A2ALL: Adult-to-Adult Living Donor Liver Transplant Cohort Study

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1. Introduction

Adult to adult living donor liver transplantation (LDLT) is a relatively new procedure increasingly used at major transplantation centers. Too few cases are performed at any one center and approaches to the patient and donor are too diverse across centers to provide reliable and generalizable information on donor and recipient outcomes from individual centers. Therefore, the National Institutes of Health has organized a network of nine leading liver transplantation centers and a data coordination center (DCC) to accrue and follow sufficient numbers of patients being considered for and undergoing LDLT to provide generalizable results from adequately powered studies. This network has established the Adult to Adult Living Donor Liver Transplantation Cohort Study (A2ALL) that will conduct both retrospective and prospective studies of LDLT.

2. Background/Significance

Over the last 20 years liver transplantation has become the standard of care and the only cure for end stage liver disease. Its success has led to over 4,000 transplants performed yearly. But there are at least 17,000 patients on the transplantation list awaiting deceased donor (DD) liver donation. As the waiting list has expanded, waiting time has also grown. As a result, patient mortality has increased while awaiting transplantation, and patients are often critically ill by the time of transplantation (*1*). Among possible remedies, living donor transplantation has become widely accepted for pediatric transplantation. Adult-to-adult LDLT is a more challenging procedure and entails potentially greater risk to the donor because of the larger portion of liver that is required (*2*). Right lobe adult-to-adult LDLT is a recently developed procedure, but nearly a thousand have already been performed in the United States. Although still a small number relative to the several thousand adult deceased donor liver transplants (DDLT) performed annually, LDLT has the potential for changing the face of liver transplantation. Not only does LDLT avoid the lengthening waiting period for a deceased donor transplant, it greatly reduces the ischemic period of the transplanted organ, allows more time for evaluation of the donor, and changes the operation from an emergency into a scheduled procedure. The major disadvantage of LDLT is that it is a difficult and potentially fatal operation for the donor. It also provides the recipient with a smaller portion of liver than would have been received with deceased donor transplantation.

The research objectives of the LDLT Cohort Study concern factors that influence the outcomes of adult-to-adult LDLT as well as a study of the biological differences between living donor (LD) and DD grafts in the recipients. Adult patients and potential donors being considered for LDLT will be recruited into this longitudinal cohort study. Recipients and their donors will be followed for sufficient time to determine outcomes related to LDLT. These outcomes will be compared with those of transplant candidates who are evaluated for but do not receive LDLT. The primary objective concerns comparison of morbidity and mortality of patients who receive LDLT with a group or groups of patients with similar illnesses and prognoses. A critical question to answer with this information is how the outcomes of LDLT compare with those of deceased donor transplantation. Transplant physicians need this information on outcomes to advise prospective recipients and donors. Therefore, sufficient recipient and donor pairs will be recruited to determine whether recipients of LDLT have substantially different survival

than non-LDLT recipients. A large number of donors and recipients from several geographically distributed institutions will be necessary to reliably determine if outcomes are different with the two approaches.

The differences between LDLT and DDLT are inherent both in the application and the biology of the procedures. By its nature DDLT includes uncertainty about both the time of transplantation and the condition of the recipient at the time of the eventual transplantation. Because the LDLT is elective, pre-transplant morbidity and mortality are minimized in the LDLT group. This means that pre-transplant morbidity and mortality are major areas in which the potential advantage of LDLT needs to be quantified. In contrast to the expected benefits of enhanced access to transplantation, the recipient of LDLT faces a procedure which is more complex than DDLT and which provides only a partial graft. Thus, the penalty paid by opting for LDLT rather than opting for DDLT also needs to be quantified.

LDLT offers a unique opportunity to study human liver regeneration and its impact on several key clinical biological issues in transplantation: the immune response, the recurrence of hepatitis C (HCV), and the approach to the treatment of hepatocellular carcinoma HCC (a growing indication for liver replacement therapy). In this protocol we plan to systematically collect clinical and biological data in recipients of LDLT and appropriate control recipients of DDLT to compare the impact of the hemigraft on these parameters. Entry into the cohort study will result in a relatively standardized clinical management protocol and the collection of the defined set of data points for all patients entered. A subset of patients may be recruited into ancillary studies that will entail a more extensive examination of focused topics.

The other major mandate in the development of the cohort study is the prospective assessment of the impact of donation on the healthy living donor. There is widespread interest in this subject among the medical community and the public at large, brought on in large measure by the recent, highly publicized death of a living donor in 2002. Concerns about the ethical issues regarding donor safety will be addressed by the organized study of the surgical, biological, and psychosocial effects of donation on donors compared to a control population of potential donors who are not selected for the procedure.

3. Study Objectives/Specific Aims

The primary study objective is to analyze the effect of choosing living donation rather than the wait for a deceased donor liver transplant. The principal hypothesis is that pursuit of a living liver allograft leads to decreased pre-transplant morbidity and mortality and better long term outcomes for patients starting from the point at which listed patients have a potential donor evaluated with at least a history and physical examination. Emerging data suggest that LDLT provides an inferior graft because of small size and technical complexity when compared to a whole liver used for DDLT. The magnitude of the disadvantage to the LD graft will be assessed by comparing results between LDLT and DDLT from the time of transplant. Finally, the study of the donor is included as a primary objective because of the tremendous importance of this issue to the patient and the public.

Secondary objectives will address selected biological and clinical issues in transplantation structured around the comparison between DDLT and LDLT.

3.1. Primary Aim 1: To quantify the impact of choosing LDLT on the candidate for transplantation.

1. The natural history of choosing to pursue LDLT versus waiting for a DDLT will be characterized. The overall survival comparison is between those receive LDLT versus those with a donor evaluated for LDLT but who do not receive LDLT. Time to transplantation and time to death will be determined.
2. Comparative analysis of pre-transplant morbidity and resource utilization will be determined by comparing the overall cohort from the time of enrollment.

3.2. Primary Aim 2: To characterize the differences between LDLT and DDLT in terms of post-transplant outcomes including patient and graft survival, surgical morbidity, and resource utilization on the recipient of a transplant.

1. Patient and graft survival analysis starting from the time of transplantation
2. Comparison of the incidence of defined medical and surgical complications after transplant between LDLT and DDLT
3. Comparison of resource utilization (hospitalization and emergency room visits) between LDLT and DDLT.

3.3. Primary Aim 3: To determine the short and long term health and quality of life (QOL) impact of donation, including (a) morbidity after liver donation, and (b) long term health-related QOL of donors compared to a control population.

1. To determine the rate of significant morbidity after liver donation.
2. To evaluate long term health-related QOL of donors compared to persons who were evaluated but did not donate.

3.4. Primary Aim 4: To standardize and assess the role of "informed consent" in affecting the decision to donate and satisfaction after living liver donation.

1. To measure the capacity of potential donors to understand information that is presented and to stratify the potential donor's capacity to understand information in general and the delivered information regarding the donation in specific.
2. To measure the motivations of the potential donors with standardized instruments and to determine if certain personality characteristics are associated with a more favorable predisposition to proceed to donation.

3. To assess whether disclosed information or life situations are the main influential factors in the potential donor's decision to proceed or withdraw from the donation process.
4. To correlate donor "satisfaction" with measurable outcomes of the donor, recipient, or perceptions of family support.
5. To measure the acceptance of adverse clinical outcomes, as a function of understanding of the disclosed risks versus the presence of life situational pressures.

3.5. Secondary Aim 1: To compare the severity of recurrence of hepatitis C between LDLT and DDLT recipients.

Primary Objective

1. To determine whether HCV disease progression differs in patients receiving LDLT compared to DDLT.

Secondary Objectives

1. To determine if recurrent HCV disease at one year (± 3 months), as observed histologically, is more frequent and severe in patients undergoing LDLT as compared to DDLT transplant.
2. To compare the rate of fibrosis progression (change in Ishak fibrosis score (*3*) per year) in LDLT and DDLT recipients by biopsies at months 3, 12, 24, and 36 after transplantation.
3. To compare time to recurrent disease between LDLT and DDLT recipients as determined by proportion of patients with histological evidence of recurrent HCV at 3 months.
4. To determine if HCV viral level at day 7 and months 1, 3, 12, 24 and 36 months differ in LDLT and DDLT recipients, and whether viral level is predictive of disease severity.
5. To determine if rejection episodes requiring treatment occur at a higher rate in HCV patients who undergo LDLT as compared to DDLT transplant and to correlate this frequency of treatment of rejection to aggressive recurrence of HCV as defined histologically.
6. To compare biochemical markers of disease activity (ALT/AST/total bilirubin) at 3 and 12 months and annually in LDLT and DDLT.
7. To determine if cholestatic hepatitis in transplanted patients with HCV occurs in a higher proportion of LDLT as compared to DDLT recipients.
8. To compare graft loss and patient survival between LDLT recipients and DDLT recipients.

3.6. Secondary Aim 2: Recurrence of HCC for DDLT versus LDLT.

Primary Objectives

1. To determine if LDLT is associated with decreased death on waiting list from progressive tumor growth versus DDLT.
2. Assess comparative HCC recurrence following LDLT or DDLT.

3. Compare long-term survival and disease free survival in patients who undergo LDLT or DDLT.

Secondary Objectives

1. Determine if LDLT recipients require a reduced number of palliative ablative procedures to control HCC when compared to those who wait for DDLT.
2. Compare rates of surgical and post-operative complication in HCC recipients of LDLT and DDLT.

3.7. Secondary Aim 3: To systematically characterize liver regeneration and function in donors and recipients.

Donors and recipients enrolled in the cohort study will be evaluated for evidence of recovery of liver mass and function following the surgical procedures (partial transplantation for recipients of LDLT, and partial hepatectomy for donors).

In the cohort protocol all donors and recipients of LDLT will undergo standardized assessments of liver volume and function to characterize the rate of restoration of the liver. In the recipient, in which the relative size of the graft will vary based on the unique donor/recipient combinations, the large sample provided in the study will permit us to correlate graft function with a number of donor and recipient parameters.

Primary Objective

1. To measure hepatic function and mass in living donors at enrollment, intraoperatively, and following hepatectomy, in order to determine whether return of hepatic function following donation correlates with rate of liver volume regeneration, biochemical impairment, and clinical events, and to see whether return of function is complete by 3 months post-resection.

Secondary Objectives

1. To correlate liver function in donors with long-term health outcomes and the incidence of clinical complications.
2. To correlate success or failure of regeneration with a series of selected clinical and laboratory variables in donors and recipients.
3. To collect liver biopsy and serum samples prospectively from a large series of donors and recipients which may form the basis for subsequent characterization of protein and gene expression of selected inflammatory and growth-related molecules.

3.8. Secondary Aim 4: To evaluate differences in the immune response to LDLT vs. DDLT grafts.

Primary objectives

1. To determine whether LDLT, which is associated with a regenerating liver, meaningfully increases the incidence of clinical rejection. In the cohort, we will compare the incidence of immunologic complications, specifically the incidence and severity of rejection between LDLT and DDLT in a defined set

of patients with a sample large enough to detect meaningful differences in the rejection rate.

2. To systematically collect serum and tissue samples that can be used to correlate clinical parameters in donors and recipients with immunologic outcomes, as defined by clinical and histologic endpoints.

3.9. Secondary Aim 5: To establish a robust data and sample repository on liver transplantation that may be used to study clinical and biological questions as new technologies and resources become available.

Primary objectives

1. To facilitate additional studies on samples and data collected in this study, thus enhancing the value of this and future investigations.
2. To ensure that samples are stored under uniform conditions, and to simplify access by other scientists to samples. Similarly, study datasets will be maintained to facilitate new analyses after the study closes.
3. To allow cost effective and high quality processing of genetic samples.

4. Investigational Plan

Potential recipients for transplantation will be evaluated and invited to participate in the study if they are eligible for LDLT using standard criteria for this procedure according to the practice of the transplant center. Recipients will enter the cohort within four weeks of the time a potential donor is scheduled for evaluation at the transplant center with an initial screening history and physical examination (H&P) (see Figure 1). Our preliminary data indicate that, after initial screening of a potential living donor, at least one-half of recipient candidates fail to receive LDLT and go on to wait for DDLT. These latter patients form the recipient control subjects of the study whose fate on the waiting list will be compared to those who undergo LDLT. The potential donors will be enrolled at the time of the initial H&P and will either go on to donate, or may serve as a control population for assessment of the impact of donation on the donors.

We will recruit additional patients (potential and actual recipients, actual donors and donor candidates who have not yet donated, but are early enough in their donation evaluation so that it is unclear whether they will go on to donation) from the A2ALL Retrospective Study (Grant 5 R01 DK62498-02) who are still alive at the start of the cohort study. We will also recruit those patients (recipients, recipient candidates, donors and donor candidates still being evaluated) whose donor evaluation occurred between the end of the Retrospective Study (2/28/2003) and the start of this prospective study at each site. Another cohort who will be approached for participation are those subjects (recipient candidates) whose date of donor evaluation occurs more than 4 weeks from the time the patient is approached. These subjects will be consented, despite the fact that they will have already passed the entry milestone of the living donor evaluation. Donor candidates who have not yet donated will be utilized as donor controls. Data from time of listing to cohort study enrollment will be collected retrospectively. Subjects will be followed prospectively according to the cohort study schedule of events, starting at the time of their enrollment. This enables a seamless capture of data and analysis of living donor transplantation from its inception into the future.

