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Regenerative medicine
brought to life.

2010 TERMIS NA, Orlando Florida

*“Bioactive Renal Cells Augment Renal Function
in the ZSF1 model of Diabetic Nephropathy”*

Rusty Kelley, PhD

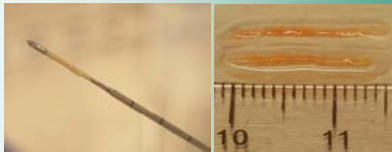
Neo-Kidney Augment (NKA) Overview

Intended to delay the need for dialysis or transplantation

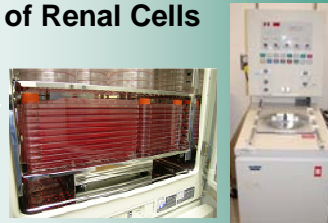
100,000 new dialysis patients each year in the US

- 350,000 currently on dialysis
- 20% annual mortality
- \$60,000 1st year cost per patient
- \$22 billion in direct US costs annually for end stage kidney disease

Renal Cells* from Kidney Biopsy



Isolation / Expansion of Renal Cells



Bioreactor System for NKA Production**



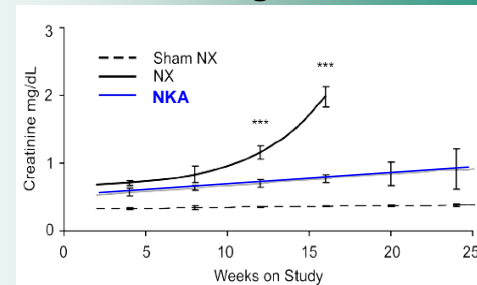
Injectable Delivery System**



In vivo Delivery



Functional Regeneration***



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*Selected Regenerative Cells used in the NKA
** In development

In Vivo Proof-of-concept Established

In three rodent models and a canine model of renal insufficiency

Proof-of-concept established for regenerative renal cells in multiple independent small animal models of chronic kidney disease (CKD)

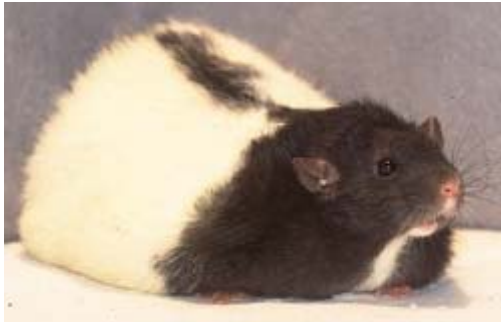
- *5/6 Nephrectomy model of terminal renal insufficiency*
 - Kelley et al, Am J Physiol Renal Physiol, 2010
- *Human cells in a nude rat model of I-R/G renal failure*
 - Wake Forest Institute of Regenerative Medicine, WFIRM
- ***ZSF1 model of metabolic syndrome and diabetic nephropathy***
 - Presnell et al, ISCT (San Francisco, Ca), 2010

Early observations from ongoing large animal studies are consistent with early small animal results

- *Isolation and delivery of renal cells from canines with renal insufficiency*
- *Early results indicate improved in vivo function with NKA Tx*

ZSF1 Rat Model of Progressive Nephropathy

Renal disease secondary to diabetes mellitus and hypertension



*Lean Female Zucker Fatty
Diabetic rat (ZDF; +/fa)*

X

*Lean Male Spontaneously
Hypertensive Heart Failure rat
(SHHF/Mcc-fa^{cp}; +/fa)*

