Athymic Rat Reduced Kidney Mass Model: A Screening Tool to Identify Human-Sourced Cell-Based Therapeutic Prototypes for Regeneration and Stabilization of Kidney Function

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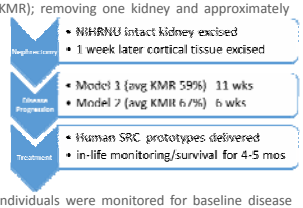
Background

Elucidating multi-modal mechanisms of action and efficacy of human-sourced autologous cell based therapeutic candidates is supported by availability of short-term preclinical models of kidney disease. The NIHRNU rat is permissive to xenogeneic transplant of human cells and Reduced Kidney Mass (RKM) models in rodents are used to identify potential CKD therapies. Selected Renal Cells (SRC) have been identified as an autologously-sourced therapeutic for CKD patients. Autologous application of rodent SRC to rodent nephrectomy and diabetic ZSF1 models have conveyed functional, structural, and survival benefits [1,2,3] A useful short-term preclinical screening model for human sourced prototypes should demonstrate similar benefits.

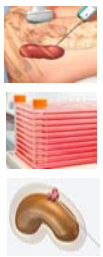
Methods

Model Generation: Proof-of-Concept athymic NIHRNU rats underwent kidney mass reduction (KMR); removing one kidney and approximately one week later, cortical tissue from remaining kidney. Surgeries were performed in 2 pilot groups with kidney weight-based reduction first averaging 59% (Model 1) then 67% (Model 2).



Model 1 and Model 2 individuals were monitored for baseline disease state utilizing serum/urine chemistries. Representatives (untreated) from each model were maintained to monitor disease progression.

Preparation and delivery of human SRC prototypes: Briefly, cells from cadaveric kidney (NDRI) biopsies were isolated, expanded in culture, and gradient selected to yield SRC. SRC were then formulated for 48 or 72 hours. Additionally, SRC were actively modified for 24 hours to form aggregates (organoids) prior to delivery. SRC prototypes as prepared were delivered to remnant kidneys via direct injection according to table below.



Nephrectomy (Nx)	Treatment (Tx) Group	Assessments
Model 1 (>59% KMR) Tx 11 weeks post-Nx Observation 204 days post-Nx 118 days post Tx	A Untreated (n=8)	Daily • Survival
	B Aggregated SRC (n=4)	Bi-Weekly • body weights • Serology and hematology • Urinalysis Panel
		Necropsy • Gross pathology observations
Model 2 (67% KMR) Tx 6 weeks post Nx Observation 183 days post Nx 116 days post Tx	A Untreated (n=8)	Terminal Histology • Assess tubular and kidney injury • HLA1 staining for human cells
	B 48 hour SRC (n=7)	
	C 72 hour SRC (n=8)	RANDOMIZATION: Untreat body weight, sCre, BUN, hct, UPC and urine specific gravity for groups of 8

In-Life Monitoring and terminal histology: Collection of blood and urine occurred bi-weekly and samples were analyzed by Marshfield Vet Labs. Animal condition/survival was monitored daily and signs of morbidity preceded humane euthanasia. Following death or terminal sacrifice, kidneys were formalin fixed and central transverse sections were stained with PAS or Masson's and evaluated by independent pathologist. Tubular, glomerular, and interstitial compartment injury from individual animal sections were observed and scored.

Results

Model Development and Disease Progression

Surgical model development proceeded with kidney mass reduction in Model 1. Following assessment of minimally altered sCre post-nephrectomy in this group, Model 2 was initiated with a more aggressive resection of pole tissue. For Models 1 and 2 respectively, 2 weeks post-KMR survival was 100% vs 64%; 24 weeks post-KMR survival was 100% vs. 5%.