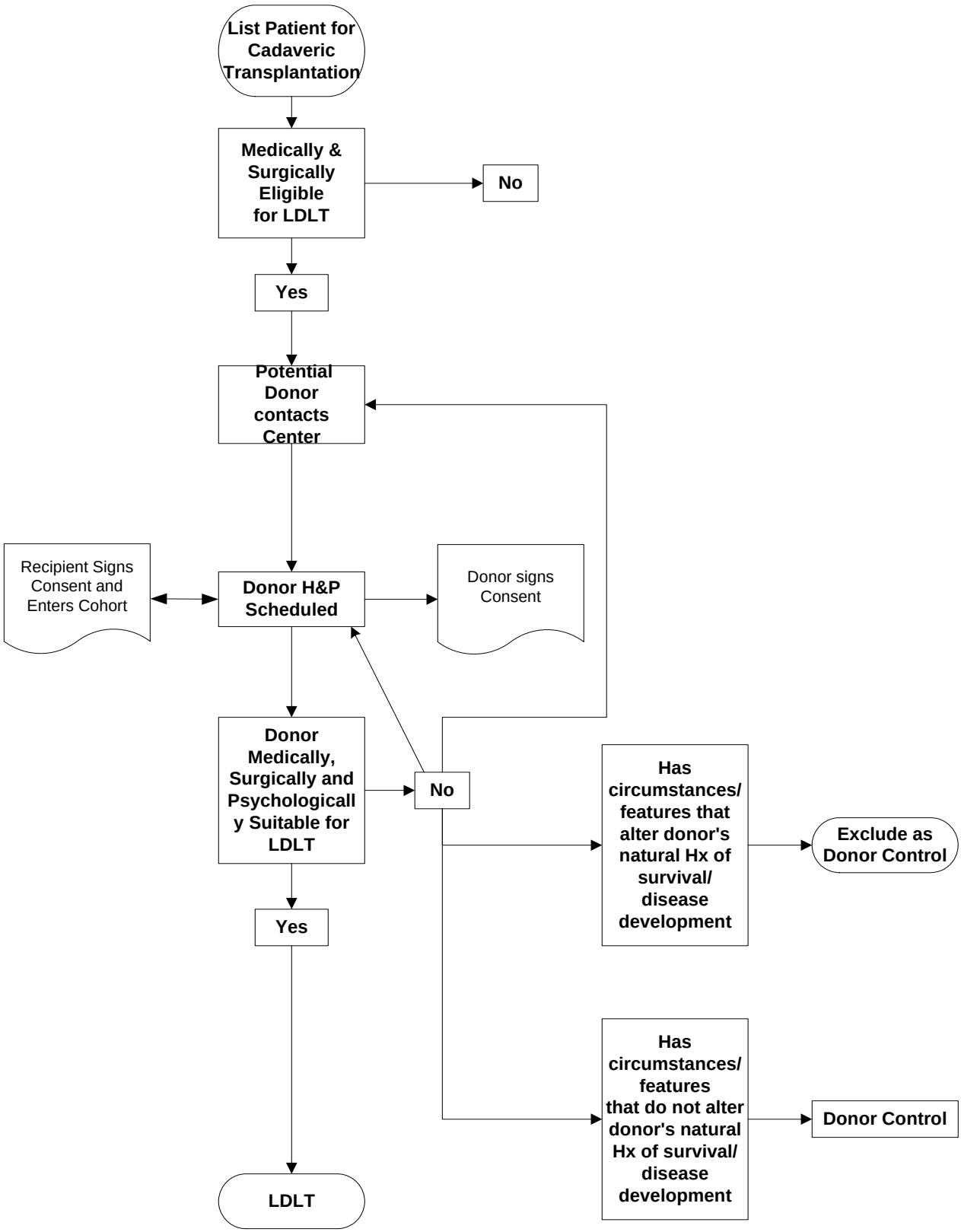


Figure 1: Flow Diagram for Entry of LDLT and DDLT Recipients into the Study

The table below summarizes the populations that will be investigated in this study for each specific aim of the cohort study. Eligibility criteria for these populations are given below.

Study Populations and Subsets Used for Each Specific Aim

		Potential Recipients				Potential Donors	
		With Donor H&P			No Donor H&P		
Primary Aims	**Brief Description**	**LDLT**	**DDLT**	**No LDLT or DDLT**	**Contemporaneous DDLT (as needed)**	**Actual Donors**	**Donor Controls**
1	LDLT vs. non-LDLT	**X**	**X**	**X**			
2	LDLT vs. DDLT	**X**	**X**		**X**		
3	QOL	**X**	**X**	**X**		**X**	**X**
4	Informed Consent					**X**	**X**
Secondary Aims							
1	Hepatitis C (at transplant)	**X**	**X**		**X**		
2	HCC (at donor H&P) LDLT vs. non-LDLT)	**X**	**X**	**X**			
	HCC (at transplant) LDLT vs. DDLT	**X**	**X**		**X**		
3	Regeneration	**X**				**X**	
4	Rejection	**X**	**X**		**X**		
5	Repository	**X**	**X**	**X**	**X**	**X**	**X**

Potential recipients with an evaluated donor must fulfill all of the following criteria:

- Potential recipient listed for single organ (liver) transplantation
- Patient is eligible for LDLT
- Age ≥ 18 years old at the time of donor history and physical exam
- Potential donor scheduled for evaluation (history and physical examination) within four weeks at the transplant center (this criteria is waived for subjects with HCV who are enrolling in the A2ALL LADR substudy. These subjects must have an identified donor who has passed telephone screening but may not have made an appointment)
- Informed consent obtained.

Patients with hepatitis C must fulfill all of the following criteria:

- Chronic hepatitis C virus infection and cirrhosis (may have concurrent HCC)
- HCV RNA positive (by qualitative or quantitative assay by local laboratory) pre-transplantation (within 6 months of transplant if not on treatment or within 2 weeks if on treatment)
- If subject joins study after transplant; subjects who are receiving treatment for HCV will be excluded from protocol biopsies.
- Informed consent obtained.

Patients with HCC must fulfill all of the following criteria:

- Diagnosis of hepatocellular carcinoma
- May have concomitant hepatitis C
- Informed consent obtained.

Contemporaneous DDLT controls must fulfill all of the following criteria:

- Potential recipient listed for single organ (liver) transplantation
- Age >= 18 years old at transplant
- Informed consent obtained.

Donor candidates and donor control candidates must fulfill all of the following criteria:

- Meet donor criteria of the transplant center
- Age >= 18 years old at donation
- Be evaluated with a history and physical examination at the transplant center
- Donor controls must have good health, or mild to moderate medical conditions that preclude donation but are not expected to impact their long-term quality of life or alter their natural history of survival/disease development compared to the normal population.

  Examples of acceptable conditions include:
  - 10 % steatosis on biopsy with normal liver tests
  - hemangioma/minor hepatic cystic disease on imaging precluding donor surgery
  - diminutive liver or diminutive left lobe
  - hepatic arterial or venous anatomical variations
  - mild pulmonary hypertension
  - hypercholesterolemia controlled with medication
  - pregnancy
  - recipient became non-LDLT candidate after donor approved.
  - history of cancer diagnosis with candidate more than 5 years post-treatment with presumed cure and no recurrence (Treated non-melanoma skin cancer is acceptable)

  Examples of unacceptable conditions include:
  - insulin dependent diabetes (controlled non insulin-dependent diabetes is acceptable)
  - hypertension
  - chronic hepatitis B or C

- hypercholesterolemia not controlled on medication
- diagnosis of cancer, excluding squamous cell and basal cell carcinoma of the skin.
- previous diagnosis of cancer with patient being less than 5 years cancer-free (or active diagnosis of melanoma)
- active substance abuse
- major EKG abnormality or structural cardiac abnormality
- moderate or severe pulmonary hypertension
- current uncontrolled, symptomatic psychiatric illness

Visit Schedule and Assessments:

After obtaining the subject's informed consent for participation in the study, the following assessments will be performed according to the visit schedule. Subjects recruited from the A2ALL Retrospective Study will join the cohort visit schedule from whatever clinical point they are at in their transplant or donation experience. Exceptions to this would be informed consent assessments for donors and protocol biopsies for subjects who are undergoing treatment for recurrent HCV post-transplant. Tables summarizing the visit schedules for donors and recipients with accompanying tests and procedures are included as Appendices A and B.

Since the study is primarily an observational investigation, it should be remembered that many of the assessments listed below are included in what is considered standard of clinical care in many institutions, and therefore would not require additional visits or sample collections from the patient.

Enrollment – Recipients:

- Medical history
- Social history
- Demographic information
- Physical examination: including weight, blood pressure, etc.
- Routine laboratory assessment: blood will be drawn to obtain values for the following tests: multichannel automated liver function tests, serum sodium, AFP, albumin, creatinine, and coagulation panels
- Blood sample will be drawn for HLA typing
- Blood sample for NIDDK Biosample Repository
- Whole blood for NIDDK Genetics Repository
- Quality of life baseline assessment
- HCV-infected subjects will undergo the following laboratory assessments in addition to those listed above:
  - HCV RNA quantitative or qualitative assay
  - HCV genotype (if not done previously)
- Subjects with HCC will also undergo the following imaging studies if not done within 3 months previous to study enrollment:
  - Bone scan
  - Chest CT

- Abdominal MRI/contrast CT

Pre-transplant Interval Assessments – Recipients:

The following assessments will be performed quarterly from the time of enrollment until transplant (or subject death if it occurs prior to transplant):

- Physical examination: including weight, blood pressure, etc.
- Routine laboratory assessment: blood will be drawn to obtain values for the following tests: multichannel automated liver function tests, serum sodium, AFP, albumin, creatinine, and coagulation panels
- Quality of life interval assessment: quarterly and one week prior to transplant
- HCV-infected subjects will undergo the following laboratory assessments in addition to those listed above:
  - HCV RNA quantitative or qualitative assay
- Subjects with HCC will also undergo the following imaging studies
  - Bone scan
  - Chest CT
  - Abdominal MRI/contrast CT

Enrollment – Donors:

- Medical history
- Social history
- Demographic information
- Physical examination: including weight, blood pressure, etc.
- Routine laboratory assessment: blood will be drawn to obtain values for the following tests: multichannel automated liver function tests, albumin, creatinine, coagulation panels, white blood cell count, hemoglobin, ferritin, platelets, blood urea nitrogen (BUN), homocysteine
- Screening lab values: CMV IgG/IgM, HIV Antibody, Hepatitis B core antibody (HBcAb), Hepatitis B surface antigen (HBsAg), Hepatitis C Antibody, HCV RNA (if HepC positive), Hepatitis D Antibody (if HepB positive)
- Blood sample for NIDDK Biosample Repository
- Liver MRI/contrast CT
- Quality of life baseline assessment
- Informed consent baseline assessment (comprehension and understanding, motivation for decision-making, and satisfaction with treatment)
- Donor controls will be asked to complete the QOL, Informed Consent and data collections. No diagnostic, imaging or invasive procedures will be performed once the decision not to donate has been made.

Day of Transplant – Recipients:

- Allograft biopsy and analysis

- Routine Laboratory Assessment: blood will be drawn to obtain values for the following tests: multichannel automated liver function tests, serum sodium, AFP, albumin, creatinine, and coagulation panels
- Explant pathology analysis
- Tissue sample for NIDDK Biosample Repository
- Blood sample for NIDDK Biosample Repository
- HCV-infected subjects will undergo the following laboratory assessments in addition to those listed above:
  - HCV RNA quantitative or qualitative assay
- Subjects with HCC will undergo the following laboratory assessments in addition to the routine laboratory assessments listed above:
  - Detailed pathological analysis of explant liver with tumor staging

Day of Donation – Donors:

- Allograft biopsy and analysis
- Routine laboratory assessment: blood will be drawn to obtain values for the following tests: multichannel automated liver function tests, albumin, creatinine, coagulation panels, white blood cell count, hemoglobin, ferritin, platelets, BUN
- Blood sample will be drawn for HLA typing
- Whole blood for NIDDK Genetics Repository
- Tissue sample for NIDDK Biosample Repository
- Blood sample for NIDDK Biosample Repository
- Quality of life assessment

Post-transplant Interval Assessments – All Recipients:

The following assessments and procedures will be performed post-operatively at Day 1, Week 1and 2, Months 1, 3, 6 and 12, and annually thereafter until the study is complete or the subject reaches a study endpoint.

- Physical examination: including weight, blood pressure, etc.
- Routine laboratory assessment: blood will be drawn to obtain values for the following tests: multichannel automated liver function tests, serum sodium, albumin, AFP, creatinine, and coagulation panels
- Blood sample for NIDDK Biosample Repository
- Quality of life interval assessments at 3, 6, and 12 months and annually thereafter
- Liver MRI/contrast CT at Month 3 only.
- Recipients of a DDLT from an HCV-antibody positive donor must have HCV genotyping done at 3 months post-transplant

Post-transplant Interval Assessments – HCV-infected Recipients:

In addition to the assessments listed above, HCV-infected recipients will undergo the following assessments at the following time points:

- Week 1, Month 1, Month 3, Year 1, 2, and 3: HCV RNA quantitative or qualitative analysis
- Month 3, Year 1, 2 and 3: Liver biopsy
- Month 3, Year 1, 2 and 3: Tissue sample for NIDDK Biosample Repository

Post-transplant Interval Assessments – Subjects with HCC:

In addition to the assessments listed above for all recipients, recipients with HCC will undergo the following assessments at Months 6, 12, 18 and 24:

- Serum AFP (at month 18 in addition to AFP's done at interval assessments for all recipients)
- Abdominal MRI/contrast CT: at months 6, 12 and 24 only.

Subjects Showing Signs and Symptoms of Allograft Rejection:

Subjects showing signs and symptoms of allograft rejection will undergo a liver biopsy with analysis to confirm the diagnosis of rejection. A biopsy will be performed to confirm each rejection episode. A tissue sample from these biopsies will also be sent for storage at the NIDDK Biosample Repository.

Post-Donation Assessments – Donors:

The following assessments will be performed for donors at Week 1, and Months 1, 3, and 12, and annually thereafter until the study is complete or the donor reaches a study endpoint or is lost to follow-up:

- Routine laboratory assessment: blood will be drawn to obtain values for the following tests: multichannel automated liver function tests, albumin, creatinine, coagulation panels, white blood cell count, hemoglobin, ferritin, platelets, BUN
- Blood sample for NIDDK Biosample Repository
- Liver MRI/contrast CT at month 3 only
- Quality of life assessment: limited to a pain survey at Week 1 and Month 1 for donors. Donor and donor controls will undergo the full battery of QOL assessments at Months 3, 12 and annually thereafter.
- Informed consent assessment (satisfaction with treatment): at Months 1, 3, and 12, and annually thereafter
- Informed consent assessment (motivation): at Week 1, Months 3 and 12, and annually thereafter.
- Donor controls will be asked to complete the QOL, Informed Consent and data collections. No diagnostic, imaging or invasive procedures will be performed once the decision not to donate has been made.

4.1. Primary Aim 1: To quantify the impact of choosing LDLT on the candidate for transplantation

4.1.1. Study Methods

Patients enter this study as candidates for transplantation, and will not necessarily become recipients. Potential outcomes for these patients may include LDLT, DDLT, withdrawal from the waiting list, or death on the waiting list. The entry point would be at initial evaluation of a potential donor that includes history and physical examination at the transplant center. Our preliminary data indicate that between one-third and two-thirds of potential LDLT candidates will actually undergo LDLT. This leads us to expect that enrollment of both donors and potential recipients at the time of donor history and physical examination will generate a number of control recipients who wait for a DDLT, as well as a cohort of potential donors who do not donate to generate a population of recipient and donor controls roughly equal in number to the LDLT population.

4.1.2. Participant Selection

See Section 4.

