Despite extensive investigation, the optimal balance between oncological results and preserving parenchyma after resection of hepatocellular carcinoma (HCC) has not been clearly elucidated.

The goal of this study was to compare the outcome after partial hepatectomy for HCC in which a margin less than or equal to 5 mm or greater than 5 mm was achieved.

### Introduction

A review of our prospective 2455 patient Hepato-Pancreatico-Biliary database was performed on all patients undergoing primary resection of HCC at a single center from December 2002 to April 2015.

Patients were stratified into resection margins 5 mm or less (“narrow”) and those greater than 5 mm (“wide”).

Primary outcome was patterns of recurrence and disease free survival (DFS).

Unpaired t-test was used to determine if each subsequent stratification was statistically significant, with alpha set at 0.05.

### Results: Baseline and Operative

<table>
<thead>
<tr>
<th>Variable</th>
<th>Margin Status</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤ 5 mm</td>
<td>&gt; 5 mm</td>
</tr>
<tr>
<td>Patients Enrolled, n</td>
<td>41</td>
<td>89</td>
</tr>
<tr>
<td>Disease characteristics:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of lesions per patient, median (range)</td>
<td>1 (1-20)</td>
<td>1 (1-20)</td>
</tr>
<tr>
<td>Mean largest tumor size, cm (SD)</td>
<td>9.9 (4.6)</td>
<td>7.3 (4.3)</td>
</tr>
<tr>
<td>Mean resection margin, mm (SD)</td>
<td>2.3 (1.7)</td>
<td>18.0 (11.9)</td>
</tr>
<tr>
<td>Positive margins on final histology, n (%)</td>
<td>8 (19.5%)</td>
<td>0 (0%)</td>
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</tbody>
</table>

### Results: Follow-up

<table>
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<tbody>
<tr>
<td></td>
<td>≤ 5 mm</td>
<td>&gt; 5 mm</td>
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<tr>
<td>Timing of Recurrence:</td>
<td></td>
<td></td>
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<tr>
<td>Recurrence at time of analysis, n (% total)</td>
<td>15 (37)</td>
<td>41 (46)</td>
</tr>
<tr>
<td>&lt;1 year from resection, n (%)</td>
<td>9 (18)</td>
<td>23 (54)</td>
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<tr>
<td>1-2 years from resection, n (%)</td>
<td>3 (6)</td>
<td>7 (17)</td>
</tr>
<tr>
<td>&gt;2 years from resection, n (%)</td>
<td>3 (6)</td>
<td>11 (27)</td>
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</table>

### Disease-free Survival

<table>
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<th>Characteristic</th>
<th>≤ 5 mm</th>
<th>&gt; 5 mm</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Intrahepatic, n (%)</td>
<td>11 (26)</td>
<td>39 (35)</td>
<td>1.00</td>
</tr>
<tr>
<td>Extrahepatic, n (%)</td>
<td>9 (22)</td>
<td>15 (17)</td>
<td>1.00</td>
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<tr>
<td>Median recurrence free survival, months</td>
<td>18.1</td>
<td>19.5</td>
<td>0.85</td>
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</table>

### Overall Survival

<table>
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<th>Characteristic</th>
<th>≤ 5 mm</th>
<th>&gt; 5 mm</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor-free survival at censorship, n (%)</td>
<td>13 (32)</td>
<td>19 (21)</td>
<td>0.27</td>
</tr>
<tr>
<td>Median overall survival, months</td>
<td>34.7</td>
<td>37.2</td>
<td>0.68</td>
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### Conclusions

- A narrow resection margin (5 mm or less) does not detract from the oncologic outcomes after partial hepatectomy for HCC.

- Tailoring resection margins may lead to greater preservation of hepatic parenchyma.

- Factors other than margin status represent the driving forces for local and systemic recurrence.

