Synthesis and Testing of MCT1 and MCT4 Inhibiting Compounds as Novel Cancer Therapeutics

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Abstract:

Triple negative breast cancers (TNBCs) account for 10-20% of breast cancers and generally affect younger patient populations (<50 years) as well as African-American/Hispanic women.[1,2] Current treatment options consist of surgical resection and radiation/chemical therapy; however, many of the drugs used are not cancer cell specific and result in widespread toxicity throughout body systems. A number of novel, small organic molecules selective towards cancer cells by inhibition of monocarboxylate transporters (MCT) were developed under the direction of Dr. Venkatram Mereddy. These compounds analogous to Alpha-cyano-4-hydroxycinnamic acid (CHC, a known MCT inhibitor) were synthesized from readily-available reagents and show high potential as lead compounds based on *in vitro* and *in vivo* studies.

Introduction:

Cancer is one of the most prominent diseases of human history. Roughly 1 in 2 men and 1 in 3 women will develop cancer at some point during their life. Despite advances made in therapeutics, in 2015 nearly 600,000 Americans are expected to die from cancer, accounting for 1 in 4 deaths. For this reason, it is crucial that improved methods be developed in cancer treatment. One common and relatively effective method has been use of chemotherapeutic agents. Three of the more common drugs used are given (Figure 1).[3]

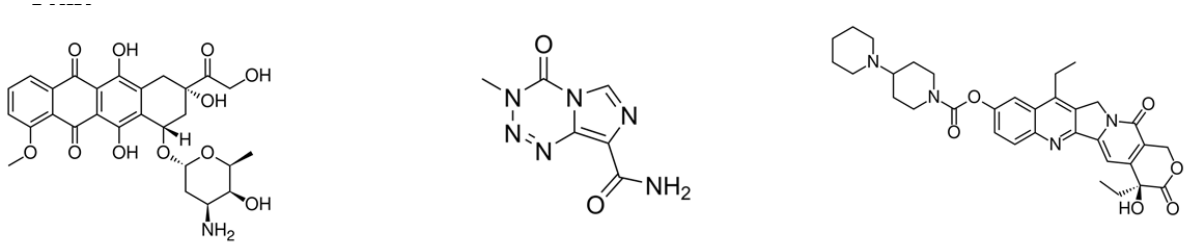


Figure 1: Doxorubicin (left), Temozolomide (middle), and Ironotecan (right) are common chemotherapeutic agents. Each of these compounds exhibits systemic cytotoxicity as a result of their non-specific methods of action.[3]

Despite their widespread use, however, each of these compounds and the majority of chemotherapeutic agents are highly toxic. Due to cancer's nature as a disease of a person's own cells, targeting cancerous cells specifically is exceedingly difficult. As a result, the search for a chemotherapeutic that is highly specific for cancerous cells remains one of the most pressing issue in science. For this reason, the Mereddy Lab at University of Minnesota Duluth is developing compounds that fit these needs.

Traditionally, cancer has been characterized as a mass of cells exhibiting sustained proliferative signaling, evasion of growth suppressors, activation of invasion and metastasis, replicative immortality, increased angiogenesis, and resistance of cell death[4]. Recently, cancer's ability to avoid immune destruction, deregulate cellular genetics, destabilize the genome, and

promote inflammation have also been identified as key components of most cancers. Each of these characteristics can be targeted in a variety of ways, as summarized in Figure 2.