4.1.3. Data Elements

A. Potential Recipient Data Collection

Pre-transplant

1. Recipient Study Enrollment
2. Listing and Transplant data
3. Recipient Demographic data
4. Recipient Condition at Enrollment
5. Recipient Condition at Listing
6. Recipient Hospitalization and Complication data

B. Recipient Data Collection

Transplant and post-transplant follow-up

1. Recipient Condition at Transplant
2. Recipient Intraoperative Data
3. Recipient Post-Surgical Morbidity
4. Recipient Complication Severity
5. Recipient Hospitalization data
6. Recipient QOL Assessment (See Appendix C)
7. Recipient Survival

C. Potential Donor Data Collection

1. Donor Evaluation
2. Donor Demographic Form

D. Donation and post-donation follow-up

1. Donor Intraoperative Data
2. Donor Post-Surgical Morbidity
3. Donor Hospitalization data

3. Donor QOL Assessment
4. Donor Survival

4.1.4. Sample Size and Power Calculations

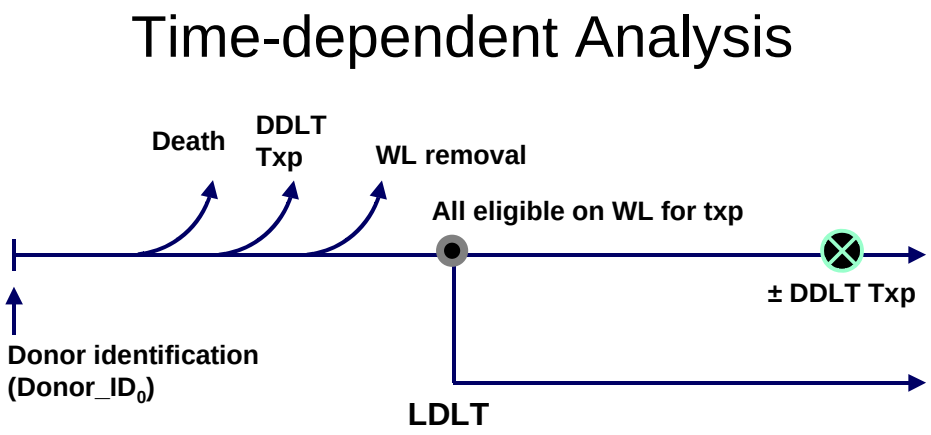
We will compare the survival experience between those who receive an LDLT and those considered for an LDLT but not receive one. Although the analysis will involve a fairly complex method of matching LDLT recipients with sets of non-recipients, for the purpose of power calculations, we will assume a much simpler 2-group design. For this analysis, we will pool the patients from the Retrospective Study and the Cohort Study for maximum power. We anticipate having at least 300 LDLT recipients and 500 non-LDLT recipients from the Retrospective Study, and 360 LDLT and 710 non-LDLT from the Cohort Study, for a total of 660 LDLT and 1210 non-LDLT recipient patients. Power calculations are based on the (two-sided) logrank test, an exponential survival distribution, approximately uniform accrual of patients for at least 7 years (from 1998) with 3 additional years of follow-up, 1% loss to follow-up, and a significance level of 0.05. Assuming a one-year survival probability of 0.875 in the LDLT group, we have 90% power to detect as significant a one-year survival probability among non-recipients lower than 0.85 or higher than 0.90.

4.1.5. Statistical Analysis

A comparison of survival between LDLT recipients and those evaluated for LDLT but not receiving a living donor organ will be made. The time origin for the survival analysis will be the date of donor evaluation. A preliminary comparison of the death rates in each group will be made using number of deaths divided by person-years at risk. For the LDLT group, time at risk will commence when the LDLT surgery is scheduled, and terminate at death, end-of-study censoring, or cancellation of the surgery. For the non-LDLT group, time at risk will commence at donor evaluation and terminate at scheduling of surgery, death, or end-of-study censoring; time at risk will also include the time interval between cancellation of a surgery, death or end-of-study censoring. This analysis estimates “overall” death rates, without considering changes in the risk of death over time. The analysis also does not incorporate covariate effects. However, it will provide a broad estimate of the potential benefit of LDLT.

A second analysis comparing these groups will use Cox regression, with the time origin at the donor evaluation, and covariate adjustment for age, gender, race, calendar year of initial evaluation, time on the waitlist, liver disease etiology and severity (including MELD (Model for End stage Liver Disease) score or Status, as applicable), comorbidities and other variables. The treatment strategies of LDLT versus no LDLT will be compared using a time-dependent indicator covariate for LDLT transplantation. In particular, this analysis will compare the risk of death at each time point after first donor evaluation for those having received an LDLT prior to that point versus those who have not. This analysis assumes that most candidates evaluated for LDLT either receive the transplant or do not receive it due to problems with the donor. In particular, it assumes that non-progression to LDLT due to DDLT transplantation or because the recipient becomes too sick to transplant is rare. Having many patients with non-progression to LDLT due to declining condition would bias the comparison by having sicker people in the non-LDLT group. These assumptions can be evaluated when the data are available.

A third, more complicated analysis will be performed that has the advantage of avoiding the assumptions mentioned above. This method will compare survival from the time of LDLT surgery among LDLT recipients to sets of controls that were evaluated for LDLT, and were alive and eligible for transplant at the same time following donor evaluation as the LDLT patient was when they received their transplant. This analysis will involve a different set of controls for each LDLT patient, with many patients re-used in several control sets. For a particular LDLT, a control group will be identified of all patients alive and awaiting transplant at the time of that LDLT. The survival experience of that LDLT will be compared with that of its control group. This set of an LDLT and its control group are illustrated in the figure below.



Those people in that control group who subsequently receive an LDLT transplant are censored from that control group, but initiate a new LDLT group with its own control group. For a new LDLT, the control group will be composed of many of the same people who were in control groups for previous LDLTs, although some of the previous controls may not be included due to death, having received a DDLT or LDLT transplant or leaving the waitlist. Comparisons between each LDLT and its control group will then be pooled in a single analysis using Cox regression. The Cox model will be stratified by the LDLT/control group set. Because many people will appear in multiple control sets, we will use a robust variance estimate based on the sandwich estimator to provide statistical adjustment for the re-use of controls in multiple control groups (*4, 5*). As a check on this method of variance adjustment, bootstrap variance estimates will also be computed. Briefly, bootstrap variance estimates are computed by resampling from the data with replacement, computing the effect estimate for each re-drawn sample, and calculating the variance of the effect estimates obtained. Covariate adjustment will include all variables listed for adjustment in the first Cox model described above. An additional assumption

must be made for this analysis: that those members of a control group who receive an LDLT (and are then censored from that control group) are not different in any systematic way from those remaining in the control group with similar covariate values. This is known as the assumption of random censoring. We can check to see if receipt of an LDLT is predicted by known covariates, and can adjust for these covariates in the survival analysis. We will not know if censoring is affected by unknown variables that may bias the analysis. To assess the impact of the assumptions of the initial analysis above [i.e., that most candidates evaluated for LDLT either receive the transplant or do not receive it due to problems with the donor], we will compare the results from the first and second methods. If a discrepancy is found, we will consider the results of the second analysis to be freer of bias and thus preferable.

In both Cox analyses described above, variables will be checked to ensure that the proportional hazards assumption is met. If non-proportional hazards are detected, particularly for the LDLT effect, they will be modeled using time-dependent covariates. Interactions between covariates and the LDLT effect will be tested.

In addition, individual components of the process will also be analyzed. We will separately estimate the distributions of time to death on the waitlist, time to transplant, and time to death following DDLT. We will use these three distributions to estimate the distribution of time to death for those not receiving a LDLT, confirming our combined estimate with the one-step estimate obtained as described above. With these estimates in place, we can then project the change in the overall survival distribution for specific changes in each of the component distributions. For example, if the distribution of time to transplant changes, say as a result of an increase in organ donations, then the effect of this change on non-LDLT survival could be estimated.

4.2. Primary Aim 2: To characterize the differences between LDLT and DDLT in terms of post-transplant outcomes including patient and graft survival, surgical morbidity, resource utilization and QOL on the recipient of a transplant.

4.2.1. Study Methods

This analysis will compare LDLT to contemporaneous deceased donor transplants beginning at the time of transplantation. The LD group would be all LD transplantations. We anticipate that the clinical comparisons between LDLT and DDLT recipients can be satisfactorily fulfilled with comparable numbers of transplants in each group for 1:1 statistical analysis. Because, by definition, DDLT candidates who are enrolled will not be transplanted immediately, no DDLT controls will be available in the cohort until DD livers become available, as their clinical condition declines over time. The need for contemporaneous DD controls may need to be met by the enrollment of recipients who may or may not have been considered for LD in the past. Since the ratio of LDLT:DDLT among our study centers varies from 1:100 to 1:3, there is a risk of selection bias unless DD controls are selected by some standardized approach. The first choice for DD controls will be patients from the A2ALL Retrospective Study who have not yet received DDLT. Ideally, DDLT transplants will be enrolled in a time frame comparable to the LDLT with whom they will be compared.

4.2.2. Participant Selection

See Section 4. for eligibility criteria. For contemporaneous DDLT controls, the following gives further details on patient selection.

The primary population of choice for recruiting DD controls would comprise those individuals from the A2ALL Retrospective study who received deceased donor transplants after 3/1/03. If there aren't enough candidates from this population, then the following method will be utilized for identifying DD contemporaneous controls:
In each center, following any LD transplant, the next eligible DDLT recipient will be approached at the time of identification of a donor (if they have not been previously enrolled), until a DDLT is enrolled. The contemporaneous control recipient will undergo all perioperative and post-transplant follow-up appropriate for the study. This step will be repeated each time an LDLT is performed until the point when patients enrolled prospectively in the cohort study who did not receive LDLT begin to be receiving DDLT by virtue of progressing on the waiting list (we estimate this will take one to two years).

Throughout the period of enrollment in the cohort study, the numbers of LDLT and DDLT will need to be comparable in each center. This will be verified by review of enrollment conducted by Data Coordinating Center every 6 months. If enrollment of DDLT falls below that of LDLT in any center during the study, the above procedure will be used to correct the deficit of DDLTs.

4.2.3. Data Elements

Recipient data collection:

1. Recipient Condition at Transplant
2. Recipient Intraoperative Data
   - Liver biopsy
3. Recipient Post-Surgical Morbidity
4. Recipient Complication Severity
5. Recipient Hospitalization data
6. Recipient QOL Assessment
7. Recipient Survival

4.2.4. Sample Size and Power Calculations

Estimation of proportions of donor complications will be made using 95% confidence intervals (CI) based on the binomial distribution. Assuming 660 donors, 95% CI widths will be no larger than +/- 0.04.

Comparisons of recipient complications after LDLT and DDLT transplants will be based on chi-square tests of equality of proportions. Assuming n=660 LDLT, n=550 DD, and alpha=0.05, we will have 83% power to detect a difference in the proportion of bile leaks, for example, of 0.18 in the DDLT group versus 0.25 in the LDLT group (a difference of 0.07). Physician estimates of this difference are closer to 0.20, so power is more than sufficient for this endpoint. If we more conservatively assume complication proportions near 0.5, we will have 93% power to detect a difference of 0.10 (such as 0.45 versus 0.55).

For comparing hospitalization between LDLT recipients and non-recipients we consider the number of hospital days in one year. Although the analysis will take into account the possibility that some patients may be included in both groups, both pre- and post-LDLT, the power calculations consider a similar but simplified design based on a two-sample t-test (2-sided, alpha=0.05), assuming 660 LDLT recipients and 710 non-recipients. We have no preliminary data on means or standard deviations (s.d.s) for number of hospital days in a year, but assuming a fairly large s.d. of 25 days, we will have 95% power to detect a difference of 5 days (effect size = 0.20 s.d.) between LDLT and non-LDLT groups.

4.2.5. Statistical Analysis

Survival from date of surgery for LDLT versus DDLT transplant will be compared using Cox regression, adjusted for prognostic variables. The distributions of time from transplant to rejection episode, or graft failure, between LDLT and DDLT transplant will be similarly compared.

Initial analysis of LDLT recipient post-operative complications will be descriptive. We will report the proportions of donors with complications such as bile leak, primary non-function, graft failure, pneumonia, and urinary tract infection, as well as any complication requiring hospital admission, re-operation, or other intervention. Confidence intervals will be included with all estimates. We will also report follow-up outcomes including wound healing, pain medications, blood laboratory values, and the proportion of patients who returned to work/school. Some attempt will be made to correlate complications with patient characteristics and operative procedures, but any such analyses will be limited by the quality of available data.
In addition, a comparison of LDLT complications with complications following DDLT will be made. Depending on the type of complication (event occurrence, time to event, or continuous outcome), a comparison of the events between LDLT and DDLT transplants will be made using logistic regression, Cox regression, or ordinary regression, respectively, each adjusted for other predictive variables as needed.

Resource utilization, particularly hospitalization (number of hospitalizations and number of hospital days) will be compared for those with and without LDLT using a repeated measures generalized linear model analysis starting at the time of donor evaluation. In addition, a comparison of hospitalization outcomes after LDLT versus DDLT will be made. Quality of life outcomes will be subscale scores calculated as weighted sums of ordinal variables, and can be analyzed as continuous variables. For comparing post-transplant quality of life for LDLT versus DDLT, we will use repeated measures regression. Treatment differences as well as treatment by time interactions will be tested.

4.3. Primary Aim 3: To determine the short and long term health and QOL impact of donation, including (a) morbidity after liver donation, and (b) long term health-related QOL of donors compared to a control population.

This study addresses the inadequacy and incompleteness of existing data to sufficiently gauge the risk to the donor. Unanswered questions exist regarding the morbidity risks to

the donor, for example: the risk that the donor will be left with inadequate hepatic reserve, the risk of biliary complications and other long-term consequences of hepatic resection. Information about quality of life and health status after adult-to-adult liver donation is even scantier; with no long-term prospective studies reported.

The analyses of these questions are critical in helping prospective donors make a true informed consent based on accurate assessment of long-term medical and psychological risks and benefits associated with LDLT.

Concerns exist regarding psychological and psychosocial problems as a result of donation that may not be adequately recognized by the transplant team. Although these concerns are largely based on anecdotes, it is an obligation of transplant programs to recognize these problems and to prevent or treat them effectively. Although various aspects of quality of life are important, there are specific post-donation concerns, including depression, sense of abandonment, body image, and sexual function. Instruments that adequately capture information on these issues will be administered to the donors.

Many hypotheses regarding donor outcomes and quality of life can be considered. Here are a few examples:

- Donor satisfaction will be related to both the medical and quality of life experiences of the recipient. This linkage will be greater for spouses.
- Pre-donation, donor physical and mental QOL will be above the norm of the general population. One or more years post-donation, donor QOL will remain in the normal range of the population.
- Donors with strong social support will feel less of a sense of abandonment.
- Donors with a better understanding of the risks of the procedure will have greater acceptance post-donation.
- Donors will have comparable QOL post-donation compared with controls who did not donate.
- Sexual satisfaction and comfort with body image will be more positive for those with strong social support and a better understanding of their post-operative course.

