



**Title:** Novel PSAT1 Small Molecule Inhibitors Decrease Breast Cancer Cell Proliferation and Synergize with Anti-Estrogen Therapies in Endocrine Resistant Cells [2015]


**Authors:** [Sarah Madison Duff](#) Brian Clem<sup>2</sup> Medicine<sup>1</sup> and Biochemistry and Molecular Biology<sup>2</sup> 2015

**Keywords:** PSAT1, Small Molecule Inhibitors, Endocrine Resistant, Triple Negative Breast Cancer, Tamoxifen, Fulvestrant, Doxorubicin, Serine Pathway

**Abstract:**

Despite aggressive treatments, a significant proportion of individuals with estrogen-receptor-positive breast cancer relapse after becoming resistant to current therapies. Therefore, the investigation of combinatorial therapies that could re-sensitize these cancers is necessary to identify further treatment options for these patients. It has been previously demonstrated that phosphoserine aminotransferase (PSAT1) is up-regulated in ER+ endocrine-resistant and triple-negative breast cancer. Thus, the serine-pathway may be necessary to protect resistant breast cancer cells from common therapeutic drugs, including anti-estrogens and cytotoxic agents. We hypothesize that specific targeting of PSAT1 in combination with common chemotherapeutic agents may prove beneficial in treating endocrine-resistant and triple-negative breast cancers. *In silico* modeling of PSAT1 identified several chemical compounds that may suppress PSAT1 activity. Further analysis revealed two molecules that exhibited inhibitory activity on recombinant PSAT1. We now examined the ability of these antagonists to decrease breast cancer cell proliferation, as well as their combinatorial effect with the anti-estrogens, Tamoxifen and Fulvestrant, and common cytotoxic chemotherapies, doxorubicin, cyclophosphamide, and paclitaxel. In LY2 ER+ endocrine-resistant and MDA-MB-468 TNBC cells, treatment with two of the putative PSAT1 inhibitors resulted in a dose-dependent inhibition of cell proliferation. Additionally, combinatorial therapy with either Tamoxifen or Fulvestrant led to a synergistic decrease in LY2 cell growth. Co-treatment with the cytotoxic chemotherapy agents did not yield any increase in suppression of MDA-MB-468 breast cancer cell growth. Our data suggest that targeting PSAT1 through small molecule inhibitors may have utility against breast cancer proliferation and may prove beneficial in combination with anti-estrogen therapies. (Supported-by-NCI-grant-R25-CA134283)

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**Title:** Role of sphingosine kinase 1 and 2 in MYC-induced leukemogenesis [2015]

**Authors:** [Alexandra Kiefer](#) Morgan Stathem Michael Scherzer Brian Wattenberg<sup>1</sup> Levi Beverly<sup>1</sup> Deanna Siow<sup>2</sup> Leah Siskind<sup>2</sup> Medicine<sup>1</sup> and Pharmacology and Toxicology<sup>2</sup>  
2015

**Keywords:** sphingosine kinase, acute myeloid leukemia

**Abstract:**

Sphingosine kinases (SKs) catalyze the conversion of sphingosine to sphingosine-1-phosphate (S1P), a lipid mediator of inflammation, cell proliferation, angiogenesis, and other pro-survival cellular processes. Altered levels of key sphingolipids have been observed in a wide variety of cancers; our lab is interested in the role of altered sphingolipid metabolism with regards to acute myeloid leukemia. Previously, our lab developed an *in vivo* screen in mice for identification of potential drivers of leukemia. Using this screen, we identified the two SK isoforms, SK1 and SK2, as cooperators with the oncogene MYC in the induction of leukemia. The long-term purpose of this project is to determine the specific domains within the SK proteins that are required for cooperation with MYC in the induction of leukemia. To this end, we created mutations in specific regions of the SKs, including the catalytic domains and phosphorylation sites.

