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## The Role of CaMK-II in the Development of Leukemia/Lymphoma in Danio Rerio

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# The Role of CaMK-II in the Development of Leukemia/Lymphoma in *Danio Rerio*

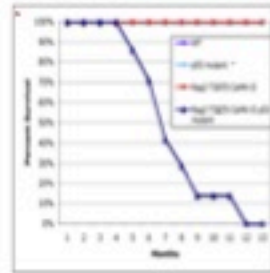
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**Introduction**

- CaMK-II is a Ca<sup>2+</sup>/calmodulin-dependent kinase expressed throughout body
- Zebrafish CaMK-II orthologs are 90-96% identical to human
- Increased CaMK-II activity seen in human patients with leukemia
- The zebrafish kidney marrow is analogous to the human bone marrow.
- Leukemic development monitored with pathology and FACS in zebrafish expressing phosphomimetic CaMK-II (T287D) in wild-type and p53 mutant backgrounds.

## Decreased survival in T287D CaMK-II p53 mutant fish than WT CaMK-II p53 mutants



**Figure 1:** Decreased survival in T287D CaMK-II p53 mutants. Rag2:T287D CaMK-II p53 mutant fish have reduced survival compared to wild type, p53 mutant, and Rag2:T287D CaMK-II fish. Disease onset is seen beginning at 4 months post fertilization.

**Methods**

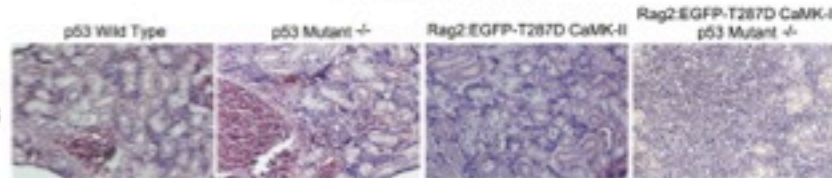
- ToI2 Gateway Technology: Transgenic Rag2:EGFP-constitutively active CaMK-II (T287D)
- Injection @ Single-Cell Stage
- Stable-line Generation: Outcross to WT and monitor EGFP expression
- Outcross the stable line to p53 mutant (M214K) zebrafish, followed by an in-cross and then genotyping for the mutation.
- FACS and Pathology: From Kidney, Spleen, and Peripheral Blood samples
- RT-PCR of FACS sorted EGFP negative and positive lymphocytes.

## EGFP Expression in Rag2:EGFP-T287D CaMK-II fish



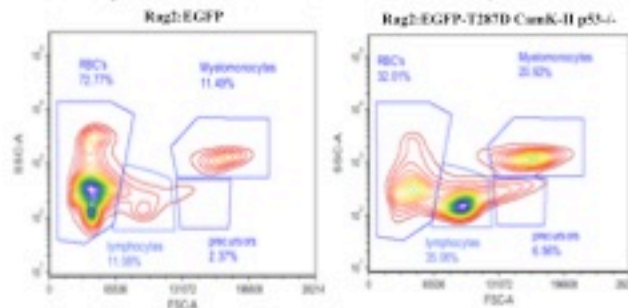
**Figure 2.** Rag2:EGFP-T287D p53 mutant fish have increased EGFP in the kidney compared to Rag2:EGFP-T287D CaMK-II wild type fish (arrow). Thymic EGFP expression is also observed (arrowhead).

## Leukemia/Lymphoma develops in EGFP-T287D CaMK-II p53 mutant fish



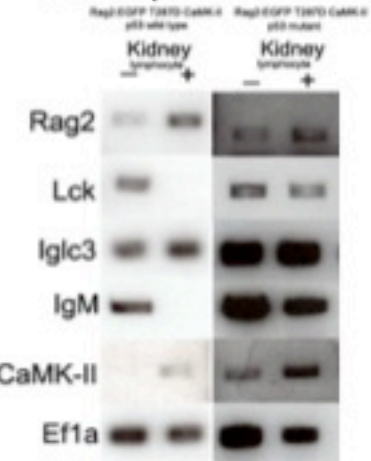
**Figure 3:** Pathology of the kidney marrow demonstrates an increase in lymphoblasts in Rag2:EGFP-T287D CaMK-II p53 mutant fish compared to wild type, p53 mutant, and wild type fish expressing EGFP-T287D CaMK-II. Kidney morphology is lost due to leukemia/lymphoma development.

## Increased lymphocytes in FACS analyzed kidney marrow of T287D CaMK-II p53 mutants



**Figure 4:** An increase in lymphocytes is observed in FACS analyzed kidney marrow of Rag2:EGFP-T287D CaMK-II p53 mutants. The decrease in erythrocytes and increase in lymphocytes is consistent with leukemia/lymphoma development.

## Lymphocyte maturation is altered in EGFP-T287D CaMK-II p53 mutant fish



**Figure 5:** EGFP positive and negative lymphocytes were analyzed for cell type. EGFP-T287D CaMK-II WT cells are immature B cells while addition of the p53 mutation yields immature and mature B cells as well as mature T cells that are EGFP positive.

**Conclusions**

- Ectopic expression of T287D CaMK-II in p53 mutant background leads to reduced survival and leukemia/lymphoma development
- Lymphocyte maturation is altered in the kidney marrow of EGFP-T287D CaMK-II p53 mutant fish.

**References**

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