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PRINCIPAL INVESTIGATOR**:** **Dr. Ramandeep Rattan**, PhD

CONTRACTING ORGANIZATION: **Henry Ford Health System**
Detroit, MI 48202-3450

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Ramandeep Rattan, Phd

E-Mail:rrattan1@hfhs.org

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14. ABSTRACT
Despite the fact that there have been significant developments in anti-cancer technology, ovarian cancer still remains one of the leading causes of gynecological cancer deaths in the United States. To contribute towards novel approaches with minimal toxicity in treatment of ovarian cancer we proposed the present work, where we are integrating the field of nanotechnology with ovarian cancer cell's unique property of overexpressing folic acid receptor alpha (FR-a) to specifically target ovarian cancer. A cerium oxide nanoparticle, called Nanoceria (NCe), that has the ability to act as an anti-oxidant in similar capacity as biological anti-oxidants was selected. NCe in our hands exhibited efficacy in inhibiting ovarian tumor growth in vivo. Taking advantage of the nanotechnology, NCe was conjugated with molecules of folic acid to specifically target ovarian tumors as they are known to over-express folate receptor. This would result in specific killing of ovarian cells by NCe while minimizing toxicity to normal tissue. Dr Seal's laboratory (UCF) was able to successfully conjugate NCe to folic acid to prepare folic acid tagged Nanoceria called FA-NCe. FA-NCe was tested in vitro and was observed to have higher anti-growth activity against ovarian cancer cells. When given in animals, FA-NCe was more potent in inhibiting ovarian tumor growth than NCe alone and enhanced the cytotoxicity of cisplatin in vivo.

15. SUBJECT TERMS
Specific targeting of ovarian cancer; nanoceria; nano-particle; folate receptor.

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INTRODUCTION

Despite the fact that there have been significant developments in anti-cancer technology, such as chemotherapy, vaccines and hormone therapy, ovarian cancer, ovarian cancer still remains one of the leading cause of gynecological cancer deaths in the United States. The current standard therapy of surgical removal followed by chemotherapy results in overall tumor reduction with survival rate less than 50%. Chemotherapy regimens for patients with ovarian cancer include cisplatin and paclitaxel for which most of the patients develop resistance and as side effect have severe toxicity to normal cells. Clearly, other novel approaches with minimal toxicity are urgently needed which may specifically target ovarian cancer cells with minimal or no effects on normal cells.

To join in this effort we proposed the present work, where we planned to integrate the field of nanotechnology with ovarian cancer cell's unique property of over-expressing folic acid receptor alpha (FR-a) specifically target ovarian cancer.

Our experimental nanoparticle is **Nanoceria (NCe)**, a cerium oxide nanoparticle. Nanotechnology-based tools and techniques are rapidly emerging in the fields of medical imaging and targeted drug delivery. Cerium oxide is a rare-earth oxide that is found in the lanthanide series of the periodic table. Nanocrystalline cerium oxide (nanoceria) exhibits a blue shift in the ultraviolet absorption spectrum, the shifting and broadening of Raman allowed modes and lattice expansion as compared to bulk cerium oxide indicating its unique properties. Nanoceria has emerged as a fascinating and lucrative material in biomedical science due to its unique ability to switch oxidation states between (III) and (IV) depending upon the environment. The ability to switch between mixed oxidation states of nanoceria is comparable to biological antioxidants. This imparts nanoceria with a very important biological property of radical scavenging which can be tuned based upon the retention of oxygen vacancies (defects) and concentration of Ce3+ species in nanoceria (1). NCe synthesized in Dr. Seal's laboratory has been tailored to retain mixed valence states (3+ and 4+) with the size in the range of 3-5nm. The reversibility of oxidation state is the key property in making NCe a potent antioxidant, thereby eliminating the need for repeated dosage (2). When generated intracellularly as a consequence of normal metabolism or pathological states, free radicals can strip electrons from cellular macromolecules and render them dysfunctional. Previous studies have demonstrated that cerium oxide nano-particles possess excellent antioxidant properties and act as potent, regenerative free radical scavengers in biological systems (1-3). These regenerative antioxidant properties are due, in part, to the valence structure of the cerium atom combined with inherent defects in the crystal lattice structure, which are magnified at the nano-scale.

Our proposal was based on our preliminary findings (Published in Plos One), where we demonstrated that NCe by itself had the ability to abate ovarian tumor growth in an in an *in vivo* nude mouse model implanted with intraperitoneal A2780 human ovarian cancer cells. In the present work we had proposed to conjugate NCe to folic acid in order to specifically target ovarian cancer cells, which over-express FR-α. Folate receptor-α (FR-α) is a folate-binding protein overexpressed in ovarian and several other epithelial malignancies that has been used as a target for imaging and therapeutic strategies. Folate or folic acid, that binds to FR-α is an essential vitamin and critical metabolite needed for the biosynthesis of amino acids, DNA, RNA and methylation reactions. Folic acid is an of importance especially for one-carbon transfer processes mediated by enzyme systems involved in DNA synthesis (4). Increased expression of FR-*α* has been described in various tumor tissues, including ovarian, endometrial and breast cancer (5). While the function of FR-*α* in cancer is not fully understood, since, folates are critical metabolites for nucleotide synthesis and methylation

reactions, its overexpression might confer a tumor growth advantage by increasing folate availability to cancer cells (5). Over 90% of nonmucinous ovarian cancers over-express FR-*α* (6). Several strategies have been employed to target the folate receptor including the use of anti -FR-*α* antibodies or folic acid (FA) conjugates.

