USING AI/ML AND MULTI-OMICS TO DETERMINE EFFICACY FOR CLINICAL NASH STUDY ENRICHMENT: HIGHLIGHTS FROM RECENTLY COMPLETED PHASE 2 TRIALS WITH RENCOFILSTAT.

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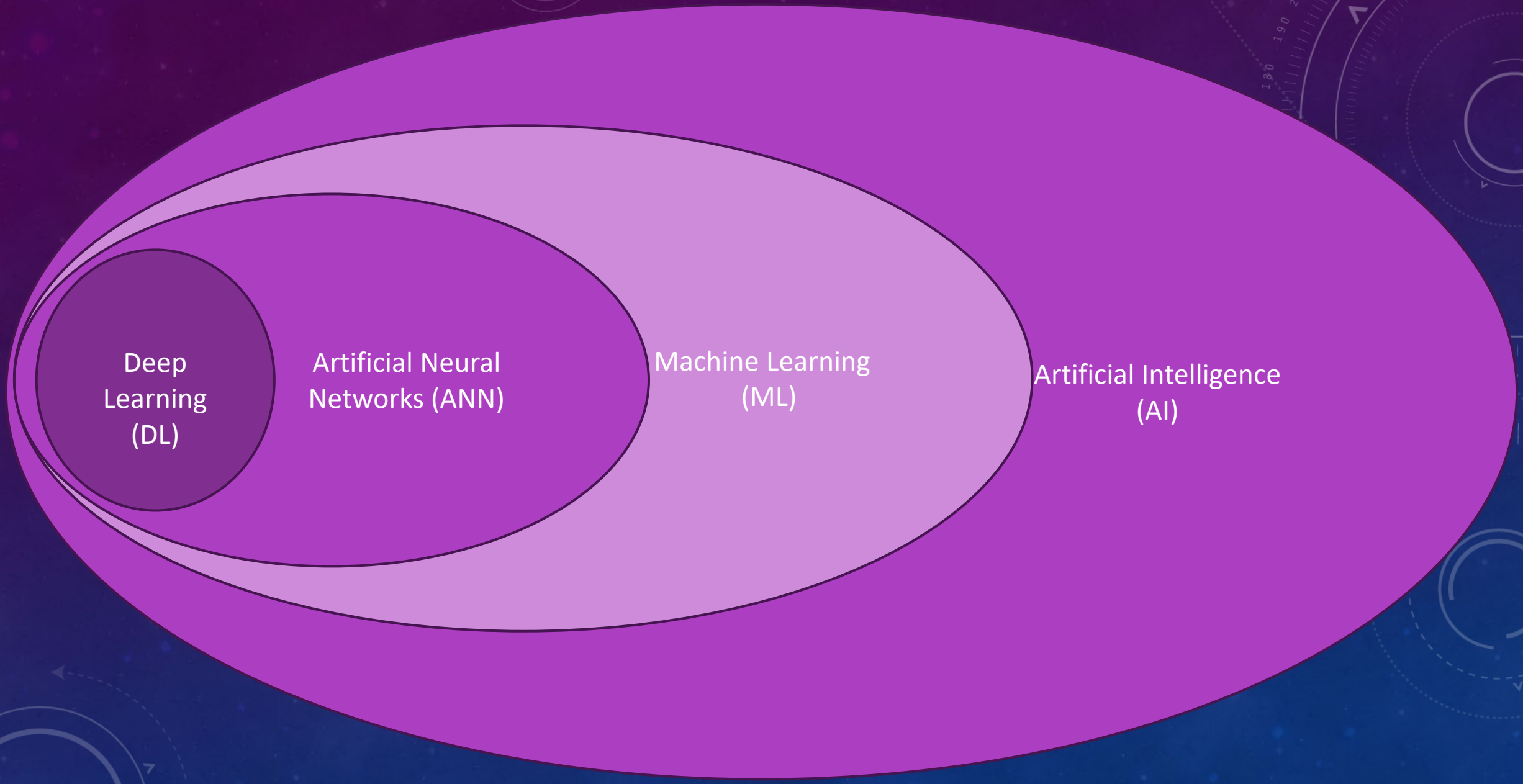
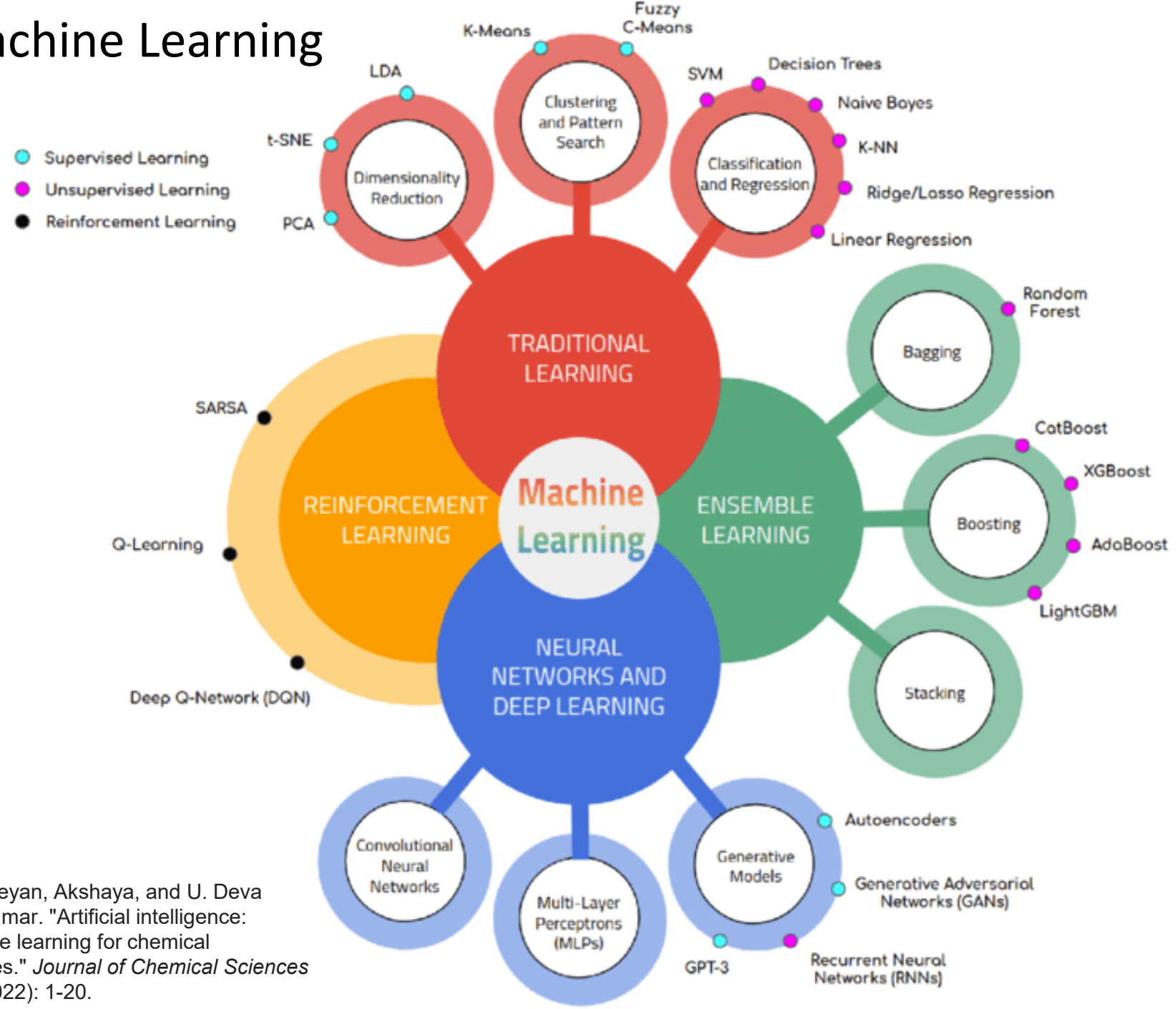
HEPION PHARMACEUTICALS

WHAT IS ARTIFICIAL INTELLIGENCE (AI)?

Narrow AI	General AI	Super AI
Stage 1	Stage 2	Stage 3
Machine Learning	Machine Intelligence	Machine Consciousness
Specialized in one area to solve one problem	A computer 'as smart as a human' in general applications!	Intellect "smarter" than human brains in all fields
Examples:		
Siri, Alexa, Cortana		?Skynet ?HAL


Deep Learning (DL)
Artificial Neural Networks (ANN)
Machine Learning (ML)
Artificial Intelligence (AI)

Machine Learning



Karthikeyan, Akshaya, and U. Deva Priyakumar. "Artificial intelligence: machine learning for chemical sciences." *Journal of Chemical Sciences* 134 (2022): 1-20.

HEPION'S AI-POWR™

- **AI** – Artificial Intelligence, machine and deep learning neural networks and Bayesian Networks
- P – Precision Medicine, individualizing treatments based on an integrative bioinformatics genetics, environment, and lifestyle
- O - Omics, including genomics, transcriptomics, proteomics, metabolomics and lipidomics
- W – World, accessing world genomic databases, and Real-World Data
- R – Response and clinical outcomes

WHY IS HEPION USING AI/ML FOR CLINICAL DRUG DEVELOPMENT

MASH/MAFLD Heterogeneity

- Disease has proven difficult develop drug therapy
- OCA?
- Resmetirom?
- Efruxifermin?
- Semaglutide?

Multi-Omic Data

- Traditional Clinical trial Data: Safety/PK-PD
- Transcriptomics
- Proteomics
- Metabolomics (Pre-clinical)
- *Microbiome*
- Can these be combined to identify patients that will respond to specific treatments *A priori*?

DATA FOR ANALYSIS

HEPA201: 28-Day Phase 2a

- N = 28 Subjects (Completed)
- **DataN = 87,473 per Subject**
- Transcriptomics
- Lipidomics
- Partial Proteomics
- Clinical Safety
- PK
- NITS
- Analysis: Traditional Safety, PK-PD, PK-PB, QSP, Bioinformatics, AI-POWR

HEPA210: 120-Day Phase 2

- N= 70 Subjects (68 completed)
- **DataN= 6,728,610 per Subject**
- Transcriptomics
- Partial Proteomics
- Clinical Safety
- PK
- NEW: HEPQUANT LIVER FUNCTION
- NITS: Fibroscan
- Analysis: Traditional Safety, PKPD, PKPB, QSP, Bioinformatics, AI-POWR

Add New Data Surveil Literature

Human Samples: Transcriptomics/Lipidomics/Proteomics/Metabolomics

Disease-Available Databases (n=1082↑ patients)	Test Drug Specific: In-House from Clinical Trials (n=616↑ patients)

Add New Data via Clinical Trials

Iterative for Validation

RNA-Seq/Proteomics/Lipidomic
PKPD/QSP/PBPK - Clinical Efficacy & Safety

Multi-Omics Analyses	PKPD-PBPK-QSP-Network Analyses

Iterative for Validation

Machine (Deep) Learning AI Pattern Recognition

Transcriptome-Interrogation Proteome-Interrogation Lipid -Interrogation	Concentration-Effect , Risk Benefit QoL Indices, Pharmacoeconomics, Pharmacovigilance

ELIMINATE THE BIOPSY!

New NDA Novel Drug with Biomarker & *A Priori* Response Prediction

BIOMARKER VS HISTOPATHOLOGY CONUNDRUM

ALT Conundrum

- ALT- ↓ ≠ ↓ HistoPath F-score.
- However:

  ALT = F-score
- ERGO: Want ALT- ↓

Is ProC3 a Better Marker?

- Higher in NASH than NAFLD
  - ↑ ballooning, inflammation, steatosis, fibrosis and NAS score
- Pro-C3 cut-offs are suggested to screen for patients and to **predict responders***

See supplementary file for Phase 2 resmetirom study, baseline Pro-C3 values of 10.0ng/mL and 17.5ng/mL have been used to analyze data

Harrison SA, Bashir MR, Guy CD, et al. Resmetirom (MGL-3196) for the treatment of non-alcoholic steatohepatitis: a multicentre, randomised, double-blind, placebo-controlled, phase 2 trial. *Lancet*. 2019;394(10213):2012-2024. doi:10.1016/S0140-6736(19)32517-6

In analysis done be Luo *et al.*, more patients had fibrosis improvement in the group with baseline PRO-C3 levels >16 ng/ml

In Phase 2a study by Hepion, we used Pro-C3 cut-off of 15.5ng/mL

WHAT HAPPENS TO MAFLD-MASH FROM F0 TO F4?

