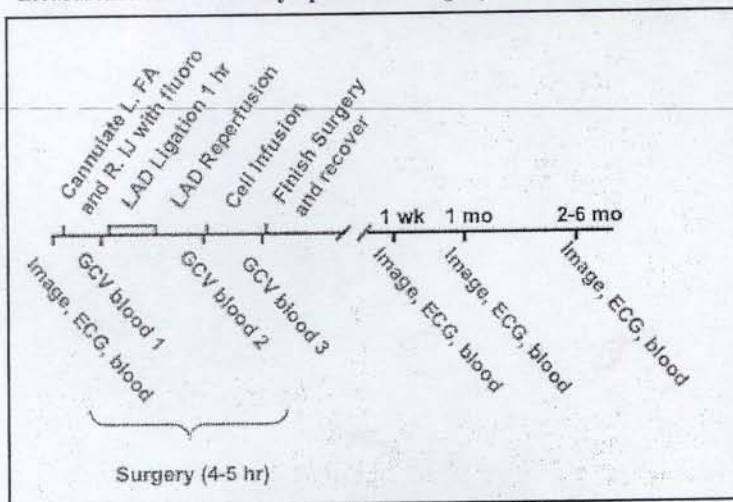


## 17. Experimental Protocol

- a) In this section describe your experimental protocols, outside of normal husbandry, to be performed on the animals. **This response should provide the committee with a clear understanding of what specifically happens sequentially to each animal or group of animals and over what time period.** It is not necessary to repeat the surgical description that is provided in question 28, but the timing of the surgery within the experiment should be indicated. Be sure to include: all drugs given, including dosage range, routes and frequency of administration; nutritional intervention; social or environmental manipulation; method and amount of biological samples taken; methods of antibody production; use of radioactive materials, blood or other fluid sampling including method and amount, etc. Specify the expected sequence, frequency and duration of these procedures. **If this protocol is to cover an animal colony, use this section to detail breeding procedures/methods.** (Append additional page(s) if necessary)

Adult male and female rhesus monkeys (age 15-22 year old) will be divided into 3 study groups of 4 animals each: control MI; MI with undifferentiated ES cells; MI with advanced primed ES cells. Prior to starting the surgery, the animals will undergo noninvasive cardiac imaging with MRI and/or echocardiography as described below for baseline measurements. ~~Two days prior to surgery the animals will be administered aspirin at a dose of 81 mg orally daily~~



**which will be continued for two weeks post-surgery to decrease the risk of thrombotic complications.** On the day of surgery, animals will be transported in a standard transport cage using the Primate Center van to the CSC, H6/385, Animal CV Cath Lab. The transport cage will be covered with surgical drape and the route of transport in CSC will avoid contact with the public as approved by LAR. The animals will then be subjected to myocardial infarction under general anesthesia using a surgical ligation of the mid left anterior descending (LAD) coronary artery (see surgery details in questions 28&29). A central venous catheter is placed via the right internal jugular into the coronary sinus to allow sampling of the great cardiac vein (GCV) at time points indicated and a second catheter is placed into the femoral artery to allow coronary angiography and ultimately cell delivery to the LAD bed. Following 1 hour for full

infarction to occur, the LAD will be reperfused. After 1 hour of reperfusion, a guide catheter will be advanced from the femoral artery insertion site to the left main coronary artery and coronary angiography will be performed to verify reperfusion of the LAD bed. Using a guidewire, the cell-delivery catheter will be advanced to the mid LAD. Primed ES cells or culture medium (sham control) will be injected in a volume of 10 ml over 10 minutes at a concentration of  $2 \times 10^6$  cells per ml for a total of  $2 \times 10^7$  transplanted cells.

Sampling of the great cardiac vein (GCV) providing venous drainage of the LAD infarct bed will be performed. Three GCV venous samples of 5 ml each (15 ml total) will be obtained: 1) before LAD ligation; 2) one hour after reperfusion prior to cell delivery; 3) during the last minute of cell delivery. Serum will be obtained from the samples and biochemical measurements of cardiac injury (CPK-MB and cTnI) will be made with the remainder of the serum frozen for future analysis. The sample during cell infusion will be collected in an EDTA tube, and then subjected to FACs sorting to determine the effluent concentration of GFP positive cells. This will give an indication of initial engraftment of the transplanted cells. Additionally, a 12 lead electrocardiograms will be performed prior to surgery, immediately after surgery and in follow-up to have another noninvasive tool to examine infarct size.

