2010 TERMIS NA, Orlando Florida

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

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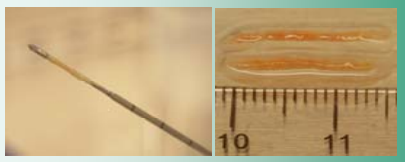
Neo-Kidney Augment (NKA) Overview

Intended to delay the need for dialysis or transplantation

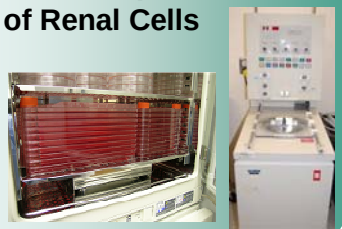
100,000 new dialysis patients each year in the US

- *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

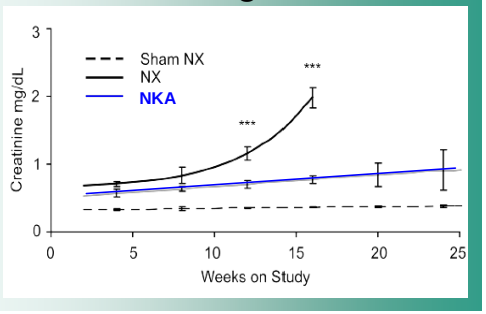


Bioreactor System for NKA Production**

Injectable Delivery System**

***In vivo* Delivery**

Functional Regeneration***



***Kelley et al, Am J Physiol Renal Physiol 2010
(Published online 9/6/2010)

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)

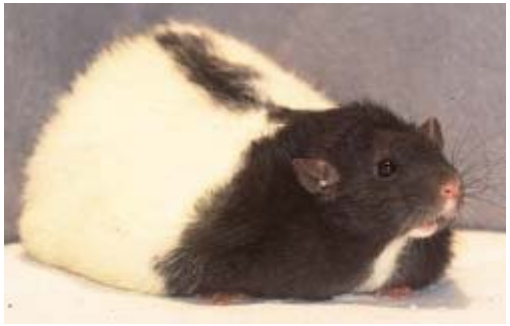
- *5/6 Nephrectomy model of terminal renal insufficiency*
  - *Kelley et al, Am J Physiol Renal Physiol, 2010*
- *Human cells in a nude rat model of I-R/G renal failure*
  - *Wake Forest Institute of Regenerative Medicine, WFIRM*
- *ZSF1 model of metabolic syndrome and diabetic nephropathy*
  - *Presnell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