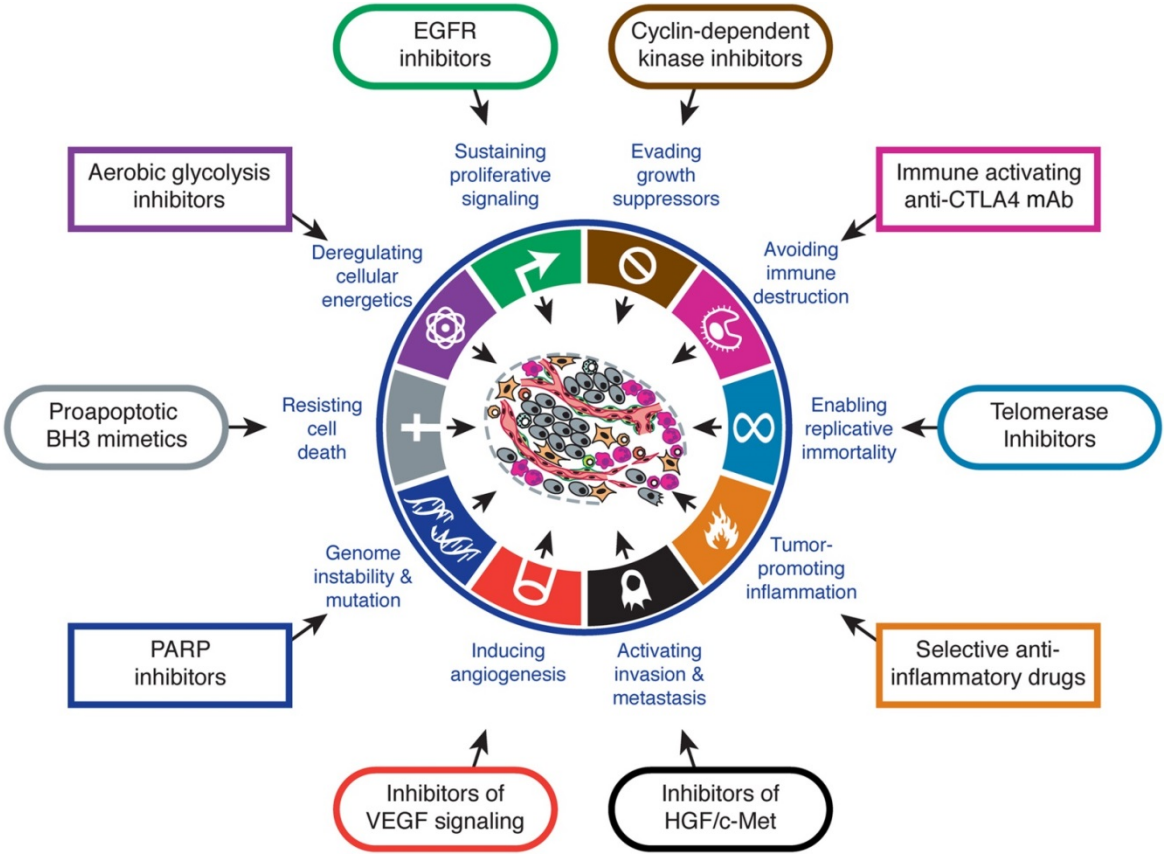


Figure 2: A summary of cancer characteristics and methods of targeting them.[4]

The Mereddy Lab has developed a number of compounds that exhibit strong potency as aerobic glycolysis inhibitors. To accomplish this, the energetics of cancerous cells must be strongly understood. Figure 3 gives a generalized depiction of metabolism exhibited by aerobic and anaerobic cells.

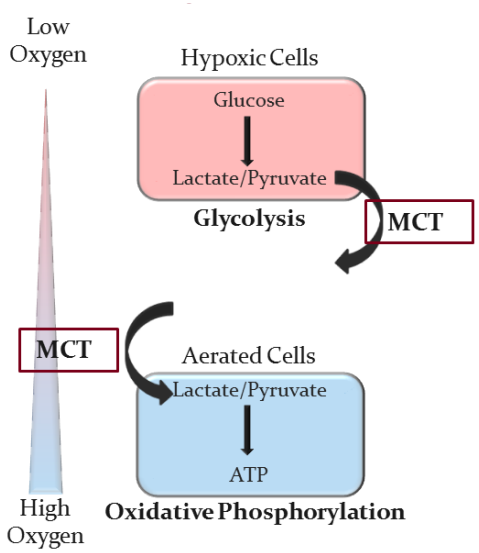


Figure 3: A generalized depiction of energy production within cells. Glycolytic cells produce lactate or pyruvate which may undergo oxidative phosphorylation in aerobic cells by transport through MCTs to yield high levels of energy (ATP).

Monocarboxylate transporters (MCTs) are a class of plasma membrane-bound proteins responsible for the transport of key metabolites (including pyruvate and lactate) into and out of cells. By use of these channels, cells may undergo the highly energy productive oxidative phosphorylation pathway by fully oxidizing pyruvate or lactate. The Mereddy Lab hypothesizes that inhibition of MCTs (specifically MCT1 and MCT4) will lead to decreased energy production of cancerous cells. Additionally, because pyruvate and lactate are acidic compounds, by preventing MCTs from regulating the pH of glycolytic cells through efflux of these compounds, the cells will likely undergo necrosis and apoptosis through acidosis (Figure 4).