MAFLD – MASH : F0 – F4 DISEASE PROGRESSION

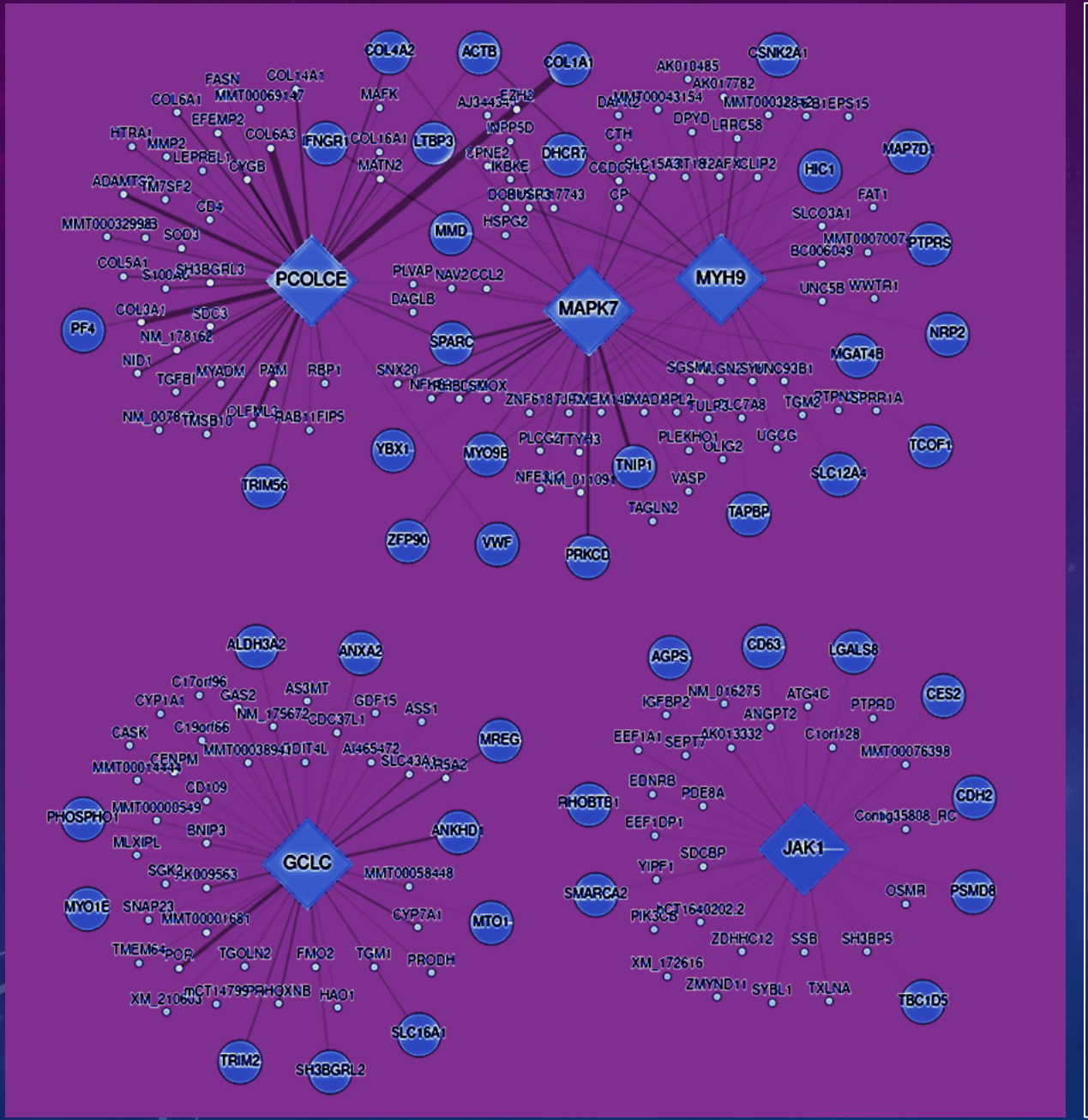
Histology	Gene	Name	Function
F0 – F4 Pooled	IL10RA	Interleukin 10 Receptor	TGFB Signaling, Proinflammatory Diseases
	COL1A1/2	Collagen Type 1A/2	Fibril Forming Collagen
F1	CFL1	Cofilin1	Actin Cytoskeleton
	VTN	Vitronectin	Wound Healing ECM
	ITIH2/3		ECM Stablization IGF Transport
F2	ANXA3	Annexin A3	Prostaglandin Regulation Ovarian/Prostate CA
	LOXL2	Lysyl Oxidase Like 2	Collagen Chain Trimerization Paralog LOXL3
	COL1A1/2	Collagen Type 1A/2	Fibril Forming Collagen
F3-F4	LYRM5(ETFRF1)	Electron Transfer flavoprotein regulatory factor1	Mitochondrial respiratory electron transport chain
	TACSTD1/EPCAM1	Epithelial cellular adhesion molecule	Lynch syndrome: Colorectal cancers

•GSE135251

PRO-C3 RESPONDER ANALYSIS

PRO-C3 IS A BIOMARKER THAT DETECTS THE FORMATION OF TYPE III COLLAGEN CAN BE USED ALONE TO PREDICT FIBROSIS OR AS PART OF A COMPOSITE SCORE

Quantitative Systems Pharmacology: RCF ProC3 Responder Network



Weighted Key Driver Analysis

- Procollagen C-endopeptidase Enhancer (PCOLCE) is the gene name for the protein Procollagen C-Proteinase Enhancer 1 (PCPE1) which has been identified as a potential biomarker and/or therapeutic target for fibrosis and liver fibrosis.
- PCPE1 regulates C-terminal procollagen processing and collagen fibril assembly.
- MYH9 acts via TGF-β1 on fibroblast-myofibroblast differentiation in lung fibrosis models.
- GCLC is a negative regulatory factor in HCV-related liver fibrosis.
- MAPK7 is part of the MAPK signaling pathway and has been shown to be modulated by CyPA and CyPD and is involved in NASH pathophysiology.
- JAK1 has been shown to possess both anti-inflammatory and antifibrotic effects in liver and lung fibrotic disease.

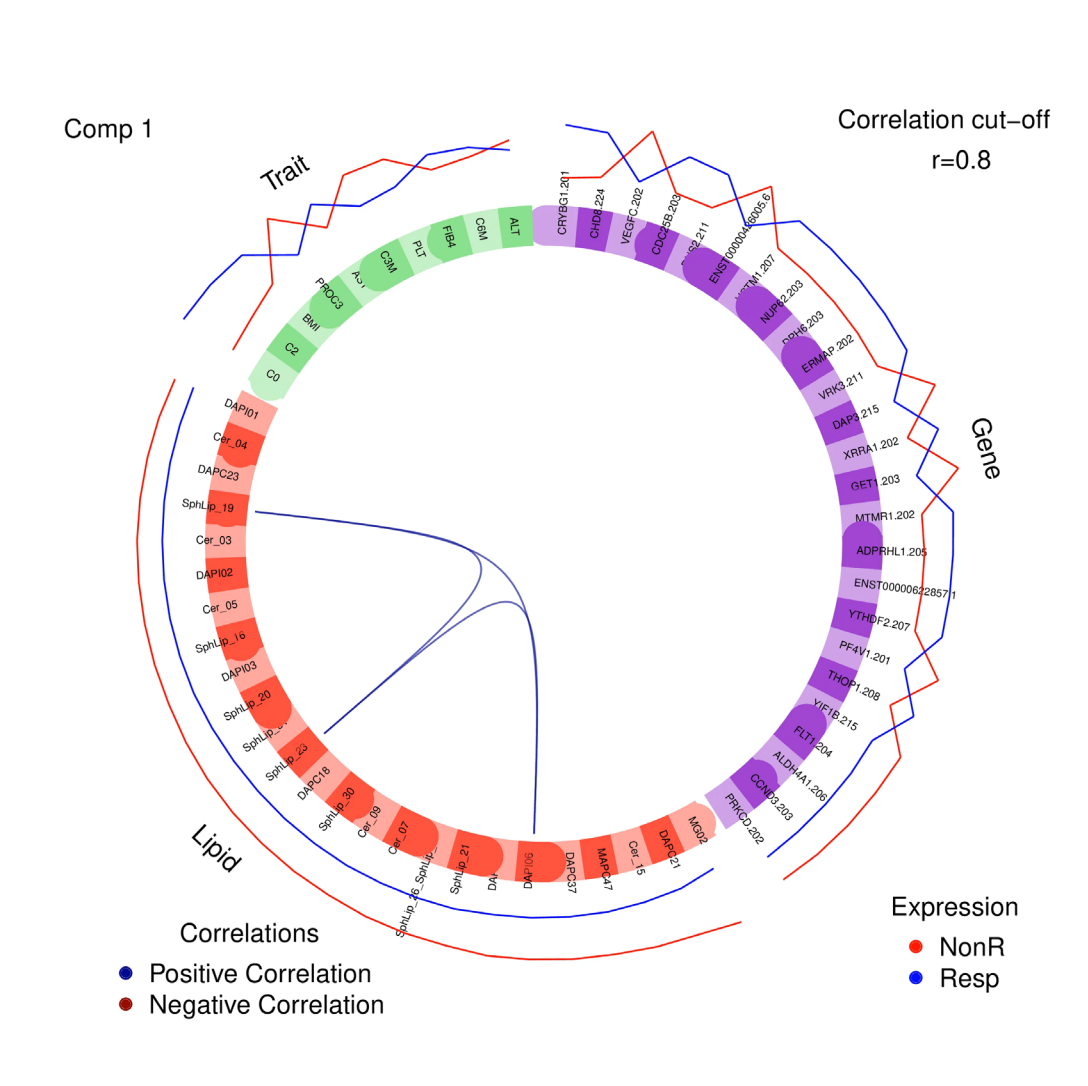
MULTI-OMIC ANALYSIS

A MIXTURE OF MACHINE LEARNING AND MULTI-VARIATE STATISTICAL ANALYSIS

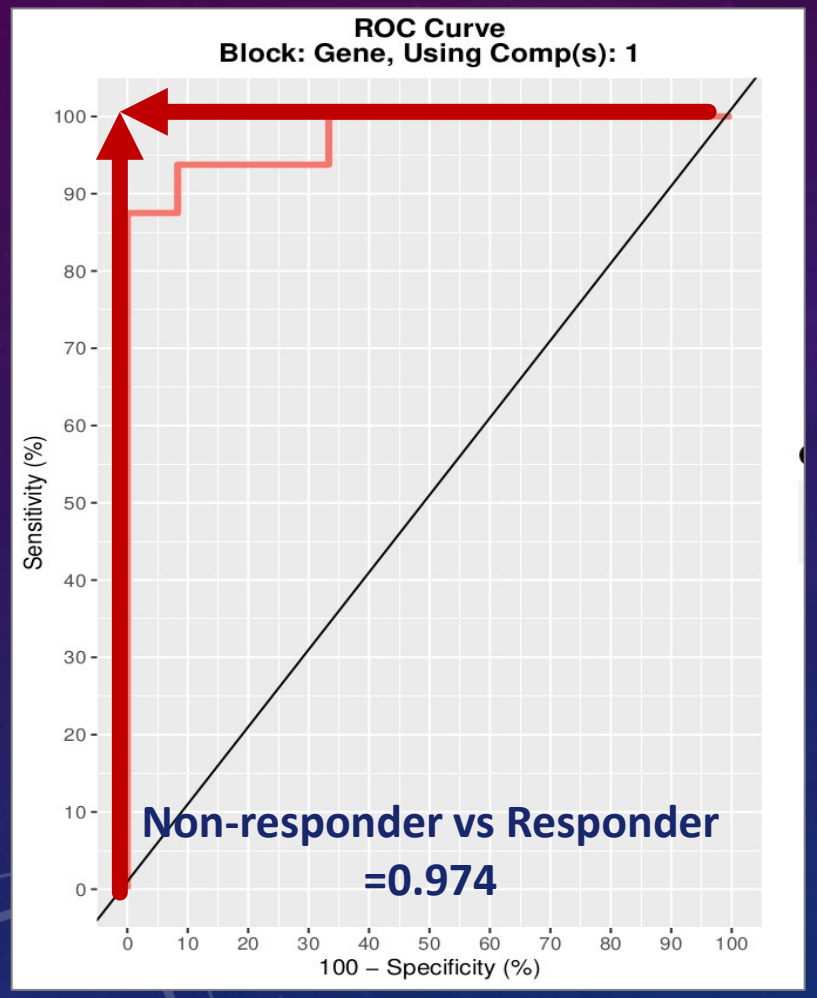
HEPA210: RCF ProC3 Responder

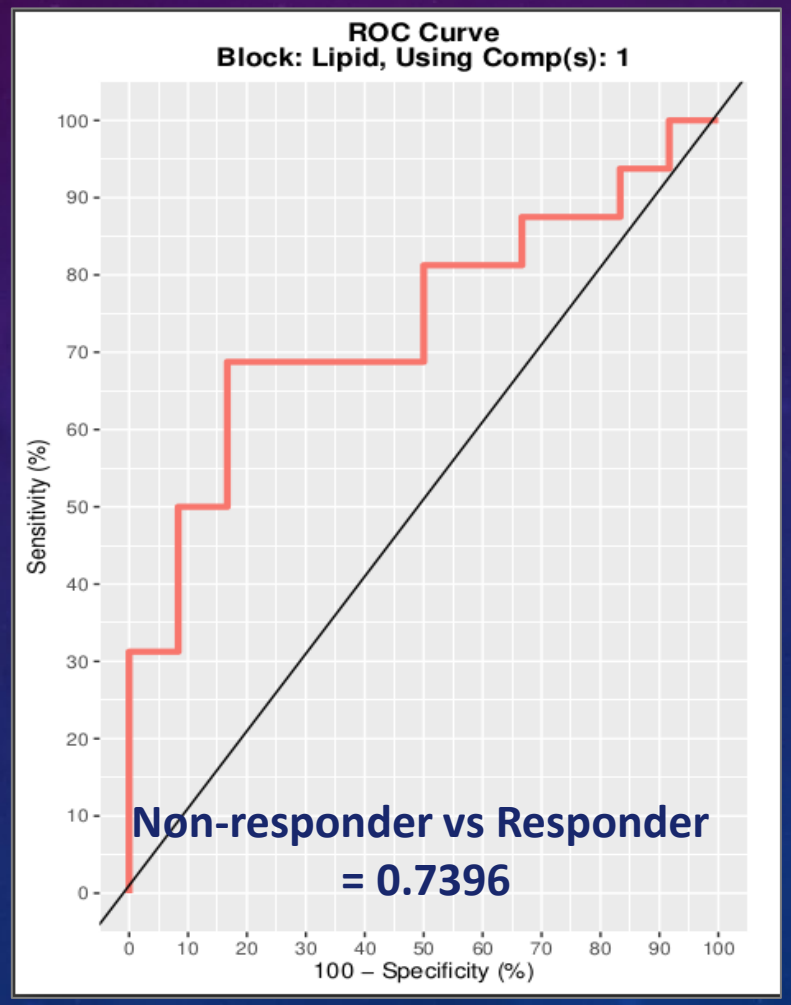
Multiblock (s)PLS-DA: ML + PKPD

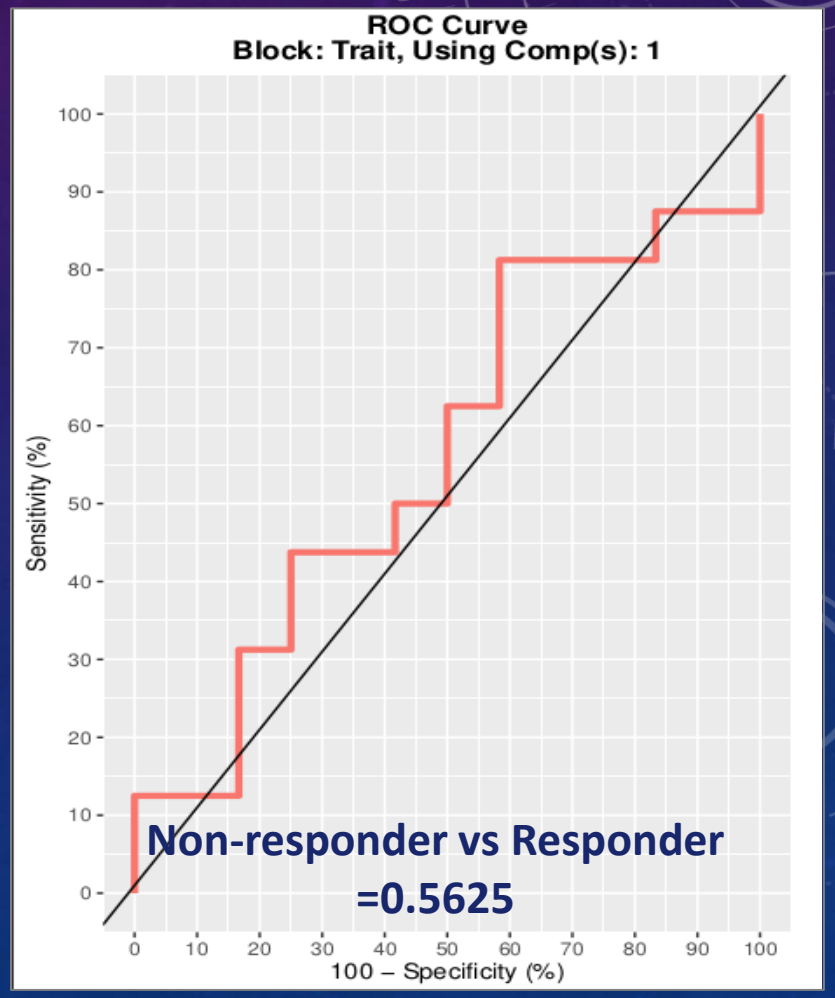
- Out of 1733 statistically significant genes 25 are predictive of ProC3 response.
- Out of 443 lipids, 25 are predictive.
- Clinical Traits ALONE did not work well to predict response.
- Response was associated with:
  - Trough Concentration = 964.2 ng/mL
  - 2-Hour Concentrations = 1160 ng/mL