=

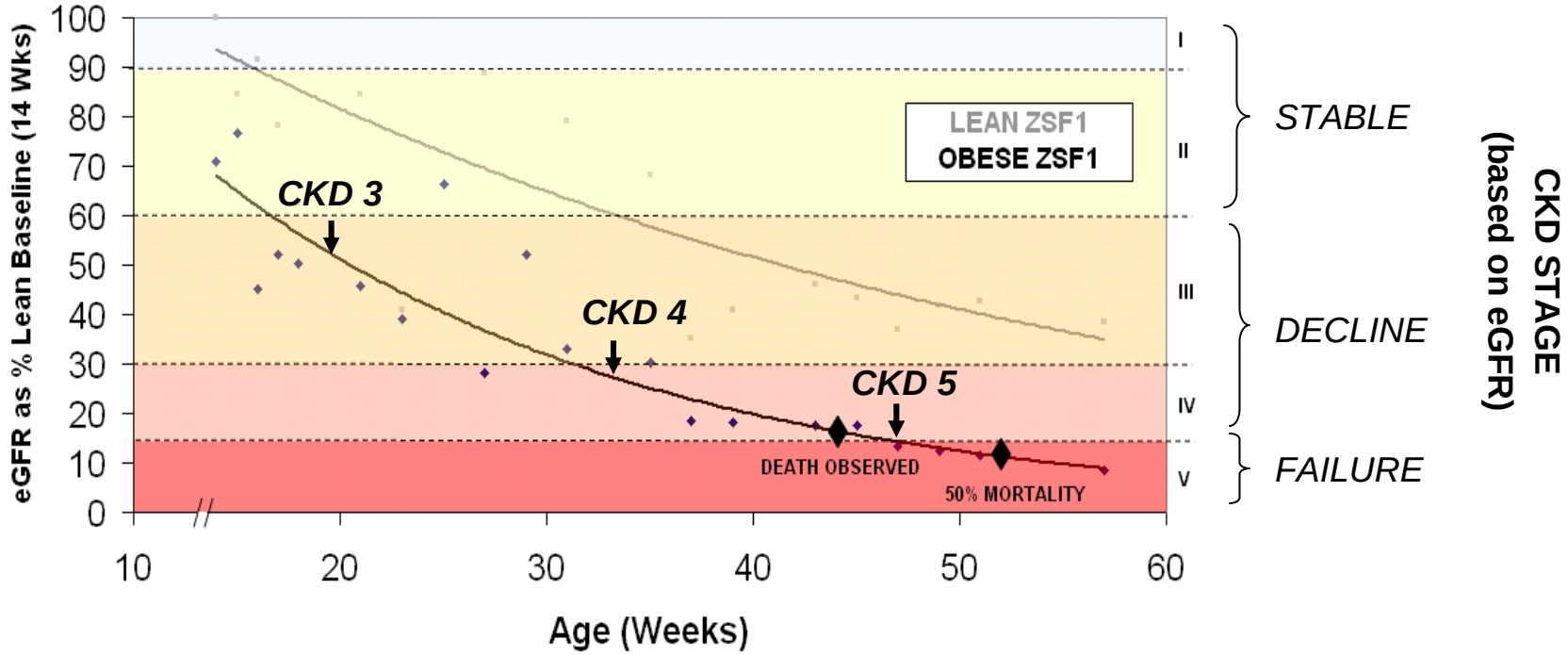
ZSF$_1$ (fa/fa^{cp}) rat

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)



