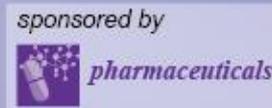




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NUC041: a Prodrug of the DNA Methyl Transferase Inhibitor (DNMTI) and Ribonucleotide Reductase Inhibitor NUC013

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NUC013 (5-aza-2',2'-difluoro-deoxycytidine) is preclinically safer and more effective than decitabine (*Pharmaceuticals* 2017, 10(3)). 5-azacytidines are hydrolyzed at the cytosine's 6-position, but in vivo, the short half-life is governed by deamination. For decitabine, attempts have been made to address these issues with continuous infusion, but use of such a regimen is limited by inconvenience and toxicity.

A hydrophobic prodrug was developed for packaging in a hydrophobic matrix to protect NUC013 from hydrolysis and deamination. In an aqueous environment, the hydrophobic moieties are readily hydrolyzed with release of NUC013. This was achieved by conjugating NUC013 with trimethylsilyl (TMS) at the 3' and 5' position (NUC041).

The half-life of NUC013 administered IV in mice is 20.1 minutes. Below, PK following administration of a dose of 3mg of NUC041 IM in a PEG-phospholipid-depot to mice:

Analyte	C _{max} (ng/mL)	T _{max} (hr)	t _{1/2} (hr)	AUC _{INF} (hr·ng/mL)	MRT _{INF} (hr)
NUC041	4210	0.5	1.7	6261	2.6
NUC013	1333	1	3.4	5813	5.1

In an ongoing study, NUC041 was administered at a dose of 3mg qwk to nude mice with NSCLC H-460 xenograft. After 3 days of treatment (n=8), tumor starting volume had decreased by 4%. However, toxicity, likely from vehicle, was also observed at this dose.

Keywords: NUC013; decitabine; NUC041; DNA methyl transferase; ribonucleotide reductase



INTRODUCTION

- NUC013 (5-aza-2',2'-difluoro-deoxycytidine) has been shown to be safer and more effective than decitabine both in vitro and in vivo (*Pharmaceuticals*. 2017, 10(3)).
- Like all 5-azacytidines, NUC013 has a short half-life, presumably due to:
 - Deamination by cytidine deaminase.
 - Hydrolysis at the 6-position of the cytosine base.
- In the case of decitabine, attempts have been made to improve efficacy through continuous infusion but these have been hampered by inconvenience and toxicity (e.g., *J Clin Oncol*. 2005; 23(17):3897-905.).

