SCIENTIFIC PROPOSAL

I. Clinical relationship: *Risk assessment, diagnosis*

The reference samples will explore the usefulness of the SCCA-IgM biomarker as:

a) Prognostic biomarker of fibrosis progression in patients with chronic hepatitis C

b) Prognostic biomarker of HCC progression in patients with cirrhosis

c) Diagnostic biomarker of HCC

II. Background and significance

Despite a marked decrease in the incidence rate of all types of viral hepatitis in the past 15 years, several thousand deaths from cirrhosis and hepatocellular carcinoma (HCC) attributable to chronic viral infection occur each year. In clinical practice, one of the most relevant goals is the early identification of the subgroup of patients with increased risk of histological progression of liver disease and HCC development. The speed rate of fibrosis progression and of HCC development can be influenced by virus, host and environmental factors, however, in individual patients no suitable markers are yet available as prognostic tools.

HCC in Mediterranean and in North American countries commonly develops on the background of a cirrhotic liver, being its frequency expected to increase in the next decades. The prognosis is very poor and the best results in terms of long term survival (up to 7 years) are achieved with patients with smaller HCC. For these reason, surveillance programs targeting patients with liver cirrhosis have been proposed. The ideal surveillance interval is unknown, although a surveillance interval of 6 months is commonly applied, based on the average tumor doubling time. Another main and still debated issue concerns which test should be applied in the surveillance. So far alpha-fetoprotein (AFP), the only serological marker approved in clinical practice, together with ultrasound tomography (US), are the only two tests commonly performed. US, although a powerful technique in terms of diagnosis, has a low positive predictive value and is still an operator skill dependent tool. AFP has a low sensitivity (ranging in the different studies from 39 to 65%) and a reliable specificity (ranging from 76 to 97%) only at high values (over 200 IU/ml). For these reasons, the need for new markers represents an absolute priority for clinicians.

In 2001 we have identified the Squamous Cell Carcinoma Antigen (SCCA1 or SERPINB3) as cell receptor for HBV (1) and these findings were confirmed by further studies (2,3). As the relationship between HBV and liver cancer development is documented largely, we explored whether this protein was expressed in liver cancer. SCCA isoforms (SCCA1 and SCCA2 or SERPINB4) has been indeed detected in the majority (>90%) of HCC samples (4,5). More recent data report high level of expression also in highly displastic nodules, underlying its role in liver carcinogenesis (6). The biological activity of SCCA *in vivo* and in particular in primary liver cancer is still under investigation. Preliminary data indicate that this serpin confers oncogenic potential since it induces resistance to apoptosis, cell proliferation and epithelial mesenchymal transition (EMT), increasing cell migration and invasion (7). SERPINB3 is also induced by hypoxia, as a Hif-2alpha and redox sensitive event (8). In addition, this serpin has been identified recently in liver progenitor cells activated after liver injury (9) and its presence in the liver stem cell compartment might play a crucial role for the their carcinogenetic potential.

III. Preliminary Data & Methods

Multivalent IgMs are typically considered the main component of the innate immunity and play an important role in the first line of defense against tumoral cells growth by the induction of apoptotic cell death. Presence of SCCA, complexed with IgM, has been detected in serum from patients with HCC, cirrhosis, and chronic hepatitis, using a recently standardized ELISA assay (Xeptagen, Italy) in which an anti-SCCA antibody is coated to the solid phase and the bound complex is then revealed by the addition of peroxidase conjugated anti-human IgM. For the determination of SCCA-IgM, optimal conditions were standardized by using SCCA-IgM immune complexes purified by gel filtration, and the amount of SCCA-IgM was expressed in arbitrary units (AU) using a calibration curve of the reference standard purified by gel filtration. The assay displayed intra-assay and inter-assay coefficients of variation of 5.5% and 6.2%, respectively. In the initial study SCCA-IgM were undetectable in serum from a healthy control population (0 of 73 controls); however, 35 of 50 patients with HCC (70%) were reactive for SCCA-IgM IC independent of etiology. No correlation was found with AFP levels, which were elevated significantly in 42% of the patients with HCC. By using an AFP cut-off value of 20 ng/mL, 96% of patients with HCC were positive for at least 1 marker. Among cirrhotic patients, the presence of circulating SCCA-IgM IC was displayed in 13 of 50 patients (26%), but at lower levels compared to HCC patients. Among 50 patients with chronic hepatitis, 18% were reactive for this biomarker (10).

