Keratin 18
in NAFLD clinical trials

The dead cells still count!

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NAFLD and NASH

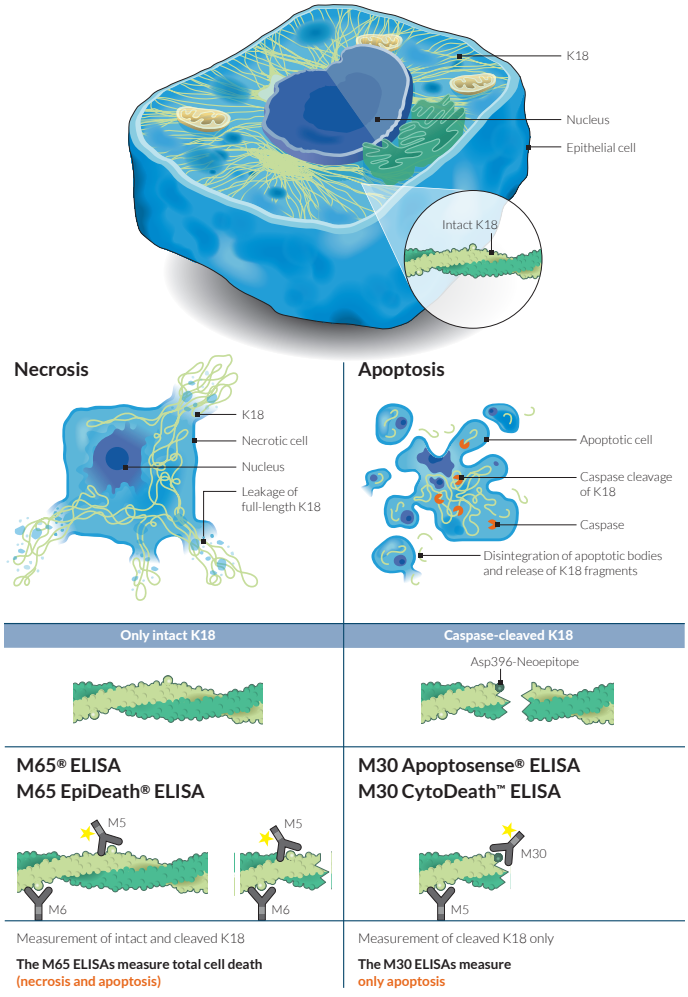
Non-alcoholic fatty liver disease (NAFLD), a condition where fat accumulates in the liver without the excess consumption of alcohol, has become the most common cause of liver disease in the Western world and is quickly rising to become the primary cause of liver transplants. Due to the rise in obesity and diabetes, NAFLD is estimated to affect 24 % of the global population, with a high prevalence on all continents. This increase is most likely related to the so-called Western lifestyle; fast food, lifestyle changes, and reduced physical activity. NAFLD starts out as steatosis which is an accumulation of fat in the liver and may further progress to non-alcoholic steatohepatitis (NASH), a more serious form of NAFLD where the liver has become inflamed. NASH is a potentially fatal condition that affects 12 % of the global adult population and can further develop into development of fibrotic tissue in the liver, with possible cirrhosis as a result.

NAFLD process

healthy liver | hepatic steatosis | steatohepatitis (NASH) | liver cirrhosis

The role of keratin 18 in NAFLD

Keratin 18 (K18) is an intermediate filament protein forming part of the cytoskeletal structure expressed in epithelial cells. Hepatocytes, a form of epithelial cell, are the main tissue cells found in the liver. When simple steatosis in NAFLD is accompanied by inflammation of hepatocytes, the disease is described as NASH. This prominent characteristic of the disease is mainly caused by hepatocyte cell death due to apoptosis and/or necrosis. Early on in the apoptosis of hepatocytes, caspases are activated which cleave the protein (K18), and the resulting fragments are subsequently released into the blood. These caspase cleaved K18 (ccK18) fragments can be efficiently quantified by the unique M30 Apoptosense® ELISA. In contrast, during hepatocyte necrosis, full length K18 may also be released into the blood without being cleaved. The M65® ELISA and the M65 EpiDeath® ELISA can quantify the amount of both ccK18 fragments and full length K18, thus measuring the amount of total hepatocyte cell death.



Did you know?

- NAFLD affects 1,9 billion people worldwide.
- There are currently no FDA or EMA approved treatments available.
- K18 is abundantly found in the liver.

VLVbio K18 assays in NAFLD Clinical Trials

Due to the high world wide prevalence of NAFLD and NASH, and the fact that there is no approved drug therapy for the disease, many clinical trials are underway to fulfill the need for pharmacological treatment options. In order to achieve clinical benefit, the required primary end-points in NAFLD clinical trials are: resolution of steatohepatitis and no worsening of fibrosis or improvement in liver fibrosis by ≥1 stage and no worsening of steatohepatitis.