### Acknowledgements

National Cancer Institute Grant R25-CA134283
Effect of Hybrid Surface-Modified Nanoparticles on HPV 18 E6 Knockdown *In Vitro*

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Department of Bioengineering1, Principal Investigator*
University of Louisville, J. B. Speed School of Engineering

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### Introduction

Cancers of the female reproductive tract have a very high incidence rate, being the third leading cause of cancer-related death in women worldwide. Specifically, cervical cancer is the leading cause of death of more than 4,000 women per year in the US alone and is associated with a very high rate of late-stage diagnosis. This is attributed to the minimal symptoms associated with human papillomavirus (HPV) 16 and 18 related cervical cancer. Although there are preventative vaccines, there are few non-invasive treatments for late-stage diagnosis. To overcome this, drug delivery vehicles, such as polymer nanoparticles (NPs), can be utilized to transport anti-invasive treatments by protecting agents during delivery, prolonging delivery, and safely locating drugs and biologics to the tumor microenvironment. In addition to these attributes, NPs can be modified to significantly enhance tumor penetration and cellular internalization. In particular, the use of NP surface modifications with both cell penetrating peptides (CPPs) and stealth ligands helps to enhance E6 expression. The long-term goals of this study were to develop polymeric-co-glycolic acid (PLGA) siRNA NPs with a variety of surface modifications to: 1) therapeutically treat HPV 18 related cervical cancer and 2) evaluate how each surface modification contributes to oncogene E6 expression as well. We hypothesize that the use of a hybrid modification—combining a stealth ligand and a CPP—will increase NP efficacy by enhancing transport through and internalization in tumor cells, respectively. We expect this will cause the knockdown of E6 to induce cancer cell senescence and apoptosis.

### Methods

#### Nanoparticle Fabrication

In this study, we synthesized and characterized PLGA NPs encapsulating siRNA, to evaluate knockdown of HPV 18 E6. To evaluate cellular uptake, the fluorescent dye Coumarin 6 (C6) was incorporated. NPs were synthesized using an oil-in-water single or water-in-oil double emulsion technique.

#### Nanoparticle Modification

Modification of nanoparticles was achieved by incorporating avidin-palmitate into the polymer matrix and subsequently reacting with biotinylated ligands.

#### Tumor Monolayer and Spheroid Formation

HeLa cervical cancer cells were the primary cell line used in the presented study. Tumor cultures were formed using the liquid overlay method.

#### Monolayer and Spheroid NP Internalization

Flow cytometry was used to quantify NP internalization. Spatial distribution of uptake was qualitatively verified using fluorescent microscopy. Analysis was performed at 1.5 hr and 24 hr incubation.

#### Quantifying HPV 18 E6 mRNA Knockdown

Cells were plated in 10-well plates, grown to 50% confluency, and were transfected with 100 nM siRNA for 3 days. RNA extraction was performed using both an RNA extraction kit and Trizol reagent. RNA was subsequently purified and validated via bioanalyzer and absorption spectra from 230-280 nm. Once pure, RNA was converted to cDNA and mRNA expression was determined using Real-Time PCR.

### Results

#### Nanoparticle Characterization

- **Figure 1**: Scanning electron microscopy (SEM) images of unmodified NPs (A-D) and histogram of size distribution (right).

#### Modified NP Surface Charge

- **Figure 2**: Nanoparticle surface charge.

#### Tumor Monolayer and Spheroid Uptake and Penetration

- **Figure 4**: Flow cytometry results quantifying NP cellular internalization at 1.5 hr and 24 hr in monolayer HeLa and VK2 cells, and HeLa tumor spheroids.

#### siRNA Design

**HPV18 E6 Oncogene:** Nucleotides 105-581

- **siRNA Targeting E6**: 5’ GAGGUAUUGUAAUUUGCAUdTdT 3’

- mRNA antisense

#### HPV 18 E6 mRNA Knockdown

- **Figure 7**: Schematic depicting NP treatment to evaluate E6 knockdown.

#### Expected (Ongoing) HPV 18 E6 Ct Values

**Table 1**: Expected critical threshold values E6 gene amplification.

### Future Studies

- **MPG and hybrid-modified NPs** show significantly higher efficacy in cellular internalization with both tumor monolayer and spheroid cells at both time points.

### Conclusions

- Therefore we hypothesize that MPG and hybrid-modified NP uptake will correlate with increased delivery and siRNA knockdown efficacy of oncogenic HPV 18 E6.

