

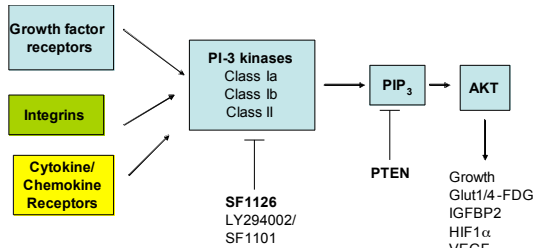
## CURE Childhood Cancer:

### “Targeted therapy for pediatric cancer”

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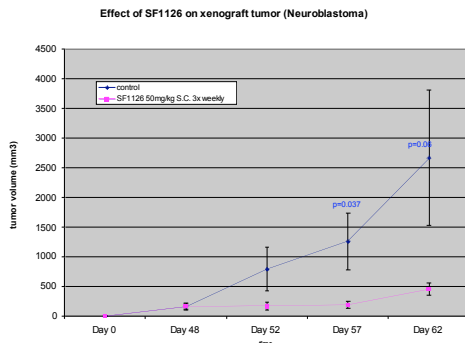
General goals: **1)** To develop targeted therapeutic agents for pediatric cancer and to learn how to use them for maximum efficacy in the pediatric cancer patient **2)** to develop a novel pan PI-3 kinase inhibitor (SF1126) for the application alone or in combination with chemotherapy, radiation or other therapies for the treatment of pediatric cancer. Below we show a schematic which outlines the rationale behind targeting all PI-3 kinases as compared to the inhibition of a single cell surface receptor or tyrosine kinase or one PI-3 kinase isoform. We continue to work on the development of **SF1126** for pediatric clinical trials. The overarching goal is to prepare this agent and other promising agents for a Phase I/II clinical trials in patients afflicted with pediatric malignancies.



**Fig. 1.** This schematic reflects the central theme of our work to develop and test a **pan** PI-3 kinase inhibitor, **SF1126** for antitumor therapeutics. The hypothesis stems from the view that a large number of cell surface receptors (growth factor receptors, chemokine and cytokine receptors, integrins, etc), pulse signals through **all** classes (class Ia, Ib, II) of PI-3 kinase to induce angiogenesis, cell growth, migration and invasion of normal brain parenchyma. A **pan** PI-3 kinase inhibitor, SF1126 will shut off a larger number of cell surface receptor input from growth factor receptors, chemokines, cytokine receptors, integrins and immunoreceptors in the tumor and stromal compartments.

### Progress in 2007:

**Summary of Research Plan of attack:** Over the past year of funding, we have addressed four important areas of investigation: **1)** we have shown that SF1126 has anti-tumor activity in neuroblastoma, rhabdomyosarcoma and high grade glioma models **2)** we have performed a genetic screen of the entire neuroblastoma genome in search of genes that may control sensitivity or resistance to SF1126 therapy in preparation of combination therapy with this agent in 2009 **3)** we have identified additional PI-3 kinase small molecule inhibitor candidates in hopes of discovering of other potent inhibitors of this pathway for application to pediatric cancer therapeutics **4)** we have evaluated 6 different agents separate or in combination with SF1126 or other therapies in an effort to develop a pipeline of agents entering Phase I or Phase II clinical trials in pediatric cancer. Below we briefly review progress in each of these individual areas of investigation.



**Fig. 1. SF1126 has potent antitumor activity in a Neuroblastoma xenograft model.** SKNBE(2) NB cells ( $5 \times 10^6$ ) were implanted sc into nude mice. Once tumor size reached  $100 \text{ mm}^3$  (day 48) treatment with SF1126 (50 mg/kg/dose given Mon/Wed/Friday) was initiated. Tumor volumes were measured (Y axis). Bars represent standard deviation of the mean.  $p < .05$  comparison of control to SF1126 treated on day 57 following the initiation of therapy. **Conclusion:** SF1126 has antitumor activity in NB xenograft model.

Our overarching goal is bring the most efficacious agents into pediatric cancer trials through the PHORG initiative at Aflac to translate them into clinical trials as rapidly as possible.

### Progress:

- 1) Over the previous funding period we have established that SF1126 has potent antitumor activity *in vitro* and *in vivo* in neuroblastoma xenograft models (Fig.1). We are currently setting up to generate SF1126 resistant neuroblastoma cell lines for a genome wide study to identify genes that encode resistance to this therapy. This is an exciting new area of therapeutics termed **chemical genomics**.
- 2) It isn't just important to develop new agents to treat pediatric cancer but to determine how to use them in an effective manner. The human genome contains 33,000 genes. We have analyzed the response of tumor cells, in this case neuroblastoma cells, to SF1126 exposure. The basic idea is to identify genes that will result in resistance to this therapy. Once identified, we will develop a strategy to get around these mechanisms of resistance in an effort to increase efficacy. Other drugs or agents to reverse resistance to SF1126 will increase the efficacy of this drug in patients. So far, we have identified about 50 potential genes which are altered by SF1126 treatment. These genes are likely candidates to regulate sensitivity to cell death events in the cancer cell upon exposure to a PI-3 kinase inhibitor e.g. p53 and bcl2 were 2 such genes observed in these microarray studies.
- 3) Drug Discovery (DD) efforts to isolate PI-3 kinase inhibitors with greater potency for pediatric cancer application (leukemia, neuroblastoma and brain tumors) continue during this funding period. Ongoing efforts using molecular modeling and high through put screening method in Durden laboratory have resulted in the identification of new compositions of matter (new drugs) which inhibit PI-3 kinase. Briefly, these experiments will utilize established PI-3 kinase enzyme assays (recombinant p85/p110 $\alpha$  protein) based on the kinase-glo system (Perkin-Elmer, Excite Envision system) to screen compounds or compound libraries obtained from the NIH or Emory University School of Medicine, Drug Discovery Core. *In silico* screening for compounds and 2 dimensional similarity searches using Dock3.0 programs and Chemnavigator *in silico* libraries for SF1126 related morpholino group containing chemical compounds allowed us to identify other candidate molecules for testing in our PI-3 kinase enzymatic assay systems. Compounds which hit on the molecular modeling screens were obtained and tested in our PI-3 kinase enzyme assays for inhibitory activity at 25 or 50  $\mu$ M concentration. The target endpoint for inhibition on the initial screen is set at  $\geq 35\%$  inhibition. SF1126 and SF1101/LY294002 were used as positive controls in these assays. We have identified 4 new classes of lead compounds which are active inhibitors of this enzyme. We are now optimizing these leads for further testing *in vitro* and *in vivo*. The ultimate long term goal is to find additional inhibitors of this pathway.
- 4) We have screened a number of agents for activity against neuroblastoma, glioma and medulloblastoma cell lines *in vitro*. So far we have tested: 1) anthracyclines 2) etoposide 3) velcade 4) rapamycin 5) dasatinib alone or in combination with each other or SF1126 in an effort to find synergistic activities. We have requested additional agents from pharmaceutical industry including: 1) sorafenib 2) sunitinib 3) BEZ235 and others to extend these studies to other agents. The next step is to take the most potentially synergistic agents into our *in vivo* animal models to test for synergy. Once *in vivo*

synergy is found we will present the data at our monthly PHORG meetings and design a Phase I clinical trial for patients.

We appreciate the support of the Cure Foundation and we believe these studies will have an impact on our patients in the near future.