CYTOKERATIN 18 (CK18) AS A BIOMARKER IN NASH CLINICAL TRIALS

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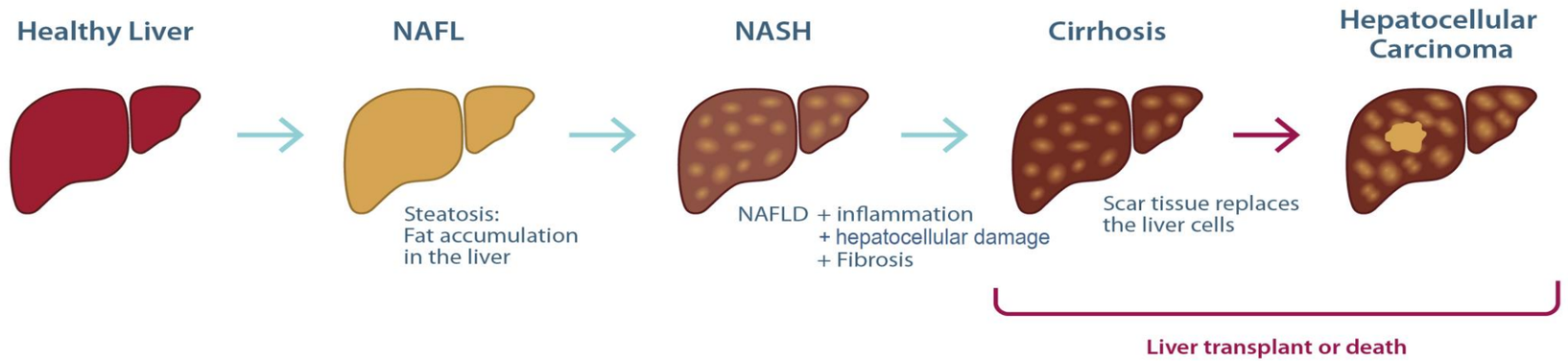
NAFLD/NASH: THE LANDSCAPE