#### PEG-modified NPs demonstrate enhanced uptake in normal VK2 cells, suggesting tumor-specific targeting. This may indicate different cell-type specific cellular uptake mechanisms.

- Twenty-four hours after NP treatment, unmodified NPs become more homogeneously dispersed throughout the tumor spheroid.

- Knockdown of HPV 18 E6 is theorized to activate tumor suppressor protein p53, resulting in an increase in cell senescence leading to tumor cell apoptosis.

### Acknowledgements

Research is supported by the University of Louisville Cancer Education Program NIH/NCI R25-CA134283.
**ABSTRACT**

Purpose: Even with the increased funding in research, pancreatic cancer still claims the highest mortality rate of all major cancers with the 5-year survival rate at a dismal 5%. This can be directly correlated to the inability to detect the tumors before they reach stage III and IV. The tumors are very tiny and are located deep in the body making contemporary imaging modalities unable to spot them. The combination of Multi-spectral Optoacoustic Tomography (MSOT) and the mesoporous silica nanoparticles offers a possible solution by providing tumor targeting using fluorescent dyes and treatment through drug delivery. In order to combat the problems faced with pancreatic adenocarcinomas, we present a colloidal mesoporous silica nanoparticle (CMSN) utilizing a chitosan coating and the V7 peptide to provide pH specific dual targeting to deliver a contrast agent.

Methods: The nanoparticle was synthesized using a modified Stober method. Water, ethanol, octyltriethoxysilane (CTAB), trimethylamine (TEA), and tetraethyl orthosilicate (TEOS) were stirred under heat for 12 hours. Then the main structure of the nanoparticle with the CTAB and silica forming the core and the TEA acting as a complexing agent limiting aggregation. Afterwards, dialysis using a 1.1 solution of ethanol (100%) to 2M acetic acid removed the CTAB from the mesoporous silica core. Additionally, the CMSN synthesis was stirred with ethanol and ammonium nitrate in order to complete the scatted removal process. Afterwards, the particles were coated with chitosan/SMCC and then conjugated with APTES. Finally, V7hPSS, an amine to sulfhydryl linker, joined the V7 pH low insertion peptides to the surface of the nanoparticles resulting in a dual acidic pH targeted system. Characterization was done using Transmission Electron Microscopy (TEM), UV-Vis spectrophotometry (NanoDrop 2000), and zeta potential/DLS (Zetasizer Nano). To determine acidic pH specificity of V7-CMSN, Panc1 and SV2P10 cell lines were incubated in cell culture medium at either pH 7.4 or 6.6 followed by treatment with V7-CMSN. Particle uptake was determined using New infrared fluorescence and tissue phantoms. Finally, for in vivo testing, the same CMSNs were injected into mice with SV2P10 pancreatic tumors. Visualization of the mice using the MSOT system was performed 8 hours later.

Results: Nanoparticles were characterized using spectrophotometry, Dynamic light Scattering (DLS), and TEM. The UV-Vis readings using the NanoDrop 2000 showed very similar absorption curves for the IR-780 dye alone and the CMSNs-IR-780 dye. V7-CMSN demonstrated acidic pH specificity in both SV2P10 and Panc 1 cells at pH 6.6 that is 2X and 8X times higher than pH 7.4, respectively. In tissue phantoms, increased pH specificity was observed, 20X and 4X, respectively, using multispectral optoacoustic tomography (MSOT). Eight hours post injection, V7-CMSN accumulated specifically within orthotropic tumors as observed using MSOT.

Conclusion: The acidic pH specific dual targeting system using chitosan and the pH-LIP V7 resulted in tumor specificity. Preferential binding and dye release was 20X higher in pH 6.6 as compared to pH 7.4 in tissue phantoms. Also, the MSOT was able to detect tumor specific accumulation of V7-CMSN in vivo. The general lack of V7-CMSN binding at pH 7.4 suggests that the particle would not accumulate in off-target organs and prevent systemic accumulation.

**RESULTS**

- **Figure 1**: TEM image of CMSN particles. The CMSN nanoparticles were placed on a copper mesh grid and grid in order to be viewed on the Transmission Electron Microscope (TEM). The nanoparticles had a size of 32.2 ± 2.8 nm.

