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14. ABSTRACT

Initial study was performed which was localized MagNaGel nanoparticles in an orthotopic ovarian cancer mouse model by MRI and also via IVIS imaging techniques. Mice were injected intraperitoneally with ovarian cancer cells tagged with luciferase gene. Tumor growth was monitored using both a 4.7T small animal MRI and IVIS Imaging system. Once tumor and ascites developed in the mice, cisplatin-loaded MagNagel particles with 10.0 nm IO core were injected into the tail-vein (IV) of the cancer bearing mice or intraperitoneally (IP) depending on the group. After set time periods, MRI and IVIS imaging was performed on the mice. Organs were harvested and drug levels determined in biodistribution studies comparing untargeted and targeted particles. Survival studies were performed using IP injected nanoparticles. Biodistribution studies confirmed high levels of the nanoparticle in the spleen and liver but without release of cisplatin in blood. Survival studies in IP treated mice did not show a survival advantage for the either untargeted or targeted (HER-2neu) nanoparticles. Further study with IV injections and with different particle formulations and targeting agents is necessary.

15. SUBJECT TERMS
Nanoparticles, ovarian cancer, orthotopic mouse model, chemotherapy

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Introduction:

Chemotherapy for ovarian cancer has changed little over the years. Sensitivity to platinum-based agents predict disease free survival. However, there are limitations of current therapies. Chemotherapy drugs do not differentiate between normal and tumor cells. Toxicities are a result of damage to normal cells and limit the dosage strength tolerated by the patient. Recent studies suggest that the costs of managing adverse events associated with toxicities can amount to 40% of the cost of treatment [1]. Thus, technologies that alleviate the toxic side effects and increase the efficacy of chemotherapy are in great demand.

Nanoparticles (10 to 100 nanometers) provide state-of-the-art technology as vehicles used in detection, diagnosis, and treatment of cancer. When loaded with chemotherapeutic agents, nanoparticle delivery to cancerous tissues relative to healthy tissues may be favorably biased by size and through the attachment of targeting ligands to the surface of the particle. Dr. Steve Barry at Alnis BioSciences, Inc has recently developed a novel Magnetic Nanoparticle Hydro-Gel (MagNaGel™) platform (under the support of NCI NO1-CO-29009 and HHSN2612200411004C). This particle is comprised of a chemotherapeutic agent, iron oxide colloids, and targeting ligands[2]. This particle platform has demonstrated high (greater that 10 wt %) chemotherapeutic loading, tumor-associated biomolecular binding, good magnetic susceptibility, and attractive toxicity and circulation profiles (see Appendix) in mouse models. These findings suggest that targeted MagNaGel™ can significantly increase efficacy and decrease toxicity in cancer treatment[2].

We hypothesize that platinum loaded MagNaGel™ particles containing specific targeting ligands, which have been identified through transcription profiling of different histological types of ovarian cancers when used in conjunction with diagnostic MRI, may play a key role in new and powerful ovarian cancer treatment regimens. We proposed: 1) developing an orthotopic mouse models both with and without ascites to simulate human ovarian cancer, 2) performing biodistribution studies of the ligand-specific cisplatin loaded MagNaGels using an ovarian cancer mouse model and 3) begin evaluating the efficacy and toxicity of selected ligand-specific cisplatin loaded MagNaGels particles.

Body:

Establishment of orthotopic ovarian cancer mouse models.

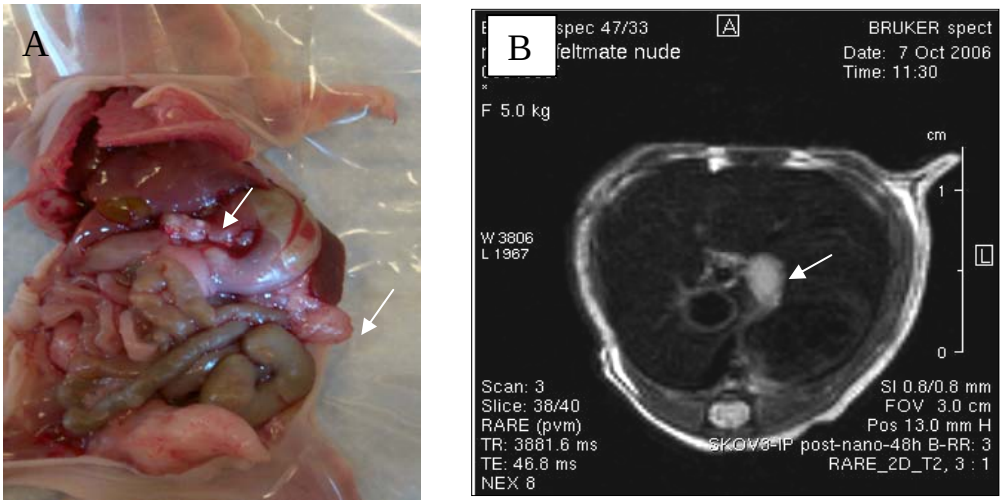


Fig. 1.(A) A photograph showing tumor masses developed in an athymic nu/nu mouse (arrows). (B) T2-weighted MRI images of the abdomen of the same mouse showing a tumor nodule (arrow) attached to the liver.