There are currently two important challenges in evaluating novel therapies for NAFLD. One of which is the difficulties in cost-effective and timely enrollment of study subjects with defined disease. The other challenge is the need to use surrogate biomarkers to monitor the early effects of therapy in order to predict histologic response. The early prediction of histologic response could decrease the need for biopsies which are invasive, expensive and carry the risk of subject morbidity.

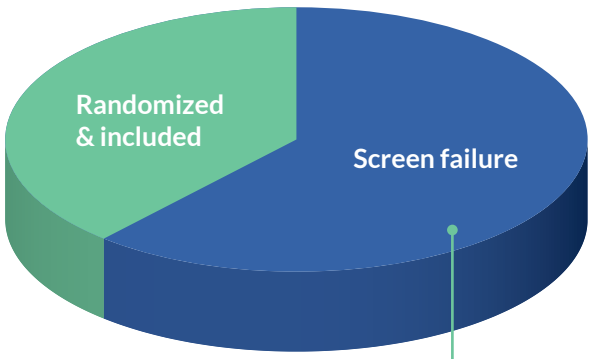
The VLVbio K18 assays; M30 Apoptosense® ELISA, M65® ELISA and M65 EpiDeath® ELISA, can aid in solving both these challenges and are currently being used in multiple active NAFLD clinical trials.

VLVbio K18 assays in Pre-Screening Subjects for Inclusion in Clinical Trials

A major challenge in studying potential therapies for NAFLD is the obtaining and pre-screening of study subjects with defined disease to be included in the trials. NAFLD clinical trials generally include subjects with fibrotic NASH, defined as a NAS of ≥4 and a fibrosis stage of F≥2. However, up to 50 % of screened subjects will not meet these eligibility criteria when assessed by liver biopsy. Traditional biochemical indicators of NAFLD may be insufficient to identify disease severity leading to the preliminary inclusion of subjects lacking the required, defined characteristics of the disease. This then leads to many subjects selected for the trial being later excluded based on biopsy findings. This adds significantly to the cost and duration of clinical trials, while causing unnecessary pain and morbidity in the subjects.

Liver cell injury is a major mechanism in NAFLD progression and the VLVbio K18 assays have been shown to correlate with steatosis and inflammation, while being elevated in NAFLD patients. Thus the VLVbio K18 assays may be used as a pre-screening tool to identify subjects with progressive disease, speeding patient recruitment and reducing the number of biopsies.

Screen failure in Therapuetic Trials



- Liver biopsy not adequate
- Histological inclusion criteria not reached

- There is a need for non-invasive methods of pre-screening in NAFLD trials.
- The VLVbio K18 biomarkers have potential for selecting and stratifying subjects with fibrotic NASH.
- The VLVbio K18 assays can reduce the need for biopsies in NAFLD trial pre-screening.

Example 1:
ccK18 has been used in several NASH trials as a pre-screening tool to select and stratify subjects for NAFLD clinical trials [1, 2]. For example, Boursier *et al.* developed and evaluated a biomarker panel, MACK-3, containing ccK18 as a tool to select subjects with fibrotic NASH.

The authors state:
"The new blood test MACK-3 accurately diagnoses fibrotic NASH. This new test will facilitate patient screening and inclusion in NAFLD therapeutic trials and will enable the identification of patients who will benefit from the treatments once approved." [2]