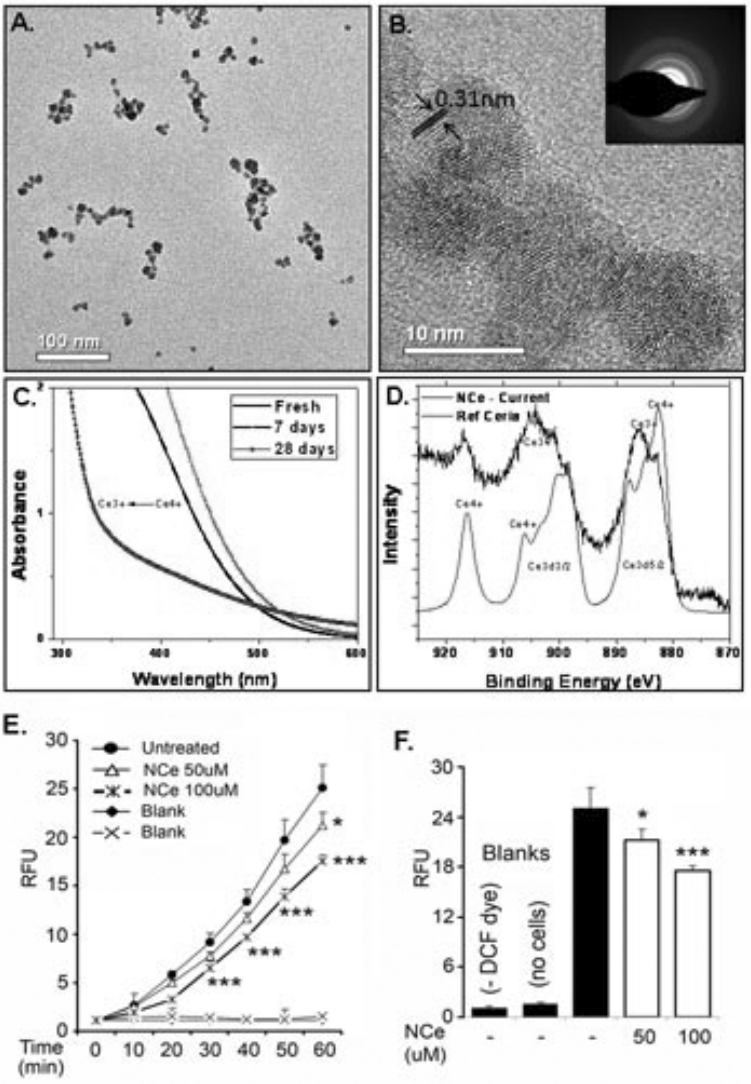
We had hypothesized that targeted delivery of folic acid (FA) conjugated nanoceria (NCe) will specifically target the ovarian cancer cell and further modification of folic acid conjugated nanoceria to cisplatin will result in specific targeting and delivery of cisplatin resulting in exclusive elimination of ovarian tumor cells *in vivo*.

Following two aims were proposed:
Aim1: ***Investigate the therapeutic efficacy of folic acid conjugated NCe (FA-NCe) in a preclinical mouse model of ovarian cancer.***

Aim2: ***Investigate the therapeutic efficacy of folic acid-Cisplatin conjugated NCe (FA-NCe-C) in a preclinical mouse model of ovarian cancer.***

BODY OF WORK

DATA:



1. Synthesis and characterization of cerium oxide nanoparticles: Cerium oxide nanoparticles used in this study contain individual crystallites of 3-5nm that are loosely agglomerated to 15-25nm. As the synthesis process is free from any organic surfactant the hard agglomeration of nanoparticles is controlled by tightly controlling the pH of the nanoparticles below 3.5 during synthesis to keep them in colloidal range. **Figure 1 A and B** shows the high resolution transmission electron micrographs (HRTEM) of NCe nanoparticles. It is evident from the image that nanoparticles are loosely agglomerated to about 15-25nm aggregates which could also be induced by the drying process. The hydrodynamic radius (37.8 nm ± 0.8) from the multimodal size distribution (volume %) analysis of DLS measurements agrees with the loose agglomerate size of the HRTEM analysis. High magnification image confirms the lattice planes of NCe in individual 3-5nm crystallites. UV-Visible spectroscopy was used to analyze the oxidation states of cerium oxide nanoparticles before and after aging treatment. **Figure 1C** shows the UV-Visible spectra from fresh and aged cerium oxide

Figure 1: Preparation and characterization of NCe: High resolution transmission electron micrographs show the presence of **A** loose agglomerates of 15-20 nm at low magnification **B** individual 3-5nm crystallites. The d spacing of 0.31 nm shows the presence of planes of ceria while the selected area electron diffraction (SAED) pattern confirms the presence of fluorite lattice of cerium oxide. The trend in oxidation state of nanoceria in **C** shows that the synthesized nanoparticles have predominance of trivalent oxidation state that undergoes a slow transformation to Ce^{+3} oxidation state over a period of 28 days **D** x-ray photoelectron spectrum from a reference ceria sample in which cerium is predominantly in +4 oxidation state is compared to the spectrum from a 4 weeks aged sample of nanoceria demonstrating the high concentration of Ce in +3 oxidation states. **E.** Basal levels of ROS in ovarian cancer cell line.
A2780 cells were treated with NCe (50-100μM) for 48h. Cells were washed with PBS and loaded with DCF-DA dye (5□M) and fluorescence was recorded at excitation 485 nm and emission 530 nm for various time periods (5-60 min). Wells containing only cells without DCFDA dye (cross) or without cells containing DCFDA dye (filled diamond) were used as a blank. **F**. Bar graph represents ROS levels at the 60 min of treatment with DCF-DA dye. Results are shown as mean ± S.D. of 4 samples. ***p< 0.001 NCe at 100uM; *p<0.05 NCe compared to untreated cells using two-tailed Student's t-test (Prism).

nanoparticles clearly indicating the predominance of Ce^{+3} oxidation state from the absorption peak at 252 nm as compared to absorption peak at 298 nm for Ce^{4+}. Further confirmation on the oxidation states of nanoparticles was obtained from XPS analysis **(Fig. 1D)**. The XPS spectrum of cerium is very complex that contains multiple peaks from the spin orbit coupling of 3d orbitals (7). Several

peaks in the Ce3d region that have been ascribed to $3d_{3/2}$ (899.5, 900.9, 903.5, 906.4 and 916.6) and $3d_{5/2}$ (880.2, 882.1, 8885, 888.1 and 898) arising from multiple valence states of cerium. The spectrum from NCe shows a predominance of cerium in +3 oxidation state as depicted by the characteristic peaks at 880.2, 885.0, 899.5 and 903.5 eV. Taken together the data from characterization of NCe is consistent with previous reports wherein cerium can be retained in trivalent oxidation by decreasing the size of the nanoparticles (1-3, 8)

2. NCe treatment inhibits production of ROS levels in A2780 cell line: Cerium oxide nanoparticles have been shown to act as free radical scavengers by inhibiting the production ofreactive oxygen species (ROS) (7, 9,10). Since, it is well established that ROS accumulation playsan important role in initiation and progression of tumorigenesis in human ovarian cancer (11,12), we examined the effect of NCe on ROS generation in ovarian cancer cell line A2780. A2780 cell line was treated with NCe (50-100□M) and post 48h of treatment, ROS generation was measured using DCFH2-DA dye followed by fluorescence reading. As shown in **figure 1E, F**, NCe treatment significantly inhibited ROS levels in A2780 cell line, suggesting that NCe treatment inhibits basal levels of oxidative stress in A2780 OvCa cell line.