4.3.1. Study Methods

This analysis will compare LDLT donors to a donor control group who underwent evaluation for donation but did not donate. Patients who are accepted as candidates for donation will be provided information regarding A2ALL and invited to participate in the donor cohort. Morbidity after liver donation will be studied by analyzing intraoperative data, rate and severity of complications, number of post-operative hospitalizations and emergency room visits, and incidence of liver failure leading to the donor's listing for transplantation. Donor quality of life will be assessed through the use of validated questionnaires, including: 1) Life Orientation Test (LOT Optimism Scale), Appendix D, 2) Mini International Neuropsychiatric Interview (MINI) [Modules: Dysthymia, Depression, Suicide, Anxiety, Post-traumatic Stress Disorder, Alcohol Dependence, Hypomania/mania, Generalized Panic Disorder and Substance Dependence], Appendix

E, 3) Medical Outcomes Study Short Form-36 (SF-36), Appendix F and 4) Brief McGill Pain Survey, Appendix G, given at enrollment and post-donation at months 3, and 12, and annually thereafter. The A2ALL Donor Survey, Appendix H, will also be administered at enrollment, post-donation months 3 and 12 and annually thereafter. Additionally, the McGill Pain Survey will be administered at Week 1 and Month 1. For each time point, the goal is to have the questionnaire administered within a window of ± 1 month of the target date.

Retention of living liver donors and donor controls is essential for the success of the study. The Data Coordinating Center will be closely tracking the progress of both donors and donor control subjects throughout the study. Regular reports will be maintained and shared with the Steering Committee and DSMB to monitor long-term participation.

Donors and donor controls will be offered $25 compensation for each completed visit after the 3 month post-donation visit (starts with the 12 month post-donation visit).

Anecdotally, transplant professionals have heard reports that donors feel "abandoned" by the transplant team post-operatively. A2ALL centers will make post-operative check-in calls to post-operative donors within the first four weeks after donation a standard of care so that satisfaction levels regarding transplant team post-operative interactions can be assessed in an unbiased manner via questionnaires.

For LDLT donors who are recruited from the A2ALL Retrospective Study, quality of life will be assessed at enrollment, and then at the subsequent times post-donation following the schedule above.

4.3.2. Participant Selection

See Section 4. for general eligibility criteria. Further details of inclusion and exclusion criteria for potential LDLT donors are given below.

Inclusion Criteria:

1. LDLT Donors: Following the formal donor evaluation, the decision to perform the donor surgery is entirely at the discretion of the transplant center and the donor. Once a donor is accepted into the donor cohort, s/he will be asked for consent to participate in the study of donor long-term outcomes, morbidity and quality of life.
2. LDLT Donor Controls: Some potential donors who fail to donate will become controls for the long-term donor study. LDLT donor controls will also be asked to consent to study their long-term outcomes. Failure to donate and subsequent inclusion in the donor control cohort can be based upon recipient issues (condition worsening or availability of DDLT) or donor health issues. In the latter case, the donors may have mild to moderate medical conditions that precluded donation, but which are not expected to impact their long-term quality of life or alter the potential donor's natural history of survival/disease development compared to the normal population. We will also utilize donor candidates from the Retrospective Study who have not donated as donor controls.

a. Assessment of donor controls will be limited to collection of morbidity and survival information, administration of Quality of Life and Informed Consent instruments post-donation.

3. The event that will switch a donor from the pre-donation assessment regimen to the donor post-donation assessment regimen or the donor control post-event assessment regimen is the occurrence of one of the following events (whichever happens first):
   a. Donation
   b. Donor ruled out (but only with mild co-morbid conditions as outlined above)
   c. Donor withdraws
   d. Recipient dies
   e. Recipient is removed from the waiting list
   f. Recipient receives DDLT

Exclusion Criteria:

1. Donors who are rejected due to serious health conditions will not be included in the cohort. The donor control exclusion criteria would include, but would not be limited to abnormalities identified during formal evaluation, which preclude donation and alter the potential donor's natural history of survival/disease development compared to the normal population.

4.3.3. Data Elements

A. Potential Donor Data Collection
   1. Donor Evaluation
   2. Donor Demographic Form

B. Donation and post-donation follow-up
   1. Donor Intraoperative Data
   2. Donor Post-Surgical Morbidity
   3. Donor Hospitalization data
   4. Donor QOL Assessment
   5. Donor Survival

4.3.4. Sample Size and Power Calculations

This analysis is based on comparisons of donors and donor controls over time in the standardized questionnaire scores. For the power calculations, we will consider the power to detect differences between donors and controls at a single time point based on a two-sample t-test. Power for the proposed repeated measures analysis will be greater, although the amount of improvement will depend on the correlation between successive measures on the same individual, which is not known at this time. We assume that the Cohort Study will yield 360 LDLT donors and 710 donor controls

Assuming a 2-sided significance level of 0.05, we will have 87% power to detect an effect size of 0.20 (i.e., a difference of 0.20 standard deviations in any given measure between donors and donor controls). Cohen has suggested that an effect size of 0.25 is a

small effect, so we will have the power to detect quite small differences between donors and donor controls.

4.3.5. Statistical Analysis

The first goal of the analysis is to estimate the mortality and morbidity after liver donation. Although we expect and hope that no donor mortality occurs, if any does, then probability of mortality will be estimated using the Kaplan-Meier estimator with confidence intervals at specific points such as one and two years. With this estimator, the probability of donor death at any time point during post-surgery follow-up can be given.

Donor morbidity will be estimated as a proportion of donors with each reported complication at relevant time points after surgery. Complications reported will include bile leak, primary non-function, graft failure, pneumonia, and urinary tract infection, as well as any complication requiring hospital admission, re-operation, or other intervention. Confidence intervals for each proportion will be given. We will also report follow-up outcomes including wound healing, pain medications, blood laboratory values, and the proportion of patients who returned to work/school. Some attempt will be made to correlate complications with patient characteristics and operative procedures, but any such analyses will be limited by the quality of available data.

The second goal of the analysis is to estimate the long-term health-related QOL of donors compared to a control population. The analysis will employ repeated measures regression analysis, implemented using SAS Proc Mixed software. Outcome measures will be validated scales from established QOL instruments. Mean differences over time between donors and controls will be estimated. Possible changing effects over time will be tested using time by transplant group interactions. These analyses will be adjusted for other variables predictive of health-related QOL, such as age, gender, and baseline comorbidities. The covariance structure of the repeated measures over time will be investigated using a full model prior to any covariate reduction. Donors recruited from the A2ALL Retrospective Study will initially be analyzed separately, and then compared with donors from the Cohort Study. If the two donor groups are similar, they may be pooled to compare with the control group. If the two donor groups are not similar, they will be analyzed as separate groups. We could then estimate any change in donor quality of life over calendar time, or by experience at the transplant center, due to improvements in transplant methods or surgeon skill.

The analysis of pain scores will parallel the repeated measures analysis described above. However, at each time point we will also collect data on the patient's expected future pain. We will compare the predicted future pain with the pain score actually obtained at the next questionnaire completion. In addition to the standardized scales, individual questions from the A2ALL Donor Survey will also be analyzed, primarily using descriptive statistics. Covariate predictors of some of these outcomes may be investigated using linear or logistic regression.

4.4. Primary Aim 4: To standardize and assess the role of "informed consent" in affecting the decision to donate and satisfaction after living liver donation.

The motivation(s) for a healthy individual to subject themselves to a potentially life altering/threatening procedure is not clearly understood. Much of the current direction in

disclosure of information about the donation process assumes that the potential donor uses a contemplative risk/benefit analysis matrix to arrive at a decision to donate. Past experience with kidney donors suggests that the contemplative risk/benefit analysis applies only to a minority of donors. The majority of donors approached the donation procedure because of a primary sense of duty. For the living liver donor, the primary motivating forces of the potential donors are unknown. Of the people screened, it is our hypothesis that certain personal characteristics (strong sense of duty or need to perform a moral good) will be present in those individuals that go through to donation, as compared to those individuals that enter the process but withdraw. Whether disclosed information or life situations are the pivotal factors in altering the decision making process for donation is unclear. It is not clear what type or amount of information would be required to alter the desire to help another by the donation of an organ. A correlation between the level of understanding and subsequent decision-making process would be desirable.

The informed consent process assumes that retention of information by the potential donor will alter the acceptance of adverse events when they occur. The measurement of the acceptance of disclosed vs. undisclosed adverse events is not certain for the previously healthy individual. We hypothesize that the process of repetitive disclosure (informed consent) of the possibility of specific adverse outcomes will make it "easier" for the donor to accept the disclosed complication, rather than the complication which occurs at a relatively low frequency that was not included in the standardized informed consent material. Whether donor satisfaction correlates with ease of donor course (as in the standard surgical procedure) or with the more complex social matrix of recipient health and family acceptance needs to be measured.

The use of healthy individuals as a source of organs for transplantation has always been controversial. It goes against the basic Hippocratic tenet of "primum non nocere" to subject a healthy person to a procedure that will produce no physical benefit. The transplant community has argued that the psychological benefits to the donor will outweigh the risks for the emotionally related individual. Family and social pressures have often resulted in individuals stepping forward to the perceived aide of an endangered loved one.

A living liver donor death in New York resulted in an intense scrutiny of the process of donor evaluation, operative and perioperative care and informed consent. The Advisory Committee on Organ Transplantation (ACOT) to Secretary Thompson issued guidelines regarding the process and information given to the potential living donor. The first ethical principle is that the donor must be competent to make a decision. Competency is a word with multiple meanings in the legal and common usage. In the common usage, competency assumes a basic level of understanding of information and possession of enough cognitive skills to derive a reasonable conclusion. However, using the legal definitions of competency (and it is unclear how much of the informed consent disclosure is to meet legal standards, ethical standards vs. best practice standards) an individual can be competent and fail to "adequately" understand the disclosed information necessary to meet a "best practice standard".

4.4.1. Study Methods

All potential donors would be given a uniform amount of information regarding the donation process and the incumbent risks associated with donation. The information disclosure and ethical principles of this process will embrace the recommendations of the ACOT. In an attempt to standardize the presentation of the materials, collaboration with Keris, Inc. (Bellevue, WA) has resulted in the development of a standardized information disclosure that utilizes multimedia technology (the Vital Link Unit) for information presentation and data capture. Incorporated into the multimedia presentation are standardized tests to measure understanding and motivation. There are three presentations addressing the donor evaluation process, the donation surgery, and post-operative expectations. The scripts for these presentations are included as Appendices I-K. In addition to the standardized media presentations, each center will interact with the individual donors in their routine standard of practice. Attempts will be made to use the Vital Link Unit for information capture in the post-donation period, however if donors cannot access the transplant center's Vital Link, questionnaires will be mailed to attempt to capture motivation/satisfaction.

Incorporated into the media presentation will be standardized assessment measures of:

1. Comprehension and understanding: Understanding by the potential donor of the material will measured by the MacArthur Competency Assessment Tool-Clinical Research (MCAT-CR), Appendix L. This tool was developed over the past decade to assess the capacity of an individual to enter into clinical research protocols. This tool was chosen rather than its therapeutic counterpart, after the informed consent subcommittee discussed the observation that the individual had no direct health benefit from donation and that measures of understanding and appreciation of lack of personal benefit needed to be assessed. These were best accomplished through the MCAT-CR. The questions for this study have been adapted by Dr. Paul Appelbaum (the primary author of the tool) into a format that can be captured through the audiovisual capture capacity of the Vital Link center. During the first visit to the transplant center (before going through the evaluation process) the individual will be asked questions, the answers will be recorded, stored at the DCC and reviewed in batches (at intervals to be determined) by the clinical psychologists at the University of Virginia. The responses will be scored for understanding, appreciation and reasoning by previously established criteria. The scores of capacity for understanding, appreciation and reasoning other "normal" individuals will be available for comparison to those from the potential living donors.
2. Motivation for decision-making: Donors are typically motivated by a sense of duty. Very little is known about the affect the information disclosure/evaluation process upon the motivation of potential donors, either kidney, liver or lung organ donors or bone marrow donors. It is anticipated that the comprehensive, standardized disclosure of information that will be given to the potential living liver donor may change the person's motivation to proceed. To test whether there is a change in motivation, standardized questions about motivation to donate will be asked at the first encounter with the center (pre-information) and at the time of donation (at the time of acquisition of informed consent for the operation). Questions will also be asked of those individuals that voluntarily withdraw from the process, those individuals that were excluded from being a donor for

discovered/medical reasons, and those whose prospective recipients either received a DDLT, were removed from the waiting list or died. The parameters of the questions are derived from previous studies in donors (kidney, bone marrow and lung). These questionnaires and a list of their measures are included as Appendices M-Q.

3. Measures of quality of life: There are a variety of quality of life issues, but relevant to the informed consent process, assessment of anxiety/depression and physical perception/pain/function are key parameters. As these perceptions change with time after the donation, capture of this information at fixed time points after the procedure (3 and 12 months and yearly) will be done. It will be important to correlate the assessment of these measures to complications associated with either the donor operation or to the recipient.
4. Measures of satisfaction.

4.4.2. Participant Selection

See Section 4. and Section 4.4.1.

4.4.3. Data Elements

These are listed above under Study Methods.

4.4.4. Sample Size and Power Calculations

The sample size for the first hypothesis on predictors of donation will include all potential donors in the Cohort Study, assumed to be 360 donors + 710 donor controls = 1070 total. Based on logistic regression with significance level 0.05 and 2-sided tests, we will have 87% power to detect a difference between a 30% donation rate at the mean level of, say, the sense of duty scale, and a 35% donation one standard deviation above the mean. This assumes a correlation of only 0.05 between the variable of interest (e.g., sense of duty) and the control variables (e.g., demographics).

The sample size for the second hypothesis on satisfaction with treatment will include only those potential donors who actually donate, approximately 360 donors. Based on standard linear regression, we will have 93% power to detect an increase of 3% in the R-squared value of the regression, assuming 5 control variables that explain a total of 10% of the variation. For the analysis of satisfaction with care at the evaluation time point, the sample size will be much larger since it will include all prospective donors and thus will have even more power.

4.4.5. Statistical Analysis

The data for this aim will include the Keris modules on comprehension and understanding, motivation for decision-making, and satisfaction with treatment. Initial analyses will provide descriptive statistics on all modules, particularly comprehension and understanding, and satisfaction with treatment. Because the ethical nature of informed consent relies on comprehension of the risks involved, low scores on the comprehension questions would bring into question the adequacy of the consent process.