- **Figure 2**: UV-Vis spectrum of V7-CMSN - IR-780 and IR-780 dye. The absorbance curve of the two graphs is similar, but a slight shift to the right can be noticed most likely due to the aggregation of the dye inside of the CMSN. This is known as a redshift. The absorbance peak for IR-780 lies around 780nm while CMSN-IR-780 lies around 760nm.

- **Figure 3**: DLS measurements showing the addition of the chitosan coat. The intensity graphs from the Malvern Zetasizer Nano showing the distribution of sizes for both the CMSN with a red line and the CMSN with chitosan coating. It can be seen from the graphs that the chitosan coating increases the size of the nanoparticles drastically. However, we hypothesize that the much higher readings on the machine are due to the fact that adding a chitosan coat causes the particles to aggregate more than they usually would due to the gelatinous properties of the chitosan.

- **Figure 4**: Odyssey imaging in order to evaluate the conjugated V7-CMSN nanoparticles in vivo. SV2P10 and Panc pancreatic cancer cells in six wells plates were treated with 7.4 and 6.6 pH media and incubated with fully conjugated CMSN+ for 20 min. Then the plates were washed 2X times for 10 min each using the correct pH media and imaged on the Odyssey infrared imaging system.

- **Figure 5**: Tissue phantoms of dye loaded and conjugated CMSN particles, SV2P10 and Panc cancer cells were incubated for 20 min and washed three times. They then scraped, centrifuged and washed again before being put into a tissue phantom (left). The phantom was then imaged in the MSOT system.

**CONCLUSION**

- CMSN’s coated with chitosan and conjugated with V7 demonstrate acidic pH tumor specificity in two different pancreatic cell lines, SV2P10 and Panc1.
- The MSOT imaging modality is able to detect the IR-780 dye in the bound conjugated CMSNs in vivo.
- The small size of the V7-CMSN improves the tumor penetration and specificity while reducing off-target accumulation.
- With improved specificity and further studies these nanoparticles could be further tested in humans in combination with the handheld MSOT to help doctors in the operating room.

**FUTURE STUDIES**

- Evaluating release of the IR-780 dye through dye release in vitro studies.
- Testing different concentrations of chitosan to achieve a smaller and more efficient coated CMSN.
- Achieving an even higher pH specificity using the V7 peptide and continuing in vivo imaging using the pancreatic adenocarcinoma in the mouse model.
- Exploring other pH low insertion peptides for better specificity
- After further analysis, more theranostic nanoparticles using pH specificity to target tumors into clinical trials

**ACKNOWLEDGEMENTS**

This work was supported by NCI Grant R25-CA134283, the Brown Cancer Center High School Program, and the University of Louisville School of Medicine.
Inhibiting the Anaphase Promoting Complex/ Cyclosome: An Innovative Approach for Cancer Chemotherapy

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Departments of 1Pharmacology and Toxicology and 2Medicine, 3Brown Cancer Center, University of Louisville, Louisville, KY

Abstract

The anaphase-promoting complex/cyclosome (APC/C) is a large, E3 ubiquitin ligase that regulates the cell cycle, in particular the metaphase to anaphase transition in mitosis and the re-entry into G1 phase. Inhibition of the APC/C results in mitotic arrest and apoptosis in cancer cells. ANAPC2 and ANAPC11 are shown to be two vital subunits for APC/C function. In silico screening of ANAPC2 identified compounds that are predicted to prevent the association of ANAPC2 and ANAPC11. Thus, we hypothesize that the relative levels of the APC/C molecular targets, securin and cyclin B, will increase in cells treated with lead compounds. To gain better insight on the inhibition of the APC/C in cancer cells, HeLa cells were treated with lead compounds 3, 8, 10, and 11 at their respective IC50s for 24 h and then harvested to make lysates. The Bradford Protein Assay was used to determine the protein concentrations in each of the samples. To examine the relative levels of securin and cyclin B, a western blot analysis was performed. Results showed that cells treated with compounds 3, 8, 10, and 11 do not have increased levels of securin or cyclin B. However, future analysis may reveal that treatment with the lead compounds causes a decrease in the levels of ubiquitinylated cyclin B and securin. This research was supported in part by University of Louisville Cancer Education Program NIH/NCI grant R25 CA134283 and a Kentucky Lung Cancer Research Program grant to JCS.