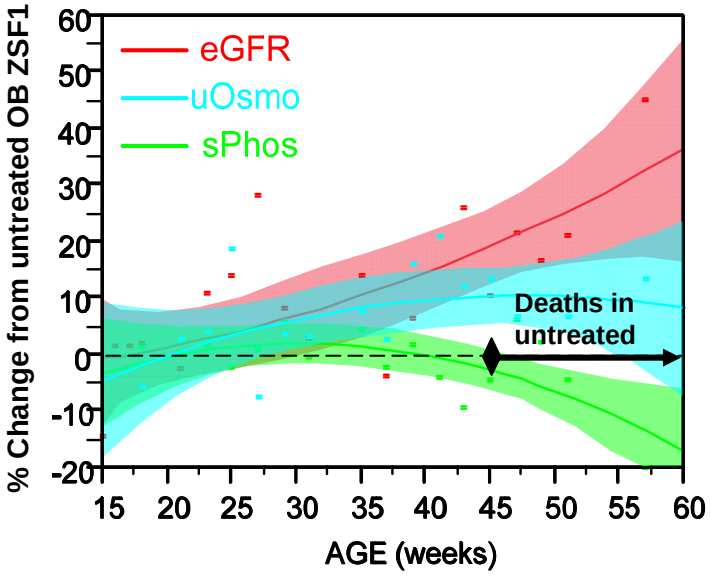
Intervention Strategy

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



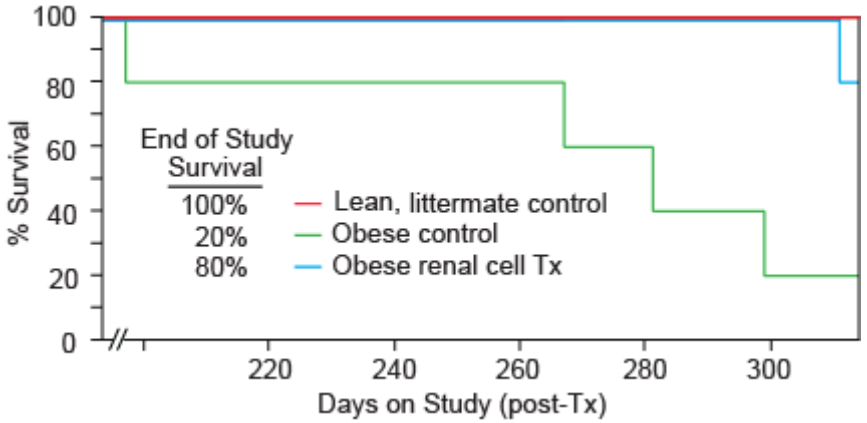
At > 1 year of age:

- ***35% improvement in eGFR***

  (Filtration)
- ***15% reduction in phosphatemia***

  (Tubular Function)
- ***10% improvement in uOSM***

  (Concentration)



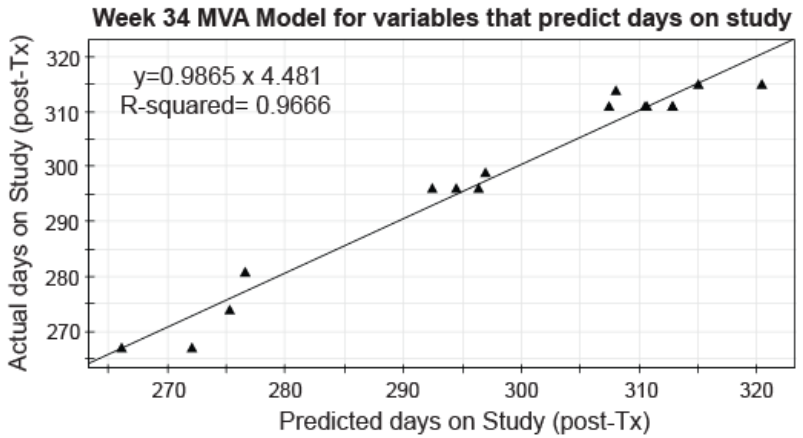
- ***Renal Cells extend survival compared to untreated Obese Controls***

Multiple Parameters Predict Survival

Filtration, protein & electrolyte balance, & urine concentration

FILTRATION
TUBULAR FXN
CONCENTRATION

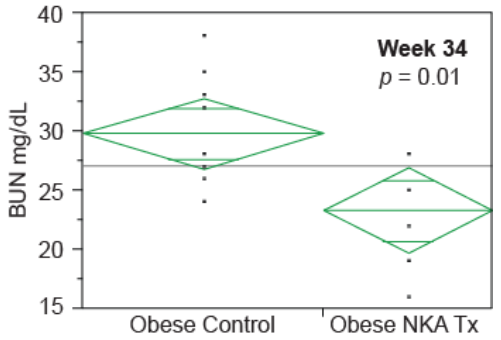
WEEK 34	WEEK 36	WEEK 40	WEEK 44	WEEK 46
CHOL mg/dL	CHOL mg/dL	CHOL mg/dL	CHOL mg/dL	uPHOS mg/dL
ALB g/dL	TRIG mg/dL	BUN mg/dL	BUN mg/dL	sCRE mg/dL
NA mmol/L	ALB g/dL	sCRE mg/dL	sCRE mg/dL	Inv sCRE
BUN mg/dL	BUN mg/dL	Inv sCRE	Inv sCRE	uCRE/sCRE
K mmol/L	uCRE/sCRE	sPHOS mg/dL	sPHOS mg/dL	BUN mg/dL
GFR 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 <u>multiple factors</u> that contribute to the survival effect:

