

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

**Cohort Study Protocol Number A2ALL-Cohort-01**

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## 1 **1. Introduction**

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

## 12 **2. Background/Significance**

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

33  
34 The research objectives of the LDLT Cohort Study concern factors that influence the  
35 outcomes of adult-to-adult LDLT as well as a study of the biological differences between  
36 living donor (LD) and DD grafts in the recipients. Adult patients and potential donors  
37 being considered for LDLT will be recruited into this longitudinal cohort study.  
38 Recipients and their donors will be followed for sufficient time to determine outcomes  
39 related to LDLT. These outcomes will be compared with those of transplant candidates  
40 who are evaluated for but do not receive LDLT. The primary objective concerns  
41 comparison of morbidity and mortality of patients who receive LDLT with a group or  
42 groups of patients with similar illnesses and prognoses. A critical question to answer  
43 with this information is how the outcomes of LDLT compare with those of deceased  
44 donor transplantation. Transplant physicians need this information on outcomes to advise  
45 prospective recipients and donors. Therefore, sufficient recipient and donor pairs will be  
46 recruited to determine whether recipients of LDLT have substantially different survival

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

50  
51 The differences between LDLT and DDLT are inherent both in the application and the  
52 biology of the procedures. By its nature DDLT includes uncertainty about both the time  
53 of transplantation and the condition of the recipient at the time of the eventual  
54 transplantation. Because the LDLT is elective, pre-transplant morbidity and mortality are  
55 minimized in the LDLT group. This means that pre-transplant morbidity and mortality  
56 are major areas in which the potential advantage of LDLT needs to be quantified. In  
57 contrast to the expected benefits of enhanced access to transplantation, the recipient of  
58 LDLT faces a procedure which is more complex than DDLT and which provides only a  
59 partial graft. Thus, the penalty paid by opting for LDLT rather than opting for DDLT  
60 also needs to be quantified.

61  
62 LDLT offers a unique opportunity to study human liver regeneration and its impact on  
63 several key clinical biological issues in transplantation: the immune response, the  
64 recurrence of hepatitis C (HCV), and the approach to the treatment of hepatocellular  
65 carcinoma HCC (a growing indication for liver replacement therapy). In this protocol we  
66 plan to systematically collect clinical and biological data in recipients of LDLT and  
67 appropriate control recipients of DDLT to compare the impact of the hemigraft on these  
68 parameters. Entry into the cohort study will result in a relatively standardized clinical  
69 management protocol and the collection of the defined set of data points for all patients  
70 entered. A subset of patients may be recruited into ancillary studies that will entail a  
71 more extensive examination of focused topics.

72  
73 The other major mandate in the development of the cohort study is the prospective  
74 assessment of the impact of donation on the healthy living donor. There is widespread  
75 interest in this subject among the medical community and the public at large, brought on  
76 in large measure by the recent, highly publicized death of a living donor in 2002.  
77 Concerns about the ethical issues regarding donor safety will be addressed by the  
78 organized study of the surgical, biological, and psychosocial effects of donation on  
79 donors compared to a control population of potential donors who are not selected for the  
80 procedure.

81

### 82 **3. Study Objectives/Specific Aims**

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

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

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

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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.

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**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.**

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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.

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**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.**

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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.

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**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.**

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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.



- 136 3. To assess whether disclosed information or life situations are the main  
137 influential factors in the potential donor's decision to proceed or withdraw  
138 from the donation process.  
139 4. To correlate donor "satisfaction" with measurable outcomes of the donor,  
140 recipient, or perceptions of family support.  
141 5. To measure the acceptance of adverse clinical outcomes, as a function of  
142 understanding of the disclosed risks versus the presence of life situational  
143 pressures.  
144

145 **3.5. Secondary Aim 1: To compare the severity of recurrence of hepatitis C**  
146 **between LDLT and DDLT recipients.**

147  
148 Primary Objective

- 149 1. To determine whether HCV disease progression differs in patients receiving  
150 LDLT compared to DDLT.  
151

152 Secondary Objectives

- 153 1. To determine if recurrent HCV disease at one year ( $\pm$  3 months), as observed  
154 histologically, is more frequent and severe in patients undergoing LDLT as  
155 compared to DDLT transplant.  
156 2. To compare the rate of fibrosis progression (change in Ishak fibrosis score (3)  
157 per year) in LDLT and DDLT recipients by biopsies at months 3, 12, 24, and  
158 36 after transplantation.  
159 3. To compare time to recurrent disease between LDLT and DDLT recipients as  
160 determined by proportion of patients with histological evidence of recurrent  
161 HCV at 3 months.  
162 4. To determine if HCV viral level at day 7 and months 1, 3, 12, 24 and 36  
163 months differ in LDLT and DDLT recipients, and whether viral level is  
164 predictive of disease severity.  
165 5. To determine if rejection episodes requiring treatment occur at a higher rate in  
166 HCV patients who undergo LDLT as compared to DDLT transplant and to  
167 correlate this frequency of treatment of rejection to aggressive recurrence of  
168 HCV as defined histologically.  
169 6. To compare biochemical markers of disease activity (ALT/AST/total  
170 bilirubin) at 3 and 12 months and annually in LDLT and DDLT.  
171 7. To determine if cholestatic hepatitis in transplanted patients with HCV occurs  
172 in a higher proportion of LDLT as compared to DDLT recipients.  
173 8. To compare graft loss and patient survival between LDLT recipients and  
174 DDLT recipients.  
175

176 **3.6. Secondary Aim 2: Recurrence of HCC for DDLT versus LDLT.**

177  
178 Primary Objectives

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

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

184

185   Secondary Objectives

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

190

191           **3.7. Secondary Aim 3: To systematically characterize liver regeneration and**  
192           **function in donors and recipients.**

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

196

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

202

203   Primary Objective

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

209

210   Secondary Objectives

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

219

220           **3.8. Secondary Aim 4: To evaluate differences in the immune response to LDLT**  
221           **vs. DDLT grafts.**

222

223   Primary objectives

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

228 of patients with a sample large enough to detect meaningful differences in the  
229 rejection rate.  
230 2. To systematically collect serum and tissue samples that can be used to  
231 correlate clinical parameters in donors and recipients with immunologic  
232 outcomes, as defined by clinical and histologic endpoints.

233 **3.9. Secondary Aim 5: To establish a robust data and sample repository on liver**  
234 **transplantation that may be used to study clinical and biological questions as**  
235 **new technologies and resources become available.**

236

237 Primary objectives

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

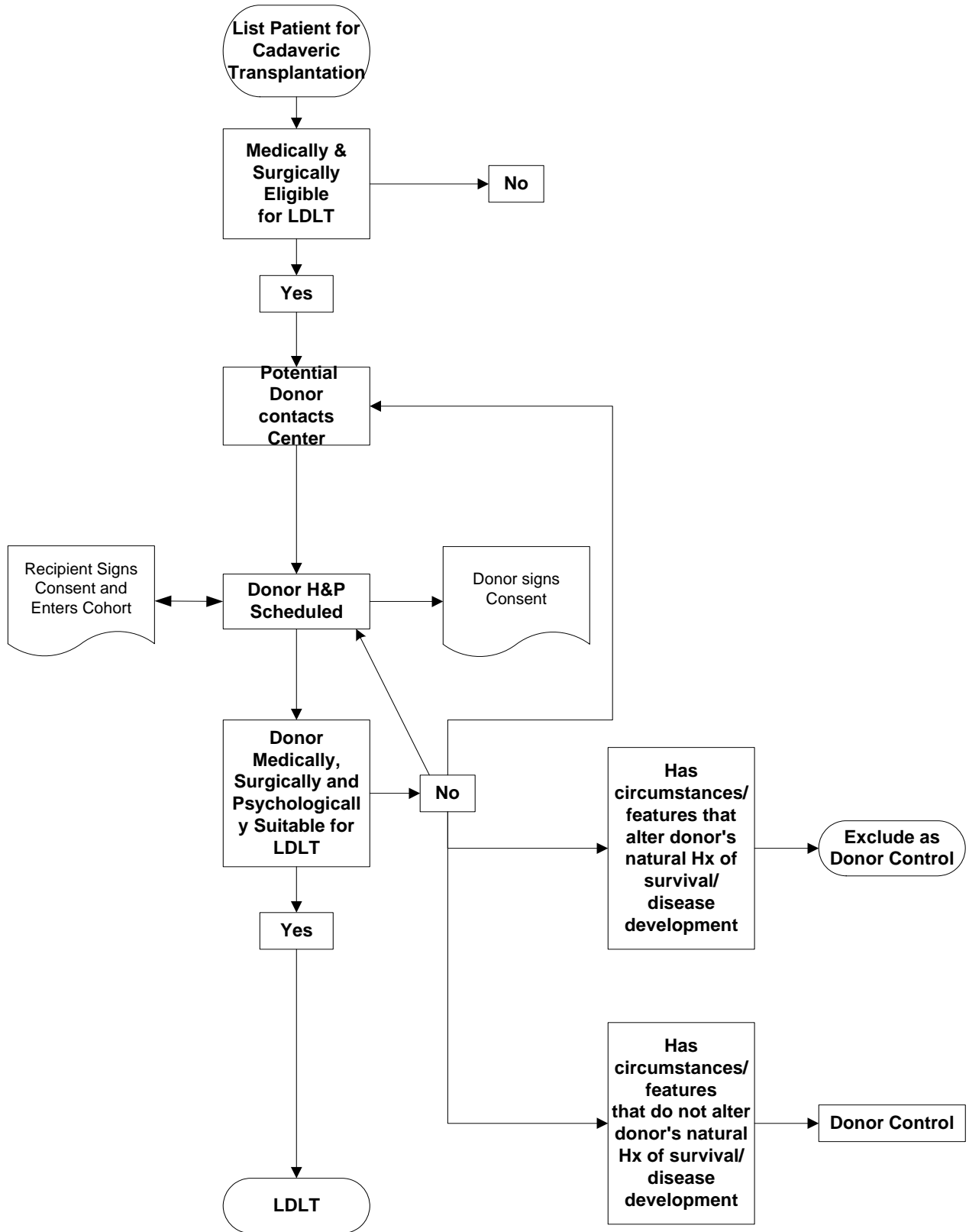
244 **4. Investigational Plan**

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

257

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



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

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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**

Primary Aims	Brief Description	Potential Recipients				Potential Donors	
		With Donor H&P			No Donor H&P	Actual Donors	Donor Controls
		LDLT	DDLT	No LDLT or DDLT	Contemporaneous DDLT (as needed)		
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
<b>Secondary Aims</b>							
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

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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.

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

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

306

307 Patients with HCC must fulfill all of the following criteria:

- 308 • Diagnosis of hepatocellular carcinoma
- 309 • May have concomitant hepatitis C
- 310 • Informed consent obtained.

311

312 Contemporaneous DDLT controls must fulfill all of the following criteria:

- 313 • Potential recipient listed for single organ (liver) transplantation
- 314 • Age  $\geq$  18 years old at transplant
- 315 • Informed consent obtained.

316

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

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

325

326 Examples of acceptable conditions include:

- 327 ○ 10 % steatosis on biopsy with normal liver tests
- 328 ○ hemangioma/minor hepatic cystic disease on imaging precluding donor
- 329 surgery
- 330 ○ diminutive liver or diminutive left lobe
- 331 ○ hepatic arterial or venous anatomical variations
- 332 ○ mild pulmonary hypertension
- 333 ○ hypercholesterolemia controlled with medication
- 334 ○ pregnancy
- 335 ○ recipient became non-LDLT candidate after donor approved.
- 336 ○ history of cancer diagnosis with candidate more than 5 years post-
- 337 treatment with presumed cure and no recurrence (Treated non-melanoma
- 338 skin cancer is acceptable)

339

340 Examples of unacceptable conditions include:

- 341 ○ insulin dependent diabetes (controlled non insulin-dependent diabetes is
- 342 acceptable)
- 343 ○ hypertension
- 344 ○ chronic hepatitis B or C

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

354

### 355 **Visit Schedule and Assessments:**

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

365

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

370

### 371 **Enrollment – Recipients:**

372

- 373 ● Medical history
- 374 ● Social history
- 375 ● Demographic information
- 376 ● Physical examination: including weight, blood pressure, etc.
- 377 ● Routine laboratory assessment: blood will be drawn to obtain values for the
- 378 following tests: multichannel automated liver function tests, serum sodium, AFP,
- 379 albumin, creatinine, and coagulation panels
- 380 ● Blood sample will be drawn for HLA typing
- 381 ● Blood sample for NIDDK Biosample Repository
- 382 ● Whole blood for NIDDK Genetics Repository
- 383 ● Quality of life baseline assessment
- 384 ● HCV-infected subjects will undergo the following laboratory assessments in addition
- 385 to those listed above:
  - 386 ○ HCV RNA quantitative or qualitative assay
  - 387 ○ HCV genotype (if not done previously)
- 388 ● Subjects with HCC will also undergo the following imaging studies if not done within
- 389 3 months previous to study enrollment:
  - 390 ○ Bone scan
  - 391 ○ Chest CT

