Exploring Syndecan-1 as a Diagnostic Marker for Hepatic Diseases in Plasma of Patients with Type 2 Diabetes

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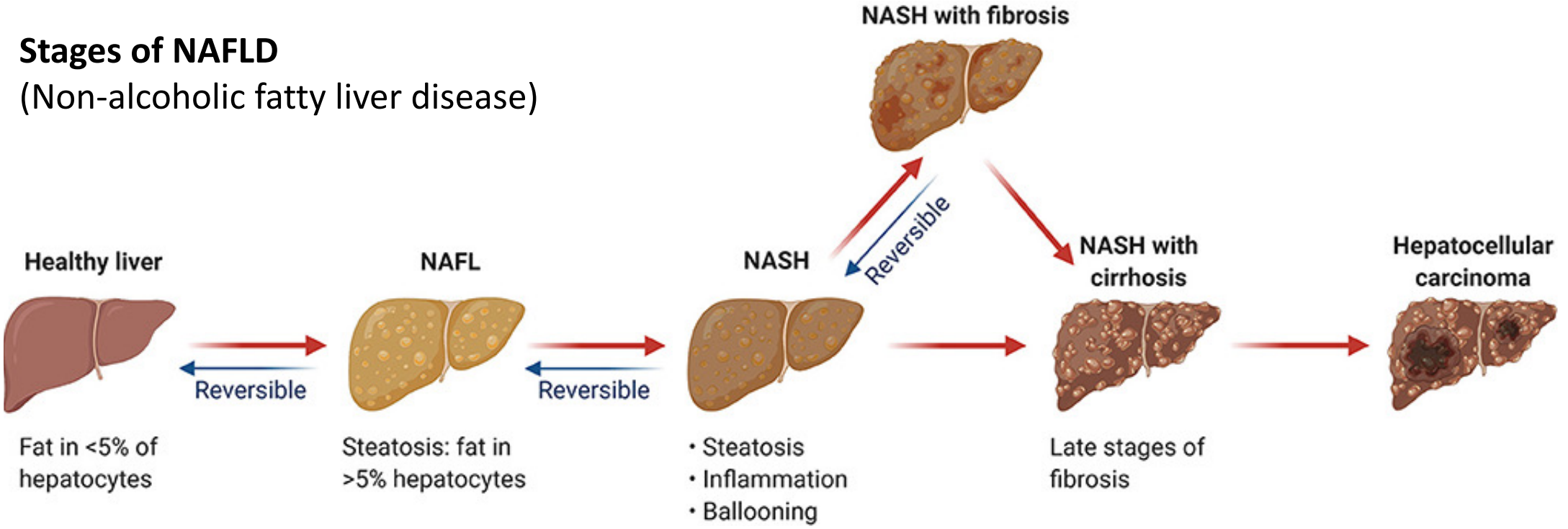
Stages of NAFLD
(Non-alcoholic fatty liver disease)



NAFLD and NASH

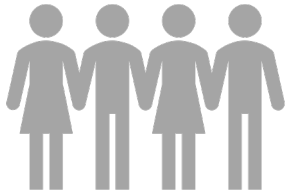
- **NAFLD** affects about 1 in 4 individuals globally
- Major cause of liver-related morbidity and mortality
- Fastest growing cause of hepatocellular carcinoma (liver cancer)
- **NASH:** Non-alcoholic steatohepatitis
  - When inflammation occurs in fatty liver

Clinical Study

Researching the correlation between different stages of NAFLD and T2DM (Type 2 Diabetes Mellitus)

Obesity and T2DM are known to be common risk factors of NAFLD

- Limited data available



Cohort of 501 T2DM patients age ≥ 50 years screened

Evaluated prevalence of NAFLD, advanced fibrosis, and cirrhosis

MRI-PDFF (Magnetic resonance imaging proton density fat fraction): Assessment of liver fat

- NAFLD described as MRI-PDFF ≥ 5%

MRE (Magnetic resonance elastography): Assessment of liver stiffness

- Advanced fibrosis and cirrhosis defined by stiffness cut points on MRE

Biopsies offered to patients with high liver fat and stiffness

Research Article
NAFLD and Alcohol-Related Liver Diseases

JOURNAL
OF HEPATOLOGY

A prospective study on the prevalence of NAFLD, advanced fibrosis, cirrhosis and hepatocellular carcinoma in people with type 2 diabetes