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

Example: BUN



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

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

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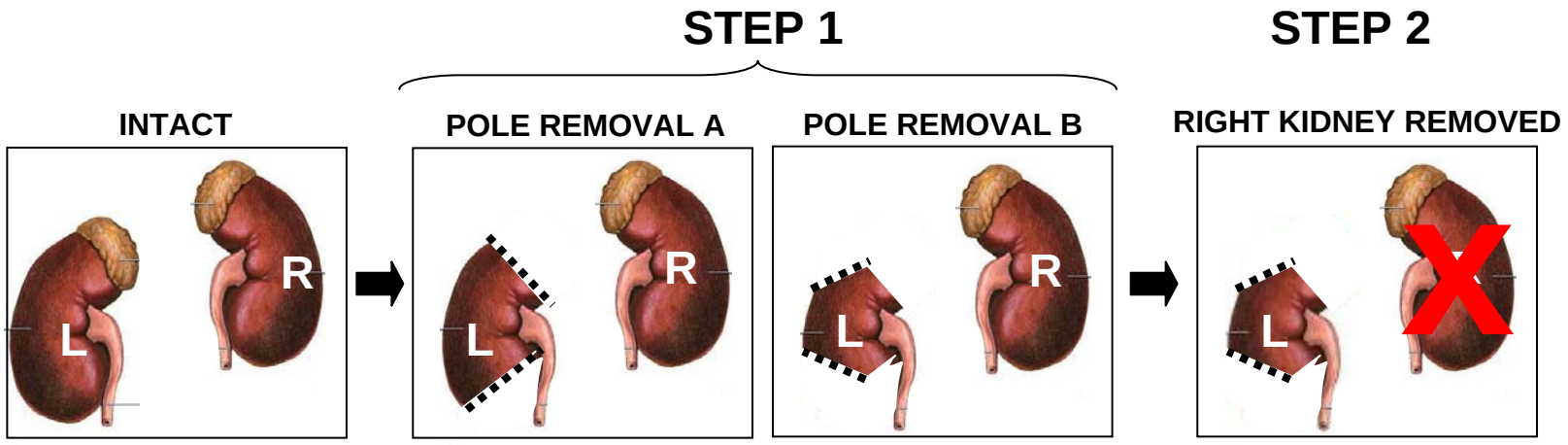
Modified 5/6 Nephrectomy Canine Model of CKD
Renal disease secondary to reduced kidney mass

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*

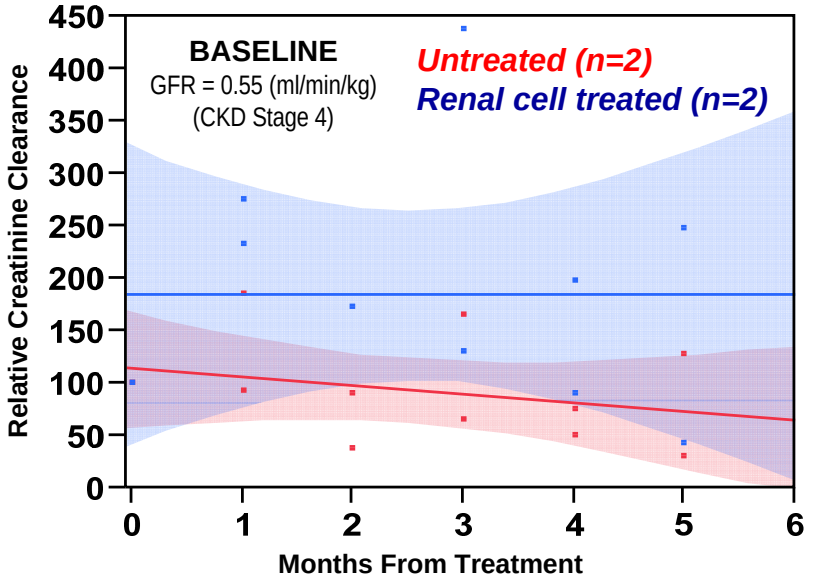


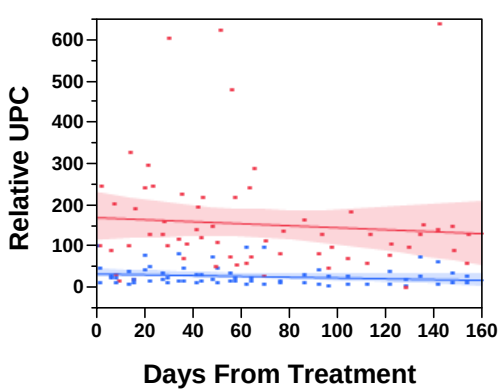
POC Study Indicates Renal Cells Improve GFR