=

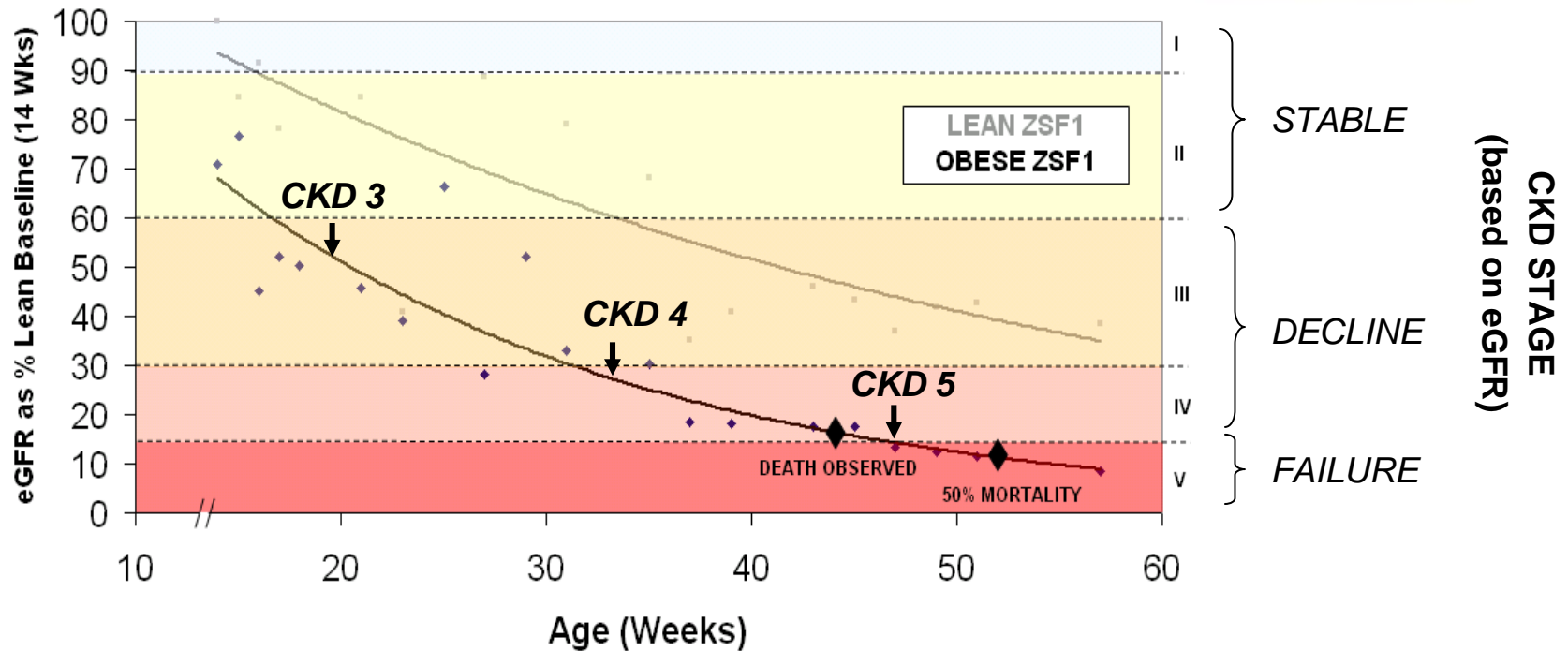
ZSF₁ (fa/fa^{cp}) rat

Aggressive Metabolic Syndrome:

- *Adiposity from leptin-receptor deficiency*
- *50% mortality at ~1yr*
- *Multiple co-morbid conditions*
 - *Morbid Obesity*
 - *Type II Diabetes (hyperglycemia)*
 - *Hypertension*
 - *Vasculopathy*
- *Progressive disease throughout the nephron*
 - *Progressive glomerular sclerosis*
 - *Progressive decline in GFR*
 - *Tubular / interstitial fibrosis*

Validating Renal Cells in Chronic Kidney Disease

Renal failure secondary to obesity and Type 2 diabetes (ZSF1)