392       ○ Abdominal MRI/contrast CT

393

394       **Pre-transplant Interval Assessments – Recipients:**

395

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

398

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

411

412       **Enrollment – Donors:**

413

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

433

434       **Day of Transplant – Recipients:**

435

- 436       • Allograft biopsy and analysis



- 437 • Routine Laboratory Assessment: blood will be drawn to obtain values for the  
438 following tests: multichannel automated liver function tests, serum sodium, AFP,  
439 albumin, creatinine, and coagulation panels  
440 • Explant pathology analysis  
441 • Tissue sample for NIDDK Biosample Repository  
442 • Blood sample for NIDDK Biosample Repository  
443 • HCV-infected subjects will undergo the following laboratory assessments in addition  
444 to those listed above:  
445 ○ HCV RNA quantitative or qualitative assay  
446 • Subjects with HCC will undergo the following laboratory assessments in addition to  
447 the routine laboratory assessments listed above:  
448 ○ Detailed pathological analysis of explant liver with tumor staging  
449

450 **Day of Donation – Donors:**

- 451  
452 • Allograft biopsy and analysis  
453 • Routine laboratory assessment: blood will be drawn to obtain values for the  
454 following tests: multichannel automated liver function tests, albumin, creatinine,  
455 coagulation panels, white blood cell count, hemoglobin, ferritin, platelets, BUN  
456 • Blood sample will be drawn for HLA typing  
457 • Whole blood for NIDDK Genetics Repository  
458 • Tissue sample for NIDDK Biosample Repository  
459 • Blood sample for NIDDK Biosample Repository  
460 • Quality of life assessment  
461

462 **Post-transplant Interval Assessments – All Recipients:**

463  
464 The following assessments and procedures will be performed post-operatively at Day 1,  
465 Week 1 and 2, Months 1, 3, 6 and 12, and annually thereafter until the study is complete  
466 or the subject reaches a study endpoint.  
467

- 468 • Physical examination: including weight, blood pressure, etc.  
469 • Routine laboratory assessment: blood will be drawn to obtain values for the  
470 following tests: multichannel automated liver function tests, serum sodium, albumin,  
471 AFP, creatinine, and coagulation panels  
472 • Blood sample for NIDDK Biosample Repository  
473 • Quality of life interval assessments at 3, 6, and 12 months and annually thereafter  
474 • Liver MRI/contrast CT at Month 3 only.  
475 • Recipients of a DDLT from an HCV-antibody positive donor must have HCV  
476 genotyping done at 3 months post-transplant  
477

478 **Post-transplant Interval Assessments – HCV-infected Recipients:**

479  
480 In addition to the assessments listed above, HCV-infected recipients will undergo the  
481 following assessments at the following time points:  
482

- 483 • Week 1, Month 1, Month 3, Year 1, 2, and 3: HCV RNA quantitative or qualitative  
484 analysis  
485 • Month 3, Year 1, 2 and 3: Liver biopsy  
486 • Month 3, Year 1, 2 and 3: Tissue sample for NIDDK Biosample Repository  
487

488 **Post-transplant Interval Assessments – Subjects with HCC:**

489  
490 In addition to the assessments listed above for all recipients, recipients with HCC will  
491 undergo the following assessments at Months 6, 12, 18 and 24:  
492

- 493 • Serum AFP (at month 18 in addition to AFP's done at interval assessments for all  
494 recipients)  
495 • Abdominal MRI/contrast CT: at months 6, 12 and 24 only.  
496

497 **Subjects Showing Signs and Symptoms of Allograft Rejection:**

498  
499 Subjects showing signs and symptoms of allograft rejection will undergo a liver biopsy  
500 with analysis to confirm the diagnosis of rejection. A biopsy will be performed to  
501 confirm each rejection episode. A tissue sample from these biopsies will also be sent for  
502 storage at the NIDDK Biosample Repository.  
503

504 **Post-Donation Assessments – Donors:**

505  
506 The following assessments will be performed for donors at Week 1, and Months 1, 3, and  
507 12, and annually thereafter until the study is complete or the donor reaches a study  
508 endpoint or is lost to follow-up:  
509

- 510 • Routine laboratory assessment: blood will be drawn to obtain values for the  
511 following tests: multichannel automated liver function tests, albumin, creatinine,  
512 coagulation panels, white blood cell count, hemoglobin, ferritin, platelets, BUN  
513 • Blood sample for NIDDK Biosample Repository  
514 • Liver MRI/contrast CT at month 3 only  
515 • Quality of life assessment: limited to a pain survey at Week 1 and Month 1 for  
516 donors. Donor and donor controls will undergo the full battery of QOL assessments  
517 at Months 3, 12 and annually thereafter.  
518 • Informed consent assessment (satisfaction with treatment): at Months 1, 3, and 12,  
519 and annually thereafter  
520 • Informed consent assessment (motivation): at Week 1, Months 3 and 12, and annually  
521 thereafter.  
522 • Donor controls will be asked to complete the QOL, Informed Consent and data  
523 collections. No diagnostic, imaging or invasive procedures will be performed once  
524 the decision not to donate has been made.  
525

526 **4.1. Primary Aim 1: To quantify the impact of choosing LDLT on the candidate**  
527 **for transplantation**

528 **4.1.1. Study Methods**

529 Patients enter this study as candidates for transplantation, and will not necessarily  
530 become recipients. Potential outcomes for these patients may include LDLT, DDLT,  
531 withdrawal from the waiting list, or death on the waiting list. The entry point would be at  
532 initial evaluation of a potential donor that includes history and physical examination at  
533 the transplant center. Our preliminary data indicate that between one-third and two-thirds  
534 of potential LDLT candidates will actually undergo LDLT. This leads us to expect that  
535 enrollment of both donors and potential recipients at the time of donor history and  
536 physical examination will generate a number of control recipients who wait for a DDLT,  
537 as well as a cohort of potential donors who do not donate to generate a population of  
538 recipient and donor controls roughly equal in number to the LDLT population.  
539

540 **4.1.2. Participant Selection**

541 See Section 4.

542 **4.1.3. Data Elements**

543 A. Potential Recipient Data Collection

544 Pre-transplant

- 545 1. Recipient Study Enrollment
- 546 2. Listing and Transplant data
- 547 3. Recipient Demographic data
- 548 4. Recipient Condition at Enrollment
- 549 5. Recipient Condition at Listing
- 550 6. Recipient Hospitalization and Complication data

551

552 B. Recipient Data Collection

553 Transplant and post-transplant follow-up

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

561

562 C. Potential Donor Data Collection

- 563 1. Donor Evaluation
- 564 2. Donor Demographic Form

565

566 D. Donation and post-donation follow-up

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

- 570 3. Donor QOL Assessment  
571 4. Donor Survival

#### 572 4.1.4. Sample Size and Power Calculations

573 We will compare the survival experience between those who receive an LDLT and those  
574 considered for an LDLT but not receive one. Although the analysis will involve a fairly  
575 complex method of matching LDLT recipients with sets of non-recipients, for the  
576 purpose of power calculations, we will assume a much simpler 2-group design. For this  
577 analysis, we will pool the patients from the Retrospective Study and the Cohort Study for  
578 maximum power. We anticipate having at least 300 LDLT recipients and 500 non-LDLT  
579 recipients from the Retrospective Study, and 360 LDLT and 710 non-LDLT from the  
580 Cohort Study, for a total of 660 LDLT and 1210 non-LDLT recipient patients. Power  
581 calculations are based on the (two-sided) logrank test, an exponential survival  
582 distribution, approximately uniform accrual of patients for at least 7 years (from 1998)  
583 with 3 additional years of follow-up, 1% loss to follow-up, and a significance level of  
584 0.05. Assuming a one-year survival probability of 0.875 in the LDLT group, we have  
585 90% power to detect as significant a one-year survival probability among non-recipients  
586 lower than 0.85 or higher than 0.90.

#### 587 4.1.5. Statistical Analysis

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

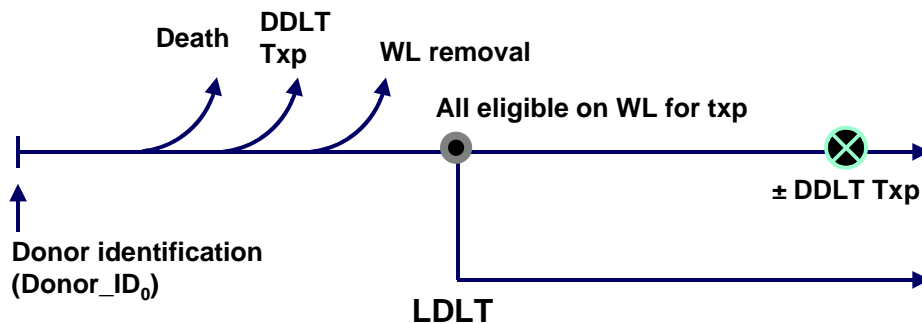
600  
601 A second analysis comparing these groups will use Cox regression, with the time origin  
602 at the donor evaluation, and covariate adjustment for age, gender, race, calendar year of  
603 initial evaluation, time on the waitlist, liver disease etiology and severity (including  
604 MELD (Model for End stage Liver Disease) score or Status, as applicable), comorbidities  
605 and other variables. The treatment strategies of LDLT versus no LDLT will be compared  
606 using a time-dependent indicator covariate for LDLT transplantation. In particular, this  
607 analysis will compare the risk of death at each time point after first donor evaluation for  
608 those having received an LDLT prior to that point versus those who have not. This  
609 analysis assumes that most candidates evaluated for LDLT either receive the transplant or  
610 do not receive it due to problems with the donor. In particular, it assumes that non-  
611 progression to LDLT due to DDLT transplantation or because the recipient becomes too  
612 sick to transplant is rare. Having many patients with non-progression to LDLT due to  
613 declining condition would bias the comparison by having sicker people in the non-LDLT  
614 group. These assumptions can be evaluated when the data are available.

615

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

626  
627  
628

## Time-dependent Analysis



629 Those people in that control group who subsequently receive an LDLT transplant are  
630 censored from that control group, but initiate a new LDLT group with its own control  
631 group. For a new LDLT, the control group will be composed of many of the same people  
632 who were in control groups for previous LDLTs, although some of the previous controls  
633 may not be included due to death, having received a DDLT or LDLT transplant or  
634 leaving the waitlist. Comparisons between each LDLT and its control group will then be  
635 pooled in a single analysis using Cox regression. The Cox model will be stratified by the  
636 LDLT/control group set. Because many people will appear in multiple control sets, we  
637 will use a robust variance estimate based on the sandwich estimator to provide statistical  
638 adjustment for the re-use of controls in multiple control groups (4, 5). As a check on this  
639 method of variance adjustment, bootstrap variance estimates will also be computed.  
640 Briefly, bootstrap variance estimates are computed by resampling from the data with  
641 replacement, computing the effect estimate for each re-drawn sample, and calculating the  
642 variance of the effect estimates obtained. Covariate adjustment will include all variables  
643 listed for adjustment in the first Cox model described above. An additional assumption  
644

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

656  
657 In both Cox analyses described above, variables will be checked to ensure that the  
658 proportional hazards assumption is met. If non-proportional hazards are detected,  
659 particularly for the LDLT effect, they will be modeled using time-dependent covariates.  
660 Interactions between covariates and the LDLT effect will be tested.

661  
662 In addition, individual components of the process will also be analyzed. We will  
663 separately estimate the distributions of time to death on the waitlist, time to transplant,  
664 and time to death following DDLT. We will use these three distributions to estimate the  
665 distribution of time to death for those not receiving a LDLT, confirming our combined  
666 estimate with the one-step estimate obtained as described above. With these estimates in  
667 place, we can then project the change in the overall survival distribution for specific  
668 changes in each of the component distributions. For example, if the distribution of time  
669 to transplant changes, say as a result of an increase in organ donations, then the effect of  
670 this change on non-LDLT survival could be estimated.

671 **4.2. Primary Aim 2: To characterize the differences between LDLT and DDLT**  
672 **in terms of post-transplant outcomes including patient and graft survival,**  
673 **surgical morbidity, resource utilization and QOL on the recipient of a**  
674 **transplant.**

#### 675 **4.2.1. Study Methods**

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

#### 690 **4.2.2. Participant Selection**

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

693  
694 The primary population of choice for recruiting DD controls would comprise those  
695 individuals from the A2ALL Retrospective study who received deceased donor  
696 transplants after 3/1/03. If there aren't enough candidates from this population, then the  
697 following method will be utilized for identifying DD contemporaneous controls:  
698 In each center, following any LD transplant, the next eligible DDLT recipient will be  
699 approached at the time of identification of a donor (if they have not been previously  
700 enrolled), until a DDLT is enrolled. The contemporaneous control recipient will undergo  
701 all perioperative and post-transplant follow-up appropriate for the study. This step will  
702 be repeated each time an LDLT is performed until the point when patients enrolled  
703 prospectively in the cohort study who did not receive LDLT begin to be receiving DDLT  
704 by virtue of progressing on the waiting list (we estimate this will take one to two years).

