Future Perspectives in Hepatology: From Basics to Clinics

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Abstracts of Invited Lectures
Poster Abstracts

Workshop

FUTURE PERSPECTIVES IN HEPATOLOGY:
FROM BASICS TO CLINICS

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Scientific Organization:
G. Gerken, Essen (Germany)

Scientific Co-Organization:
A.E. Canbay, Magdeburg (Germany)
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Session I

Viral Hepatitis / HCC
Immune regulation in chronic viral hepatitis

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Chronic inflammation contributes to the progression of viral hepatitis to liver cirrhosis and hepatocellular carcinoma. The rate of disease progression varies greatly among infected individuals and is thought to be driven by host immune responses.

Virus-specific T cells are either chronically exhausted and functionally impaired or specific for viral sequences that have already mutated, and therefore cannot eliminate all infected target cells in chronic hepatitis. Here, we asked to which extent innate immune cell populations (monocytes, macrophages, NK cells) and innate-like T cell subpopulations with evolutionary conserved invariant T cell receptors (NKT and MAIT cells) contribute to chronic inflammation. These cells can be activated both by virus-induced cytokines and/or by bacteria and bacterial products that translocate from the gut and reach the liver via the portal vein.

To examine the relative contribution of virus-induced cytokines and microbial products to innate immune cell activation, inflammation and severity of liver disease, we studied innate immune cells from liver, portal vein and systemic blood of 29 hepatitis C patients by multicolor flow cytometry and plasma markers for immune cell activation by ELISA. Monocytes, natural killer (NK) cells and MAIT cells were more activated in the liver than in the systemic and the portal vein blood. Further, NK cells and MAIT cells displayed a more cytotoxic phenotype in the liver than in the other two compartments. Markers for monocyte/macrophage activation (soluble CD14 and CD163) were higher in systemic than in portal plasma consistent with shedding by intrahepatic monocytes/macrophages. Conversely, levels of iFABP, a marker for intestinal barrier integrity, were higher in portal than in systemic plasma, consistent with its production by enterocytes and decreased intestinal barrier integrity in chronic liver disease.

To investigate how antiviral therapy affects innate immune cell activation, we studied paired liver biopsies prior to and at week 4 of sofosbuvir/velpatasvir therapy. HCV RNA levels declined by about 6 log_{10} IU/ml and were undetectable at week 4 in all patients studied. Likewise, liver inflammation (HAI score) decreased significantly. This was accompanied by a decrease in the activation of blood and liver monocytes and the levels of monocyte-derived IL-18, along with a decrease in activation and cytotoxicity of intrahepatic NK cells and MAIT cells.

Collectively, these data show that innate immune cell activation and inflammation are rapidly reversible with antiviral therapy, thus appear to be driven predominantly by virus-induced cytokines in patients with compensated HCV-related liver disease.
Novel antiviral strategies to eradicate hepatitis B

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Hepatitis B virus (HBV) establishes a stable nuclear persistence form, the so-called cccDNA, in infected hepatocytes. Unlike HCV, which can be eliminated with directly acting antivirals, cccDNA prevents elimination of HBV. Therefore, currently used antivirals such as nucleos(t)ide analogous efficiently control HBV replication but don’t affect its persistence via cccDNA. Thus, hepatitis B still requires long-term treatment.

Virus elimination or at least functional hepatitis B cure, however, is desired for the 240–300 million carriers at high risk to develop liver disease and hepatocellular carcinoma. Eliminating HBV cccDNA is a critical issue if we want to cure HBV infection. Looking at the HBV life cycle, there are however only limited options to target the nuclear HBVcccDNA. Capsid assembly modifiers, non-nucleosidic polymerase and entry inhibitors and siRNAs under development don’t target cccDNA directly. Talen- or CRISPR/Cas based targeting of cccDNA are limited by the fact that they require gene therapy and evoke safety concerns.

Interferon (IFN) α that is approved for therapeutic application can clear HBV infection in 20–25% of chronic carriers but dosing is limited by severe side effects. Lymphocytes can eliminate HBV by killing infected cells, and have also been described to control HBV in a non-cytolytic fashion via secreted cytokines. Moreover, transplantation of HBV-immune bone marrow has been shown to safely cure hepatitis B. Thus, immune stimulation seems a promising approach to cure hepatitis B, and most promising approaches at the moment are a combination of antiviral drugs with immune activation.

Immune activation can either be achieved in an antigen-unspecific fashion or in an HBV-specific fashion. Approaches to activate immune responses in a non-specific fashion that are currently developed include toll-like receptor agonists and checkpoint inhibitors. HBV-specific immune activation is possible by therapeutic vaccination, adoptive T cell therapy or targeting of lymphocytes using antibodies. HBV-specific B- and T-cell activation mimics naturally occurring clearance of HBV infection, and allows for cytolytic and non-cytolytic activity of lymphocytes targeting HBV persistence.
Antiviral therapy in harder-to-treat HCV patients

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The revolution of hepatitis C treatment allows now to cure almost all patients infected with HCV. The phenomenal success of novel therapies is based on the combination of direct acting antivirals (DAA) targeting different steps in the HCV lifecycle. Since January 2014, 10 DAAs have been approved by EMA with some of the compounds being formulated as fixed-dose combination tablets. Sofosbuvir-based regimens (in combination either with a NS5A inhibitor or with simeprevir) can be differentiated from NUC-free regimens containing combinations of a HCV protease inhibitor plus a NS5A inhibitor with or without a non-nucleoside polymerase inhibitor. Antiviral efficacy is high for all approved regimens with sustained virological response rates above 95% if drugs are applied according to the label. SVR rates are dependent on the HCV genotype (slightly lower responses in genotypes 3 and 1a), the stage of liver disease and the presence of variants carrying resistance-associated substitutions (RASs). The addition of ribavirin has been shown to reduce the risk of treatment failure and relapse for some regimens when negative response factors are present. Thus, pre-treatment testing for RASs may be considered e.g. in cirrhotic patients with HCV genotype 3 infection or after failure of an earlier DAA-containing therapy. This will allow to select optimal DAA combinations and also reduce treatment durations and costs.

Despite the high virological response rates, there are still relevant and yet unresolved challenges in HCV treatment. HCV nucleotides such as sofosbuvir cannot be used in patients with advanced kidney disease (e.g. GFR < 30 ml/min) which is a problem in particular for HCV genotype 3 infections where all currently available regimens are based on sofosbuvir. Even though liver function parameters and liver stiffness values improve after viral clearance, treatment is difficult in patients with decompensated cirrhosis when the clinical benefit is limited. Here, antiviral therapy after liver transplantation may be a reasonable alternative approach. It is also unclear to what extent extrahepatic manifestations recover when HCV is eliminated. We recently showed that the inflammatory milieu as well altered immune cell populations are not completely restored in SVR patients. Another challenge may be co-morbidities and potential drug-drug interactions between ongoing medications with distinct HCV-DAAs. Finally, it is important to note that hepatocellular carcinoma still develop and a regular monitoring after therapy is required also in HCV RNA-negative individuals. Effects of HCV clearance on immune surveillance of HCC remain to be determined in more detail.
Selected references from our group on the topic (2015–16):

- Hengst et al. DAA-induced HCV clearance does not completely restore the altered cytokine and chemokine milieu in patients with chronic hepatitis C. J Infect Dis. 2016 epub.


- Owusu Sekyere et al. Type I interferon elevates co-regulatory receptor expression on CMV- and EBV-specific CD8 T cells in chronic hepatitis C. Front Immunol. 2015; 6:270.


Global hepatitis vaccination programs to prevent HCC

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Hepatocellular carcinoma (HCC) is a significant public health problem worldwide with an annual death rate of ~850,000. The incidence of HCC is highest in Asia and Africa but has been declining following introduction of universal vaccination against hepatitis B. In contrast, the initially low incidence of HCC in the Western hemisphere is on the rise. Recognition of the many risk factors associated with development of HCC and improved surveillance of patients at risk are leading to better control and prevention. Control of HCC requires proper surveillance of patients at risk and recognition of risk factors including cirrhosis and chronic liver disease, irrespective of etiology, persistent HBV and HCV infection, non-alcoholic fatty liver disease, metabolic syndromes incl. diabetes, hyperlipidemia and obesity, excessive intake of alcohol and exposure to aflatoxin. Current EASL, AASLD and APASL guidelines recommend similar surveillance measures including ultrasound screening of the liver, and monitoring of biomarkers at ~6 month intervals in patients at risk. Prevention of hepatitis B virus (HBV) infection has made an enormous progress in the past 35 years. Vaccination is the most effective means for reduction of the global incidence and burden of HBV infection. Over a billion doses of HBV vaccines have been administered so far in > 180 countries with an excellent record of safety and efficacy. Following a full course of vaccination, seroprotection rates to anti-HBs are close to 100% in children and almost 95% in healthy adults. In Taiwan, the impact of universal vaccination against HBV of neonates and children introduced in 1984, lead to a significant reduction in HCC incidence in children. Similar results were also reported by other countries endemic for HBV. Anti-viral treatment against HBV has been shown to reduce the risk of HCC in patients with chronic HBV infection, although the risk is not completely abolished. In a similar manner, anti-viral treatment against HCV with interferon and ribavirin has lead to a decline of HCC in patients with chronic HCV infection. It is too early yet to assess the effect of the recently introduced direct anti-viral agents against HCV.

In conclusion: Prevention of HCC through vaccination against HBV, development of new antivirals and controlling the metabolic syndrome is expected to lead to a reduction in HCC worldwide. Although the means for controlling HCC differ between various continents and countries, the major progress in developing means for prevention, are expected to contribute the decline in incidence of HCC, especially in regions with endemic HBV where introduction of universal vaccination using low-cost and effective vaccines has had already a significant impact.

Reference:

A look into the future: Immune therapy of HCC

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Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide, with a continuously high mortality. Thus, the development of new therapeutic strategies is crucial to decrease recurrence rates and to improve the overall survival rates of HCC patients. The rationale for immunotherapy is based on the findings of several studies showing specific CD8(+) T-cell responses against various tumor-associated antigens (TAAs) in HCC patients and a clinical benefit of T-cell infiltration in the tumor tissue. In addition, different vaccination strategies have led to induction of TAA-specific immunity and at least some tumor control. The factors responsible for the failure of TAA-specific immune responses to fully control tumor growth and immune evasion are not completely understood. Most likely, several different mechanisms contribute to the failure of the TAA-specific immune responses, e.g. the expression of inhibitory receptors such as PD-1 and CTLA-4 on TAA-specific T cells, the action of suppressive cell populations such as regulatory T cells or myeloid derived suppressor cells or the tolerogenic liver microenvironment. The aim of immune-based therapies is to overcome these mechanisms of T-cell failure and to induce or boost TAA-specific CD8(+) and CD4(+) T-cell responses. Several preclinical and clinical studies of immune-based therapeutic approaches show encouraging results. For example, recent data indicate that immune checkpoint inhibitors may show at least partial response also in HCC. It can be expected that a better understanding of the mechanisms responsible for TAA specific failure and its restoration will lead to the development of novel immune based treatment approaches that are currently being evaluated in preclinical and in early clinical settings. Indeed, immune checkpoint blockade along with adoptive immune cell therapy and vaccine approaches are currently being tested either alone or in combination with other treatments. Here, we provide an overview for the rationale of immunotherapy in HCC, summarise ongoing studies and provide a perspective for immune based approaches in patients with HCC.
The role of the liver in the metabolic syndrome: A global challenge

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The talk will cover the emerging data on global prevalence of NAFLD/NASH and the associated healthcare burden. An overview of the pathogenesis of NAFLD will be given with attention given to the role the liver plays in driving systemic IR.
The role of microbiota in the gut liver axis in NASH

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The gut-liver axis plays an important role in the pathogenesis and progression of non-alcoholic fatty liver disease (NAFLD) along the disease spectrum ranging from simple (bland) steatosis, steatohepatitis (NASH) to advanced fibrosis, cirrhosis and cancer (HCC). Changes in gut microbiota (dysbiosis, i.e. qualitative changes with increased proportion of potentially harmful bacteria and reduced levels of beneficial bacteria, a loss in diversity or quantitative changes in the amount of bacteria with bacterial overgrowth) and alterations in gut integrity with increased intestinal permeability ('leaky gut') may play an important role in the pathogenesis and progression of NAFLD to NASH ('multihit' pathogenesis of NASH). A range of studies in humans and animals has demonstrated intriguing associations between intestinal dysbiosis and NAFLD, but the causality and the mechanistic links still need to be fully established. Nevertheless, microbial changes may offer exciting new opportunities for development of diagnostic/prognostic biomarkers and novel therapeutics for NASH.