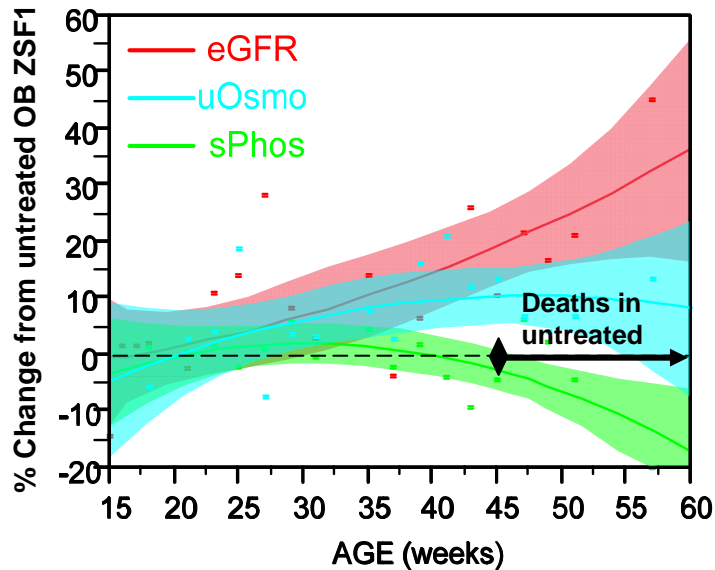
Intervention Strategy

Syngeneic Diseased ZSF1 donors (littermates)

- Treated (Tx) both kidneys at Stage 3 CKD (16-18 weeks)
- Glucose control (insulin) introduced at 31-34 weeks
- Subset of Tx subjected to re-treatment (Re-Tx) at 34 weeks
- Lean ZSF1 littermates = "healthy" controls

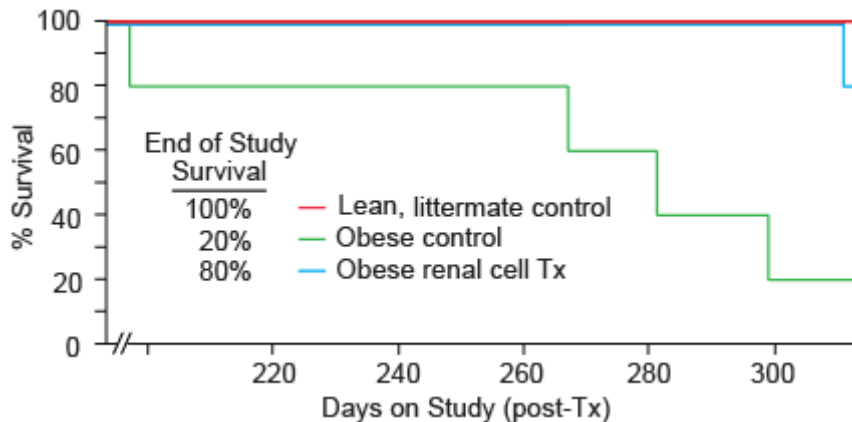
Renal Cells Improved Kidney Function

Detected throughout the nephron and whole organism



At > 1 year of age:

- **35% improvement in eGFR**
(Filtration)
- **15% reduction in phosphatemia**
(Tubular Function)
- **10% improvement in uOSM**
(Concentration)



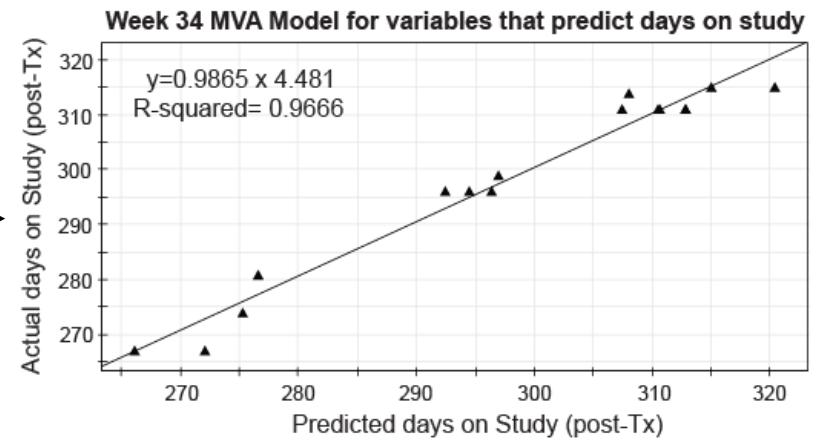
- **Renal Cells extend survival compared to untreated Obese Controls**