Authors

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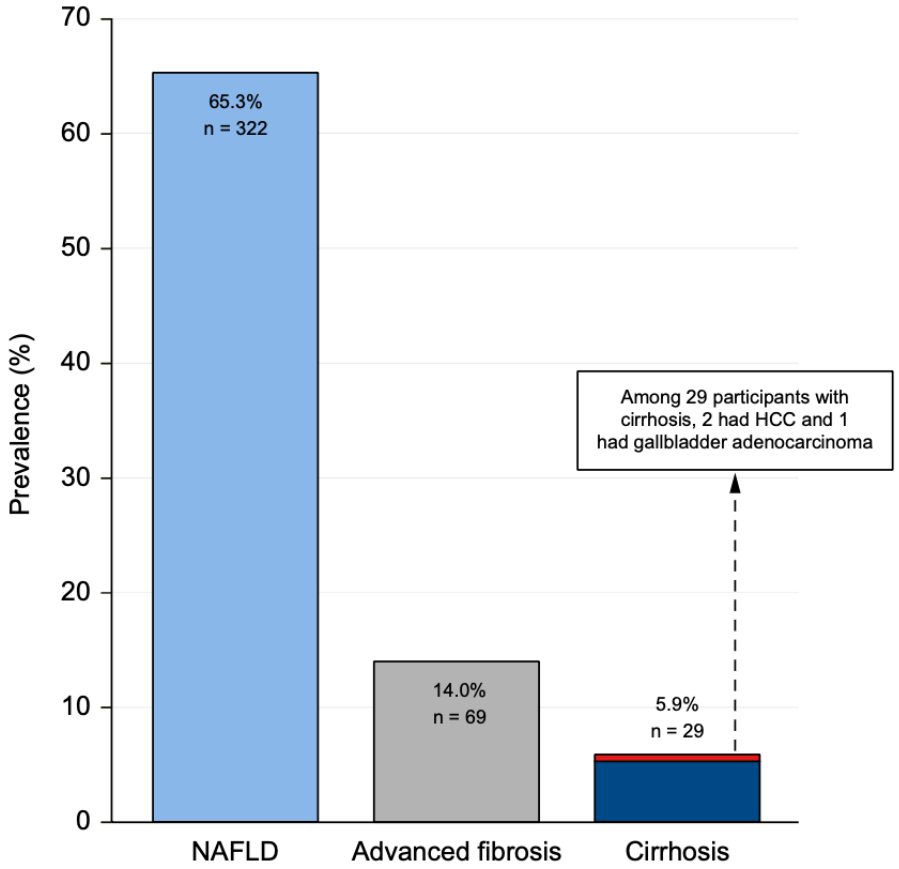
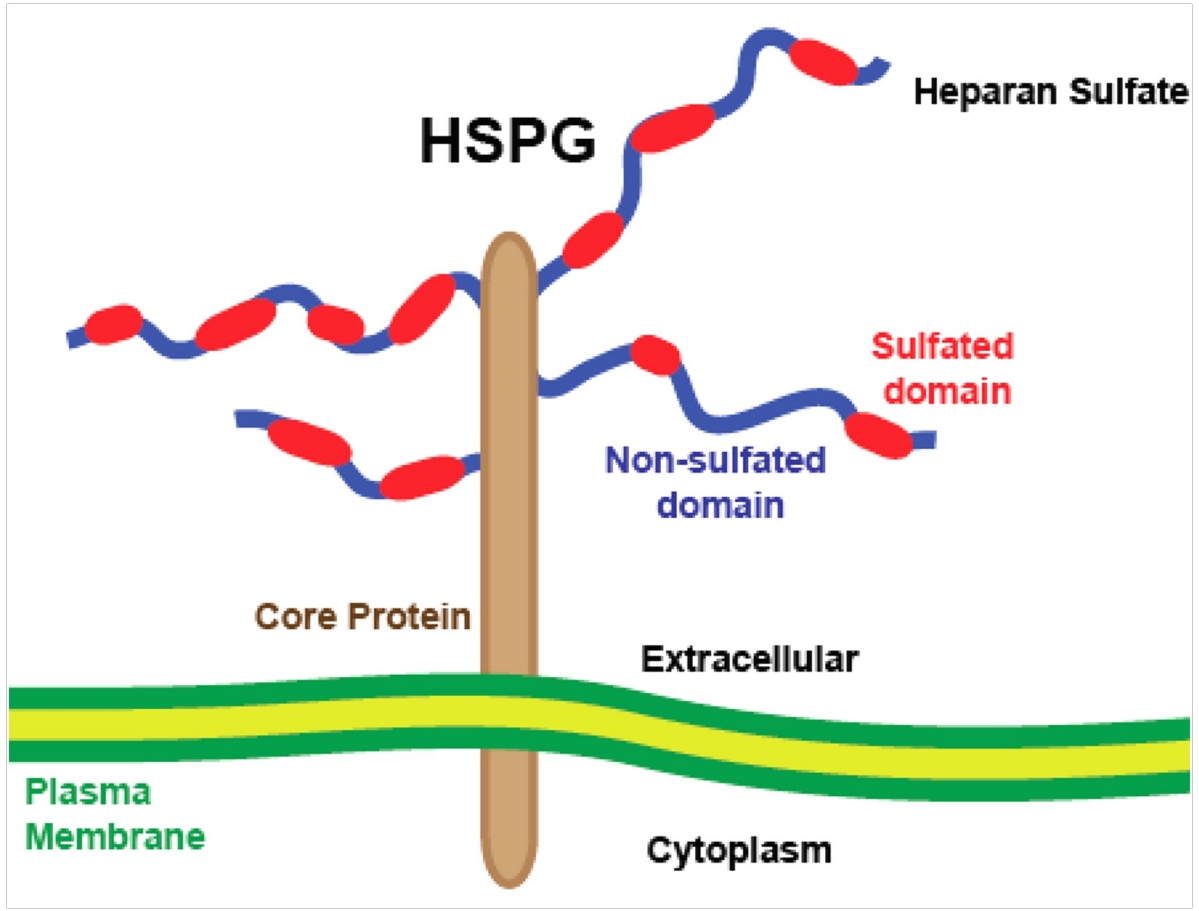