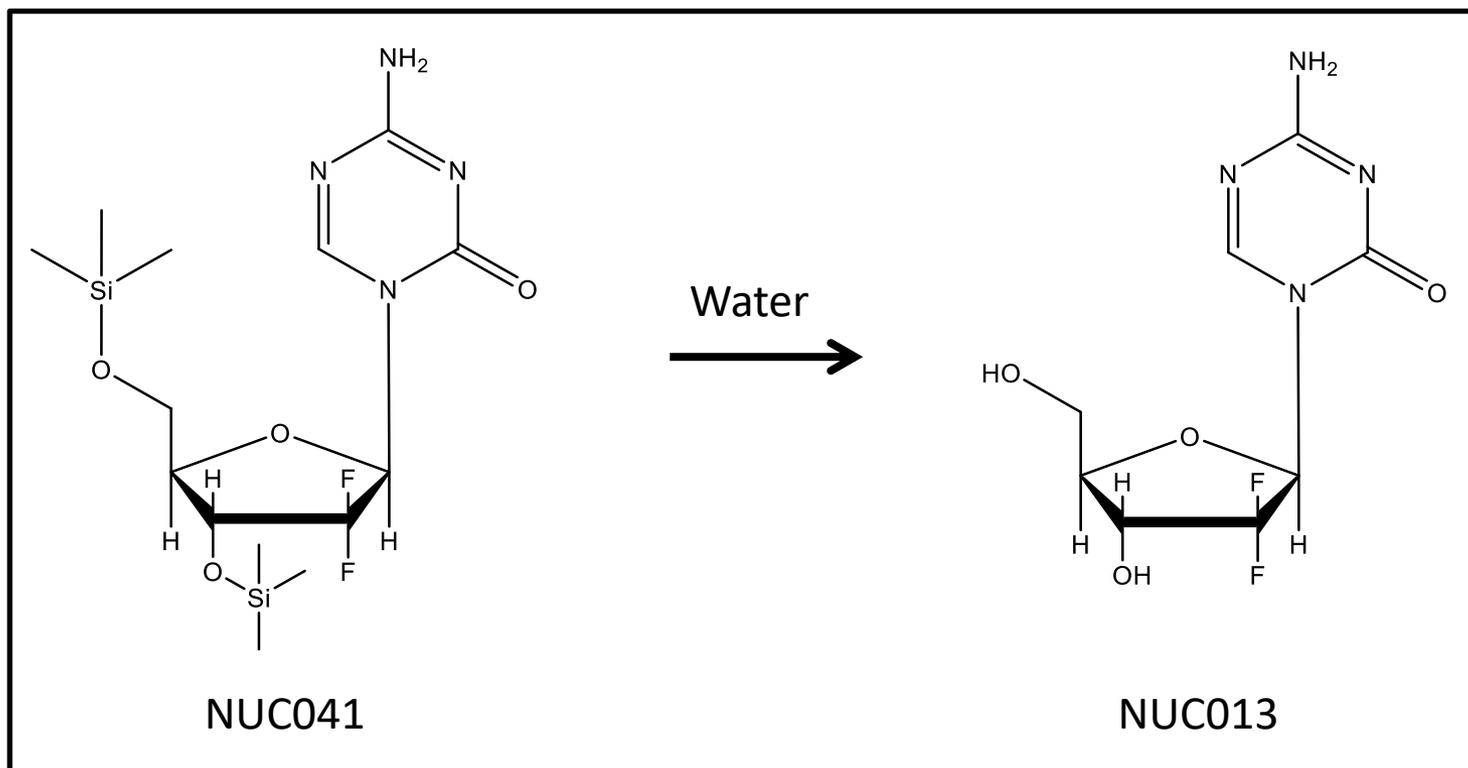


Prodrug design

- Classical cytidine prodrug motifs, such as alterations at the 4-position amino (e.g., *Antiviral. Res.* 2005; 67(1):1-9) may further destabilize 5-azacytidines.
- NUC041 is based on a different approach:
 - The goal was to make NUC013 hydrophobic, such that it could be packaged and protected in a hydrophobic matrix.
 - NUC013 was conjugated with trimethylsilyl (TMS) at the 3' and 5' positions.
 - Upon release from the hydrophobic matrix, the TMS residues are hydrolyzed and release NUC013, before NUC013 undergoes degradation.



NUC041 is hydrolyzed to NUC013



TMS toxicology

Extensive toxicologic studies performed by Dow Corning for NASA demonstrate TMS safety in animals