705  
706 Throughout the period of enrollment in the cohort study, the numbers of LDLT and  
707 DDLT will need to be comparable in each center. This will be verified by review of  
708 enrollment conducted by Data Coordinating Center every 6 months. If enrollment of  
709 DDLT falls below that of LDLT in any center during the study, the above procedure will  
710 be used to correct the deficit of DDLTs.

#### 711 **4.2.3. Data Elements**

712 Recipient data collection:

- 713 1. Recipient Condition at Transplant
- 714 2. Recipient Intraoperative Data
  - 715 o Liver biopsy
- 716 3. Recipient Post-Surgical Morbidity
- 717 4. Recipient Complication Severity
- 718 5. Recipient Hospitalization data
- 719 6. Recipient QOL Assessment
- 720 7. Recipient Survival

721

#### 722 **4.2.4. Sample Size and Power Calculations**

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

726

727 Comparisons of recipient complications after LDLT and DDLT transplants will be based  
728 on chi-square tests of equality of proportions. Assuming n=660 LDLT, n=550 DD, and  
729 alpha=0.05, we will have 83% power to detect a difference in the proportion of bile leaks,  
730 for example, of 0.18 in the DDLT group versus 0.25 in the LDLT group (a difference of  
731 0.07). Physician estimates of this difference are closer to 0.20, so power is more than  
732 sufficient for this endpoint. If we more conservatively assume complication proportions  
733 near 0.5, we will have 93% power to detect a difference of 0.10 (such as 0.45 versus  
734 0.55).

735

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

745

#### 746 **4.2.5. Statistical Analysis**

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

751

752 Initial analysis of LDLT recipient post-operative complications will be descriptive. We  
753 will report the proportions of donors with complications such as bile leak, primary non-  
754 function, graft failure, pneumonia, and urinary tract infection, as well as any complication  
755 requiring hospital admission, re-operation, or other intervention. Confidence intervals  
756 will be included with all estimates. We will also report follow-up outcomes including  
757 wound healing, pain medications, blood laboratory values, and the proportion of patients  
758 who returned to work/school. Some attempt will be made to correlate complications with  
759 patient characteristics and operative procedures, but any such analyses will be limited by  
760 the quality of available data.

761 In addition, a comparison of LDLT complications with complications following DDLT  
762 will be made. Depending on the type of complication (event occurrence, time to event, or  
763 continuous outcome), a comparison of the events between LDLT and DDLT transplants  
764 will be made using logistic regression, Cox regression, or ordinary regression,  
765 respectively, each adjusted for other predictive variables as needed.

766

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

775

#### 776 **4.3. Primary Aim 3: To determine the short and long term health and QOL** 777 **impact of donation, including (a) morbidity after liver donation, and (b) long** 778 **term health-related QOL of donors compared to a control population.**

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



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

785  
786 The analyses of these questions are critical in helping prospective donors make a true  
787 informed consent based on accurate assessment of long-term medical and psychological  
788 risks and benefits associated with LDLT.

789  
790 Concerns exist regarding psychological and psychosocial problems as a result of donation  
791 that may not be adequately recognized by the transplant team. Although these concerns  
792 are largely based on anecdotes, it is an obligation of transplant programs to recognize  
793 these problems and to prevent or treat them effectively. Although various aspects of  
794 quality of life are important, there are specific post-donation concerns, including  
795 depression, sense of abandonment, body image, and sexual function. Instruments that  
796 adequately capture information on these issues will be administered to the donors.

797  
798 Many hypotheses regarding donor outcomes and quality of life can be considered. Here  
799 are a few examples:

- 800
- 801 • Donor satisfaction will be related to both the medical and quality of life  
802 experiences of the recipient. This linkage will be greater for spouses.
  - 803 • Pre-donation, donor physical and mental QOL will be above the norm of the  
804 general population. One or more years post-donation, donor QOL will remain in  
805 the normal range of the population.
  - 806 • Donors with strong social support will feel less of a sense of abandonment.
  - 807 • Donors with a better understanding of the risks of the procedure will have greater  
808 acceptance post-donation.
  - 809 • Donors will have comparable QOL post-donation compared with controls who  
810 did not donate.
  - 811 • Sexual satisfaction and comfort with body image will be more positive for those  
812 with strong social support and a better understanding of their post-operative  
813 course.
- 814

#### 815 **4.3.1. Study Methods**

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

827 E, 3) Medical Outcomes Study Short Form-36 (SF-36), Appendix F and 4) Brief McGill  
828 Pain Survey, Appendix G, given at enrollment and post-donation at months 3, and 12,  
829 and annually thereafter. The A2ALL Donor Survey, Appendix H, will also be  
830 administered at enrollment, post-donation months 3 and 12 and annually thereafter.  
831 Additionally, the McGill Pain Survey will be administered at Week 1 and Month 1. For  
832 each time point, the goal is to have the questionnaire administered within a window of  $\pm$   
833 1 month of the target date.

834  
835 Retention of living liver donors and donor controls is essential for the success of the  
836 study. The Data Coordinating Center will be closely tracking the progress of both donors  
837 and donor control subjects throughout the study. Regular reports will be maintained and  
838 shared with the Steering Committee and DSMB to monitor long-term participation.

839  
840 Donors and donor controls will be offered \$25 compensation for each completed visit  
841 after the 3 month post-donation visit (starts with the 12 month post-donation visit).

842  
843 Anecdotally, transplant professionals have heard reports that donors feel “abandoned” by  
844 the transplant team post-operatively. A2ALL centers will make post-operative check-in  
845 calls to post-operative donors within the first four weeks after donation a standard of care  
846 so that satisfaction levels regarding transplant team post-operative interactions can be  
847 assessed in an unbiased manner via questionnaires.

848  
849 For LDLT donors who are recruited from the A2ALL Retrospective Study, quality of life  
850 will be assessed at enrollment, and then at the subsequent times post-donation following  
851 the schedule above.

### 852 **4.3.2. Participant Selection**

853  
854 See Section 4. for general eligibility criteria. Further details of inclusion and exclusion  
855 criteria for potential LDLT donors are given below.

#### 856 **Inclusion Criteria:**

- 857  
858
- 859 1. LDLT Donors: Following the formal donor evaluation, the decision to perform the  
860 donor surgery is entirely at the discretion of the transplant center and the donor. Once  
861 a donor is accepted into the donor cohort, s/he will be asked for consent to participate  
862 in the study of donor long-term outcomes, morbidity and quality of life.
  - 863 2. LDLT Donor Controls: Some potential donors who fail to donate will become  
864 controls for the long-term donor study. LDLT donor controls will also be asked to  
865 consent to study their long-term outcomes. Failure to donate and subsequent  
866 inclusion in the donor control cohort can be based upon recipient issues (condition  
867 worsening or availability of DDLT) or donor health issues. In the latter case, the  
868 donors may have mild to moderate medical conditions that precluded donation, but  
869 which are not expected to impact their long-term quality of life or alter the potential  
870 donor’s natural history of survival/disease development compared to the normal  
871 population. We will also utilize donor candidates from the Retrospective Study who  
872 have not donated as donor controls.

- 873 a. Assessment of donor controls will be limited to collection of morbidity and  
874 survival information, administration of Quality of Life and Informed Consent  
875 instruments post-donation.
- 876 3. The event that will switch a donor from the pre-donation assessment regimen to the  
877 donor post-donation assessment regimen or the donor control post-event assessment  
878 regimen is the occurrence of one of the following events (whichever happens first):
- 879 a. Donation  
880 b. Donor ruled out (but only with mild co-morbid conditions as outlined above)  
881 c. Donor withdraws  
882 d. Recipient dies  
883 e. Recipient is removed from the waiting list  
884 f. Recipient receives DDLT  
885

886 **Exclusion Criteria:**

- 887
- 888 1. Donors who are rejected due to serious health conditions will not be included in the  
889 cohort. The donor control exclusion criteria would include, but would not be limited  
890 to abnormalities identified during formal evaluation, which preclude donation and  
891 alter the potential donor's natural history of survival/disease development compared  
892 to the normal population.  
893

894 **4.3.3. Data Elements**

- 895 A. Potential Donor Data Collection
- 896 1. Donor Evaluation  
897 2. Donor Demographic Form  
898
- 899 B. Donation and post-donation follow-up
- 900 1. Donor Intraoperative Data  
901 2. Donor Post-Surgical Morbidity  
902 3. Donor Hospitalization data  
903 4. Donor QOL Assessment  
904 5. Donor Survival  
905

906 **4.3.4. Sample Size and Power Calculations**

907 This analysis is based on comparisons of donors and donor controls over time in the  
908 standardized questionnaire scores. For the power calculations, we will consider the  
909 power to detect differences between donors and controls at a single time point based on a  
910 two-sample t-test. Power for the proposed repeated measures analysis will be greater,  
911 although the amount of improvement will depend on the correlation between successive  
912 measures on the same individual, which is not known at this time. We assume that the  
913 Cohort Study will yield 360 LDLT donors and 710 donor controls  
914

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

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

#### 920 **4.3.5. Statistical Analysis**

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

926  
927 Donor morbidity will be estimated as a proportion of donors with each reported  
928 complication at relevant time points after surgery. Complications reported will include  
929 bile leak, primary non-function, graft failure, pneumonia, and urinary tract infection, as  
930 well as any complication requiring hospital admission, re-operation, or other intervention.  
931 Confidence intervals for each proportion will be given. We will also report follow-up  
932 outcomes including wound healing, pain medications, blood laboratory values, and the  
933 proportion of patients who returned to work/school. Some attempt will be made to  
934 correlate complications with patient characteristics and operative procedures, but any  
935 such analyses will be limited by the quality of available data.

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

951  
952 The analysis of pain scores will parallel the repeated measures analysis described above.  
953 However, at each time point we will also collect data on the patient's expected future  
954 pain. We will compare the predicted future pain with the pain score actually obtained at  
955 the next questionnaire completion. In addition to the standardized scales, individual  
956 questions from the A2ALL Donor Survey will also be analyzed, primarily using  
957 descriptive statistics. Covariate predictors of some of these outcomes may be  
958 investigated using linear or logistic regression.

959

#### 960 **4.4. Primary Aim 4: To standardize and assess the role of “informed consent” in** 961 **affecting the decision to donate and satisfaction after living liver donation.**

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

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

977

978

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

989

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

997

998 A living liver donor death in New York resulted in an intense scrutiny of the process of  
999 donor evaluation, operative and perioperative care and informed consent. The Advisory  
1000 Committee on Organ Transplantation (ACOT) to Secretary Thompson issued guidelines  
1001 regarding the process and information given to the potential living donor. The first  
1002 ethical principle is that the donor must be competent to make a decision. Competency is  
1003 a word with multiple meanings in the legal and common usage. In the common usage,  
1004 competency assumes a basic level of understanding of information and possession of  
1005 enough cognitive skills to derive a reasonable conclusion. However, using the legal  
1006 definitions of competency (and it is unclear how much of the informed consent disclosure  
1007 is to meet legal standards, ethical standards vs. best practice standards) an individual can  
1008 be competent and fail to “adequately” understand the disclosed information necessary to  
1009 meet a “best practice standard”.

1010

1011 **4.4.1. Study Methods**

1012 All potential donors would be given a uniform amount of information regarding the  
1013 donation process and the incumbent risks associated with donation. The information  
1014 disclosure and ethical principles of this process will embrace the recommendations of the  
1015 ACOT. In an attempt to standardize the presentation of the materials, collaboration with  
1016 Keris, Inc. (Bellevue, WA) has resulted in the development of a standardized information  
1017 disclosure that utilizes multimedia technology (the Vital Link Unit) for information  
1018 presentation and data capture. Incorporated into the multimedia presentation are  
1019 standardized tests to measure understanding and motivation. There are three presentations  
1020 addressing the donor evaluation process, the donation surgery, and post-operative  
1021 expectations. The scripts for these presentations are included as Appendices I-K. In  
1022 addition to the standardized media presentations, each center will interact with the  
1023 individual donors in their routine standard of practice. Attempts will be made to use the  
1024 Vital Link Unit for information capture in the post-donation period, however if donors  
1025 cannot access the transplant center's Vital Link, questionnaires will be mailed to attempt  
1026 to capture motivation/satisfaction.