Pathophysiological implications: The intestinal microbiome is an important factor in the development of obesity. Microbiota determine energy extraction from food (e.g. by conversion of fibers to fatty acids), modulate energy metabolism and storage in adipose tissue, perhaps even food intake and produce microbial products such as endotoxin ('metabolic endotoxemia') as well as metabolites. Such metabolites include fatty acids and secondary bile acids which can act as signaling molecules along the gut liver axis in NASH via activation of G-protein coupled receptors and nuclear (hormone) receptors. Dysbiosis also contributes to breakdown of the intestinal barrier, translocation of microbial products and intestinal, hepatic as well as systemic inflammation. Importantly, the contribution of the intestinal microbiome in NASH not only involves the translocation of bacterial products such as endotoxin but also microbial metabolites produced in a dysbiotic intestinal environment that can promote hepatic injury and inflammation.

Microbiota metabolize (dehydroxylate) primary into secondary bile acids and are responsible for their deconjugation (allowing passive diffusion), which altogether profoundly alters the signaling function and distribution/access of bile acids to cells and tissues. Since bile acids have important signaling function in the regulation of metabolism and inflammation, microbiota are important modulators of bile acid signaling in the host. Conversely, bile acids play an important role in determining bacterial growth and intestinal integrity.

Importantly, microbiota undergo major modification in response to diet and certain dietary patterns have been linked to specific gut microbial enterotypes. Notably, diet fat-induced taurine-conjugation of bile acids may promote expansion of pathobionts such as *Bilophila wadsworthia*. Moreover, microbiota may drive inflammation in NASH through conversion of dietary phosphatidylcholine into precursors which are then further metabolized in liver into trimethylamine oxide (TMAO) resulting in macrophage activation and progression of atherosclerosis. Importantly, increased fructose
consumption as a potential driver of hepatic fibrogenesis in NASH has also been associated with bacterial overgrowth, dysbiosis and leaky tight junctions.

**Diagnostic and prognostic implications:** A range of studies have shown alterations of microbiota in NASH which appear to differ from the changes seen in obesity or simple steatosis. A recent study has linked NAFLD severity with gut dysbiosis and a shift in metabolic function of the gut microbiota. As such, *Bacteroides* were identified as independently associated with NASH and *Ruminococcus* with significant fibrosis. Other studies have shown an increased abundance of endotoxin and alcohol-producing bacteria in NASH microbiomes (the latter explaining mildly elevated blood-ethanol concentration in (even pediatric) NASH patients) and a reduction of beneficial bacteria such as *Faecalibacterium prausnitzii*. In the further progression to liver cirrhosis, a reduction of Lachnospiraceae and Ruminococcaceae and 7-alpha dehydroxylating bacteria (*Blautia*) with an increase of potentially pathogenic Enterobacteria have been observed. Moreover, an obesity-induced gut microbial bile acid metabolite (the secondary bile acid deoxycholic acid) promotes cellular senescence and favors HCC formation in mice. Thus, analysis of gut microbiota (and their metabolites) may add important diagnostic/prognostic information to traditional predictors of NAFLD severity and may identify novel therapeutic targets.

**Therapeutic implications:** Dysbiosis in NASH may represent a valuable target for therapeutic interventions. Manipulation of the intestinal microbiome can be principally achieved by antibiotics, prebiotics, probiotics, and fecal microbiota transplantation (FMT). Interestingly, some of the beneficial effects of bariatric surgery have also been at least partly attributed to associated changes in gut microbiota. While preclinical evidence from a range of experimental animal (mainly mouse) studies suggests, that modulation and/or transfer of microbiota can ameliorate/cure NASH (and vice versa), the clinical effects are so far not convincing enough to recommend manipulation of gut microbiota as treatment of NAFLD and NASH. A proof of concept FMT study has resulted in improvement of metabolic parameters (most notably insulin resistance) in individuals with metabolic syndrome. Importantly, several novel bile acid based therapeutics may have a major impact on gut microbiota which could at least in part explain their some of their therapeutic effects. The same may also hold true for other therapies altering/shifting bile acid signaling in the gut such as ABST inhibitors and resins which both result in increased colonic exposure and microbial metabolism of bile acids.

**Further reading:**


Molecular pathogenesis of NASH

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The progression of non-alcoholic fatty liver disease (NAFLD) to the more advanced stage disease called non-alcoholic steatohepatitis (NASH) and/or fibrosis-cirrhosis is an end-result of a complex interplay of genetic, epigenetic, and environmental factors. Recent studies show that signals from the gut, adipose tissue, brain, and endocrine organs also contribute to modulating NAFLD outcomes.

Although ectopic fat accumulation in the liver is generally considered benign, multi-parallel hits from lipotoxicity, oxidative, mitochondrial, endoplasmic reticulum, and immune dysfunction can potentially promote progression to NASH and fibrosis. NASH and fibrosis in essence, reflects a perturbed liver repair process, which results in liver cell death and the deposition of scar tissue.

Understanding the molecular and cellular mechanisms which drive NAFLD progression (or regression) would allow us to identify new biomarkers of disease, and enable the development of novel therapeutic targets. Cumulative reports suggest that injured or dying hepatocytes secrete factors which stimulate surrounding hepatic stellate cells, endothelial cells, and liver progenitors to evoke a repair process, which comprise a ductular reaction (progenitor response), fibrogenesis, and an accumulation of various immune cells including inflammatory monocytes/macrophages. Macrophage subsets orchestrate liver repair by secreting pro-inflammatory or pro-restorative factors. Despite initial controversy, cumulative evidence suggests that epithelial-mesenchymal transition and mesenchymal-epithelial transition participate in this tissue repair process.

Although early studies had focused on prototypical cytokines such as TNFα, adipokines, and LPS (microbiota), there is growing recognition of the roles of developmental morphogens (such as hedgehog, Wnt, or Notch), matricellular proteins (osteopontin, SPARC), DAMPs/PAMPs (HMGB1), and nuclear receptor signaling (RXR, FXR, LXR, PXR, Vitamin D and thyroid hormone receptors).

In this presentation, we will discuss the roles of some of these molecules/signaling pathways, and will highlight relevant translational studies.
Emerging new pharmacotherapy for NAFLD and NASH

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Non-alcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease worldwide, which is associated with fibrosis and hepatocellular carcinoma and may require liver transplantation (LTx). Although the primary treatment of NAFLD is weight loss and lifestyle modification, these are rarely achieved permanently. Thus, the need for pharmacotherapy is still high. The recent gains of knowledge on the pathophysiology of NASH led to development of new approaches for medication. One target is the basis of NAFLD with drugs aimed to counter hepatic fat accumulation and consequently metabolic stress. Among these substances are peroxisome proliferator-activator receptor agonists (eg, pioglitazone, elafibranor, saroglitazar), medications targeting the bile acid-farnesoid X receptor axis (obeticholic acid), inhibitors of de novo lipogenesis (aramchol, NDI-010976), incretins (liraglutide) and analogues of the fibroblast growth factors (FGF)-21 or FGF-19. Another approach targets the consequences of metabolic stress: oxidative stress, inflammation and cellular and tissue injury. Drugs aimed against these processes are antioxidants (vitamin E), drugs affecting the tumour necrosis factor α pathway (emricasan, pentoxifylline) and immune modulators (amlexanox, cenicriviroc). Targeting the gut is another independent approach to treat NAFLD. Medications to influence gut-associated factors of NAFLD include antiobesity agents as orlistat or gut microbiome modulators (IMM-124e, fecal microbial transplant, solithromycin). In cases with long exposure to NAFLD or NASH antifibrotics (sintuzumab and GR-MD-02) will be an important element of therapy, to reduce severe fibrosis and associated the liver-related morbidity and mortality. In the near future practitioners will hopefully have some of these therapeutic options to assist lifestyle modifications for NAFLD treatment. Though, the new challenge will be to identify the ideal regimen for each individual patient.
Perspectives of gene therapy in liver diseases

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Gene therapy for liver disease opens a therapeutic window which is not amenable to other forms of treatments. Specifically inborn errors to metabolism, hemophilia but also cancer and infectious diseases might be curable with these new types of treatments. Viruses which transport the genetic information should specifically target hepatocytes without causing severe side effects. To achieve this goal several treatments strategies have been formulated in the past and are currently examined within pre-clinical and clinical studies: a) replacement of a mutated gene with a healthy copy i.e. classical gene therapy b) deleting a mutated gene to abolish erroneous protein production c) insertion of new genetic information into the hepatocyte to allow production of a beneficial protein to treat a disease d) using a combined genetic and pharmacological strategy to treat diseases i.e. cancer of the liver.

As precise targeting remains a challenge but the introduction of adeno-associated viral (AAV) vectors might offer a number of advantages which could help to overcome these problems i.e. enhanced transduction of liver cells, reduced prevalence of neutralizing antibodies, and diminished immune responses to capsid structures. Hemophilia B emerges as an exiting target for liver directed gene therapy as an AAV8 vector achieved FIX levels of 20–25% that have been sustained for more than 6 months since undergoing hepatic gene transfer. Liver cancer could become another example of successful gene therapy especially as we learn to combine such treatments with other interventions i.e. immune checkpoint blockade.
Session III

Fibrosis / Cirrhosis / CCC
Acute-on-chronic liver failure: A new entity

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Acute-on-chronic liver failure (ACLF) is now increasingly recognized as a distinct clinical entity in patients with liver diseases. The EASL-CLIF consortium defines ACLF as a syndrome that develops in patients with cirrhosis and is characterized by acute decompensation, organ failure and high short-term mortality. It thereby differs from “decompensated cirrhosis” (= no organ failure) or “acute liver failure” (= no underlying cirrhosis/chronic liver disease). The core concept is the acute deterioration with subsequent (multi-)organ failure in a patient with cirrhosis (or chronic liver disease) in response to an acute insult. Typical precipitating events that cause ACLF in the setting of cirrhosis are bacterial infections, alcohol abuse, drug toxicity or viral hepatitis. The pathogenesis of ACLF is characterized by an initial cytokine burst, oftentimes presenting as systemic inflammatory response syndrome (SIRS), followed by compensatory anti-inflammatory response and later immune paralysis. These mechanisms predispose to sepsis and multi-organ failure (e.g., liver, renal, coagulation, brain, heart or pulmonary failure). It is critical to recognize the diagnosis and severity of ACLF, which is staged according to the number of organ failures as ACLF grades 1–3, as early as possible in cirrhotic patients. The 28-day-mortality rate ranges between 20–80% in ACLF. A multidisciplinary approach including hepatologists, intensivists, transplant surgeons, infectious disease specialists and others is required to offer optimal therapy (e.g., antibiotics, renal replacement therapy, antivirals) and identify selected patients that benefit from liver transplantation. Hematopoietic growth factors, modulation of gut microbiota, cell-based therapies or augmentation of regeneration are future directions for novel approaches to the prevention or treatment of ACLF.
New treatment options for liver fibrosis: From bench to bedside

