Appendix 1
Statistical analysis and power considerations
    The primary end point is a combined end point of prevention of cortical interstitial expansion and graft loss from IF/TA. We hypothesize that Angiotensin II receptor blockade with losartan will reduce the percentage of patients who double their cortical interstitial volume or lose their graft from biopsy IF/TA by 60% in a 5-year prospective study. The only information available regarding the effect of RAAS blockade on rates of expansion in the interstitial compartment comes from Cordonnier et al (5).In this study an ACE inhibitor given for two years to proteinuric type 2 diabetic patients completely prevented expansion of the interstitial compartment. Therefore, our estimation of a reduction in the combined endpoint of prevention of interstitial expansion and graft loss from IF/TA over an even a longer time period (5 years) by 60% is rather conservative.

    In addition to the estimated difference between the two groups the following were also considered in estimating the sample size:

1.) Based on data in diabetic transplant recipients there is a progressive expansion in the cortical interstitial volume over a 5 year period, more specifically, 60% of participants in this cohort had at least doubling of their cortical interstitial volume in 5 years. (12)

2.) Twenty percent of patients lost their grafts in the first 5 years of transplantation; 18% of these graft losses were due to biopsy proven IF/TA.

Based on the above, the control group rate for the combined endpoints is = $(.18)(.20)+(.6)(.75) \cong 50\%$. Thus, we expect the losartan group to have 40 % the rate of the control group $\cong 20\%$. With 58 participants in each group, the study will have 90% power to detect this difference with a two-sided Fisher's Exact test at $\alpha = 0.05$.

        Other sample size considerations: In the largest study of losartan in transplant recipients the discontinuation rate due to side effects was 4%. Drop outs may also occur for other reasons. Based on our experience with trials of similar design, a 10% drop out rate is projected. This will inflate the total sample size to 129 patients. Reviewing our transplant population survival data, we found that the proportion that survives 5 years is 96.5%. Thus the total sample size is, further, inflated by 6 to account for these deaths. Given these considerations, in order to maintain 90% power, the final sample size was determined to be 68 participants/group (136 total).

The DSMB, at year 2 of the study, recommended increasing the sample size to 154 subjects considering that the number of inadequate baseline biopsies exceeded expectations and due to the fact that many subjects were prescribed an ACE inhibitor. The table and graph below also depict the power situation as originally planned, after DSMB recommendation to inflate sample size and the achieved enrollment.

