Bridge to Liver Regeneration
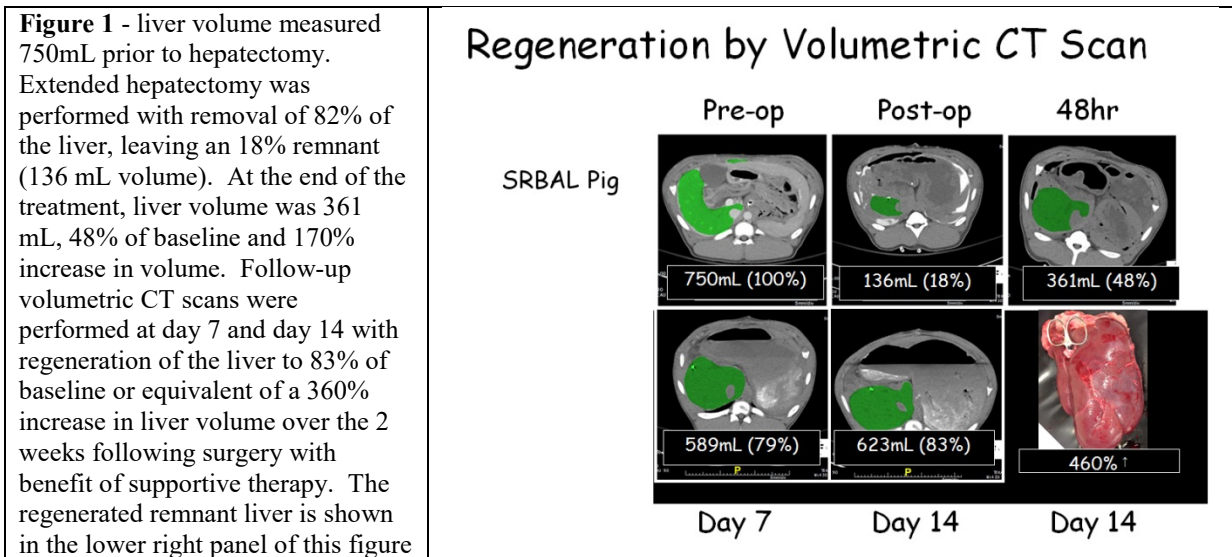**1-yr Progress report**
Minnesota Regenerative Medicine Grant
(Grant #MRM 2015 2692)

Principal Investigator: Scott Nyberg, M.D., Ph.D.
Project Timeline: May 1, 2015 to April 30, 2016

Progress to Date:

Specific Aim #1: Demonstrate enhanced liver regeneration after supportive therapy in a porcine model of post-resection ALF.

> **Summary of progress to date:** Although our study is ongoing and final results are not expected for another 12 months, we have clearly shown enhanced liver regeneration after supportive therapy with the spheroid reservoir bioartificial liver (SRBAL) device. This progress is best demonstrated using volumetric CT analysis (see Fig. 1). The animal in Figure 1 was treated with a bioartificial liver from 24 to 48 hours post resection and survived to the study endpoint of Day 14. In contrast, an 80% hepatectomy animal treated with no cell bioartificial liver developed elevated ammonia with cerebral herniation and did not survive beyond the 32-hour time point.

Figure 1 - liver volume measured 750mL prior to hepatectomy. Extended hepatectomy was performed with removal of 82% of the liver, leaving an 18% remnant (136 mL volume). At the end of the treatment, liver volume was 361 mL, 48% of baseline and 170% increase in volume. Follow-up volumetric CT scans were performed at day 7 and day 14 with regeneration of the liver to 83% of baseline or equivalent of a 360% increase in liver volume over the 2 weeks following surgery with benefit of supportive therapy. The regenerated remnant liver is shown in the lower right panel of this figure

Regeneration by Volumetric CT Scan

Histology studies showing increased staining of the mitotic marker Ki67 during liver regeneration are shown in Figure 2. Further molecular studies will be obtained to confirm enhanced liver regeneration. These studies consist of tissue samples which have been collected and are currently being saved for batch analysis at the end of the study. These molecular studies will be performed in the lab of Dr. Jeff Albright at the VA Medical Center.