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Prevention or reversal of cirrhosis has become a primary endpoint for trials in patients with chronic liver disease. Significant progress has been made in understanding the mechanisms of liver fibrosis. This includes its dynamic nature, the plasticity of all liver cell populations, especially immune cell subsets, endowing them with fibrogenic or fibrolytic properties, opening the potential of inducing pharmacological reversal even of advanced fibrosis and cirrhosis. Since we have learned that even a cirrhotic human liver can regress to a non-cirrhotic stage with highly effective antiviral therapy for chronic hepatitis B or C, there is hope to develop pharmacological therapies that prevent further progression of speed up reversal in patients with viral and non-viral fibrotic liver diseases, in whom current treatment is less effective. Promising strategies address upstream signals, e.g., by lipoapoptotic or otherwise injured hepatocytes, or more directly macrophages changing their functional polarization towards fibrolysis, activated cholangiocytes or activated hepatic stellate cells/myofibroblasts. There is an increasing number of drugable molecular and cellular targets, with agents such as small molecules, blocking antibodies, siRNA, or antisense oligonucleotides that can inhibit fibrosis progression or induce fibrosis reversal. Some antifibrotic agents have already entered phase 1–2 clinical trials, with a study design that is still based on histological readouts, but increasingly complemented by a number of biologically plausible surrogate markers. Some of the challenges ahead are to better understand the heterogeneity of the fibrotic response in different individuals with variant liver diseases and to develop better noninvasive methods to assess the dynamics of fibrogenesis and fibrolysis. Notably, remarkable progress for novel serum markers of fibrogenesis and fibrolysis has been made. This will permit shorter and smaller proof-of-concept trials, and personalized therapies. Such therapies will employ an individualized dose titration and use of drug combinations for improved efficacy and reduced side effects.
Endoscopic imaging and intervention in hepatobiliary disorders

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Nearly five decades ago, William McCune cannulated the papilla of Vater using a flexible endoscope for the first time, thus pioneering a technique which subsequently developed into the gold standard of minimal invasive treatment of (among others) biliary disorders. The use of guidewires in Seldinger technique and the introduction of accessories that could be passed through an endoscope’s working channel quickly expanded the potential of the method. Parallel to the advent of magnetic resonance imaging of the biliary system, endoscopic retrograde cholangiopancreatography (ERCP) evolved from a primarily diagnostic tool to an interventional modality with a leading role in the treatment of both benign and malignant diseases including post-surgical complications.

While fluoroscopy is still the basis of endoscopic biliary imaging, delineating the lumen of extra- and intrahepatic biliary ducts and facilitating the differential diagnosis of filling defects, contemporary endoscopic techniques include direct cholangioscopic visualization of the biliary epithelium and inflammatory or neoplastic alterations. The latest developments in this regard are single-use digital endoscopes that can be passed deeply into intrahepatic ducts and facilitate tissue retrieval from suspicious lesions. Therapeutic options include the removal of intraductal stones \( (in\ toto) \), or, in larger stones, after either mechanical or electrohydraulic fragmentation or laser-lithotripsy), drainage of obstructed ducts or segments via the placement of biliary stents, treatment of benign strictures and leaks.

Endoscopically administered palliation of cholangiocarcinoma has gained interest in recent years, either by means of photodynamic therapy (PDT) with laser irradiation after photosensitization or by intraductal radiofrequency ablation using heat-inducing catheters.

In recent years, endoscopic ultrasound (EUS) likewise changed from a purely diagnostic instrument to a therapeutic technique with a distinct spectrum of indications. Transmural access to ( bile) ducts not accessible otherwise is gained via EUS and facilitates internal biliary drainage when the papilla cannot be reached or cannulated. Newly developed endoscopically placed lumen-apposing metal stents (LAMS) serve to create durable artificial fistulae between the digestive tract and biliary ducts or the gall bladder in patients with life-threatening septic biliary disorders that are unfit for surgical treatment.

Both endoscopic imaging and interventions follow an evolutionary path other endoscopic techniques have gone before that leads to reduced invasiveness, improved accuracy in the diagnosis of neoplastic lesions and expanding therapeutic possibilities, individualized and in multimodal treatment concepts.
Pathogenesis and management of cholangiocarcinoma in the future

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Cholangiocarcinoma can be classified as intrahepatic cholangiocarcinoma (iCCA), perihilar cholangiocarcinoma (pCCA), and distal cholangiocarcinoma (dCCA) depending upon its anatomic site of origin.1 In this overview, I will review emerging concepts regarding diagnosis and therapy for iCCA and pCCA. iCCA is unique amongst gastrointestinal adenocarcinomas in that 10–15% of these cancers contain fibroblast growth factor receptor 2 (FGFR2) fusion genes, and another 15–20% are characterized by isocitrate dehydrogenase 1 or 2 mutations.2–4 Targeted therapy of these mutations may be efficacious in iCCA. Trials are currently ongoing using FGFR2 inhibitors for these cancers, and one agent, BGJ398, shows promise in a phase II trial.5 Interestingly, synthetic lethality experiments suggest Src-family kinase inhibitors may be useful for patients with IDH mutations.4 One Src family kinase inhibitor is Dasatinib which is clinically available.

pCCA is difficult to diagnose, and hence most efforts have focused on new diagnostic technologies for this disease. Technologies include advanced cytologic techniques employing fluorescent in situ hybridization (FISH), and next generation sequencing from cells obtained via brush cytology during ERCP.6 Recently, we have been exploring liquid biopsies for peripheral blood for the diagnosis of pCCA using methylation markers specific for epigenetic changes in pCCA. We have identified a strong signature using two differentially methylated regions. Treatment for pCCA remains challenging with liver transplantation remaining curative for select patients.7–10 We look forward to future advances for this enigmatic and devastating cancer.

References:


Session IV

Liver Transplantation
Living donor liver transplantation in children and adults: Obstacles and visions for the future

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Living donor liver transplantation (LDLT) is the only alternative to deceased donor liver transplantation (DDLT) currently. Unfortunately, LDLT is a more complicated procedure mainly because of its technical complexity and different physiological requirements for regeneration of a partial graft. Moreover, donor safety continues to be a major problem in LDLT with a low but definite donor risk. Interestingly, living donor deaths have generated far more media coverage and public attention than other medical complications which have affected LDLT programs worldwide. However continuous technical advances and challenges are enabling us to overcome the technical obstacles in LDLT.

The Milan criteria have served as the gold standard for liver transplantation for hepatocellular carcinoma. However, these criteria are based on DDLT and focus on maximizing efficacy in using limited public resources. However liver grafts for LDLT are accepted as a private gift. These guidelines have been criticized for being overly strict and restrictive. Most Asian LDLT centers reported comparable long-term survival outcomes using expanded selection criteria. However, whether these new criteria can replace the Milan criteria remains unclear.

Despite technical advances in LDLT, the high incidence of biliary complications remains the most intractable problem. Biliary complications might reflect the anatomical and physiological limitations associated with LDLT, such as multiple or small-sized graft duct openings, arterial hypoperfusion of the liver graft secondary to portal hypertension, edematous bowel loops, and poor blood supply to bile duct. Technical factors such as biliary drainage tube, suture material, suture method, among others might be of less importance to account for biliary complications.

In the majority of cases, living-related transplants register an excellent outcome for pediatric recipients, due to the possibility of performing the transplant before the child’s clinical condition deteriorates. Centers with most experience in this area report survival rates between 80 and 90% after 1 year.
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POSTER ABSTRACTS

Poster Numbers 1 – 33

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Risk factors and outcome of autoimmune hepatitis-induced acute liver failure

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Introduction: Autoimmune hepatitis (AIH) is a relatively rare cause of liver dysfunction and may lead in some cases to acute liver failure (ALF). Aim of our study was to evaluate the clinical course and outcome of patients with AIH induced ALF.

Methods: We retrospectively enrolled 32 patients with AIH induced ALF and 92 age and sex matched patients with chronic AIH (cAIH). All patients were enrolled at the University Clinic Essen from 1988 to 2014. Clinical data, laboratory parameters, and liver histology were assessed. All ALF patients were treated with corticosteroids after diagnosis.

Results: Overweight, higher γ-globulin levels, absence of SMA and HLA B8, and presence of AMA and HLA DR7 were risk factor for an ALF vs chronic manifestation. Liver histology was significantly more often typical for AIH in ALF setting than in cAIH. Spontaneous survival rate was 91% and 97% in ALF and cAIH patients respectively at 6 months after diagnosis and only one patient in the ALF group developed sepsis under therapy.

Discussion/Conclusion: Liver biopsy in an AIH mediated ALF setting was both safe and effective in diagnosing AIH. Corticosteroid therapy was not associated with high mortality or sepsis. Our findings suggest that treatment at an early stage of AIH mediated ALF may improve the outcome.
Clinical course and core variability in HBV-infected patients without detectable anti-HBc antibodies

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Introduction: Anti-HBc antibodies indicate direct encounter of the immune system with hepatitis B virus (HBV) and are even the best serological marker to detect occult HBV infection. Anti-HBc appear normally during the first few weeks after infection and remain positive lifelong. The aim of our study was to scrutinize HBV infected individuals who tested negative for anti-HBc.

Methods: From 2006 to 2016, 29 patients tested positive for HBV DNA but negative for anti-HBc and were included in this study. In 21 cases a partial or full HBV genome analysis was possible. As control group we used 23 HBV chronically infected patients.

Results: Patients with detectable HBV-DNA in the absence of anti-HBc were diagnosed with acute HBV infection (n = 4, 13.8%), HBV reactivation (n = 14, 48.3%) and chronic hepatitis B (CHB) (n = 11, 37.9%). Eleven remained anti-HBc-negative during the entire follow up period. HBV genotyping revealed the presence of Genotype (GT)-A (n = 11), GT-B (n = 1), GT-D (n = 7), GT-E (n = 1) and GT-G (n = 1). Compared to the control group, HBV variants from anti-HBc-negative patients showed less variability in the core region and especially less frequently mutations in known B-lymphocyte response regions (amino acids 76–87 and 105–116).

Discussion/Conclusion: In this study HBV-DNA in the absence of anti-HBc was most often found after HBV reactivation under immunosuppression. Core sequences found in anti-HBc-negative patients had fewer mutations in total and in known B lymphocyte response regions compared to sequences of anti-HBc-positive patients.
Modulation of the *unfolded protein response* (UPR) in HBV mouse model by activation of transcription factor ATF6

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**Introduction**: Accumulation of HBV envelope proteins (HBs) in the endoplasmic reticulum (ER) of hepatocytes in patients suffering from chronic HBV infection may cause a direct, intrinsic mechanism of cell damage. *Unfolded protein response*, ER-Stress, and apoptosis could be the outcome of intracellular HBs accumulation. The synthetic chaperone 4-PBA (phenylbutyric acid) dissolves cell-damaging aggregates in different models of protein aggregation diseases and has been used therapeutically in the clinic. ATF6 is an ER-Stress regulating transcription factor. Accumulation of misfolded proteins in the ER causes proteolytic cleavage of ATF6. The cleavage product acts as a transcription factor for formation of chaperones. The aim of the present study was to investigate the effect of the chemical chaperone 4-PBA on the mechanism of ATF6 in HBV mouse model.