1027

1028 Incorporated into the media presentation will be standardized assessment measures of:

- 1029 1. Comprehension and understanding: Understanding by the potential donor of the  
1030 material will be measured by the MacArthur Competency Assessment Tool-Clinical  
1031 Research (MCAT-CR), Appendix L. This tool was developed over the past  
1032 decade to assess the capacity of an individual to enter into clinical research  
1033 protocols. This tool was chosen rather than its therapeutic counterpart, after the  
1034 informed consent subcommittee discussed the observation that the individual had  
1035 no direct health benefit from donation and that measures of understanding and  
1036 appreciation of lack of personal benefit needed to be assessed. These were best  
1037 accomplished through the MCAT-CR. The questions for this study have been  
1038 adapted by Dr. Paul Appelbaum (the primary author of the tool) into a format that  
1039 can be captured through the audiovisual capture capacity of the Vital Link center.  
1040 During the first visit to the transplant center (before going through the evaluation  
1041 process) the individual will be asked questions, the answers will be recorded,  
1042 stored at the DCC and reviewed in batches (at intervals to be determined) by the  
1043 clinical psychologists at the University of Virginia. The responses will be scored  
1044 for understanding, appreciation and reasoning by previously established criteria.  
1045 The scores of capacity for understanding, appreciation and reasoning other  
1046 "normal" individuals will be available for comparison to those from the potential  
1047 living donors.
- 1048 2. Motivation for decision-making: Donors are typically motivated by a sense of  
1049 duty. Very little is known about the affect the information disclosure/evaluation  
1050 process upon the motivation of potential donors, either kidney, liver or lung organ  
1051 donors or bone marrow donors. It is anticipated that the comprehensive,  
1052 standardized disclosure of information that will be given to the potential living  
1053 liver donor may change the person's motivation to proceed. To test whether there  
1054 is a change in motivation, standardized questions about motivation to donate will  
1055 be asked at the first encounter with the center (pre-information) and at the time of  
1056 donation (at the time of acquisition of informed consent for the operation).  
1057 Questions will also be asked of those individuals that voluntarily withdraw from  
1058 the process, those individuals that were excluded from being a donor for

1059 discovered/medical reasons, and those whose prospective recipients either  
1060 received a DDLT, were removed from the waiting list or died. The parameters of  
1061 the questions are derived from previous studies in donors (kidney, bone marrow  
1062 and lung). These questionnaires and a list of their measures are included as  
1063 Appendices M-Q.  
1064 3. Measures of quality of life: There are a variety of quality of life issues, but  
1065 relevant to the informed consent process, assessment of anxiety/depression and  
1066 physical perception/pain/function are key parameters. As these perceptions  
1067 change with time after the donation, capture of this information at fixed time  
1068 points after the procedure (3 and 12 months and yearly) will be done. It will be  
1069 important to correlate the assessment of these measures to complications  
1070 associated with either the donor operation or to the recipient.  
1071 4. Measures of satisfaction.  
1072

#### 1073 **4.4.2. Participant Selection**

1074 See Section 4. and Section 4.4.1.  
1075

#### 1076 **4.4.3. Data Elements**

1077 These are listed above under Study Methods.  
1078

#### 1079 **4.4.4. Sample Size and Power Calculations**

1080 The sample size for the first hypothesis on predictors of donation will include all  
1081 potential donors in the Cohort Study, assumed to be 360 donors + 710 donor controls =  
1082 1070 total. Based on logistic regression with significance level 0.05 and 2-sided tests, we  
1083 will have 87% power to detect a difference between a 30% donation rate at the mean  
1084 level of, say, the sense of duty scale, and a 35% donation one standard deviation above  
1085 the mean. This assumes a correlation of only 0.05 between the variable of interest (e.g.,  
1086 sense of duty) and the control variables (e.g., demographics).  
1087

1088 The sample size for the second hypothesis on satisfaction with treatment will include  
1089 only those potential donors who actually donate, approximately 360 donors. Based on  
1090 standard linear regression, we will have 93% power to detect an increase of 3% in the R-  
1091 squared value of the regression, assuming 5 control variables that explain a total of 10%  
1092 of the variation. For the analysis of satisfaction with care at the evaluation time point, the  
1093 sample size will be much larger since it will include all prospective donors and thus will  
1094 have even more power.

#### 1095 **4.4.5. Statistical Analysis**

1096 The data for this aim will include the Keris modules on comprehension and  
1097 understanding, motivation for decision-making, and satisfaction with treatment. Initial  
1098 analyses will provide descriptive statistics on all modules, particularly comprehension  
1099 and understanding, and satisfaction with treatment. Because the ethical nature of  
1100 informed consent relies on comprehension of the risks involved, low scores on the  
1101 comprehension questions would bring into question the adequacy of the consent process.

1102 Levels of patient satisfaction with treatment will be of interest to all participating centers,  
1103 whose programs depend on good patient care.

1104  
1105 The first hypothesis involves predictors of the decision to donate. We will use logistic  
1106 regression to investigate variables potentially predictive of donation. Demographic  
1107 variables will be included to adjust for any confounding. The primary variables of  
1108 interest are questions related to sense of duty and need to perform a moral good. Also of  
1109 interest is whether the level of comprehension and understanding of the information  
1110 presented is predictive of the decision to donate.

1111  
1112 The second hypothesis involves predictors of donor satisfaction with treatment, and will  
1113 be restricted to potential donors who actually go through donation after the evaluation  
1114 time point. The outcome measure will be a summary of several Likert-scale questions  
1115 related to patient satisfaction, and will be a continuous variable for practical purposes.  
1116 Linear regression will be used to investigate predictors of patient satisfaction with  
1117 treatment at each time point from donation. Demographic variables will be included to  
1118 adjust for confounding. Several variables are of interest. First we will test variables  
1119 related to ease of the post-surgical course (e.g., number and severity of post-surgical  
1120 complications, number of days in the hospital). Second we will test variables measuring  
1121 the patients' foreknowledge of adverse events that they experienced; we will include in  
1122 the model both an indicator variable reflecting whether all the patient's adverse events  
1123 were listed on the consent form, and also an indicator variable reflecting whether the  
1124 patient thought they had been informed of the particular adverse event(s). (It will also be  
1125 interesting to see whether patients report not being informed of adverse events that are  
1126 given in the consent form, and if so, whether some events are more likely than others to  
1127 have been missed by the patient.) Third, we will test variables related to recipient health  
1128 for their effect on patient satisfaction with treatment, because one might imagine that the  
1129 donor would feel better about donation if the recipient were doing well. Recipient health  
1130 will be measured by number and severity of recipient adverse events, recent  
1131 hospitalization, liver function tests, and quality of life. Finally, we will test measures of  
1132 family acceptance and appreciation based on questions from the A2ALL Donor Survey.

1133 **4.5. Secondary Aim 1: To compare the severity of recurrence of Hepatitis C**  
1134 **between LDLT and DDLT recipients.**

1135  
1136 Hepatitis C virus (HCV) infection is the most common indication for DD and live donor  
1137 liver transplantation in North America. Reinfection of the graft is universal in patients  
1138 who are viremic pre-transplantation and recurrent disease is more rapidly progressive  
1139 post-transplantation than in the non-transplant setting. Among DDLTs, graft survival is  
1140 reduced for patients with HCV disease compared to patients with other causes of chronic  
1141 liver disease except malignancy. Chronic rejection, recurrent malignancy and recurrent  
1142 HCV are the most common causes of late graft loss. Factors most consistently associated  
1143 with progressive disease and recurrent cirrhosis: are year of transplantation, donor age,  
1144 pre-transplant level of viremia, absence of HBV coinfection and acute rejection.

1145  
1146 Preliminary studies of LDLT recipients suggest that allograft injury due to HCV occurs  
1147 earlier and is more severe compared to recipients of DD livers. In a retrospective study  
1148 from the University of Colorado and Mount Sinai Hospital in New York, the short-term



1149 outcome of 42 LDLT HCV patients was compared to 86 HCV patients undergoing  
1150 DDLT. Mean AST and ALT were higher at each follow-up interval reaching statistical  
1151 significance at months 1 and 3 for AST and month 1 for ALT. The percentage of patients  
1152 with aminotransferase > 100 IU/or bilirubin > 2 mg/dl was higher at each time point for  
1153 live donor liver transplant recipients. The time to histologic recurrence was significantly  
1154 shorter in live donor recipients (3.5 months) vs. DDLT recipients (6.7 months), (p=0.01)  
1155 but protocol biopsies were not performed. While these results suggest that HCV may  
1156 recur earlier and be associated with higher serum aminotransferase levels in live donor  
1157 liver transplant patients compared to DDLT recipients, the longer term outcomes (risk of  
1158 progressive fibrosis and graft loss) are unknown. The current study will examine the rate  
1159 and severity of HCV disease in a large prospective cohort of live donor and DDLT  
1160 recipients over a 3-year period. Liver biopsies obtained at 3 months and annually will be  
1161 compared between groups to assess differences in liver fibrosis and total  
1162 necroinflammatory activity, controlling for other factors known to affect disease  
1163 progression (e.g. donor age, acute rejection episodes, baseline viral level).  
1164

#### 1165 **4.5.1. Study Methods**

1166 This is a prospective cohort study of patients with chronic HCV undergoing LDLT.  
1167 Contemporaneous DDLT recipients matched (as in main prospective study) will be used  
1168 as controls. Potential DDLT recipients listed for liver transplantation who are likely to be  
1169 transplanted within the next 3 months will be approached and consented for study. The  
1170 primary endpoint of the study is the severity and rate of histological disease progression.  
1171 Antiviral therapy will not be used until the patient achieves a level of significant disease  
1172 severity that has been defined by four histological endpoints with/without clinical criteria.  
1173 Protocol biopsies at 3 months and annually will be used to assess rate of recurrence and  
1174 severity of disease. Patients will be followed for up to 3 years. Data and specimens will  
1175 be collected to evaluate the effect of specific clinical, virological and immunological  
1176 factors on disease progression in LDLT recipients and controls.  
1177

#### 1178 **Study Endpoints**

1179 The primary and some of the secondary endpoints are based upon liver histology. Any  
1180 liver biopsy performed post-transplantation will be reviewed for the HCV histological  
1181 endpoints (i.e. both protocol biopsies and biopsies done as part of clinical care).  
1182

#### 1183 **Primary Endpoint**

- 1184 1. Proportion of patients with one of the following four endpoints indicative of  
1185 “progressive” disease:
  - 1186 a. Ishak fibrosis score of  $\geq 3$  (some bridging fibrosis)
  - 1187 b. Ishak fibrosis score of  $\geq 2$  plus total bilirubin  $> 5.0$  mg/dL
  - 1188 c. Ishak fibrosis score of  $\geq 2$  plus Knodell score (necroinflammatory indices)  
1189  $\geq 9$
  - 1190 d. Diagnosis of cholestatic hepatitis

1191  
1192 Additional causes of liver test elevation must be excluded. Specifically, there must be:

- 1193 e. Absence of rejection (acute and chronic)
- 1194 f. Absence of biliary disease

1195 g. Sepsis

1196

1197 **Secondary Endpoints**

- 1198 1. Rate of fibrosis progression (Ishak fibrosis score) – change in fibrosis score per  
1199 year  
1200 2. Total and necroinflammatory scores at 3 months, 1 year, 2 years and 3 years  
1201 (Knodell score)  
1202 3. Time to recurrence of disease determined by proportion of patients with  
1203 histological evidence of recurrent HCV at 3 and 12 months  
1204 4. Viral level at day 7 and months 1, 3, and 12, 24 and 36– comparison of LD and  
1205 DDLT recipients and prediction of post-LT disease progression.  
1206 5. Proportion with cholestatic hepatitis  
1207 6. Graft and patient survival

1208

1209 **4.5.2. Participant Selection**

1210 See Section 4. for general eligibility criteria, and Section 4.2.2 for further details on  
1211 selection of contemporaneous controls.

1212 **4.5.3. Data Elements**

1213 Potential Recipients positive for HCV

- 1214 1. HCV at enrollment  
1215 2. HCV survey

1216

1217 Recipients positive for HCV

- 1218 1. HCV intraoperative sample collection  
1219 2. HCV at Transplant  
1220 3. HCV Post-operative Recurrence and Rx data  
1221 4. Liver biopsy and histology data

1222

1223 Histological Evaluation:

1224 Local pathologists will use a standardized form for scoring all liver biopsies on HCV  
1225 study participants performed after the first 4 weeks post-transplantation. The Knodell  
1226 system (See Appendix R) will be used. To be an evaluable biopsy, in terms of HCV-  
1227 related endpoints, there must be an absence of other concurrent conditions such as acute  
1228 rejection, biliary obstruction and CMV hepatitis. Both protocol liver biopsies (month 3  
1229 and annually) and non-protocol biopsies will be scored for evidence of HCV recurrence.

1230 **4.5.4. Sample Size and Power Calculations**

1231 We estimate the total number of transplant patients available (based on the recent survey  
1232 of A2ALL transplant centers) to be 360 LDLT and 355 DDLT. We assume that 30% of  
1233 waitlist patients have HCV, or approximately  $(0.30 \times 360 =)$  108 LDLT and  $(0.30 \times 355 =)$   
1234 107 DDLT. All calculations below assume two-sided testing with a significance level of  
1235 0.05.

1236

1237 1. The primary endpoint upon which the study will be powered is histological severity of  
1238 disease at 1 year. We base sample size calculations on a comparison of binomial  
1239 proportions. If we assume that 10% of DDLT patients achieve the composite endpoint of  
1240 (Ishak  $\geq 3$ , etc), we have 82% power to detect an increase in the LDLT proportion to 25%.