3. Synthesis and characterization of Ceria –Folic acid (FA-NCe):

Cerium oxide nanoparticles were synthesized by using precipitation method and as described above (13). Final nanoparticles were dried overnight under vacuum at 60-80°C. Cerium oxide nanoparticles (CNPs) obtained by this method were then functionalized with amine (CO2-NH2) by using 3-aminopropyl-trimethoxysilane as described elsewhere (14). Folic acid were conjugated to the amine functionalized by using 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC) and *N*-hydroxysulfosuccinimide (Sulfo-NHS) coupling chemistry. Molar ratio of folic acid:CeO2-NH2:Sulfo-NHS-EDC was 1:1:2.2:11.2. Reaction

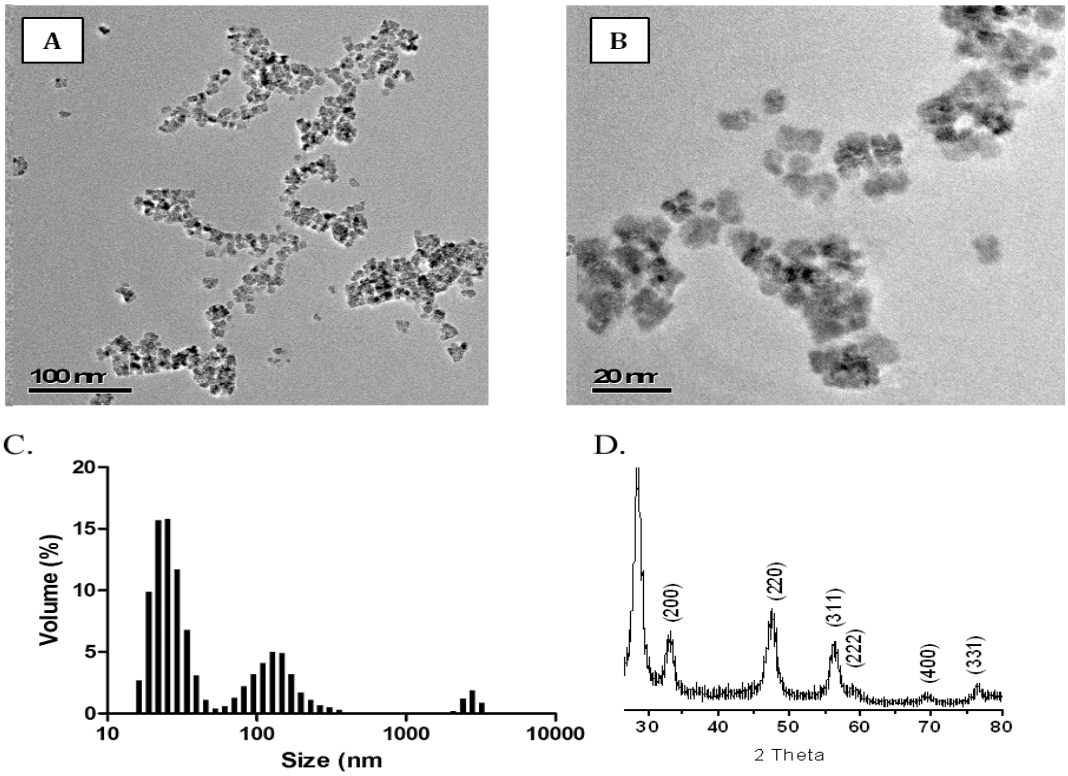


Figure 2: Characterization of FA-NCe. (A) and (B) are the HRTEM images of CNPs-FA conjugate, show particles size in the range of 8-10nm. (C) Hydrodynamic radii of the CNP-FA conjugates are shown. (D) XRD diffraction pattern of CNPs-FA conjugate.

was carried for 24hr and then washed in both DMSO and water to remove EDC, Sulfo-NHS and unbound folic acid. Finally, folic acid conjugated CeO2 **(FA-NCe)** were dried under vacuum at 40°C. CeO2-FA was then characterized by using high resolution transmission electron microscopy (HRTEM), X-ray diffraction (XRD) and dynamic light scattering (DLS). HRTEM images show the size of the CeO2-FA is ~10nm **(Fig. 2A, B)**. Hydrodynamic radii of CeO2 were found in approximately ~30nm nanometers with small percentage having 150nm agglomerated particles (Fig 2C). The surface charge of the CeO2-FA has been also analyzed and found to be 25mV. XRD data confirm microcrystalline nature of the particles (Fig 2D).

4. FA-NCe inhibits ovarian cancer proliferation *in vitro*.

Our previous data with NCe had shown no effect on proliferation of ovarian cancer cells. Therefore we wanted to determine if FA-NCe has the ability to affect growth on ovarian cancer cells in vitro. For this cisplatin sensitive A2780 and resistant C200 were plated in 96 well plates at $4x10^3$ cells/well and treated in triplicates with various concentrations of NCe (10-100 μM). Cell viability was determined at 72hrs by MTT assay as described before (15). As shown in figure 3, NCe treatment had no significant effect on the proliferation or survival of ovarian cancer cell lines both A2780 **(Fig 3A)** or C200 **(Fig. 3B)**. While FA-NCe showed significant inhibition in proliferation/cell death in both the cell lines (black bars).

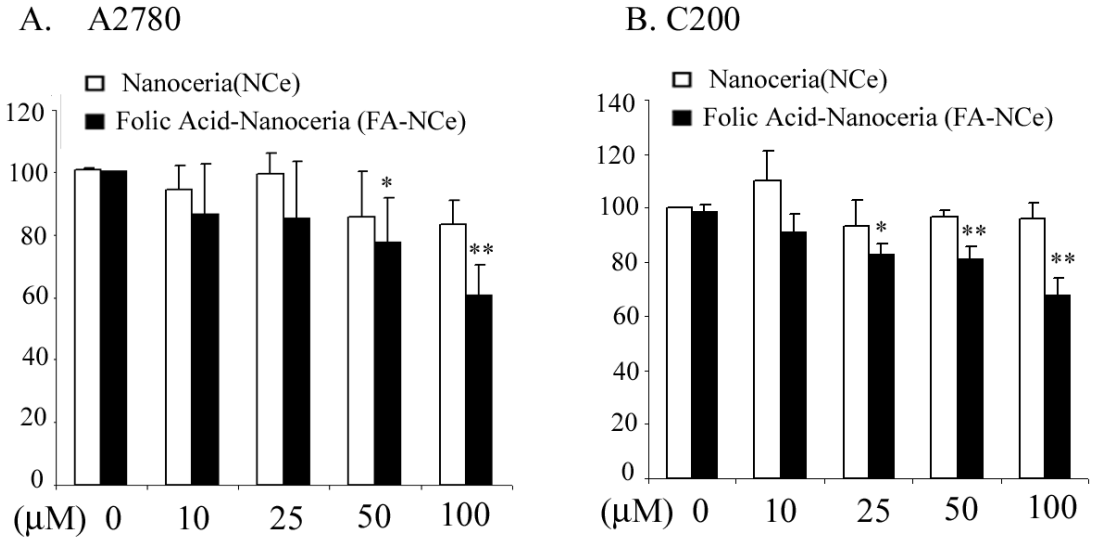


Figure 3: FA- NCe treatment inhibits proliferation in ovarian cancer cell lines.
Percent viability of **A**. A2780 and **B.** C200 cells treated with indicated doses of NCe (10-100μM) as determined by MTT assay. The data is represents three individual experiments done in triplicates. * $p<0.001$ **$p<0.05$ compared to untreated (0) cells at respective time point using two-tailed Student's t-test (Prism).