**Methods**: Stable-HBs expressing cell lines (HuH7, NIH3T3 und AML12) were established in order to study the effect of PBA. The HBs transgenic mice C57BL/6J-tg(Alb1HBV)44Bri/J were treated continuously with PBA (in drinking water) over a period of 1–8 weeks. Cell culture experiments and animal model were analyzed by immunohistochemistry, mRNA-Array, qRT-PCR and Western blot.

**Results**: PBA causes a distribution or dissolving of aggregated HBV envelope proteins in HBs transgenic cell lines. The HBs transgenic mouse model also shows an altered intracellular aggregation pattern with a consecutive distribution of the transgene. Moreover, PBA increased the expression of ATF6 and triggered increased expression, proteolytic activation, and nuclear translocation of acute phase response proteins (serum Amyloid, lipocalin, metallothionein).

**Discussion/Conclusion**: Dissolving intracellular aggregates of HBV envelope proteins *in vitro* and in animal model has been successfully carried out by PBA. The activation of ATF6 induced an increased expression of acute phase proteins. This targeted immunomodulation could open new perspectives for HBV therapy by using synthetic chaperones. The identified properties of the results of our study increased the knowledge about cellular pathophysiology induced by HBV surface proteins. PBA might be important for the development of new therapeutic options for the treatment of HBV.
KITENIN expression is critical for progression of cholangiocarcinoma

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Introduction: Cholangiocarcinoma (CC) is the second most common primary hepatic malignancy and worldwide incidence and mortality rates are rising. Molecular mechanisms of CC carcinogenesis are not fully discovered and new therapeutic approaches are of utmost need. KAI1 C-terminal interacting tetraspanin (KITENIN), a member of the tetraspanin family, is expressed in different solid cancers and known to be important for tumor progression and metastasis. The aim of the present study is to explore the function of KITENIN in human CC cell lines (SZ-1, TFK-1), tissues and mouse model (Alb-Cre/KRASG12D/p53L/L).

Methods: KITENIN expression was determined by immunohistochemistry, immunofluorescence and Western Blot. We analyzed the effect of siRNA KITENIN on proliferation and epithelial plasticity on CC cell lines by using MTT-, migration- and invasion-assay. Western Blot was used to study epithelial-mesenchymal transition (EMT).

Results: KITENIN is highly expressed in human CC cell lines (n = 2), human CC (n = 14) and murine CC (n = 5). First, silencing of KITENIN impaired proliferation, migration and invasion in both intra- and extra-hepatic human CC cells (p < 0.05). Second, silencing of KITENIN down-regulated EMT markers (N-cadherin, Vimentin, Slug and Snail).

Discussion/Conclusion: Our study demonstrates that KITENIN plays a crucial role for CC carcinogenesis and that targeting of KITENIN might become a potential therapeutic target for human CC.
Interfering with TGF-β2: The next approach of targeted CLD therapy?

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Aim: We targeted TGF-β2 expression using antisense oligonucleotides (AONs) in MDR2-KO mice to attenuate fibrogenesis and translated our findings to human PBC and PSC patients.

Methods: In 16 weeks old MDR2-KO mice, TGF-β2 expression was targeted using AONs for 4 weeks. Therapeutic efficacy was evaluated by cell-type specific immuno-fluorescent analysis of AON biodistribution, tissue morphology, and liver parameters. Expression of TGF-β2 and markers for fibrosis and inflammation were investigated by RT-PCR, immunoblot and immunohistochemistry (IHC). TgfB2 mRNA and TGF-β2 expression were determined in livers and serum of PSC and PBC patients using RT-PCR, IHC and ELISA and correlated to clinicopathological parameters.

Results: In MDR2-KO liver tissue, TGF-β2 is expressed in fibroblasts and areas of proliferating bile ducts. AONs targeting TGF-β2 expression accumulated in non-parenchymal cells. While ALT, AST and body weight were not affected, TgfB2 levels, hydroxyproline content, collagen deposition and αSMA protein expression were downregulated in mouse livers upon AON treatment. Further, in line with an induction of PparG expression, inflammatory infiltrates were significantly reduced in AON treated mice. Similarly to MDR2-KO mice, TGFB2 expression was upregulated in PSC and PBC patients compared to normal livers. Especially PBC patients (GSE79850) classified with high risk (no treatment response, liver transplantation requirement) were significantly correlated with increased TGFB2 expression. Preliminary results indicate that in PBC and PSC, like in the Mdr2-/- mice, TGF-β2 is localized in areas of proliferating bile ducts. TGF-β2 protein levels in corresponding sera are currently investigated.

Conclusions: TGF-β2-directed AON application attenuated fibrogenesis and inflammation in MDR2-KO mice. A corresponding upregulation of TGF-β2 in PSC and PBC patients unveils TGF-β2 as an interesting target for treatment of human biliary diseases.
Induction of epithelial-mesenchymal transition in hepatocellular carcinoma using 12-O-tetradecanoylphorbol-13-acetate for investigation of metastasis

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Introduction: Hepatocellular carcinoma (HCC) is one of the deadliest cancers worldwide. The main reason for a dismal prognosis in patients with advanced disease is the common occurrence of metastasis. However, metastasis-related epithelial-mesenchymal transition (EMT) is still poorly understood regarding functional and genotypic changes. A better understanding of altered gene expression and regulation that results in EMT will foster the investigation of novel therapeutic approaches for patients. Therefore, the aim of this study was to establish EMT in HCC cells by using 12-O-tetradecanoylphorbol-13-acetate (TPA) for further gene expression experiments.

Methods: For this study, two different human HCC cell lines were used (PLC and Hep3B). TPA was used in non-toxic concentrations (100 nM) and different incubation times (4, 24, and 48 hours) to trigger EMT in HCC cells. Microscopy, qRT-PCR analysis, Western blot analysis, and immunofluorescence were used to analyze changes in cell morphology and gene expression. Boyden chamber assay was used to quantify cell migration.

Results: TPA was sufficient to induce marked changes in cell morphology with loss of cell-cell-contacts already after 4 hours of treatment. Accordingly, qRT-PCR analysis showed strong down-regulation of E-Cadherin, and up-regulation of EMT-related Slug, Snail, S100a4, and Vimentin mRNA expression as compared to controls. Western blot and immunofluorescence analysis confirmed loss of E-cadherin on protein level. Boyden chamber assay showed strong enhancement of migratory potential in HCC cells after TPA-triggered induction of EMT.

Discussion/Conclusion: TPA as a small molecule drug mimicking diacylglycerol (DAG) leading to activation of protein kinase C (PKC) is a known tumor promoter in different cancer cells. Here, we show that TPA is able to markedly induce EMT-like phenotype, function, and gene expression in HCC cell lines. Therefore, as compared to EMT-inducing “cocktails” including recombinant TGF-β and antibodies against E-Cadherin, TPA treatment could represent a time- and cost-effective alternative model to investigate EMT in HCC.
Increased histone deacetylase 7 (HDAC7) expression in hepatic fibrosis

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Introduction: Recent evidence has highlighted a pathological imbalance in hepatic fibrosis between the acetylation and deacetylation of histone proteins regulated by histone deacetylases (HDACs). However, the role of individual HDACs in liver fibrosis is almost completely unknown. Recently, we identified that HDAC7 can bind to the promoter of the hepatocyte growth factor (HGF) gene, and herewith, suppresses the expression of this anti-fibrogenic factor in activated hepatic stellate cells.

The aim of this study was to get further insight into the expression and function of HDAC7 in chronic liver disease.

Methods and results: HDAC7 expression was significantly increased in different murine models of chronic liver injury (bile duct ligation, thioacetamide intoxication and diet induced non-alcoholic steatohepatitis (NASH)) as well as in liver specimens from patients with different liver disease compared to healthy control liver tissue. HDAC7 expression revealed a significant correlation with the expression of collagen type I as well as alpha-smooth muscle actin, a marker of hepatic stellate cell (HSC) expression. In line with this, HDAC7 mRNA and protein expression markedly increased during in vitro activation of murine as well as human HSCs.

Conclusion: Our study indicates activated HSCs as major source of increased HDAC7 expression in liver fibrosis. Together with our previous finding that HDAC7 suppresses anti-fibrogenic HGF expression these data indicate HDAC7 as critical orchestrator of the pathologically impaired histone (de)acetylation in liver fibrosis.
Outcome after liver resection for hepatocellular carcinoma

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Introduction: Hepatocellular carcinoma (HCC) is one of the most common malignant diseases worldwide. Curative therapeutic options are hepatic resection, liver transplantation and ablation. Due to individual patient characteristics and in the era of organ shortage liver transplantation is only reserved for a small cohort of patients and hepatic resection becomes more and more important. However preoperative morbidity and mortality after liver resection is comparably high. In this study the histories of patients who underwent liver resection for HCC in our center were assessed. The aim of this study is to detect parameters influencing long term survival and risk factors for complications after hepatic resection.

Methods: Medical records of 69 HCC patients who underwent curative liver resections between 1999 and 2005 in our center were reviewed retrospectively. The 1-, 3- and 5-year survival rates and correlations with clinical, laboratory, and pathological data were analyzed using uni- and multivariable analysis.

Results: Cirrhosis was the most common underlying liver disease (60–90%). The overall mortality rate was 14%. 1-, 3- and 5-year survival rates were 93%, 75% and 70%, respectively. Hämangiosis showed to be an independent predictor of survival. Preoperatively decreased albumin blood levels, the intraoperative performance of Pringle manoeuvre and postoperative bile leak as well as tumor size, staging, lymphangiosis, resection status and the extent of resection revealed to influence long term prognosis.

Discussion/Conclusion: Long-term results after hepatic resection for HCC are comparable to the results of other centers published in the literature. The indication for major resections should be evaluated restrictively especially in in old patients. Individual patient, tumor and laboratory characteristics as well as reasonable therapeutic alternatives have to be assessed before performing hepatic resections.
Analysis of the CMV-specific cellular immunity in kidney and liver transplant patients


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Cytomegalovirus (CMV) is one of the most important infectious complications after transplantation. Methods detecting the CMV-specific T-cell immune response have shown promise in detecting patients at risk of developing CMV disease.

Kidney and liver transplant patients were enrolled in a prospective, longitudinal study, from the 6th of December 2015 to the 6th of June 2016. Patients were stratified according to their CMV- IgG serostatus and divided into two groups: preemptive therapy (D-/R+, D+/R+), universal prophylaxis (D+/R-). The CMV T-cell immune response was determined using the Quantiferon (Qiagen) and ELISpot (Lophius, Oxford Immunotec) assays, at different time points during the follow-up period (3–6 months). The primary endpoint was determining a cutoff for the cellular immune response, which protects against CMV disease. Secondary endpoints included: evaluation of the performance of the three tests and comparison of the CMV-specific immune response in kidney vs. liver transplant patients.
The role of adiponectin signaling in hepatocyte-like cells derived from steatosis patients’ induced pluripotent stem cells (iPSCs)

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Introduction: Metabolism in hepatocytes is highly susceptible to nutritional cues. In the presence of abundant calories derived from fat and carbohydrates, hepatocytes store fatty acids as triacylglycerides in lipid droplets (LDs). This effect is increased by the action of insulin and results in the development of non-alcoholic fatty liver disease (NAFLD), the hepatic manifestation of the metabolic syndrome. While early stages of NAFLD are benign and reversible, many patients develop fibrosis (NASH), cirrhosis and even hepatocellular carcinoma (HCC). The adipokine adiponectin has been associated with many positive aspects on metabolism which result in increased insulin sensitivity as well as reduced gluconeogenesis and LDs in the liver. Adiponectin plasma levels are reduced in obesity, type 2 diabetes and insulin resistance in contrast to healthy individuals. Hepatocytes express adiponectin receptors 1 and 2 (AdipoR 1+2) which are involved in regulating glucose and lipid metabolism, inflammation and oxidative stress. The metabolism-associated adiponectin signaling activates the AMP-activated protein kinase (AMPK) and peroxisome proliferator-activated receptor \(\alpha\) (PPAR\(\alpha\)) pathways which both increase fatty acid oxidation. In addition, AMPK is also involved in reducing gluconeogenesis.