Introduction

• Adjuvant chemotherapy has increased cancer survival rate
• However, people are still dying from this disease, justifying the need for new chemotherapeutics
• Taxanes are chemotherapeutics that disrupt microtubule function, leading to mitotic arrest and apoptosis in cancer cells
• However, taxanes like paclitaxel and docetaxel are known to be ineffective with cancer cells lacking functional Spindle Assembly Checkpoints or containing mutant tubulin and are often in short supply
• This project is directed towards developing new mitosis disrupting drugs

The Anaphase Promoting Complex/Cyclosome (APC/C)

The anaphase-promoting complex/cyclosome (APC/C) is a large, E3 ubiquitin ligase that regulates the cell cycle. It promotes the degradation of proteins and two essential substrates: Cdh1 and Cdc34. APC/C dysfunction is vital for cell proliferation and inhibition of the APC/C results in mitotic arrest and apoptosis in cancer cells.

• Homology structure of ANAPC2 was screened in silico against a small compound library to identify lead compounds to inhibit complex formation.
• The ability of lead compounds to stabilize APC/C targets was determined.

Materials and Methods

Lead Compounds:

These 4 compounds were dissolved in DMSO solution.

HeLa cells were plated in 6 cm dishes and were allowed to attach at the same time. Cells were harvested and protein extracts were prepared.

Cell Culture:

Conducted to determine protein concentration of lysates.

Performed Western Blot to detect levels of securin and cyclin B extracted from HeLa cells.

Materials and Methods

Step 1

Hypothesis

If lead compounds bind to ANAPC2 and inhibit the binding of ANAPC11, thus the APC/C molecular targets, cyclin B and securin, will accumulate in the treated cells.

Results

Figure 3. Poncova-S Stained blots

Membranes were stained in Poncova-S solution for 10 minutes on a shaker after proteins were transferred. They correspond to blots in Figure 4.

Conclusions

• There was no substantial change between the relative levels of cyclin B and securin in the HeLa cells treated with the lead compounds.
• Cyclin B and securin were stabilized by novel compounds.
• These data suggest that lead compounds 3, 8, 10, and 11 are not inhibiting the APC/C, under these conditions.

Acknowledgements

This research was supported in part by University of Louisville Cancer Education Program NIH/NCI grant R25 CA134283 and Kentucky Lung Cancer Research Program grant to JCS.
TARGETING ATP-BINDING CASSETTE TRANSPORTER (ABCB5) IN BRAF INHIBITOR RESISTANT MELANOMA

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DEPARTMENT OF SURGERY, UNIVERSITY OF LOUISVILLE SCHOOL OF MEDICINE

Abstract

Melanoma is the most dangerous form of skin cancer. More than 50% of metastatic melanoma patients have a specific mutation in the serine/threonine kinase BRAF. This results in constitutive activation of the RAS-RAF-MEK-ERK-MAP kinase pathway, which causes uncontrolled cell growth. Vemurafenib (also known as PLX4032) is an oral chemotherapy agent that targets the specific mutation V600E in the BRAF protein. It has shown very promising results, but melanoma cells rapidly develop resistance to the BRAF inhibitor PLX and disease progression ensues. The mechanisms by which melanomas develop BRAF inhibition resistance remain unknown, but the overexpression of ABCBS oncprotein, an ATP-binding cassette (ABC) transporter, has been shown to efflux anti-cancer drugs from melanoma. We hypothesize that ABCBS contributes to the PLX resistance of melanoma by effluxing anti-cancer drugs. Our goal is to determine whether ABCBS is highly expressed in BRAF inhibitor resistant melanoma cell lines and to demonstrate that inhibition of ABCBS may overcome BRAF inhibition resistance. We first established three PLX resistant melanoma cell lines, SK-28PLX, A2058PLX, and A375PLX. We showed that ABCBS was overexpressed in SK-28PLX and A2058PLX cell lines, but not A375PLX cells, and that ABCBS overexpression is associated with activation of p-ERK status. Knockdown of ABCBS by siRNA resulted in the re-sensitizing of PLX in A2058PLX resistant cells. These results confirm that overexpression of ABCBS may be one of the causes for resistance to the BRAF inhibitor in melanoma cells. It provides a starting point for personalized treatment strategy in targeting ABCBS in BRAF inhibitor resistant melanomas.