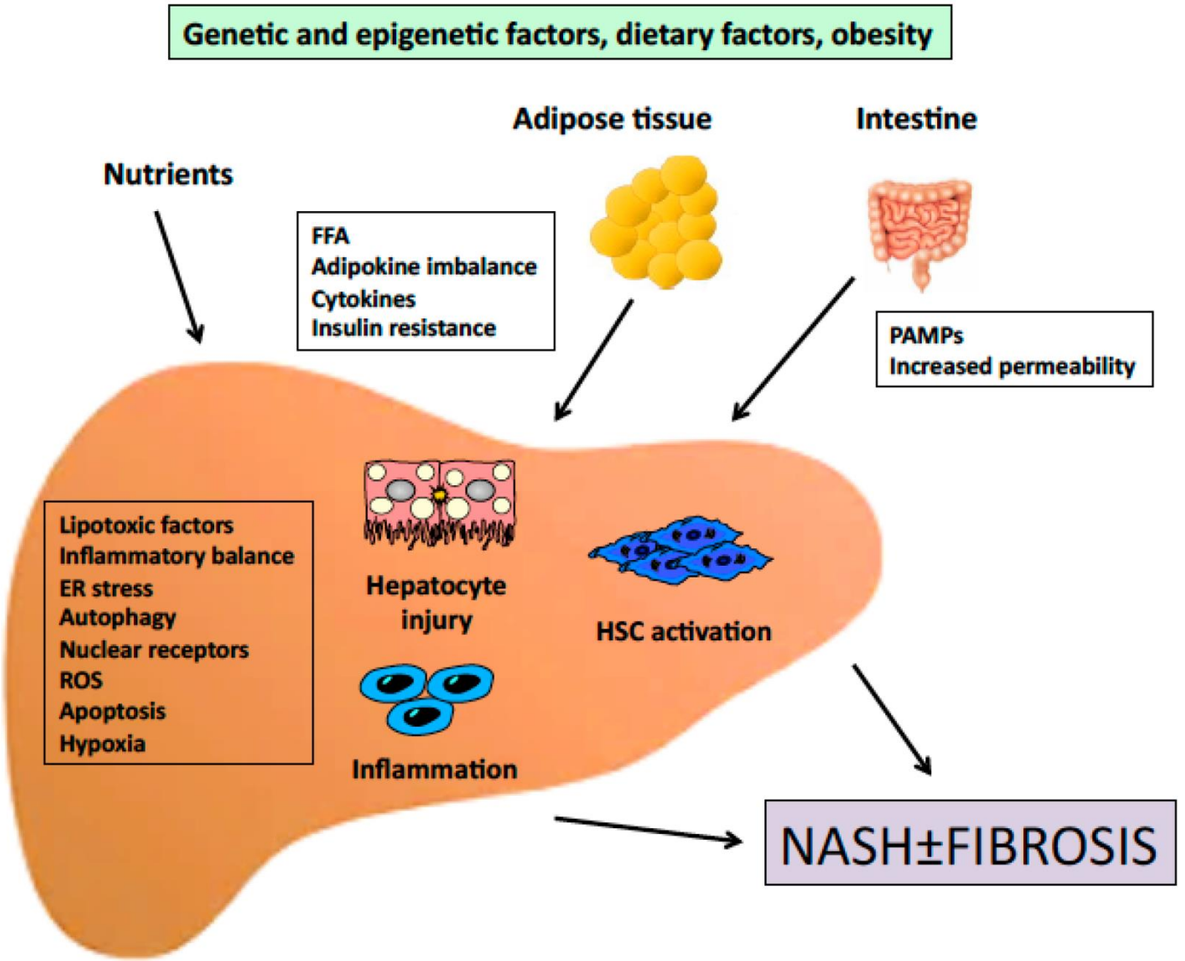


US Prevalence:	~65M	~15M	~1-3M	~70-200K

Source: cn-bio.com

- NAFLD: Non-alcoholic fatty liver disease
- NASH: Non-alcoholic steatohepatitis
- NAFLD is the most common cause of liver disorder in Western countries
- It is strongly connected to the epidemic increase of obesity and type 2 diabetes
- This year (2020), NASH is projected to overtake Hepatitis C as the leading cause for liver transplants in the U.S.

NASH PATHOGENESIS

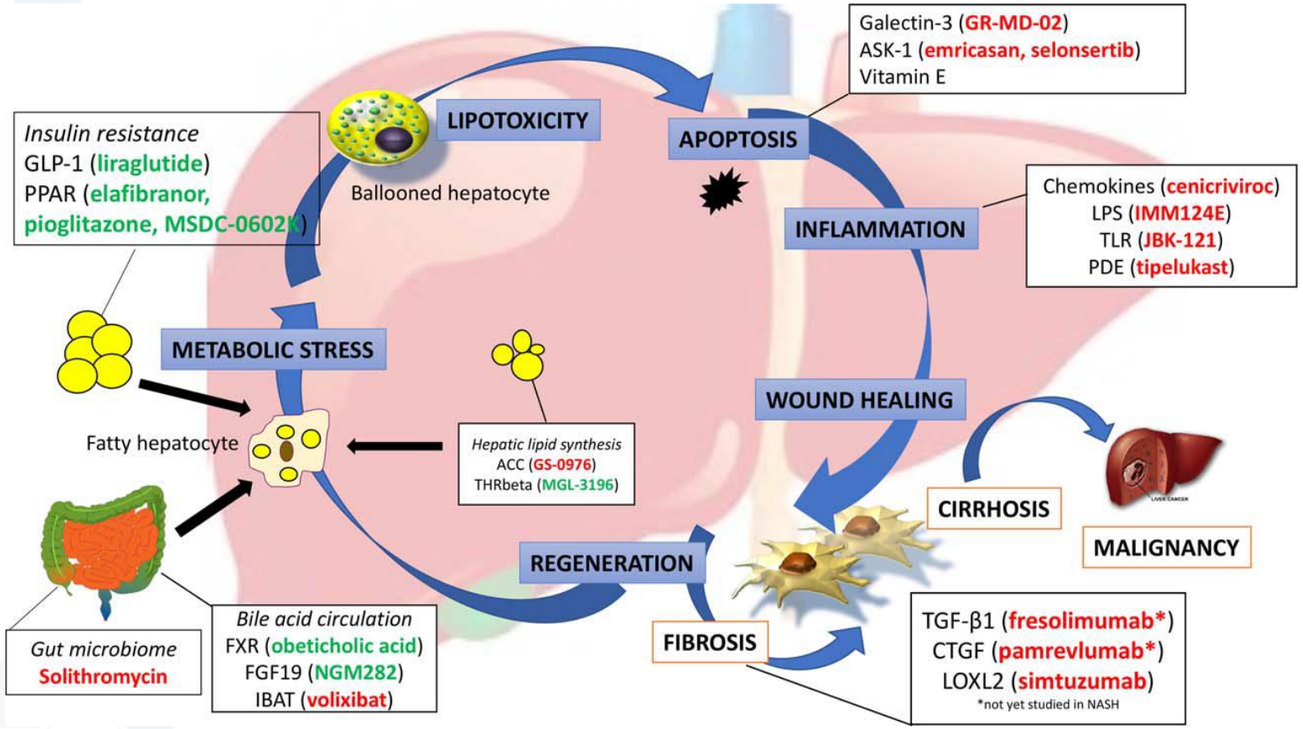


There are currently no FDA-approved pharmacologic therapies for NASH

Source: Caligiuri et al *Int. J. Mol. Sci.* (2016)

NASH CLINICAL TRIALS

NASH Therapeutic Targets



Source: Wegerman et al. *Clin Liver Dis* (2018)

