## Pilot & Feasibility Program

### Use of an animal model of Epidermal Nevus Syndrome to understand the mechanism of FGFR signaling in renal phosphate wasting syndromes

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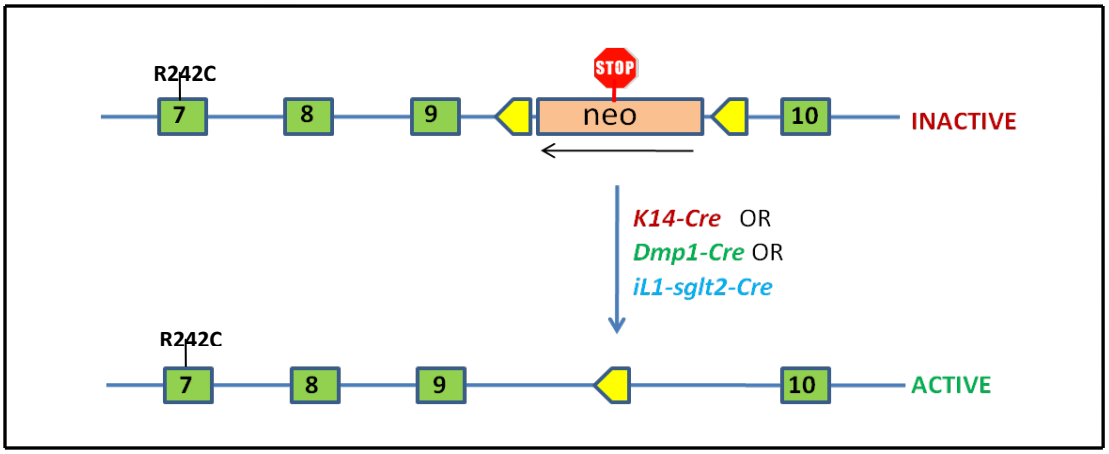
Veraragavan Eswarakumar focused on a rare phosphate wasting syndrome associated with FGF receptor dysfunction. Dr. Eswarakumar notes, “Because Epidermal Nevus Syndrome (ENS) is due to mosaicism resulting from a postzygotic mutation of FGFR3, we hypothesized that cells other than keratinocytes harbor the mutation and contribute to the skeletal abnormalities and phosphate wasting observed in ENS patients. Our goal was to create an animal model of ENS by introducing a lethal FGFR3 mutation into the mouse germline using a loxP-flanked stop sequence to overcome prenatal lethality and then to selectively activate the mutation in specific tissues including skin, bone, and kidney. Identifying tissues that harbor the mutant receptor will contribute to an improved understanding of the mechanism of FGFR signaling in phosphate homeostasis and ENS.”

### Findings to Date

We have successfully generated embryonic stem (ES) cells for the lethal Fgfr3-R242C mutation by gene targeting via homologous recombination. Four germline transmitting knock-in chimeric mutant mice have been generated from two independent ES clones. The mutation is kept dormant by insertion of a *neo* gene in reverse orientation, which changes the reading frame of the mRNA creating several stop codons and premature termination of the receptor transcript. However, this cassette is flanked by two directly repeated *loxP* (locus of crossover [x] in P1) elements, as shown below.




**Schematic of the Fgfr3-R242C mutant construct before and after Cre-mediated recombination.** The presence of neo gene in reverse orientation prevents expression of the mutant gene. After Cre-mediated recombination, the neo gene is excised allowing the expression of the mutant gene. Arrow denotes the direction of neo transcription. Yellow boxes represent loxP target sites for Cre-mediated recombination.

We now plan to use this animal model to conditionally activate the Fgfr3-R242C mutant receptor in keratinocytes (as a model for ENS), osteoblasts (source of FGF23), and in the nephrons (site of phosphate absorption) using the K14-Cre, DMP1-Cre and iL1-sglt2-Cre mice, respectively. The effects of the mutation on phosphate homeostasis will then be evaluated in three activated animal models.