices
- Lower levels of circulating miR-30d associated with higher recurrence risk, despite prior findings showing high miR-30d in tumor tissue (Gaziel-Sovran et al., 2011)
- Circulating miRNA may not always be shed from tumor tissue
- **Further study the functional roles of miR-30d and the other signature miRNAs regarding their levels in circulating fluids**