A retrospective, longitudinal study was conducted in a cohort of prospectively followed cirrhotic patients. Two groups with similar clinical profile at presentation were studied: group A included 16 patients who developed HCC during a median follow up of 4 years. Group B included 17 patients who did not develop HCC during the same time interval. At presentation similar levels of SCCA-IgM complexes and of AFP were detected in both groups. The increase over time of SCCA-IgM, assessed within at least one year before clinical diagnosis of HCC, was remarkably higher in group A than in group B, while AFP increase was not significantly different. ROC curves were plotted for the rate of change in the levels of both markers and the diagnostic accuracy measured as AUROC was higher for SCCA-IgM (0.821) than for AFP (0.654). This study documented that the progressive increase of SCCA-IgM over time was associated with liver tumor development, suggesting that monitoring the behaviour of SCCA-IgM might became useful to identify cirrhotic patients at higher risk of HCC development (11).

The ELISA assay was used also to determine the presence of SCCA-IgM in 188 patients with chronic hepatitis and in 100 controls. An additional serum sample was available after a median period of 6 years in 57 untreated patients: these patients were subdivided in group A, including 8 patients with a fibrosis score increase ≥ 2 in a second liver biopsy and group B, including 49 patients without fibrosis progression during a similar follow up. SCCA-IgM complexes were detectable in 63/188 (33%) patients but in none of the controls. A significant increase of SCCA-IgM levels over time was observed in patients with fibrosis progression, but not in those without histologic deterioration, suggesting that monitoring SCCA-IgM levels over time could be a useful approach to identify patients with chronic hepatitis at higher risk for cirrhosis development (12).

The reference panel will be used to explore the behaviour over time of the biomarker SCCA-IgM in serial serum samples of cirrhotic patients in relation to HCC development or disease progression, including cirrhosis complications (ascites, encephalopathy, variceal bleeding, hepatorenal syndrome).

In serial sera of individual patients the increase of SCCA-IgM over time (ϕ) will be evaluated using the following formula: (ϕ) =[SCCA-IgM (Tx) – SCCA-IgM (T0)] / [(TX-T0)months] where T0 refers to the time at presentation and Tx refers to the follow up time points.

IV. Data Analysis plan

Statistical Analysis will be performed by an independent data monitor committee which will include experts in statistical analysis multivariate and in epidemiological analysis.

The requested reference set, including approximately 50 patients who developed HCC and up to 2 samples of patients with the same characteristics who were not known to develop HCC during follow-up is large enough to demonstrate the utility of the biomarker.

V. Future Plans

If the biomarker is found to have promising performance characteristics:

- I will plan to approach EDRN for funding and collaboration in proceeding to a phase II validation study.
- I am amenable to working within the collaborative framework of EDRN in proceeding to phase II studies
- I will be amenable to including my biomarker into a larger panel of biomarkers for phase II validation, if deemed beneficial
- If refinements will improve the performance of the biomarker test, I will concur with further development of the test. The advantage of inclusion of EDRN resources for this purpose will be discussed.

VI. References

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VII. Human Subject Approval

Samples were collected under the Saint Louis University approved IRB protocol #10863 for study entitled "Hepatitis C Antiviral Long-term Treatment against Cirrhosis (HALT-C): A Randomized Controlled Trial to Evaluate the Safety and Efficacy of Long-Term Peginterferon alfa-2a for Treatment of Chronic Hepatitis C in Patients who failed to Respond to Previous Interferon Therapy". The samples were stripped of any identifiers before being transferred to a central repository. Any additional IRB approvals or exemptions will be provided at the time of grant award notification if necessary.