5. FA-NCe show higher efficacy that NCe in inhibiting ovarian cancer colony forming ability.

To confirm our previous result we also performed the colony formation assay as it considered a better assay to determine drug effect on cancer cells. For this, 2000 A2780 and C200 cells were plated in triplicates in 6-well plates and treated with indicated concentrations of NCe. The cells were allowed to form colonies for up to 2weeks and media was replaced every fourth day. Colonies were stained with MTT and counted as described before (15). The clonogenic assay also showed similar results where

NCe treatment showed no significant change, while FA-NCe significantly inhibited colony formation of both A2780 and C200 cell lines **(Fig. 4; black bars)**.

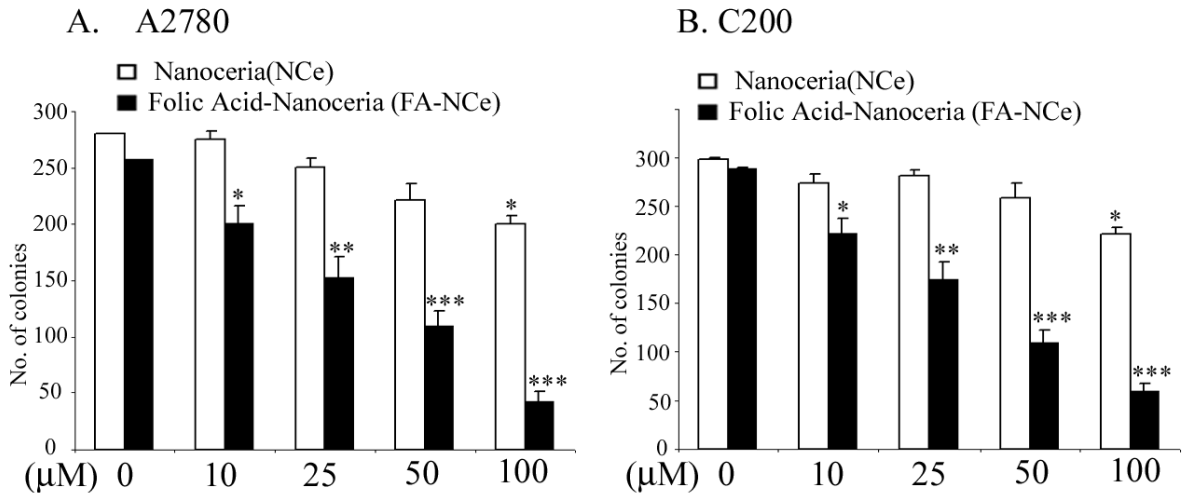


Figure 4: FA- NCe treatment inhibits colony formation in ovarian cancer cell lines.
Number of colonies formed in **A**. A2780 and **B.** C200 cells treated with indicated doses of NCe (10-100μM) as determined by visual counts after staining them with MTT dye. The data is represents three individual experiments done in triplicates. * $p<0.001$ **$p<0.05$ compared to untreated (0) cells at respective time point using two-tailed Student's t-test (Prism).

6. Internalization of FA-NCe in the cells: To assess that FA-NCe can be uptaken by the cells the amount of FA-NCe inside the cells was determined by MS. Cells were treated with a broad range of

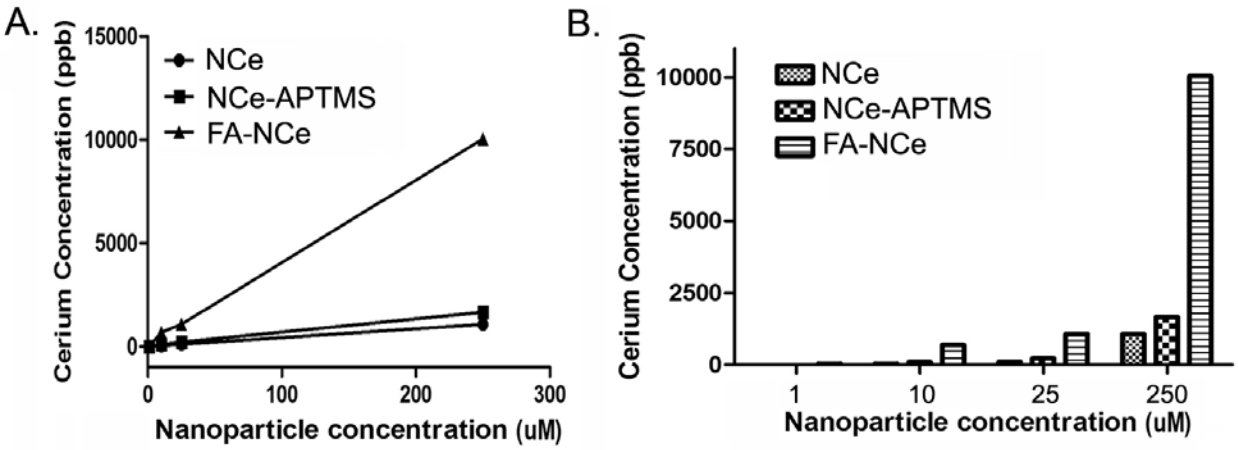


Figure 5: Internalization of NCe formulations. Cells were treated with indicated doses of NCe, NCe-APTMS and FA-NCe) **A**. 0-300μM and **B.** 1 -250μM for 24h. Cells were extensively washed, pelleted and subjected to mass spectroscopy to determine the nanoparticle concentration inside the cell. concentration is expressed as parts per billion (ppb).

NCe alone, functionalized NCe with amine (CO2-NH2) by using 3-aminopropyl- trimethoxysilane (APTMS) (14) and the functionalized NCe conjugated with FA (FA-NCe) at a dose range of 0 -300μM. After 24h cells were extensively washed to remove excess nanopartices or any attached to the surface of the cells. Cell pellet was subjected to Mass Spectroscopy to determine the amount of nanoparticles inside the cells in terms of parts per billion (ppb). A dose dependent intake inside the

cells was quantified (**Fig. 5A**). To further confirm that nanoparticles were internalized at lower doses used for experimentation, the internalized nanoparticles were measures at 1 to 250μM (**Fig. 5B**). These data show that the nanopartices (NCe, NCe-APTMS, FA-NCe), all are increasingly internalized by the cells corresponding to the increasing doses being treated. FA-NCe had significantly higher uptake by the cells, indicating that (i) the conjugation with FA, indeed makes NCe more targeted to the cancer cells (ii) cells are more receptive to NCe when tagged with FA and (iii) the conjugation and the fictionalization modifications of the original NCe does not alter its functional properties.