1241  
1242 2. The secondary endpoints also capture rate of disease progression. If we assume 50%  
1243 of DDLT patients will achieve the composite endpoint at 3 years, we will have 85%  
1244 power to detect an increase in the LDLT proportion to 70%. Thus, based on dichotomous  
1245 endpoints (progression or not), we can only detect a difference between LDLT and  
1246 DDLT with high power only if the difference is at least 15-20%.

1247  
1248 Alternatively, we can look at differences in fibrosis progression using a numeric scale.  
1249 With an estimate of SD=0.25 for the yearly rate of increase in fibrosis score, we have  
1250 82% power to detect an increase or decrease of 0.10 compared to 0.45 (DDLTLT)  
1251 [Berenguer 2000 -- for DDLT 0.3-0.48/year on Desmet scale 0-4)]. Power will be  
1252 greater to detect LDLT rates above 0.55 or below 0.35.

1253

#### 1254 **4.5.5. 4.5.5 Statistical Analysis**

1255 The primary objective is to compare HCV progression probabilities in LDLT versus  
1256 DDLT, where HCV progression is defined above. This comparison will be tested using  
1257 logistic regression, with HCV progression (yes/no) at a given time point as the outcome  
1258 variable, and LDLT versus DDLT as the covariate of interest. This comparison will be  
1259 adjusted for other variables associated with HCV progression. If HCV progression is  
1260 evaluated at regular points over time, then discrete time survival analysis will be used to  
1261 compare the time until HCV progression in the two groups.

1262

1263 Secondary objectives will involve several analyses. First, both the rate of fibrosis  
1264 progression (change in Ishak score over time) and change in the necroinflammatory score  
1265 (Knodell score) will be analyzed using repeated measures regression. A difference in  
1266 slopes of the Ishak or Knodell scores over time for LDLT and DDLT will be tested with  
1267 an interaction term between LDLT/DDLT and follow-up time. Second, the proportion of  
1268 patients with histologic evidence of recurrent HCV at 3 months and 1 year after LDLT  
1269 versus DDLT will be compared using logistic regression. This analysis will parallel the  
1270 analysis for the primary objective described above. Because recurrent disease occurs  
1271 prior to progressive disease, these two analyses will not be independent. Third, analysis  
1272 of viral level at day 7 and months 1, 3, 6, and 12 will be performed using repeated  
1273 measures regression, with the primary comparison between LDLT and DDLT.

1274 Differences in the pattern of viral levels over time will be tested using a time by  
1275 LDLT/DDLT interaction. As a second step, HCV disease severity will be added to the  
1276 model to see if viral level is significantly predicted by disease severity. Fourth, the  
1277 incidence of cholestatic hepatitis in LDLT and DDLT will be compared using logistic  
1278 regression, adjusted for any other prognostic factors. Finally, graft and patient survival  
1279 will be analyzed using Kaplan-Meier estimators, logrank tests comparing LDLT and  
1280 DDLT, and Cox regression to compare groups adjusted for other variables.

1281 **4.6. Secondary Aim 2: Recurrence of HCC for deceased donor versus LDLT.**

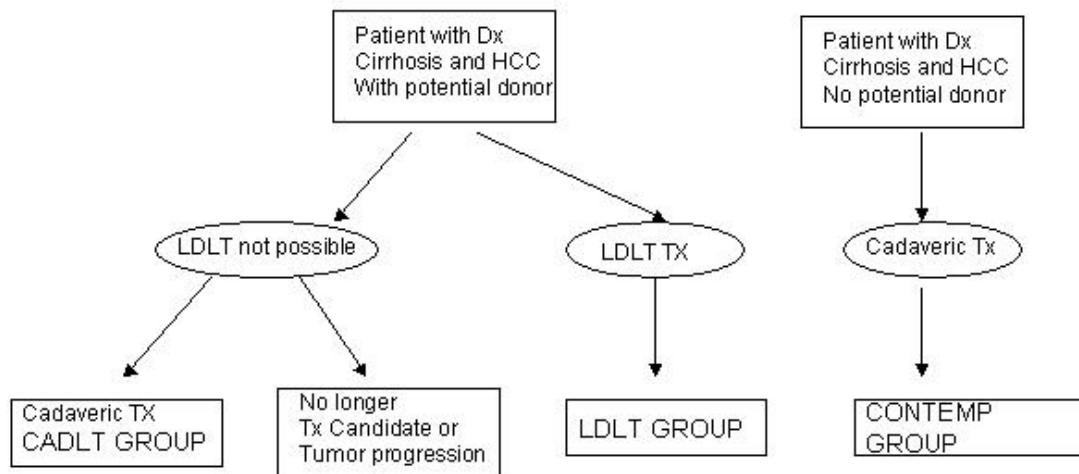
1282 The utility of liver transplantation for patients with cirrhosis and hepatocellular  
1283 carcinoma with stage 1 or 2 disease has clearly been demonstrated. The key to this  
1284 therapy is timing of transplantation, and progression of disease while a patient is awaiting  
1285 transplantation is a potential hazard. The near immediate availability of a graft from a  
1286 living donor opens up the possibility of intervening with transplant before the  
1287 complication of progression of disease is encountered. However, to date there has been  
1288 no clear evidence that the benefits of this mode of therapy are superior to standard  
1289 deceased donor transplantation as it relates to patient survival and cancer free survival. A  
1290 prospective study to compare outcomes of these two treatment modalities is therefore  
1291 needed.  
1292

1293 **4.6.1. Study Methods**

1294 This is a prospective cohort study which will collect data elements specific to a subset of  
1295 patients enrolled in the A2ALL Cohort Study, who carry a diagnosis of cirrhosis and  
1296 hepatocellular carcinoma. This cohort will include all LDLT recipients who consent to  
1297 enrollment, have cirrhosis, and carry a diagnosis of hepatocellular carcinoma, or develop  
1298 hepatocellular carcinoma while waiting for transplant. To address the Primary Aims and  
1299 utilization of ablation, patients who undergo LDLT procedures (**LDLT GROUP**) will be  
1300 compared with recipients who were enrolled but ultimately could not undergo a live  
1301 donor transplant secondary to recipient or donor factors, and remained on the waiting list  
1302 or eventually underwent deceased donor liver transplant (**DDLTL GROUP**).  
1303

1304 Since there will be a period of time before a suitable number of DDLTL GROUP patients  
1305 accumulate, a third group consisting of contemporaneous deceased donor controls will  
1306 also be recruited (**CONTEMP GROUP**). These patients carry a diagnosis of cirrhosis  
1307 and hepatocellular carcinoma, will be selected by the Data Coordinating Center, and  
1308 enrolled at the time of transplant. This CONTEMP GROUP will serve as a control to  
1309 address the question of operative and post-operative complication rates. It is anticipated  
1310 that the CONTEMP GROUP will only be needed for the first two years of the study,  
1311 depending on number of patients enrolled.  
1312

1313 A schematic for patient flow through the study is below.  
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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 (DDLTL GROUP).

- 1336 5. Patients with evidence of local recurrence of HCC during this waiting period may  
1337 undergo repeat ablation in an attempt to limit disease progression prior to  
1338 undergoing DDLT or LDLT.  
1339 6. Patients who develop evidence of metastatic disease or vascular invasion will be  
1340 dropped from the study protocol and followed until death.  
1341 7. The liver explants of all patients who undergo LDLT or DDLT will be evaluated  
1342 for presence of HCC under a defined pathologic protocol : EXPLANT  
1343 PATHOLOGY:  
1344 a. Explant liver will be sliced at 4-5mm intervals and all suspicious nodules  
1345 for HCC processed for light microscopy.  
1346 b. Tumors will be measured and inspected for encapsulation, invasion into  
1347 adjacent liver, and vascular structures. Sections from the tumor/tumors  
1348 will be fixed in formalin and processed for light microscopy. The size,  
1349 multiplicity, grade (G1,G2,G3), degree of mitosis (<10/HPF or  
1350 >=10/HPF), infiltration into adjacent liver, encapsulation, and vascular  
1351 invasion (micrometer vs. millimeter portal or hepatic vein ) will be  
1352 determined for all tumors.  
1353 8. All patients will be followed in the LDLT cohort main study protocol prior to and  
1354 following LDLT and DDLT  
1355 9. All patients who undergo LDLT or DDLT will be followed post transplant for  
1356 evidence of recurrent or metastatic disease. Serum AFP will be determined at  
1357 post-transplant month 3,6,12,18, 24 and every six months until the conclusion of  
1358 the study. Patients will also undergo either an abdominal CT or MRI at months 6,  
1359 12 and 24 post-transplant to document absence of recurrence. Criteria for disease  
1360 recurrence include radiologic imaging demonstrating a lesion with characteristics  
1361 of tumor (date and type of study to be recorded, possibilities include bone scan,  
1362 PET scan, CT or MRI.) Tissue diagnosis is also acceptable but not required for  
1363 diagnosis of recurrence. An elevated AFP in the absence of a documented lesion  
1364 on imaging will not be accepted as evidence of recurrence.  
1365

1366 Primary endpoints will be patient survival, cancer-free patient survival.

1367  
1368 Secondary endpoints will include loss of candidacy while on list due to tumor  
1369 progression, overall use of ablative therapies, complications of ablative therapies,  
1370 accuracy of preoperative imaging modalities, surgical and postoperative complication  
1371 rates.  
1372

#### 1373 **4.6.2. Participant Selection**

1374 See Section 4. for overall eligibility criteria, and Section 4.2.2 for further details on  
1375 selection of contemporaneous controls. The specific inclusion and exclusion criteria for  
1376 patients with HCC are given below.  
1377

##### 1378 *Inclusion Criteria:*

- 1379  
1380 1. Suspected or confirmed HCC which meet the UNOS definition for being listed  
1381 for Stage I or Stage II priority.  
1382 a. A single mass lesion on imaging studies < 5 cm in diameter

- 1383            b. Multiple lesions on imaging studies, but no greater than three lesions and  
1384            no single lesion > 3 cm.  
1385            c. A tissue diagnosis of HCC.  
1386            d. An AFP greater than > 500 ng/ml.  
1387        2. Patients with greater than Stage II disease will also be enrolled, as long as they  
1388            were accepted by the individual transplant center as acceptable for liver  
1389            transplant.  
1390        3. Patients with history of ablation prior to listing will also be eligible, as long as  
1391            one of the features in section 1 above was present prior to the ablative procedure.  
1392

1393        *Exclusion criteria :*

- 1394  
1395        1. Evidence of metastatic disease based upon chest CT, bone scan and abdominal  
1396            MRI or contrast CT.  
1397        2. No tumor found in explant and no history of prior ablative therapy.  
1398        3. Patients with synchronous cholangiocarcinoma.  
1399

1400            **4.6.3. Data Elements**

1401        In addition to the data elements collected for the A2ALL Cohort study, specific data  
1402        elements will be collected in these patients with HCC. These include:

1403  
1404        Potential Recipients positive for HCC

- 1405            1. HCC data at Listing  
1406            2. HCC data at Enrollment

1407  
1408        Recipients positive for HCC

- 1409            1. HCC Data Immediately Prior to Transplant  
1410            2. HCC Explant Assessment  
1411            3. HCC Post-operative Recurrence and Treatment Data

1412

1413            **4.6.4. Sample Size and Power Calculations**

1414        It is anticipated (based on 1998-2002 data and trends) that approximately 75 patients  
1415        (25% of all 300 LDLTs) will have undergone LDLT for HCC over the three-year period  
1416        of the Retrospective study at the A2ALL study centers. Similarly, there should be  
1417        approximately 125 patients (25% of all 500 non-LDLT) in the non-LDLT group to serve  
1418        as controls. In addition, approximately 15% of Cohort patients are estimated to have  
1419        HCC ( $0.15 \times 360 =$ ) 54 LDLT and ( $0.15 \times 710 =$ ) 106 non-LDLT. Thus, the total cohort for  
1420        endpoints collected in both Retro and Cohort studies will be ( $75 + 54 =$ ) 129 LDLT and  
1421        ( $125 + 106 =$ ) 231 non-LDLT. With this number of patients, there will be an 95% power to  
1422        detect a 20% difference (e.g., 40% versus 60%) in recurrence of HCC (loss of transplant  
1423        candidacy or post-transplant recurrence) between the LDLT group and non-LDLT group.  
1424        These numbers should also allow for adequate comparison of patient survival, since  
1425        tumor recurrence typically leads to patient death.