Levels of patient satisfaction with treatment will be of interest to all participating centers, whose programs depend on good patient care.

The first hypothesis involves predictors of the decision to donate. We will use logistic regression to investigate variables potentially predictive of donation. Demographic variables will be included to adjust for any confounding. The primary variables of interest are questions related to sense of duty and need to perform a moral good. Also of interest is whether the level of comprehension and understanding of the information presented is predictive of the decision to donate.

The second hypothesis involves predictors of donor satisfaction with treatment, and will be restricted to potential donors who actually go through donation after the evaluation time point. The outcome measure will be a summary of several Likert-scale questions related to patient satisfaction, and will be a continuous variable for practical purposes. Linear regression will be used to investigate predictors of patient satisfaction with treatment at each time point from donation. Demographic variables will be included to adjust for confounding. Several variables are of interest. First we will test variables related to ease of the post-surgical course (e.g., number and severity of post-surgical complications, number of days in the hospital). Second we will test variables measuring the patients' foreknowledge of adverse events that they experienced; we will include in the model both an indicator variable reflecting whether all the patient's adverse events were listed on the consent form, and also an indicator variable reflecting whether the patient thought they had been informed of the particular adverse event(s). (It will also be interesting to see whether patients report not being informed of adverse events that are given in the consent form, and if so, whether some events are more likely than others to have been missed by the patient.) Third, we will test variables related to recipient health for their effect on patient satisfaction with treatment, because one might imagine that the donor would feel better about donation if the recipient were doing well. Recipient health will be measured by number and severity of recipient adverse events, recent hospitalization, liver function tests, and quality of life. Finally, we will test measures of family acceptance and appreciation based on questions from the A2ALL Donor Survey.

4.5. Secondary Aim 1: To compare the severity of recurrence of Hepatitis C between LDLT and DDLT recipients.

Hepatitis C virus (HCV) infection is the most common indication for DD and live donor liver transplantation in North America. Reinfection of the graft is universal in patients who are viremic pre-transplantation and recurrent disease is more rapidly progressive post-transplantation than in the non-transplant setting. Among DDLTs, graft survival is reduced for patients with HCV disease compared to patients with other causes of chronic liver disease except malignancy. Chronic rejection, recurrent malignancy and recurrent HCV are the most common causes of late graft loss. Factors most consistently associated with progressive disease and recurrent cirrhosis: are year of transplantation, donor age, pre-transplant level of viremia, absence of HBV coinfection and acute rejection.

Preliminary studies of LDLT recipients suggest that allograft injury due to HCV occurs earlier and is more severe compared to recipients of DD livers. In a retrospective study from the University of Colorado and Mount Sinai Hospital in New York, the short-term

outcome of 42 LDLT HCV patients was compared to 86 HCV patients undergoing DDLT. Mean AST and ALT were higher at each follow-up interval reaching statistical significance at months 1 and 3 for AST and month 1 for ALT. The percentage of patients with aminotransferase > 100 IU/or bilirubin > 2 mg/dl was higher at each time point for live donor liver transplant recipients. The time to histologic recurrence was significantly shorter in live donor recipients (3.5 months) vs. DDLT recipients (6.7 months), (p=0.01) but protocol biopsies were not performed. While these results suggest that HCV may recur earlier and be associated with higher serum aminotransferase levels in live donor liver transplant patients compared to DDLT recipients, the longer term outcomes (risk of progressive fibrosis and graft loss) are unknown. The current study will examine the rate and severity of HCV disease in a large prospective cohort of live donor and DDLT recipients over a 3-year period. Liver biopsies obtained at 3 months and annually will be compared between groups to assess differences in liver fibrosis and total necroinflammatory activity, controlling for other factors known to affect disease progression (e.g. donor age, acute rejection episodes, baseline viral level).

4.5.1. Study Methods

This is a prospective cohort study of patients with chronic HCV undergoing LDLT. Contemporaneous DDLT recipients matched (as in main prospective study) will be used as controls. Potential DDLT recipients listed for liver transplantation who are likely to be transplanted within the next 3 months will be approached and consented for study. The primary endpoint of the study is the severity and rate of histological disease progression. Antiviral therapy will not be used until the patient achieves a level of significant disease severity that has been defined by four histological endpoints with/without clinical criteria. Protocol biopsies at 3 months and annually will be used to assess rate of recurrence and severity of disease. Patients will be followed for up to 3 years. Data and specimens will be collected to evaluate the effect of specific clinical, virological and immunological factors on disease progression in LDLT recipients and controls.

Study Endpoints

The primary and some of the secondary endpoints are based upon liver histology. Any liver biopsy performed post-transplantation will be reviewed for the HCV histological endpoints (i.e. both protocol biopsies and biopsies done as part of clinical care).

Primary Endpoint

1. Proportion of patients with <u>one</u> of the following <u>four</u> endpoints indicative of "progressive" disease:
    a. Ishak fibrosis score of ≥3 (some bridging fibrosis)
    b. Ishak fibrosis score of ≥2 plus total bilirubin >5.0 mg/dL
    c. Ishak fibrosis score of ≥2 plus Knodell score (necroinflammatory indices) ≥ 9
    d. Diagnosis of cholestatic hepatitis

Additional causes of liver test elevation must be excluded. Specifically, there must be:

    e. Absence of rejection (acute and chronic)
    f. Absence of biliary disease

g. Sepsis

Secondary Endpoints

1. Rate of fibrosis progression (Ishak fibrosis score) – change in fibrosis score per year
2. Total and necroinflammatory scores at 3 months, 1 year, 2 years and 3 years (Knodell score)
3. Time to recurrence of disease determined by proportion of patients with histological evidence of recurrent HCV at 3 and12 months
4. Viral level at day 7 and months 1, 3, and 12, 24 and 36– comparison of LD and DDLT recipients and prediction of post-LT disease progression.
5. Proportion with cholestatic hepatitis
6. Graft and patient survival

4.5.2. Participant Selection

See Section 4. for general eligibility criteria, and Section 4.2.2 for further details on selection of contemporaneous controls.

4.5.3. Data Elements

Potential Recipients positive for HCV

1. HCV at enrollment
2. HCV survey

Recipients positive for HCV

1. HCV intraoperative sample collection
2. HCV at Transplant
3. HCV Post-operative Recurrence and Rx data
4. Liver biopsy and histology data

Histological Evaluation:
Local pathologists will use a standardized form for scoring all liver biopsies on HCV study participants performed after the first 4 weeks post-transplantation. The Knodell system (See Appendix R) will be used. To be an evaluable biopsy, in terms of HCV-related endpoints, there must be an absence of other concurrent conditions such as acute rejection, biliary obstruction and CMV hepatitis. Both protocol liver biopsies (month 3 and annually) and non-protocol biopsies will be scored for evidence of HCV recurrence.

4.5.4. Sample Size and Power Calculations

We estimate the total number of transplant patients available (based on the recent survey of A2ALL transplant centers) to be 360 LDLT and 355 DDLT. We assume that 30% of waitlist patients have HCV, or approximately (0.30*360=) 108 LDLT and (0.30*355=) 107 DDLT. All calculations below assume two-sided testing with a significance level of 0.05.

1. The primary endpoint upon which the study will be powered is histological severity of disease at 1 year. We base sample size calculations on a comparison of binomial proportions. If we assume that 10% of DDLT patients achieve the composite endpoint of (Ishak ≥3, etc), we have 82% power to detect an increase in the LDLT proportion to 25%.

2. The secondary endpoints also capture rate of disease progression. If we assume 50% of DDLT patients will achieve the composite endpoint at 3 years, we will have 85% power to detect an increase in the LDLT proportion to 70%. Thus, based on dichotomous endpoints (progression or not), we can only detect a difference between LDLT and DDLT with high power only if the difference is at least 15-20%.

Alternatively, we can look at differences in fibrosis progression using a numeric scale. With an estimate of SD=0.25 for the yearly rate of increase in fibrosis score, we have 82% power to detect an increase or decrease of 0.10 compared to 0.45 (DDLT) [Berenguer 2000 -- for DDLT 0.3-0.48/year on Desmet scale 0-4)]. Power will be greater to detect LDLT rates above 0.55 or below 0.35.

4.5.5. 4.5.5 Statistical Analysis

The primary objective is to compare HCV progression probabilities in LDLT versus DDLT, where HCV progression is defined above. This comparison will be tested using logistic regression, with HCV progression (yes/no) at a given time point as the outcome variable, and LDLT versus DDLT as the covariate of interest. This comparison will be adjusted for other variables associated with HCV progression. If HCV progression is evaluated at regular points over time, then discrete time survival analysis will be used to compare the time until HCV progression in the two groups.

Secondary objectives will involve several analyses. First, both the rate of fibrosis progression (change in Ishak score over time) and change in the necroinflammatory score (Knodell score) will be analyzed using repeated measures regression. A difference in slopes of the Ishak or Knodell scores over time for LDLT and DDLT will be tested with an interaction term between LDLT/DDLT and follow-up time. Second, the proportion of patients with histologic evidence of recurrent HCV at 3 months and 1 year after LDLT versus DDLT will be compared using logistic regression. This analysis will parallel the analysis for the primary objective described above. Because recurrent disease occurs prior to progressive disease, these two analyses will not be independent. Third, analysis of viral level at day 7 and months 1, 3, 6, and 12 will be performed using repeated measures regression, with the primary comparison between LDLT and DDLT. Differences in the pattern of viral levels over time will be tested using a time by LDLT/DDLT interaction. As a second step, HCV disease severity will be added to the model to see if viral level is significantly predicted by disease severity. Fourth, the incidence of cholestatic hepatitis in LDLT and DDLT will be compared using logistic regression, adjusted for any other prognostic factors. Finally, graft and patient survival will be analyzed using Kaplan-Meier estimators, logrank tests comparing LDLT and DDLT, and Cox regression to compare groups adjusted for other variables.

4.6. Secondary Aim 2: Recurrence of HCC for deceased donor versus LDLT.

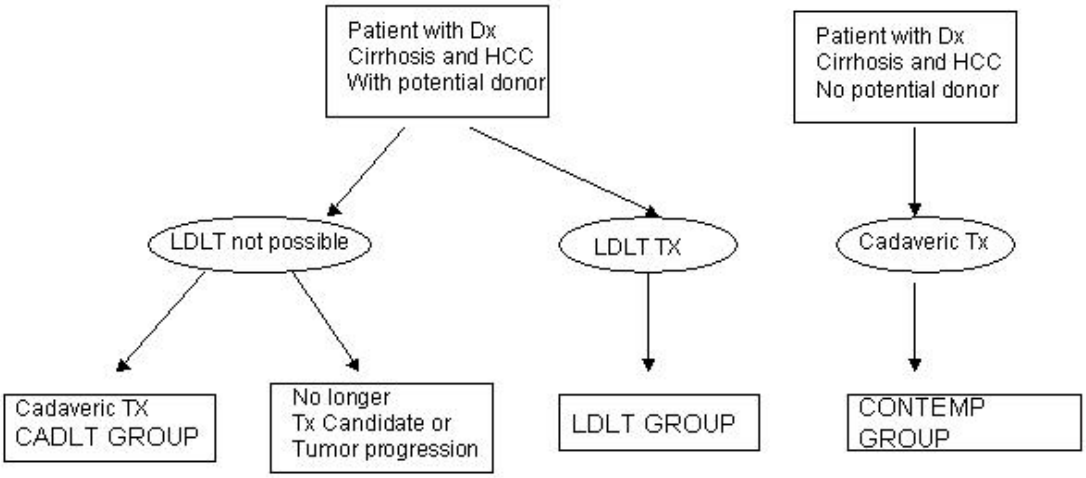
The utility of liver transplantation for patients with cirrhosis and hepatocellular carcinoma with stage 1 or 2 disease has clearly been demonstrated. The key to this therapy is timing of transplantation, and progression of disease while a patient is awaiting transplantation is a potential hazard. The near immediate availability of a graft from a living donor opens up the possibility of intervening with transplant before the complication of progression of disease is encountered. However, to date there has been no clear evidence that the benefits of this mode of therapy are superior to standard deceased donor transplantation as it relates to patient survival and cancer free survival. A prospective study to compare outcomes of these two treatment modalities is therefore needed.

4.6.1. Study Methods

This is a prospective cohort study which will collect data elements specific to a subset of patients enrolled in the A2ALL Cohort Study, who carry a diagnosis of cirrhosis and hepatocellular carcinoma. This cohort will include all LDLT recipients who consent to enrollment, have cirrhosis, and carry a diagnosis of hepatocellular carcinoma, or develop hepatocellular carcinoma while waiting for transplant. To address the Primary Aims and utilization of ablation, patients who undergo LDLT procedures (**LDLT GROUP**) will be compared with recipients who were enrolled but ultimately could not undergo a live donor transplant secondary to recipient or donor factors, and remained on the waiting list or eventually underwent deceased donor liver transplant (**DDLT GROUP**).

Since there will be a period of time before a suitable number of DDLT GROUP patients accumulate, a third group consisting of contemporaneous deceased donor controls will also be recruited (**CONTEMP GROUP**). These patients carry a diagnosis of cirrhosis and hepatocellular carcinoma, will be selected by the Data Coordinating Center, and enrolled at the time of transplant. This CONTEMP GROUP will serve as a control to address the question of operative and post-operative complication rates. It is anticipated that the CONTEMP GROUP will only be needed for the first two years of the study, depending on number of patients enrolled.

A schematic for patient flow through the study is below.



As patients are enrolled in the study multiple data elements will be collected as part of the A2ALL Cohort Study. Specific data elements related to the subset of patients with hepatocellular carcinoma, as well as their management while on the list is outlined below.

1. All enrolled patients with a confirmed or suspected diagnosis of HCC will first undergo evaluation to exclude metastatic disease and/or vascular invasion (chest CT, bone scan and abdominal MRI or contrast CT). These staging modalities and results will be recorded at 3-month intervals as required by UNOS.
2. All enrolled patients, who will wait > 3 months for transplant, will then undergo ablation of each lesion(s) with whatever technique(s) are currently utilized at each of the individual centers participating in this study (RFA, cryotherapy, alcohol ablation, chemoembolization, etc). The specific technique and all complications will be recorded.
3. Patients able to undergo LDLT (LDLT GROUP) will proceed with this procedure 3 months following ablation or as soon thereafter as is possible.
4. Patients unable to undergo LDLT will be followed at periodic intervals and receive testing to assess for disease progression (chest CT, bone scan and abdominal MRI or contrast CT) every 3 months until they are able to undergo DDLT (DDLT GROUP).