NASH Drug Studies by Phase (2020)	
Phase 1	40+
Phase 2	~30
Phase 3	6

Source: NASHBIOTECHS.COM

NASH CLINICAL TRIAL ENDPOINTS

NASH CRN Scoring System:

- **NAS** = Steatosis + lobular inflammation + ballooning
- **NFS** = F0 (no fibrosis) → F4 (cirrhosis)

Surrogate endpoints for accelerate/conditional approval:

i. Resolution of NASH without worsening of fibrosis
ii. Improvement in fibrosis without worsening of NASH
iii. Both

NASH activity grade: grade = total score: S + L + B (range 0–8)

Steatosis	S score	Lobular inflammation	L score	Hepatocyte ballooning	B score
< 5%	0	None	0	None	0
5–33%	1	< 2	1	Few ballooned cells	1
34–66%	2	2–4	2	Many ballooned cells	2
> 66%	3	> 4	3		

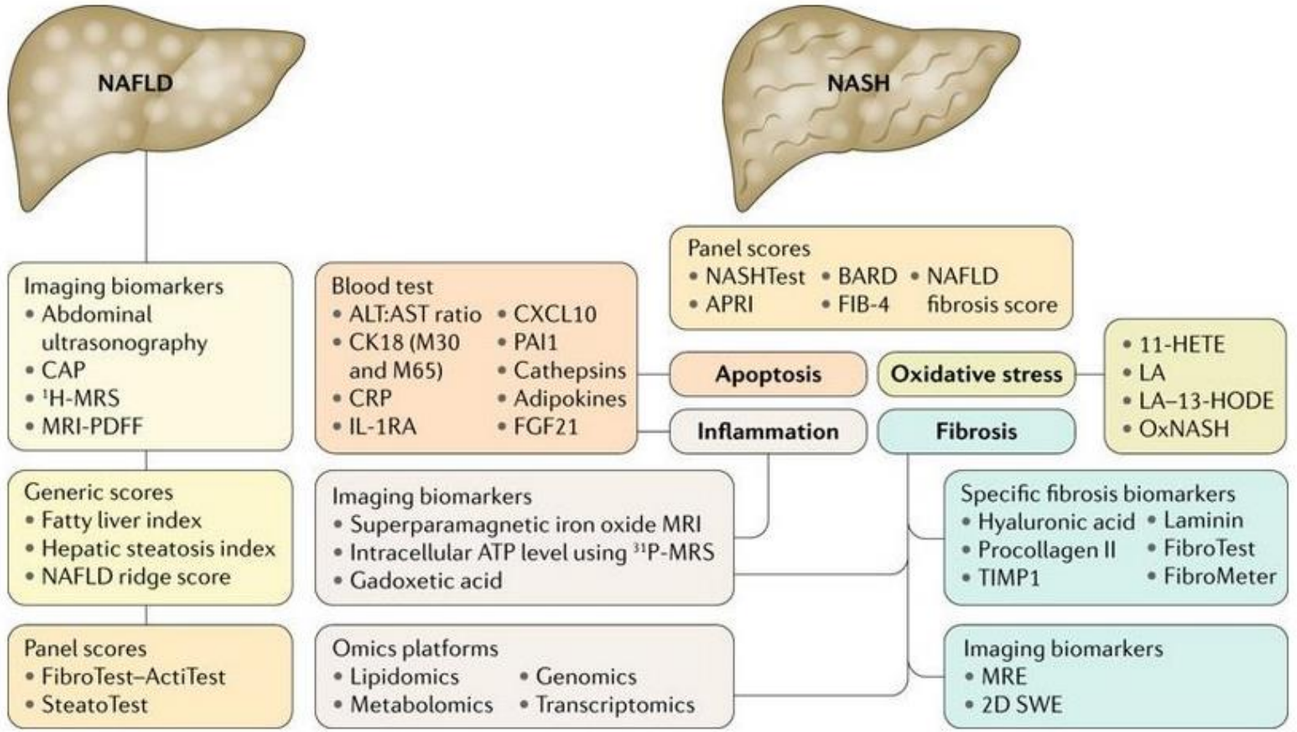
NASH fibrosis stage	Stage
None	0
Mild, zone 3 perisinusoidal fibrosis	1a
Moderate, zone 3 perisinusoidal fibrosis	1b
Portal/periportal fibrosis only	1c
Zone 3 perisinusoidal and portal/periportal fibrosis	2
Bridging fibrosis	3
Cirrhosis	4