1426

1427        The lowest power will be available for comparisons between LDLT and DDLT among  
1428        Cohort patients only: an estimated 54 LDLT and 54 DDLT. For these comparisons,

1429 continuous outcomes will have more power than dichotomous outcomes. We will have  
1430 87% power to detect an effect size (number of standard deviations different) of 0.60  
1431 between groups. Cohen considers an effect size of 0.50 to be moderate, and 0.90 to be  
1432 large.  
1433

#### 1434 **4.6.5. Statistical Analysis**

1435 Initial analysis of HCC will be primarily descriptive. The characteristics of HCC patients  
1436 described will include TNM explant pathologic stage, use of ablation pre-transplant, and  
1437 pre transplant ablation method (i.e. chemoembolization, RFA, etc.). The proportion of  
1438 HCC recurring within one year will be presented, with 95% CI. Predictors of one-year  
1439 recurrence will be explored using logistic regression for patients with at least one year of  
1440 follow-up. The difference between recurrence (or presence) proportions for LDLT versus  
1441 non-LDLT patients will also be estimated using a 95% confidence interval. A comparison  
1442 of survival between these two groups will be performed using Cox regression, adjusted  
1443 for various prognostic covariates. A comparison of survival between LDLT and  
1444 deceased donor transplant recipients will also be performed.  
1445 Secondary outcomes include comparisons of numbers of ablative procedures for LDLT  
1446 versus waiting for DDLT, and numbers of surgical complications for LDLT versus  
1447 DDLT. Differences in numbers of ablative procedures will be analyzed using Poisson  
1448 regression, assuming that multiple ablative procedures may be necessary for some  
1449 patients. Differences in surgical complications will be analyzed using logistic regression,  
1450 assuming each complication either does or does not occur for each patient in a given time  
1451 frame.

#### 1452 **4.7. Secondary Aim 3: To systematically characterize liver regeneration and** 1453 **function in donors and recipients.**

1454 Information about liver regeneration after liver transplantation remains descriptive and is  
1455 limited by our inability to directly sample liver tissue in the days after liver surgery.  
1456 Following donation and transplantation, it has been observed that hepatic function of  
1457 living liver donors returns to normal soon after right hepatic lobe resection using standard  
1458 serum tests of liver function. Recipients of living donor liver transplants also have rapid  
1459 return of function, however there may be a reduction and delay in recovery of hepatic  
1460 metabolism following LDLT compared to whole DDLT, which has been observed with  
1461 respect to the metabolism of immunosuppressive compounds.  
1462

1463 Regeneration may fail if the graft is excessively small or damaged in other ways though  
1464 the factors associated with graft function have not been adequately studied. Finally, it  
1465 remains uncertain when and if the liver volume returns to its baseline following this  
1466 procedure.  
1467

1468 Initiation of molecular pathways associated with liver regeneration previously identified  
1469 in rodent models is presumably necessary for hepatic recovery in the human living donor  
1470 liver transplant setting in both donors and recipients. It is presumed that failure of  
1471 regeneration is a consequence of a failure to initiate these events though limitations in our  
1472 ability to directly assess liver tissue over time has prevented confirmation of these  
1473 expectations.  
1474



1475 Primary Objectives

- 1476 • To measure hepatic function and mass in living donors at study entry,  
1477 perioperatively, and following hepatectomy, and determine whether return of  
1478 hepatic function following donation correlates with rate of liver regeneration,  
1479 biochemical impairment, and clinical events, and if return of function is complete  
1480 by 3-6 months post-resection.
- 1481 • To correlate liver function in donors with long-term health outcomes and the  
1482 incidence of clinical complications.
- 1483 • To correlate success or failure of regeneration with a series of selected clinical  
1484 and laboratory variables in donors and recipients
- 1485 • To collect liver biopsy and serum samples prospectively from a large series of  
1486 donors and recipients which may form the basis for subsequent characterization of  
1487 protein and gene expression of selected inflammatory and growth-related  
1488 molecules.  
1489

1490 **4.7.1. Study Methods**

1491  
1492 A. Restoration of liver mass and function:  
1493

1494 In this cohort we will limit our observations of restoration of liver mass to measurement  
1495 of liver volume at defined interval after transplantation and donation and clinical and  
1496 standard laboratory assessment at standardized intervals.  
1497

1498 B. Tissue and serum collection:  
1499

1500 Tissue and sera will be collected and stored prospectively for eventual analysis using  
1501 molecular and proteomic techniques to detect selected molecules associated with  
1502 regeneration and repair.  
1503

1504 Liver biopsies will be collected prior to hepatectomy in the living donors, on the back-  
1505 table, and after reperfusion of the liver graft in recipients. These will be processed and  
1506 stored for eventual analysis. In addition, sera will be collected and stored at defined  
1507 intervals from donor and recipients which will be available for subsequent studies of gene  
1508 and protein expression.  
1509

1510 a. Tissue samples  
1511

1512 i) In the living donor setting, one Tru-cut core biopsy of the right lobe of the donor liver  
1513 will be performed prior to the removal of the right lobe (control in situ biopsy), and one  
1514 on the back table after flushing with preservation solution (cold ischemia biopsy), for  
1515 baseline assessment. Approximately 30-60 minutes after reperfusion of the right lobe into  
1516 the recipient, the donated portion of the liver will also have one Tru-cut core biopsy  
1517 performed (post-reperfusion biopsy). All biopsy specimens will be split in the operating  
1518 room, with two-thirds placed in RNAlater to be processed for RNA or immunohistology,  
1519 and one-third placed in paraffin block for later section/H&E histology.  
1520

1521 ii) In the DDLT donor setting, biopsies will be obtained from the donor liver on the back  
1522 table (cold ischemia biopsy), and then approximately 30-60 minutes after implantation  
1523 (post-reperfusion biopsy), as described above. The control DDLT arm is important for  
1524 comparison and correlation of molecular events with ischemic injury and/or graft to  
1525 recipient size ratio.

1526  
1527 Serum samples will be obtained at baseline (preoperatively), intraoperatively prior to  
1528 resection or transplantation and at the time of final biopsy, on post-operative days (PODs)  
1529 1 and 7, and at 2, 4, 12 and 24 weeks.

#### 1530 **4.7.2. Participant Selection**

1531 See Section 4. for eligibility criteria.

1532

#### 1533 **4.7.3. Data Elements**

1534 Serum tests of liver function at defined times will be tabulated and compared sequentially  
1535 for donors and LDLT recipients. Mean values for comparable time points will be  
1536 compared between DDLT and LDLT.

1537

1538 To correlate liver function in donors with long-term health outcomes as determined by  
1539 data collection at defined intervals as noted above in Aim 3 and the incidence of clinical  
1540 complication

1541

1542 To correlate success or failure of regeneration with a series of selected clinical and  
1543 laboratory variables in donors and recipients. Liver volume at defined intervals will be  
1544 correlated with a selection of pre, peri, and post surgical clinical and laboratory variables

#### 1545 **4.7.4. Sample Size and Power Calculations**

1546 Power analyses are based on estimation of correlation coefficients and coefficient of  
1547 determination between measures of liver regeneration and liver function. We anticipate  
1548 having 360 donors and 360 recipients in the Cohort study. We assume a two-sided test  
1549 for a non-zero correlation with significance level 0.05. With these assumptions, we can  
1550 detect a correlation of 0.17 or greater with 90% power in either the donors or the  
1551 recipients. In a multiple regression analysis, we will have 85% power to detect an  
1552 increase in the R-squared of 2%, assuming 5 variables already in the model explaining  
1553 20% of the variance.

#### 1554 **4.7.5. Statistical Analysis**

1555 Analysis of liver regeneration will be performed using repeated measures regression. We  
1556 will follow the mean course of hepatic function and mass in living donors from baseline  
1557 through 3 months post surgery. We will test for a difference between liver function at  
1558 baseline and function at 3 months post surgery to determine whether liver function has  
1559 returned to normal. We will also model liver function as predicted by liver regeneration  
1560 volume, biochemical impairment, and the occurrence of certain clinical events. We will  
1561 also compare the return of hepatic function in LDLT versus DDLT recipients using  
1562 repeated measures regression.

1563

1564 **4.8. Secondary Aim 4: To evaluate differences in the immune response to LDLT**  
1565 **vs. DDLT grafts.**

1566

1567 Living donor liver transplantation presents a unique immunological setting that is  
1568 determined by three major variables that are different from the DDLT setting, with the  
1569 potential to impact on short and long-term graft and patient survival, as well as recurrent  
1570 disease:

- 1571 1. Regeneration may be associated with different pattern of lymphocyte trafficking  
1572 in and out of the graft resulting in a differential repopulation of the liver with  
1573 donor cells, and unknown effects on the extent of peripheral chimerism.
- 1574 2. Transplantation of a lobe from a living donor is done under conditions allowing  
1575 extremely short cold ischemic time (60 vs. 500 minutes), a variable that may  
1576 affect the severity of the inflammatory and immune response.
- 1577 3. We assume that 40% of LDLT are done between genetically related individuals,  
1578 resulting in a potentially more favorable HLA matching.

1579

1580 Preliminary data demonstrate a potentially different alloimmune and antigen-specific  
1581 immune response in recipients undergoing LDLT. Previous single center studies have  
1582 suggested a reduced rate of rejection after adult-to-adult LDLT. Interestingly, there is a  
1583 trend toward more rapid recurrence of hepatitis C infection, a phenomenon that may be  
1584 related to liver regeneration and/or inhibition of immune related anti-HCV response. The  
1585 prospective cohort study will be designed to address whether LDLT affects mechanistic  
1586 issues of liver transplantation immunobiology. The clinical findings will be correlated  
1587 with specific laboratory assays, and will be compared with a control group undergoing  
1588 DDLT transplantation. These findings will determine the opportunities for appropriate  
1589 clinical modifications in the immunosuppression protocol, aiming at better outcomes for  
1590 graft rejection and reduced frequency of recurrent disease. The clinical data and the  
1591 specimens that are collected will be used to further support hypothesis-driven  
1592 investigations, aiming to determine patterns of immune response and the potential  
1593 development of favorable induction of tolerance, and those that are aiming to reduce the  
1594 recurrence of the primary liver disease.

1595

1596 To determine whether recipients of LDLT develop better immunological acceptance of  
1597 the allograft when compared to recipients of DDLT allografts, the alloimmune response  
1598 will be followed by a set of well-described clinical variables and pathology findings that  
1599 are associated with acute and chronic rejection of the allograft:

1600

- 1601 1. Time to rejection
- 1602 2. Frequency of rejection and recurrent rejection
- 1603 3. Severity of rejection as reported by liver biopsy
- 1604 4. Frequency of steroid resistant rejection necessitating antibody therapy.
- 1605 5. The development of chronic rejection and graft loss.

1606

1607

1608 **4.8.1. Study Methods**

1609 Common immunosuppression and rejection protocols:

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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.
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 1639

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.

1640  
 1641  
 1642

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 adjusted to those recommended in Table 1 once kidney function improves

1643

#### 1644 **4.8.2. Participant Selection**

1645 See Section 4. for eligibility criteria.

1646

#### 1647 **4.8.3. Data Elements**

1648 Recipient Post-transplant:

1649 1. Recipient Baseline Immunosuppression & Rejection

1650 2. Rejection Episodes & Treatment

1651 3. Liver biopsy

1652 Liver biopsy is required in all patients who are diagnosed with acute and/or persistent  
1653 rejection. The biopsy will be obtained prior to or within 24 hours after treatment for  
1654 rejection.

1655

#### 1656 **4.8.4. Sample Size and Power Calculations**

1657

1658 Power analyses are based on the proportion of organs that have experienced at least one  
1659 rejection episode in the first year after transplant, with LDLT and DDLT compared using  
1660 a test of binomial proportions. We assume 360 LDLT and 355 DDLT patients, two-sided  
1661 testing and a significance level of 0.05. We will have 93% power to detect a difference  
1662 between the LDLT and DDLT proportions of 13% (e.g., 43% versus 56%), and will have  
1663 even greater power if the same 13% difference is closer to zero or one.

#### 1664 **4.8.5. Statistical Analysis**

1665 The goal of this specific aim is to find predictors of organ rejection, and in particular to  
1666 see if rejection rates differ between LDLT and DDLT. We will first use Cox regression,  
1667 comparing time from transplant to organ rejection in each group. We will adjust for  
1668 patient demographics, recipient and donor ABO and HLA typing, and whether the donor  
1669 is related to the recipient. To test the effect of liver regeneration on organ rejection, we  
1670 will include clinical and biochemical indicators of regeneration in the model.

1671 Radiological and pathological correlates, and extent of organ damage will also be tested.

1672 We will also consider frequency of rejection in cases of recurrent rejection. If there are  
1673 enough cases of recurrent rejection, we will compare LDLT with DDLT using a multiple  
1674 event Cox regression. Severity of rejection in LDLT versus DDLT will also be  
1675 considered, restricted only to cases with rejection.

1676 **4.9. Secondary Aim 5: To establish a robust data and sample repository on liver**  
1677 **transplantation that will be used to study clinical and biological questions as**  
1678 **new technologies and resources become available.**

1679 **4.9.1. Study Methods**

1680  
1681 The NIDDK Central Repositories are three separate contract-funded components that  
1682 work together to store data and samples from significant NIDDK-funded studies. One  
1683 component is the Biosample Repository, which will gather, store and distribute biological  
1684 samples from studies. The second component is the Genetics Repository, which will  
1685 receive and process blood samples to allow genetic analyses. The Genetics Repository  
1686 will create immortalized cell lines or cryopreserve nucleated cells for future  
1687 immortalization, and prepare DNA from one or both of these sources. The third  
1688 component is a Database Repository that will gather, store and distribute the incremental  
1689 or finished datasets from studies.