Results: We have established an \textit{in vitro} model for NAFLD based on the differentiation of human induced pluripotent stem cells (iPSCs) into hepatocyte like cells (HLCs). Treatment of HLCs with oleic acid (OA) induces the formation of LDs in parallel with an increase of the LD-coating protein PLIN2. In addition, we observed regulated expression of metabolism related genes. The influence of an individual’s genetic background on LD incorporation and metabolism changes are assessed by using iPSCs derived from dermal fibroblasts from NAFLD patients and lean controls. Within this model, we investigate the beneficial effects of adiponectin on steatotic HLC metabolism using a small molecule analogue called AdipoRon. Metabolic adaptations are monitored by gene expression analysis, quantification of LDs, measurement of reactive oxygen species (ROS) and analysis of mitochondrial integrity.
Interleukin-13 depletion normalized the enterohepatic circulation of bile salts in Abcb4 knockout mice

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Introduction: IgG4-related cholangitis is associated with a biliary and hepatic Th2-specific cytokine profile. In this regard it has been shown that Interleukin-13 (IL-13) disrupts tight junction-associated biliary epithelial cell barrier function. Furthermore, regurgitation of bile acids from leaky bile ducts causes intrahepatic cholangitis in Abcb4 knockout mice. With the current project the therapeutic value of IL-13 depletion in Abcb4\textsuperscript{-/-} mice was demonstrated.

Methods: Serum, liver, and ileum of female hybrids from IL-13\textsuperscript{-/-} and Abcb4\textsuperscript{-/-} mice aged 8, 26, and 52 weeks were analyzed by qRT-PCR, serum analysis, Western blot, and histology for ALT, bile acids, inflammation and fibrogenesis.

Results: Depletion of IL-13 in Abcb4\textsuperscript{-/-} mice recovered intrahepatic biliary architecture and in consequence caused a nearly tenfold decrease of total serum bile acid concentrations. Decrease of serum bile acid concentration went along with relative enhancement of fecal bile excretion. Thus, enterohepatic circulation of bile salts as well as hepatic and ileal integrity was nearly normalized in IL-13\textsuperscript{-/-}/Abcb4\textsuperscript{-/-} hybrids aged 8 weeks. The beneficial effects were lost with maturity but hybrids aged 52 weeks still exhibited reduced fibrosis.

Discussion/Conclusion: Recovery of bile duct integrity by IL-13 depletion stopped hepatic regurgitation of bile acids from leaky bile ducts and consequently reduced intrahepatic cholangitis and ileitis in Abcb4\textsuperscript{-/-}-mice aged 8 weeks. Anyway, IL-13\textsuperscript{-/-}-dependent stabilization of biliary epithelial cell barrier only caused a transient recovery from cholangitis as the underlying cause of liver injury in Abcb4\textsuperscript{-/-} mice, the lack of phospholipids in bile, was not altered. Nevertheless, pharmacological blocking of hepatic IL-13 signaling might improve therapy of cholangitis.
Recovery of CYP2E1 after intoxication is a detrimental factor for liver pathogenesis

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Introduction: Drug-induced hepatotoxicity is a major health concern. Little is known about how exposure to toxic chemicals influences CYP2E1 expression. Here we report that the liver protects itself against toxicity of repeated doses of carbon tetrachloride (CCl\textsubscript{4}) by down regulating CYP2E1 expression.

Methods: Male C57BL/6N mice received 1.6 g/kg of CCl\textsubscript{4} intraperitoneally at either 8 or 30 day intervals. Hepatotoxicity was analyzed at 2, 5, 8 and 30 days following four successive doses in both intervals (n = 3–4/group/time point). Blood and livers were collected for analysis of ALT and AST to assess hepatic parenchymal injury and livers were subjected to histopathological investigations.

Results: ALT and AST levels showed a peak at day two and returned to normal by day 5 after a single dose of CCl\textsubscript{4}. With 8 day intervals after 2 to 4 doses of the toxic insult, the levels of these markers decreased in a time-dependent manner. In contrast, if the intervals were extended to 30 days, the levels of ALT and AST were comparable to the first doses. Furthermore, histological analysis and quantification of the pericentral necrosis by HE staining showed similar trends. Two days after a single challenge, all CYP2E1 expressing hepatocytes were killed. Administration of a single dose of CCl\textsubscript{4} was associated with pericentral lesions and recovered within one week. However, deposition of extracellular matrix and collagen were observed upon Picro-Sirius red staining after repeated exposure. Moreover, fibrogenesis was confirmed by alpha-SMA (collagen-producing cell marker) in the pericentral regions. The recovery of protein and mRNA levels of CYP2E1 was analyzed by immunohistochemistry and RT-PCR, respectively. After administration of a single dose of CCl\textsubscript{4}, CYP2E1 required one month to completely recover.

Conclusion: We can conclude that the slow recovery of the metabolizing enzyme CYP2E1 after chronic CCl\textsubscript{4} administration in mice is associated with less destruction and more extracellular matrix and collagen deposition.
ABCB5\(^+\) mesenchymal stem cells as a potential therapy in liver diseases

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**Aim:** ABCB5\(^+\) cells are mesenchymal stem cells isolated from human skin. Stem cells in general have the capacity to differentiate in any type of cell. Their therapeutic potential has already been shown by significantly reducing chronic venous ulcers. Stem cells provide a promising therapy option for patients with end stage liver disease since there is no other chance of survival than liver transplantation.

**Methods:** Mdr2\(^{-/-}\) mice develop different stages of liver damage from fibrosis with inflammation to HCC. The mice were immunosuppressed by implanting an osmotic pump steadily releasing Tacrolimus. 24 hours post implantation, 5 x 10\(^5\) ABCB5\(^+\) cells were injected into the tail vein. After two days, livers were resected to detect ABCB5\(^+\) cells in the liver, and after two and four weeks to measure any therapeutic effect in the damaged livers of Mdr2\(^{-/-}\) mice. Improvements of liver damage, in particular on inflammatory and fibrosis, were evaluated on mRNA level, tissue morphology and plasma.

**Results:** Application of ABCB5\(^+\) stem cells showed no toxic effect on liver plasma parameters (ALT, AST) and a tendency of reducing alkaline phosphatase values. Preliminary results indicate no obvious difference between untreated Mdr2\(^{-/-}\) mice and stem cell transplanted animals 2 and 4 weeks later, when analysing typical fibrosis and inflammation markers at the mRNA level. However, when analysing the morphology of the livers, fibrosis and inflammation reduction, as analysed by Sirius red and macrophage marker S100A4 staining was evident 2 weeks after stem cell transplantation. Further, this reduction increased from about 4% to around 20% after 4 weeks.

**Conclusions:** Mdr2\(^{-/-}\) mice tolerated the ABCB5\(^+\) stem cells very well and we see a first beneficial effect, which will be further consolidated. We are currently analyzing hydroxyproline assays to quantify the collagen concentration and are increasing the number of transplanted ABCB5\(^+\) stem cell numbers in a new set of experiments to enhance the observed effect.
A cholesterol-containing modified western diet inducing steatohepatitis (NASH) with insulin resistance in wildtype B6 mice

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**Introduction**: Obesity is associated with insulin resistance and type II diabetes but also with non-alcoholic fatty liver disease (NAFLD) and steatohepatitis (NASH) that are hepatic manifestations of the metabolic syndrome. One of the most relevant factors contributing to obesity is diet. Feeding studies with rodents using diets like high-fat, methionine-choline-deficient or paigen diet often did not induce the same phenotype like in human metabolic syndrome. Here we designed a new high-fat-containing western-diet that caused obesity, insulin resistance and NASH in mice.

**Methods**: Male C57BL/6 mice were fed chow, high-fat diet (HFD) (25 g/100 g lard) or western-type diet containing high fat (25 g/100 g soybean oil) without (WD) or with cholesterol (WD-C) for 20 weeks.

**Results**: Mice fed a western-diet with cholesterol high-fat-diet significantly gained weight, increased their body fat mass and were glucose intolerant and slightly hyperinsulinemic compared to chow-fed mice. Serum parameters for liver inflammation were elevated after feeding a WD-C but not after feeding a WD or HFD. Histological scoring of the liver revealed steatohepatitis with fibrosis (NASH) in WD-C-fed mice and only simple steatosis without inflammation in WD- and HFD-fed mice. Gene expression analysis detected an up-regulation of chemokines, pro-inflammatory cytokines, immune cell infiltration and induction of markers for fibrosis and apoptosis only in livers of WD-C-fed mice. Serum level of triglycerides and cholesterol were slightly elevated, but liver triglycerides and cholesterol were highly increased in WD-C-fed mice compared to chow-fed mice. Cholesterol induced expression of chemotactic and inflammatory cytokines in cultured Kupffer cells and rendered cultured hepatocytes more susceptible to TNFa/actinomycin D-induced apoptosis.

**Discussion/Conclusion**: Mice fed a western-type diet with high amounts of unsaturated fatty acids and cholesterol developed obesity, insulin resistance as well as hepatic inflammation and fibrosis and therefore are a potential better model for human metabolic syndrome and NASH than mice fed a HFD.
Secondary sclerosing cholangitis – Characterization of a patient collective from the University Hospital Essen

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Introduction: Secondary sclerosing cholangitis (SSC) is a chronic cholestatic disease affecting predominantly intrahepatic bile ducts. The disease is progressive and is associated with biliary cirrhosis and/or cholestatic liver failure. Pathophysiology of SSC is still not fully understood. However, SSC is linked with critical illness and treatment in intensive care unit (ICU).

Methods: We retrospectively analyzed 45 patients diagnosed with SSC by endoscopic retrograde cholangiopancreatography (ERCP) between 2002 and 2015. The patients were either admitted to our hospital for further treatment of SSC or newly diagnosed in our centre.

Results: Male patients were more often affected than females (27 [60%] vs. 18 [40%]). The mean age at diagnosis was 46 years (range 17–69). In 43/45 patients an intensive care treatment prior to onset of SSC was documented. The most frequent causes for treatment on the ICU were polytrauma (17/45) and pneumonia (14/45). Characteristic laboratory findings were elevated levels of bilirubin (mean 10.5 mg/dl [range 0.4–31.9 mg/dl]), alkaline phosphatase (mean 864 U/l [range 93–3807 U/l]) and gamma-glutamyltransferase (mean 824 U/l [range 80–3563 U/l]). Coagulation tests were not affected (thromboplastin time: mean 101% [range 56–120%]). Mean number of ERCPs performed per patient was 5, with a range between 1 and 24. 44 patients received additional treatment with ursodeoxycholic acid. 17 patients died, 10 were listed for liver transplantation and 4 of those patients were transplanted.

Discussion/Conclusion: SSC developing in patients after ICU stay is of growing interest. Indeed SSC often progresses towards liver cirrhosis and consequently failure, therefore liver transplantation is the only causative treatment. In some patients, biliary interventions by ERCP can mitigate the course and help to stabilize liver function and disease progression.
Expression of fibrogenic markers at time of transplantation correlates with recurrence of HCC in patients undergoing liver transplantation

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Background and aims: Liver transplantation remains the only curatively intended treatment for patients with non-resectable hepatocellular carcinoma (HCC). One of the common underlying features of HCC development is liver cirrhosis. Up to 25% patients show a tumor recurrence following transplantation. The correlation of fibrogenic markers prior to liver transplantation with HCC recurrence following liver transplantation has not been characterized. We explored the expression of b-PDGF receptor and other fibrogenic markers in tumor tissue and tumor-surrounding liver tissue in patients undergoing liver transplantation and correlated these findings with tumor recurrence.