Source: Kleiner et al., *Hepatology* 2005

NON-INVASIVE BIOMARKERS FOR NAFLD/NASH

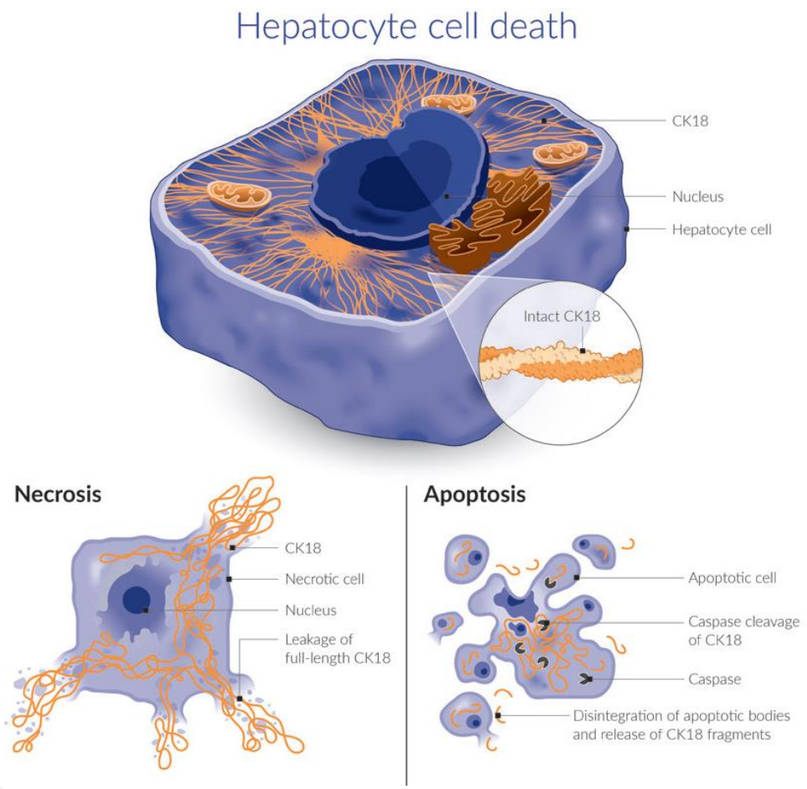
Liver Biopsy

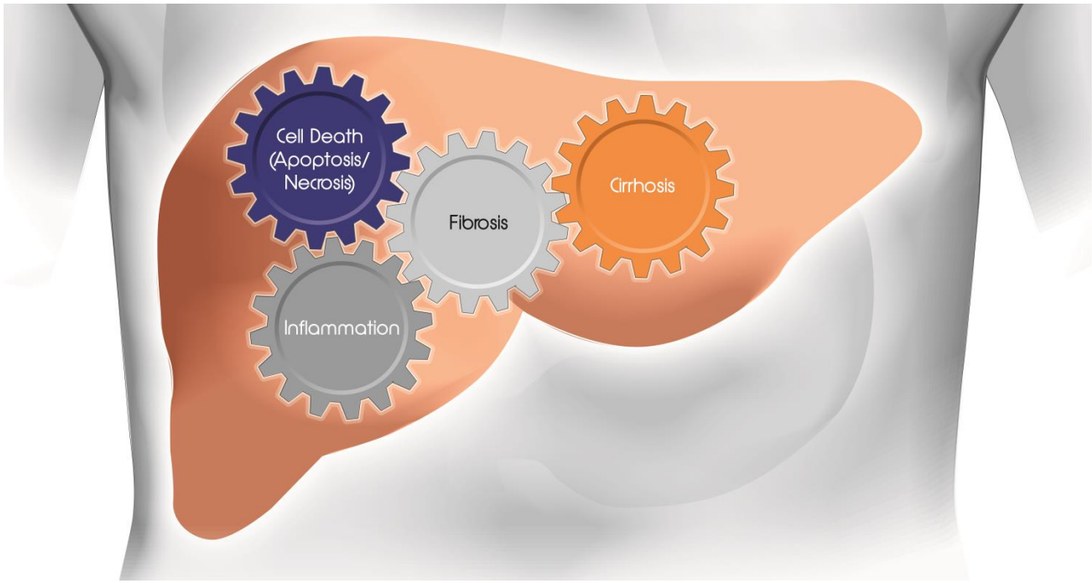
- Invasive
- Costly
- Medical complications
- Sampling errors



Source: Wong et al. *Nat Rev Gastroenterol Hepatol* (2018)