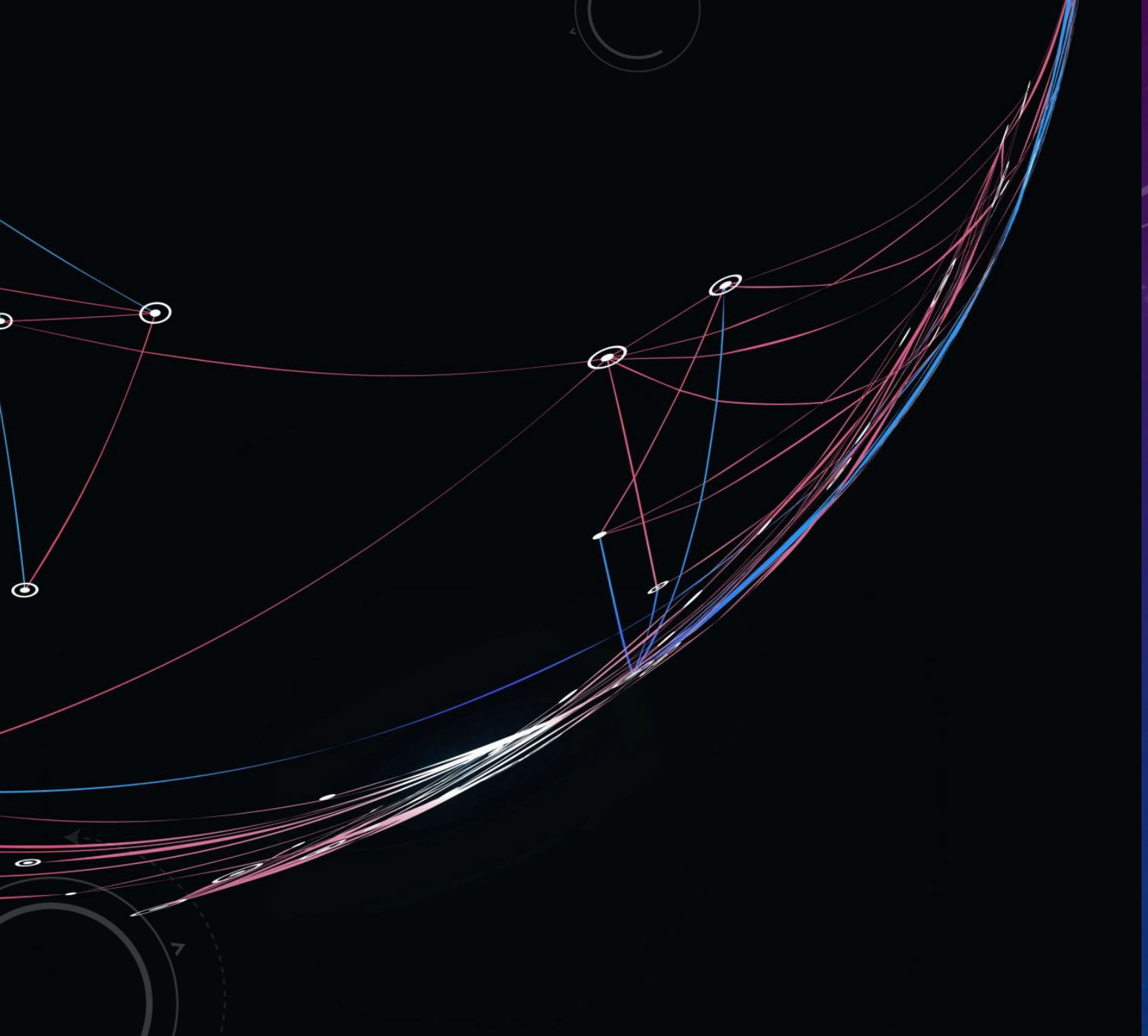


Multi-omics: Pro-C3 Responder ROC Curve





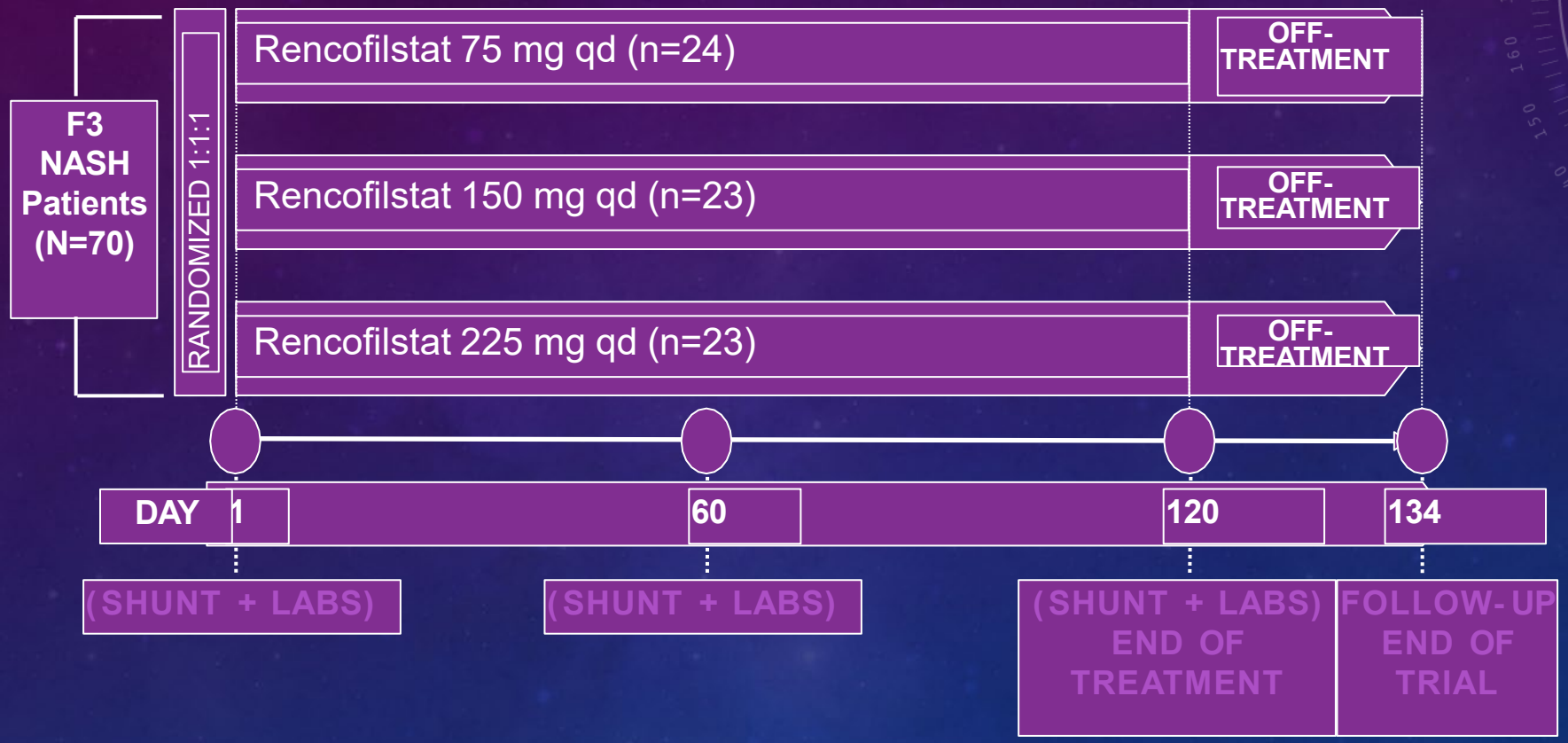




HEPQUANT

A MEASURE OF LIVER FUNCTION

HEPA210: HEPQUANT LIVER FUNCTION STUDY



HepQuant DuO Results in the 225 mg/day Rencofilstat Arm

Parameter	Baseline, N=23	60 Days, N=21	120 Days, N=18
	Mean(SD)	Mean(SD)	Mean(SD)
DSI	16.44 (3.3)	14.98 (4.1)**	14.79 (3.4)**
SHUNT (%)	24.98(4.9)	22.52 (5.3)**	23.15 (4.6)*
Hepatic Reserve (%)	87.86 (7.5)	90.77 (8.5)**	91.60 (7.5)**
Portal HFR (mL/min/kg)	16.52 (5.5)	20.44 (11.8)*	18.83 (5.2)*
Systemic HFR (mL/min/kg)	3.91 (0.6)	4.09 (0.7)**	4.17 (0.6)**
RISK ACE	2.41	2.07****	1.92****

Change from baseline by paired t-test: *p<0.10, **p<0.05, ***p<0.01, ****p<0.001.

DSI: Disease Severity Index (0-50); HFR: Hepatic Filtration Rate; RISK ACE: Risk of clinical events per person-year

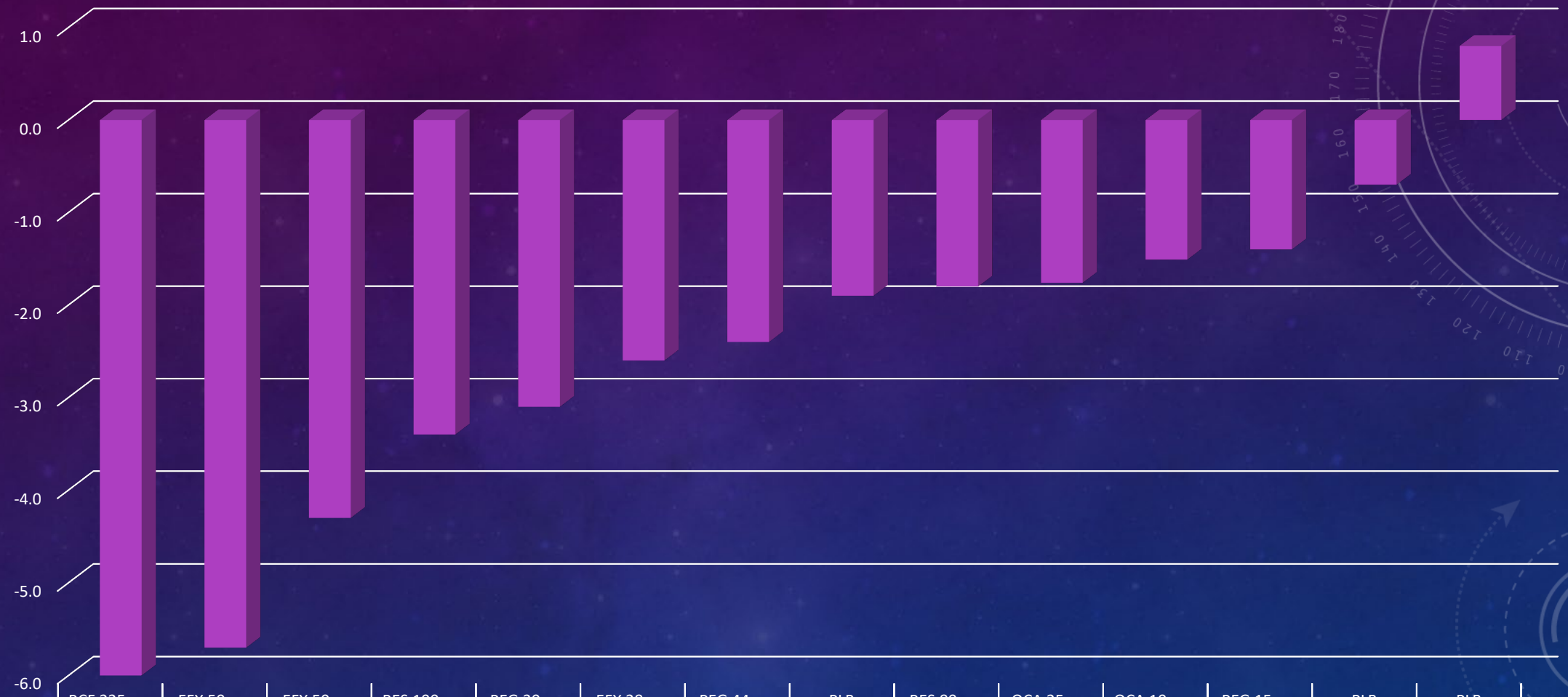
MARKERS OF FIBROSIS

Biomarkers of Fibrosis and Liver Function in the 225 mg/day Rencofilstat Arm (Day 120)		
	225 mg RCF n=21	225 mg RCF (Baseline Pro-C3 ≥ 37.5 ng/mL) (n=6)
	% Change from Baseline	
AST (U/L)	4.68 ± 31.92*	-11.34 ± 38.54*
ALT (U/L)	-21.63 ± 32.8*	-37.78± 31.42*
ELF	-2.51 ± 6.85*	-5.31 ± 7.02*
Fibroscan LSM (kPa)	-28.84 ± 7.39**	-33.62 ± 19.74**
Pro-C3 (ng/mL)	-9.58 ± 31.56*	-16.23 ± 22.59*
Fib-4	17.90 ± 41.91	-3.5 ± 47.6

* Different from Baseline, Friedman ANOVA (data non-normally distributed).