Methods: The correlation of fibrogenic marker expression in the explanted liver and HCC recurrence following liver transplantation was assessed using tumor and tumor-surrounding liver tissue from patients undergoing liver transplantation at our center between 08/2010 and 02/2012. Tissue was analyzed for the expression of fibrogenic proteins and genes, as well as collagen deposition into the extracellular matrix. Results were correlated with HCC recurrence and disease-free survival.

Results: Following liver transplantation, patients with recurrent HCC exhibited increased levels of fibrogenic markers on both protein and RNA level within the non-tumorous liver tissue in comparison to the tumor tissue itself. Patients who did not develop tumor recurrence up to four years after liver transplantation showed a reversed expression pattern of fibrogenic markers with higher levels of b-PDGFR, collagen 1 and aSMA in their tumor tissue versus tumor-surrounding liver tissue at time of liver transplantation. These findings correlated with analysis of collagen deposition in the liver.

Conclusions: The expression of fibrogenic markers is a key feature during the progression of liver disease to HCC due to various etiologies. Here, we demonstrate a correlation between differential expression patterns of fibrogenic markers within tumor and non-tumorous tissue in patients prior to liver transplantation with tumor recurrence following liver transplantation.
The *ABCB4* p.T175A allele might be associated with increased liver injury: Analysis of two independent cohorts of patients with chronic liver diseases and NAFLD

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**Introduction**: Hepatic fibrosis represents the uniform response to chronic liver injury. Lately large-scale whole-genome sequencing identified the common variant c.711A>T of the hepatobiliary lecithin transporter ABCB4 as risk factor for hepatobiliary diseases and cirrhosis (Gudbjartsson et al. *Nat Genet.* 2015). Thus we now assess this variant along with the less frequent procholestatic *ABCB4* p.T175A mutation (Delaunay et al. *Hepatology*. 2015) for their involvement in liver injury in two large cohorts of patients.

**Methods**: The first cohort comprised 678 patients (age 50.2 ± 12.7 years, 414 men) with chronic liver diseases and liver fibrosis quantified using transient elastography (Fibroscan) (Krawczyk et al. *J Hepatol.* 2011). Liver biopsy results were available in 150 patients. The second cohort was composed of 516 patients with NAFLD (48.2 ± 13.4 years, 239 men) recruited in the framework of the German NAFLD CSG group. In this cohort liver biopsy was performed in 309 individuals. The *ABCB4* variants c.711A>T and p.A175T were genotyped using Taqman assays.

**Results**: In a total of 1,194 genotyped patients, 30 carried the p.T175A variant. The c.711A>T procholestatic polymorphism was present in 798 individuals. In the first cohort, carriers of the p.A175T variant presented with significantly increased TE levels (p = 0.02) as compared to patients with the common genotype. Among five biopsied carriers of the risk allele, three presented with fibrosis stage F4. In the NAFLD group, seven of the nine carriers of the minor p.A175T allele (78%) had been scheduled for liver biopsy. This variant was however not associated with histological fibrosis stages or liver function tests (all p > 0.05). Presence of the *ABCB4* c.711 variant was in turn not associated with liver stiffness, results of liver biopsy, or liver function tests (all p > 0.05).
**Discussion/Conclusion:** Carriers of the *ABCB4* p.A175T risk allele who suffer from chronic liver disease might be at increased risk of progressive liver injury and fibrosis. This effect appears to be less pronounced in NAFLD patients. Our observation points to a sensitizing role of procholestatic mutations and underscores the value of elastography for integrated assessment of pathogenic pathways in chronic liver disease.
Iso-alpha acids protect from alcohol-induced liver injury

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Introduction: Alcoholic mediated liver injury is one of most frequent causes of liver disease worldwide. Iso-alpha-acids (IAA) are the main bitter acids in beer derived from hops. Recent studies showed that IAA exhibit beneficial effects on glucose and lipid metabolisms and have anti-inflammatory properties.

The aim of this study was to analyze the effect of IAA on alcohol-induced liver injury in \textit{in vitro} and \textit{in vivo} models.

Methods and results: Primary human hepatocytes (PHH) were incubated with serial concentrations of IAA (up to 25 µg/ml) for 24 h. Subsequently, cells were exposed to alcohol concentrations up to 300 mM for 24 h. Under these conditions, alcohol induced some toxic effects on hepatocytes as indicated by increased levels of transaminases in the supernatant. In addition, alcohol induced hepatocellular triglyceride accumulation and the expression of genes involved in \textit{de novo} lipogenesis (fatty acid synthase [FASN] and stearoyl-CoA desaturase-1 [SCD-1]) and pro-inflammatory factors (IL-8 and ICAM-1). These alcohol-induced effects were inhibited by IAA pre-incubation in dose-dependent manner. Next, we analyzed the effect of IAA in a model of acute-alcohol induced liver injury in mice. IAA (100 mg/kg) was applied by oral gavage. Control mice received vehicle only. After three hours, both groups received a single high dose of alcohol (6 g/kg) by oral gavage. IAA treated mice showed significantly reduced levels of serum transaminases, hepatic steatosis and expression of pro-inflammatory genes (plasminogen activator inhibitor-1 [PAI-1]).

Conclusion: IAA protect human hepatocytes and mouse livers from (acute) alcohol-induced injury. Together with previous studies showing the safety of IAA applications in men our data suggest IAA as promising therapeutic agent for the prevention and treatment of alcoholic liver disease.
Effects of radiation and/or tumor necrosis factor-alpha on cell damage in a healthy liver: A role for PECAM-1

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Introduction: The liver is considered to be radiosensitive; however, the mechanisms of radiation-induced liver damage are poorly understood. Platelet endothelial cell adhesion molecule 1 (PECAM-1/CD31) is an adhesion molecule and expressed mainly in blood cells and endothelial cells. Its expression is decreased during inflammatory processes. Tumor necrosis factor (TNF)-alpha, which is induced by radiation, is known to downregulate PECAM-1.

Methods: The aim of the current study was to investigate if combined treatment with TNF-alpha and irradiation would enhance liver damage through regulation of the PECAM-1 signaling pathway. This was studied \textit{in-vivo} in mouse models of single-dose selective liver irradiation w/wo TNF-alpha administered intraperitoneally shortly before irradiation.

Results: Both irradiation and TNF-alpha administration alone induced elevated aspartate aminotransferase (AST)-levels (hepatic damage) in serum, compared to sham-irradiated mice (control). This hepatic damage was further enhanced in mice that received combined treatment with irradiation and TNF-alpha. In parallel to hepatic damage, a time-dependent decrease in the expression level of hepatic PECAM-1 was found in mice that received each single irradiation or TNF-alpha treatment. The administration of irradiation together with TNF-alpha showed additional decline in the expression of PECAM-1. In contrast, increased expression of hepatic lipocalin-2 (LCN-2), an acute phase protein, was detected at mRNA and protein levels after irradiation or TNF-alpha treatment. The level of LCN-2 was further increased in mice that received combined treatment with TNF-alpha and irradiation, compared to irradiation or TNF-alpha alone. This induction seems to be mediated by the activation of the signal transducer and activator of transcription (STAT)-3 signaling pathway. In order to study the role of PECAM-1 in hepatic damage, the liver of both wild-type (WT) and PECAM-1 knock-out (KO)-mice were selectively irradiated (25 Gy). PECAM-1 KO mice showed higher liver damage in parallel to increased LCN-2 expression compared to WT-mice at RNA and protein levels. By means of Western blotting, an increased level of cell death-related proteins (SOD-1, BAX) was observed after irradiation in both WT- and PECAM-1 KO mice. However, the level of Cyt-C was reduced only in PECAM-1 KO mice after irradiation.

Discussion/Conclusion: Our study shows a synergistic effect of radiation and TNF-alpha on hepatic cell-damage, probably through regulation of PECAM-1. Our results may help to develop protective strategies to reduce radiation-induced defects in normal liver tissue, as well as strategies, which may increase the effects of radiation on tumor tissue.
Involvement of caveolin-1 in liver cancer metabolism

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Introduction: Cancer cells have aberrant metabolic activities, which they use to gain survival and proliferative advantages. Caveolin-1 (CAV1) is a plasma membrane protein that has been shown to influence metabolism, notably in hepatocytes, adipocytes, cancer-associated fibroblasts, and cancer cells, and is often overexpressed in hepatocellular carcinoma (HCC). The aim of this study is to investigate the contribution of CAV1 in the metabolic activities of HCC.

Methods: We analyzed the expression pattern of CAV1 in clinical datasets from HCC patients. In addition, we knocked down Cav1 in freshly isolated murine hepatocyte, followed by microarray to generate a training set for investigating the effect of CAV1 on metabolic genes. With this array, we also performed gene functional annotation analysis. In HCC cells, we stably overexpressed CAV1 in a well-differentiated cell (HUH7) that has low basal level of its protein, and knocked down its expression in a poorly differentiated cell (HLE) in which it is constitutively overexpressed. Other experimental methods applied include immunoblotting, qPCR, biochemical, proliferation/viability and migration assays.

Results: We found CAV1 to be overexpressed in 5 of 8 HCC analyzed. Functional annotation upon Cav1 knockdown revealed the deregulation of genes involved in fatty acid, amino acid, and carbohydrate metabolism. In HCC cells, CAV1 expression also correlated with their migratory and ‘Warburg effect’ phenotypes (i.e. increase glucose consumption and lactate output), which were high in HLE compared to low-CAV1 expressing HUH7. We found that the induced overexpression of CAV1 in the low-expressing cells led to increased proliferation, ATP production, as well as enhanced glucose consumption and lactate output.

Conclusion: Our study suggests that CAV1 overexpression could contribute to tumor metabolic phenotype, especially in early HCC.
Both quality and quantity of the diet are important determinants in induction of obesity, fatty liver, and metabolic syndrome

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**Background:** Daily consumption of high-calorie enriched diet is associated with obesity, metabolic syndrome, T2DM and non-alcoholic fatty liver (NAFLD). The definition of NAFLD allows consumption of moderate amount (20–30 g/day) of alcoholic beverage.

**Methods:** Changes in glucose and lipid metabolism were studied in rats (160 g) which were given a regular chow, prepared fresh-daily Lieber-DeCarli (high-fat diet [HFD]), or HFD+ 30% kcal Ethanol + 30% Fructose (HF-EF) diet for 8 weeks. Body weights (BW), liver weights (LW), total caloric intakes, fasting C-peptide levels and insulin resistance were assessed. Pancreatic and hepatic mRNA was isolated for subsequent RT-PCR analyses.

**Results:** After 8 weeks, the average BW gain of chow group was 324 ± 12 g/rat, LW was 14.5 ± 0.3 g, and they had consumed 5420 ± 200 kcal/rat. In the HFD-fed rats, BW gain was 305 ± 10 g/rat, LW 15.8 ± 0.7 g, and diet intake was 5270 ± 250 kcal/rat. The BW-gain of HF-EF-red rats was 254 ± 10 g/rat, LW 17.5 ± 0.4 g, and calorie consumption was 4730 ± 220 kcal/rat ($p < 0.05$). At one week of feeding, both the HFD and HF-EF diets increased hepatic TG which increased further over time, as did the chow diet.