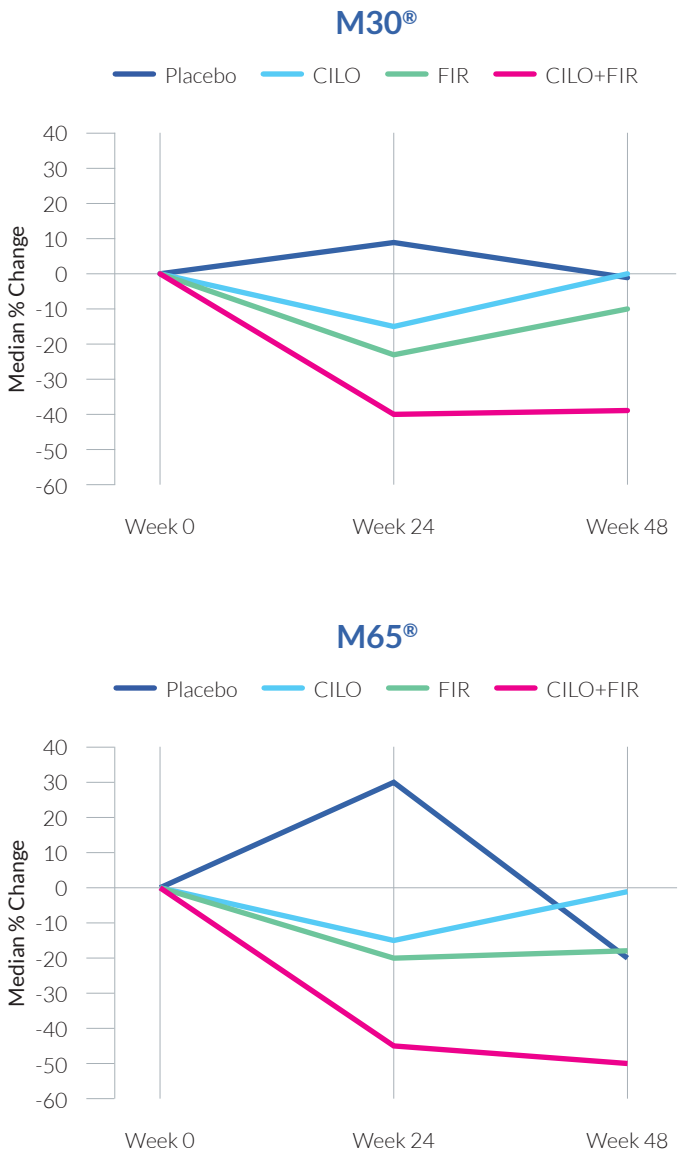
VLVbio K18 assays as an Endpoint in Clinical Trials

Histological improvement is the gold standard in NAFLD therapeutic trials. However, it is of importance to use biomarkers of hepatic injury in order to predict histological improvement as it can take a long time to occur. Furthermore, early phase studies are generally of limited duration (12 to 18 months), and histological endpoints may be difficult to meet in this timeframe. Sanyal *et al.* highlight the need for better diagnostic and therapeutic modalities for NASH, recommending a decrease in markers of hepatic injury or cell death such as the VLVbio K18 assays, as a secondary endpoint for early phase NASH trials[3].

The VLVbio K18 assays may be useful as a cost-effective surrogate biomarker to demonstrate response to treatment, providing a clearer picture of the subjects' status at the start of therapy, and to enable the results of therapy to be seen earlier and more clearly. The VLVbio K18 assay levels have been shown to correlate with improvement in histology in many published NAFLD clinical trials.

VLVbio K18 assays in clinical trials:

- Can provide a serological biomarker of therapy response.
- Can differentiate responses obtained with active compounds from placebo.
- Can potentially distinguish between more and less effective combination therapies.
- May indicate therapeutic response in advance of changes in biopsy scores.
- Are translational biomarkers, applicable to in-vitro, rodent and human studies.



Adapted from Kowdley *et. al.* AASLD, 2020

Example 2: The ATLAS Trial
Loomba *et al*[4]. and Kowdley *et al*[5]. presented the results from the ATLAS trial where 392 Patients with advanced liver fibrosis were randomized to receive: placebo; or combination therapy with either Cilofexor+Selonsertib, Firsocostat+Selonsertib, or Cilofexor+Firsocostat for 48 weeks.

Findings:
At 48 weeks, changes in baseline levels compared with placebo of serum ccK18 and total K18, measured by the M30 Apoptosense® ELISA and the M65® ELISA respectively, were more significant for subjects treated with the Cilofexor + Firsocostat combination than monotherapies. This group also had a significantly higher proportion of subjects with a ≥2-point reduction in their NAS.

Changes in serum ccK18 and total K18 levels at 48 weeks compared with baseline were greater for therapies containing Firsocostat, both alone and in combinations.

These results indicate the potential for serum ccK18 and total K18 values to distinguish between the relative effectivity of different therapy combinations.

Kowdley et al state:
"Reductions in these biomarkers of cell death were greatest in histological responders, suggesting that plasma CK18 M30® and M65® are potentially useful markers of treatment response."

Example 3: Resmetirom (MGL-3196) for the treatment of non-alcoholic steatohepatitis: a multicentre, randomised, double-blind, placebo-controlled, phase 2 trial

Harrison *et al*[6] presented the results from a phase II NAFLD trial where 84 adults with biopsy confirmed NASH and a hepatic fat fraction of at least 10 % at baseline were randomly assigned to receive daily Resmetirom or placebo. Hepatic fat measurements were made at weeks 12 and 36, and a second liver biopsy was obtained at week 36.

The authors state:

"Serum cytokeratin-18 fragments, detected using the M30 Apoptosense® ELISA, and which might reflect hepatocyte apoptosis, were statistically significantly reduced within groups (weeks 12 and 36) and relative to placebo at week 36."