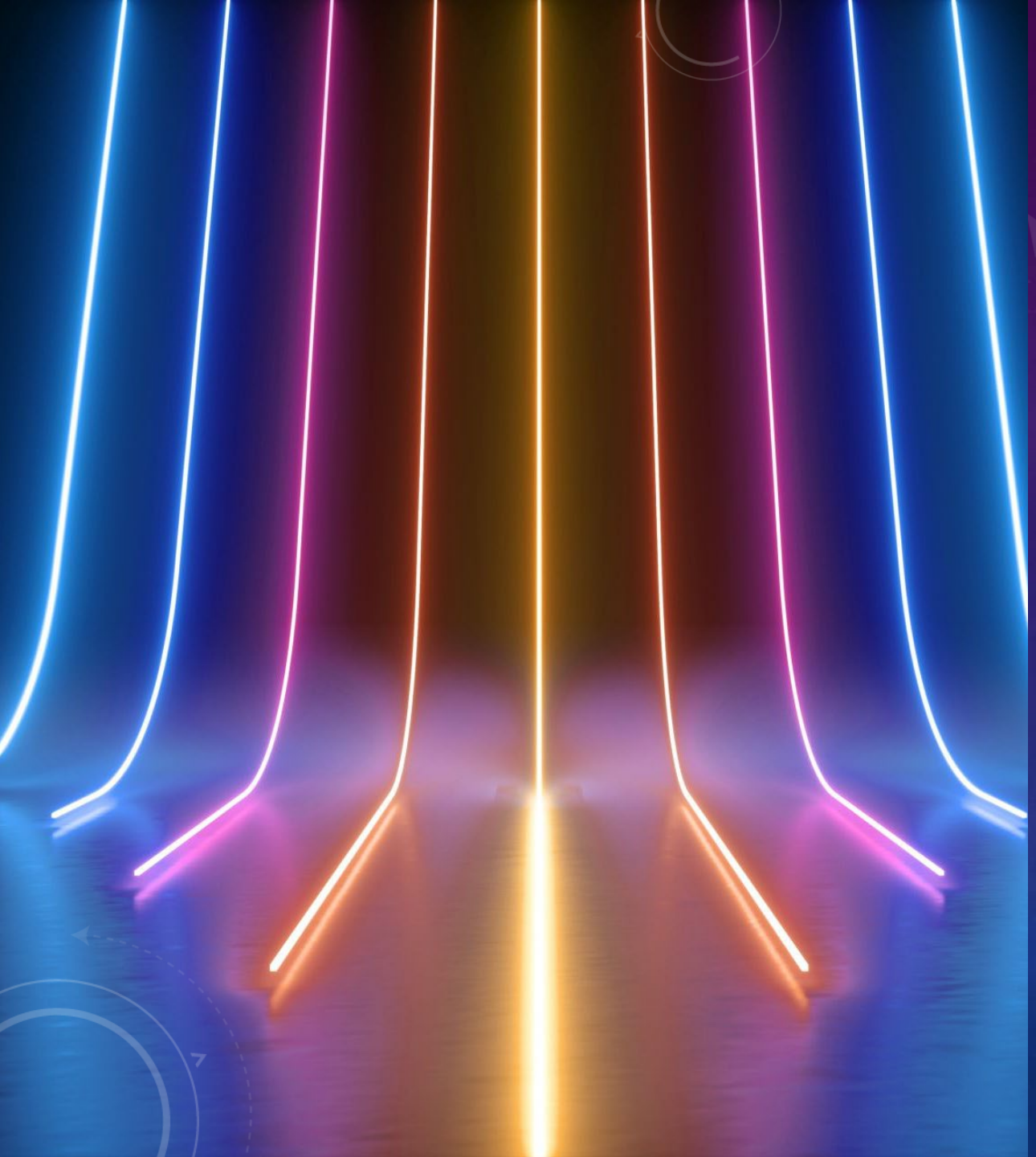
** LSMeans, Different from Baseline

Fibroscan Liver Stiffness Absolute Change from Baseline (kPa)



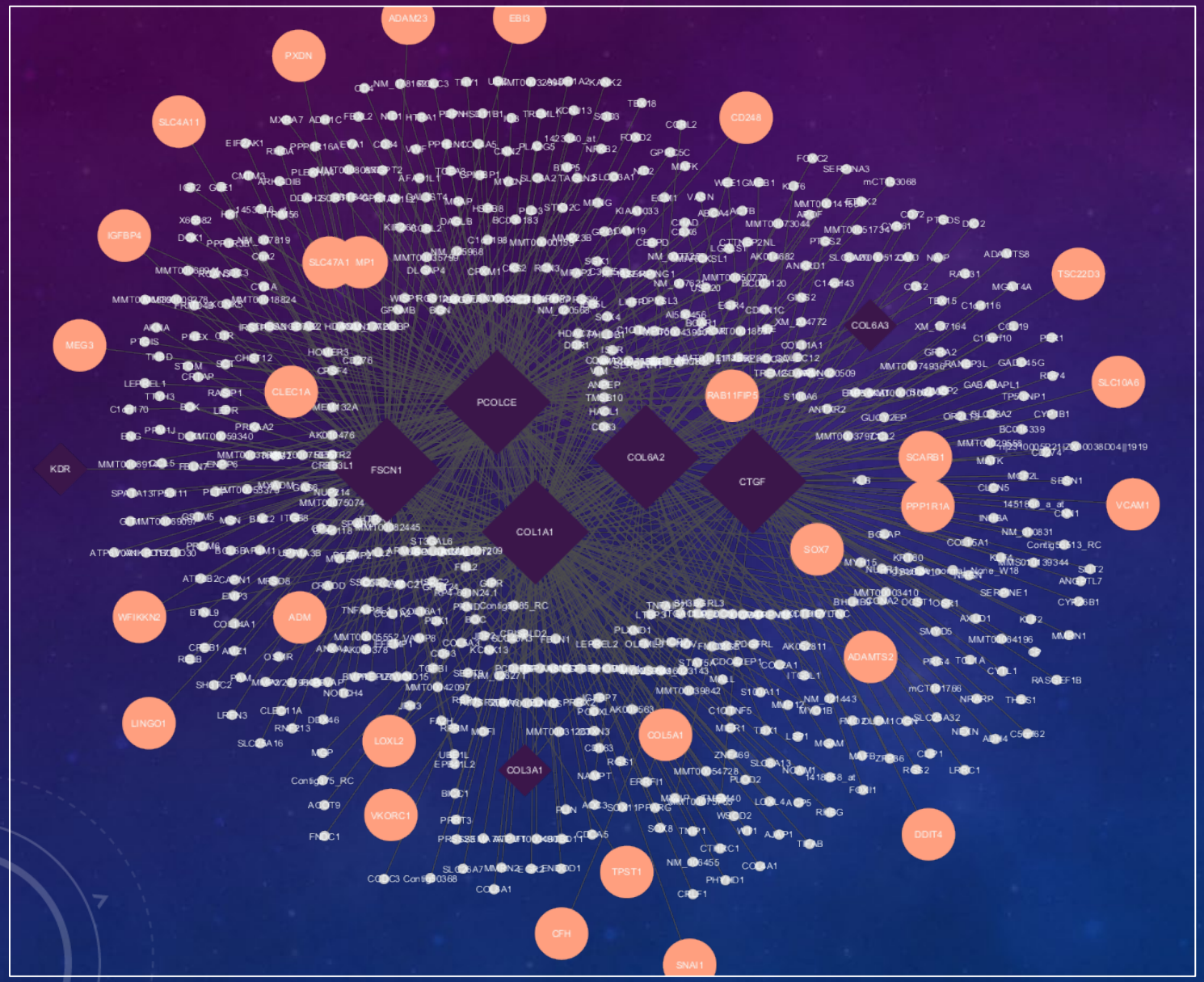
	RCF 225 mg QD	EFX 50 mg QW	EFX 50 mg QW	RES 100 mg QD	PEG 30 mg QW	EFX 28 mg QW	PEG 44 mg Q2W	PLB	RES 80 mg QD	OCA 25 mg QD	OCA 10 mg QD	PEG 15 mg QW	PLB	PLB
Absolute CFB	-6.0	-5.7	-4.3	-3.4	-3.1	-2.6	-2.4	-1.9	-1.8	-1.8	-1.5	-1.4	-0.7	0.8