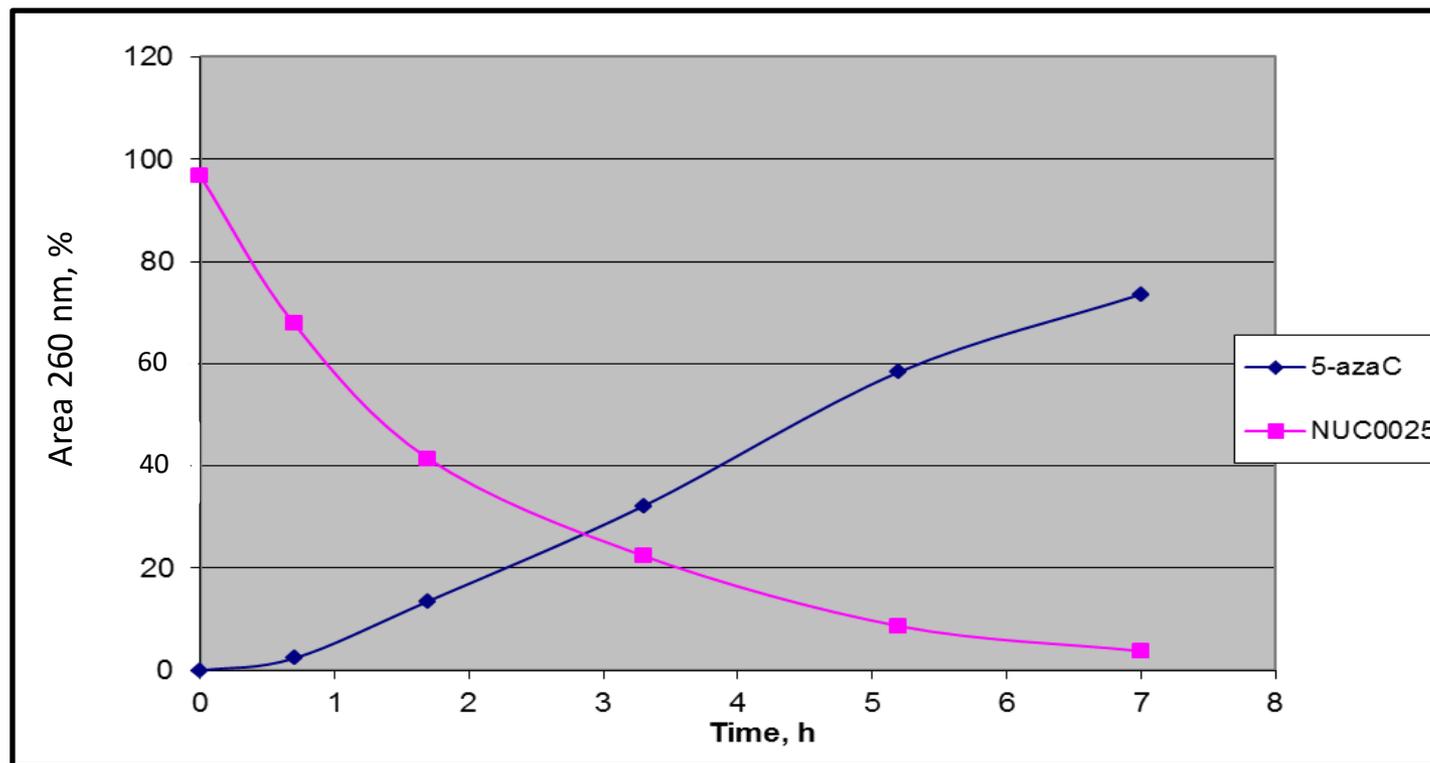
(<https://ntrs.nasa.gov/archive/nasa/casi.ntrs.nasa.gov/19950006466.pdf>):

- IP or IV administration of TMS to rats, guinea pigs and rabbits in doses of 100-200 mg/kg produced light to moderate anesthesia.
- Oral subchronic and chronic studies at doses of up to 250 mg/kg showed no significant toxic effects.



RESULTS

Hydrolysis of TMS prodrug of 5-azacytidine (NUC025)



Initial feasibility studies were performed with the TMS prodrug of 5-azacytidine (5-azaC) and showed efficient release of 5-azaC from NUC025 at 20°C in PBS.



TMS-prodrug has similar activity to nucleoside in vitro

Compound	Breast MDA-MB-231 (μM)	Colon HCT-116 (μM)	Leukemia L1210 (μM)
5-azaC	1.64	0.62	1.7-2.4
NUC025	3.28	1.08	1.18

5-azaC and NUC025 have similar GI_{50} in cell lines MDA-MB-231, HCT-116 and L1210.