HEPATOCYTE CELL DEATH IN NASH



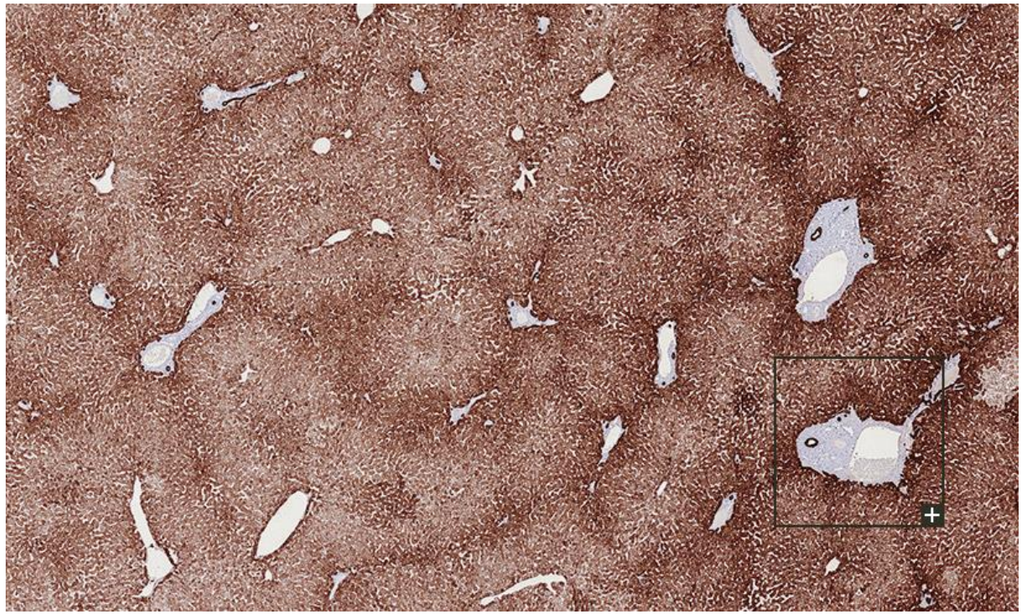


- Hepatocyte apoptosis is considered a hallmark of NASH
- Hepatocyte apoptosis contributes to inflammation, liver injury, fibrosis
- Hepatocyte apoptosis is thought to be a driver in the progression of NAFLD

CYTOKERATIN 18 (CK18) IN THE LIVER

- Cytokeratin 18 (CK18, Keratin 18, K18) is the major intermediate filament protein in the liver
- Cytoprotective function
- Present in hepatocytes and cholangiocytes
- Hepatocyte ballooning is associated with a loss of cytoplasmic CK18 immunostaining
- Component of Mallory-Denk bodies (MDBs)
- During hepatocyte apoptosis, CK18 is cleaved by caspases (9, 3, and 7)
- Upon hepatocyte cell death, CK18 is released into the blood

➢ **CK18 is a serum biomarker for hepatocyte cell death**



Normal liver showing the expression of cytokeratin 18 (KRT18) positive cells. Positive staining is seen in epithelial cells including both hepatocytes and bile ducts.

Source: proteinatlas.org

CK18 CELL DEATH ASSAYS

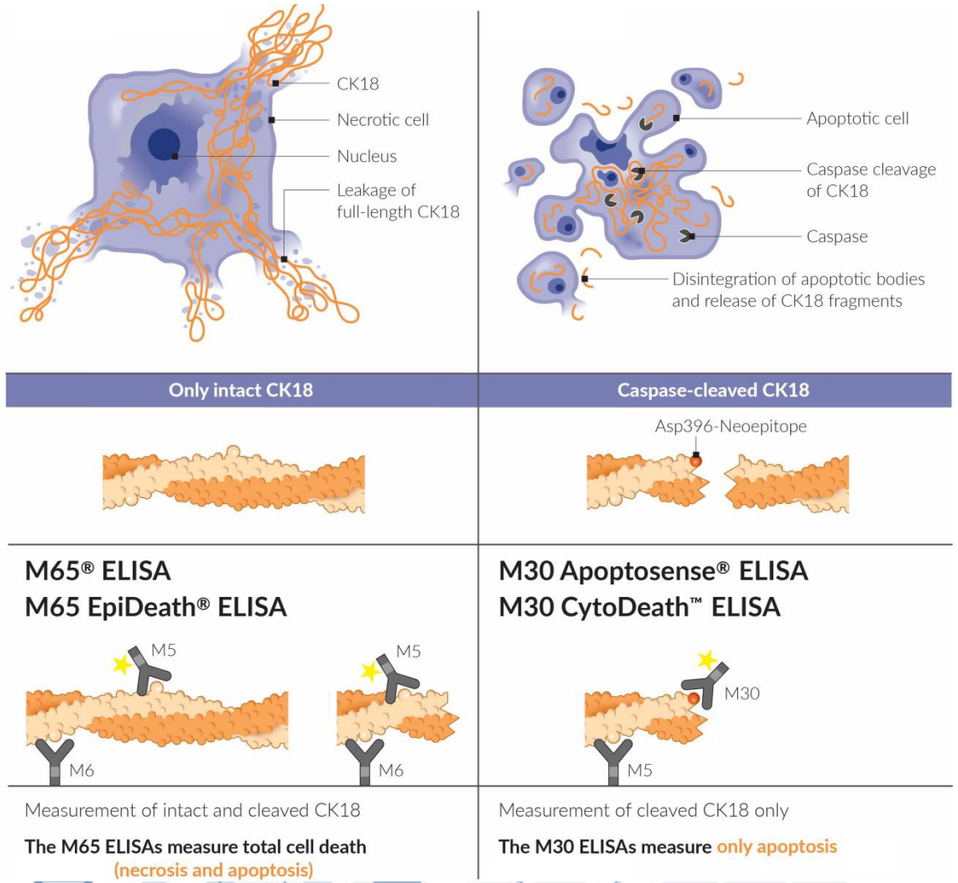
- Standard ELISA format
- Serum or plasma samples

