

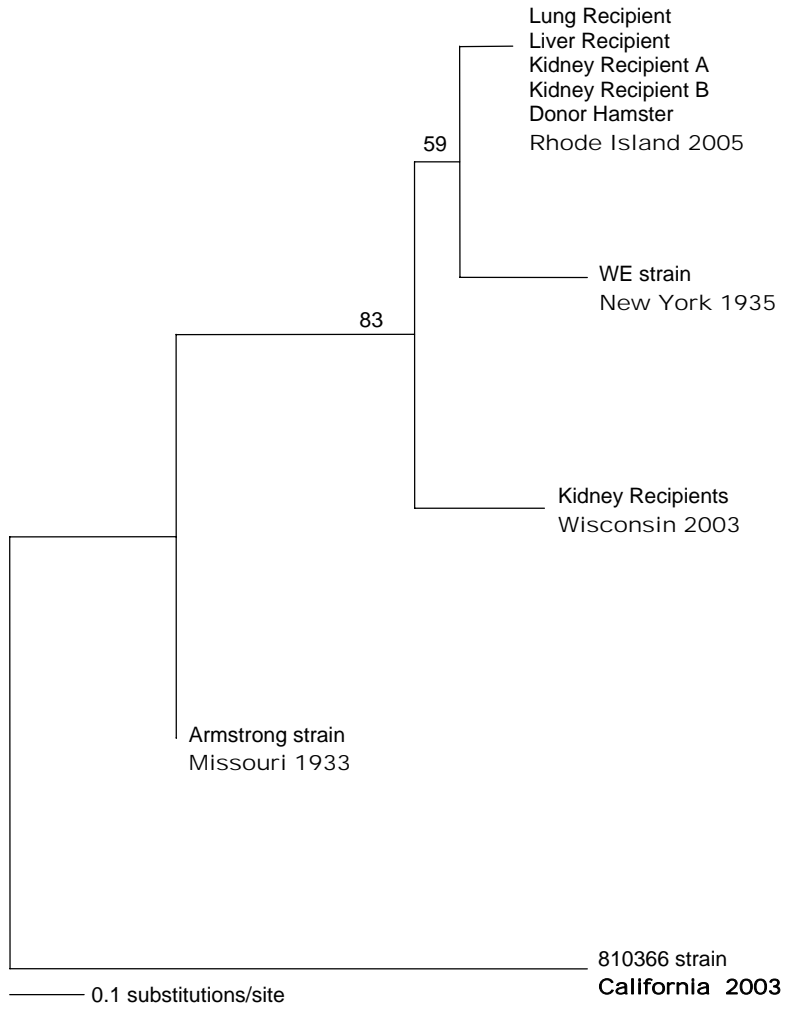
## Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Fischer SA, Graham MB, Kuehnert MJ, et al. Transmission of lymphocytic choriomeningitis virus by organ transplantation. *N Engl J Med* 2006;354:2235-49.

**Supp Figure 1. Phylogenetic relationship of LCMV isolated in 2003 and 2005 transplant recipient clusters relative to previously characterized virus strains.** Total RNA from the samples was isolated using ABI 6100 RNA extraction protocol with slight modification. In brief, 50- $\mu$ l samples (blood, sera, tissue homogenate, or tissue culture supernatant) were added to 300  $\mu$ l of 2X non-cellular lysis buffer (Applied Biosystems). Samples were diluted to 1X with water containing 20  $\mu$ g of poly-A (Sigma) and allowed to sit 1 hour at room temperature to facilitate poly A-RNA binding. RNA was then extracted, using the 6100 Nucleic Acid Preparation Station (Applied Biosystems) according to the manufacturer's protocol with minor modifications. After the final wash, RNA was eluted by using 150  $\mu$ l of elution buffer. For RT-PCR detection of LCMV, cDNA was synthesized at 42°C for 1 hour, using an Access RT-PCR Kit (Promega) and L gene primer Arena 4160F (5'GCA GAR TTY AAA TCI AGA TT). Resulting cDNA was then amplified by PCR using FastStart PCR (Roche) and the Arena L gene primers 4160F and 4393R (5'CCR TYI ASC CAR TCT ITI ACA TC). Amplification was carried out by denaturation at 95°C for 2 minutes, followed by 36 cycles of 1 minute each at 95°C, 45°C (annealing), and 72°C (extension) and with a final extension at 72°C for 10 minutes. Product DNA was resolved on 1 percent agarose gels and visualized using ethidium bromide staining. The DNA fragments of appropriate size (233 nt) were excised and sequenced using the primers originally used for amplification. Sequences obtained from various samples were analyzed using the Sequencher 4.1 program (Gene Codes Corp.). Phylogenetic relatedness of LCMV strains was estimated by maximum likelihood analysis of nucleotide sequence differences, using the Wisconcin Package Version 10.3. (Accelrys) and PAUP (Sinauer Associates) software packages. GenBank accession numbers for the LCMV strains include Armstrong (J04331), WE (AF004519), 810366 (DQ182707), transplant recipients from Wisconsin donor (DQ182706), transplant recipients from Rhode Island donor (DQ182703).

Figure 5



**Supp Figure 2. Clinical course and treatment of LCMV infection in  
surviving renal transplant recipient, 2005 cluster.**

Abbreviations: IV = intravenous; RT-PCR = reverse transcriptase-polymerase  
chain reaction for LCMV; IHC = immunohistochemical staining for LCMV.

Diarrhea

Allograft pain, abdominal wall erythema

Fever (temperature > 100.3 ° F)

Thymoglobulin

Ribavirin  
IV oral IV oral

Mycophenolate mofetil

Tacrolimus

Prednisone (10 mg daily)

Urine (RT-PCR + culture)

Blood (RT-PCR + culture)

Tissue, kidney (IHC)

Tissue, colon (IHC)

Serum IgM

+

+

-

+

+

+

+

+

-

-

+

-

+

-

-

-

-

-

+

0

12

24

Days post transplant

48

60

72

**Supp Table 1. Clinical and laboratory findings in organ transplant recipients with LCMV infection, 2003 and 2005 clusters.**

	<b>Wisconsin and Minnesota, 2003</b>				<b>Rhode Island and Massachusetts, 2005</b>			
	<b>Lung</b>	<b>Liver</b>	<b>Kidney 1</b>	<b>Kidney 2</b>	<b>Lung</b>	<b>Liver</b>	<b>Kidney A</b>	<b>Kidney B</b>
Fever	+	+	+	+	-	+	+	+
Altered mental status	-	+	+	+	+	+	+	+
Nuchal rigidity	-	-	-	+	-	-	-	-
Diarrhea	-	-	+	+	+	-	+	+
Abdominal pain	-	-	+	+	+	+	+	+
Rash*	-	+	+	+	+	-	+	+
Hypotension	+	+	-	-	-	+	-	-
Seizure activity	-	-	+	+	-	+	-	-
Arrhythmia	-	-	-	+	-	+	+	-
Renal failure	+	-	-	+	+	+	-	-
Hypoxia	+	-	-	-	+	+	+	+
Pulmonary infiltrates	+	+	-	-	+	-	-	-
Respiratory failure	+	+	+	+	+	+	-	-
WBC count	↑	↓	↓	↓	↑	↓	normal	↑
Thrombocytopenia	+	+	+	+	+	+	+	+
↑ AST/ALT	-	+	+	+	+	+	+	+
↑ PT	-	+	+	-	+	+	+	+
Outcome (Day post-transplant)	Death (9)	Death (17)	Death (53)	Death (76)	Death (23)	Death (26)	Alive	Death (23)

\* manifestations included peri-incisional erythema , petechial eruption, diffuse erythroderma, and pustular rash