Figure 2 – Increased Ki67 Staining during Liver Regeneration

| Baseline | 48 hours post hepatectomy |
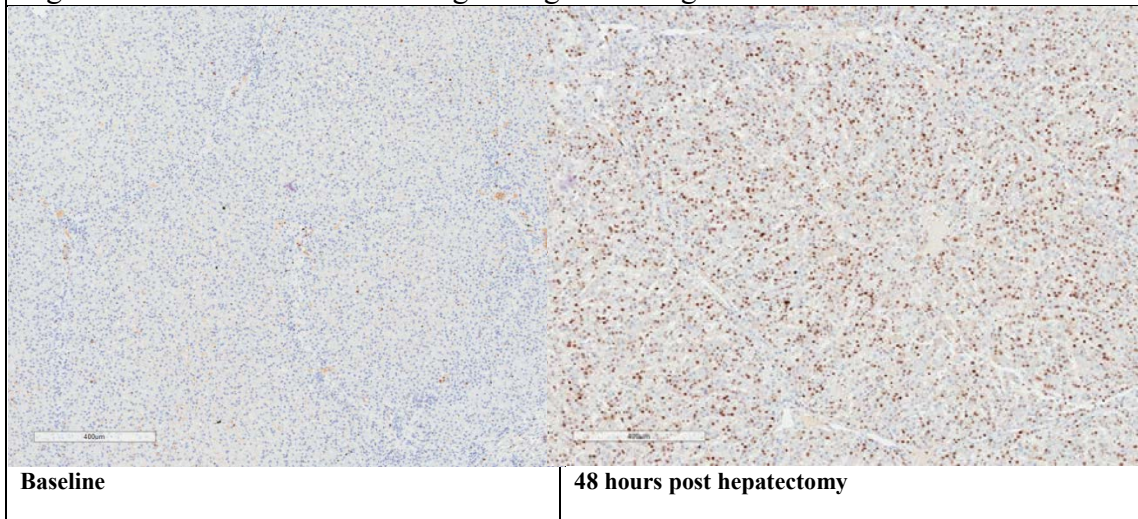
In addition, we have performed 50% hepatectomy on 6 pigs and collected either serum or plasma from these pigs to assess their regenerative activity in an ex vivo model. These supplemental studies demonstrate that serum from the 50% hepatectomy pigs significantly enhances hepatocyte proliferation under ex vivo conditions. We utilized a decellularized pig liver matrix in the studies, and the peak level of enhanced regeneration was seen at 48 hours following the hepatectomy. These studies will be pursued further in collaboration with a local company, Miromatrix, in attempts to seed their decellularized porcine grafts for creation of a transplantable pig liver graft.

Specific Aim #2: Demonstrate prolonged survival after supportive therapy in a porcine model of post-resection ALF.

Summary of progress to date: To date we have treated a total of 9 animals under either controlled conditions without supportive therapy or supportive therapy with the bioartificial liver containing cells 200 grams per treatment or no cells. Although these studies are ongoing, preliminary evidence suggests improvement in survival with the SRBAL treatment. To date, no deaths with 100% survival in the SRBAL treatment group (Fig. 3), while both control groups (no supportive therapy and no cell BAL groups) have experienced 50% mortality. SRBAL treatments have been associated with stable hemodynamics by the post-resection pig, clearance of ammonia, and prevention of cerebral herniation. Based on these results, we expect 8 to 12 animals to confirm reproducibility of these results.

Figure 3
SRBAL pig shown at 90 hours post-hepatectomy. This pig was up ambulating immediately following the SRBAL treatment and was electively euthanized at 14 days.

SRBAL Pig#1
t=90hr
after 82% hepatectomy
+ 24hr SRBAL treatment

Specific Aim #3: Demonstrate a neuroprotective effect of supportive treatment in a porcine model of post-resection ALF.

> **Summary of progress to date:** Along with the improved survival using the SRBAL device, improved survival has correlated with a reduction in serum ammonia and reduced intracranial pressure during SRBAL treatment. Reduced intracranial pressure was shown previously in our earlier study (Glorioso et al, J Hepatology 2015, 63:388-398) to be the result of reduced cerebral edema with the mechanism of cerebral edema formation to be from a combination of elevated ammonia levels and systemic inflammation. The ability of SRBAL therapy to lower serum ammonia is illustrated in Figure 4. . Of note, both animals in Figure 4 had approximately the same serum ammonia level at 24 hours post-hepatectomy. When placed on the SRBAL device the ammonia of the treated animal was reduced to a normal range.