The impact that these mutations had on expression and activity was analyzed *in vitro* in HEK293 cells. Expression of WT and mutant forms of SK1 and SK2 were evaluated via real-time qRT-PCR and western analysis and compared to the enzymatic activities that were quantified using a radiolabeled assay. Now that our mutant forms of SK1 and SK2 have been characterized, future experiments will involve co-expressing them with MYC in our Tet-O-MYC mouse model of leukemia. Further characterizing the SK-MYC cooperation will provide an understanding of leukemogenesis mechanisms for identification of potential therapeutic targets. This research was supported by National Cancer Institute grant R25-CA134283.

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**Title:** Small Dual Surfactant Mesoporous Silica Nanoparticles Demonstrate Acidic pH Specificity [2015]


**Authors:** Matthew Neal Lacey McNally Medicine<sup>1</sup> 2015

**Keywords:** mesoporous silica, nanoparticles, pancreatic cancer, pH specificity, drug delivery, MSOT

**Abstract:**

Localized delivery of drug and contrast agent has the potential to address the current inadequacies of pancreatic adenocarcinoma diagnosis and treatment. With a five-year survivability of 6%, this phenotype is the deadliest form of cancer. The poorly ordered vasculature of pancreatic tumors combined with the shortcomings of current imaging modalities make this disease difficult to diagnose and treat effectively. We created 25 nm, pH responsive, peptide targeted, mesoporous silica nanoparticles (MSNs) to act as a diagnostic nanodelivery system that preferentially delivers contrast to pancreatic tumors. The MSNs were conjugated with chitosan and V7 pH low insertion peptide to impart pH responsiveness and pancreatic tumor targeting, respectively. It was determined that loading the MSNs with IR 780 iodide dye did not appreciably alter the absorbance peak (dye: 780 nm; loaded dye: 804 nm), though signal attenuation was observed. Pancreatic cell lines Panc1 and S2VP10 were assessed for MSN binding at physiological pH (7.4) and at cancerous pH (6.6). It was found that the MSNs showed 45.6x the fluorescence at pH 6.6 that was seen at pH 7.4, confirming pH responsive cell binding. These findings suggest that this type of nanodelivery system is a good candidate for future use with *in vivo* models of pancreatic adenocarcinoma. Funding for this research was provided by the National Cancer Institute grant R25-CA134283.

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**Title:** Genetic polymorphisms in 5-FU related enzymes predict complete pathologic response in rectal cancer [2015]

**Authors:** [Bailey Nelson](#)<sup>1</sup> Jane Carter<sup>1</sup> Maurice R. Eichenberger<sup>1</sup> Uri Netz<sup>1</sup> Susan Galandiuk<sup>1</sup> Price Institute of Surgical Research<sup>1</sup> 2015

**Keywords:** 5-FU, rectal cancer, genetic polymorphisms, thymidylate synthase

**Abstract:**

**Introduction:** Patients with rectal cancer undergo preoperative neoadjuvant chemoradiation with approximately 70% exhibiting pathologic downstaging in response to treatment. There is currently no accurate test to identify patients who are complete responders to therapy. 5-Fluorouracil (5-FU) is used in the neoadjuvant treatment of rectal cancer. Genetic polymorphisms affect the activity of thymidylate synthase (TS), an enzyme involved in 5-FU metabolism, and this may account for differing responses to neoadjuvant treatment seen patients. Detection of such polymorphisms might identify patients likely to have a complete response to neoadjuvant therapy.

**Methods:** DNA was isolated from whole blood taken from patients with newly diagnosed rectal cancer who received neoadjuvant therapy (n=52). Patients were given a tumor regression score based on cancer staging. PCR was performed targeting the promoter region of TS. PCR products were separated by electrophoresis to visualize if a patient was homozygous for a double-tandem repeat (2R), a triple-tandem repeat (3R), or heterozygous (2R/3R). A single nucleotide polymorphism (SNP) may also be present in the second repeat unit of the 3R allele. Restriction fragment length polymorphism assays were performed on patients with at least one 3R allele using HaeIII.

**Results:** Patients with at least one TS 3G allele were more likely to have complete or partial pathological response to 5-FU neoadjuvant therapy (OR 9.6 95% CI 1.2 – 75.7) (p=0.02).