Multiple Parameters Predict Survival

Filtration, protein & electrolyte balance, & urine concentration

FILTRATION
TUBULAR FXN
CONCENTRATION

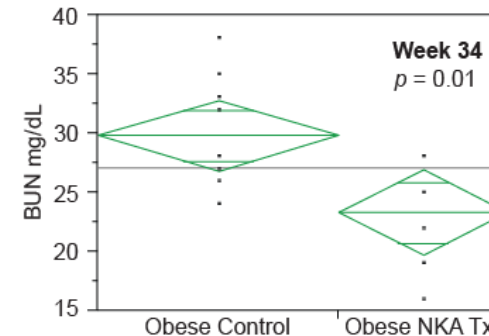
WEEK 34	WEEK 36	WEEK 40	WEEK 44	WEEK 46
CHOL mg/dL	CHOL mg/dL	CHOL mg/dL	CHOL mg/dL	uPHOS mg/dL
ALB g/dL	TRIG mg/dL	BUN mg/dL	BUN mg/dL	sCRE mg/dL
NA mmol/L	ALB g/dL	sCRE mg/dL	sCRE mg/dL	Inv sCRE
BUN mg/dL	BUN mg/dL	Inv sCRE	Inv sCRE	uCRE/sCRE
K mmol/L	uCRE/sCRE	sPHOS mg/dL	sPHOS mg/dL	BUN mg/dL
GFR ml/min/kg	sCRE mg/dL	uCRE/sCRE	uCRE/sCRE	GLU mg/dL
uSG	Inv sCRE	uCRE mg/dL	uCRE mg/dL	sCA mg/dL



MVA predicts multiple factors that contribute to the survival effect:

- Filtration: CHOL, **BUN**, GFR, CREA
- Protein handling: Albumin
- Electrolyte Balance: K, Na, Phos
- Urine Concentration: Specific Gravity

Example: **BUN**



*Multivariable linear regression using 'Treatment', 'Age', and 'Animal' to predict Days on Study.
P values = Effects Test for Treatment.

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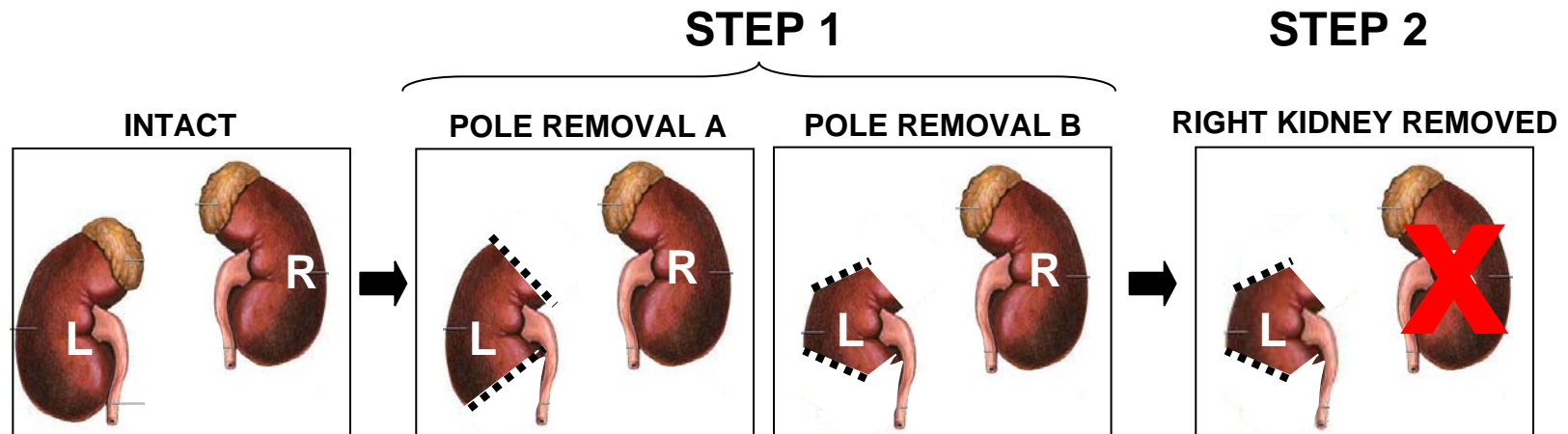
Modified 5/6 Nephrectomy Canine Model of CKD