7. FA-NCe inhibits ovarian cancer *in vivo*: To determine the *in vivo* efficacy of FA-NCe, 2 million A2780 ovarian cancer cells were injected into 30 nude mice. At day 5, treatments were initiated. Group 1 (n=10) received vehicle (PBS, 100μl), Group 2 received NCe (0.1mg/kg bd wt) (n=10), and Group 3 received FA-NCe (0.1mg/kg bd wt) (n=10), by intra-peritoneal injections twice a week. At end of 4 weeks mice were sacrificed and tumor growth and burden determined. As seen in figure 6, mice treated with FA-NCe showed slow tumor growth as reflected by less weight gain compared to NCe alone and vehicle treated mice (**Fig. 6A**). Measurement of excised tumor weight also showed that FA-NCe was more effective in inhibiting tumor growth (**Fig. 6B**), indicating that conjugation of FA increased the efficacy of NCe.

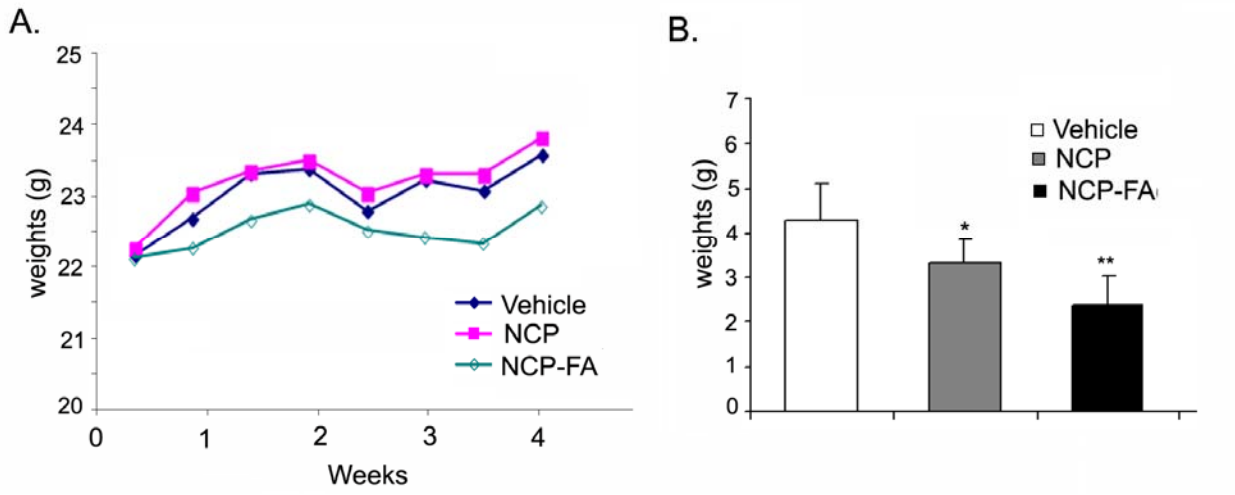


Figure 6: FA- NCe inhibits ovarian cancer growth more effectively that NCe. 2 million cells were injected into nude mice and treated with the indicated treatments as described in the text. **(A)** Average weight progression of different groups. (**B)** Excised tumor weights of various groups showing FA-NCe to be most efficient in inhibiting tumor weight.

8. FA-NCe treated mice had less ovary-associated tumors: In the A2780 mouse model, the cancer cells home to the ovary and form ovary associated tumors, along with spread in other organs. Ovary associated tumors were excised individually. Pictograph in **figure 7** shows that FA-NCe associated tumors were less in size. Also, involvement of one ovary was associated more with FA-NCe treated mice, with some mice showing no ovary involvement.

9. Tumor burden score: Tumor burden and spread was enumerated by assigning a score of 0, if no tumor nodules were observed; 1 if 1-2 nodules were observed; 2 if 2-4 nodules were seen and 3 in case of extensive involvement. Vital organs known to be associated with ovarian metastasis were examined for tumor burden and scored. All organs examined (ovary, peritoneum, bowel, stomach, liver and kidney) from FA-NCe treated showed significantly lower score compared to vehicle and NCe treated mice (**Figure 8**). The greatest difference was observed in the score of kidney and liver, indicating that FA-NCe has the potential to inhibit metastatic spread of ovarian cancer, which could be in part due to restriction of tumor size.

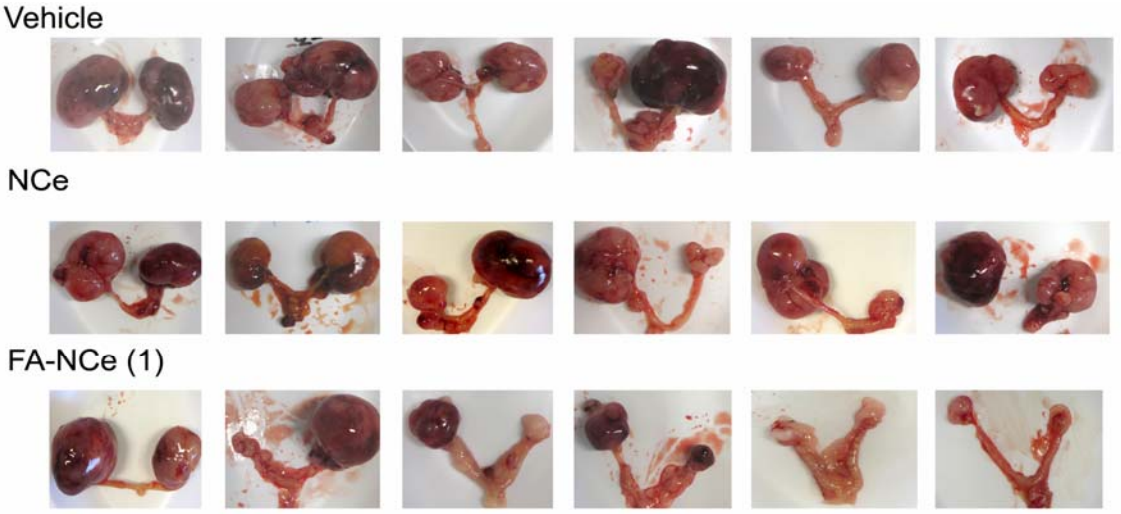


Figure 7: FA- NCe restricts ovary associated tumor. Ovaries and its associated tumors were excised from each mouse and photographed. A representation of ovaries from 6 mice from each group are shown (n=10)