Overarching Goal

- Explore the correlation of SDC-1 (Syndecan-1) and differing levels of NAFLD
- Potential diagnostic marker
  - Non-invasive test (measured in the blood) compared to biopsies
- Overall clinical significance of SDC-1



SDC-1

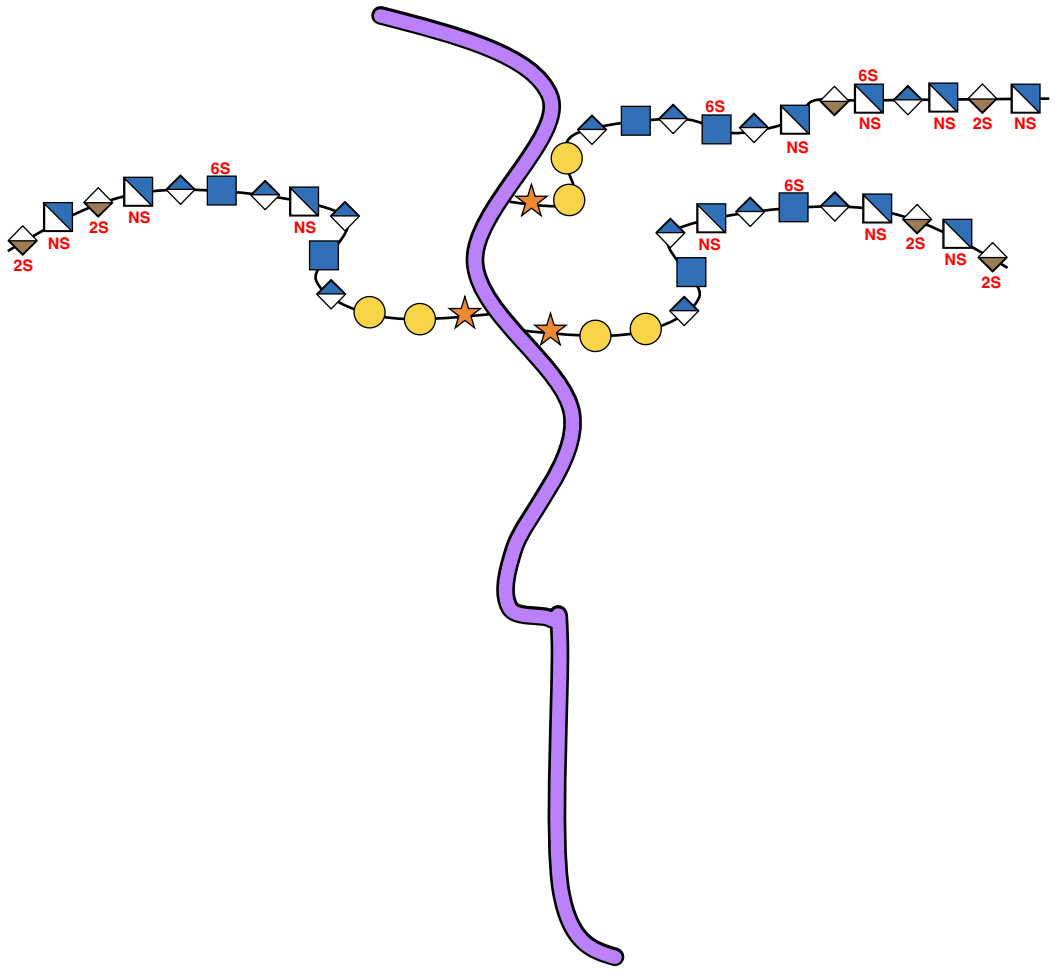
SDC-1 (Syndecan-1): heparan sulfate proteoglycan of the cell surface, abundant in hepatocytes