**Conclusion:** Identification of patients with specific genetic polymorphisms in enzymes involved in 5-FU metabolism appear to predict the likelihood of pathologic response of rectal cancer to preoperative neoadjuvant therapy.

**Funding:** National Cancer Institute grant R25-CA134283

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**Title:** Combined Therapy of Oncolytic Adenovirus and Temozolomide Enhances Lung Cancer Virotherapy In Vitro and In Vivo [2015]

**Authors:** Jonathan Nitz<sup>1</sup> Stephen Wechman<sup>1</sup> Eric Riedinger<sup>1</sup> Rajesh Sharma<sup>2</sup> Heshan Zhou<sup>1</sup> Kelly McMasters<sup>1</sup> Jorge Gomez-Gutierrez<sup>1</sup> Surgery<sup>1</sup> and Medicine<sup>2</sup> 2015

**Keywords:** Lung, Cancer, Oncolytic, Adenovirus, temozolomide

**Abstract:**

Oncolytic virotherapy has made significant progress in recent years; however, widespread approval of virotherapeutics is still limited. Primarily, this is due to the fact that currently available virotherapeutics are primarily tested in monotherapeutic clinical trials exclusively (i.e., not in combination with other therapies). At present, only small and often clinically insignificant responses have been achieved. Therefore, scenarios with combination partners are postulated to be more effective. Combinatory settings would help to achieve a rapid stabilization, or even reduction, of tumor masses while providing enough time for achieving therapeutic success. For this reason, combination strategies of virotherapy with highly genotoxic regimens, such as chemotherapy, are of major interest. There are two types of lung cancer cells in terms of OAd replication efficiency: permissive or semi-permissive. In permissive cancer cells, OAd replication is greater than in semi-permissive cancer cells. Therefore, improving the therapeutic efficacy in both types of lung cancer cells is needed to be able to eliminate heterogeneous lung tumors. In this study, we investigated whether TMZ-induced autophagy could enhance virotherapy in both permissive and semi-permissive lung cancer cells. We found that semi-permissive lung cancer cells are efficiently destroyed by OAd in combination with TMZ. This enhanced killing effect was due to apoptosis, virus replication, and autophagy. In a lung cancer xenograft model, combined therapy had a superior tumor growth suppression than each treatment alone. We have provided an experimental rationale to test OAd in combination with TMZ in a lung cancer clinical trial.

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**Title:** Development of Immunomodulatory Exosomal Nanocarriers to Treat Melanoma [2015]

**Authors:** [Thomas Noel](#)<sup>1</sup> Joshua Hood<sup>1</sup> Pharmacology and Toxicology<sup>1</sup> 2015

**Keywords:** Melanoma, Exosome, Melittin, Nanocarrier

**Abstract:**

Melanoma induction of pro-tumor M2-like macrophages facilitates tumor survival and metastasis. One way melanoma communicates with immune cells is by release of tumor exosomes. Exosomes are natural biological nanovesicles analogous to non-replicating retroviruses. Previously we discovered that melanoma exosomes home to and create pre-metastatic niches in lymph nodes.

The cytolytic bee venom peptide melittin possesses Th1 immune adjuvant properties required for viral or tumor vaccine efficacy. Further, we showed that melittin attenuates HIV infectivity by disrupting HIV lipid membranes. Given the structural similarity between HIV and exosome membranes, here we test the hypothesis that melanoma exosomes can be formulated as melittin nanocarriers to modulate macrophage pro-tumor cytokine expression. Melittin loaded into the membrane of exosomes allows for the release of potentially pathogenic RNA content while keeping the exosome structure largely intact.

Our results show that melittin loading does not significantly alter exosome size or electrokinetic mobility properties necessary for lymph node homing. Melittin loaded into B16F10 melanoma exosomes at low concentrations does not decrease cell proliferation of RAW264.7 macrophages contrasted with free melittin's cytolytic effects. Unmodified B16 exosomes and B16 exosomes modified with low concentrations of melittin both increase the viability of RAW264.7 macrophages. Melittin modified melanoma exosomes decrease the expression of pro-angiogenic IL-1 $\alpha$  and induce no expression of the immunosuppressive M2 cytokine IL-10 compared to unmodified exosomes. These findings demonstrate the successful conversion of melanoma exosomes into non-cytotoxic melittin nanocarriers with the potential to antagonize induction of pro-tumor macrophages.