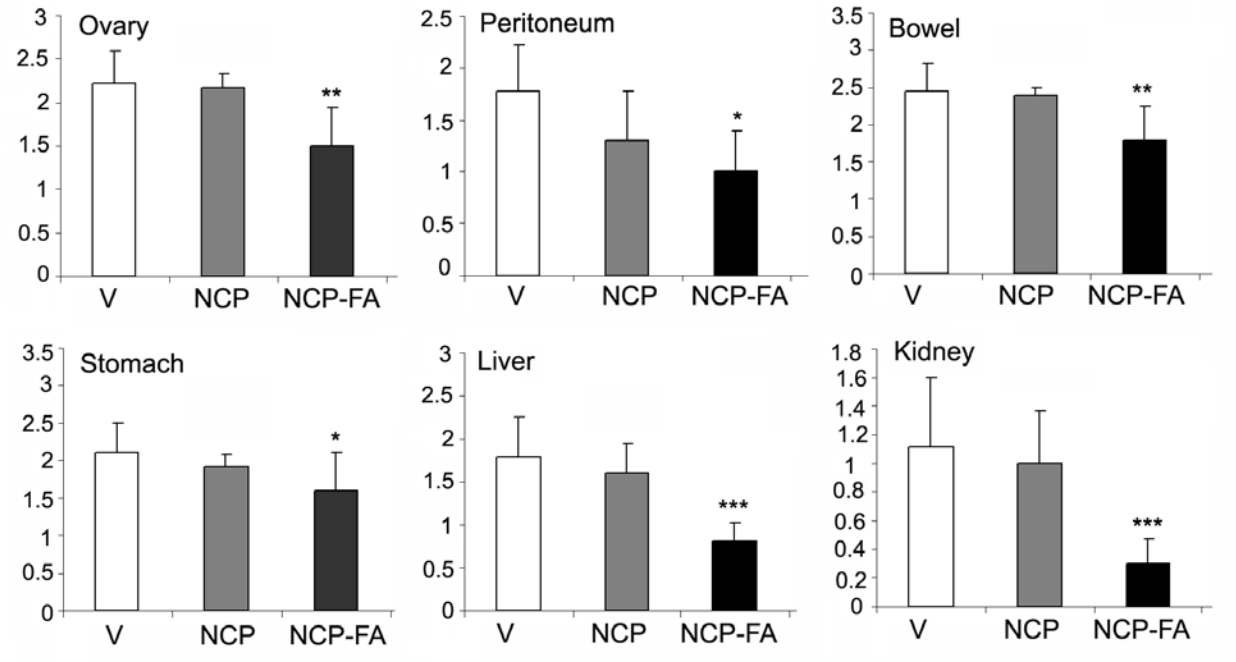


Figure 8: Tumor score of various organs. Tumor burden was scored as explained in the text in all the 10 mice from each group. FA-NCe group showed lower tumor burden in all vital organs.

10. Toxicity: To determine the cellular toxicity of FA-NCe *in vivo*, the vital organs (liver heart, spleen, kidney and lungs) were excised from untreated and NCe treated mice at the end of the study and formalin fixed. H&E staining of the various sections revealed that the morphology of all organs from treated and untreated mice appeared to be normal and no necrosis was observed (data not shown). Analysis of liver function (aspartate aminotransferase, AST; alanine aminotransferase, ALT; albumin) and kidney function (creatinine; urea ; albumin, uric acid) in plasma collected, showed no significant difference in the untreated and treated mice. All values were found within the normal limits in both

groups (**Fig 9**). These data show that FA-NCe treatment twice a week for 4 weeks at the dose of 0.1 mg/kg is safe and does not result in tissue cytotoxicity or any abnormal physiological vital functions.

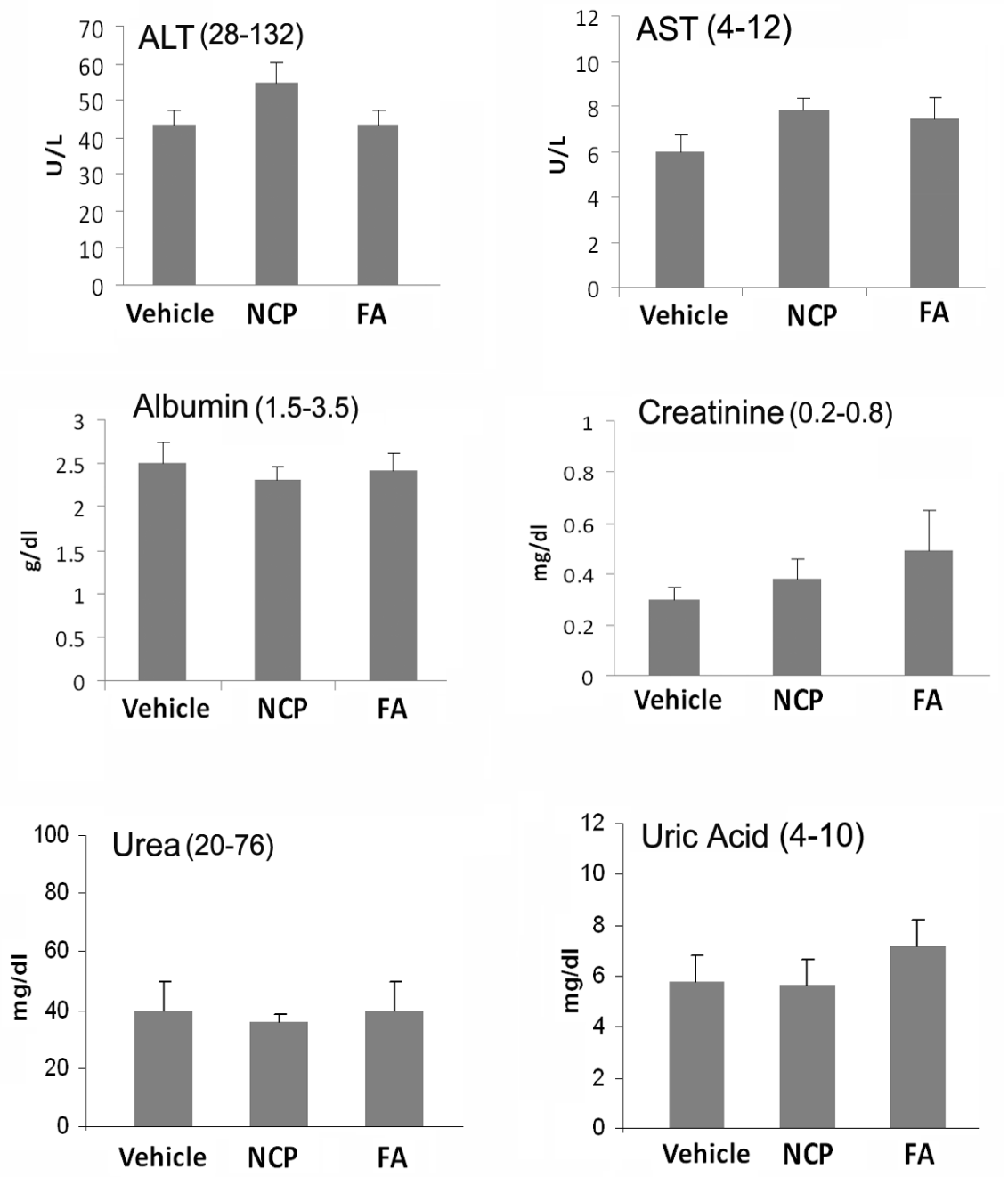


Figure 9: FA-NCe treatment is non-toxic in nude mice. After sacrificing animal groups (n = 10) at the end of the study (4 weeks), blood was collected in heparin coated tubes and plasma was prepared. A panel of liver and kidney cytotoxic tests were performed in plasma prepared from control, NCe and FA-NCe (labelled as FA) treated mice as per the manufacturer's instructions, ALT (Alanine Transaminase), AST (Aspartate Transaminase), Albumin, Creatinine, urea and uric acid.