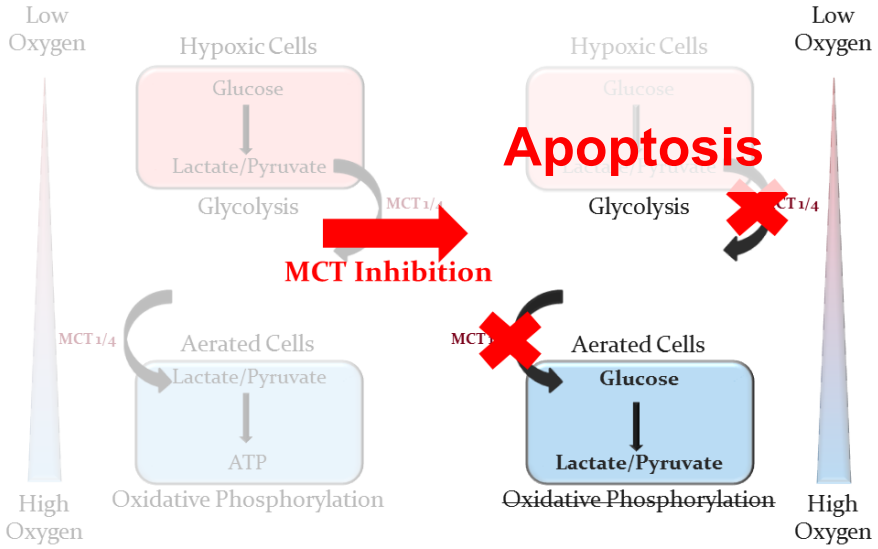


Figure 4: MCT inhibition may lead to apoptosis of cells.

In order to accomplish inhibition of MCT1 and MCT4, a library of compounds was synthesized that are analogous to a known MCT inhibitor, α-cyano-4-hydroxycinnamic acid (CHC). CHC and a sample of the 250+ compounds that were synthesized are shown in Figure 5.

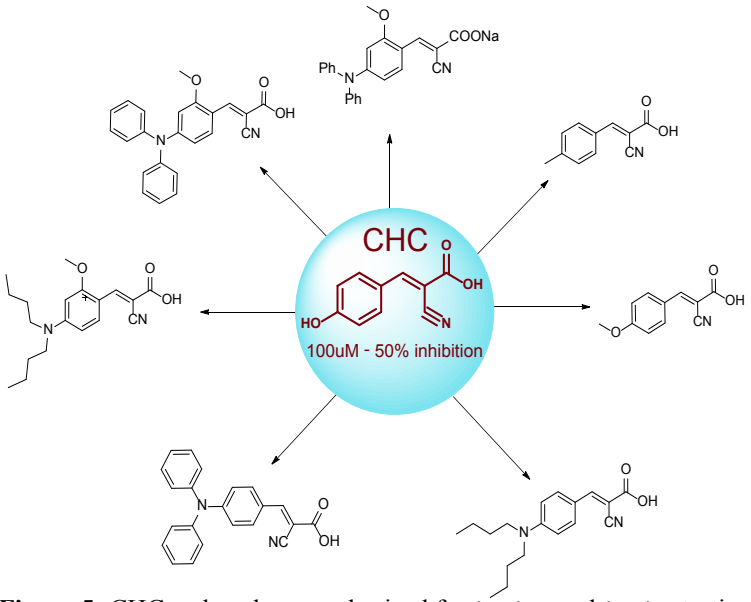


Figure 5: CHC and analogs synthesized for *in vitro* and *in vivo* testing.

By use of relatively simple and inexpensive chemistry (Figure 6), the series of dialkylated cyanoamines was created.