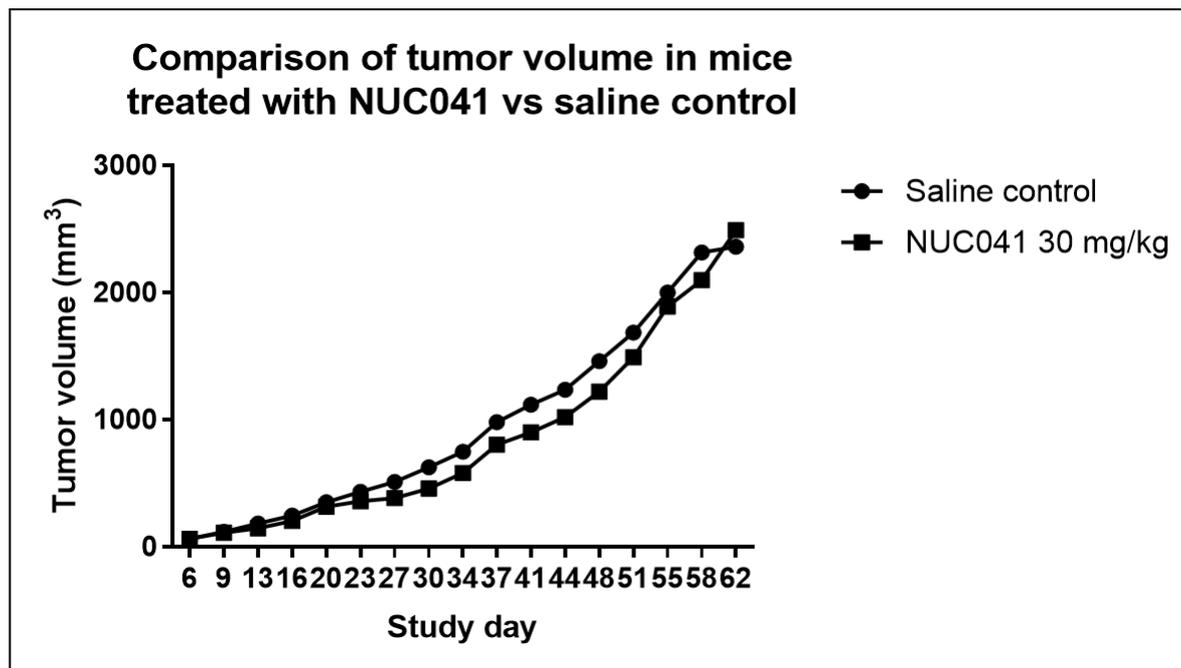


Initial studies with NUC041

- The pharmacology of NUC041 is highly depend on the vehicle.
 - Initial studies were carried out with a NUC041 nanoemulsion.
 - NUC041 nanoemulsion resulted in:
 - Half-life of NUC013 derived from IV administration of NUC041 equal to 15 minutes.
 - Compares to half-life of IV NUC013 of 20.1 minutes.
 - On an equimolar basis, the area under the curve (AUC) of NUC013 derived from IV NUC041 was only 5.1% of IV NUC013.
 - The volume of distribution at a steady state (V_{ss}) of NUC041 was 4,431 ml/kg.
 - High volume of distribution suggested that NUC041 nanoemulsion might be taken up by RES or that NUC041 might be taken up by adipose tissue.



NUC041 nanoemulsion was safe and moderately effective in a pilot study in a mouse xenograft model of colon cancer (LoVo)



30 mg/kg NUC041 nanoemulsion administered IV qd for 3 consecutive days per week for 3 weeks, was tolerated and significantly inhibited the growth of LoVo ($p < 0.05$) on study days 27 and 30 compared to saline control.



Formulation goals following experience with nanoemulsion

1. Improve circulating half-life
2. Decrease volume of distribution
3. Increase AUC

A decision was made to attempt to address these issues with a pegylated phospholipid depot of NUC041 (PPD-NUC041).

- An intramuscular (IM) route was chosen instead of a subcutaneous route to increase the efficiency of delivery and minimize localization of NUC041 to adipose tissue.
- Half-life of depot formulations is volume dependent and the maximum IM volume that can be administered to a mouse at one site is 0.05 ml.