Renal disease secondary to reduced kidney mass

Canine 5/6 Nx-induced model of renal failure

Chronic renal insufficiency

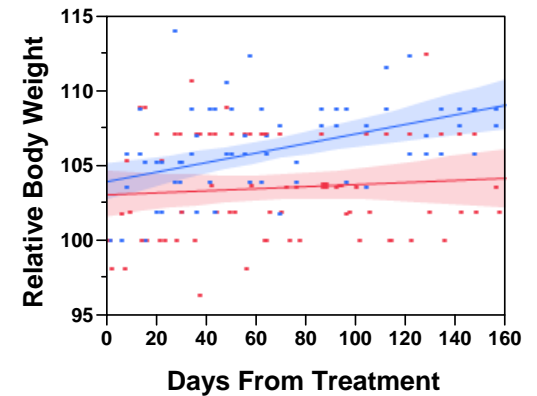
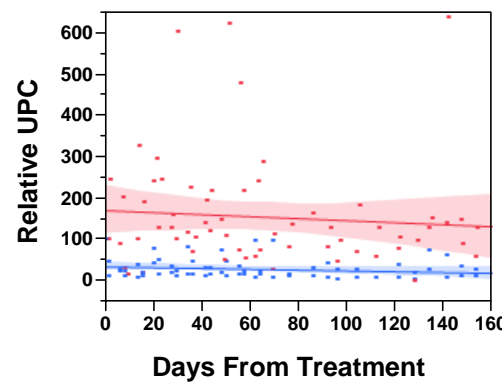
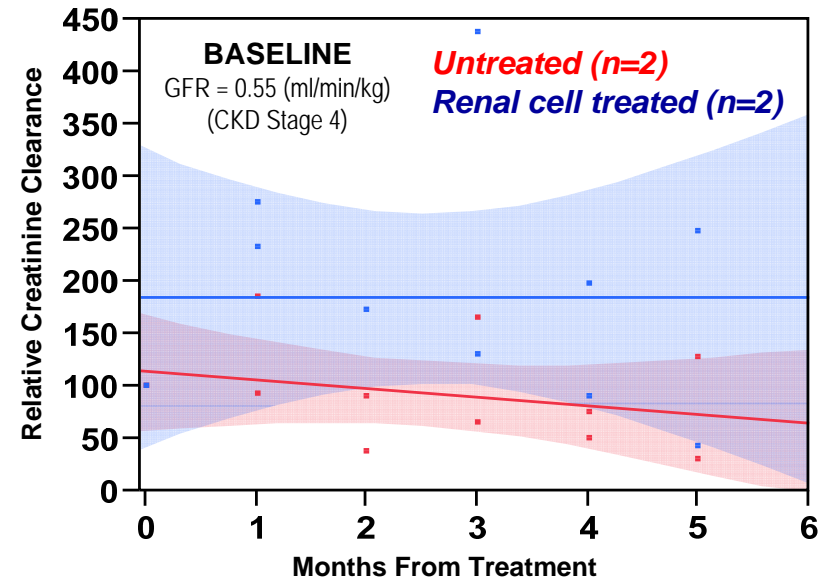
- *>50% reduction in GFR*
- *Mild hypertension*
- *Uremia*
- *Mild anemia*
- *Progressive proteinuria*
- *Gradual weight loss*



POC Study Indicates Renal Cells Improve GFR In 5/6-Nephrectomized dog model at five (5) months (ACTIVE)

Treatment with Renal Cells shows trends of efficacy:

- *Increased Creatinine Clearance (CCL)*
- *Reduced Urine Protein:Creatinine Ratio (uPC)*
- *Increased Body Weight*