Table 1. Model Generation

NIHRNU 9-10 Week Male	Model 1	Model 2
First Surgery		
Number of Animals	28	39
Animal Wt (g)	207 ± 20	211 ± 23
Excised Kidney Wt. (g)	907± 86	943 ± 108
Second Surgery		
Animal Weight (g)	220 ± 17	221 ± 23
Excised Pole Wt. (mg)	155 ± 30	317 ± 47
% KMR (wt. based)	59 ± 1.5	67 ± 2
Two Weeks Post		
Number of Animals	28	25
Survival (%)	100	64
Animal Wt. (g)	241 ± 17	245 ± 25
24 Weeks Post		
Number of Animals	28	2
Survival (%)	100	5

Figure 1. Clinical Pathology Comparison of Model 1 Vs Model 2.

Representative chemistries from Model 1 (solid lines) demonstrate significantly increasing proteinuria and hypophosphatemia while Model 2 (broken lines) exhibit increasing proteinuria, cholesterolemia, azotemia, hyperphosphatemia, and anemia. (α = 0.01)

Significant [a] Differences Model 1 Vs Model 2

Week 3 Post-Nx
uPro, Chol, BUN, sCre, uCre

Last In-Life/End of Study
Chol, BUN, UPC, sPhos, sCre, uCre, Hgb

Significant [b] Change Week 3 Post-Nx vs End Of Study

Model 1
uPro, UPC, sPhos

Model 2
uPro, Chol, BUN, UPC, sPhos, sCre, uCre, Hgb

[a] 2-tailed t-test p-value ≤0.01
[b] Paired t-test p-value ≤0.01

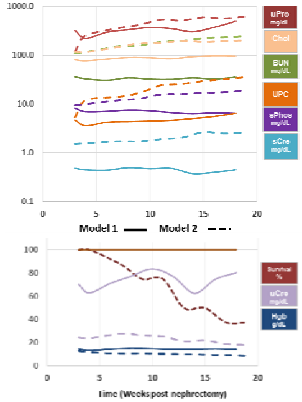
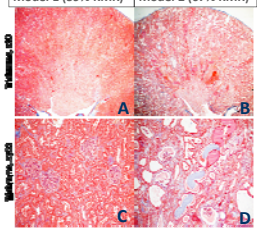


Figure 2. Greater Tubular and Glomerular Pathology in Model 2



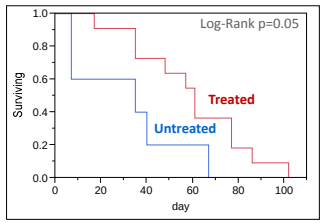
Panels A&B are low-magnification (10x) Masson's Trichrome stained sections demonstrating greater tubule dilatation and cast formation in Model 2. Higher magnification (100x) shown in Panels C&D reveal prominent glomerular sclerosis in Model 2. Kidney Injury scoring for untreated performed as described previously[1] with injury scaled from 0 = normal to 4 = marked.

Model	Tubulo-Interstitial Injury Score (mean)	Glomerular Injury Score (mean)
1	1.4 ± 0.4	0.8 ± 0.3
2	2.5 ± 0.2	3.0 ± 0.4
t-test	p<0.0001	P<0.0001

Prototype Testing In Proof-of-Concept RKM Models

Results from Model 2 were stratified by pre-treatment (Tx) sCre level for analysis as starting sCre effected survival (p<0.0001). No significant difference in survival was noted for 48 vs 72 hr of formulation and these Models were combined for further analyses. In rats with starting sCre ≥ 1, delivery of human SRC prototypes (n=11) provided significant survival benefit (p=0.05) and stabilization of clinically relevant biomarkers (Table 2; p<0.05, one-tailed t-test) associated with independent functional niches i.e.: waste removal, protein and cholesterol handling, RBC production.

Figure 3. Survival Benefit With Delivery of SRC to Model 2



Pre-Tx sCre	Survival (Days post-Tx) SRC Prototypes	Un-Tx
>2	17	35
	35	7
1 to 2	48	77	35
	57	77	40
	61	86	67
	61	102	
<1	72	146*	94
	95	146*	104
			123

* End of study

Table 2. Human SRC Treatment Improved Relevant Functional Markers in Comparison to Untreated Controls in RKM Model 2

Serum and urine chemistries from last time point all individuals from stratification group were in-life were utilized to compare untreated and SRC prototype treated. Significant p-values highlighted green.