SDC-1 regulates physiological and pathological processes

Cell proliferation, wound healing, tumorigenesis

SDC-1

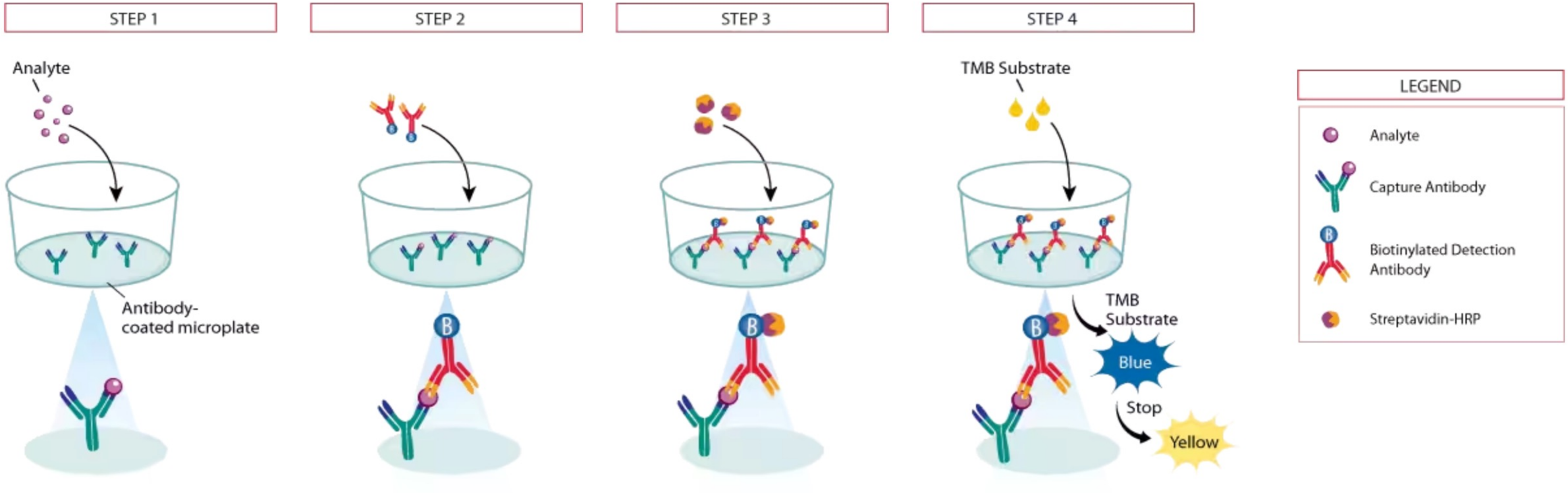
- **Liver disease** can be characterized by elevated SDC-1 expression
  - Good marker for Hepatitis C based hepatocellular carcinoma and liver fibrosis, increasing with liver dysfunction (Regos et al., Springer, 2019.)
- **SDC-1 shedding** occurs during hepatic pathophysiology, leading to its expression in the plasma as a soluble form



ELISA

- **ELISA** (Enzyme-Linked Immunosorbent Assay)
- Sandwich ELISA
  - Two antibodies are used (indirect capture and detection)
  - Highly sensitive and specific
  - Used for biological samples
- **SDC-1** is the target protein (analyte)
- DuoSet ELISA Development Systems Assay (R&D)

ELISA



Overnight incubation of capture antibody.

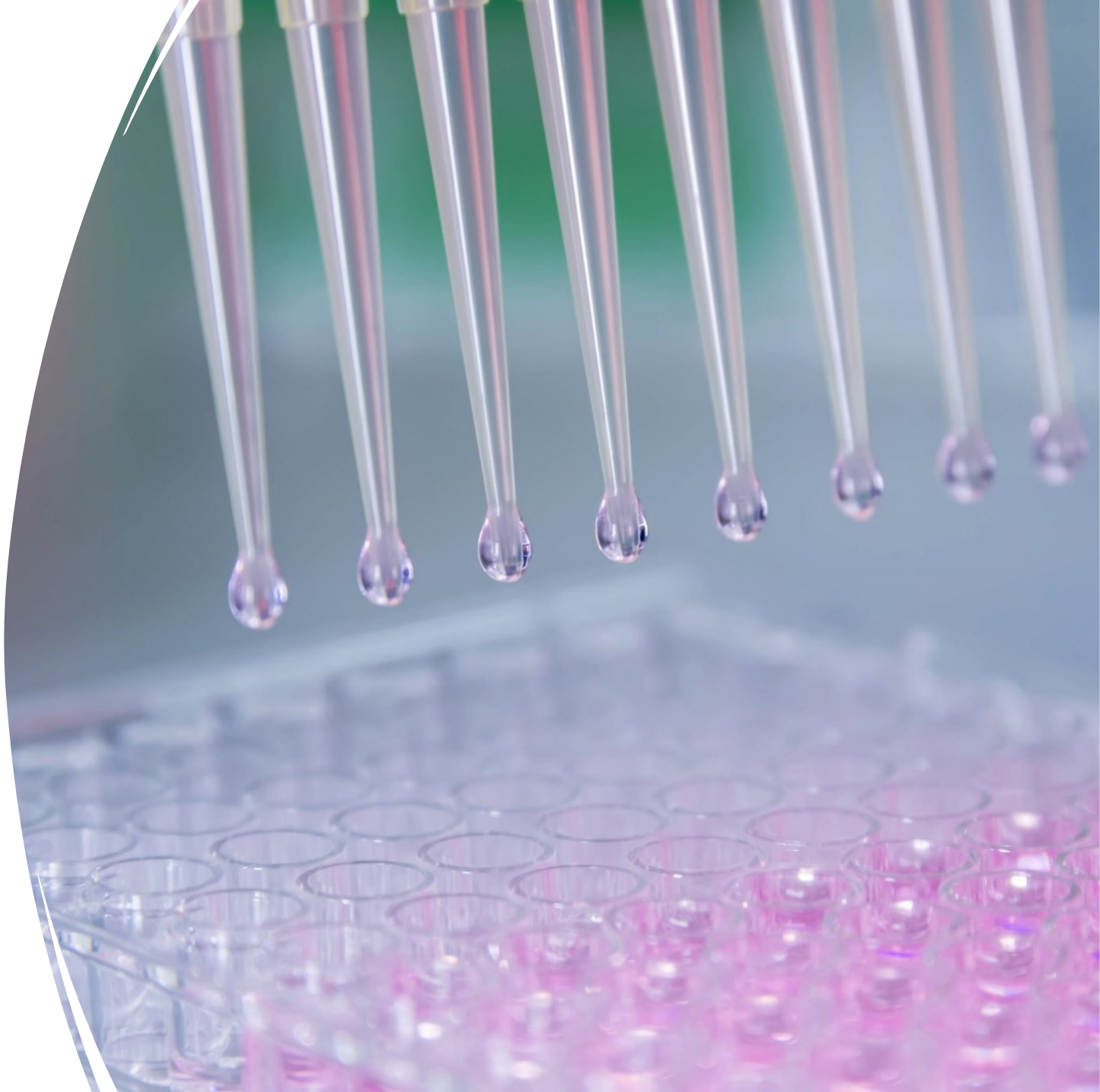
Standards/samples added, incubation of detection antibody.

Streptavidin-HRP binds to biotinylated detection antibody, amplifying the signal.

TMB Substrate solution is added, a blue color is developed. Stop solution turns color to yellow. Absorbance at 450 nm is measured.

Experiment

- 17 ELISA Plates, 481 Human plasma samples
- Able to obtain 408 samples in range
- Data found by interpolating the measured absorbances using a standard curve (Prism 9)

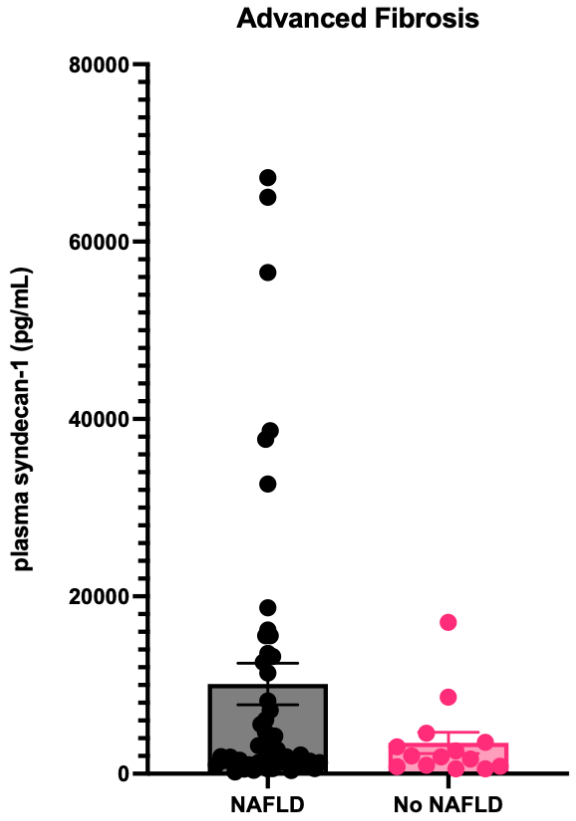


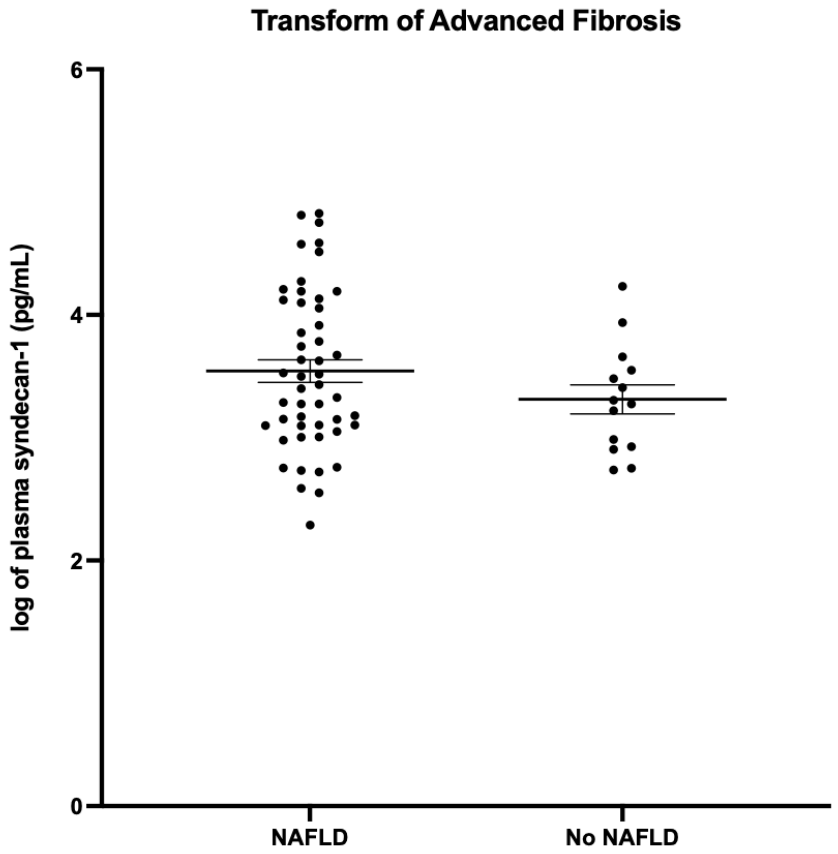
Troubleshooting / Optimizing

- Optimized ELISA through multiple tests
  - Different dilutions with reagent diluent (to fit the range of the standard curve)
    - 1:100 and 1:50 -> low absorbance
    - Started with 1:10 -> low absorbance
    - Switched to 1:2 -> more in range, high absorbances
    - Low repeats at 1:2 / no dilution
    - High repeats at 1:50

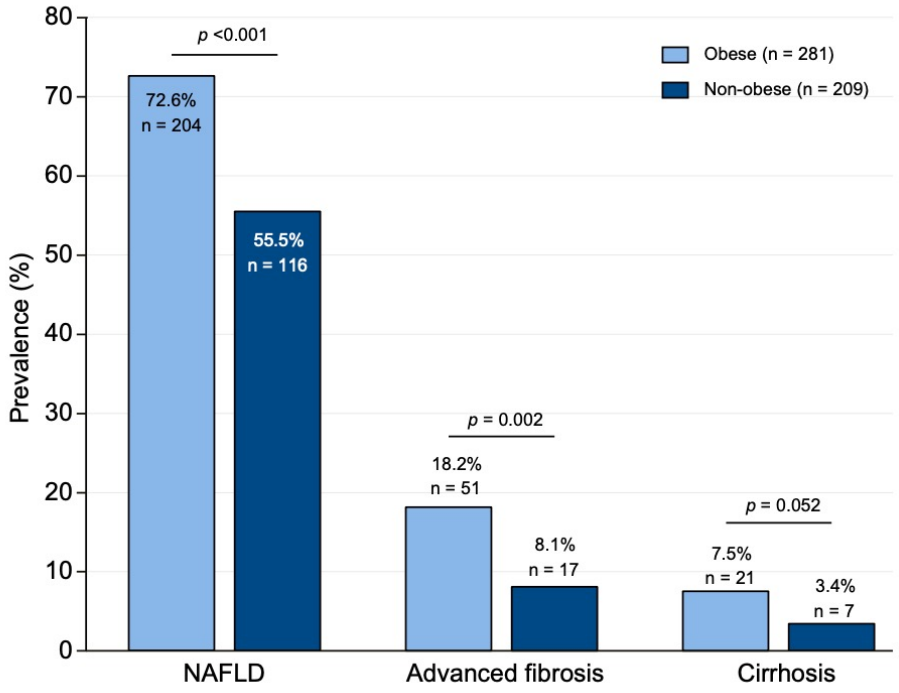
T	U	V	W	X	Y	Z