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**Title:** Can Cancer Cell Lines Clarify Molecular Mechanisms of Hereditary Non-Polyposis Colorectal Cancer? [2015]

**Authors:** [Henry Roberts](#)<sup>1</sup> Michael McClain<sup>1</sup> Jonathan Rice<sup>1</sup> Jane Carter<sup>1</sup> James Burton<sup>1</sup> Susan Galandiuk<sup>1</sup> Surgery<sup>1</sup> 2015

**Keywords:** Colorectal Cancer, microRNAs, miR-99a, HNPCC, mTOR

## Abstract:

### Introduction

HCT116 is a widely used experimental Dukes' D colon cancer (CC) cell line not commonly known to be derived from a HNPCC patient. A characteristic of HNPCC is inherited mutations in DNA mismatch repair (MMR). We have demonstrated different microRNA (miR) expression patterns between HCT116 and other sporadic CC cell lines with respect to miR-99a. This miR is an established inhibitor of mammalian Target of Rapamycin (mTOR). Increases in mTOR have been shown to increase cell growth, proliferation, migration, invasion and decrease apoptosis. We hypothesize the influence of miR-99a on the mTOR pathway, with respect to cell proliferation and motility, is dissimilar between MMR proficient (MMR+) and deficient (MMR-) CC cell lines.

### Methods

Dukes C and Dukes D MMR-(HCT15, HCT116), MMR+ (HT29, T84) and normal colon epithelium (CCD841) cell lines were transfected with miR-99a mimic (99M) and a negative control(M-). qPCR and western blot were used to measure RNA and protein levels, respectively. Functional assays were performed measuring cell migration and invasion.

### Results

All cell lines were successfully transfected and showing significant upregulation of miR-99a ( $p < 0.001$ ). After transfection with 99M, total mTOR protein was decreased as compared to M- for all cell lines. Migration decreased after transfection with 99M for all cell lines as compared to M- except for HCT116.


### Conclusion



We intend to investigate further to identify different pathways involved in HNPCC cancers that may permit development of more effective adjuvant therapy for MMR-cancer patients with advanced disease.

Support: R25CA134283.

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**Title:** Development of a plasma miRNA panel in detecting response to treatment of colorectal adenoma and colorectal cancer [2015]

**Authors:** [Vanessa States](#)<sup>1</sup> Jane Carter<sup>2</sup> Robert Eichenberger<sup>2</sup> Jianmin Pan<sup>3</sup> Shesh Rai<sup>4</sup> Susan Galandiuk<sup>2</sup> Surgery<sup>1</sup> Surgery<sup>2</sup> Biostatistics<sup>3</sup> and Biostatistics<sup>4</sup> 2015

**Keywords:** miRNA, colorectal cancer, colorectal adenoma

**Abstract:**

**Introduction:** Colorectal adenomas (CRA) develop into sporadic colorectal cancer (CRC) due to mutational activation of oncogenes and inactivation of tumor suppressor genes.

The carcinoembryonic antigen (CEA) test is a widely used non-invasive test used to screen for recurrent disease. Due to its less than optimum sensitivity and specificity, there is need for a more accurate noninvasive biomarker. MiRNAs are heavily investigated potential biomarkers due to their stability and dysregulation in disease states.

**Methods:** Plasma was isolated from 5 patients with advanced CRA and 5 patients with stage II or III CRC prior to treatment and 4-6 weeks following surgical treatment. RNA was extracted and 768 miRNAs were screened using microfluidic array technology. Data were analyzed using paired t-tests after normalizing raw cycle threshold data to endogenous RNU6.


**Results:** Significant differential expression of plasma miRNAs was observed between pre- and post-treatment samples. There was generalized miRNA upregulation in CRA and down-regulation in CRC.

