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Activity of Vitamin E Phosphate (VEP) Prodrugs of Gemcitabine in a Xenograft Model of NSCLC (NCI-H460)

Richard Daifuku[1]

[1] Epigenetics Pharma, Mercer Island, WA, 98040, USA

***** Corresponding author: rdaifuku@yahoo.com

Abstract: VEP nucleosides bypass two mechanisms of tumor resistance: nucleoside transport and kinase downregulation. Isoforms of VE have shown activity against solid and hematologic tumors. Gemcitabine was conjugated at the 5' position to either δ-tocopherol-MP (NUC050) or δ-tocotrienol-MP (NUC052).
NUC050 has been demonstrated to deliver gemcitabine-MP intracellularly. Its half-life IV in mice is 3.9 compared to 0.28 hours for gemcitabine (*European J Cancer.* 2016. 61(Suppl. 1):S119).
When tumors in nude mice reached 32 to 75 mg mm^3 (day 4) treatment was initiated with gemcitabine (120 mg/kg IP q3dx9), NUC050 or NUC052 (both 40 mg/kg qwkx4) and compared to saline control (SC).
Gemcitabine inhibited tumor growth but was not tolerated. NUC050 resulted in inhibition to tumor growth on days 11-31 ($p<0.05$), with a nadir of -73% compared to SC. Median survival was 25.5 days (SC) vs 33 days (NUC050) ((hazard ratio) HR=0.24, p=0.017). NUC052 had the dose increased to 50 mg/kg after 2 doses. NUC052 resulted in inhibition to tumor growth on days 14-27 ($p<0.05$), with a nadir of -45%, and median survival was 34 days (HR=0.27, p=0.033).
NUC050 and NUC052 have been shown to be safe and effective in a NSCLC xenograft. Studies have been initiated in a pancreatic cancer xenograft.

Keywords: gemcitabine; tocopherol; tocotrienol; xenograft; resistance

INTRODUCTION

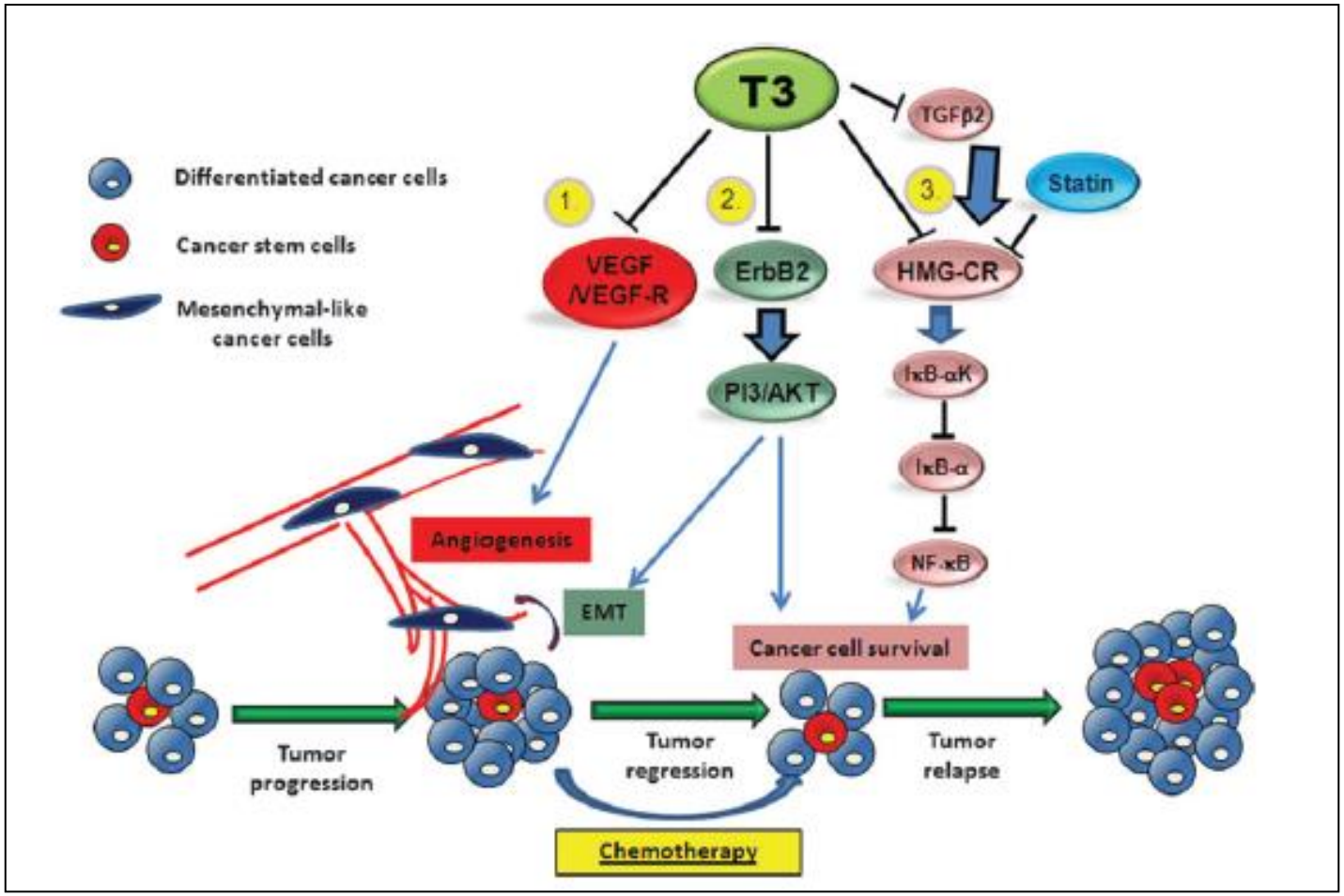
- VEP nucleoside prodrugs are designed to provide the following advantages:
  - Certain vitamin E isoforms have substantial anti-tumor activity.
  - Bypass two major mechanisms of tumor resistance to nucleosides, namely:
    - Nucleoside transport downregulation.
    - Kinase downregulation.
  - Prolong the half-life of the nucleoside analog:
    - In the case of cytidine analogs, VEP prodrugs are unlikely to be substrates for cytidine deaminase, the enzyme responsible for the short half-life of cytidines.
- For ease of synthesis and relevance, gemcitabine was conjugated with VEP as a model system.