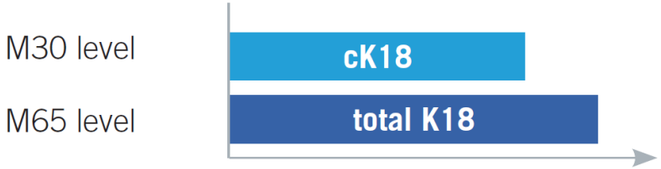
Total Cell Death (M65®)

- M65® ELISA
- M65® EpiDeath® ELISA
- M5 and M6 antibodies
- Measures total CK18
- Total cell death
  - Apoptosis and necrosis

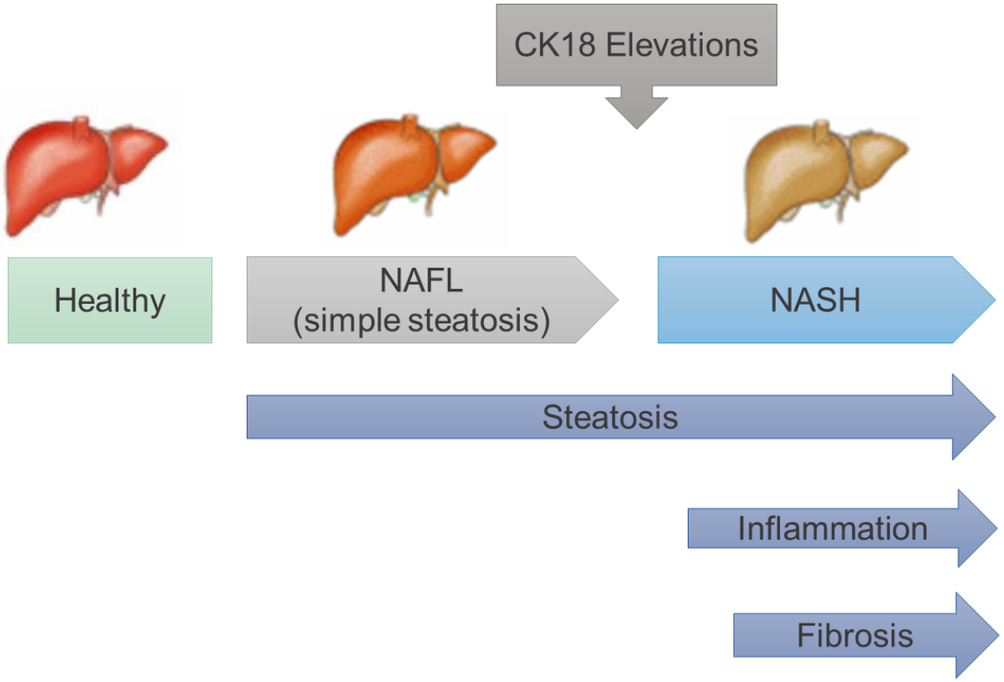
Apoptosis (M30®)

- M30® Apoptosense®
- M30® CytoDeath™
- M30® Antibody
- Measures CK18 fragments (cCK18)
- Apoptosis only





