

analytical specificity and reliable quantification. Here we developed the first Selected Reaction Monitoring (SRM) assay for the multiplexed measurement of cancer biomarkers in human bile. For this purpose, 8 potential biomarker candidates previously highlighted by proteomic analysis were selected. Equal volumes of bile collected from patients presenting with malignant and non-malignant biliary stenosis were stacked on the top of a SDS-PAGE gel. Proteins were then digested in-gel with trypsin and proteotypic peptides of each candidate biomarker were quantified by nanoLC-SRM on a 5500-QTrap mass spectrometer (ABSciex) using heavy synthetic peptides as standards (PEPotecTM, ThermoFisher). SRM data were finally analysed using Skyline software and manual validation. The developed assay proved to be valuable and reliable to quantify all the selected candidates. Moreover, the results confirmed the simultaneous overexpression of some of the proteins in bile samples from malignant stenoses. Overall, our data demonstrate the ability of SRM to quantify cancer biomarkers in human bile and emphasize the interest of using multiplexed SRM assays to assess the diagnostic potential of a panel of bile biomarkers in differentiating biliary stenoses. Work supported by the PRIME-XS consortium.

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## The changing face of epidemiology of systemic fungal infections

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### Abstract

Invasive fungal diseases (IFDs) are an increasingly common complication in critically ill patients in Europe and are frequently fatal. Because of changes in treatment strategies and the increased use of antifungal prophylaxis, the epidemiology of IFDs has changed substantially in recent years and infections due to *Candida* species are no longer the majority in many institutions. In contrast, the emergence of non-*Candida* IFDs such as aspergillosis, *ucrm*ycosis and fusariosis has increased. Rates of IFD-related mortality in Europe depend on the pathogen, geographical location and underlying patient characteristics, with rates ranging from 28 to 59% for *Candida* infections and from 38 to 80% for invasive aspergillosis. Early initiation of antifungal therapy is critical for improving outcomes; however, this is complicated by the difficulty in diagnosing IFDs rapidly and accurately. Choice between agents should be based on a variety of factors, including spectrum of activity, adverse events, drug interactions, route of administration, clinical efficacy of individual agents and local epidemiology.

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## Anti-tumor effects of the human monoclonal anti-nuclear antibody on the HEp-2 cells

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### Abstract

Function of autoantibodies from patients with autoimmune diseases in malignancies development is not clear yet. It has been reported that a cell-penetrating lupus autoantibody, 3E10, which was isolated from a mouse model of systemic lupus erythematosus (SLE), has been a potential targeted

therapy for DNA-repair deficient malignancies. We have got four human monoclonal antinuclear antibodies from patients with autoimmune disease, 3B5, 3C1, 3E8 and 4F3. Our data showed that four antibodies could combine HEp-2 cells and display different nuclear types as antinuclear antibody (ANA). Also, these four ANAs can inhibit HEp-2 cells proliferation. We think these antibodies may be potential antibody drugs to cancer therapy. However, the function and mechanism are not clear. Further study, we want to clarify the effects of four ANAs on proliferation of various cancers cells and to investigate the mechanism of four ANAs affecting various cancers cells proliferation and their targets. This may be a new mechanism of malignancies development in patients with autoimmune diseases, and provide novel angle of autoantibody function study.

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## Rapid saline test for varicella zoster virus

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### Abstract

Varicella zoster virus (VZV) is a ubiquitous human herpesvirus typically causing childhood varicella (chickenpox) at which time a life-long latent infection is established in ganglionic neurons throughout the neuraxis. Reactivation of latent virus, typically in the elderly and immunocompetent usually causes zoster (shingles) but can also result in serious neurologic disease. In cases of vasculopathy, meningoencephalitis and myelitis where VZV is suspected, diagnosis requires detection of virus DNA or antibody in CSF. In collaboration with NASA, VZV DNA was found in saliva of health astronauts suggesting asymptomatic virus reaction due to the stress of spaceflight. This led to a series of studies indicating virus DNA can be found in saliva of patients with VZV associated neurologic disease. With the goal of eliminating the need for lumbar puncture to diagnose VZV associated neurologic disease; we developed a rapid saliva test for the detection of VZV DNA in saliva that can be used in space as well as on Earth. Herein the test and its potential application will be present.

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## Novel approaches for the supportive extracorporeal therapy of sepsis: Towards personalized treatment

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### Abstract

Sepsis and sepsis-associated multiple organ failure are associated with extensive tissue damage caused by over-activation of the innate immune system and by the excessive release of inflammatory mediators. The development of targeted therapies for sepsis remains a major challenge due to the complex network of inflammatory mediators involved in the septic process.

Early detection and timely therapeutic intervention are crucial for improved outcome of patients with sepsis. Currently however, the diagnosis of sepsis mostly relies on general symptoms. Taking into account the extreme heterogeneity of septic patients, the application of supportive extracorporeal therapies to modulate the concentration of inflammatory mediators requires diagnostic tools to monitor the inflammatory profile of the patients in order to identify the optimal time window for application of supportive therapies.

Here, we report on the development of extracorporeal adsorption systems for cytokine modulation and on the development and validation of a novel array technology to detect markers of inflammation (interleukins 6 and 10,

C-reactive protein, procalcitonin, serum amyloid A) in a bedside-approach (detection from whole blood samples within 30 min). We demonstrate that the modulation of inflammatory mediators in septic plasma by means of selective adsorption significantly reduces endothelial activation in a cell culture model. We also discuss the role of extracellular microvesicles as markers and as potential targets for therapy.