Concentration						
	Absorbance		pg/mL			1 to 5?
DOS0147	0.0757	134.73	1347.272498	LOW		LOW
DOS0148	0.1676	302.69	3026.856187			
DOS0149	2.33975	6486.8	64867.76474			
DOS0150	3.24845	11604	116037.0387		HIGH	
DOS0151	0.07265	129.24	1292.366683	LOW		LOW
DOS0152	0.12145	217.73	2177.262114	LOW		
DOS0153	3.23545	11510	115097.7379		HIGH	
DOS0154	0.1023	182.84	1828.378046	LOW		
DOS0155	0.18065	326.94	3269.386111			
DOS0156	0.11015	197.11	1971.13621	LOW		
DOS0157	0.2752	505.74	5057.441819			
DOS0159	0.53395	1024.5	10245.48433			
DOS0160	0.1131	202.49	2024.876357	LOW		
DOS0161	0.6427	1256.5	12565.17587			
DOS0162	2.32935	6441.4	64414.39783			
DOS0163	0.2177	396.35	3963.528982			
DOS0164	0.1139	203.95	2039.458652	LOW		
DOS0165	0.05855	103.92	1039.226374	LOW		LOW
DOS0166	3.2877	11892	118920.6315		HIGH	
DOS0167	0.1276	228.98	2289.757832	LOW		
DOS0168	3.3084		0		HIGH	
DOS0169	3.29415		0		HIGH	