RCF: LSMeans Absolute Change from Baseline



HEPA210: FIBROSCAN RESPONDER

FIBROSCAN RESPONDER TRANSCRIPTOMIC NETWORK

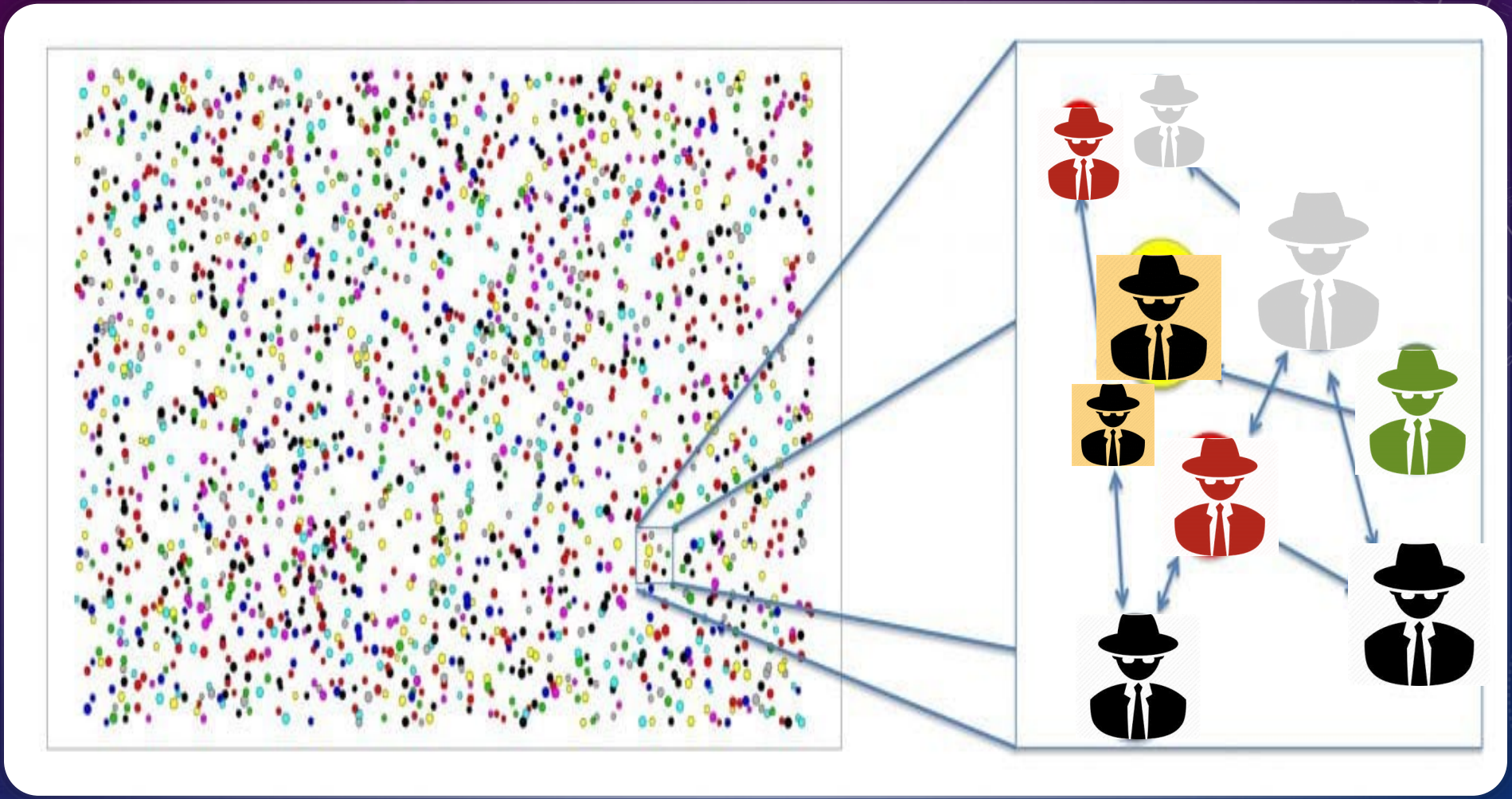


Weighted Key Drivers

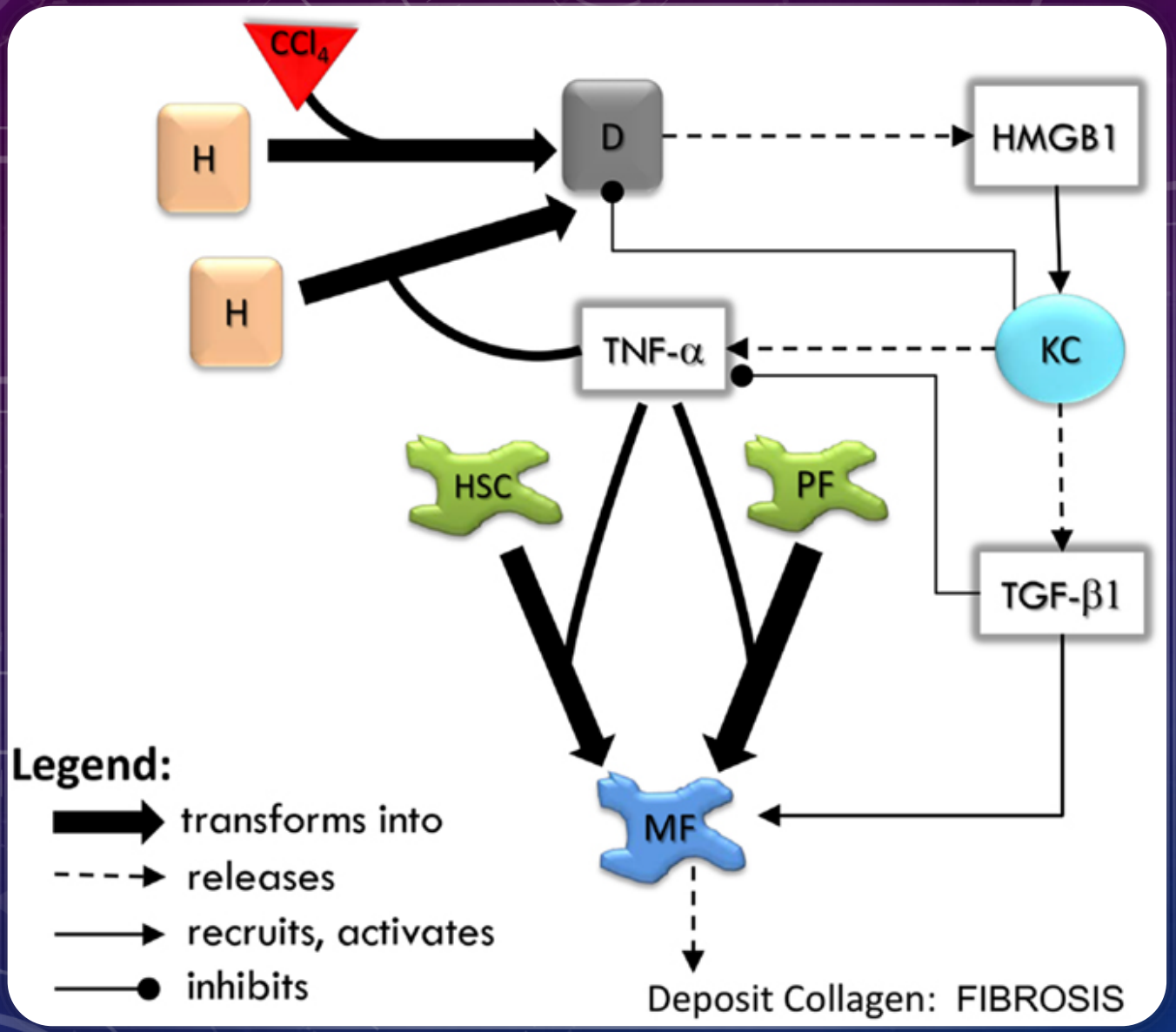
PCOLCE
COL1A1
COL6A2
FSCN1
CTGF

Drivers
COL3A1
COL6A3

QSP: AGENT BASED MODELING



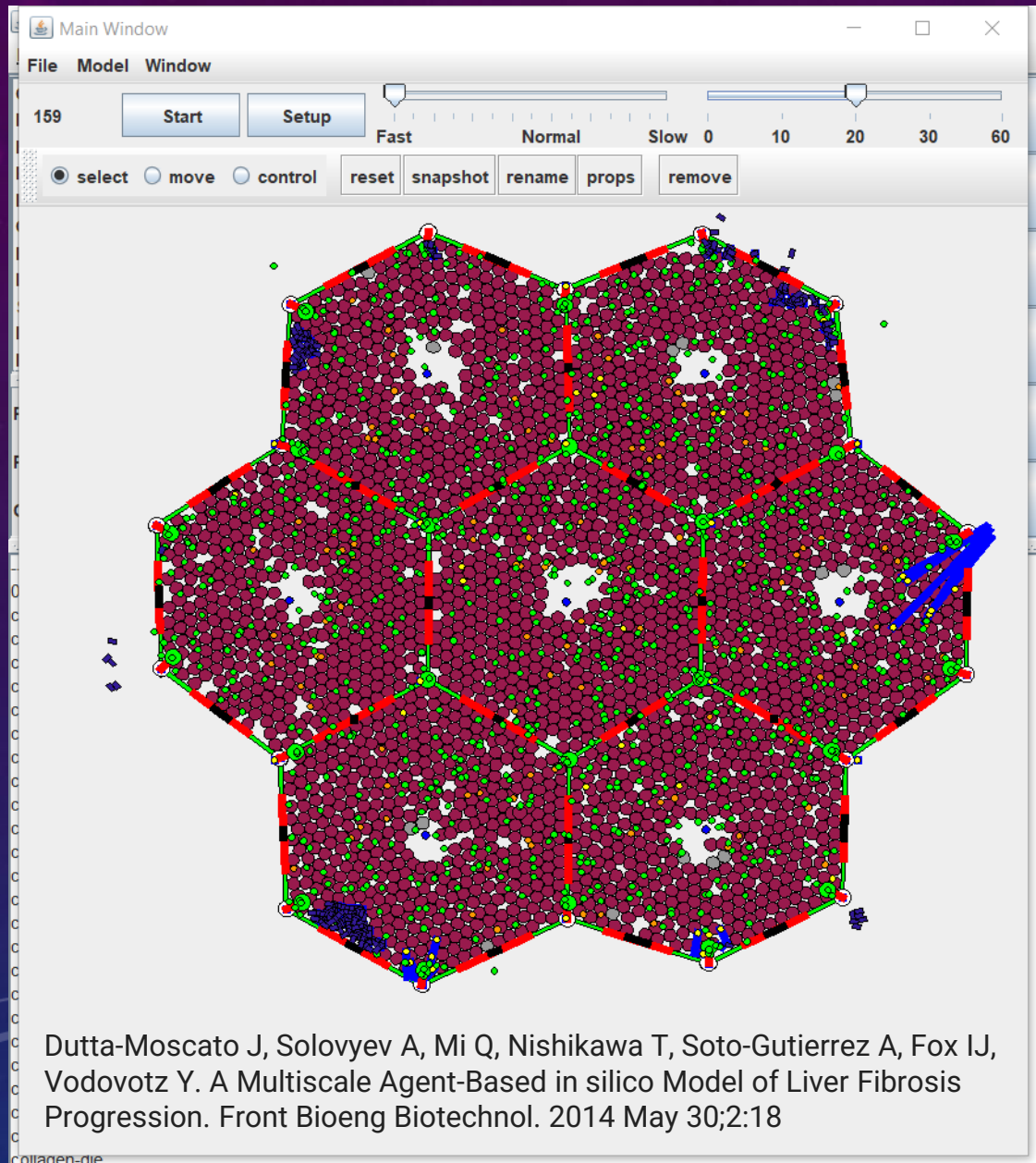
AGENTS ARE CREATED, PROCESSED AND **DESTROYED**



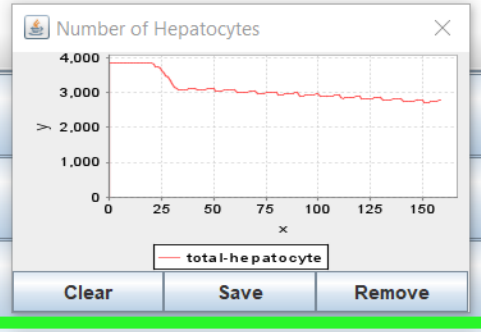
A MULTISCALE AGENT-BASED IN SILICO MODEL OF LIVER FIBROSIS PROGRESSION

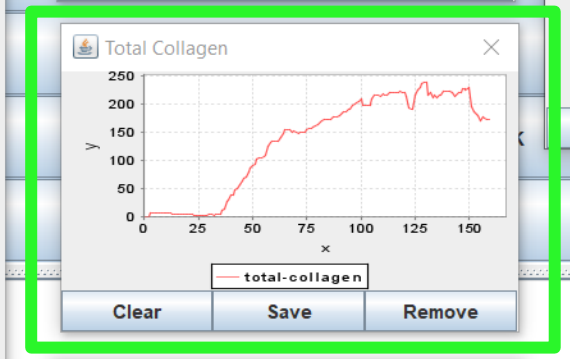
Dutta-Moscato, Joyeeta, et al. "A multiscale agent-based in silico model of liver fibrosis progression." *Frontiers in bioengineering and biotechnology* 2 (2014): 18.

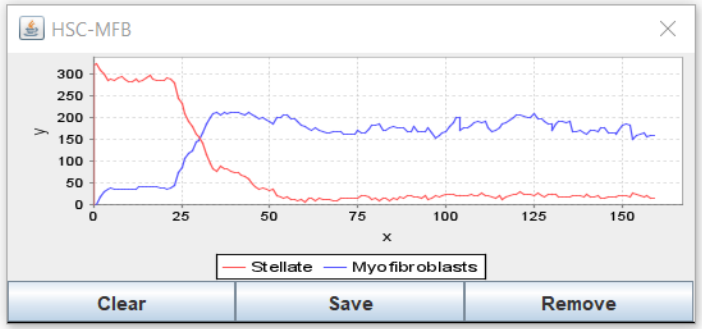
ORIGINAL CCL_4 MODEL: 159 'CLICKS'



Dutta-Moscato J, Solovyev A, Mi Q, Nishikawa T, Soto-Gutierrez A, Fox IJ, Vodovotz Y. A Multiscale Agent-Based in silico Model of Liver Fibrosis Progression. Front Bioeng Biotechnol. 2014 May 30;2:18

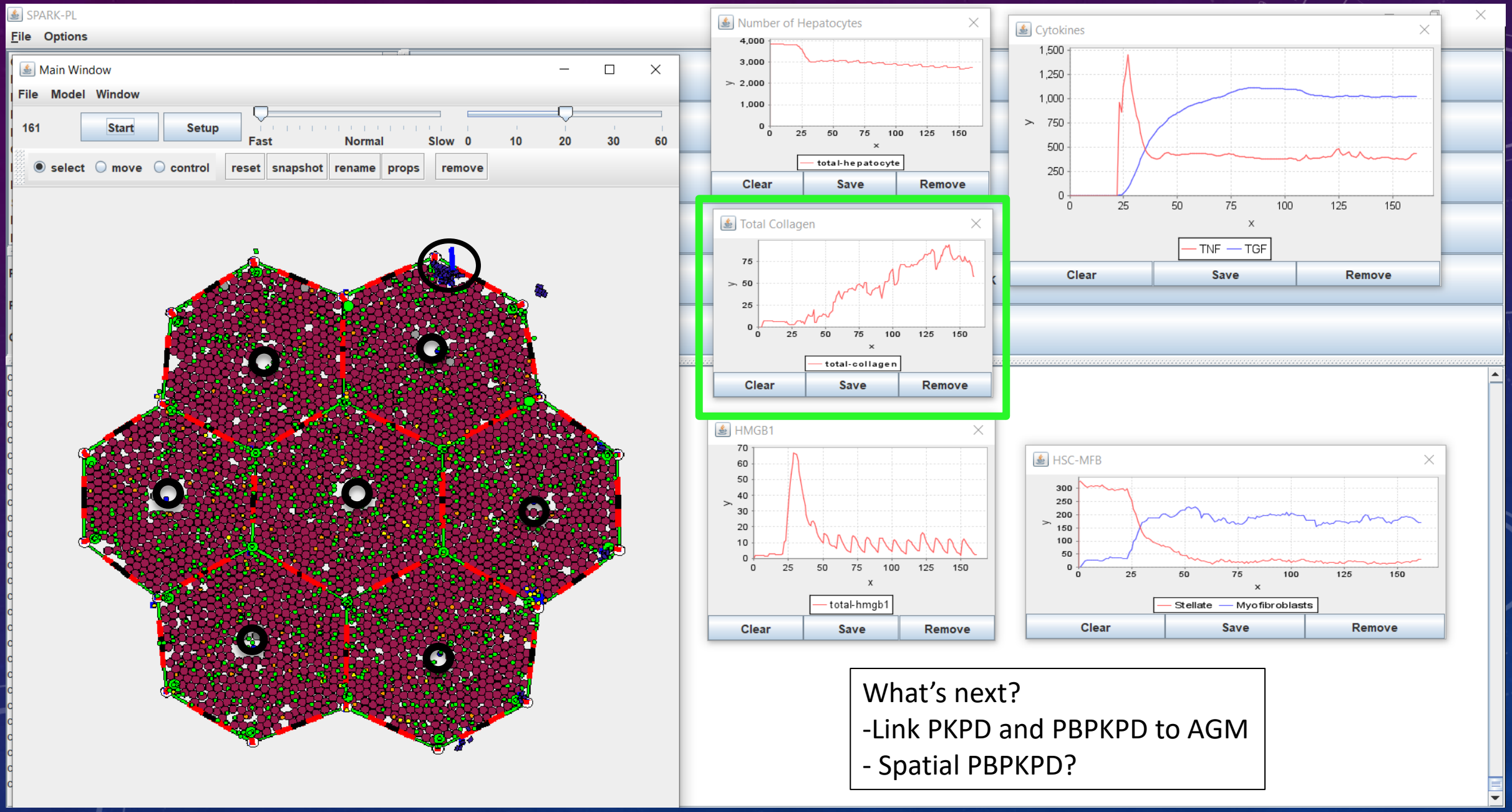






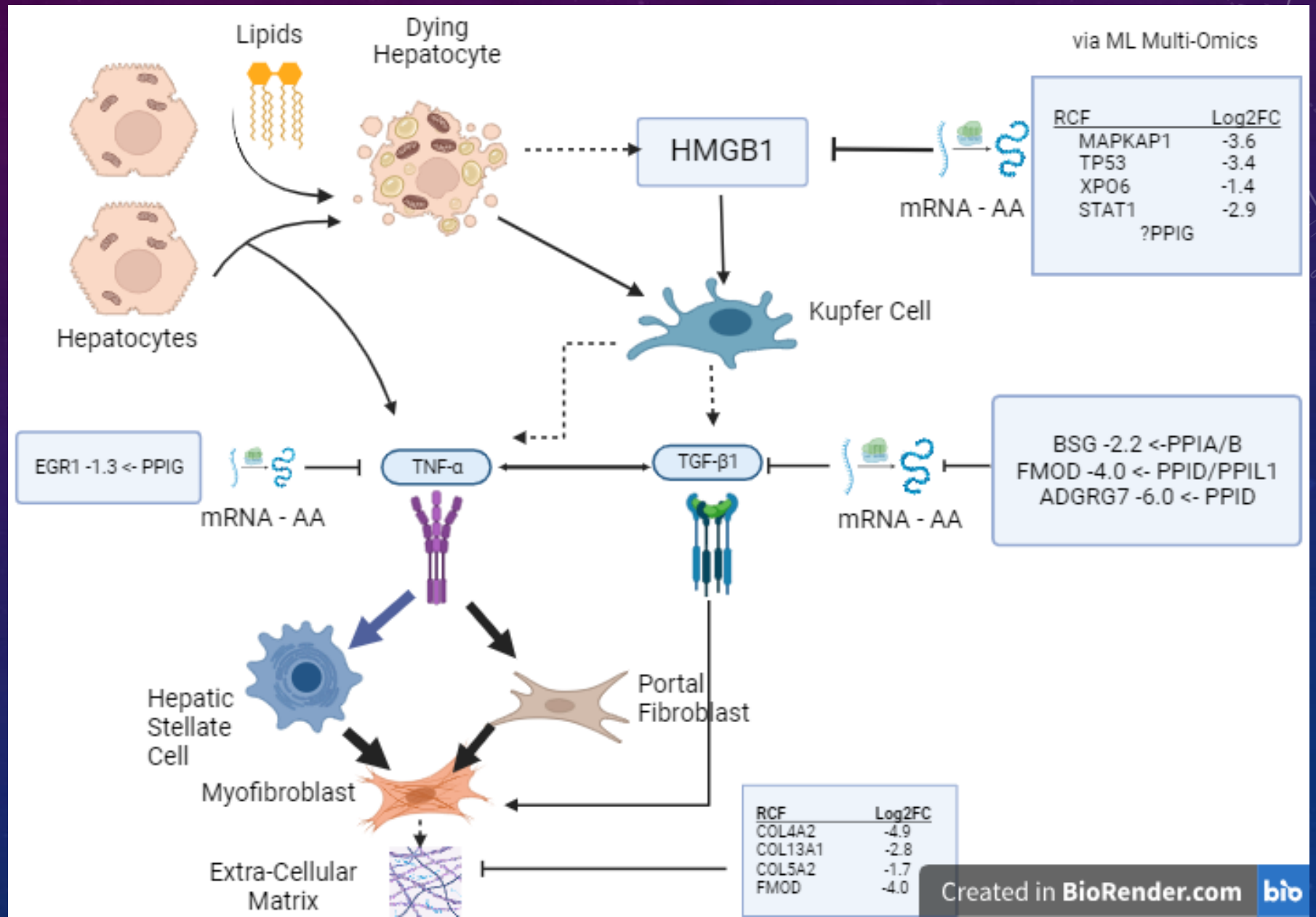
Myofibroblasts : Orange , Stellate Cells: Yellow
Dead Cells: Grey, Macrophages: Green
Collagen: Blue

