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

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**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 γ-tocopherol had no effect on cell proliferation.
  • Growth was inhibited 50% (IC₅₀) 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
    • γ-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

Tocotrienols (T3) target multiple signaling pathways in cancer

Carcinogenesis. 2012; 33:233–239
Gemcitabine and γ-tocotrienol are additive in a pancreatic cancer xenograft.
Proposed intracellular metabolism of VEP prodrugs

VEP-nucleoside → Nucleoside-MP → Vitamin E

Phosphatase? Phosphodiesterase?
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 study 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.

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VEP prodrugs of gemcitabine tested in H460 model of NSCLC

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

D-5-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-\textit{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.
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:
  • 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
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