Long-term chow *ad libitum* intake increased HOMA-IR values, fasting serum insulin and glucose levels compared to those observed at wk1 or wk4. Plasma glucose, insulin and triglyceride levels were increased significantly over time in all groups, with the greatest increase being observed in the HF-EF rats.

**Conclusion:** Independently of diet compositions (chow or HFD), increased amount of daily caloric intake induced obesity, hepatic steatosis and the metabolic syndrome. Although caloric intake of the HF-EF group was lower and induced a lower body weight gain, it produced the highest liver weight and the greatest hallmarks of metabolic syndrome, together with the highest insulin production. Other factors that may reduce insulin sensitivity of hepatocytes in the HF-EF-fed rats remain to be determined.
Nucleic acid-based polymers that are effective against hepatitis B virus infection in patients do not harbor immune stimulatory properties in primary isolated blood or liver cells

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Introduction: Nucleic acid polymers (NAPs) block the release of HBsAg from infected hepatocytes in vivo. Although this mechanism is likely responsible for the activity of NAPs against hepatitis B virus (HBV) in patients, the role of potential immune-stimulatory effects have not been explored. In this study, the immune stimulatory properties of NAPs were examined in primary isolated human blood and parenchymal and non-parenchymal liver cells.

Methods: Human peripheral blood mononuclear cells (PBMCs) and primary isolated hepatocytes (PHH) and Kupffer cells (KC) were treated with the following NAPs: REP 2006, the prototypic degenerate NAP (dN)₄₀, contains residual CpG (TLR-9 stimulatory) content, REP 2055 is clinically active having a sequence (dAdC)₂₀ devoid of CpG content and REP 2139 (also clinically active) and REP 2165 are REP 2055 analogues further rendered immunologically inactive by replacing cytidine with 5-methylcytidine replaces and 2'-O methylation of riboses. Immune responsiveness was confirmed with known ligands for TLR-3 (poly I:C), TLR-7 (ssRNA) or TLR-9 (CpG ODN2216). Total RNA was isolated and quantitative RT-PCR was performed to analyse gene expression indicating antiviral (IFNA₄, IFNB₁, IFNG, IFNL₂) and inflammatory (TNF, IL6, and IL10) effects. The intracellular uptake of CY3-labeled NAPs was confirmed using fluorescence microscopy.

Results: REP 2006, REP 2139 and REP 2165 induced pro-inflammatory responses in PBMCs but displayed no significant antiviral activity. In PHH, no significant inflammatory or antiviral responses were detected for any NAP. In KC, pro-inflammatory activity (restricted to TNF) was observed with REP 2006 and REP 2055, whereas a weak but significant induction of interferon genes (INFA and IFNL2) was only observed with REP 2006 at the highest concentration. These signals were comparable to those induced by ODN2216 stimulation.

Discussion/Conclusion: The data presented here suggest that NAPs optimized to treat hepatitis B virus infection in patients do not induce antiviral responses in primary isolated blood or parenchymal and non-parenchymal liver cells. We therefore hypothesize that the antiviral activity of NAPs against HBV infection cannot be explained by direct induction of innate antiviral responses.
Transgene induced unfolded protein response (UPR) accelerated cholestatic liver damage and carcinogenesis in Abcb4\(^{-/-}\) mice

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**Introduction:** Chronic HBV infection with concurrent immunosuppression has been shown to enhance the risk of developing fibrosing cholestatic hepatitis, an aggressive and mostly fatal form of viral hepatitis. High-level expression of hepatitis B viral surface proteins (HBs) has been associated with fibrosing cholestatic hepatitis. Clinical studies as well as laboratory experiments demonstrated the cytotoxic effect of HBs expression. Here, pathologic consequence of HBs expression without viral infection was characterized in a mouse model of intrahepatic cholestasis.

**Methods:** Serum and liver of hybrids from HBs transgenic and Abcb4\(^{-/-}\) mice were analyzed by qRT-PCR, serum-analysis, Western blot, and histology for ALT, bile acids, inflammation and fibrogenesis, UPR as well as tumorigenesis associated pathway regulation.

**Results:** Abcbc4\(^{-/-}\)/HBs\(^{+/-}\) hybrids showed accelerated cholestatic liver disease and tumorigenesis. Increased total serum bile acid levels as well as decreased concentrations of the protective bile acid TUDCA went along with enhanced liver damage and fibrogenesis. HBs induced UPR was augmented in hybrids although HBs expression was reduced.

**Discussion/Conclusion:** Remarkably, the current study demonstrates aggravation of liver disease by intracellular HBs accumulation without viral infection in case of simultaneous cholestatic labefaction. This observation suggests that a specific reduction of HBs expression in chronically infected HBV patients might improve therapy and outcome.
Therapeutic vaccination of chronically HBV-infected patients with low-level of HBsAg using a third generation PreS/S vaccine (Sci-B-Vac™) result in seroconversion to anti-HBs

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Introduction: Chronic hepatitis B (CHB) is currently treated with IFN-α and nucleos(t)ide analogues (NUCs), however, rarely reduce the total number of cccDNA copies in hepatocytes. Therefore, cytolytic and non-cytolytic approaches are needed to eliminate cccDNA. Effective virus-specific T and B cells remain crucial in eliminating cccDNA-carrying hepatocytes. Reduction of HBV viremia by NUCs however, provides a window for the reconstitution of HBV-specific immune response. Our previous studies in transplant patients indicate that NUCs, in combination with a third generation PreS/S vaccine (Sci-B-Vac™), may lead to the control of HBV viremia during the drug-off period in patients.

Methods: We immunized 4 HBsAg-positive patients with chronic hepatitis B infection 4 to 6 times with Sci-B-Vac™ and determined HBsAg quantitative and anti-HBs titers before and after each vaccination. All patients treated with NUCs were at least for two years HBV-DNA negative prior to vaccination.

Results: HBsAg concentrations of the 4 immunized patients were 19865, 448, 20.2 and 19.2 IU/l before the first vaccination. All patients remained HBV DNA negative for the whole period of observation (at least 2 years). After three vaccinations 3 of the 4 patients seroconverted to anti-HBs. One year after starting vaccinations, the anti-HBs titers where 75, 77 and 152 IU/l respectively. The patient with an initial high concentration of HBsAg did not seroconvert. CD4 and CD8 T-cell response is under investigation and will be presented at the meeting.

Discussion/Conclusion: The HBV carriers with low-level HBsAg titers and negative results for HBV DNA under NUC treatment vaccinated with the PreS/S vaccine (Sci-B-Vac™) seroconverted to anti-HBs and showed declining concentrations of HBsAg. This treatment may have reduce the tolerizing effect of HBsAg with respect to an appropriate T and B cell response. Further studies are needed to determine whether HBV cccDNA may be reduced or even eliminated in these patients.
Epigenetic modification of HepG2 cells improves its role as an \textit{in vitro} drug screening test system

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\textbf{Introduction}: The wide use of human hepatocytes (PHH) in drug metabolism testing of pharmaceuticals are met with limitations due to limited availability, variability and dedifferentiation property. In our study, as an alternative, we used HEPG2- cells though its drug metabolizing activity is low. Till date several strategies to improve the function of HEPG2 cells have been unsuccessful. Studies have shown that epigenetic regulators influence the expression and function of some phase I/II enzymes by altering crucial transcription factors responsible for drug metabolism. Consequently, we modified the epigenetic status of these cells by 5-azacytidine (5-AZA) and vitamin C treatment to improve its function.

\textbf{Methods}: HEPG2 cells were stimulated with 5-AZA and Vitamin C, following RNA and Protein expression of EMT markers and phase I/II enzymes was quantified using PCR/WB. Subsequently, the activity of some phase I/II enzymes were tested.

\textbf{Results}: We observed epigenetic modification after treatment of cells with 5-AZA and Vitamin C which resulted in significant changes of EMT, and improved the expression and activity of some phase I/II genes and enzymes. After stimulation with 5-AZA and vitamin C the expression of HNF4α and E-cadherin, which are key regulators of liver-specific gene expression were increased by 70%, three fold when compared to untreated control cells. Similarly, the EMT marker gene Snail significantly decreased after stimulation. Furthermore, the RNA/protein expression and the activity of some phase I/II enzymes were increased. For example, we achieved a 2 to 4.5 fold increase in basal activity, depending of the tested enzyme.

\textbf{Discussion/Conclusion}: Our study showed that epigenetic modification of the HEPG2 cells resulted in the increased phase I/II gene expression and enzyme activity. This increase is associated with a reduction of EMT. Enhancement of liver specific functions in HEPG2 using epigenetic modifiers opens new opportunities for drug development, toxicity screening.
Iso-alpha-acids from hops inhibit proliferation and interleukin-8 expression of hepatocellular carcinoma cells

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Introduction: Chronic inflammation represents a hallmark in the development of hepatocellular carcinoma (HCC). The inflammatory process is involved in the formation of a microenvironment that promotes growth and survival of cancer cells. Thus, anti-inflammatory components might exhibit antitumor activities. Iso-alpha-acids (IAA) from hops are formed during the brewing process and provide the bitter taste of beer. Since IAA are known to possess anti-inflammatory properties, they might be effective against HCC development and progression. The aim of this study was to analyze the effects of IAA on proliferation of HCC cells and on expression of genes that play a role in inflammation and HCC progression.

Methods: The effects of IAA on the proliferation of the human HCC cell lines Hep3B and HepG2 were investigated using XTT assay. Furthermore, expression levels of cyclin D1 and IL-8 were analyzed by qPCR.

Results: IAA inhibited proliferation of HCC cells in a dose-dependent manner. In line with this, IAA treatment resulted in a dose-dependent reduction of mRNA expression of cyclin D1 which is critical for the regulation of the cell cycle. Furthermore, IAA significantly inhibited expression of IL-8.

Discussion/Conclusion: Our data indicate that IAA inhibit the tumorigenicity of HCC cells and the expression of genes involved in HCC progression. These data indicate IAA as promising agents for the treatment of HCC.
Analysis of effects of ethanol on expression of factors promoting hepatic metastasis in hepatoma and melanoma cells

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Introduction: The liver is an attractive "soil" for metastasis of several primary tumors, including malignant melanoma. However, the molecular mechanisms and risk factors that affect hepatic metastasis are mostly unknown. (Chronic) ethanol consumption induces pathological changes in the liver, including enhanced expression of growth factors and pro-angiogenic factors known to induce tumor progression. Still, the influence of alcohol on (hepatic) metastasis is widely unknown.

The aim of this study was to analyze the effect of ethanol on factors promoting hepatic metastasis in vitro.

Methods: Conditioned medium (CM) was generated from the human hepatoma cell line HepG2-E47 (stably expressing Cyp2e1) stimulated with ethanol (50 or 100 mM) for 24 h or control medium. Subsequently, human melanoma cell lines were exposed to CM of control and ethanol-treated hepatoma cells, as well as ethanol (50 or 100 mM) alone. Cell proliferation was detected by electrical impedance measurement applying the xCELLigence system (Roche). Additionally, mRNA levels of genes relevant for melanoma metastasis were analyzed in ethanol-treated (125 or 250 mM) melanoma cells by qPCR.

Results: CM of ethanol-treated HepG2-E47 cells induced a higher proliferation rate in melanoma cells than control CM or ethanol stimulation alone. Moreover, also ethanol stimulation alone induced a proliferation of melanoma cells compared to cells in control medium. Furthermore, exposure to ethanol induced increased expression levels of VEGF, ICAM-1 and RANTES.