Athymic Nu/Nu female mice were utilized to establish orthotopic ovarian cancer mouse models. Both SKOV3 and SKOV3-IP-Luc as well as HEYA8 ovarian cells were grown in tissue culture. $2x10^6$ cells were utilized in 200 µl intraperitoneal (IP) injection. The SKOV3 model was developed as a tumor model without ascitic fluid while HEYA8-injected mice rapidly developed both tumor and ascites. Figure 1 demonstrates an example of tumor-bearing mouse after SKOV3 injection. MRI and IVIS Imaging systems were utilized to monitor tumor growth and response and as weight was not a sensitive indicator.

Accumulation of MagNaGel in tumor tissue and ascites in an orthotropic ovarian cancer mouse model

In our pilot study we localized the MagNaGel in an ascites-forming orthotopic ovarian cancer mouse model by MRI, mice were injected intraperitoneally with $1x10^6$ ovarian cancer cells HeyA8. Tumor growth was monitored using a 4.7T small animal MRI (Bruker Daltonics, Billerica, MA) at the Harvard Medical School MRI Core Facility. After 17 days when tumor and ascites developed in the mice, 200 µl of cisplatin-loaded MagNagel particles with 10.0 nm IO core (untargeted) were injected into the tail-vein of the cancer bearing mice. After 24 hr, MRI was performed on the mice. Iron oxide particle in the size range of MagNaGels are known as Ultrasmall Super-Paramagnetic Iron Oxide (USPIO) particles and typically darken compartments in which they are concentrated in T2-weighted images. Figure 2 shows tumor accumulation of MagNagel particles 24 hours after tail-vein injection. In addition, the lighten area in the T2-weighted images, which represented ascites in the pelvic and the abdomen, showed decreased in contrast compared to the surrounding organs after the injection of nanoparticles suggesting that presence of MagNagel particles in this compartment. Both kidneys and spleen did not show an increase in contrast. These results indicate the MagNaGel particles were effective contrast agents for MRI and the feasibility of using MRI to localize and quantify MagNagel particles in the orthotopic ovarian cancer mouse model.

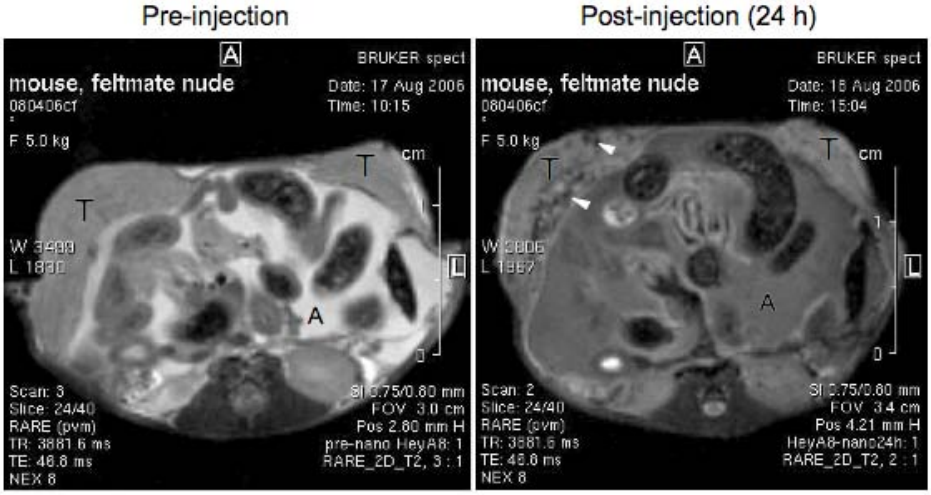


Fig. 2. T2-weighted MRI images of the mouse abdomen before, and 24 hours after injection of 200 µl of a 20 mM Fe MagNaGel nanoparticle solution. FFE 3D minimum TE, partial echo, pulse sequence. Tumors (T) and ascites (A) darkened 24 hours after tail-vein injection. Arrow heads indicate accumulation of MagNaGel particles in tumor tissues.