Pre-Tx sCre	Treatment (Tx)		sCre	BUN	Chol	Phos	UPC	Hct
High >2	Un-Tx (n=2)	Avg 1 wk post-Tx	2.8	356	144	24.7	11.5*	18.9
	SRC (n=3)		2.5	154	155	7.9	20.9	30.8
		t-test p-value	0.32	0.04	0.42	0.007	n/a	0.09
Mid 1 to 2	Un-Tx (n=3)	Avg 1 mo post-Tx	2.9	190	225	17.6	37.6	29.2
	SRC (n=8)		1.6	130	176.25	8.25	19.8	35.3
		t-test p-value	0.01	0.03	0.05	0.003	0.02	0.02
Low <1.0	Un-Tx (n=3)	Avg 2 mo post-Tx	1.5	112	164	7.0	19.4	35.8
	SRC (n=4)		1.5	119	181	7.4	21.8	36.3
		t-test p-value	0.90	0.84	0.61	0.69	0.83	0.86
Observed Effects in Rodent CKD Models with Autologous SRC ZSF1 and Lewis Nephrectomy [4]			lower sCre	lower BUN	lower Chol	lower Phos	lower UPC	higher Hct

* Single measure

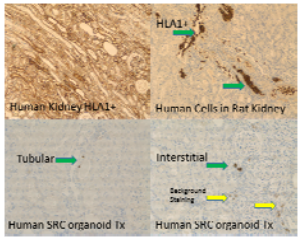
Table 3. Significant Changes in Clinical Pathology Measures Associated with Decline in Renal Function in RKM Model 1

p-Values paired T-test (Pre-Treatment and End of Study)							
Treatment	sCre	Hct	Phos	Chol	UPC	uCre	spGrav
Untreated	0.60	<0.0001	0.15	0.01	<0.0001	0.06	0.02
Aggregated SRC	0.30	0.26	0.48	0.005	0.01	0.87	0.73

Human SRC prototype effects detected in Model 1 were fewer in comparison to Model 2. Survival benefit was not discernable as all survived until end of study. Untreated animals did exhibit some statistically significant changes in renal markers associated with functional decline that were not noted in prototype treated animals.

Results (cont'd)

Figure 4. HLA1 Staining Consistent with Presence of Human Cells at 4 months Post-Treatment



At end of study kidney tissues were paraffin embedded, sectioned (5 micron), and stained for HLA1 to identify the presence of human cells. 1-2 cells were visualized per review of 4 sections within tubules and interstitium .

Conclusions

1. **Extent of KMR (59 vs 67%) in NIHRNU rats significantly impacts rate of disease progression, as assessed by survival, clinical pathology and histopathology, when creating short-term screening model.**
2. **In this xenogeneic model, detection of human SRC prototypes by HLA1 staining (Models 1 and 2) and stabilization of kidney function as assessed by sCre, BUN, cholesterol, phosphorus, hematocrit, UPC, and survival (Model 2) is consistent with that demonstrated in autologous/longer term rodent models. Prior to study entry, consideration should be given to implementing a minimum sCre threshold (sCre of 1 or higher).**
3. **Delivery of an aggregated SRC prototype to the minimally progressing Model 1 demonstrated few discernable treatment effects and may, in future work, be better compared to unmodified SRC in Model 2.**
4. **Current POC study demonstrates the potential application of Model 2 for screening human-sourced SRC-based therapeutic prototypes.**

References

[1]Kelley, R. et al. (2010). Am Journal of Physiology Renal Phys. 299:1026-1039.
[2]Presnell, Sharon, et al. (2011). Tissue Eng Part C: Methods. 17(3): 261-273.
[3]Kelley, R. et al. (2013). Cell Transplantation. 22(6):1023-1039.

Acknowledgements & Notes

The animal use was reviewed, approved and monitored by NAMSA IACUC prior to and during the course of the study. Animals were housed in accordance with criteria outlined in the "Guide for the Care and Use of Laboratory Animals" (National Academy Press, 1996).

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