Post-operative recovery for the animals will be closely monitored as described in question 29. After the initial 5-day postoperative recovery, the animals will be monitored at least daily. Observation of the animals' activity level, breathing rate and pattern, peripheral edema, signs of pains (e.g. facial grimace, limited movement), and eating pattern will be made. We anticipate that all animals will recover from the surgery and controlled myocardial infarction. Based on results in other animal myocardial infarction models and clinical studies, it is anticipated that the large myocardial infarction will result in progressive ventricular remodeling over the following six months with the greatest changes occurring in the first two months. The resulting cardiomyopathy will make the animals prone to heart failure which in its milder forms may be manifest by exercise intolerance and exertional dyspnea. It is possible that some animals will develop more severe heart failure which will be treated with diuretics (furosemide) as described in Question 20.



At three time points (1 wk, 1 mo, 2-6 mo) following surgery, the animals will undergo noninvasive MRI and/ or echocardiographic imaging of the heart. This imaging will be used to determine cardiac chamber size and function as well as to examine for transplanted ES cells. Echocardiography will be performed on ketamine sedated (15 mg/kg IM) animals at the Primate Center. Animals will be transported to the CSC for this imaging in the research MRI scanner when MRIs are done. To enable this imaging, animals will be sedated with ketamine, 15 mg/kg IM, intubated and ventilated, then placed under general anesthesia using isoflurane inhalant 0.6-1.5% depending on eye, jaw, and pedal reflexes. During this period of anesthesia, routine blood samples will be obtained using standard phlebotomy techniques from the antecubital vein to obtain 5 ml of blood. These blood samples will measure electrolytes, CSA level, complete blood count, as well as serum markers for heart failure including brain natriuretic peptide (BNP). A simple twelve lead electrocardiograms will also be obtained.

After obtaining the 2-6 month data, the animals will be sacrificed and undergo complete autopsy at the Primate Center. Cardiac pathology evaluation will include a variety of immunohistochemistry studies to examine for regenerated myocardium and GFP positive transplanted cells.

The protocol is amended to include 2 additional pilot animals to allow optimization of the surgical procedure. The first animal will be sacrificed at the end of the surgical procedure, and the second animal will be allowed to recover for 48 hr before sacrifice to optimize post-surgical monitoring. The protocol is also amended to provide an alternative manner of administration of immunosuppression to prevent rejection of the allografted cells. As experience of others has suggested difficulty in rhesus monkeys routinely taking oral cyclosporin A, we plan to use daily IV injection of cyclosporin (Sandimmune 50mg/ml solution) at a daily dosage of 10 mg/kg for the duration of the study (up to 6 months). The animals may be sedated with ketamine 10 mg/kg IM to allow IV access to be obtained. The protocol is further amended to make cyclosporin dosing optional at this stage given recent data suggesting that it may not be necessary in mouse models.

b) Do any animals undergo any type of restraint beyond normal housing methods? **NO** If YES, indicate method, length of restraint, and justification for such restraint. If the design of the study requires continuous restraint for longer than 12 hours without the opportunity for exercise, be sure the justification addresses need for such an extended period and include the maximum length of time the animals will be restrained. Include any plans for providing additional enrichment and any steps taken to avoid physical discomfort during the restraint. (See Campus Policy on Non-human Primate Chairing if applicable - available on the web at: [www.rarc.wisc.edu](http://www.rarc.wisc.edu))

c) Are any animals subjected to fluid or food restriction? **YES** If YES, discuss level of restriction, expected consequences, and justification for such restrictions

Food will be withheld overnight and water for two hours prior to surgery. No consequences anticipated.

d) Will any animals require nonstandard husbandry exemption (e.g. exercise exemption, extended cage cleaning periods, etc.) **Yes** If YES, indicated nonstandard husbandry required and justification for this practice.

Animals will be housed individually for the duration of the study. The need for individual housing is due to the fact that some monkeys could develop heart failure and be unable to function in a normal non-human primate social setting.