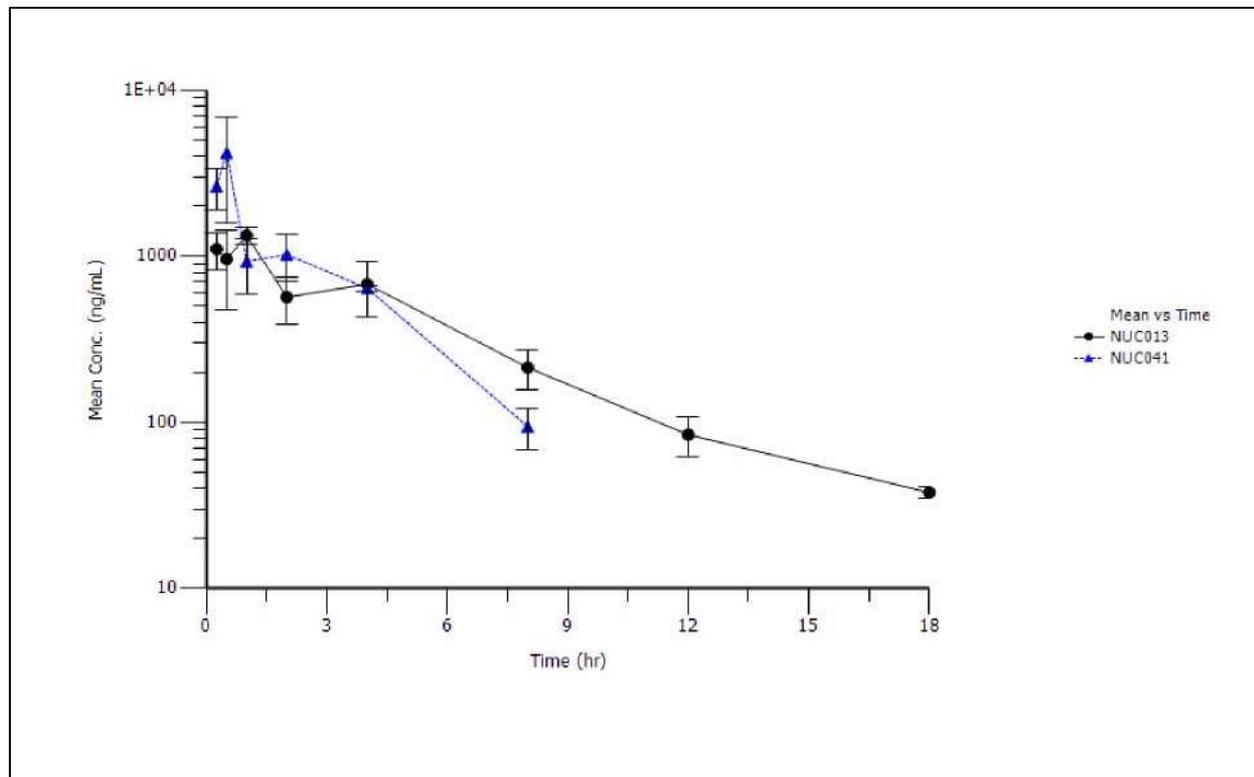
PPD-NUC041 pharmacokinetics

Analyte	C_{\max} (ng/mL)	T_{\max} (hr)	$t_{1/2}$ (hr)	AUC_{INF} (hr·ng/mL)	MRT_{INF} (hr)
NUC041	4210	0.5	1.7	6261	2.6
NUC013	1333	1	3.4	5813	5.1

Mice were injected IM with 3 mg NUC041 formulated in 0.05 ml PPD.



Time vs concentration plot of NUC041 and NUC013



Mean concentrations (\pm standard deviations) of NUC-041 and NUC-013 in blood over time following IM administration of NUC-041 (3 mg/mouse) to mice in PPD.



PPD-NUC041 pharmacokinetics

- Pharmacokinetics of NUC013 derived from IM PPD-NUC041 are dramatically improved over NUC013 from IV nanoemulsion NUC041.
 - Half-life of NUC013 derived from IM PPD-NUC041 is 10.1-times longer than IV NUC013.
 - AUC of NUC013 from PPD-NUC041 on an equimolar basis is 8.6% of IV NUC013.
 - V_z/F (apparent volume of distribution) NUC041 = 1,172 ml.



Pilot mouse tolerability study

- Two mice were given a single dose of 3 mg NUC041 IM and survived without weight loss.
- Approximately one month later, these same mice were administered 3 mg IM qd x 4 and died.
 - Issue as to whether toxicity was primarily due to NUC041 or vehicle.