5. Patients with evidence of local recurrence of HCC during this waiting period may undergo repeat ablation in an attempt to limit disease progression prior to undergoing DDLT or LDLT.
6. Patients who develop evidence of metastatic disease or vascular invasion will be dropped from the study protocol and followed until death.
7. The liver explants of all patients who undergo LDLT or DDLT will be evaluated for presence of HCC under a defined pathologic protocol : EXPLANT PATHOLOGY:
    a. Explant liver will be sliced at 4-5mm intervals and all suspicious nodules for HCC processed for light microscopy.
    b. Tumors will be measured and inspected for encapsulation, invasion into adjacent liver, and vascular structures. Sections from the tumor/tumors will be fixed in formalin and processed for light microscopy. The size, multiplicity, grade (G1,G2,G3), degree of mitosis (<10/HPF or >/=10/HPF), infiltration into adjacent liver, encapsulation, and vascular invasion (micrometer vs. millimeter portal or hepatic vein) will be determined for all tumors.
8. All patients will be followed in the LDLT cohort main study protocol prior to and following LDLT and DDLT
9. All patients who undergo LDLT or DDLT will be followed post transplant for evidence of recurrent or metastatic disease. Serum AFP will be determined at post-transplant month 3,6,12,18, 24 and every six months until the conclusion of the study. Patients will also undergo either an abdominal CT or MRI at months 6, 12 and 24 post-transplant to document absence of recurrence. Criteria for disease recurrence include radiologic imaging demonstrating a lesion with characteristics of tumor (date and type of study to be recorded, possibilities include bone scan, PET scan, CT or MRI.) Tissue diagnosis is also acceptable but not required for diagnosis of recurrence. An elevated AFP in the absence of a documented lesion on imaging will not be accepted as evidence of recurrence.

Primary endpoints will be patient survival, cancer-free patient survival.

Secondary endpoints will include loss of candidacy while on list due to tumor progression, overall use of ablative therapies, complications of ablative therapies, accuracy of preoperative imaging modalities, surgical and postoperative complication rates.

4.6.2. Participant Selection

See Section 4. for overall eligibility criteria, and Section 4.2.2 for further details on selection of contemporaneous controls. The specific inclusion and exclusion criteria for patients with HCC are given below.

Inclusion Criteria:

1. Suspected or confirmed HCC which meet the UNOS definition for being listed for Stage I or Stage II priority.
    a. A single mass lesion on imaging studies < 5 cm in diameter

b. Multiple lesions on imaging studies, but no greater than three lesions and no single lesion > 3 cm.
c. A tissue diagnosis of HCC.
d. An AFP greater than > 500 ng/ml.

2. Patients with greater than Stage II disease will also be enrolled, as long as they were accepted by the individual transplant center as acceptable for liver transplant.
3. Patients with history of ablation prior to listing will also be eligible, as long as one of the features in section 1 above was present prior to the ablative procedure.

Exclusion criteria :

1. Evidence of metastatic disease based upon chest CT, bone scan and abdominal MRI or contrast CT.
2. No tumor found in explant and no history of prior ablative therapy.
3. Patients with synchronous cholangiocarcinoma.

4.6.3. Data Elements

In addition to the data elements collected for the A2ALL Cohort study, specific data elements will be collected in these patients with HCC. These include:

Potential Recipients positive for HCC

1. HCC data at Listing
2. HCC data at Enrollment

Recipients positive for HCC

1. HCC Data Immediately Prior to Transplant
2. HCC Explant Assessment
3. HCC Post-operative Recurrence and Treatment Data

4.6.4. Sample Size and Power Calculations

It is anticipated (based on 1998-2002 data and trends) that approximately 75 patients (25% of all 300 LDLTs) will have undergone LDLT for HCC over the three-year period of the Retrospective study at the A2ALL study centers. Similarly, there should be approximately 125 patients (25% of all 500 non-LDLT) in the non-LDLT group to serve as controls. In addition, approximately 15% of Cohort patients are estimated to have HCC (0.15*360=) 54 LDLT and (0.15*710=) 106 non-LDLT. Thus, the total cohort for endpoints collected in both Retro and Cohort studies will be (75+54=) 129 LDLT and (125+106=) 231 non-LDLT. With this number of patients, there will be an 95% power to detect a 20% difference (e.g., 40% versus 60%) in recurrence of HCC (loss of transplant candidacy or post-transplant recurrence) between the LDLT group and non-LDLT group. These numbers should also allow for adequate comparison of patient survival, since tumor recurrence typically leads to patient death.

The lowest power will be available for comparisons between LDLT and DDLT among Cohort patients only: an estimated 54 LDLT and 54 DDLT. For these comparisons,

continuous outcomes will have more power than dichotomous outcomes. We will have 87% power to detect an effect size (number of standard deviations different) of 0.60 between groups. Cohen considers and effect size of 0.50 to be moderate, and 0.90 to be large.

4.6.5. Statistical Analysis

Initial analysis of HCC will be primarily descriptive. The characteristics of HCC patients described will include TNM explant pathologic stage, use of ablation pre-transplant, and pre transplant ablation method (i.e. chemoembolization, RFA, etc.). The proportion of HCC recurring within one year will be presented, with 95% CI. Predictors of one-year recurrence will be explored using logistic regression for patients with at least one year of follow-up. The difference between recurrence (or presence) proportions for LDLT versus non-LDLT patients will also be estimated using a 95% confidence interval. A comparison of survival between these two groups will be performed using Cox regression, adjusted for various prognostic covariates. A comparison of survival between LDLT and deceased donor transplant recipients will also be performed.
Secondary outcomes include comparisons of numbers of ablative procedures for LDLT versus waiting for DDLT, and numbers of surgical complications for LDLT versus DDLT. Differences in numbers of ablative procedures will be analyzed using Poisson regression, assuming that multiple ablative procedures may be necessary for some patients. Differences in surgical complications will be analyzed using logistic regression, assuming each complication either does or does not occur for each patient in a given time frame.

4.7. Secondary Aim 3: To systematically characterize liver regeneration and function in donors and recipients.

Information about liver regeneration after liver transplantation remains descriptive and is limited by our inability to directly sample liver tissue in the days after liver surgery. Following donation and transplantation, it has been observed that hepatic function of living liver donors returns to normal soon after right hepatic lobe resection using standard serum tests of liver function. Recipients of living donor liver transplants also have rapid return of function, however there may be a reduction and delay in recovery of hepatic metabolism following LDLT compared to whole DDLT, which has been observed with respect to the metabolism of immunosuppressive compounds.

Regeneration may fail if the graft is excessively small or damaged in other ways though the factors associated with graft function have not been adequately studied. Finally, it remains uncertain when and if the liver volume returns to its baseline following this procedure.

Initiation of molecular pathways associated with liver regeneration previously identified in rodent models is presumably necessary for hepatic recovery in the human living donor liver transplant setting in both donors and recipients. It is presumed that failure of regeneration is a consequence of a failure to initiate these events though limitations in our ability to directly assess liver tissue over time has prevented confirmation of these expectations.

Primary Objectives

- To measure hepatic function and mass in living donors at study entry, perioperatively, and following hepatectomy, and determine whether return of hepatic function following donation correlates with rate of liver regeneration, biochemical impairment, and clinical events, and if return of function is complete by 3-6 months post-resection.
- To correlate liver function in donors with long-term health outcomes and the incidence of clinical complications.
- To correlate success or failure of regeneration with a series of selected clinical and laboratory variables in donors and recipients
- To collect liver biopsy and serum samples prospectively from a large series of donors and recipients which may form the basis for subsequent characterization of protein and gene expression of selected inflammatory and growth-related molecules.

4.7.1. Study Methods

A. Restoration of liver mass and function:

In this cohort we will limit our observations of restoration of liver mass to measurement of liver volume at defined interval after transplantation and donation and clinical and standard laboratory assessment at standardized intervals.

B. Tissue and serum collection:

Tissue and sera will be collected and stored prospectively for eventual analysis using molecular and proteomic techniques to detect selected molecules associated with regeneration and repair.

Liver biopsies will be collected prior to hepatectomy in the living donors, on the back-table, and after reperfusion of the liver graft in recipients. These will be processed and stored for eventual analysis. In addition, sera will be collected and stored at defined intervals from donor and recipients which will be available for subsequent studies of gene and protein expression.

a. Tissue samples

i) In the living donor setting, one Tru-cut core biopsy of the right lobe of the donor liver will be performed prior to the removal of the right lobe (control in situ biopsy), and one on the back table after flushing with preservation solution (cold ischemia biopsy), for baseline assessment. Approximately 30-60 minutes after reperfusion of the right lobe into the recipient, the donated portion of the liver will also have one Tru-cut core biopsy performed (post-reperfusion biopsy). All biopsy specimens will be split in the operating room, with two-thirds placed in RNAlater to be processed for RNA or immunohistology, and one-third placed in paraffin block for later section/H&E histology.

ii) In the DDLT donor setting, biopsies will be obtained from the donor liver on the back table (cold ischemia biopsy), and then approximately 30-60 minutes after implantation (post-reperfusion biopsy), as described above. The control DDLT arm is important for comparison and correlation of molecular events with ischemic injury and/or graft to recipient size ratio.

Serum samples will be obtained at baseline (preoperatively), intraoperatively prior to resection or transplantation and at the time of final biopsy, on post-operative days (PODs) 1 and 7, and at 2, 4, 12 and 24 weeks.

4.7.2. Participant Selection

See Section 4. for eligibility criteria.

4.7.3. Data Elements

Serum tests of liver function at defined times will be tabulated and compared sequentially for donors and LDLT recipients. Mean values for comparable time points will be compared between DDLT and LDLT.

To correlate liver function in donors with long-term health outcomes as determined by data collection at defined intervals as noted above in Aim 3 and the incidence of clinical complication

To correlate success or failure of regeneration with a series of selected clinical and laboratory variables in donors and recipients. Liver volume at defined intervals will be correlated with a selection of pre, peri, and post surgical clinical and laboratory variables

4.7.4. Sample Size and Power Calculations

Power analyses are based on estimation of correlation coefficients and coefficient of determination between measures of liver regeneration and liver function. We anticipate having 360 donors and 360 recipients in the Cohort study. We assume a two-sided test for a non-zero correlation with significance level 0.05. With these assumptions, we can detect a correlation of 0.17 or greater with 90% power in either the donors or the recipients. In a multiple regression analysis, we will have 85% power to detect an increase in the R-squared of 2%, assuming 5 variables already in the model explaining 20% of the variance.

4.7.5. Statistical Analysis

Analysis of liver regeneration will be performed using repeated measures regression. We will follow the mean course of hepatic function and mass in living donors from baseline through 3 months post surgery. We will test for a difference between liver function at baseline and function at 3 months post surgery to determine whether liver function has returned to normal. We will also model liver function as predicted by liver regeneration volume, biochemical impairment, and the occurrence of certain clinical events. We will also compare the return of hepatic function in LDLT versus DDLT recipients using repeated measures regression.

4.8. Secondary Aim 4: To evaluate differences in the immune response to LDLT vs. DDLT grafts.

Living donor liver transplantation presents a unique immunological setting that is determined by three major variables that are different from the DDLT setting, with the potential to impact on short and long-term graft and patient survival, as well as recurrent disease:

1. Regeneration may be associated with different pattern of lymphocyte trafficking in and out of the graft resulting in a differential repopulation of the liver with donor cells, and unknown effects on the extent of peripheral chimerism.
2. Transplantation of a lobe from a living donor is done under conditions allowing extremely short cold ischemic time (60 vs. 500 minutes), a variable that may affect the severity of the inflammatory and immune response.
3. We assume that 40% of LDLT are done between genetically related individuals, resulting in a potentially more favorable HLA matching.

Preliminary data demonstrate a potentially different alloimmune and antigen-specific immune response in recipients undergoing LDLT. Previous single center studies have suggested a reduced rate of rejection after adult-to-adult LDLT. Interestingly, there is a trend toward more rapid recurrence of hepatitis C infection, a phenomenon that may be related to liver regeneration and/or inhibition of immune related anti-HCV response. The prospective cohort study will be designed to address whether LDLT affects mechanistic issues of liver transplantation immunobiology. The clinical findings will be correlated with specific laboratory assays, and will be compared with a control group undergoing DDLT transplantation. These findings will determine the opportunities for appropriate clinical modifications in the immunosuppression protocol, aiming at better outcomes for graft rejection and reduced frequency of recurrent disease. The clinical data and the specimens that are collected will be used to further support hypothesis-driven investigations, aiming to determine patterns of immune response and the potential development of favorable induction of tolerance, and those that are aiming to reduce the recurrence of the primary liver disease.

To determine whether recipients of LDLT develop better immunological acceptance of the allograft when compared to recipients of DDLT allografts, the alloimmune response will be followed by a set of well-described clinical variables and pathology findings that are associated with acute and chronic rejection of the allograft:

1. Time to rejection
2. Frequency of rejection and recurrent rejection
3. Severity of rejection as reported by liver biopsy
4. Frequency of steroid resistant rejection necessitating antibody therapy.
5. The development of chronic rejection and graft loss.

4.8.1. Study Methods

<u>Common immunosuppression and rejection protocols:</u>

Guidelines:

1. A common immunosuppression protocol will be used in order to allow an analysis of recipient outcomes as related to graft and patient survival, as well as recurrent disease.
2. The proposed protocol is based on standard immunosuppression drugs that are currently being used in the liver transplant setting.
3. Patient safety may require further adjustment for the proposed immunosuppression protocol.

The standard immunosuppression protocol for the A2ALL study is shown in Table 1. The protocol includes adjustment for patients suffering from HCV infection.

The standard treatment for steroid-sensitive and steroid-resistant rejection is shown in Table 2. The protocol includes adjustment for patients suffering from HCV infection.

Adjustment in the calcineurin inhibitors (CNI) for patients who are suffering from renal insufficiency are presented in Table 3.

Table 1.

	Non-HCV		HCV	
	Tacrolimus (ng/ml)?	Prednisone (mg/d)?	Tacrolimus (ng/ml)	Prednisone (mg/d)
OR		500		500
1 week	10	20	10	20
1 month	10	15	10	10
2 months	10	5	10	5
3 months	10	5	10	D/C
6 months	8	D/C for all patient except: PSC, Autoimmune, PBC who are to continue on 5 mg for the first year, and 2.5 mg for the second year.	8	
1 year	5-7		5-7	

Comments		The Prednisone is tapered in the first week (see below) to 20 mg/day. Reduction in Prednisone is by 5 mg every month.		The Prednisone is tapered in the first week (see below) to 20 mg/day. Reduction in prednisone is by 2.5 mg every one to two weeks.

Tacrolimus will be started within the first 12 hours after surgery with the aim to achieve levels within the first 3 days.