Comparative activity of tocopherols and tocotrienols in tissue culture

- Comparative effects of tocopherols and tocotrienols on preneoplastic (CL-S1), neoplastic (-SA), and highly malignant (+SA) mouse mammary epithelial cell growth and viability in vitro.
  - Treatment with 0-120 µM α- and ɣ-tocopherol had no effect on cell proliferation.
  - Growth was inhibited 50% (IC_{50}) as compared with controls by treatment with the following in CL-S1, -SA and +SA cells, respectively :
    - δ-tocopherol: 55, 47, and 23 µM
    - α-tocotrienol: 12, 7, and 5 µM
    - ɣ-tocotrienol: 8, 5, and 4 µM
    - δ-tocotrienol: 7, 4, and 3 µM
  - Highly malignant +SA cells were the most sensitive and preneoplastic CL-S1 cells were the least sensitive to the antiproliferative and apoptotic effects of δ-tocopherol and tocotrienols

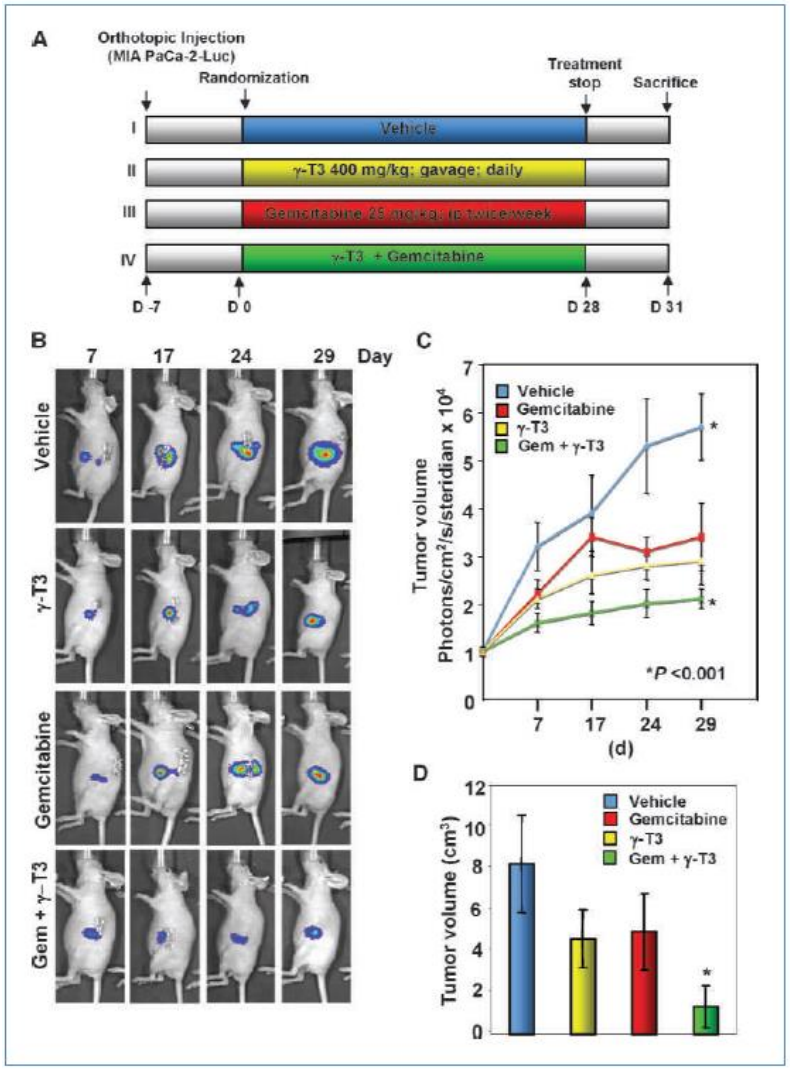
Proc Soc Exp Biol Med. 2000; 224(4):292-301