1690  
1691 The NIDDK conducts and supports much of the clinical research on the diseases of  
1692 internal medicine and related subspecialty fields. Many of the large clinical studies  
1693 funded by the NIDDK collect biospecimens from subjects for analysis and store the  
1694 samples for future study in a study-specific repository. The samples from the A2ALL  
1695 Cohort Study will be collected over several years from carefully chosen subjects and are  
1696 present in a finite quantity. Each sample is unique and cannot be replaced if lost,  
1697 damaged, or contaminated. Therefore, it is essential that the samples be stored under  
1698 optimal conditions, which vary from sample type to sample type. To this end, the NIDDK  
1699 has established a Biosample Repository.

1700  
1701 Discovery of disease related genes requires a population of individuals with the genetic  
1702 variant, as well as a population of control (unaffected) individuals. Thus, a repository of  
1703 DNA samples, immortalized cell lines, and accompanying clinical and pedigree data is  
1704 clearly an invaluable resource for the research community studying liver disease and  
1705 issues relating to transplant such as regeneration, immunology, HCC and HCV. The  
1706 NIDDK has established a Genetics Repository for DNA samples for the study of the  
1707 impact of genetics on disease.

1708  
1709 In addition to the Biosample and Genetics Repositories, the NIDDK has established a  
1710 Data Repository that will store, maintain, perform quality control assessments, and  
1711 distribute data related to the studies storing materials at the Biosample and Genetics  
1712 Repositories. The Data Repository will foster the development of highly usable public  
1713 data sets, thus allowing re-analysis of these data and, where relevant, of specific  
1714 biosamples, thus optimizing use of study data and samples.

1715  
1716 The collection of patient and control biosamples and DNA samples from this and other  
1717 studies for storage in the Biosample, Genetics and Data Repositories has the potential to  
1718 become a resource with which researchers can rapidly validate clinical hypotheses and  
1719 algorithms for clinical decision. The collections will also advance the development of  
1720 prognostics, markers, and therapeutics. To date, no such collection has been available to  
1721 the investigators interested in studying liver disease and transplant issues. The

1722 repositories will allow storage, maintenance, and quality control, and equitable, ethical  
1723 distribution of biosamples and other resources important to the study of liver transplant.  
1724 This will allow sharing of resources, thus encouraging work by junior investigators,  
1725 investigators with novel approaches, and others not included in current collaborations,  
1726 without excluding those who are established in their fields. In addition, the Genetics  
1727 Repository may increase the sample size and the resulting power of a study to identify  
1728 genetic determinants of a disease. It will ensure that research participants will be making  
1729 a maximal contribution, and will decrease duplicative sampling efforts. A2ALL is  
1730 committed to sharing the resources collected in this study with current and future  
1731 researchers via the use of the NIDDK repositories.  
1732

#### 1733 **4.9.2. Participant Selection**

1734 All eligible Cohort Study subjects will be presented with information and approached for  
1735 consent to have their biosamples, genetic material and non-identified data stored in the  
1736 NIDDK repositories for future study.

#### 1737 **4.9.3. Data Elements**

##### 1738 **Sample Repository and Genetics Repository**

1739 Specimen collection:

1740 Collection of blood and tissue for immediate and/or future analysis of mechanistic issues  
1741 in LDLT immunobiology will be done as part of the cohort study, but will require  
1742 separate consent form(s), some of which will be developed in an investigator initiated  
1743 hypothesis-driven studies.  
1744

1745 The following is a preliminary recommendation for collection of blood and tissue from  
1746 LDLT and DDLT recipients and donors at specific intervals prior and after  
1747 transplantation. Some of the fresh specimens will be used for assays, whereas other will  
1748 be stored:  
1749

##### 1750 **Pre-transplant, Intraoperative and Post-operative**

1751  
1752 Donor and recipient whole blood for genetic repository (24 ml collected at enrollment for  
1753 recipients and on the day of donation surgery for the donor)

1754 Donor and recipient blood for biorepository (15 ml drawn at enrollment, day of surgery  
1755 and post-transplant day 1, weeks 1 and 2\*, months 1, 3, 6\*, 12 and yearly through month  
1756 60). Those subjects who have joined the study later in their transplant/donation  
1757 experience may have blood drawn for the repository at annual visits from Year 5 through  
1758 Year 10. However, because they will have entered the study after some collection  
1759 timepoints, the total amount of blood drawn from these subjects will be similar to or less  
1760 than that drawn from subjects who entered the study at the time of donor evaluation.  
1761

1762 \*Donors will not have blood drawn at week 2 or month 6. Total blood drawn during the  
1763 five-year study will be 219 ml for donors and a maximum of 304 ml for recipients.  
1764

1765  
1766 Additional collection of blood and tissue will be coordinated with other ancillary studies,  
1767 and correlated with liver function tests.



1768 **5. Human Subjects**

1769 **5.1. Protection of Human Subjects**

1770 **5.1.1. Institutional Review Board**

1771 This study and analysis will be performed under Institutional Review Board (IRB)  
1772 oversight. Prior to the initiation of the study, an IRB approval for study of human  
1773 subjects will be obtained separately from the IRB of each of the participating transplant  
1774 centers and the DCC. Revisions to the study protocol and changes in the study design  
1775 will also be submitted to the individual IRBs for approval prior to implementation.

1776  
1777 Patients will be enrolled in the cohort study with full informed consent which will include  
1778 the gathering of privileged health information (PHI), the collection of blood and tissue  
1779 specimens beyond that normally performed for transplant clinical care, and the collection  
1780 of medical and quality of life information at defined intervals prior to and after the  
1781 transplant in donors and recipients.

1782

1783 **5.1.2. Patient Confidentiality**

1784 Special procedures for ensuring patient confidentiality will be implemented. Data  
1785 transmission and the distributed data systems have multiple layers of security as  
1786 discussed below in the study management section. Each study subject will be assigned an  
1787 identification number. Only this number will be used to identify subjects in any  
1788 individual tabulation. The PHI that is collected will represent the minimal necessary to  
1789 successfully execute the study. Since this study plans to establish a link to the Scientific  
1790 Registry of Transplant Recipients (SRTR) database, to reduce the data burden on the  
1791 study coordinators, SRTR PX-ID numbers will be collected. The PX-ID does not include  
1792 PHI. The only PHI that will be entered into the electronic data system is date of birth. It  
1793 is expected that only group data will be published. If individual subject data are to be  
1794 published, no identifying information will be included. The study files will be maintained  
1795 in a secure location as described above. Access to computerized data will be restricted to  
1796 study personnel. Password authorization will be enforced. Previous use of this security  
1797 system and secured server indicates that this technique is very successful in assuring the  
1798 protection of confidential information.

1799

1800 Authorized representatives of the Sponsor, the National Institute of Diabetes, Digestive  
1801 and Kidney Diseases (NIDDK), National Institute of Health (NIH), participating clinical  
1802 institution, DCC monitoring staff, as well as the IRB, have access to medical records and  
1803 records from participation in this study. Such access is necessary to ensure the accuracy  
1804 of the findings.

1805 **5.1.3. Risks to the Patient**

1806 Patients enrolled in this study will experience more than the normal amount of testing  
1807 which is customary for this complicated medical and surgical procedure. Additional time  
1808 will be required both before and after the transplant for the gathering of medical and  
1809 quality of life information. Blood will be collected and stored for special tests which are

1810 not normally performed for this procedure. During and after the surgery liver biopsies  
1811 will be collected which add a small risk of bleeding.

#### 1812 **5.1.4. Unauthorized Data Release**

1813 The data sets will be stored on a secure server with restricted access (requires a unique  
1814 username and password) at the DCC and every precaution will be taken to keep the  
1815 information private. However, there is always the possibility of unauthorized release of  
1816 data about subjects. Such disclosure would be extremely unlikely to involve a threat to  
1817 life, health, or safety, since the only PHI that will be collected is date of birth. It is  
1818 conceivable that such disclosure could have psychological, social, or legal effects on the  
1819 patient. Using the standard security procedures (described above under patient  
1820 confidentiality) can effectively minimize the risk of unauthorized disclosure of data. All  
1821 study personnel who have access to patient data will be educated regarding the need to  
1822 protect confidentiality and the procedures to be followed to ensure such protection. All  
1823 staff will also be required to sign a standard medical record confidentiality agreement.  
1824 The computer system on which data are maintained uses standard password protection  
1825 procedures to limit access to authorized users.  
1826

#### 1827 **5.1.5 Adverse Event Monitoring and Reporting**

##### 1828 **Definition of Adverse event**

1829 An adverse event is any untoward medical occurrence or unfavorable and unintended  
1830 sign in a research subject that occurs during or as a result of research procedure.  
1831

1832 For this observational study, the majority of the procedures are standard clinical care and  
1833 adverse effects of clinical care will be tracked as complications but not be considered  
1834 adverse study events. Each center will review the list of study procedures and identify  
1835 the specific procedures that are NOT standard-of-care at their institution and these will be  
1836 considered research procedures. Complications that are a result of research procedures  
1837 will be reported and tracked as adverse events.  
1838

##### 1839 **Assessment of Adverse Event Severity and Relationship to Treatment**

1840 The modified World Health Organization (WHO) grading system will be used for  
1841 grading severity of AEs (Appendix D). For AEs not covered by the modified WHO  
1842 grading system, the following definitions will be used:  
1843  
1844

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

1845  
1846  
1847  
1848

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   |

1849

#### **Definition of Serious Adverse Events**

1850

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

1851

- 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.

1852

1853

1854

1855

1856

1857

1858

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.

1863

1864

#### **Reporting responsibility**

1865

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.

1866

1867

1868

1869

1870

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.

1876

1877

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

1878

1879

1880 within 7 working days of the investigator receiving the information; however, important  
1881 follow-up information must be submitted within 24 hours. All deaths connected to a  
1882 study procedures must be reported to the DCC within 24 hours of the investigator being  
1883 informed of the event.  
1884

## 1885 **5.2. Benefits to the Patients**

1886 There are no direct benefits to the patients for participation in the study.

## 1887 **5.3. Inclusion of Women**

1888 This is a multi-center study drawing on a clinical population from nine transplant  
1889 institutions across the United States. The demographics of the study population are pre-  
1890 determined due to the retrospective all-inclusive nature of the study. Women will be  
1891 included in the retrospective study as living liver donors and recipients. It is anticipated  
1892 that the representation of women will correspond to the fraction of females in the living  
1893 liver donor and recipient population.

## 1894 **5.4. Inclusion of Minorities**

1895 This is a multi-center study drawing on a clinical population from nine transplant  
1896 institutions across the United States. The demographics of the study population are pre-  
1897 determined due to the retrospective all-inclusive nature of the study. Racial and ethnic  
1898 minority groups will be included in the donor and recipient components of the  
1899 retrospective study and will be proportional to their representation in the donor and  
1900 recipient population.

## 1901 **5.5. Inclusion of Children**

1902 The Adult-to-adult living donor liver transplantation cohort study specifically excludes  
1903 children. By definition this study is designed to examine the risks, benefits and outcomes  
1904 of Adult-to-Adult living donor liver transplantation. Adult-to-Adult transplants have  
1905 only recently been performed and this study is an attempt to collect systematic data on  
1906 this procedure.

## 1907 **5.6. Data and Safety Monitoring Plan**

1908 Accepted principles of data and safety monitoring will be observed throughout the  
1909 conduct of the A2ALL cohort study. The NIH will appoint an independent Data Safety  
1910 and Monitoring Board (DSMB) that will provide study oversight. The DSMB will  
1911 approve the study protocol prior to enrollment and will also approve all subsequent  
1912 protocol revisions.

1913  
1914 Each transplant center principal investigator will be responsible for monitoring the  
1915 enrollment of subjects and submission of data to the DCC. The DCC will be responsible  
1916 for monitoring for effective conduct of the protocol and accurate and timely data  
1917 submission.

1918  
1919 IRBs will be provided feedback on a regular basis.  
1920

1921 Training of study coordinators and study monitoring activities will be conducted by the  
1922 DCC to ensure patient confidentiality and privacy and to maximize the reliability,  
1923 accuracy, and timeliness of study data.

## 1924 **6. Study Organization**

### 1925 **6.1. Clinical Transplant Centers**

1926 The participating Clinical Centers will have primary responsibility for developing the  
1927 study protocol, maintaining high rates of follow-up and data collection, obtaining data of  
1928 high quality, and interpreting, presenting, and publishing findings from the study.