Results

BRAF mutation cell lines Culture in PLX 4032 BRAF inhibitor resistant cell lines

| SK-MEL-28 | 0.5 µM | SK-MEL-28 PLX |
| A2058 | 10 µM | A2058 PLX |
| A375 | 1 µM | A375 PLX |

Figure 1. Establishment of PLX resistant melanoma cell lines.

Figure 2. IC50 determination in PLX resistance melanoma cells.

Figure 3. Differential expression of ABCBS in 3 PLX resistant cell lines by real time RT-PCR and Western Blot.

Figure 4. Differential expression of ERK and p-ERK in three PLX resistant cell lines.

Figure 5. Downregulation of Akt and upregulation of p-Akt in three PLX resistant cell lines.

Figure 6. ABCBS knockdown in A2058PLX cell line by real time RT-PCR.

Figure 7. PLX re-sensitization after ABCBS knockdown in A2058 PLX resistant cell line.

Conclusions

- ABCBS was differentially expressed in PLX-resistant melanoma cell lines.
- ABCBS upregulation in PLX-resistant cell lines may not be associated with Akt and p-Akt levels.
- ABCBS upregulation in PLX-resistant cell lines may be associated with p-ERK levels.
- ABCBS knockdown in PLX resistant cells may re-sensitize PLX resistant cells to PLX.
- These findings may lead to personalized treatments for chemoresistant melanoma.

Future directions

- Examine effect of ABCBS knockdown in SK-28 PLX cell lines
- Examine change in ERK and p-ERK levels after ABCBS knockdown
- Evaluate association between ABCBS expression and drug resistance
- Differentiate the roles of glycosylated and unglycosylated ABCBS in chemoresistance

Acknowledgements

This research was supported by NCI R25 grant to the University of Louisville Cancer Education Program NIH/NCI (R25-CA134283).

I am grateful to the Cancer Education Program and the McMasters’s Lab of the University of Louisville for their support.

Exploring Energy Metabolism Changes in Vinyl Chloride Induced Non-Alcoholic Fatty Liver Disease (NAFLD)

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ABSTRACT

Background. Vinyl chloride (VC) is a ubiquitous environmental contaminant and ranks 4th on the ATSDR Inorganic Substances Priority List. We have previously reported increased histopathologic lesions in mice exposed to VC. Here we examined VinCI-induced changes in liver histology and cytokines in a mouse model. Materials and Methods. The VinCI-exposed mice were sacrificed at 16 weeks and livers were collected for histology and cytokine measurements. Results. When compared to controls, VinCI-exposed mice had increased liver injury by histology and cytokines. Conclusion. These data suggest that VC exposure may be linked to the development of NAFLD.

MATERIALS AND METHODS

Animals and treatments. Six week old CD1/luc mice were obtained from Jackson Laboratory (Bar Harbor, ME). Animals were fed either a low fat diet (LFD, 10% kcal) or a high fat diet (HFD, 42% kcal) for 10 weeks. At the end of this period, animals were given VC or chloroethanol (ClEtOH, 7% w/v) for 7 days.

Biochemical analysis and histology. Plasma levels of ALT, AST were determined using kits (Thermo Scientific, Waltham, MA). Livers were removed, weighed, and snap-frozen in liquid nitrogen. The liver samples were homogenized in 1X PBS buffer (10% w/v, Sigma-Aldrich Microwave, St. Louis, MO) and centrifuged at 12,000 rpm for 10 min. All samples were then prepared for vitamin and cholesterol analysis. Liver samples were extracted with chloroform/methanol (2:1, vol/vol) and the resulting extracts were measured for TAG, cholesterol, and bile acid.

REFERENCES