Tocotrienols (T3) target multiple signaling pathways in cancer



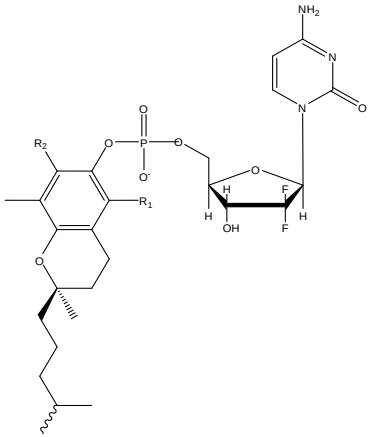
Carcinogenesis. 2012; 33:233–239

Gemcitabine and γ-tocotrienol are additive in a pancreatic cancer xenograft



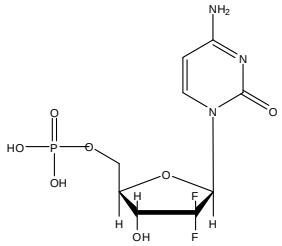
Cancer Res. 2010; 70(21):8695-8705

Proposed intracellular metabolism of VEP prodrugs

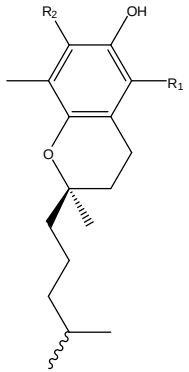


VEP-nucleoside

Phosphatase?
Phosphodiesterase?



Nucleoside-MP



Vitamin E

VEP-gemcitabine bypasses two mechanisms of resistance to gemcitabine

- In vitro, NUC050 has shown:
  - That cellular penetration is independent of nucleoside transport.
  - Intracellular delivery of gemcitabine monophosphate.

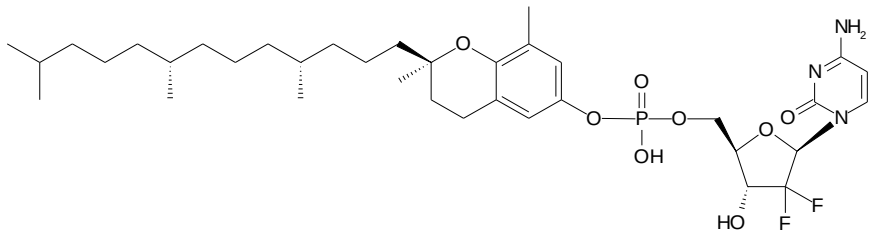
- In mice, NUC050 (δ-tocopherol phosphate gemcitabine) has shown:
  - When administered IV, a half-life of 3.9 hours.
    - Gemcitabine half-life is reported to be 0.28 hours.