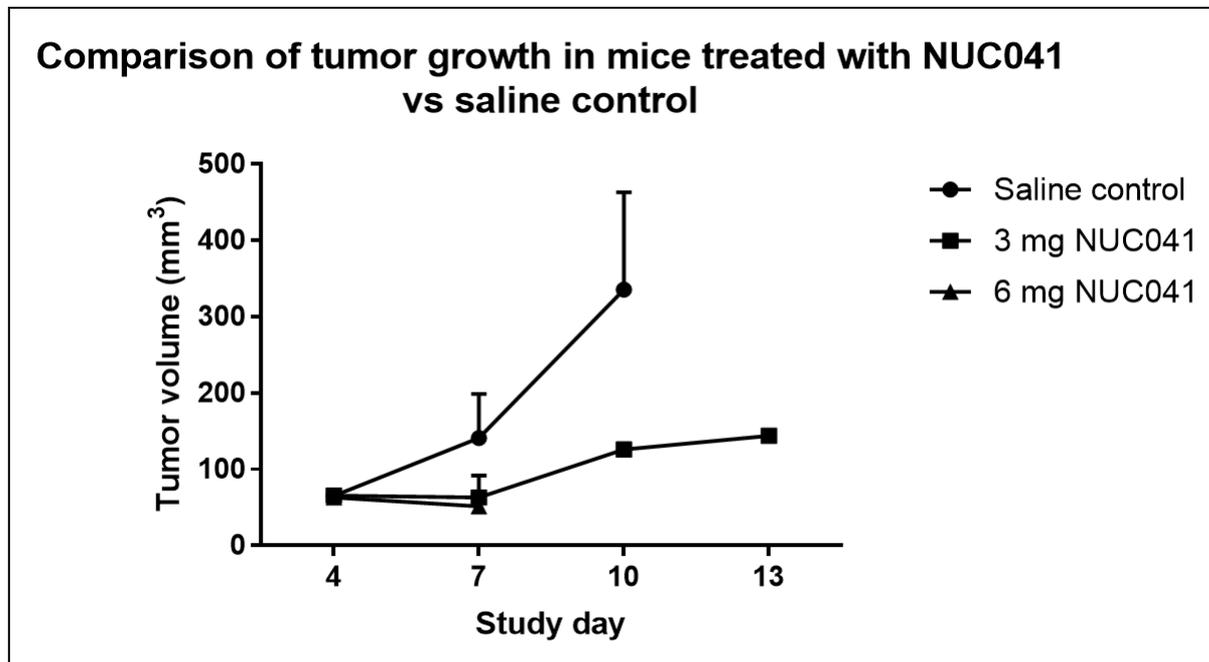


NSCLC Study 1 methods

- 10^7 tumor cells from culture in Matrigel™ of H460 non small cell lung cancer (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 mm³.
 - Mice (n= 10/group) received either:
 - Normal saline (negative control).
 - NUC041 3 mg IV qod (mean dose of 132 mg/kg).
 - NUC041 6 mg IV qod (mean dose of 268 mg/kg).
 - Dose held if weight loss > 5% pretreatment weight.



PPD-NUC041 inhibits tumor growth but is toxic in a mouse xenograft model of NSCLC



- Two mice in 6 mg NUC041 group received a single dose and died.
- Eight mice received single 3 mg dose:
 - Seven mice died.
 - One mouse was redosed after 6 days and died.