11. Investigation of cisplatin conjugated FA-NCe in vivo (FA-NCe-C): To determine the *in vivo* efficacy of FA-NCe-C, 2 million A2780 ovarian cancer cells were injected into nude mice. At day 5, treatments were initiated in groups of 10 mice each. Group 1: vehicle (PBS, 100ml), Group 2: NCe (0.1mg/kg bd wt), Group 3: FA-NCe (0.1mg/kg bd wt), Group 4: FA-NCe-C (0.1mg/kg bd wt) Group 5: Cisplatin (4mg/kg bd wt). Both drugs were given by intra-peritoneal injections, nanoparticles were given twice a week and cisplatin treatments once a week. At end of 4 weeks mice were sacrificed and tumor growth and burden determined. **Figure 10** depicts the average weight of excised tumors. As expected NCe and FA-NCe showed slow tumor growth, with FA-NCe being more effective.

Unfortunately, the cisplatin conjugated FA-NCe-C, did not show any inhibition of tumor growth compared to untreated growth, indicating that conjugation of cisplatin to the complex somehow made the cisplatin lose its anti-tumor property.

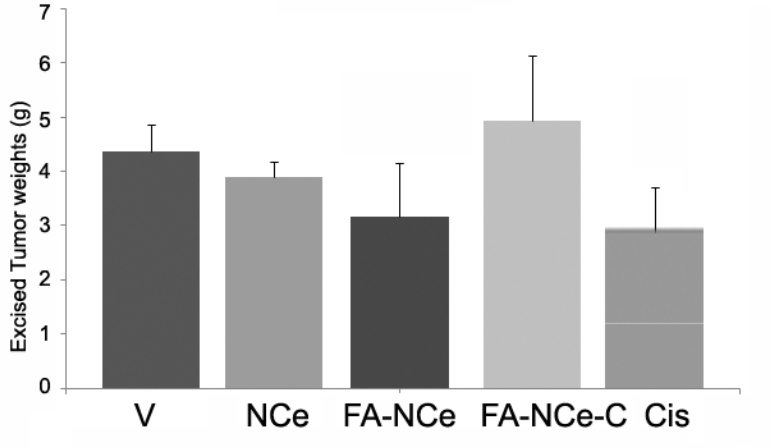


Figure 10: FA- NCe-C does not have any effect on ovarian cancer growth *in vivo*. 2 million cells were injected into nude mice and treated with the indicated treatments as described in the text. Tumors were excised and weighed. NCe and FA-NCe inhibited tumor growth alone and in combination with cisplatin while FA-NCe-C had no effect on tumor growth.

Unfortunately the numerous attempts of cisplatin conjugation nanoparticle did not work. We figured out that this is due to the loss of the potency of cisplatin, as the chemical cisplatin is very unstable. Dr. Seal is attempting to re-design the conjugated particle for our testing, where we plan to try this using pharmacology grade cisplatin that has been stabilized or try paclitaxel. Hence, we will be continuing to work to achieve this goal.

11. FA-NCe enhances cisplatin cytotoxicity: Next we investigated if combination of FA-NCe and cisplatin will be more effective in inhibiting ovarian cancer growth. A2780 xenografts were generated as before. At day 5, treatments were initiated in groups of 10 mice each. Group 1: vehicle (PBS, 100ml), Group 2: FA-NCe (0.1mg/kg bd wt), Group 3:, Group 4: FA-NCe-C (0.1mg/kg bd wt) Group and cisplatin (4mg/kg bd wt) and group 5: Cisplatin (4mg/kg bd wt). Both drugs were given by intra-peritoneal injections, nanoparticles were given twice a week and cisplatin treatments once a week. At end of 4 weeks mice were sacrificed and tumor growth and burden determined. **Figure 11** depicts the average weight of excised tumors. As before FA-NCe inhibited tumor growth, which was enhanced by combination with cisplatin (Fig. 11).

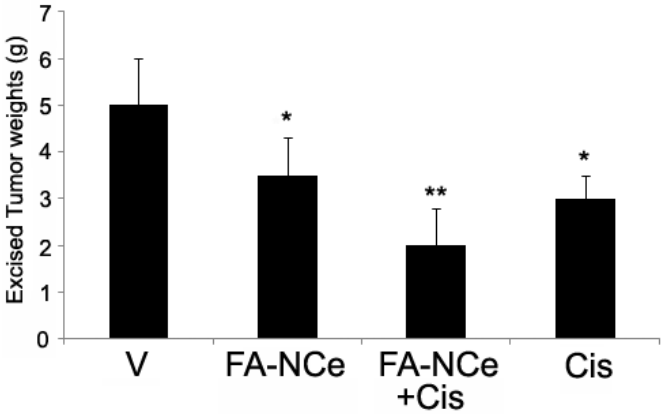


Figure 11: FA- NCe enhances cicplatin cytotoxicity . 2 million cells were injected into nude mice and treated with the indicated treatments as described in the text. Tumors were excised and weighed. FA-NCe inhibited tumor growth alone and in combination with cisplatin.

12. Tumor burden score: Tumor burden and spread was enumerated as before. Vital organs known to be associated with ovarian metastasis were examined for tumor burden and scored. All organs examined (ovary, peritoneum, bowel, spleen, liver and kidney) from FA-NCe treated showed significantly lower score compared to vehicle and NCe treated mice in almost all organs which was lowered further by combination with cisplatin (**Figure 12**). Cisplatin by itself all showed significant difference.