- MTD of NUC050 was established at 40 mg IV qwk.

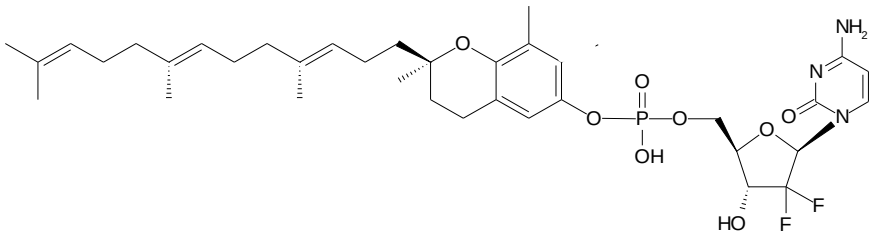
- A small pilot sudy in nude mice suggested efficacy of NUC050 against colon cancer (LoVo).
  - Two mice demonstrated maximum tumor weight reduction of 50.6% compared to 5 saline matched controls.

European J Cancer. 2016. 61(Suppl. 1):S119

VEP prodrugs of gemcitabine tested in H460 model of NSCLC



D-δ-tocopheryl phosphate-5'-gemcitabine triethylammonium salt (NUC050)



D-δ-tocotrienyl phosphate-5'-gemcitabine triethylammonium salt (NUC052)

METHODS

- 10^7 tumor cells from culture in Matrigel™ of H460 NSCLC were implanted subcutaneously in the flank of NCr-*nu/nu* mice.

- Study initiation began when the required number of mice had tumors of approximately 32 to 75 mm^3.
  - Mice (n= 10/group) received either:
    - Normal saline (negative control).
    - Gemcitabine 120 mg/kg IP q3d (positive control) x 9.
    - NUC050 40 mg/kg IV qwk x 4.
    - NUC052 40 mg/kg IV qwk x 4.

- Mice were euthanized per protocol when:
  - Their weight decreased more than 30% from the weight on the first day of treatment.
  - Their tumor reached 4,000 mm^3 in volume, ulcerated or sloughed off.
  - The animal was moribund.

STUDY CONDUCT

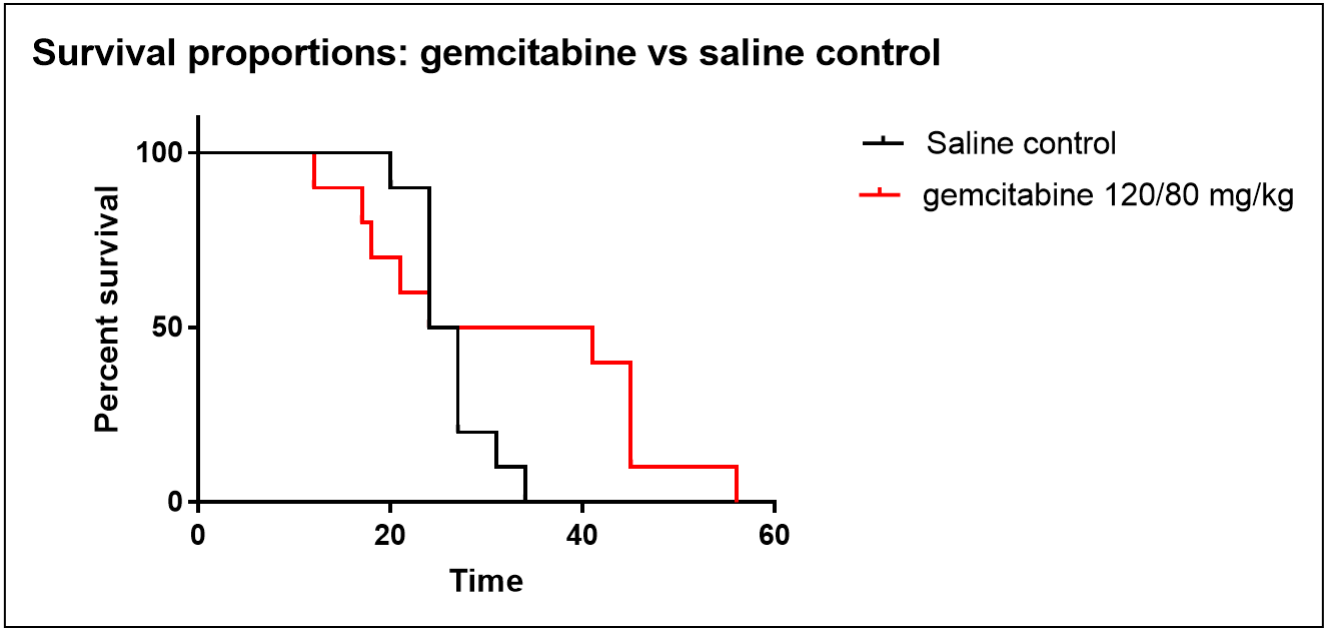
Mice treated with NUC050 and NUC052 were subdivided into two groups (n = 5), with one group administered normal saline (NS) as the vehicle and the other a nano-emulsion developed for NUC050.