Figure 4 - The benefit of SRBAL therapy is demonstrated versus no cell BAL therapy. The control (no cell BAL) animal developed an elevated serum ammonia and herniated approximating 32 hours after hepatectomy. In contrast, the hepatectomy animal treated with SRBAL containing 200 grams of hepatocytes did not develop intracranial hypertension and survived to the 90-hour time point and continued to do well out to final end point of 14 days. This animal was shown in Figure 3
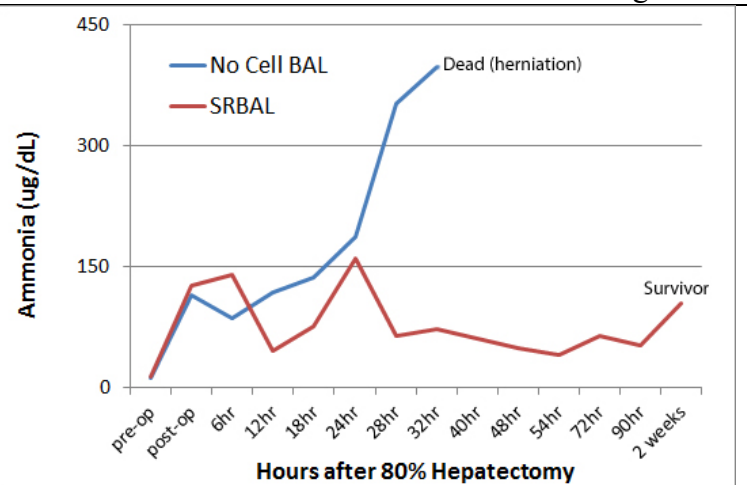
List any publications or manuscripts: To date there have been no manuscripts published since this study is not yet complete. The data has been included in several presentations including the International Liver Transplant Annual Congress in Seoul, Korea, May 1, 2016; Transplant Grand Rounds at University of Minnesota, January 2016, and the Mayo Clinic Transplant Grand Rounds February 2016. The SRBAL technology has previously been patented by Mayo Clinic. We are currently in the process of licensing this patent with at least one strongly interested investor group. At the time of this progress report, the patent has not yet been licensed. Furthermore, a new patent application is in preparation regarding the use of post-resection pig serum to enhance ex vivo expansion of hepatocytes in a decellularized liver graft.

Budget Update: The studies appear to be on schedule and within 20% of the estimated budget. One of the surgeons (Jian Yang MD) involved in the study is from Chengdu China. Of note, Dr. Yang's VISA was delayed and he did not arrive to begin work until August 2015, roughly 4 months after the initial start date of this project. As a result, we are a few months behind schedule with regard to the study animals. We are performing hepatectomies at 2-week intervals, as originally planned, so roughly 2 animals per month. We anticipate completion of the large experimentation by July 1, 2017. The molecular and histology studies will follow. Therefore, an extension of funding will be needed beyond the current deadline of April 30, 2017.

Overall Overview: "Bridge to Liver Regeneration" is well under way and on course to complete the proposed studies a few months later than originally planned, in mid-year 2017. Our model of 80% hepatectomy is highly reproducible; blood loss <500mL per animal is routinely achieved by our experienced team of hepatobiliary surgeons. In fact, average blood loss of our most recent 6 animals has been below 200 mL per animal. This level of blood loss has been associated with stable hemodynamics so the benefit of SRBAL treatment can be assessed and not biased by a hemodynamically unstable animal following excessive blood loss. As stated earlier, a total of 15 animals have been studied, including 6 animals to assess serum and plasma in the setting of a regenerative liver. These studies clearly show that sera collected 48 hours after a major hepatectomy enhance the proliferation of hepatocytes in an ex vivo system. These new studies will be further tested using a decellularized liver model in collaboration with a Minnesota company, Miromatrix, Inc. The goal of the new studies with Miromatrix is to produce a transplantable liver graft to be used in clinical transplantation. These studies are preliminary, however we are quite excited by the preliminary results and encouraged for the prospect of a transplantable tissue engineered liver. With regard to the primary aims of the study, we are on course as described above. Nine animals have satisfied the study requirements of less than 500 cc blood loss and these results appear reproducible. The SRBAL technology has been patented by Mayo Clinic, and we are in the process of identifying major investors to fund production of a clinical-grade device under FDA oversight. This device would be tested in phase 1, 2, and 3 clinical trials. That process is well under way with the FDA. Current study will strengthen the IND application at FDA and introduce a novel indication (post-resection-acute liver failure) for the SRBAL device. The current indication for the device is drug toxicity acute liver failure based on our earlier study (Glorioso et al, Journal of Hepatology 2015). Therefore, our new studies continue to follow the goals of the Minnesota Regenerative Medicine program, both to improve the health of Minnesotans with liver disease, as well as patients throughout the United States. Translation of our large animal pre-clinical data into a clinical trial is also in progress and major investor funding appears to be on the horizon.