CK18 IN NASH

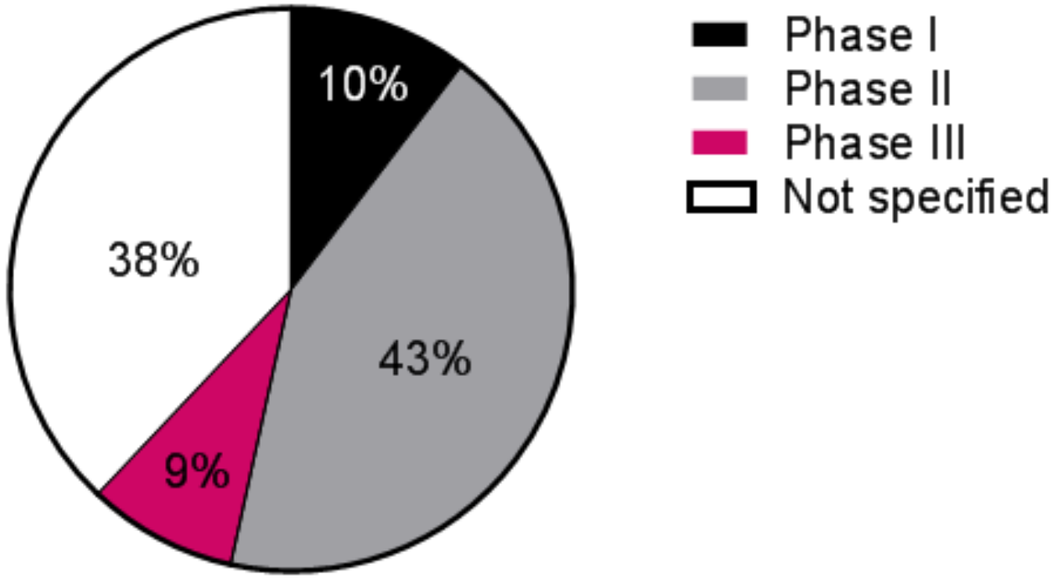


- CK18 elevations may represent active NASH
- CK18 levels have been show to increase with increases in the NAS and fibrosis stage
- **Monitoring changes in serum CK18 levels may be useful for assessing treatment response in NASH clinical trials**
- **A reduction in cell death markers (eg. CK18) was recommended as a secondary endpoint for NASH clinical trials by an AASLD-FDA Joint Workshop (2015)**

CK18 IN CLINICAL TRIALS

- 115 clinical trials have applied CK18 as a biomarker
- 60 clinical trials with a NAFLD/NASH indication

CK18 Biomarker Use in NASH Clinical Trials by Phase



Source: Clarivate, Cortellis search CK18, filtered by NAFLD/NASH studies, 7-Aug-2018

CK18 IN EARLY PHASE NASH DRUG TRIALS

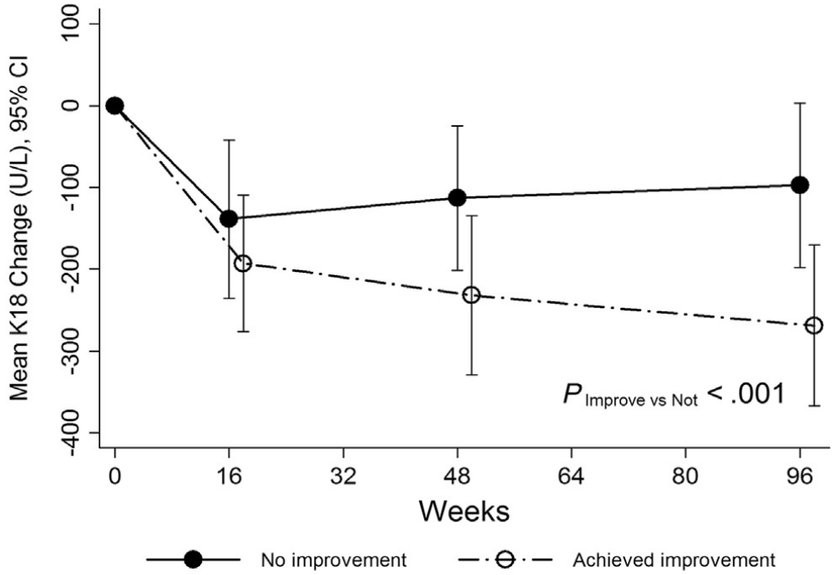
Drug	Selonsertib (Gilead)	Elafibranor (Genfit)	MGL-3196 (Madrigal)	PF-05221304 (Pfizer)	Tirzepatide (Eli Lilly)	MSDC-0602K (Cirius)	IMM-124E (Immuron)	AXA1125 (Axcella)	Emricasan (Conatus)	DUR-928 (Durect)
Mechanism of Action/Target	ASK-1 inhibitor	PPAR a/d agonist	THRbeta agonist	ACC 1/2 inhibitor	GIP/GLP antagonist	MCP modulator	Antibody, multifactorial	Metabolic modulator	Pan-caspase inhibitor	Nuclear receptor modulator
CK18 Biomarker	M30® M65®	M30® M65®	M30®	M30® M65®	M30®	M30®	M65®	M65®	M30® M65®	M30® M65®
Trial Phase	2	2b	2	2a	2b	2b	2	1	1	1
Length of study	24 weeks	52 weeks	36 weeks	16 weeks	26 weeks	26 weeks	24 weeks	12 weeks	48 hours	24 hours