In the course of the study, the following adjustments were made:

- After 4 doses, the gemcitabine dose was decreased to 80 mg/kg IP q3d because of weight loss and one death attributed to drug toxicity.
- After receipt of two doses of NUC050, it was noted that mice treated with NUC050 in NS had better outcomes than those treated with emulsion:
  - Tumor mean volume 183.4 mm^3 vs 513.0 mm^3 ($p = 0.031$, student t-test);
  - Mean mouse weight 20.9 vs 18.3 g ($p = 0.014$, student t-test).
  - Protocol was amended and all mice received NS.
- No vehicle toxicity was noted for NUC052, however, the mice receiving emulsion were switched to NS on the same study day and the dose increased to 50 mg/kg.

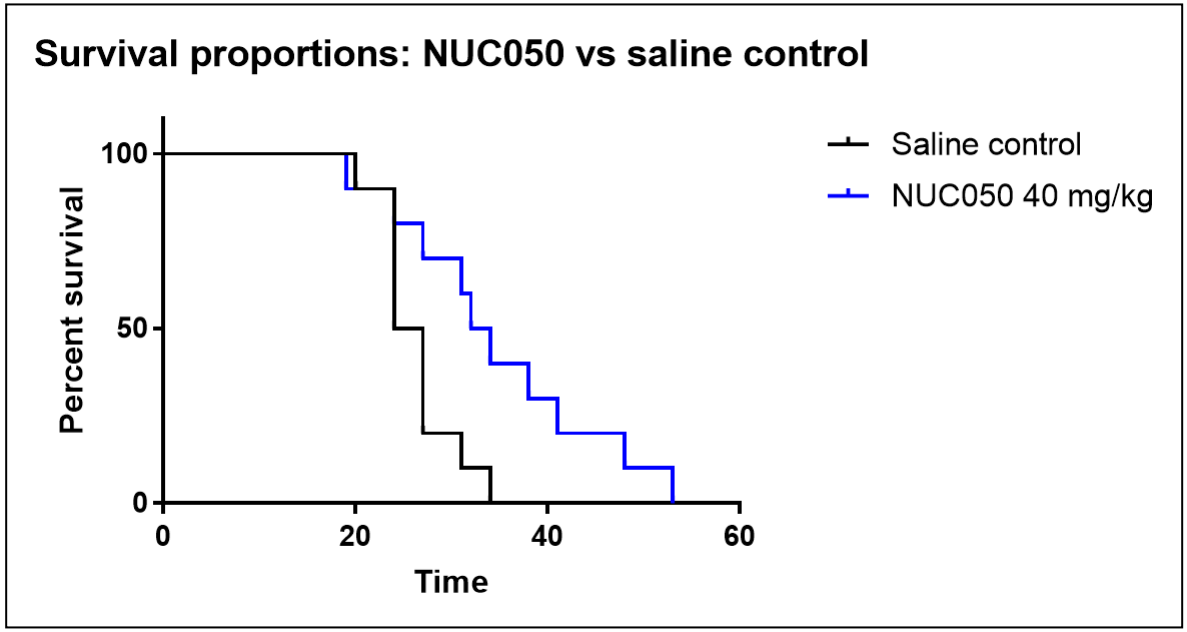
RESULTS AND DISCUSSION

Gemcitabine was toxic at doses tested



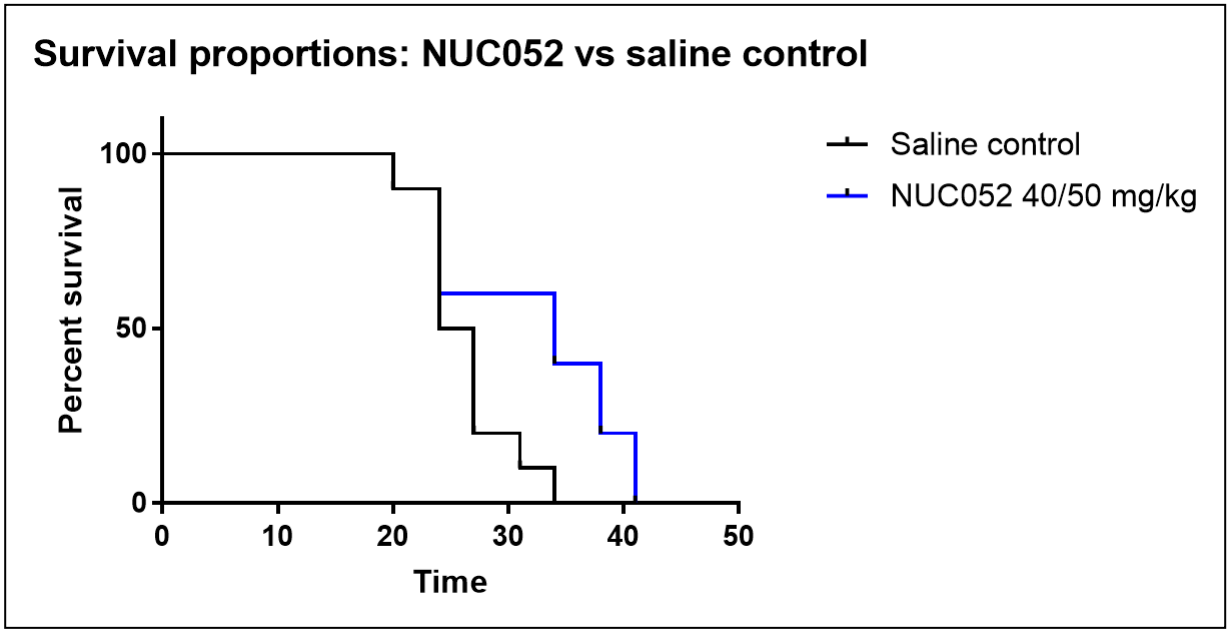
Median survival saline 25.5 days, gemcitabine 32.5 days, (hazard ratio) HR = 0.46 (p = 0.18). {Percent survival = [10 – (deaths + mice euthanized)] x 10}

NUC050 significantly improved survival of mice in xenograft model of NSCLC



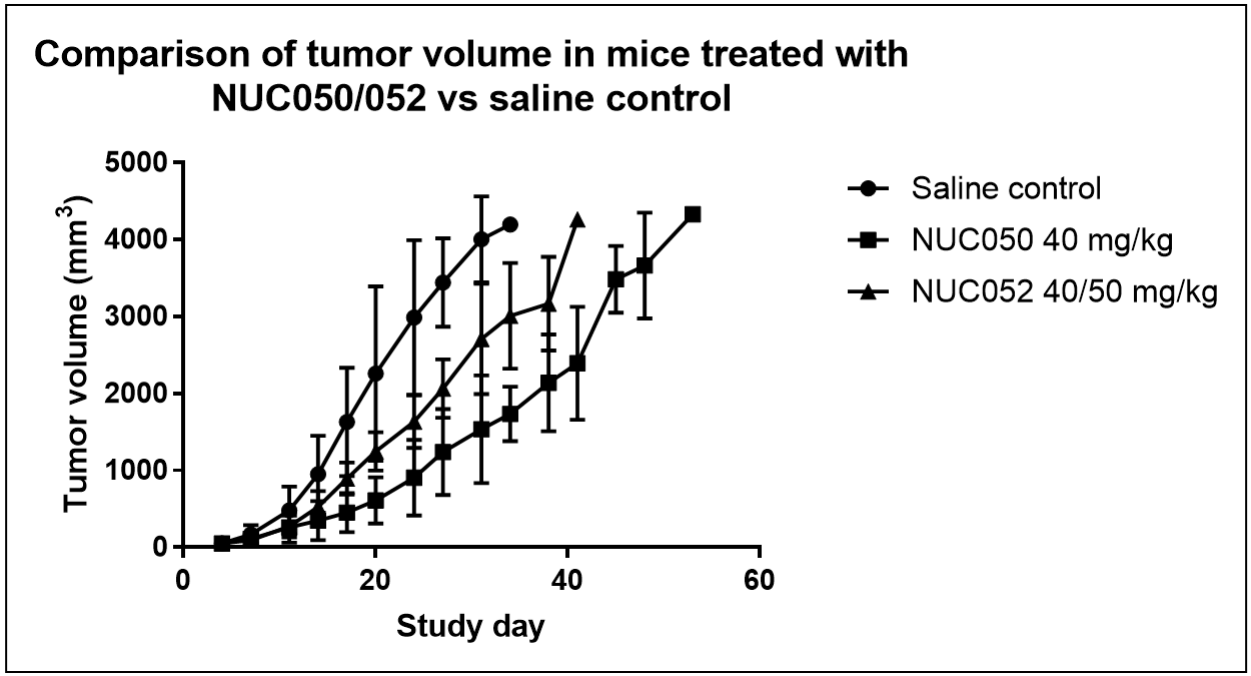
The median survival for NUC050 was 33 days compared to 25.5 days for saline, HR = 0.24 (p = 0.039).