Discussion/Conclusion: Our in vitro data suggest that ethanol induces different mechanisms that affect (hepatic) metastasis. In hepatocytes, it induces the secretion of promitogenic factors. Furthermore, it directly induces proliferation of melanoma cells and the expression of factors known to promote (hepatic) metastasis. In vivo models and epidemiological studies are needed to further investigate the connection between alcohol consumption and (hepatic) metastasis, which would have important implication for the guidance and recommendations for tumor patients.
Generation of a 3D model to better mimic NAFLD in vitro

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Introduction: Non-alcoholic fatty liver disease (NAFLD) has become one of the major risks for the development of hepatocellular carcinoma (HCC). Simple steatosis, which is the first stage of NAFLD, is characterized by abnormal lipid accumulation in hepatocytes. As the molecular processes which lead to steatosis and further progression to non-alcoholic steatohepatitis (NASH), fibrosis, cirrhosis and HCC, are currently not completely understood, many in vivo and in vitro models have been established. However, current existing in vitro models are wrought with limitations. Liver-biopsy derived primary hepatocytes have several limitations: They (i) are rare with a low number of healthy donors, (ii) have high inter-donor variability, (iii) show limited expansion in culture and (iv) rapid decline in function. Thus, the generation of hepatocyte like cells (HLCs) from induced pluripotent stem cells (iPSCs) can provide an alternative cell source. So far these cells lack full maturity even though they express ALBUMIN and cytochrome P450 family members. Mature HLCs are needed to maximize the relevance of the experimental outcome and applicability of these cells for toxicology and drug screening. Improved maturity and functionality of human iPSC-derived HLCs has been achieved employing three-dimensional (3D) approaches incorporating MSCs and endothelial cells.

Methods: Our preliminary proof of principle experiments involved mixing of iPSC-derived mesenchymal stem cells (iMSCs) with human umbilical vein endothelial cells (HUVECs) and HepG2 cells to generate 3D in vitro liver organoids. Furthermore it is planned to generate MSCs, HLCs and endothelial cells from the same iPSC line (same genetic background). Additionally spinner flasks were used to provide better medium flow and to improve liver bud growth. These liver buds were then challenged with high levels of glucose and insulin to mimic steatosis.

Results: Within three weeks these cells aggregated and formed vascularized liver buds when cultured on artificial extracellular matrices. These buds secrete urea, express ALBUMIN, VIMENTIN (MSC marker) and CD31 – an endothelial specific marker. Fat droplet formation after challenging with glucose and insulin resulted in activated expression of steatosis-associated genes.

Discussion/Conclusion: These iPSC-derived liver organoids have the added advantage of having present mesenchymal and endothelial cells from the same individual. Further studies are underway to better characterize these liver buds both molecular and biochemically for liver associated genes, pathways and functions. This 3D iPSC-based approach is a good model to study steatosis and complements our current iPSC-based 2D model.
Inhibition of the epigenetic modifier, lysine demethylase LSD1, affects the myofibroblastic differentiation of hepatic stellate cells

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Introduction: After chronic liver injury, Hepatic Stellate Cells (HSCs) differentiate into myofibroblasts, which are central players of liver fibrogenesis, characterized by accumulation of extracellular matrix. Recently, it was shown, that epigenetic changes are involved in liver fibrogenesis. LSD1 is a H3K9 and H3K4 demethylase, which acts as an epigenetic writer in carcinogenesis, but its function in liver fibrosis is unknown. Hereby, we aim to study the role of LSD1 and its relative epigenetic modified function in liver fibrosis.

Methods: We inhibited LSD1 in the HSC-T6 by HCI-2509 (C12), and silencing the LSD1 by a short hairpin (sh) LSD1 through pSuper.Retro viral transduction system which to get the stable polyclonal and monoclonal shLSD1-HSC-T6 cell lines. The expression of fibrogenic components (collagens, SMA, PPARγ) and anti-fibrogenic epigenetic “Writers” and “Erasers” (HDAC4, EZH2, and Mecp2) were investigated by qPCR, and the methylation status of histone were determined by Western blot. Then the miRNA-array was done to analysis the miRNA profile of these stable monoclonal cells.

Results: Since the LSD1 level was silenced in HSC-T6, the expression of miRNA29a was upregulated and fibrogenic components were modified. And the LSD1 also have impact on expression of HDAC4, EZH2, and Mecp2. The miRNA- array shows that parts of miRNAs, which are involved in fibrogenic, angiogenesis and inflammatory responses, are also dysregulated by silencing LSD1 in HSC-T6.

Discussion/Conclusion: Our studies have evidenced that LSD1 plays a central role in HSCs contributes to liver fibrosis. Post-transcriptional knockdown of LSD1 suggested an increased antifibrotic function in transgenic HSC-T6 cells, not only through the affection on the miRNAs but also the influencing on other anti-fibrogenic epigenetic roles.
Targeting the TGF-β1 pathway for normalization of chronic liver disease

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Background and aims: The concept of reversing chronic liver disease (CLD) has been intensively studied over the past decade. Indeed, as the prevalence of end-stage liver disease is constantly on the rise, the lack of established anti-fibrotic therapies is a considerable unmet need in clinical practice. The transforming growth factor (TGF)-β has been identified as a master regulator of liver fibrogenesis. Thus, targeting TGF-β1 pathway might modulate CLD development and/or progression in murine model. galunisertib is an oral small molecule targeting TGF-β type I receptor (ALK-5). It is worth to mention that this inhibitor is already in clinical trials phase II to treat the hepatocellular carcinoma patients (NCT02423343).

Methods: CLD development and progression were investigated in the MDR2 KO mice. 4 months old mice were daily exposed to galunisertib via oral gavage for 14 consecutive days. Two days after the last dosage, blood plasma and livers were harvested for standard CLD assessments.

Results: No changes were observed in body weight of galunisertib-exposed mice compared to vehicle treated group. pSMAD2 immunostaining showed a reduction in the number of positive hepatocytes after galunisertib administration, indicating efficacy of the treatment in targeting the TGF-beta pathway. Upon galunisertib treatment, liver transaminases, namely ALT and AST were reduced. Moreover, galunisertib reduced the level of α-SMA expression as tested by IHC staining and immunoblotting. Currently, the treatment effect will be further examined at the molecular and morphological levels using real time RT-PCR and immunohistochemistry/immunofluorescent stainings.

Conclusion: We can conclude so far that galunisertib shows a trend in the direction of CLD regression. Further experiments are ongoing to determine the optimal settings for galunisertib to normalize liver diseases.
HCV therapy reduced not only the liver-related but the overall mortality too – Results in the Leipzig anti-D cohort after 38 years

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Introduction: The long-term natural history of hepatitis C (1b) infection is still not fully understood. So far, there are few long-term studies with a known date of infection which include all those infected persons without bias. According to the initial reports it was assumed that after 20 years, 30% of patients would have been developed liver cirrhosis. A new issue is to what extent the prognosis after more than three decades has been influenced by the anti-HCV therapy.

Methods: Between August 1978 and March 1979 it came to the administration of 14 hepatitis C-contaminated anti-D immunoglobulin batches at 2867 East German women to prevent Rh isoimmunization. This HCV cohort is of particular interest because there are only a few cohorts with known HCV infection time that would allow precise statements about the spontaneous course and therapy results. Our data were collected in 15 study centers in East Germany since the beginning. The Leipzig anti-D cohort as part of the total cohort includes 356 women, of whom now 181 were followed up after 38 years. 79 women were treated with IFN-based therapies, 7 women with IFN-free regimen.

Results: After 38 years, 85% of the 181 women in the HCV ELISA were positive. 33% were viremic (HCV PCR positive). Only 11 (8.3%) of viremic women had liver cirrhosis, 9 (5.4%) had advanced fibrosis. In the last 15 years a continuous, but slow rise of advanced fibrosis score was observed. HCC has not been diagnosed. 48 women with an IFN-based therapy and all women with an IFN-free regimen were treated successfully (SVR). Since 1978 six HCV RNA-positive women of the Leipzig cohort died; eight women died after viral clearance. The overall mortality in the therapeutic SVR group was lower than in the treatment-naïve group.

Discussion/Conclusion: Young women without comorbidity eliminate HCV (1b) infection spontaneously in approximately half of the infected cases. After 38 years, a continuous but relatively low progression with regard to final states such as cirrhosis, HCC or death could be confirmed in this cohort. IFN-free therapies should be recommended because the overall mortality after successful therapy (SVR) was lowest.
Different mechanisms control the loading of miR-198 into exosomes

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Background and aim: miR-198 has been proven as a tumor suppressor in different cancer types, inhibiting cell growth and proliferation. Previous studies have shown that miR-198 is the most downregulated miRNA during liver diseases progression. Therefore we aimed to study the regulatory function of miR-198 in liver cancer cells.

Methods: miR-198 expression cassette was cloned under doxycycline induced tet-on promoter and stably transfected into liver cancer cells. RNA was isolated and real time PCR was performed to analyze miR-198 expression level. As well, the supernatants were collected and after serial centrifugation the exosomes were precipitated. The isolated exosomes were further characterized by Western Blotting and real time PCR. Immunoprecipitation method was used to pull down miR-198 binding proteins. And mass spectrometry will be adopted to identify the vital proteins responsible for miR-198 loading.

Results: Intracellular miR-198 level was at first strikingly upregulated after doxycycline treatment, which was followed by significantly decrease. Exosomes have been isolated from supernatant and characterized by exosome markers, CD63 and HSP70, and miR-198 is enormously enriched in exosomes. Both Ago2 and ubiquitin immunoprecipitation have shown that miR-198 is binding with Ago2 proteins and strongly associated with ubiquitin. Furthermore, mass spectrometry results have revealed that Ago2 proteins are ubiquitinated intracellularly. Overexpression of ubiquitin in the cells promotes miR-198 exosomal secretion.

Conclusion: In liver cancer cells, intracellular miR-198 is tightly controlled and is recognized by Ago2 proteins and secreted via exosomes, which could offer possible explanation why miR-198 is downregulated in liver cancer cells.
An unusual case of acute-on-chronic liver failure

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Introduction: This case study presents the case of a male patient with an unusual case of acute-on-chronic liver failure.

Case presentation: A fifty-seven-year-old man arrived at the emergency department with the following symptoms: fatigue, jaundice, abdominal distention, and lower-leg edema. He had a history of hepatitis C virus (HCV) infection and past alcohol and intravenous drug abuse. He was known to have liver cirrhosis with Child-Pugh score B. The HCV infection had successfully been treated with direct-acting antiviral therapy one year ago.

The laboratory results of the patient at the emergency department showed an acute hepatic failure: ALT 670 IU/l (reference < 41 IU/l), Bilirubin 27 mg/dl (ref. < 1.2 mg/dl), INR 2.99 (ref. 0.9–1.25), MELD-Score 40.

Management of outcome: The patient was admitted to the hospital and underwent conservative therapy of acute hepatic failure. However, in the following days, he developed progressive hepatic encephalopathy, hepatorenal syndrome and coagulation dysfunction.

Meanwhile different causes of acute on chronic liver failure such as drug or alcohol abuse, toxic liver failure, HCV relapse, hepatocellular carcinoma, and portal vein thrombosis were ruled out.

Several days after hospital admission, laboratory results were positive for hepatitis E virus (HEV) IgG and IgM. Positive HEV PCR confirmed the diagnosis of an acute hepatitis E infection.

Because of fulminant liver failure the patient was listed and underwent transplantation. Due to primary non-function, the patient was re-transplanted four days later. This time, transplantation was successful. One month later, the patient was discharged from the clinic.

Discussion/Conclusion: In patients without preexisting liver diseases, the course of an acute Hepatitis E infection is in general asymptomatic and self-limiting. There are cases of fulminant infection with liver failure in patients with preexisting liver disease, alcohol abuse or immune suppression. Testing for Hepatitis E should be routinely undertaken in cases of acute and acute-on-chronic liver failure.
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