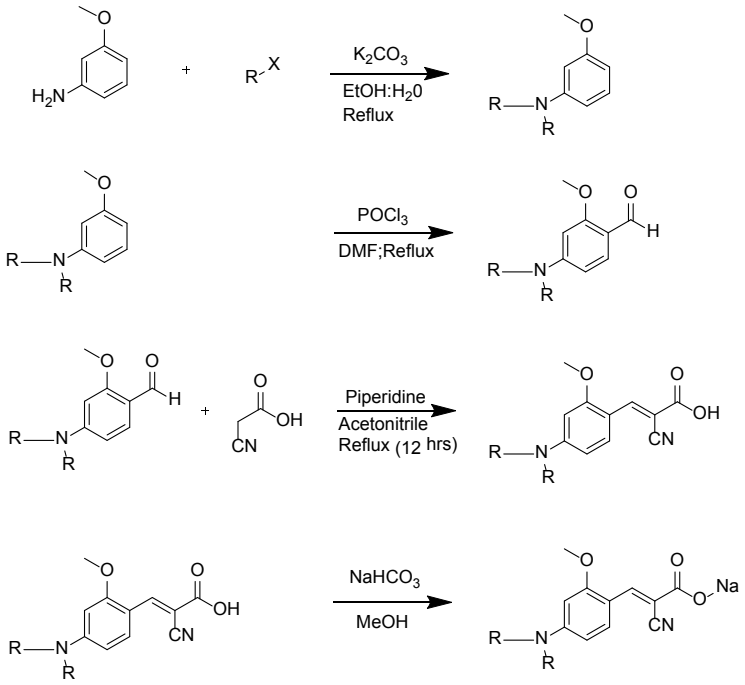


Figure 6: General method of synthesis of CHC analogs, where "R" represents any alkylated compound.

The efficacy of these compounds as MCT inhibitors was accomplished by use of an inhibition assay on a variety of MCT-expressing cell lines, including RBE4 (a noncancerous rat brain endothelial cell line) and MDA-MB-231 (a triple-negative breast cancer cell line) following the procedure detailed in the methods section of this report. The most potent inhibitors were selected for use in immunocompromised imprinting control region (ICR) mouse trials following IACUC protocol for laboratory mice. Following mice trials, an assessment of the efficacy and toxicity of the library of compounds was determined.

Methods:

Organic Synthesis

Procedural protocol related to the synthesis of CHC analogs followed the standard operating procedures of the Mereddy Lab and the University of Minnesota.

Inhibition Assays

MCT inhibition assay was performed on RBE4 and MDA-MB-231 cell lines using ^{14}C lactate (3μM radioactive; 2 μM non-radioactive). Approximately $1x10^5$ cells/well were seeded in a 24-well plate and incubated in 5% CO_2 and 37°C atmosphere for 18 -24 hours. Following incubation, cells were washed with HEPES buffer (pH 7.4). The buffer was removed and test compounds in ^{14}C lactate solution were added to facilitate transport of lactate for 60 minutes. The test compounds were removed and ice-cold stop buffer (100 μM CHC in HEPES) was added. Cells were lysed with 5% triton-X in 0.1M NaOH solution. Uptake values of the lysed cells were obtained in dpm (disintegrations per minute) using Scintillation counter.

Systemic Toxicity in ICR Mice

Mouse trials were conducted following the protocol dictated by IACUC, the FDA, the CDC, University of Minnesota, OSHA, the EPA, and all related governing agencies. Doses were given based on mass of mice either orally or intraperitoneally 2-4 times per day for a period of 3 weeks.

Results:

Inhibition assays of the CHC analogs yielded the inhibition concentration necessary to induce 50% inhibition (IC50) of MCTs. Figure 7 shows the most potent compounds.