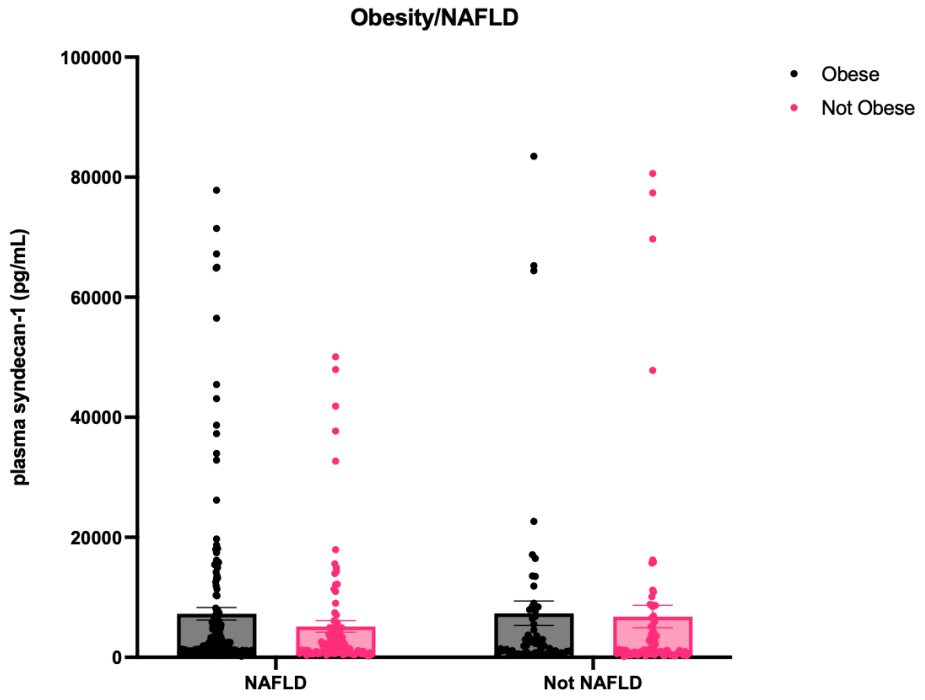
Results


Advanced Fibrosis
plasma syndecan-1 (pg/mL)
80000
60000
40000
20000
0
NAFLD
No NAFLD

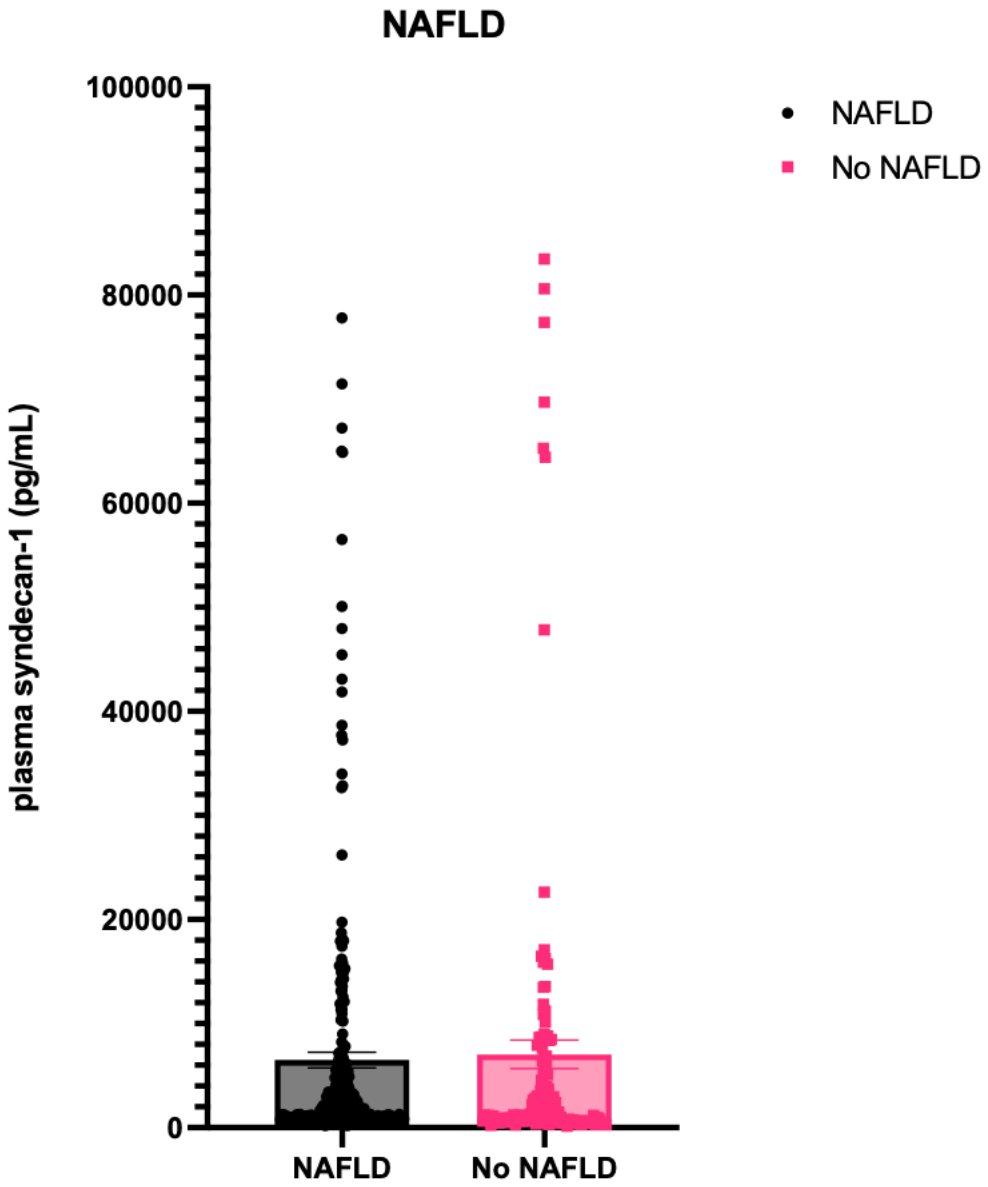

Transform of Advanced Fibrosis
log of plasma syndecan-1 (pg/mL)
6
4
2
0
NAFLD
No NAFLD