Severe recurrence of hepatitis C may necessitate more rapid withdrawal of Prednisone and/or lowering tacrolimus levels to below the recommended above.

Table 2: Rejection Protocol

	Prednisone
Prednisone For biopsy proven rejection	In hospital: 1000-1500 mg total over a maximum of 3 days Outpatient taper: 200mg 160mg 120mg 80mg 40mg 20mg Maintenance: follow the protocol as proposed in table 1 for HCV+ and non-HCV patients.
Steroid-resistant rejection (patient should have biopsy proven persistent rejection)	Thymoglobulin: 1.5mg/kg daily for 5-7 days Thymoglobulin should be adjusted as per Center protocol for reduced platelet and/or WBC counts.

Table 3: Adjustment of immunosuppression in the presence of renal insufficiency

1. Creatinine levels between 2-3 will be managed by reduction of tacrolimus levels to the range of 5-7, and MMF at 1gr BID.
2. Creatinine >3 will be managed by calcineurin-free interval for 3 days with MMF 1.5gr

BID. After that time, tacrolimus level will be adjusted to achieve a level of 5-7, and MMF will be reduced to 1 gr BID.
3. Tacrolimus levels will be ajusted to those recommended in Table 1 once kidney function improves

4.8.2. Participant Selection

See Section 4. for eligibility criteria.

4.8.3. Data Elements

Recipient Post-transplant:

1. Recipient Baseline Immunosuppression & Rejection
2. Rejection Episodes & Treatment
3. Liver biopsy

Liver biopsy is required in all patients who are diagnosed with acute and/or persistent rejection. The biopsy will be obtained prior to or within 24 hours after treatment for rejection.

4.8.4. Sample Size and Power Calculations

Power analyses are based on the proportion of organs that have experienced at least one rejection episode in the first year after transplant, with LDLT and DDLT compared using a test of binomial proportions. We assume 360 LDLT and 355 DDLT patients, two-sided testing and a significance level of 0.05. We will have 93% power to detect a difference between the LDLT and DDLT proportions of 13% (e.g., 43% versus 56%), and will have even greater power if the same 13% difference is closer to zero or one.

4.8.5. Statistical Analysis

The goal of this specific aim is to find predictors of organ rejection, and in particular to see if rejection rates differ between LDLT and DDLT. We will first use Cox regression, comparing time from transplant to organ rejection in each group. We will adjust for patient demographics, recipient and donor ABO and HLA typing, and whether the donor is related to the recipient. To test the effect of liver regeneration on organ rejection, we will include clinical and biochemical indicators of regeneration in the model. Radiological and pathological correlates, and extent of organ damage will also be tested. We will also consider frequency of rejection in cases of recurrent rejection. If there are enough cases of recurrent rejection, we will compare LDLT with DDLT using a multiple event Cox regression. Severity of rejection in LDLT versus DDLT will also be considered, restricted only to cases with rejection.

4.9. Secondary Aim 5: To establish a robust data and sample repository on liver transplantation that will be used to study clinical and biological questions as new technologies and resources become available.

4.9.1. Study Methods

The NIDDK Central Repositories are three separate contract-funded components that work together to store data and samples from significant NIDDK-funded studies. One component is the Biosample Repository, which will gather, store and distribute biological samples from studies. The second component is the Genetics Repository, which will receive and process blood samples to allow genetic analyses. The Genetics Repository will create immortalized cell lines or cryopreserve nucleated cells for future immortalization, and prepare DNA from one or both of these sources. The third component is a Database Repository that will gather, store and distribute the incremental or finished datasets from studies.

The NIDDK conducts and supports much of the clinical research on the diseases of internal medicine and related subspecialty fields. Many of the large clinical studies funded by the NIDDK collect biospecimens from subjects for analysis and store the samples for future study in a study-specific repository. The samples from the A2ALL Cohort Study will be collected over several years from carefully chosen subjects and are present in a finite quantity. Each sample is unique and cannot be replaced if lost, damaged, or contaminated. Therefore, it is essential that the samples be stored under optimal conditions, which vary from sample type to sample type. To this end, the NIDDK has established a Biosample Repository.

Discovery of disease related genes requires a population of individuals with the genetic variant, as well as a population of control (unaffected) individuals. Thus, a repository of DNA samples, immortalized cell lines, and accompanying clinical and pedigree data is clearly an invaluable resource for the research community studying liver disease and issues relating to transplant such as regeneration, immunology, HCC and HCV. The NIDDK has established a Genetics Repository for DNA samples for the study of the impact of genetics on disease.

In addition to the Biosample and Genetics Repositories, the NIDDK has established a Data Repository that will store, maintain, perform quality control assessments, and distribute data related to the studies storing materials at the Biosample and Genetics Repositories. The Data Repository will foster the development of highly usable public data sets, thus allowing re-analysis of these data and, where relevant, of specific biosamples, thus optimizing use of study data and samples.

The collection of patient and control biosamples and DNA samples from this and other studies for storage in the Biosample, Genetics and Data Repositories has the potential to become a resource with which researchers can rapidly validate clinical hypotheses and algorithms for clinical decision. The collections will also advance the development of prognostics, markers, and therapeutics. To date, no such collection has been available to the investigators interested in studying liver disease and transplant issues. The

repositories will allow storage, maintenance, and quality control, and equitable, ethical distribution of biosamples and other resources important to the study of liver transplant. This will allow sharing of resources, thus encouraging work by junior investigators, investigators with novel approaches, and others not included in current collaborations, without excluding those who are established in their fields. In addition, the Genetics Repository may increase the sample size and the resulting power of a study to identify genetic determinants of a disease. It will ensure that research participants will be making a maximal contribution, and will decrease duplicative sampling efforts. A2ALL is committed to sharing the resources collected in this study with current and future researchers via the use of the NIDDK repositories.

4.9.2. Participant Selection

All eligible Cohort Study subjects will be presented with information and approached for consent to have their biosamples, genetic material and non-identified data stored in the NIDDK repositories for future study.

4.9.3. Data Elements

Sample Repository and Genetics Repository

Specimen collection:
Collection of blood and tissue for immediate and/or future analysis of mechanistic issues in LDLT immunobiology will be done as part of the cohort study, but will require separate consent form(s), some of which will be developed in an investigator initiated hypothesis-driven studies.

The following is a preliminary recommendation for collection of blood and tissue from LDLT and DDLT recipients and donors at specific intervals prior and after transplantation. Some of the fresh specimens will be used for assays, whereas other will be stored:

Pre-transplant, Intraoperative and Post-operative

Donor and recipient whole blood for genetic repository (24 ml collected at enrollment for recipients and on the day of donation surgery for the donor)
Donor and recipient blood for biorepository (15 ml drawn at enrollment, day of surgery and post-transplant day 1, weeks 1 and 2*, months 1, 3, 6*, 12 and yearly through month 60). Those subjects who have joined the study later in their transplant/donation experience may have blood drawn for the repository at annual visits from Year 5 through Year 10. However, because they will have entered the study after some collection timepoints, the total amount of blood drawn from these subjects will be similar to or less than that drawn from subjects who entered the study at the time of donor evaluation.

*Donors will not have blood drawn at week 2 or month 6. Total blood drawn during the five-year study will be 219 ml for donors and a maximum of 304 ml for recipients.

Additional collection of blood and tissue will be coordinated with other ancillary studies, and correlated with liver function tests.

5. Human Subjects

5.1. Protection of Human Subjects

5.1.1. Institutional Review Board

This study and analysis will be performed under Institutional Review Board (IRB) oversight. Prior to the initiation of the study, an IRB approval for study of human subjects will be obtained separately from the IRB of each of the participating transplant centers and the DCC. Revisions to the study protocol and changes in the study design will also be submitted to the individual IRBs for approval prior to implementation.

Patients will be enrolled in the cohort study with full informed consent which will include the gathering of privileged health information (PHI), the collection of blood and tissue specimens beyond that normally performed for transplant clinical care, and the collection of medical and quality of life information at defined intervals prior to and after the transplant in donors and recipients.

5.1.2. Patient Confidentiality

Special procedures for ensuring patient confidentiality will be implemented. Data transmission and the distributed data systems have multiple layers of security as discussed below in the study management section. Each study subject will be assigned an identification number. Only this number will be used to identify subjects in any individual tabulation. The PHI that is collected will represent the minimal necessary to successfully execute the study. Since this study plans to establish a link to the Scientific Registry of Transplant Recipients (SRTR) database, to reduce the data burden on the study coordinators, SRTR PX-ID numbers will be collected. The PX-ID does not include PHI. The only PHI that will be entered into the electronic data system is date of birth. It is expected that only group data will be published. If individual subject data are to be published, no identifying information will be included. The study files will be maintained in a secure location as described above. Access to computerized data will be restricted to study personnel. Password authorization will be enforced. Previous use of this security system and secured server indicates that this technique is very successful in assuring the protection of confidential information.

Authorized representatives of the Sponsor, the National Institute of Diabetes, Digestive and Kidney Diseases (NIDDK), National Institute of Health (NIH), participating clinical institution, DCC monitoring staff, as well as the IRB, have access to medical records and records from participation in this study. Such access is necessary to ensure the accuracy of the findings.

5.1.3. Risks to the Patient

Patients enrolled in this study will experience more than the normal amount of testing which is customary for this complicated medical and surgical procedure. Additional time will be required both before and after the transplant for the gathering of medical and quality of life information. Blood will be collected and stored for special tests which are

not normally performed for this procedure. During and after the surgery liver biopsies will be collected which add a small risk of bleeding.

5.1.4. Unauthorized Data Release

The data sets will be stored on a secure server with restricted access (requires a unique username and password) at the DCC and every precaution will be taken to keep the information private. However, there is always the possibility of unauthorized release of data about subjects. Such disclosure would be extremely unlikely to involve a threat to life, health, or safety, since the only PHI that will be collected is date of birth. It is conceivable that such disclosure could have psychological, social, or legal effects on the patient. Using the standard security procedures (described above under patient confidentiality) can effectively minimize the risk of unauthorized disclosure of data. All study personnel who have access to patient data will be educated regarding the need to protect confidentiality and the procedures to be followed to ensure such protection. All staff will also be required to sign a standard medical record confidentiality agreement. The computer system on which data are maintained uses standard password protection procedures to limit access to authorized users.

5.1.5 Adverse Event Monitoring and Reporting

Definition of Adverse event

An adverse event is any untoward medical occurrence or unfavorable and unintended sign in a research subject that occurs during or as a result of research procedure.

For this observational study, the majority of the procedures are standard clinical care and adverse effects of clinical care will be tracked as complications but not be considered adverse study events. Each center will review the list of study procedures and identify the specific procedures that are NOT standard-of-care at their institution and these will be considered research procedures. Complications that are a result of research procedures will be reported and tracked as adverse events.

Assessment of Adverse Event Severity and Relationship to Treatment

The modified World Health Organization (WHO) grading system will be used for grading severity of AEs (Appendix D). For AEs not covered by the modified WHO grading system, the following definitions will be used:

Mild:	awareness of sign, symptom, or event, but easily tolerated
Moderate:	discomfort enough to cause interference with usual activity and may warrant intervention
Severe:	incapacitating with inability to do usual activities or significantly affects clinical status, and warrants intervention
Life-threatening:	immediate risk of death

The investigator must also assess the relationship of any adverse event to the research procedure, based on available information, using the following guidelines:

Unlikely related:	no temporal association, or the cause of the event has been identified; or the procedure cannot be implicated
Possibly related:	temporal association, but other etiologies are likely to be the cause; however, involvement of the procedure cannot be excluded
Probably related:	temporal association; other etiologies are possible, but unlikely

Definition of Serious Adverse Events

A serious adverse event (SAE) is any adverse experience that results in any of the following outcomes:

- death;
- life-threatening AE (i.e., one that places the subject, in the view of the investigator, at immediate risk of death from the AE as it occurs);
- persistent or significant disability/incapacity;
- required in-patient hospitalization, or prolonged hospitalization;
- congenital anomaly or birth defect.

Additionally, **important medical events** that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse event when, if based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

Reporting responsibility

All adverse events must be recorded. The onset and end dates, severity and relationship to study procedure(s) will be recorded for each adverse event. Any action or outcome (e.g., hospitalization, additional therapy, etc.) will also be recorded for each adverse event. Subjects will be questioned and/or examined by the investigator or his/her designee for evidence of adverse events.

All AEs and SAEs must be reported by the investigator to the A2ALL Data Coordinating Center (DCC). The DCC will review reports of all related SAEs and other relevant immediately, and may request additional information from sites for analysis of these events. Sites will report serious adverse events according to the time frames outlined below.

All events that are serious and related (possibly or probably) must be reported to the DCC within 24 hours of the investigator being informed of the event. Follow-up information about a previously reported serious and related adverse event may be reported to the DCC

within 7 working days of the investigator receiving the information; however, important follow-up information must be submitted within 24 hours. All deaths connected to a study procedures must be reported to the DCC within 24 hours of the investigator being informed of the event.

5.2. Benefits to the Patients

There are no direct benefits to the patients for participation in the study.

5.3. Inclusion of Women

This is a multi-center study drawing on a clinical population from nine transplant institutions across the United States. The demographics of the study population are pre-determined due to the retrospective all-inclusive nature of the study. Women will be included in the retrospective study as living liver donors and recipients. It is anticipated that the representation of women will correspond to the fraction of females in the living liver donor and recipient population.

5.4. Inclusion of Minorities

This is a multi-center study drawing on a clinical population from nine transplant institutions across the United States. The demographics of the study population are pre-determined due to the retrospective all-inclusive nature of the study. Racial and ethnic minority groups will be included in the donor and recipient components of the retrospective study and will be proportional to their representation in the donor and recipient population.

5.5. Inclusion of Children

The Adult-to-adult living donor liver transplantation cohort study specifically excludes children. By definition this study is designed to examine the risks, benefits and outcomes of Adult-to-Adult living donor liver transplantation. Adult-to-Adult transplants have only recently been performed and this study is an attempt to collect systematic data on this procedure.

5.6. Data and Safety Monitoring Plan

Accepted principles of data and safety monitoring will be observed throughout the conduct of the A2ALL cohort study. The NIH will appoint an independent Data Safety and Monitoring Board (DSMB) that will provide study oversight. The DSMB will approve the study protocol prior to enrollment and will also approve all subsequent protocol revisions.

Each transplant center principal investigator will be responsible for monitoring the enrollment of subjects and submission of data to the DCC. The DCC will be responsible for monitoring for effective conduct of the protocol and accurate and timely data submission.

IRBs will be provided feedback on a regular basis.