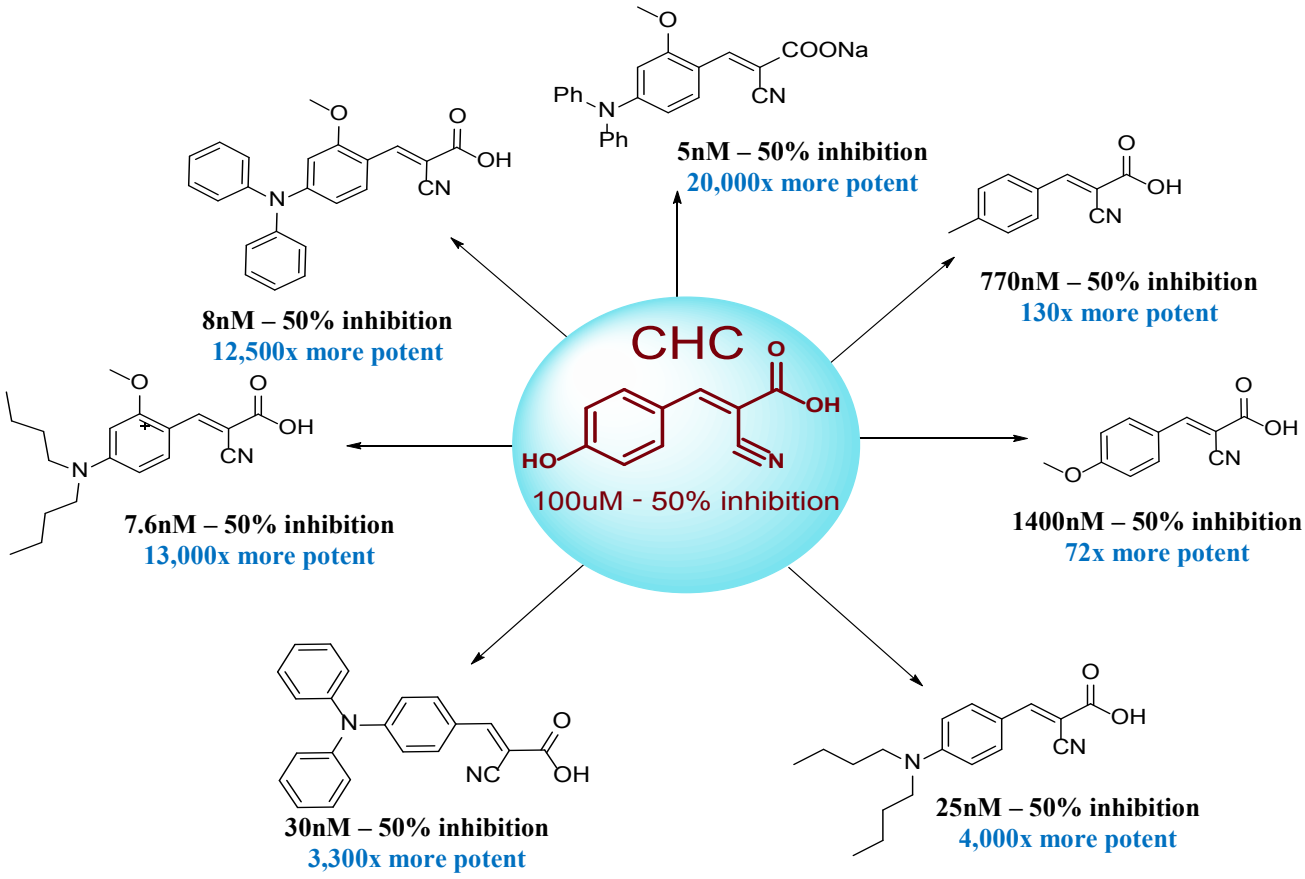
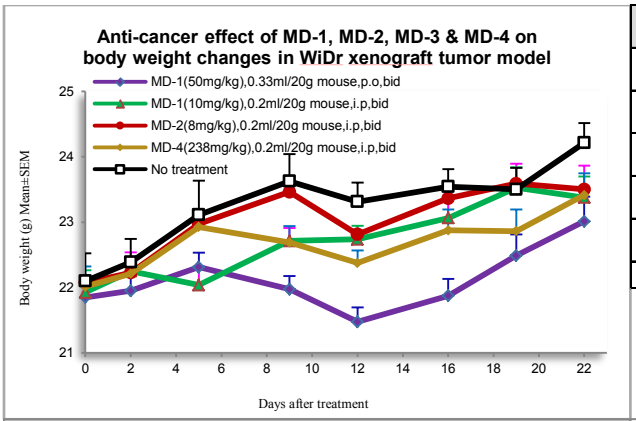
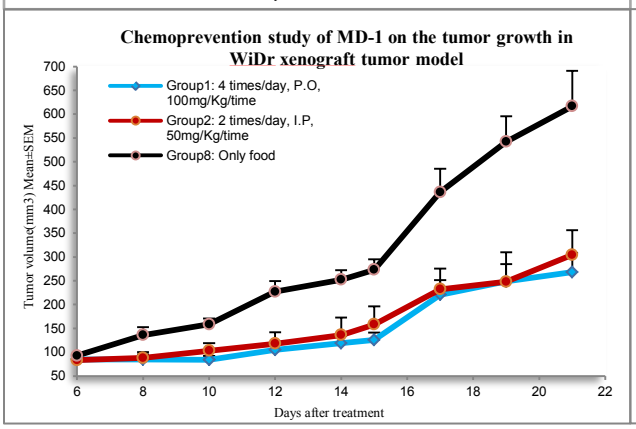


Figure 7: CHC and the most potent analogs synthesized. IC50 values (black) for the compounds as well as potency relative to CHC (blue) are given.