Source: clinicaltrials.gov

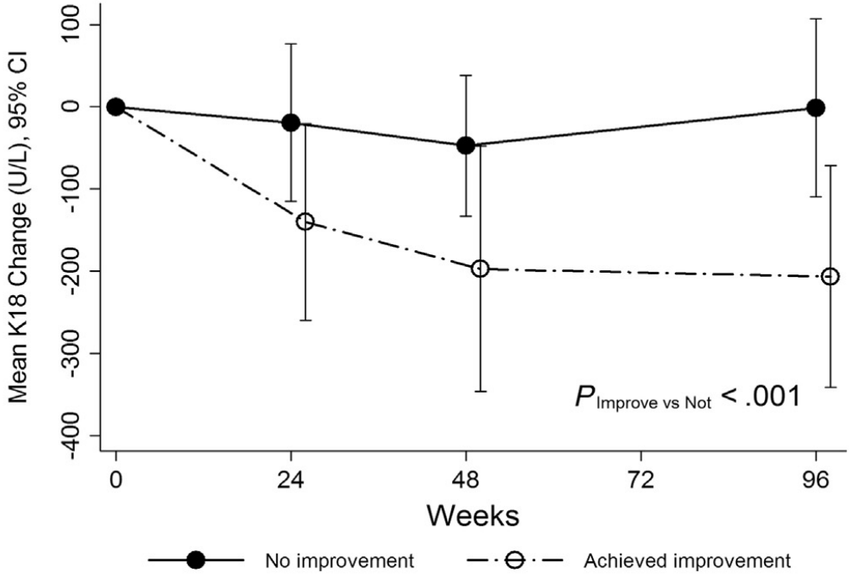
- Statistically significant changes in CK18 have been observed as early as 6 weeks of treatment
- For direct caspase inhibitors, changes can occur as early as 12 hours post-dose
- The observed time course is dependent on the mechanism of action of the drug

CORRELATION BETWEEN CHANGES IN CK18 AND CHANGES IN LIVER HISTOLOGY

PIVENS
(Vit E/pioglitazone, adults, NASH)



TONIC
(Vit E/metformin, children, NAFLD)



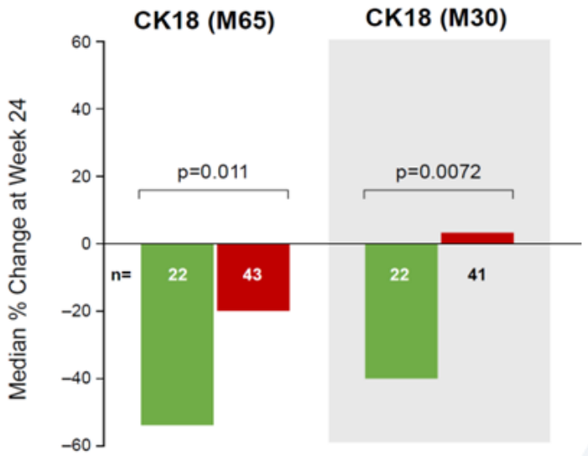
Source: Vuppalanchi et al. *Clin Gastroenterol Hepatol*. 2014

- **Decreases in CK18 were strongly associated with improved liver histology**

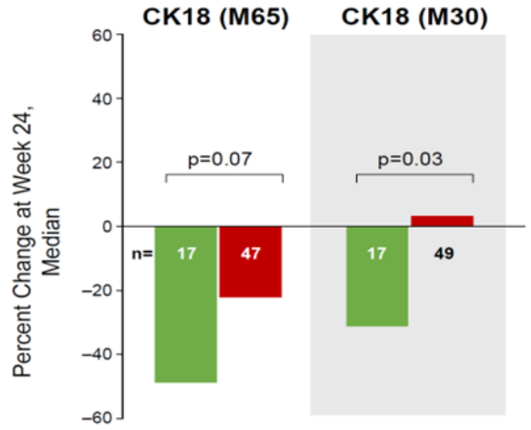
CORRELATION BETWEEN CHANGES IN CK18 AND CHANGES IN LIVER HISTOLOGY (CONT.)

- Selonsertib/simtuzimab in subjects with NASH (NAS ≥5, F 2-3)
- 24 weeks

Change in CK18 according to Fibrosis Response



Change in CK18 according to Lobular Inflammation Response



responders

nonresponders

Source: Loomba et al. *Hepatol*. (2018).

- **Histologic responders had greater reductions in CK18 (M30® and M65®)**

CK18 ASSAYS AS A PRESCREENING TOOL IN NASH CLINICAL TRIALS

- Problem: high screen-failure rate in NASH clinical trials (>55%)
  - Many screened subjects to not have NASH upon biopsy, or they do not meet study enrollment criteria (NAS ≥4 and F1-F3)
  - Adds to cost and duration of studies
- CK18 levels correlate with ballooning
- Subjects with high CK18 levels are more likely to have ballooning, inflammation, and steatosis, and therefore are more likely to have a NAS of ≥4
- **CK18 assays may be useful for prescreening subjects recruited for NASH clinical trials prior to biopsy**

SUMMARY

- NASH is a global epidemic. It is a slowly progressing chronic liver disease that can lead to cirrhosis, hepatocellular carcinoma, and other medical complications
- There are currently no approved drugs for the treatment of NASH, but many clinical trials are underway to find an effective pharmacotherapy
- One of the biggest challenges for NASH drug development is the selection of surrogate endpoints that predict clinical outcomes
- Monitoring changes in serum CK18 levels treatment may be useful for assessing treatment response, especially in early phase NASH studies
- Improvements in CK18 correlate with improvements in liver histology
- Using CK18 assays as a prescreening tool prior to biopsy may decrease the screen fail rate in NASH clinical trials

THANK YOU!

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