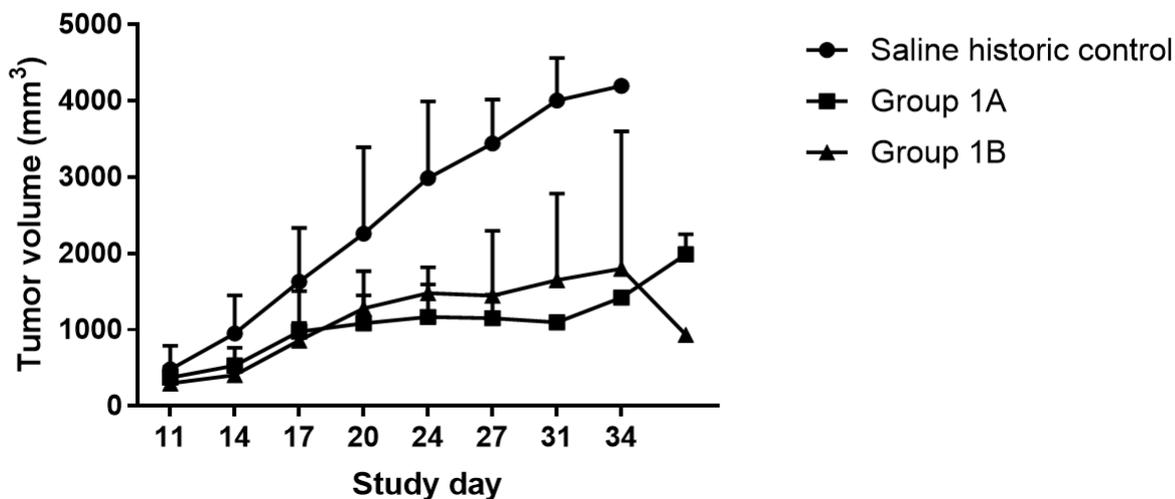
NCSL Study 2 methods

- Study began with mice from saline control group on study day 10.
 - Mice had tumors of ranging from 172 to 527 mm³.
 - Mice received either (n = 5):
 - Group 1A: NUC041 1.8 mg IM (mean dose of 73 mg/kg).
 - Group 1B: NUC041 1.2 mg IM (mean dose of 52 mg/kg).
 - Dose held if weight loss > 5% pretreatment weight.
- Study 2 is ongoing.



PPD-NUC041 significantly inhibits tumor growth in a mouse xenograft model of NSCLC

Comparison of tumor growth in mice treated with NUC041 vs historic saline control



All mice in control group died (n = 1) or were euthanized (n = 9) per protocol by study day 34 as a result of tumor growth or tumor ulceration.



- **Study 2 conduct and results Group 1A**

- Group 1A (n = 5):
 - Three mice died between study days 15-17.
 - Remaining mice (n = 2) had weight loss of 10.9% for 2 days following injection of vehicle alone on study day 19.
 - On study day 26, mice were administered 50 µg dexamethasone IP 30 minutes prior to 3 mg NUC041 IM.
 - Tumor growth was significantly inhibited compared to historic control ($p < 0.05$) on study days 17, 24, 27 and 31.
 - Tumor regression observed between study days 24 and 31.



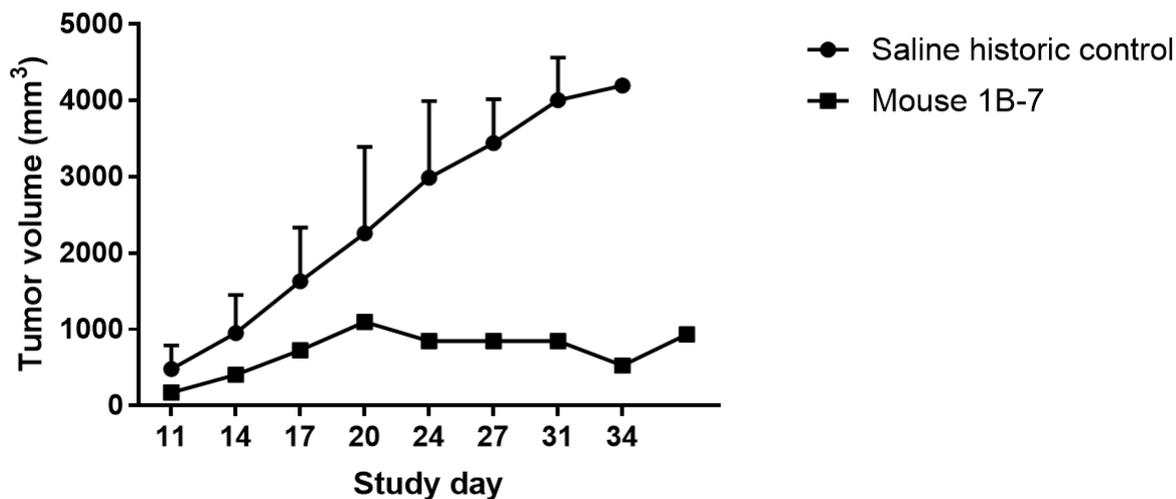
Study 2 conduct and results Group 1B

- Group 1B (n = 5):
 - One mouse died on study day 15.
 - Remaining mice (n = 4) were pretreated with dexamethasone 30 minutes before NUC041 1.2 mg on study days 19, 21 and 23.
 - On study day 19, pretreated with 50 µg dexamethasone IP.
 - Study day 21: 2.5% weight gain.
 - On study day 21 and 23 pretreated with 25 µg dexamethasone IP.
 - Study day 23: 9.9% weight loss.
 - Per protocol, study drug should have been held on study day 23.
 - Two mice died on study day 26.
 - One mouse euthanized on study day 34 for tumor ulceration.
 - Tumor growth was significantly inhibited compared to historic control ($p < 0.05$) on study days 24 and 27.
 - Tumor regression noted between study days 24 and 27.