Significantly dysregulated miRNAs were used to create a panel for the detection of CRA or CRC recurrence following resection. The resulting panel was able to differentiate between pre- and post-treatment samples for CRA (miR-186 and miR-623) with an AUC of 0.94 (95% CI 0.76 – 1.00) and for CRC (miR-324-5p, miR-30d and miR-766) with an AUC of 0.88 (95% CI 0.63 – 1.00).

**Conclusion:** Current findings suggest a difference in miRNA expression between CRA and CRC. Further validation is needed to develop a miRNA panel for monitoring recurrent disease.

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**Title:** The Role of DAB2IP in RASSF-mediated Tumor Suppression [2015]

**Authors:** [Desmond Stewart](#)<sup>1</sup> Geoffrey Clark<sup>1</sup> Lee Schmidt<sup>1</sup> Pharmacology & Toxicology<sup>1</sup>  
2015

**Keywords:** Ras, cancer, senescence, NORE1A, DAB2IP

**Abstract:**

Ras is the most frequently mutationally activated oncogene found in human cancers, and can be detected in over 33% of all human tumors. While Ras is powerfully transforming, Ras also has the paradoxical ability to induce cell cycle arrest and apoptosis; however, the mechanism by which this occurs has not been fully characterized. In part, Ras appears to activate these growth suppression mechanisms via its effector, NORE1A. Ras binds NORE1A directly to promote senescence and apoptosis, and expression of NORE1A is frequently lost in human tumors. Unfortunately, the mechanism of action remains unclear. Recent work discovered that NORE1A functions as a Ras regulated scaffolding molecule, where NORE1A is key in the stabilization and activation of the SCF-b-TrCPubiquitin ligase complex. Thus, the potential exists for NORE1A to act as a scaffold for other tumor suppressor proteins. DAB2IP, a Ras-GAP, has also been implicated in tumor suppression. DAB2IP is frequently inactivated in human prostate cancer, and a study using knockout mice has shown that DAB2IP inactivation is sufficient for prostate adenocarcinoma development. Here we now show that NORE1A and DAB2IP form a novel, direct, Ras-regulated protein complex. Furthermore, NORE1A and DAB2IP cooperate to potentially activate oncogene-induced senescence (OIS), which is a key protection mechanism against hyperactive Ras signaling. Thus, loss of NORE1A and/or DAB2IP impairs the ability of the cell to protect itself against oncogenic Ras mutations, thus removing a key tumor suppressive mechanism. Support: R25CA134283.

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**Title:** Paraoxonase-2 mediates a homoserine lactone-induced apoptosis in breast cancer cells [2015]

**Authors:** Nicole Stivers<sup>1</sup> Aaron Whitt<sup>1</sup> Aaron Neely<sup>1</sup> Guoping Zhao<sup>1</sup> Chi Li<sup>1</sup> Medicine, Pharmacology and Toxicology<sup>1</sup> 2015

**Keywords:** paraoxonase-2, apoptosis, homoserine lactone, breast cancer

**Abstract:**

Paraoxonase-2 (PON2) is an enzyme involved in the hydrolysis of organophosphates and lactones. PON2 has been reported to be highly expressed in multiple cancers, and to contribute to the resistance of conventional chemotherapeutic drugs. Our recent study found that N-(3-oxododecanoyl)-L-homoserine lactone (C12) from the bacterium *Pseudomonas aeruginosa* induced apoptosis in mammalian cells dependent on PON2. However, the detailed molecular mechanism of PON2 in C12-induced apoptosis in breast cancer was unknown. In this study, we first screened a panel of human breast tissues for PON2 expression from patients with conditions ranging from normal to malignant. It was found that higher levels of PON2 were expressed in breast tissues from patients with malignant forms of breast cancer. Furthermore, upon stably reducing PON2 expression in T47-D and MDA-MB-231 breast cancer cells, significantly less cell death and caspase-3/7 activation was observed, and less release of cytochrome c was observed in T47-D cells following C12-treatment. Overall, our results demonstrate that PON2 plays a vital role in C12-induced apoptosis in breast cancer cells, which indicates PON2 might be a new target for breast cancer therapy.

This research was supported by R25-CA134283 from the National Cancer Institute.

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