Results


80
70
60
50
40
30
20
10
0
Prevalence (%)
p <0.001
Obese (n = 281)
Non-obese (n = 209)
72.6%
n = 204
55.5%
n = 116
p = 0.002
18.2%
n = 51
8.1%
n = 17
p = 0.052
7.5%
n = 21
3.4%
n = 7
NAFLD
Advanced fibrosis
Cirrhosis


Obesity/NAFLD
100000
80000
60000
40000
20000
0
plasma syndecan-1 (pg/mL)
Obese
Not Obese
NAFLD
Not NAFLD

Results


NAFLD
100000
80000
60000
40000
20000
0
plasma syndecan-1 (pg/mL)
NAFLD
No NAFLD
NAFLD
No NAFLD

Future Directions

- Obtain information of the 2 patients with hepatocellular carcinoma, focus and evaluate SDC-1 levels
- Explore the correlation between SDC-1 and other demographics such as sex, age, hypertension, and cholesterol levels
- Rerun samples out of range

Acknowledgements

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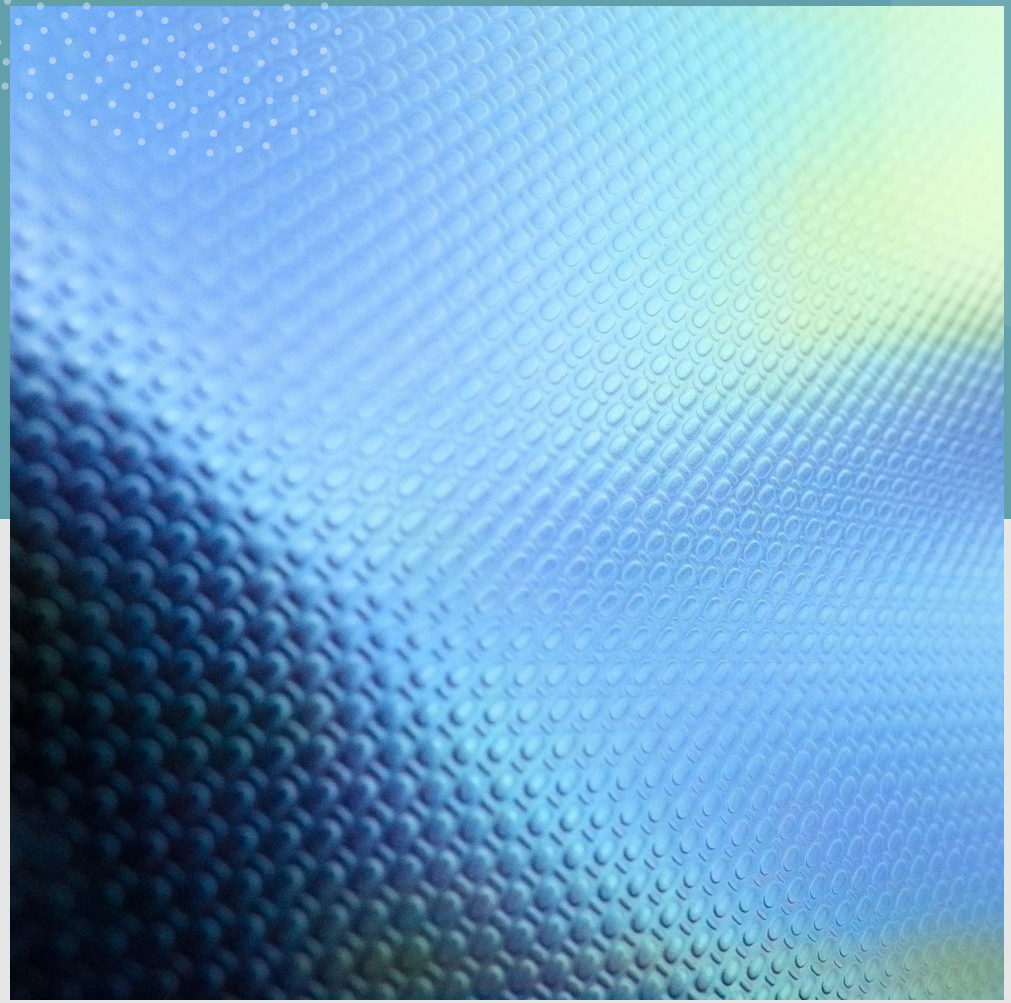

Thank You!
Questions?