Patients Per Group	Power	Description
45	80.4%	Enrollment needed for 80% power
58	90.3%	Enrollment needed for 90% power
68	94.5%	Enrollment after initial inflation due to projected dropout
76	96.6%	Final enrollment after second inflation due to higher than expected baseline biopsies (DSMB recommendation)
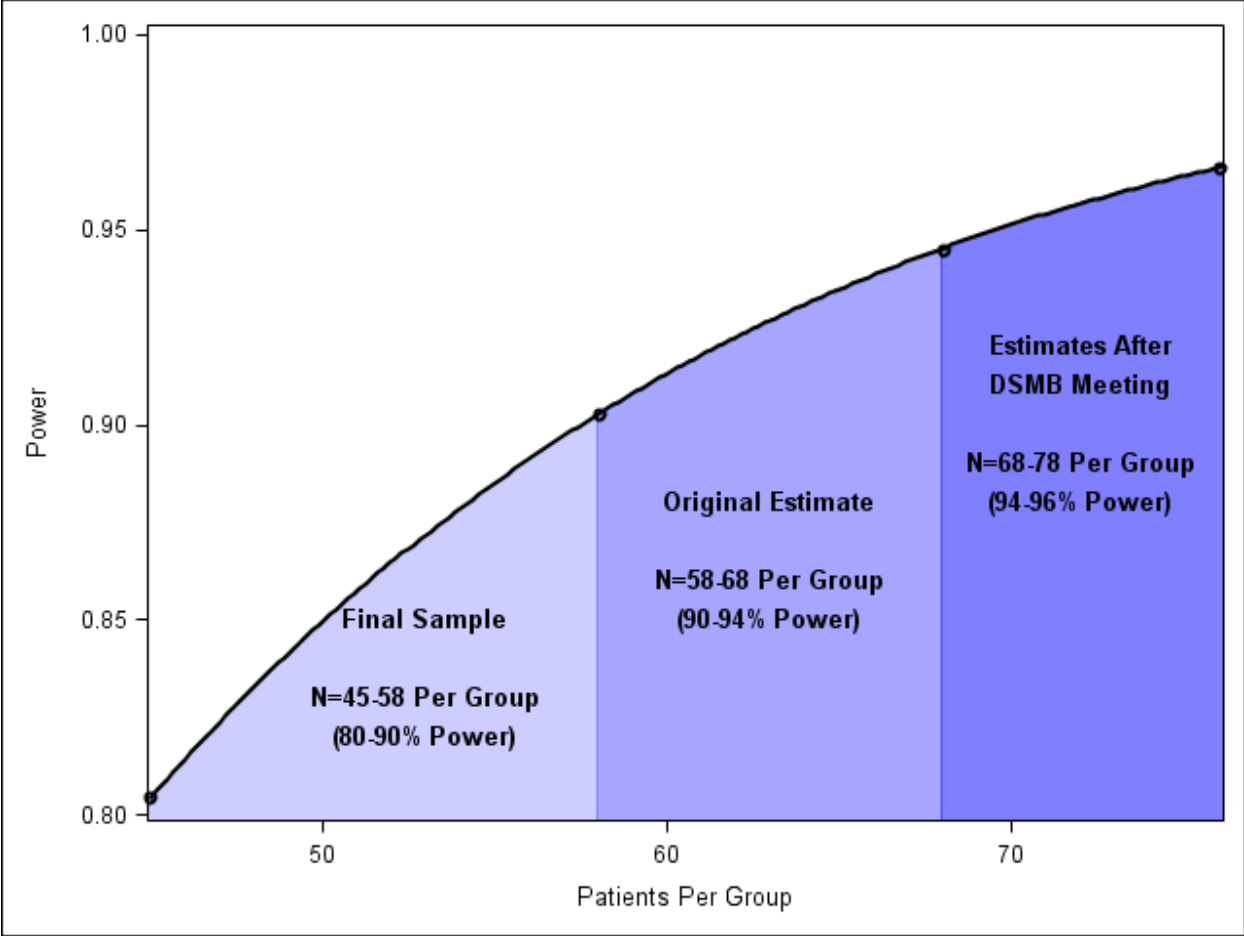
APPENDIX 2: Measurement of cortical interstitial volume; the primary endpoint

Evaluation of the adequacy of specimens:

1.   Objective: To determine if there is enough renal cortex without artifact available in a biopsy for Vv(Int/C) estimation.

2.   Method: An arbitrary slide from the stack of serial sections which is not folded and contains an almost full face of all available biopsy fragments is examined with a light microscope with 20X objective lens.

Sampling slides and sections:

1.   Objective: To sample sequential sections 100 μm apart; the average diameter of the glomerulus. This interval between sequential sections minimizes the effect of glomerulosclerosis on Vv(Int/C) at multiple levels and reduces the between-sections dependency of Vv(Int/C).

2. Method:

- Each slide has 4 sections. One section from slide #1 is selected.

- Every 5th slide from the stack of serial sections (5 μm thick) is then sampled to the end of the stack.

Digital imaging:

1.   Objective: To obtain sequential non-overlapping digital images of the sampled sections.

2.   Method: Digital images of non-overlapping fields from cortex of each entire selected section are obtained at 25x objective magnification, using a Spot digital camera and Spot advanced software.

Estimation of Vv(Int/C), using point counting:

1. Sampling:

- Objective: To count about $\geq$60 systematically and uniformly distributed fine points on interstitium of each biopsy. Since the size of the available cortical tissue is variable in each biopsy, the fraction of cortex that is sampled can increase by a factor of 4 without losing the principle of systematic and uniform sampling.

- Rounds: Point counting is performed in up to 4 sequential rounds.

Round #1:   Sequential odd images, the right ½ of the grid P4-4 on odd numbered sections and the left ½ of the grid on even numbered sections. Images from each section are recognizable by annotations on the start and end image of sections.

Round #2:   Sequential even images, the right ½ of the grid on odd numbered sections and the left ½ of the grid on even numbered sections.

Round #3:   Sequential odd images, the left ½ of the grid on odd numbered sections and the right ½ of the grid on even numbered sections.

Round #4:   Sequential even images, the left ½ of the grid on odd numbered sections and the right ½ of the grid on even numbered sections.

Schematic demonstration of sampling rounds: