Multi-analyte Liquid Biomarkers For Lung Cancer Detection and Risk Assessment

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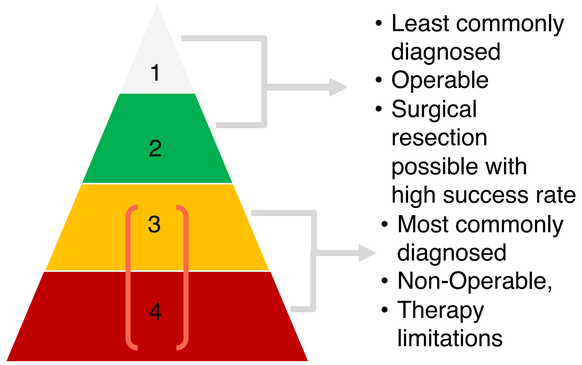
Lung Cancer by the Numbers

Lung cancer is the leading cause of cancer-associated accounting (>25% of all cancer deaths)

- **Histological subtypes include:**
  - Small Cell Lung Cancer (makes up 15% of all lung cancers)
  - Squamous Cell Lung Cancer (makes up ~30% of all Non small cell lung cancers (NSCLC)
  - Non Small Cell Lung Cancer (NSCLC)
    - **Makes up 80% to 85% of all lung cancers**
    - **Largely asymptomatic in early stages, 55% of patients advanced stage.**

- **Screening includes**
  - Low Dose Computed Tomography (LDCT) images findings classified as Lung-RADS scores (1-4; size & localization)
    - 25% rate of false positive using LDCT

- **Treatments**
  - Targeted therapy
    - Biopsies to match patient genetic driver/ drug resistance mutations (i.e. EGFR) to therapy
  - Chemotherapy
  - Immunotherapy

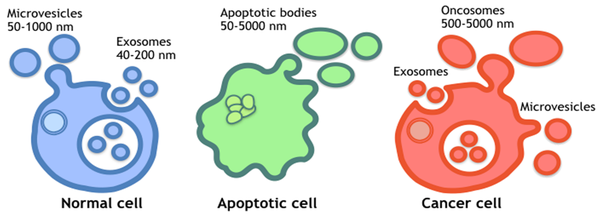


Lung RADS Score

- **Approach**
  - We are using a multi-analyte approach to identify biomarkers that can be used in liquid biopsies to decrease false positives and improve prognostics of how a patient will react to a treatment.

Most patients are diagnosed in the later stages, 3 and 4, where the outcome is poor, A logical approach to tackling this disease is to diagnose at earlier stages for a better outcome. However, the current tools, specifically the Lung RADs is not adequate. The rate of false positives is high at 25% especially in early stages; therefore, an alternative is needed.

Extracellular Vesicles (EVs)



Kosaka, N. *et al. . Exp Mol Med* (2019).

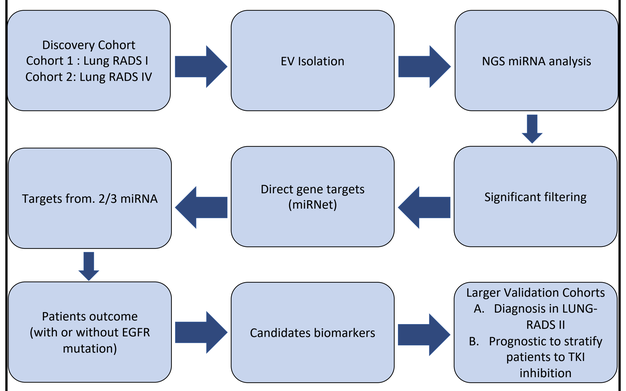
- All cells produce Extracellular Vesicles (EVs) as shown in this figure. Cancer cells also produce these vesicles, but have different cargo compared to that of a normal cell. This cargo includes Proteins; mRNA, and miRNA

- miRNA was the main focus in this study to uncover it's potential as a liquid biopsy biomarker

Objective

- NSCLC stage IV- specific EV morphometrics (Size, density)
- NSCLC stage IV- specific miRNA (from EV and directly from plasma)
- Combinatorial EV/Plasma miRNA signatures (Robustness)
- Test identified markers in stage II patients for early detection
- Prognostic value (Response to TKI inhibition)

Methods



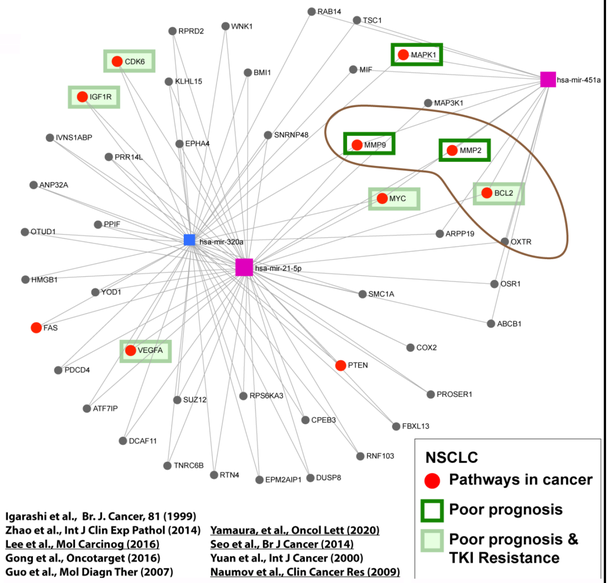
- Two discovery cohorts of 20 patients each with a comparable risk profile as heavy smokers.
- Cohort 1 included patients with Lung RADs I
- Cohort 2 contained patients with Lung RADs IV, including patients with false positives.

Results

miRNA	logFC	PValue	FDR(<5%)
hsa-miR-423-5p	-1.20	0.001	0.039
hsa-miR-451a	-3.58	0.00	0.01
hsa-miR-21-5p	-1.40	0.00	0.01

EV miRNA **Plasma miRNA**

Direct target genes [>2 (3 miRNA)]



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Gong et al., Oncotarget (2016)
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Yamaura, et al., Oncol Lett (2020)
Seo et al., Br J Cancer (2014)
Yuan et al., Int J Cancer (2000)
Naumov et al., Clin Cancer Res (2009)

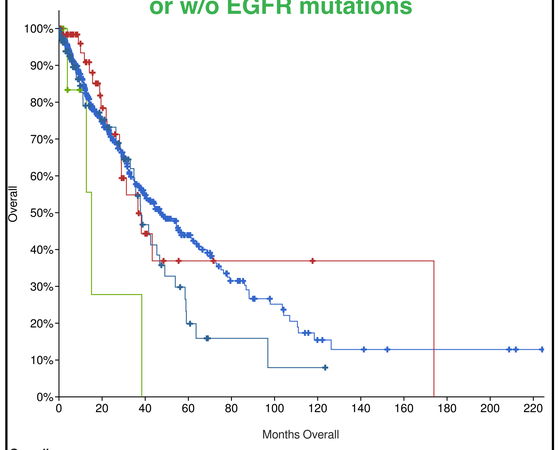
KEGG Classification

Name	Hits	Pval	adj.Pval	Color
Pathways in cancer	10	1.44e-7	0.000012672	
Glioma	4	0.000139	0.006116	
Prostate cancer	4	0.00043	0.01261333	
MAPK signaling pathway	6	0.000615	0.01353	
mTOR signaling pathway	3	0.000845	0.014872	
Colorectal cancer	3	0.00109	0.01558857	
Focal adhesion	5	0.00124	0.01558857	
p53 signaling pathway	3	0.0028	0.025696	
Melanoma	3	0.0028	0.025696	
Pancreatic cancer	3	0.00292	0.025696	
Chronic myeloid leukemia	3	0.00343	0.02744	
Progesterone-mediated oocyte maturatic	3	0.00445	0.03012308	
Small cell lung cancer	3	0.00445	0.03012308	
Thyroid cancer	2	0.00614	0.03859429	
GnRH signaling pathway	3	0.00698	0.04094933	

- We identified 2 miRNA from the EV and 1 from the plasma, and focused on target genes that have at least 2 of the 3 miRNA.
- The list of genes were ran through KEGG classification to produced pathways that were cancer relevant and validated the choosing of the specific miRNA as they all met statistical significance

Pan-Lung Cancer (TCGA, Nat Genet 2016) 1144 samples/patients

Patients with miRNA target gene GOF/CNA with or w/o EGFR mutations



Overall

- Unaltered group
- Patient positive for miRNA target group with EGFR
- Patient positive for miRNA target group only
- EGFR only

	N	5 Year Survival	Median Months Overall
Unaltered Group	750	43.91%	46.91
Patient Positive for miRNA Target Group with EGFR	10	N/A	15.1
Patient Positive for miRNA Target Group Only	73	36.92%	36.64
EGFR only	119	19.86%	37.83

Conclusions

- miRNA biomarker targets predict poor overall survival (OS) for patients with EGFR mutations.

- Future Direction
  - Test these potential liquid biopsy biomarkers (3miRNAs) in larger cohort
  - Diagnosis (stage II patients)
  - Prognosis (OS of stage III/IV patients) receiving TKI treatment

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