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## Prognosticative biomarker clusters for polycystic kidney disease

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### Abstract

Polycystic kidney disease (PKD), in its autosomal recessive (AR) or autosomal dominant (AD) form, is characterized by the formation and expansion of numerous fluid-filled cysts within the kidneys. Quite often, the disease spreads to extrarenal territories including the liver. In addition to cyst formation, interstitial collagen deposition or scarring is sometimes observed in both kidney and liver. Progressive enlargement of the kidneys via replacement of the renal parenchyma with cysts and decreasing renal function makes ADPKD the leading genetic cause of renal transplantation. Highly aggressive fibrocystic kidney and liver disease in ARPKD means that many children with this form of disease do not live past the age of ten years. Using the PCK rat model of PKD, we have identify a minimally invasive biomarker cluster with high correlative value for fibrocystic disease progression. These results are important in that patient compliance, disease prognosis, interventional decisions and outcomes can be further and vastly improved by identification of minimally invasive or non-invasive biomarkers that are prognosticative of disease progression. Furthermore, rather than rely on a single biomarker, clinical outcomes may be better predicted by identification of a cluster of disease-relevant biomarkers which would bring increased correlation with disease progression. Clinical trials of therapeutics for chronic fibrotic diseases would also benefit from identification of such biomarkers given Big Pharma's reluctance to invest in trials wherein endpoints could be years away with no interim hint of success/failure. Identification of minimally invasive or non-invasive biomarkers in proliferative fibrocystic disease can better stratify children waitlisted for scarce kidneys and/or livers. The tangible outcome/technology/product that will result from the proposed research is biomarker-cluster chips designed to read urine or serum samples to determine disease progress or remission from disease. It is anticipated that these chips can eventually be mass produced in a relatively inefficient fashion and would have the predictive power  $\geq$  imaging technologies but at far lesser cost and far lesser inconvenience. Eventually, this paradigm and the resulting technology may be extended to other diseases.

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## Proteomic profiling to identify markers of bacterial meningitis

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### Abstract

Bacterial meningitis is usually fatal without treatment and prompt and accurate diagnosis coupled with the timely administration of parenteral

antibiotics are necessary in order to save lives. Despite the availability of highly effective antibiotics, the complications from bacterial meningitis (such as deafness, hydrocephalus, seizures and cerebral palsy) remain high. In areas with a high incidence of human immunodeficiency virus infection, *Streptococcus pneumoniae* is the commonest cause of bacterial meningitis. The diagnosis of bacterial meningitis can sometimes be delayed whilst samples are analysed in a laboratory using traditional methods of microscopy and antigen testing. We used cutting-edge high definition and quantitative mass spectrometry to identify specific protein signatures in cerebrospinal fluid associated with *Streptococcus pneumoniae* infection which could lead to the development of assays or point-of-care devices to improve the speed and accuracy of diagnosis, and consequently to enhance the prognosis of adults and children with bacterial meningitis. A range of samples (cases and controls,  $n=12$ ) from Malawian children has been analysed. Our data indicate some clear trends, and confirm that quantitative proteomics analysis will be successful in generating a comprehensive protein list from which markers might be nominated. We identified a total of 519 proteins in data dependent discovery proteomics and obtained quantitative data for 161 proteins using data independent Hi3 quantification. Using Progenesis LCMS we obtained a list of 202 potential candidates using data dependent acquisition approach and 109 using data independent acquisition, 82 proteins being common to both workflows. The protein profiles clearly differentiated cases and controls and have the potential to inform diagnosis and management of bacterial meningitis, especially in the developing world where the disease burden and mortality is greatest.

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## Claudin expression in animal models of IBD and human disease

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### Abstract

Claudins are transmembrane proteins constituting one of three tight junction protein families. There are 24 members of the claudin family identified, differently expressed in various cells/tissues and among species. In patients with inflammatory bowel disease (IBD), site and disease activity dependant changes in expression of certain claudins has been noted.

The aim of the study was to explore expression of claudins in the mouse models of IBD and to compare it to claudin expression in human disease.

The expression of several sealing claudins that are present in colon of humans and rodents has been evaluated by immunohistochemistry.

A decrease in claudin 1 expression was observed in a chronic mouse DSS model and adoptive transfer model of colitis, as it has been reported in human disease. Claudin 3 expression was not altered in the non-inflamed mucosa. Nevertheless, a subset of claudin 3 was internalized into cytoplasm of absorptive cells in inflamed mucosa in a chronic DSS model. In an adoptive transfer model of IBD, 8 weeks post-transfer, a reduction in claudin 3 expression was noted in surface colon epithelium as noticed in patients with Crohn's disease and ulcerative colitis. Claudin 8 expression decreased in the upper part of crypts, as is reported in patients with Crohn's disease. A subset of crypt base cells became strongly positive. Finally, a decrease in claudin expression in inflamed mucosa of human biopsies from patients with ulcerative colitis (UC) was observed.

In conclusion, it was shown that claudin 1, 3 and 8 expression pattern/intensity is altered in the mouse models of IBD in a same manner as observed in human biopsies from UC patients and described in patients with Crohn's disease.

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