NUC041 may lead to tumor regression

Comparison of tumor growth in mouse treated with NUC041
vs historic saline control



- Group 1B mouse 1B-7 was last treated on study day 23.
- Tumor volume remained stable or regressed over a period of 14 days, up to 11 days following last drug injection.



DISCUSSION

- By design, NUC041 pharmacology is highly dependent on formulation.
 - This has complicated determination of NUC041 safety and efficacy.
 - Search for a formulation providing desirable PK without toxicity.
- PPD-NUC041 has been shown to provide a prolonged half-life for NUC013 in mice.
 - Half-life of NUC041 in such depot formulations is dependent on the injected volume. Hence, it is likely that half-life of NUC013 would be significantly further prolonged in larger animals or humans.



- NUC041 has shown effectiveness at suppressing NSCLC tumor growth:
 - Assessment of efficacy has been compromised by the toxicity of PPD.
 - Limited data suggest that tumor growth suppression or even regression may persist for days beyond single treatment.
 - Tumor regression observed despite large starting tumor size.
- Pharmacodynamic response is not directly related to AUC of NUC013 but may be related, at least in part, to duration of exposure.
 - NUC013, as all azacytidines, has a short half-life as a result of deamination and hydrolysis.
 - Azacytidines need to be phosphorylated and incorporated in DNA to be active as DNMTI.
 - Longer duration of exposure to drug will allow replicating tumor cells opportunity for such incorporation.



- In later stages of treatment, there is evidence of tumor regression.
 - These results are compatible with results from ex vivo experiments with 72 hour exposure to nM doses of decitabine (*Cancer Cell*. 2012; 21:430-446).
 - Such exposure may result in loss of tumor stem cells.
 - Epigenetic changes are maintained in the target cells after drug removal and accumulate with each low dose treatment until there is a complete loss of cancer stem cell potential.



- PPD has been shown to be toxic to mice:
 - Injection of formulation alone led to > 10% weight loss.
 - Dexamethasone premedication appears to be able to mitigate toxicity at a dose of 50 µg/mouse but not 25 µg/mouse.
 - These findings are compatible with those from a study of mice injected with Staphylococcal enterotoxin, where 50 µg/mouse of dexamethasone protected mice but 10 µg/mouse did not (*Antimicrob Agents Chemother.* 2006; 50(1):391-395).
 - Toxicity is most likely due to activation of complement by PEG-phospholipid (e.g., *Toxicology.* 2005; 216:106-121).
- Contribution, if any, of NUC041 to the observed toxicity of PPD in mice has not yet been established:
 - NUC013 MTD has not been established but is > 120 mg/kg IV for 3 consecutive days per week (*Pharmaceuticals.* 2017; 10(3)) but as noted above for activity, toxicity could also be related to the duration of exposure.



CONCLUSIONS

- NUC041 formulated in PPD for IM injection has shown significant improvement in the half-life of the active, NUC013, in mice.
- NUC041 has been shown to suppress tumor growth even in large tumors, such tumor growth suppression appears to persist for days following a single injection and may lead to tumor regression.
 - Further studies are necessary to confirm NUC041 safety and efficacy.
- The PPD formulation has been shown to be toxic to mice.
 - Toxicity is most likely related to complement activation.
 - Toxicity may be mitigated by premedication with dexamethasone.



- The potential contribution of NUC041 to the observed toxicity is currently unknown.
 - NUC013 at higher doses than administered in this study has not been shown to be toxic.
- A possible avenue to further improve the AUC of NUC013 might be an IV formulation of NUC041 in a vehicle such as a PEG-liposome.



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