- 1929
- 1930 1. Columbia University Health Sciences
- 1931 New York, NY
- 1932 Principal Investigator: Jean Emond, MD
- 1933 2. Northwestern University
- 1934 Chicago, IL
- 1935 Principal Investigator: Michael Abecassis, MD
- 1936 3. University of Pennsylvania
- 1937 Philadelphia, PA
- 1938 Principal Investigator: Abraham Shaked, MD
- 1939 4. University of Colorado Health Sciences
- 1940 Denver, CO
- 1941 Principal Investigator: James Trotter, MD
- 1942 5. University of California, Los Angeles
- 1943 Los Angeles, CA
- 1944 Principal Investigator: R. Mark Ghobrial, MD
- 1945 6. University of California, San Francisco
- 1946 San Francisco, CA
- 1947 Principal Investigator: Christopher Freise, MD
- 1948 7. University of North Carolina
- 1949 Chapel Hill, NC
- 1950 Principal Investigator: Jeff Fair, MD
- 1951 8. University of Virginia
- 1952 Charlottesville, VA
- 1953 Principal Investigator: Carl Berg, MD
- 1954 9. Virginia Commonwealth University
- 1955 Richmond, VA
- 1956 Principal Investigator: Robert Fisher, MD

### 1957 **6.2. Data Coordinating Center**

1958 The Data Coordinating Center (DCC) contributes content area expertise and shares in  
1959 scientific leadership of the research group. The DCC has developed a communication  
1960 infrastructure that includes meetings, teleconferences, electronic mail and bulletins,  
1961 interactive web-based encounters and written correspondence. The DCC assists in  
1962 protocol development and preparation of scientific publications. The DCC has the major  
1963 responsibility of creating a database and data collection systems for the transplant  
1964 centers, ongoing evaluation of data quality and performance monitoring of the transplant  
1965 centers and statistical analyses of the data. The DCC will also create a comprehensive

1966 Manual of Operations (MOO) that will govern the conduct of the study. The manual will  
1967 detail the protocols, protocol clarifications and amendments, summary of the regulatory  
1968 requirements for the study, instructions for enrollment, data collection, data management,  
1969 visit schedules and detailed instructions on the use of the electronic data submission.  
1970

1971 University of Michigan  
1972 Ann Arbor, MI  
1973 Principal Investigator: Robert M. Merion, MD

### 1974 **6.3. Steering Committee**

1975 The primary governing body of the study is the Steering Committee, comprised of each  
1976 of the Principal Investigators of the transplant centers, the Principal Investigator of the  
1977 DCC and the NIDDK Project Scientist. The Steering Committee develops policies for  
1978 the study pertaining to access to patient data and specimens, ancillary studies,  
1979 performance standards, and publications and presentations. They develop the study  
1980 protocol and meet to discuss the progress of the study and to consider problems arising  
1981 during its conduct. The Steering Committee may establish subcommittees to further  
1982 develop specific components of the study protocol and propose ancillary areas of study.  
1983 Small working groups may be established to prepare manuscripts and presentations.

### 1984 **6.4. Cohort Study Subcommittees**

1985 The following subcommittees have been established to address specific issues in the  
1986 Cohort study.

- 1987 • Cohort Study Protocol Design
- 1988 • Hepatitis C Virus (HCV) Workgroup
- 1989 • Hepatocellular Carcinoma (HCC) Workgroup
- 1990 • Regeneration and Function Workgroup
- 1991 • Clinical Immunology Workgroup
- 1992 • Outcomes/Endpoints/Definitions Workgroup
- 1993 • Informed Consent Workgroup
- 1994 • QOL workgroup
- 1995 • Publications

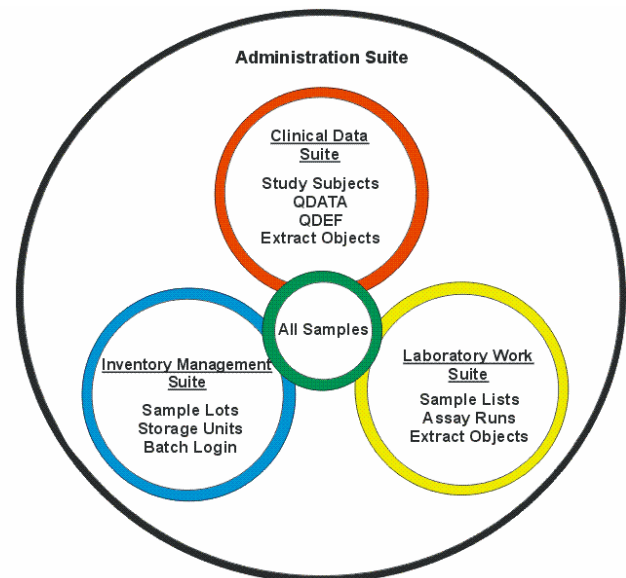
1996  
1997 Other possible subcommittees include:

- 1998 • A2ALL Study Policies
- 1999 • Ancillary Study Policy
- 2000 • Access to Study Data
- 2001 • Others as required

## 2002 **7. Study Management**

### 2003 **7.1. Data collection, Data Collection** 2004 **Forms, and Data Entry – BioDBx**

2005 The DCC will utilize the web-based BioDBx  
2006 program as the data management nucleus for  
2007 the A2ALL studies.



2008 This system was developed specifically for multicenter clinical trials management at the  
2009 University of Michigan. Briefly, BioDBx is a highly flexible database application that  
2010 allows investigators to organize their research operations and perform common actions  
2011 on research data within a single database. There are three main suites: the Clinical Data  
2012 Suite, which manages clinical data, the Inventory Management Suite, which manages  
2013 inventory such as acquired specimens, and the Laboratory Workspace, which manages  
2014 laboratory operations. An Administrative Suite is the overall manager for the foregoing  
2015 three suites.

2016  
2017 The Clinical Data Suite manages clinical data within a defined hierarchy, the highest  
2018 level of which is termed Active Studies. From Active Studies, study subjects and study  
2019 data are managed and clinical data can be viewed. There is a QDef module where study  
2020 set-up occurs and a QData module where clinical data are entered after being defined by  
2021 QDef. The Study Subjects module is used to enter and manage demographic data for  
2022 study participants and the Study Samples module allows entry and edit of data for  
2023 samples obtained from the subjects in the study.

2024  
2025 The primary mechanism by which a study is set up in BioDBx is through a four-  
2026 component QDef (Question Definition) module. The four components are: 1) definition,  
2027 2) validation, 3) extraction, and 4) navigation. Definition functions to determine where  
2028 and why a variable or question appears. Validation determines acceptable values for a  
2029 variable or acceptable answers to a question. Extraction defines where the data from a  
2030 particular element will go for statistical analysis. Navigation is a characteristic that  
2031 determines what data element is requested next.

2032  
2033 QData is the module within the Clinical Data Suite used to enter and edit data from Case  
2034 Report Forms or study questionnaires. QData can only be utilized for a given study when  
2035 the entire battery of questions for a study have been defined (in QDef) and tested. For the  
2036 complement of data defined, QData prepares individual data entry screens for users to  
2037 key responses for specific study subjects. After responses have been entered, they can be  
2038 printed and reviewed or extracted into extract objects for statistical analysis.

2039  
2040 The DCC will utilize the BioDBx QDef module to create electronic case report forms to  
2041 capture all relevant study data for the main A2ALL cohort study, the study of previously  
2042 transplanted A2ALL recipients, the A2ALL donor study, and all investigational/research  
2043 protocols that are developed and implemented during the course of the study. The  
2044 BioDBx system allows real-time monitoring of study data for protocol adherence, quality  
2045 assurance, adverse event reporting, discrepancy reporting, and other trends.

## 2046 **7.2. Data Management**

2047 All study data will be entered into the BioDBx electronic data entry system by study  
2048 coordinators at each study site. This data will be encrypted and transferred to the DCC  
2049 and stored on a secure server at the University of Michigan. Access to the server and  
2050 BioDBx system is limited and requires a unique username and password combination.  
2051 The servers are backed up daily and physically stored in a locked facility.

2052  
2053 All analysis of the data sets will utilize de-identified (coded) data sets.

### 2054 **7.3. Quality Control and Database Management**

2055 The first steps in ensuring protocol compliance are good protocol design and careful  
2056 orientation of study personnel. Following final agreement on protocols, and prior to  
2057 study initiation at any of the transplant centers, the DCC will organize a Training and  
2058 Certification session for transplant center study coordinators/data entry personnel.  
2059

2060 The BioDBx electronic data entry system will have built-in data checks as part of study  
2061 quality assurance. Protocol compliance will be assessed by monitoring the submission of  
2062 data at required intervals. Data inconsistencies and discrepancy reports will be reviewed  
2063 by the Clinical Monitor so that necessary queries can be generated and sent to the  
2064 transplant center study sites for verification and resolution.  
2065

2066 Periodic requests may be generated for the submission of random source documents to  
2067 assess the quality of data acquisition and data entry at each site. In addition, the Clinical  
2068 Monitor or Project Manager will visit each site at least once to review source documents,  
2069 monitor regulatory compliance, and assess protocol adherence.  
2070

2071 In addition to source document verification, the Clinical Monitor and Project Manager  
2072 will produce reports from the BioDBx system to look for inconsistencies in submitted  
2073 data, particularly for repeated measures data elements, even if data do not fall outside of  
2074 built-in validation routines.  
2075

2076 Studies of intra-subject and inter-subject data variability by transplant center as well as  
2077 intra-transplant center and inter-transplant center data variability will be used to further  
2078 ascertain random or systematic data quality issues.  
2079

2080 Comparisons of major endpoints from the current study to national data from the SRTR  
2081 will be used to assess the extent to which participants in the A2ALL study are  
2082 representative of the general population of patients undergoing these procedures in the  
2083 United States.

### 2084 **7.4. Data Security/Data Transfer**

2085 Personnel at each study center will collect and enter data into BioDBx, a web-based data  
2086 entry system. Authentication is currently enabled from Oracle Developer Form Server to  
2087 the Oracle database. Between the web server and client browser, secure socket layer  
2088 technology is in place. This will ensure safety and confidentiality of data by using secure  
2089 encrypted data transmission from the transplant centers to the BioDBx database server.  
2090

2091 The database logs every modification of every cell in the database to ensure the ability to  
2092 monitor access to the data and audit transactions. The system is accessible only via an  
2093 established account with a logon and password for security and confidentiality.

2094 Authorized study personnel will be assigned an account on the system.  
2095

2096 The BioDBx database server is located in a locked cabinet in a locked room at the  
2097 University of Michigan Medical Center. The hardware administrator and his designated  
2098 backup are the only individuals who have keys. The data will be transferred via the  
2099 secure network to the Kidney Epidemiology Cost Center (KECC) at the University of



2100 Michigan. The A2ALL project staff is physically located in the KECC office suite. The  
2101 office suite is kept locked with entry control 24 hours a day to prohibit unauthorized  
2102 entry.

2103  
2104 The computer system at KECC currently is used for research projects that involve  
2105 processing large volumes of identified and re-identifiable patient-specific data. The  
2106 KECC system has a comprehensive security plan based on the guidelines in OMB  
2107 Circular A-130, "Security of Federal Automated Information Resources" and NIST  
2108 Publication 800-18 "Guide for Developing Security Plans for Information Technology  
2109 Systems." This plan has undergone extensive review by HRSA for security certification  
2110 for maintaining patient-identified data. The A2ALL project will be covered by this  
2111 security plan and will be required to comply.

## 2112 **8. Electronic Data Submission**

2113 BioDBx will be utilized for electronic submission of data for this study. Detailed  
2114 instructions on the use of BioDBx, data element definitions and a code list will be  
2115 provided in a Manual Of Operations (MOO). Each study site will be provided a copy of  
2116 the MOO and the entire manual will be available on the study web site.

2117 References

2118

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2135

2136 **APPENDICES**

2137 **Appendix A: Schedule of Recipient Visits, Tests and Assessments**

Visit Type	Pre Transplant Period	Transplant Hospitalization	Post Transplant Follow-up Period											
	Quarterly from Enrollment	Transplant OP Day 0	PTD1	PTW1	PTW2	PTM1	PTM3	PTM6	PTM12	PTM18	PTM24	PTM36	PTM48	PTM60
<b>Recipients</b>														
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							
LFTs	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

2138

2139 **Appendix B: Schedule of Donor Visits, Tests and Assessments**

Visit Type	Pre-Donation Period	Donation Hospitalization	Post Donation Follow-up Period										
	Enrollment	Donation OP Day 0	PDD1	PDW1	PDM1	PDM3	PDM6	PDM12	PDM18	PDM24	PDM36	PDM48	PDM60
<b>Donors*</b>													
Donor liver biopsy		ALL											
HLA Typing		ALL											
Liver MRI	ALL					ALL							
LFTS	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												
<b>QOL Assessments</b>	ALL			McGill Pain Survey Only	McGill Pain Survey Only	ALL		ALL		ALL	ALL	ALL	ALL
<b>Informed Consent Assessments</b>	ALL					ALL		ALL		ALL	ALL	ALL	ALL

2140 \*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  
 2141 for data collection, QOL and informed consent assessments at the visits listed in the above schedule