Training of study coordinators and study monitoring activities will be conducted by the DCC to ensure patient confidentiality and privacy and to maximize the reliability, accuracy, and timeliness of study data.

6. Study Organization

6.1. Clinical Transplant Centers

The participating Clinical Centers will have primary responsibility for developing the study protocol, maintaining high rates of follow-up and data collection, obtaining data of high quality, and interpreting, presenting, and publishing findings from the study.

1. Columbia University Health Sciences
   New York, NY
   Principal Investigator: Jean Emond, MD
2. Northwestern University
   Chicago, IL
   Principal Investigator: Michael Abecassis, MD
3. University of Pennsylvania
   Philadelphia, PA
   Principal Investigator: Abraham Shaked, MD
4. University of Colorado Health Sciences
   Denver, CO
   Principal Investigator: James Trotter, MD
5. University of California, Los Angeles
   Los Angeles, CA
   Principal Investigator: R. Mark Ghobrial, MD
6. University of California, San Francisco
   San Francisco, CA
   Principal Investigator: Christopher Freise, MD
7. University of North Carolina
   Chapel Hill, NC
   Principal Investigator: Jeff Fair, MD
8. University of Virginia
   Charlottesville, VA
   Principal Investigator: Carl Berg, MD
9. Virginia Commonwealth University
   Richmond, VA
   Principal Investigator: Robert Fisher, MD

6.2. Data Coordinating Center

The Data Coordinating Center (DCC) contributes content area expertise and shares in scientific leadership of the research group. The DCC has developed a communication infrastructure that includes meetings, teleconferences, electronic mail and bulletins, interactive web-based encounters and written correspondence. The DCC assists in protocol development and preparation of scientific publications. The DCC has the major responsibility of creating a database and data collection systems for the transplant centers, ongoing evaluation of data quality and performance monitoring of the transplant centers and statistical analyses of the data. The DCC will also create a comprehensive

Manual of Operations (MOO) that will govern the conduct of the study. The manual will detail the protocols, protocol clarifications and amendments, summary of the regulatory requirements for the study, instructions for enrollment, data collection, data management, visit schedules and detailed instructions on the use of the electronic data submission.

University of Michigan
Ann Arbor, MI
Principal Investigator: Robert M. Merion, MD

6.3. Steering Committee

The primary governing body of the study is the Steering Committee, comprised of each of the Principal Investigators of the transplant centers, the Principal Investigator of the DCC and the NIDDK Project Scientist. The Steering Committee develops policies for the study pertaining to access to patient data and specimens, ancillary studies, performance standards, and publications and presentations. They develop the study protocol and meet to discuss the progress of the study and to consider problems arising during its conduct. The Steering Committee may establish subcommittees to further develop specific components of the study protocol and propose ancillary areas of study. Small working groups may be established to prepare manuscripts and presentations.

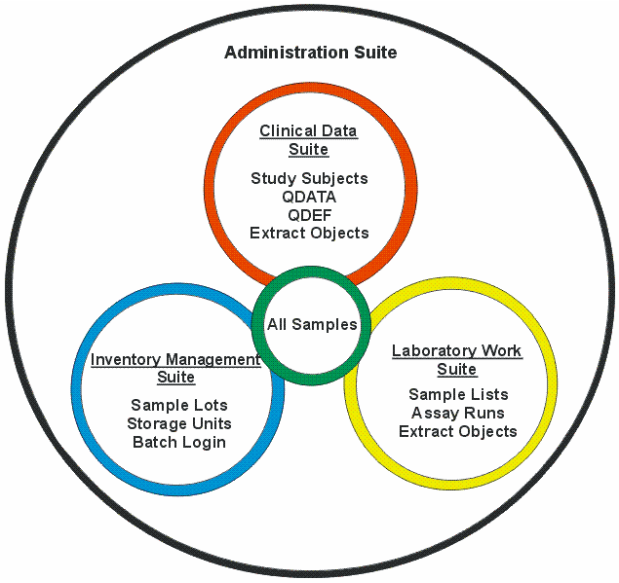
6.4. Cohort Study Subcommittees

The following subcommittees have been established to address specific issues in the Cohort study.

- Cohort Study Protocol Design
- Hepatitis C Virus (HCV) Workgroup
- Hepatocellular Carcinoma (HCC) Workgroup
- Regeneration and Function Workgroup
- Clinical Immunology Workgroup
- Outcomes/Endpoints/Definitions Workgroup
- Informed Consent Workgroup
- QOL workgroup
- Publications

Other possible subcommittees include:

- A2ALL Study Policies
- Ancillary Study Policy
- Access to Study Data
- Others as required



7. Study Management

7.1. Data collection, Data Collection Forms, and Data Entry – BioDBx

The DCC will utilize the web-based BioDBx program as the data management nucleus for the A2ALL studies.

This system was developed specifically for multicenter clinical trials management at the University of Michigan. Briefly, BioDBx is a highly flexible database application that allows investigators to organize their research operations and perform common actions on research data within a single database. There are three main suites: the Clinical Data Suite, which manages clinical data, the Inventory Management Suite, which manages inventory such as acquired specimens, and the Laboratory Workspace, which manages laboratory operations. An Administrative Suite is the overall manager for the foregoing three suites.

The Clinical Data Suite manages clinical data within a defined hierarchy, the highest level of which is termed Active Studies. From Active Studies, study subjects and study data are managed and clinical data can be viewed. There is a QDef module where study set-up occurs and a QData module where clinical data are entered after being defined by QDef. The Study Subjects module is used to enter and manage demographic data for study participants and the Study Samples module allows entry and edit of data for samples obtained from the subjects in the study.

The primary mechanism by which a study is set up in BioDBx is through a four-component QDef (Question Definition) module. The four components are: 1) definition, 2) validation, 3) extraction, and 4) navigation. Definition functions to determine where and why a variable or question appears. Validation determines acceptable values for a variable or acceptable answers to a question. Extraction defines where the data from a particular element will go for statistical analysis. Navigation is a characteristic that determines what data element is requested next.

QData is the module within the Clinical Data Suite used to enter and edit data from Case Report Forms or study questionnaires. QData can only be utilized for a given study when the entire battery of questions for a study have been defined (in QDef) and tested. For the complement of data defined, QData prepares individual data entry screens for users to key responses for specific study subjects. After responses have been entered, they can be printed and reviewed or extracted into extract objects for statistical analysis.

The DCC will utilize the BioDBx QDef module to create electronic case report forms to capture all relevant study data for the main A2ALL cohort study, the study of previously transplanted A2ALL recipients, the A2ALL donor study, and all investigational/research protocols that are developed and implemented during the course of the study. The BioDBx system allows real-time monitoring of study data for protocol adherence, quality assurance, adverse event reporting, discrepancy reporting, and other trends.

7.2. Data Management

All study data will be entered into the BioDBx electronic data entry system by study coordinators at each study site. This data will be encrypted and transferred to the DCC and stored on a secure server at the University of Michigan. Access to the server and BioDBx system is limited and requires a unique username and password combination. The servers are backed up daily and physically stored in a locked facility.

All analysis of the data sets will utilize de-identified (coded) data sets.

7.3. Quality Control and Database Management

The first steps in ensuring protocol compliance are good protocol design and careful orientation of study personnel. Following final agreement on protocols, and prior to study initiation at any of the transplant centers, the DCC will organize a Training and Certification session for transplant center study coordinators/data entry personnel.

The BioDBx electronic data entry system will have built-in data checks as part of study quality assurance. Protocol compliance will be assessed by monitoring the submission of data at required intervals. Data inconsistencies and discrepancy reports will be reviewed by the Clinical Monitor so that necessary queries can be generated and sent to the transplant center study sites for verification and resolution.

Periodic requests may be generated for the submission of random source documents to assess the quality of data acquisition and data entry at each site. In addition, the Clinical Monitor or Project Manager will visit each site at least once to review source documents, monitor regulatory compliance, and assess protocol adherence.

In addition to source document verification, the Clinical Monitor and Project Manager will produce reports from the BioDBx system to look for inconsistencies in submitted data, particularly for repeated measures data elements, even if data do not fall outside of built-in validation routines.

Studies of intra-subject and inter-subject data variability by transplant center as well as intra-transplant center and inter-transplant center data variability will be used to further ascertain random or systematic data quality issues.

Comparisons of major endpoints from the current study to national data from the SRTR will be used to assess the extent to which participants in the A2ALL study are representative of the general population of patients undergoing these procedures in the United States.

7.4. Data Security/Data Transfer

Personnel at each study center will collect and enter data into BioDBx, a web-based data entry system. Authentication is currently enabled from Oracle Developer Form Server to the Oracle database. Between the web server and client browser, secure socket layer technology is in place. This will ensure safety and confidentiality of data by using secure encrypted data transmission from the transplant centers to the BioDBx database server.

The database logs every modification of every cell in the database to ensure the ability to monitor access to the data and audit transactions. The system is accessible only via an established account with a logon and password for security and confidentiality. Authorized study personnel will be assigned an account on the system.

The BioDBx database server is located in a locked cabinet in a locked room at the University of Michigan Medical Center. The hardware administrator and his designated backup are the only individuals who have keys. The data will be transferred via the secure network to the Kidney Epidemiology Cost Center (KECC) at the University of

Michigan. The A2ALL project staff is physically located in the KECC office suite. The office suite is kept locked with entry control 24 hours a day to prohibit unauthorized entry.

The computer system at KECC currently is used for research projects that involve processing large volumes of identified and re-identifiable patient-specific data. The KECC system has a comprehensive security plan based on the guidelines in OMB Circular A-130, "Security of Federal Automated Information Resources" and NIST Publication 800-18 "Guide for Developing Security Plans for Information Technology Systems." This plan has undergone extensive review by HRSA for security certification for maintaining patient-identified data. The A2ALL project will be covered by this security plan and will be required to comply.

8. Electronic Data Submission

BioDBx will be utilized for electronic submission of data for this study. Detailed instructions on the use of BioDBx, data element definitions and a code list will be provided in a Manual Of Operations (MOO). Each study site will be provided a copy of the MOO and the entire manual will be available on the study web site.

References

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APPENDICES

Appendix A: Schedule of Recipient Visits, Tests and Assessments

Visit Type	Pre Transplant Period Quarterly from Enrollment	Transplant Hospitalization Transplant OP Day 0	Post Transplant Follow-up Period PTD1	PTW1	PTW2	PTM1	PTM3	PTM6	PTM12	PTM18	PTM24	PTM36	PTM48	PTM60
Recipients														
Allograft Biopsy		ALL					HCV		HCV		HCV	HCV		
HLA Typing	ALL (ONCE)													
HCV Genotyping	HCV													
HCV RNA quant/qual	HCV	HCV		HCV		HCV	HCV		HCV		HCV	HCV		
Chest CT	HCC													
Bone Scan	HCC													
Abdominal MRI/Contrast CT	HCC							HCC	HCC		HCC			
HCC Explant Histologic Analysis		HCC												
AFP	ALL	ALL					ALL	ALL	ALL	HCC	ALL	ALL	ALL	ALL
Explant biopsy		ALL												
Liver MRI							ALL							
LFT's	ALL	ALL		ALL	ALL	ALL	ALL	ALL	ALL		ALL	ALL	ALL	ALL
Albumin	ALL	ALL		ALL	ALL	ALL	ALL	ALL	ALL		ALL	ALL	ALL	ALL
INR	ALL	ALL		ALL	ALL	ALL	ALL	ALL	ALL		ALL	ALL	ALL	ALL
Serum creatinine	ALL	ALL		ALL	ALL	ALL	ALL	ALL	ALL		ALL	ALL	ALL	ALL
Serum Sodium	ALL	ALL		ALL	ALL	ALL	ALL	ALL	ALL		ALL	ALL	ALL	ALL
PT/PTT	ALL	ALL		ALL	ALL	ALL	ALL	ALL	ALL		ALL	ALL	ALL	ALL
Blood for Sample Repository	ALL	ALL	ALL	ALL	ALL	ALL	ALL	ALL	ALL		ALL	ALL	ALL	ALL
Tissue for Sample Repository		ALL					HCV		HCV		HCV	HCV		
Cells for Genetics Repository	ALL (at Enrollment only)													
QOL Assessments	ALL						ALL		ALL		ALL	ALL	ALL	ALL

Appendix B: Schedule of Donor Visits, Tests and Assessments

	Pre-Donation Period	Donation Hospitalization	Post Donation Follow-up Period										
Visit Type	Enrollment	Donation OP Day 0	PDD1	PDW1	PDM1	PDM3	PDM6	PDM12	PDM18	PDM24	PDM36	PDM48	PDM60
Donors*													
Donor liver biopsy		ALL											
HLA Typing		ALL											
Liver MRI	ALL					ALL							
LFT'S	ALL	ALL		ALL	ALL	ALL		ALL		ALL	ALL	ALL	ALL
Albumin	ALL	ALL		ALL	ALL	ALL		ALL		ALL	ALL	ALL	ALL
INR	ALL	ALL		ALL	ALL	ALL		ALL		ALL	ALL	ALL	ALL
Serum creatinine	ALL	ALL		ALL	ALL	ALL		ALL		ALL	ALL	ALL	ALL
PT	ALL	ALL		ALL	ALL	ALL		ALL		ALL	ALL	ALL	ALL
PTT	ALL	ALL		ALL	ALL	ALL		ALL		ALL	ALL	ALL	ALL
BUN	ALL	ALL		ALL	ALL	ALL		ALL		ALL	ALL	ALL	ALL
Hgb	ALL	ALL		ALL	ALL	ALL		ALL		ALL	ALL	ALL	ALL
Platelets	ALL	ALL		ALL	ALL	ALL		ALL		ALL	ALL	ALL	ALL
Ferritin	ALL	ALL		ALL	ALL	ALL		ALL		ALL	ALL	ALL	ALL
WBC	ALL												
Homocysteine	ALL												
CMV IgG/IgM	ALL												
HIV Antibody	ALL												
HBsAg	ALL												
HBcAb	ALL												
HepD Antibody (If HepB+)	ALL												
HepC Antibody	ALL												
HCV RNA (if HepC+)	ALL												
Blood for Sample Repository	ALL	ALL	ALL	ALL	ALL	ALL		ALL		ALL	ALL	ALL	ALL
Tissue for Sample Repository		ALL											
Cells for Genetics Repository	ALL												
QOL Assessments	ALL			McGill Pain Survey Only	McGill Pain Survey Only	ALL		ALL		ALL	ALL	ALL	ALL
Informed Consent Assessments	ALL					ALL		ALL		ALL	ALL	ALL	ALL

*Donor controls will not have any laboratory, clinical or blood testing performed after decision not to donate has been made. They will continue to be followed for data collection, QOL and informed consent assessments at the visits listed in the above schedule