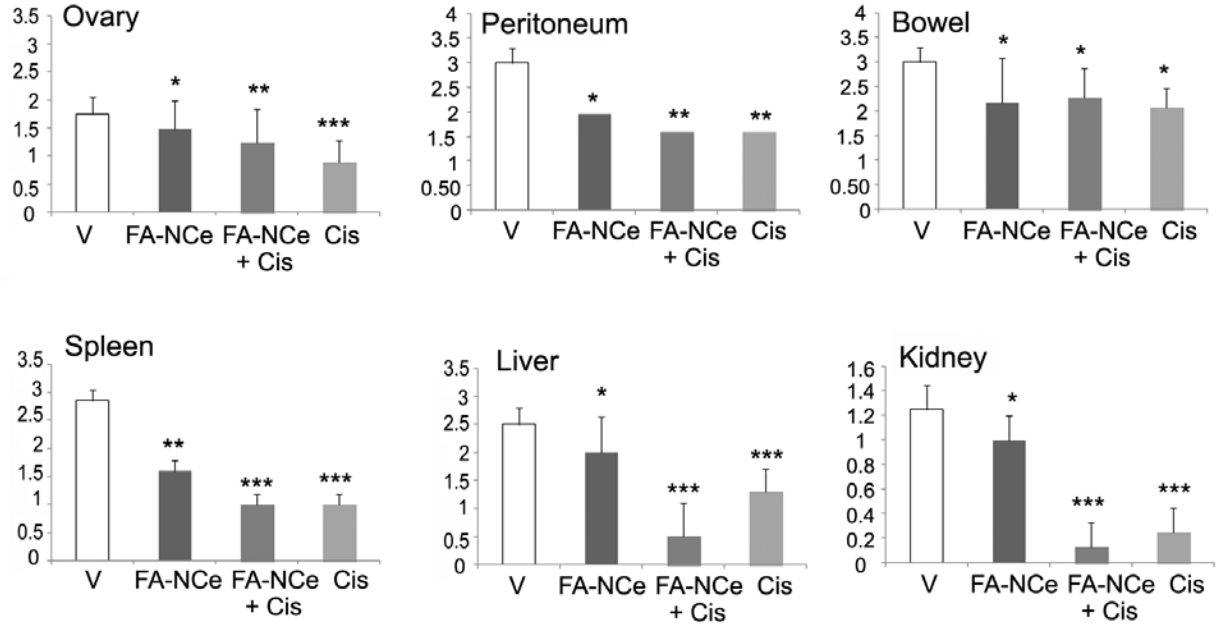


Figure 12: Tumor score of various organs. Tumor burden was scored as explained in the text in all the 10 mice from each group. FA-NCe group showed lower tumor burden in all vital organs.

13. Decreased proliferation and angiogenesis by FA-NCe: To look at the proliferation of cancer

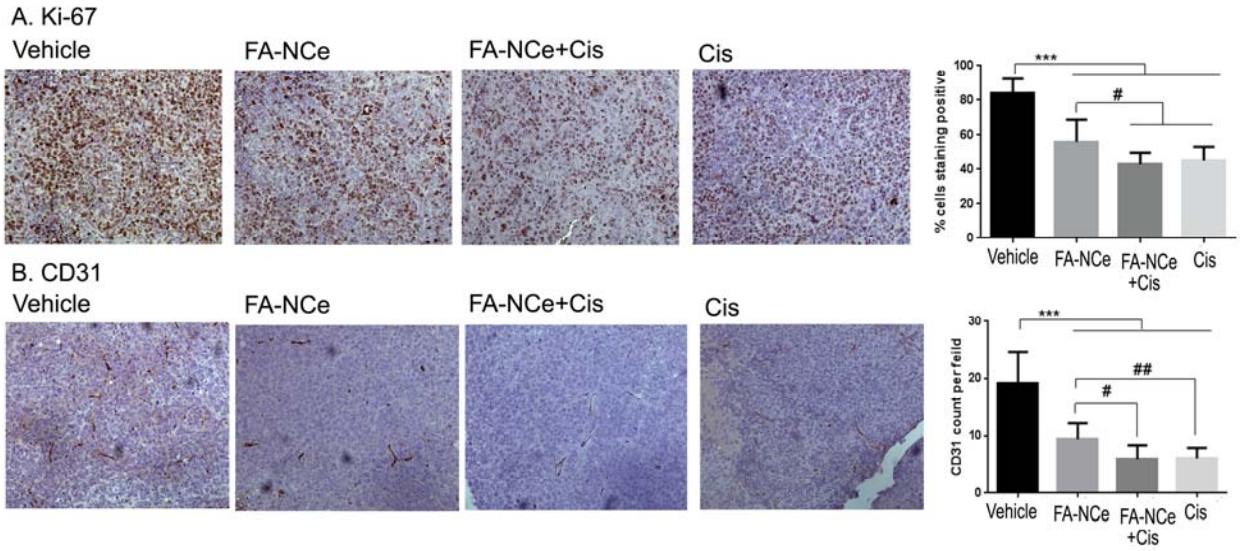


Figure 13: Representative immuno-stains. (A). Proliferation index as determined by quantification of Ki-67 was decreased under FA-NCe treatments and further enhanced by combination of cisplatin. (B) Level of angiogenesis was determined by staining for CD31, a marker for endothelial cells. CD31 positive cells were decreased under FA-NCe treatments and further enhanced by combination of cisplatin.

cells, immuno-histochemical analysis of Ki67 was performed. Significant difference was observed in the number of cells staining positive for Ki-67. Enumeration of Ki-67 positive cells counted over 5 high power fields of five sections from each group also showed significant less Ki-67 positive cells in FA-NCe treated xenografts compared to untreated group (expressed in %age; Figure 13A), indicating that less number of cells were proliferating under FA-NCe treatment. The proliferation was further

decreased when FA-NCe was combined with cisplatin. Together, these data indicate that FA-NCe has the ability to restrict ovarian tumor growth *in vivo* due to decreased proliferation of ovarian cancer cells.

Since previously we have shown that NCe inhibits angiogenesis by targeting endothelial specifically, we also performed CD31 staining. CD31 staining FA-NCe treated A2780 tumors showed significantly less number of CD31 positive micro-vessels (Figure 13B) compared to untreated mice. Quantification of positively stained vessels at high power from 5 different fields of five sections from each group is shown in the bar gpah. The count did not change much with cisplatin treatments.

Work Left: We are still awaiting data on the tissue distribution of FA-NCe from one of our collaborators. We were delayed due to our collaborator's technical problem with his instrument. When we get the data, we will also be completing our manuscript.

Key Research Accomplishments:

1. Nanoceria with consistent anti-oxidant properties can be successfully synthesized.

2. Nanoceria can be successfully conjugated with folic acid.

3. Nanoceria conjugated with folic acid appears to have higher anti-tumor activity against cisplatin sensitive and resistant ovarian cancer cells *in vitro*.

4. Nanoceria conjugated with folic acid shows higher anti-tumor activity against A2780 generated tumors in nude mice, compared with control and NCe treated mice.

5. Nanoceria conjugated with folic acid inhibition of ovarian tumor in vivo is associated with decreased ovarian involvement and containment of tumor spread as seen by lower clinical score.

6. FA-NCe treatment twice a week at the dose of 0.1 mg/kg is safe and does not result in tissue cytotoxicity or any abnormal physiological vital functions.

7. FA-NCe enhances cisplatin's cytotoxicity *in vivo*.

REPORTABLE OUTCOMES:

1. Nanoceria can be successfully conjugated with folic acid

2. Nanoceria conjugated with folic acid appears to have higher anti-tumor activity against cisplatin sensitive and resistant ovarian cancer cells *in vitro*.

3. Nanoceria conjugated with folic acid shows higher anti-tumor activity *in vivo*, which is associated with decreased spread of tumor to other organs.

4. Nanoceria conjugated with folic acid shows no toxicity.

5. Nanoceria conjugated with folic acid when combined with cisplatin results in greater tumor inhibition.

CONCLUSION

Nanoceria conjugated with folic acid appears to have higher anti-tumor activity against cisplatin sensitive and resistant ovarian cancer cells *in vitro*. This is validated in vivo, in the A2780 nude mouse xenografts, where it significantly inhibited tumor growth and enhanced the cytotoxicity of cisplatin when given in combination.

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