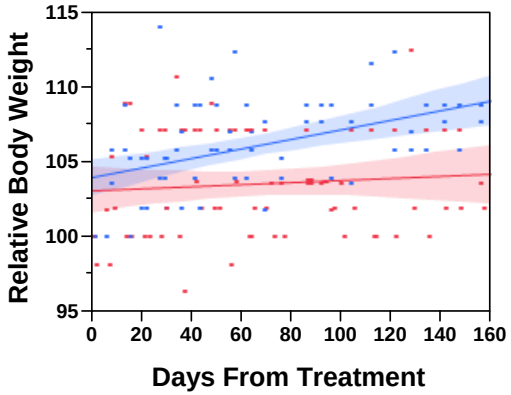
n 5/6-Nephrectomized dog model at five (5) months (ACTIVE)

Treatment with Renal Cells shows trends of efficacy:

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



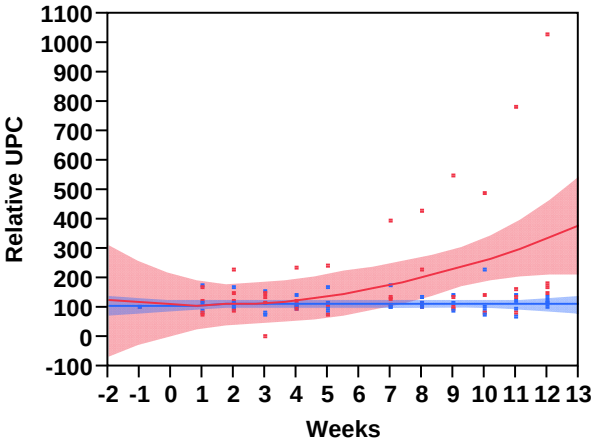


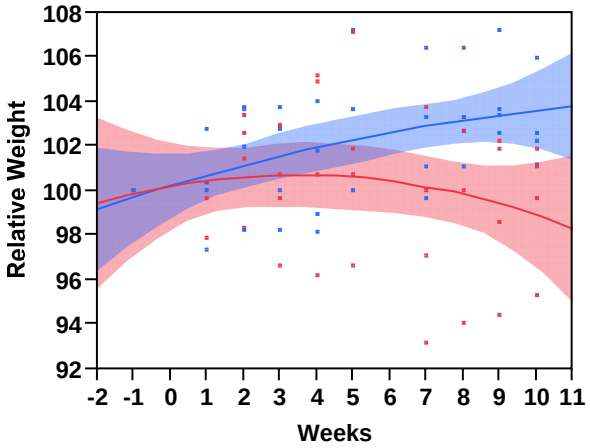


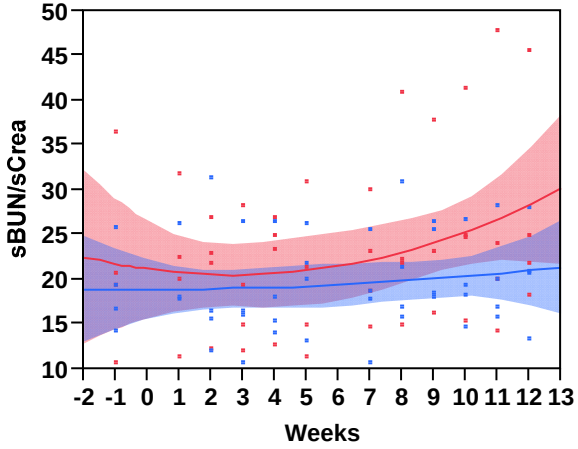
Large Animal Study Confirms Renal Cell Function

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 rine 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*