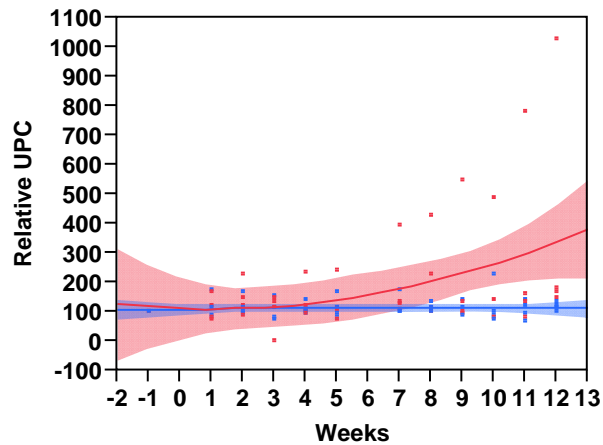


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Large Animal Study Confirms Renal Cell Function

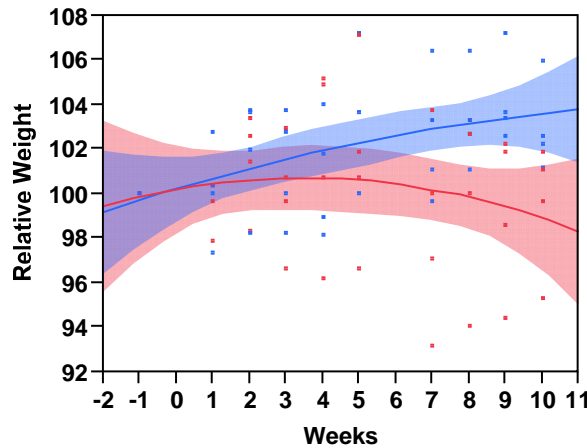
Significant improvement 13 weeks post-treatment

Untreated (n=4)
Renal cell treated (n=4)



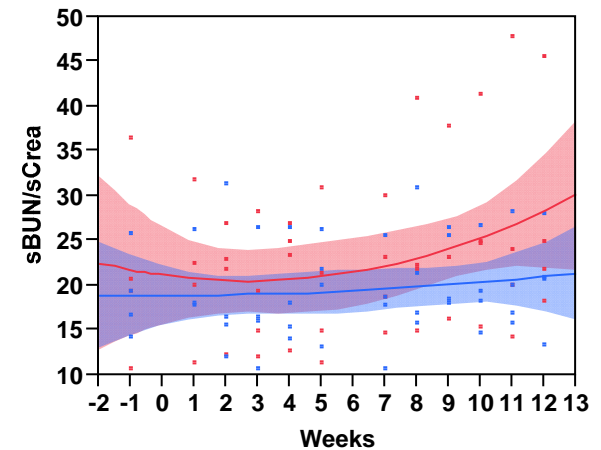
Protein balance improves with treatment

(UPC, $p = 0.0281$)*



Treatment promotes weight gain vs. weight loss

(BW, $p < 0.0001$)*



Renal filtration stabilizes with treatment

(BUN/sCre, $p < 0.0001$)*

***Multivariable linear regression using 'Treatment', 'Age', and 'Animal' to predict BUN/sCre, UPC & Body Weight.
P values = Effects Test for Treatment.**

In Vivo Function of Selected Renal Cells

Demonstrated in rodents and dogs with CKD

Renal cells function in a model of CKD secondary to obesity and Type 2 Diabetes (ZSF1) (Active)

- *Renal cells provide regenerative stimulus that preserves functional renal mass*
- *Treatment at Stage 3-4 CKD (17-34 weeks) delivers significant benefits beyond 1 year durability*
 - *Improved filtration*
 - *Improved protein balance*
 - *Restoration of electrolyte exchange*
 - *Improved urine concentration*
 - *Survival*

Renal cells function in a Canine model of CKD (Active)

- *5 mo results indicate in vivo function of regenerative cellular components*
 - *Stabilized filtration*
 - *Restoration of protein balance*
 - *Prevention of weight loss*