RCF LIVER MODEL 1: RCF AFTER 161 'CLICKS'



What's next?
-Link PKPD and PBPKPD to AGM
- Spatial PBPKPD?

201-210 PRO-C3 RESPONDER AGM ANALYSIS



THE HEPION AI-POWR™ PROPRIETARY CLINICAL PROCESS

Extrinsic

Input
All
Data

←AI-POWR™→
[Generative ANN]
[Deep Convoluted ANN]
[Bayesian Causal ANN]

Validation
S.o.u.p
Ensemble

Output
Right Patient
Right Dose

Compare

Intrinsic

AI-POWR™

<THE DATA>

PKPD/PBPKPD/QSP
+ Bioinformatics
+Multi-omics
+ QoL
+Safety
+ Deep Learning AI
=Digital
Biomarkers
Responders

Biomarkers/Patient Selection/
Simulation
1. AI/ML popPKPD
2. AI/ML QSP
3. AI/ML PBPKPB (dev)
4. AI/ML Bioinformatics -PKPD

AI/ML 1,2,3,4
- Responder Identification
-Phase III Enrichment
-Predict Clinical Outcome
Deep Learning Individual Response Model
Drug-Disease Interaction Model

-Digital Biomarkers
-Eliminate Biopsy
-Patient Specific Dosing
-Clinical Monitoring
-Reimbursement

Phase1
(1st in Human)

Phase II
(Proof of
Concept)

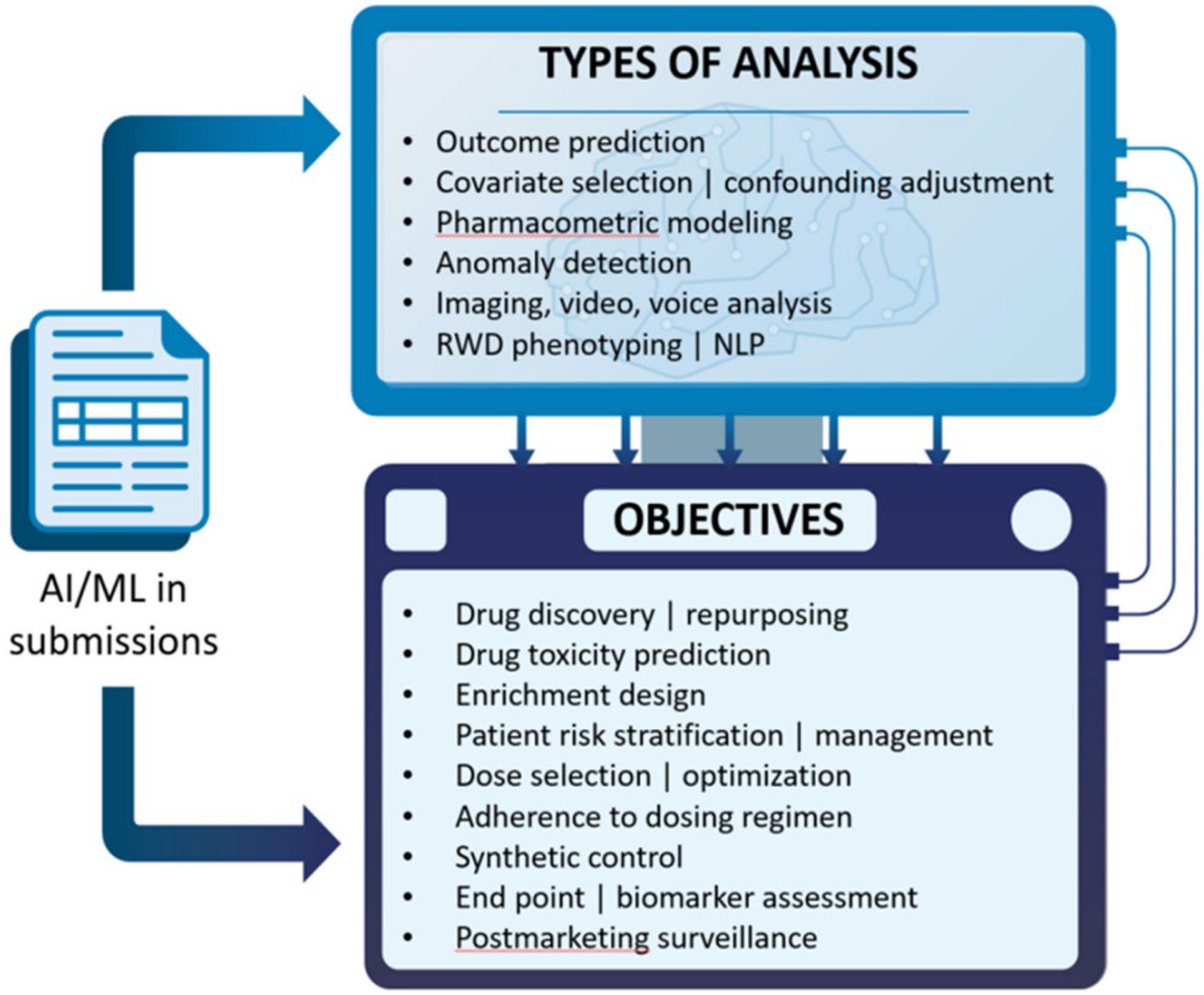
Phase III
(Multi-
Centre Trials)

Clinical
Practice

Pre-Clinical Data
Informs Development

Identify New Therapeutics via

REGULATORY PATH IS OPEN: THE FDA HAS AN AI-ML TEAM ALREADY IN PLACE FOR IND/NDA



The FDA has already assessed IND/NDA Applications with AI/ML components.

AI-POWR™ Analyses

1. Outcome Prediction
2. Covariate Selection
3. Pharmacometric Modelling
4. Real World Data Phenotyping with NLP & NN's
5. Synthetic Data Generation for Modelling & Simulation

AI-POWR™ Objectives

1. Study Enrichment
2. Dose Selection/Optimization
3. Synthetic Control
4. Endpoint|Biomarkers
5. Post-Marketing Clinical Decision Making

Liu, Qi, et al. "Landscape analysis of the application of artificial intelligence and machine learning in regulatory submissions for drug development from 2016 to 2021." *Clinical pharmacology and therapeutics* (2022).

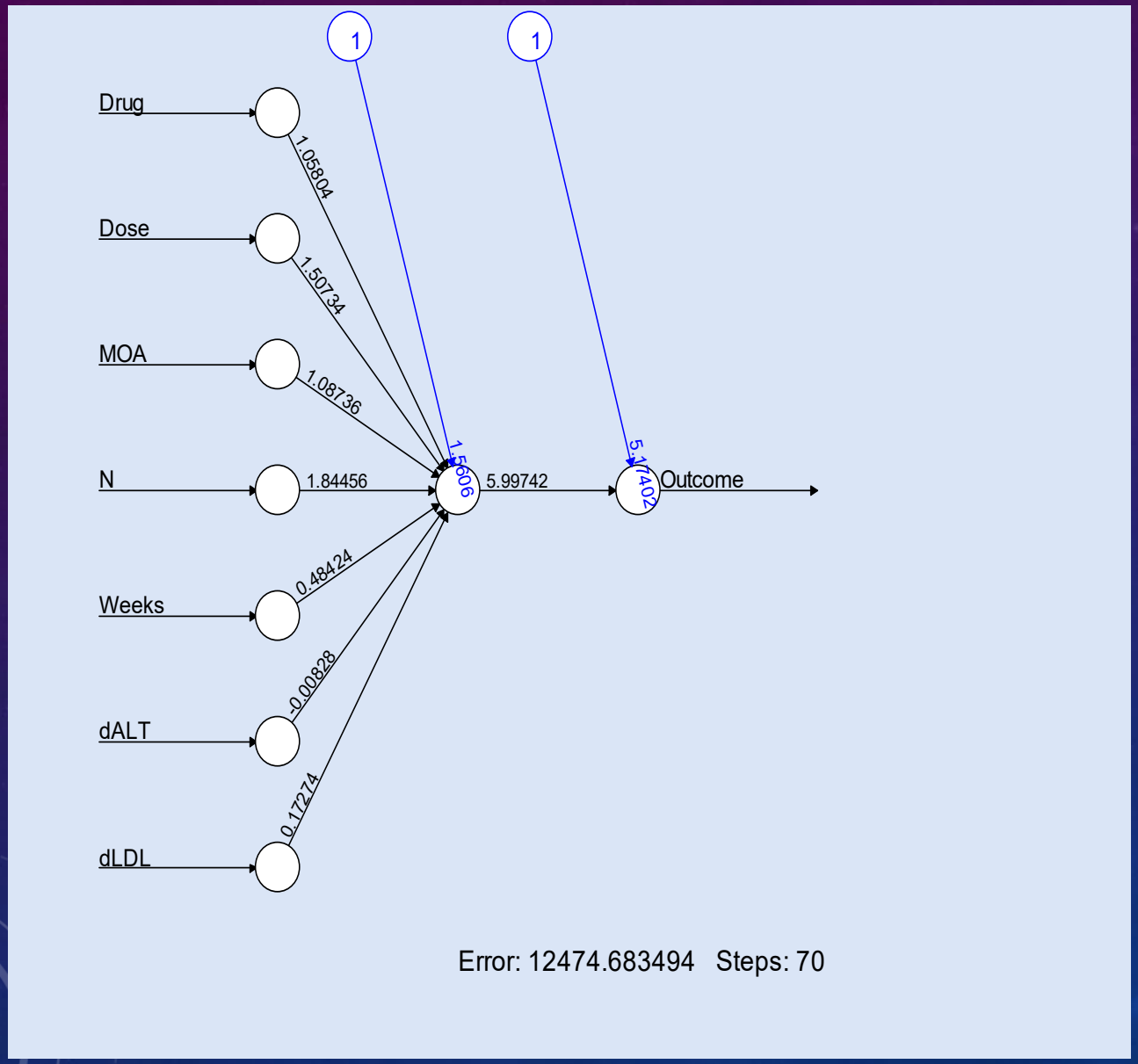
THANK YOU FOR ALLOWING US TO SHARE THE HIGHLIGHTS OF HEPION'S ANALYTICAL PATHWAYS

EXTRA SLIDES

AI-POWR™

84,710 DISCRETE DATA POINTS PER PATIENT

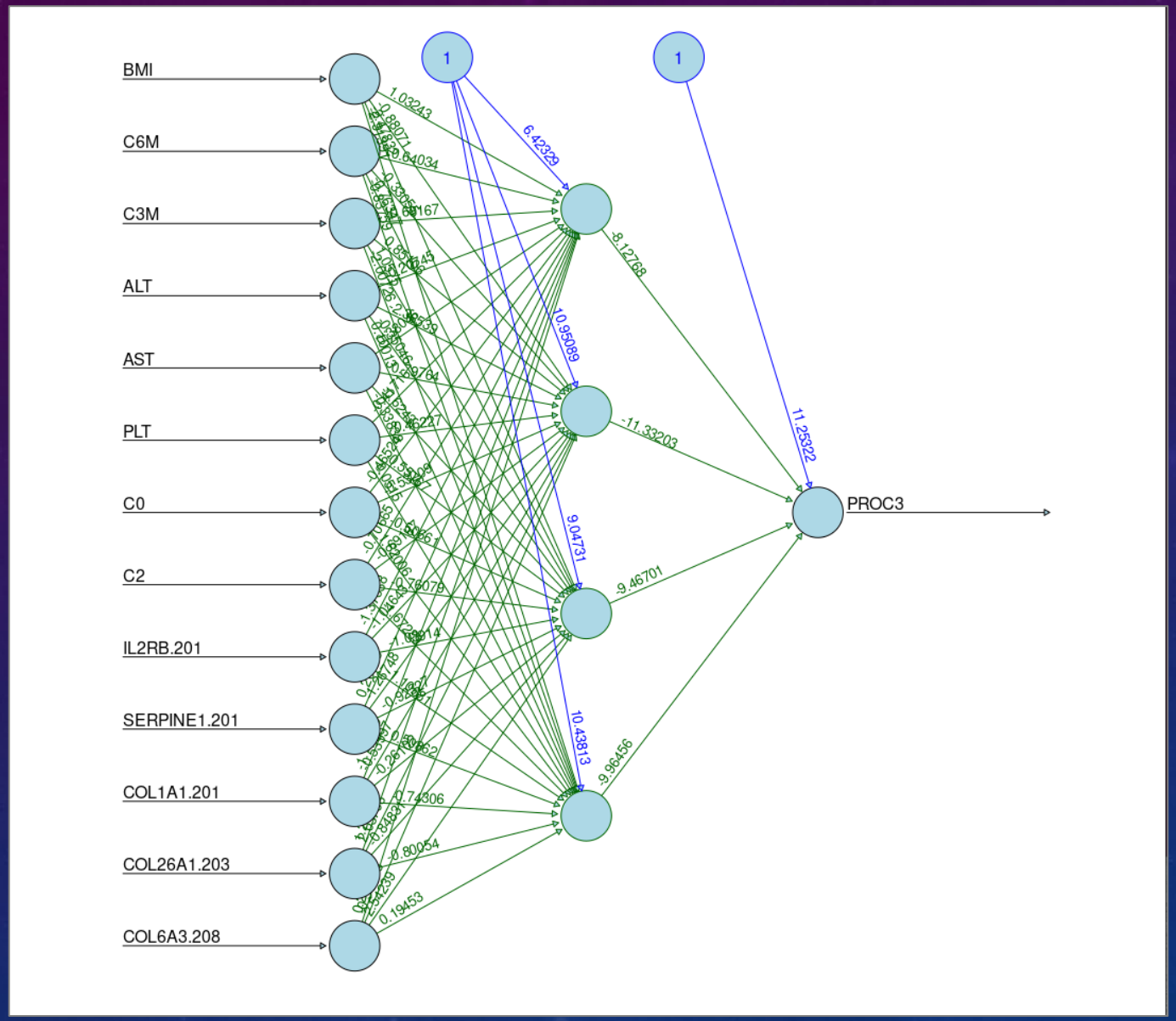
THE CONCEPT: SHALLOW ANN TO PREDICT CLINICAL OUTCOME



Limited data, small sample size

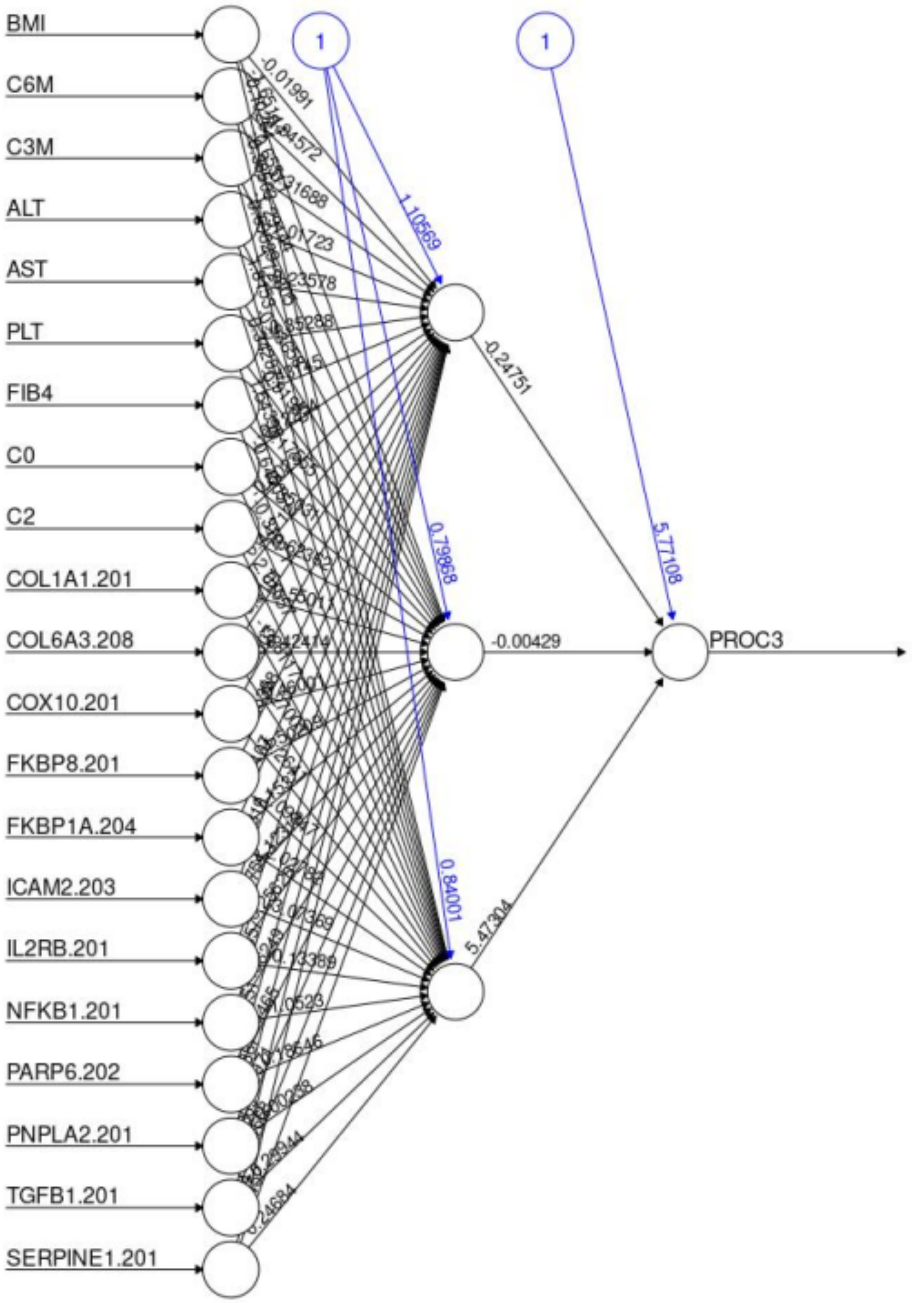
Fit: ~60% Responder vs Non-Responder

Early ANN Predicts RCF ProC3 Response



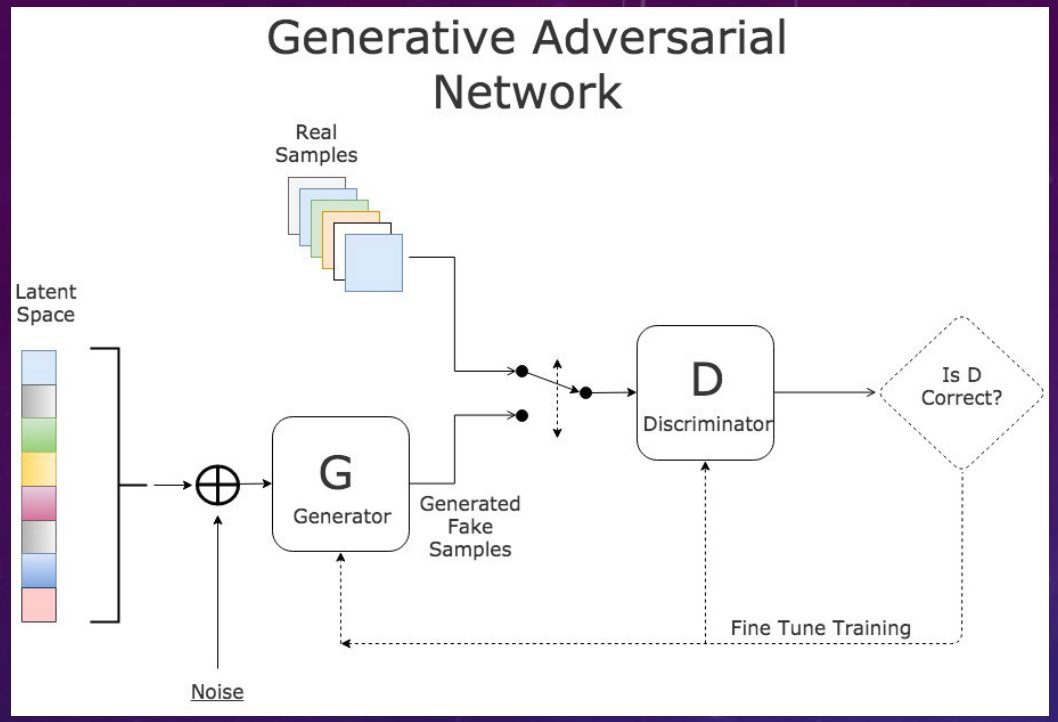
Note:
-5 Genes
-2 Diagnostic collagens
-3 Clinical labs (most scores use these)
-RCF Concentration

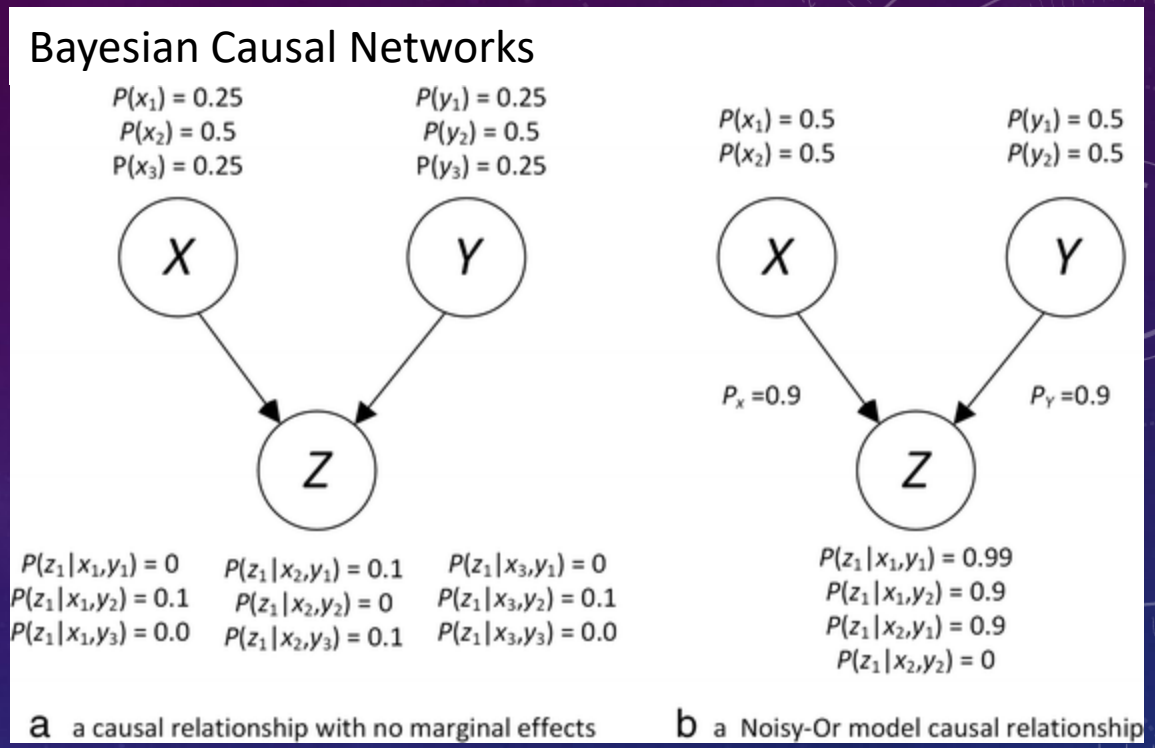
- ❑ Predicted Responders 100%
- ❑ Quantitatively Poor

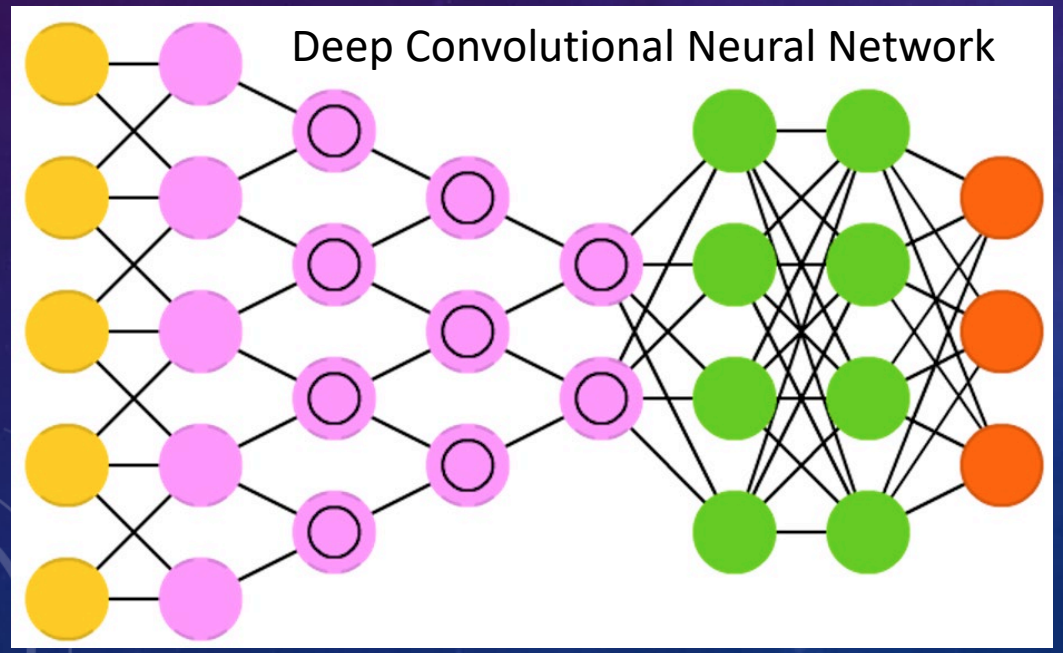


Node	WT_Hid1	WT_Hid2	WT_Hid3
Intercept Hid1	1.11	0.8	0.84
BMI	-0.02	-1.65	-0.1
C6M	-0.95	-0.66	-0.39
C3M	-0.32	-1.29	-0.7
ALT	-1.02	1.73	1.82
AST	-0.24	-0.94	0.34
PLT	-0.35	-0.92	1.54
FIB4	1.43	-0.18	-0.65
C0	-0.12	1.35	-0.32
C2	-0.8	-0.62	2.01
COL1A1	0.45	-0.55	-1.28
COL6A3	0.01	0.42	-0.27
COX10	0.38	0.46	-0.26
FKBP8	-0.25	-1.5	1.09
FKBP1A	1.21	1.15	2.03
ICAM2	1.03	-2.12	-3.07
IL2RB	-0.66	-0.26	-0.13
NFKB1	0.59	0.86	-1.05
PARP6	0.94	1.03	0.19
PNPLA2	-2.18	0.28	0
TGFB1	-2.04	-0.25	0.3
SERPINE1	1.36	0.43	0.25

INCREASING COMPLEXITY







AI-POWR™

- Biomarker Selection
- Patient Selection
- Synthetic Data Generation
- Phase IV Patient/Dose Selection
- Compare GAN v DCNN v BCN v PKPD

Regulatory Perspective of AI/ML

CDER AI

FDA's CDER has seen a rapid increase in drug regulatory submissions with AI/ML components

FDA

Count of regulatory submissions for drug development with key terms "machine learning" or "artificial intelligence" from 2016 to 2021

	Year					
Submission Type (n)	**2016**	**2017**	**2018**	**2019**	**2020**	**2021**
IND	1	1	2	5	11	128
NDA, ANDA, BLA	-	-	1	2	2	2
DDT, CPIM	-	-	-	-	1	2

	Year					
Drug Development Stage (n)	**2016**	**2017**	**2018**	**2019**	**2020**	**2021**
Discovery and Development	-	-	-	-	1	3
Preclinical Research	-	-	-	-	-	8
Clinical Research	1	1	3	5	12	118
Post-Market Safety Monitoring	-	-	-	2	1	3

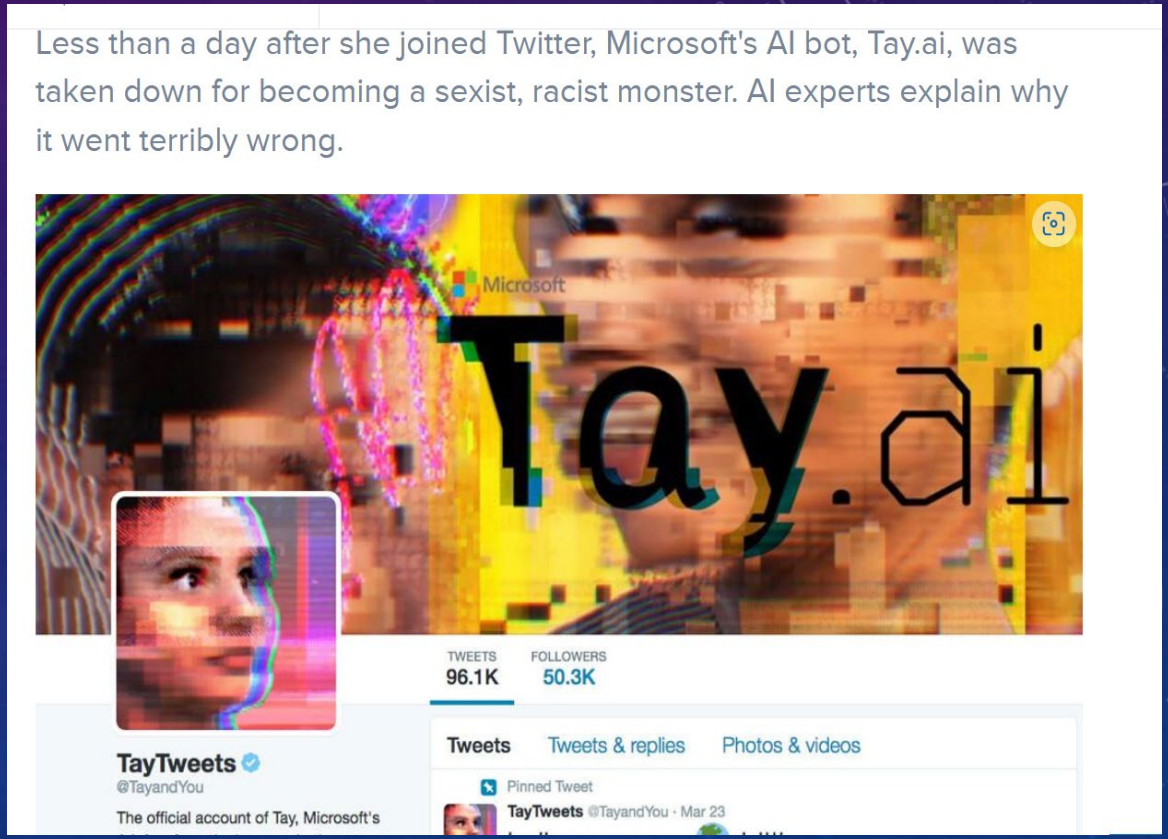
ABBREVIATIONS: Investigational New Drug (IND); New Drug Application (NDA), Abbreviated New Drug Application (ANDA), Biologics License Application (BLA); Drug Development Tool (DDT) Qualification Programs, Critical Path Innovation Meeting (CPIM)

SOURCE: Internal databases maintained by the FDA Center for Drug Evaluation and Research (CDER)

Qi Liu, R. H., Julie Hsieh, Hao Zhu, Mo Tivari, Guansheng Liu, Daphney Jean, M. Khair ElZarrad, Tala Fakhouri, Steven Berman, Billy Dunn, Matthew Diamond, and Shiew-Mei Huang (2022). Landscape Analysis of the Application of Artificial Intelligence and Machine Learning in Regulatory Submissions for Drug Development from 2016 to 2021. Clinical Pharmacology & Therapeutics. (Accepted May 2022)

Stolen from presentation: Regulatory Considerations for the Use of AI in Drug Development

AI SYSTEMS CAN AMPLIFY BIAS

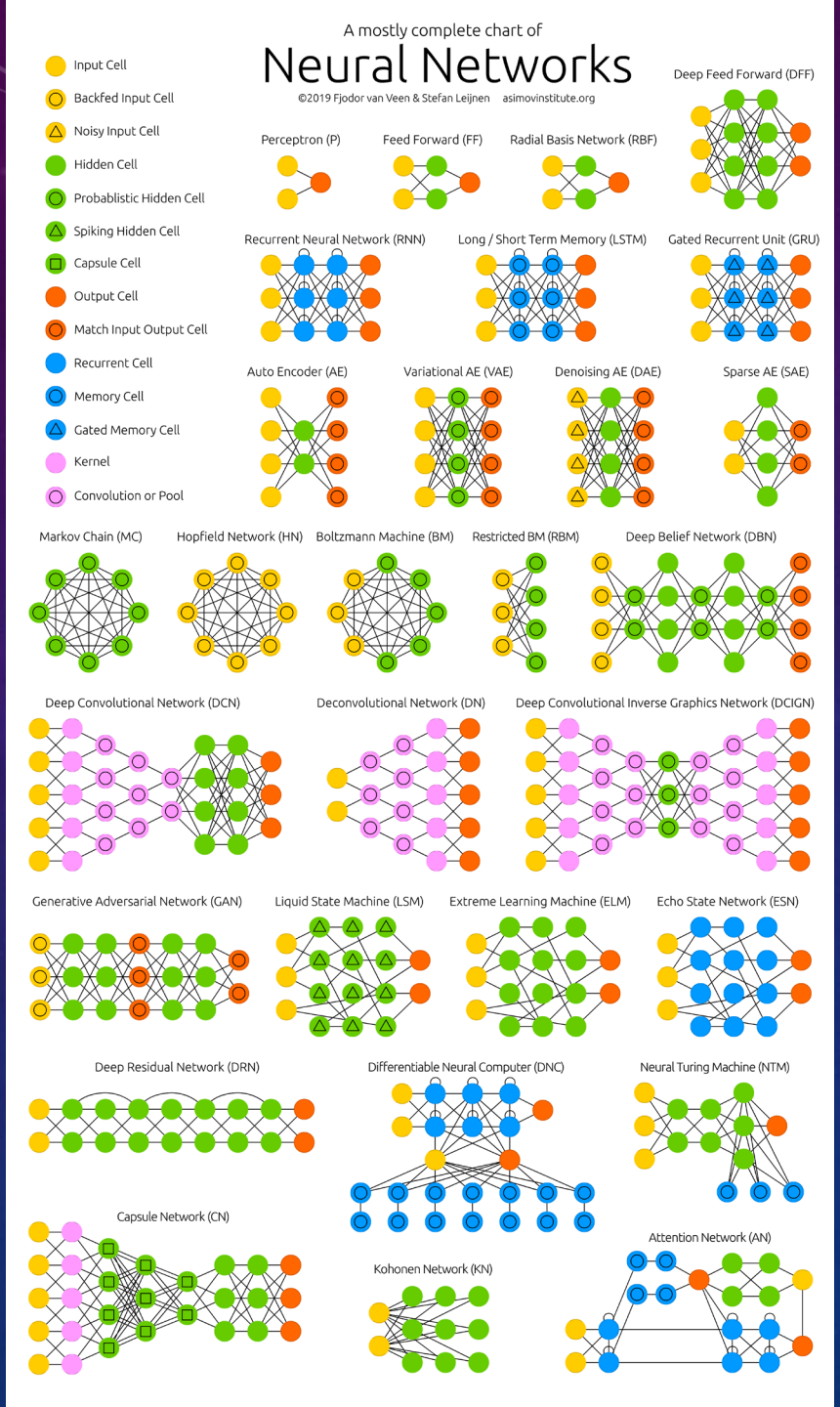


LET'S ASK AN AI FOR THE ANSWERS TO THAT

- Data quality and availability
- Integration with existing processes and regulations
- Technical challenges
- Ethical and legal concerns
- Expertise and talent shortage
- Cost and investment
- Resistance to change

POSSIBLE SOLUTIONS

- Build partnerships and collaborations
- Develop standards for data collection and analysis
- Develop expertise and talent
- Address ethical and legal concerns
- Start with small projects and scale up
- Invest in advanced technologies and infrastructure
- Promote a culture of innovation and collaboration


A mostly complete chart of
Neural Networks
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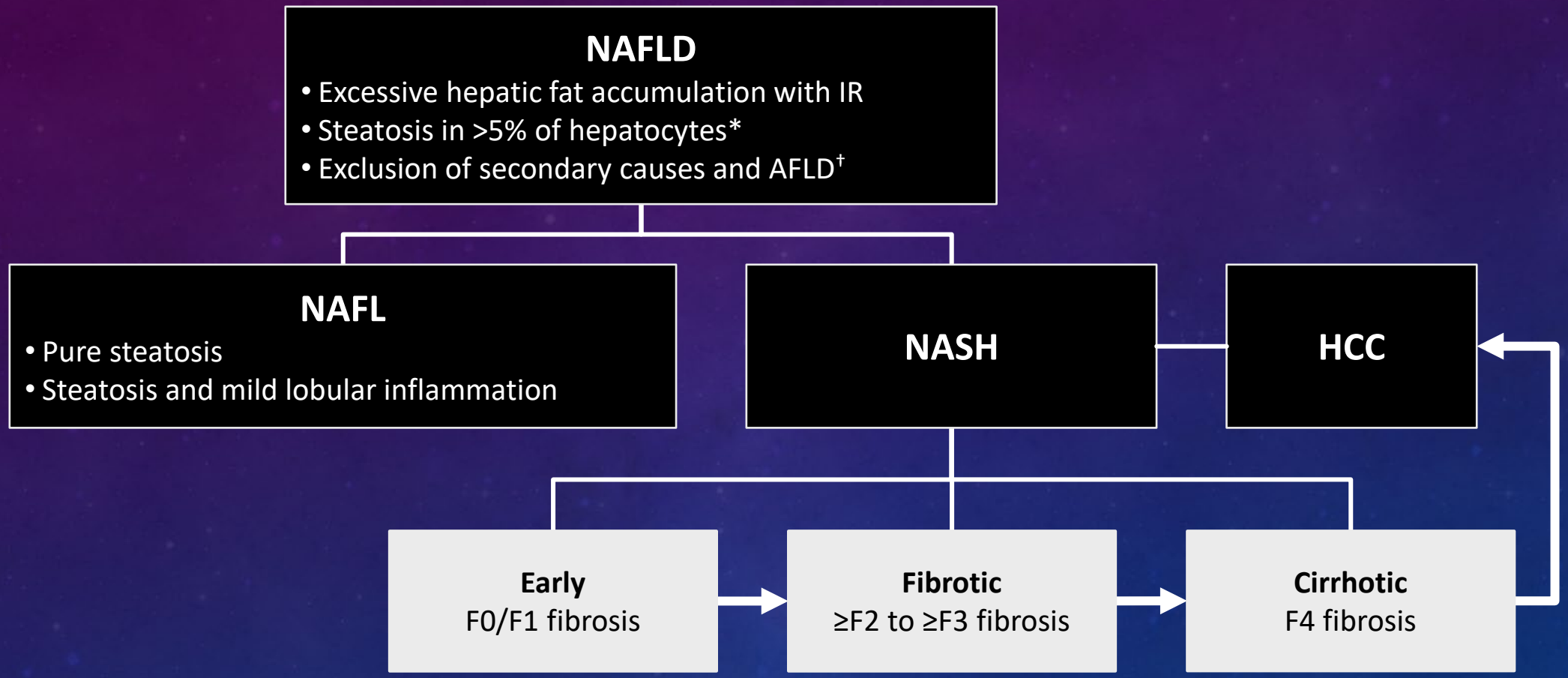
Input Cell
Backfed Input Cell
Noisy Input Cell
Hidden Cell
Probablistic Hidden Cell
Spiking Hidden Cell
Capsule Cell
Output Cell
Match Input Output Cell
Recurrent Cell
Memory Cell
Gated Memory Cell
Kernel
Convolution or Pool
Perceptron (P)
Feed Forward (FF)
Radial Basis Network (RBF)
Deep Feed Forward (DFF)
Recurrent Neural Network (RNN)
Long / Short Term Memory (LSTM)
Gated Recurrent Unit (GRU)
Auto Encoder (AE)
Variational AE (VAE)
Denoising AE (DAE)
Sparse AE (SAE)
Markov Chain (MC)
Hopfield Network (HN)
Boltzmann Machine (BM)
Restricted BM (RBM)
Deep Belief Network (DBN)
Deep Convolutional Network (DCN)
Deconvolutional Network (DN)
Deep Convolutional Inverse Graphics Network (DCIGN)
Generative Adversarial Network (GAN)
Liquid State Machine (LSM)
Extreme Learning Machine (ELM)
Echo State Network (ESN)
Deep Residual Network (DRN)
Differentiable Neural Computer (DNC)
Neural Turing Machine (NTM)
Capsule Network (CN)
Kohonen Network (KN)
Attention Network (AN)

THE PROFILE



Definitive diagnosis of NASH requires a liver biopsy

*According to histological analysis or proton density fat fraction or >5.6% by proton MRS or quantitative fat/water-selective MRI;

†Daily alcohol consumption of ≥30 g for men and ≥20 g for women

EASL–EASD–EASO CPG NAFLD. J Hepatol 2016;64:1388–402