NUC052 significantly improved survival of mice in xenograft model of NSCLC



NUCC052, median survival was 34 days compared to 25.5 days for saline, HR = 0.27 (p = 0.033).

NUC050/052 significantly inhibited tumor growth in a mouse xenograft model of NSCLC



- Tumor weights were significantly lower than saline control ($p < 0.05$) for NUC050 on Study days 14 through 31, while the same is true for NUC052 on study days 14 through 27.
- Tumor weights were significantly lower ($p < 0.05$) for NUC050 compared to NUC052 on study days 17 through 34.

Discussion

- NUC050 significantly improved survival and inhibited tumor growth after 4 weeks of treatment:
  - The median survival for NUC050 was 33 days compared to 25.5 days for saline, HR = 0.24 ($p = 0.039$).
  - There was significant inhibition of tumor growth ($p < 0.05$) compared to saline on study days 14-31.
  - NUC050 was significantly better at inhibiting tumor growth on study days 17-34 than NUC052.
  - All deaths (3) occurred in the subgroup that used the nano-emulsion as a vehicle.
    - Cause of deaths is unknown but may be related to uptake of the drug by the reticuloendothelial system.

- NUC052 significantly improved survival and inhibited tumor growth after 4 weeks of treatment:
  - median survival was 34 days compared to 25.5 days for saline, HR = 0.27 ($p = 0.033$).
  - There was significant inhibition of tumor growth ($p < 0.05$) compared to saline on study days 14-27.

Discussion (continued)

- Gemcitabine was used as a positive control, however it was toxic at the doses tested:
  - Dose and regimen based on literature for treatment of H460 (*Anticancer Res*. 2014, 34:6951-6960).
  - There were 7 animal deaths noted on study, which resulted in no significant improvement in animal survival compared to saline control.
    - 1 deaths on dose of 120 mg/kg.
    - 6 deaths on dose of 80 mg/kg.
  - Toxicity complicates assessment of tumor growth inhibition.
    - There was significant inhibition of tumor growth ($p < 0.05$) on study days 11-31.

CONCLUSIONS

- NUC050 (40 mg/kg IV qwk) and NUC052 (40-50 mg/kg IV qwk) were safe and effective when administered in saline in a xenograft model of NSCLC
  - Both NUC050 and NUC052 significantly improved survival and inhibited tumor growth.
  - Despite literature suggesting that δ-tocotrienol is more effective than δ-tocopherol at inhibition of tumor growth, NUC050 may be more effective than NUC052.
  - The nanoemulsion developed for NUC050 was toxic.
    - It is likely that efficacy would have been improved had all mice only received saline as vehicle.
  - Conclusions about the relative efficacy of NUC050 or NUC052 compared to gemcitabine cannot be drawn because of gemcitabine toxicity.

Future directions

A study has been initiated in a mouse pancreatic cancer xenograft model with a tumor moderately resistant to gemcitabine, MiaCaPa-2

This study is comparing:

- Gemcitabine 60 mg/kg IP q3d
- NUC050 40 mg/kg IV qwk
- NUC052 50 mg/kg IV qwk

ACKNOWLEDGMENTS

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