Figure 8 summarizes the results of testing of the compounds in ICR mice.



Groups	Drug	Dose (mg/kg) & Route	Schedule	No. of Mice	Death
1	MD-1	50mg/kg,p.o	bid,21 days	12	0
2	MD-1	6.67mg/kg,i.p	bid,21days	12	0
3	MD-2	18mg/kg,p.o	bid,21days	12	0
4	MD-2	5.3mg/kg,i.p	bid,21days	12	0
5	MD-3	18mg/kg,i.p	bid,21 days	12	0
6	No treatment			12	0



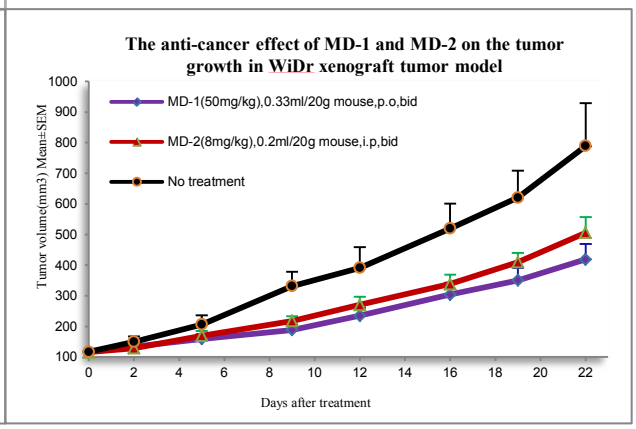


Figure 8: Results from ICR mice trials is depicted. Body weight changes in a WiDr xenograft tumor model (top left) show minimal weight loss throughout testing, as summarized by the table (top right). Chemoprevention studies involving compound "MD-1" administered orally (P.O) and intraperitoneally (i.p.) show a significant reduction in tumor volume (bottom left). Analysis of "MD-1" and "MD-2" yielded similar results (bottom right).

Discussion:

In order to improve outcomes of cancer diagnosis, improved therapeutics must be developed. Further, the chemotherapeutic compounds currently available are generally highly cytotoxic. To improve quality of life and life expectancy of patients, less toxic drugs must be made available. The Mereddy Lab successfully synthesized a library of compounds that show high promise as lead compounds for future cancer studies. The acquired results support MCT targeting as an effective and minimally toxic method of chemotherapy. Chemical synthesis successfully created a library of more than 250 CHC analogs for *in vitro* testing. Several compounds showed potency high enough to merit *in vivo* study in ICR mice. MD-1, MD-2, and MD-3 showed tumor volume

reductions as great as 50% while killing no mice. Mice weights upon conclusion of trials were within 5% of that of control mice, further confirming the conclusion that the compounds exhibited minimal toxic effects. Further, MD-1 exhibited significant tumor reduction capability whether administered orally or intraperitoneally, suggesting that multiple methods of drug delivery in human subjects would be possible. Future studies related to MCT inhibition will use a wider variety of compounds as well as numerous other cell lines to confirm the achieved results. In order to fully understand and utilize the compounds developed, a strong understanding of the pharmacodynamics involved in inhibition of MCTs will be determined. Orthotopic studies followed by survival advantage studies will yield information crucial to determining whether testing in higher order organisms should be initiated.

References:

1. Elias, A. D. (2009). Triple-Negative Breast Cancer: a short review Am. J. Clin. Oncol. 33:637-645.

2. Schneider, B. P., Winer, E. P., Foulkes, W. D., Garber, J., Perou, C. M., Richardson, A., Sledge, G. W., Carey, L. A. (2008). Triple-negative breast cancer: risk factors to potential targets. Clin Cancer Res. 14:8010-8018.

3. American Cancer Society. Cancer Facts and Figures 2015. 2015. Web. Accessed Apr 2015. doi: http://www.cancer.org/research/cancerfactsstatistics/cancerfactsfigures2015/index

4. Hanahan, Douglas et al.Hallmarks of Cancer: The Next Generation. Cell , Volume 144 , Issue 5 , 646 - 674