Example 4: Relationship Between Changes in Serum Levels of Keratin 18 and Changes in Liver Histology in Children and Adults With Nonalcoholic Fatty Liver Disease

Vuppalanchi *et al*[7]. demonstrated the results from two separate randomized NAFLD clinical trials. ccK18 was measured using the M30 Apoptosense® ELISA in serum samples collected at baseline and various time points from adults with NASH and children with NAFLD. Liver biopsy specimens were collected at baseline and week 96.

Findings:

- ✓ There were greater decreases in serum levels of ccK18 in adults with histologic improvement at week 96.
- ✓ There were greater decreases in serum levels of ccK18 in children with histologic improvements at both week 48 and week 96.
- ✓ Decreases in serum ccK18 values are associated strongly with improved liver histology in adults or children with NAFLD.

Example 5: Obeticholic Acid (OCA) Improves Noninvasive Markers of Fibrosis in Patients With Nonalcoholic Steatohepatitis (NASH): A Secondary Analysis of the Phase 3 REGENERATE Study

Anstee *et al*[8]. presented the secondary analysis results of the phase III REGENERATE trial where 2400 Patients were randomized to placebo, Obeticholic Acid (OCA) 10 mg, or OCA 25 mg once daily. At month 18, fibrosis status was classified into 3 categories: 1) Improvement of Fibrosis by ≥1 Stage, 2) No Change and 3) Worsening of Fibrosis by ≥1.

Findings:

OCA significantly reduced serum ccK18 levels, measured by the M30 Apoptosense® ELISA, which correlated with the histologic resolution of definite NASH.

The authors state:

"These data support the use of easily accessible non-invasive markers of fibrosis and steatohepatitis to monitor NASH patients treated with OCA."

The VLVbio K18 assays have not been cleared or approved by the U.S. Food and Drug Administration.

VLVbio™ Product Line	
ELISA Products	**Prod. No**
M30 Apoptosense® ELISA	10011
M30 CytoDeath™ ELISA	10900
M65® ELISA	10020
M65 EpiDeath® ELISA	10040
M65 EpiRat™ ELISA	10060

How to order

VLVbio is collaborating with distributors all over the world to provide fast, reliable and convenient service for you. Please visit www.vlvbio.com or email order@vlvbio.com to find your local distributor.

References:

[1] Nier *et al*, 2020. Adipokines and Endotoxemia Correlate with Hepatic Steatosis in Non-Alcoholic Fatty Liver Disease (NAFLD). Nutrients. 12(3), 699.

[2] Boursier *et al*, 2018. Screening for therapeutic trials and treatment indication in clinical practice: MACK-3, a new blood test for the diagnosis of fibrotic NASH.Alimentary Pharmacology & Therapeutics. 47(10),1387–96.

[3] Sanyal *et al*, 2015. CHALLENGES AND OPPORTUNITIES IN DRUG AND BIOMARKER DEVELOPMENT FOR NONALCOHOLIC STEATOHEPATITIS: FINDINGS AND RECOMMENDATIONS FROM AN AMERICAN ASSOCIATION FOR THE STUDY OF LIVER DISEASES (AASLD) - FOOD AND DRUG ADMINISTRATION (FDA) JOINT WORKSHOP. Hepatology. 61(4) 1392-1405.

[4] Loomba *et al*, 2021. Combination therapies including cilofexor and firsocostat for bridging fibrosis and cirrhosis due to NASH. Hepatology. 73(2) 625-43.

[5] Kowdley *et al*, 2020. Combination Treatment With Cilofexor And Firsocostat Normalizes Cell Death Marker Cytokeratin 18 (CK18) In Patients With Advanced Fibrosis Due To NASH. Poster presented at AASLD: The Liver Meeting®. Digital Experience, November 13-16 2020.

[6] Harrison *et al*, 2019. Resmetirom (MGL-3196) for the treatment of non-alcoholic steatohepatitis: a multicentre, randomised, double-blind, placebo-controlled, phase 2 trial. The Lancet. 394, 2012–24.

[7] Vuppalanchi *et al*, 2014. Relationship Between Changes in Serum Levels of Keratin 18 and Changes in Liver Histology in Children and Adults With Nonalcoholic Fatty Liver Disease. Clinical Gastroenterology and Hepatology. 12(12), 2121–2130.

[8] Anstee *et al*, 2019. Obeticholic Acid (OCA) Improves Noninvasive Markers of Fibrosis in Patients With Nonalcoholic Steatohepatitis (NASH): A Secondary Analysis of the Phase 3 REGENERATE Study. Presented at The Liver Meeting® of the American Association for the Study of Liver Diseases (AASLD). November 8-12, 2019.

VLVbio

Hästholmsvägen 32, 131 30 Nacka, Sweden
Telephone: +46 (0) 8 122 053 00
E-mail: info@vlvbio.com • www.vlvbio.com