Uptake of nanoparticles by tumor cells *in vitro*

To evaluate whether cisplatin-loaded MagNagel nanoparticles were up-taken by ovarian cancer cells *in vitro*, SKOV3 cells growing in 8-well chamber slides were treated with Cy5.5-labeled cisplatin-loaded MagNagel nanoparticles for 8, 16, and 24 hours. The results showed accumulation of Cy5.5 positive granules in the cells after 24 hours (Fig. 3) suggesting that MagNagel nanoparticles were uptaken by cells through the endocytotic process.

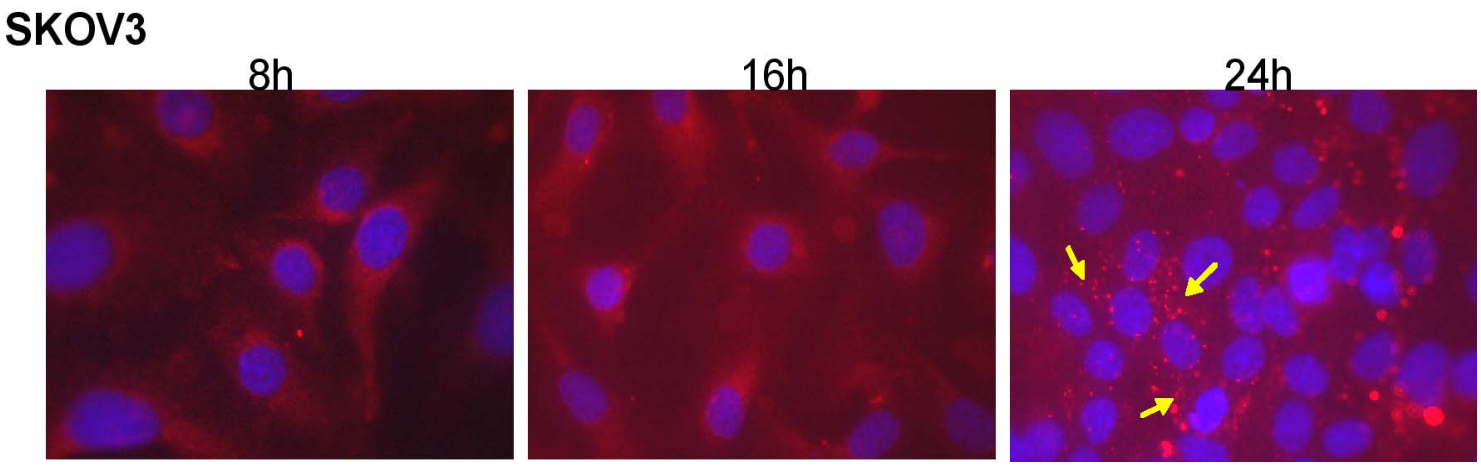


Fig. 3. Micrographs showing SKOV3 cells cultured in medium with Cy5.5 labeled MagNaGel. A total of 1 x 10^4 cells were seeded into each well of the 8-chambed slide. They were then treated with 10 µl of a 20 mM Fe MagNaGel nanoparticle solution or with control buffer in 200 µl of culture medium. At 8, 16, and 24 hours after treatment, cells were washed with PBS twice, stained with DAPI, and observed under a Leica DMIRE2 fluorescent microscope with the Cy5.5 filter cube set. Arrows indicate Cy5.5 positive granules accumulation in the cytoplasm of the cells. 200X

Biodistribution Study

The biodistribution of the labeled cisplatin nanoparticles was determined by using an orthotopic ovarian cancer mouse model. A total of $1x10^6$ cells (OVCAR3) in 200 µl were introduced into each female nude mouse by IP injection. After 24-28 days elapsed, tumor was confirmed by MRI. Initial studies utilized 3 mice/group and these were injected intravenously (through tail-vein) with cisplatin-containing nanoparticles. At timepoints: 1hr, 6hr, 24 hr, 48 hr, 72 hr and 120hr mice were weighed, sacrificed, representative organs (e.g. liver, kidneys, heart, brain, and lung specimens), blood and tumor regions were removed/collected, snap-frozen and sectioned. Tissue was processed for platinum (Pt) levels. Results show an initial rapid peak and fall of blood levels. Tissue levels rose and declined more slowly in liver and lung. Findings of high levels in the spleen are under further investigation. We suspect that the splenic architecture facilitated accumulation of untargeted nanoparticles. Tumor tissue held fairly stable levels of cisplatin from 24-72 hours, much longer than that of blood. Kidney cisplatin levels remained low and cleared after 72 hours. Additonal studies augmenting particles with hyperthermia (water bath to 40°C) have shown improved cisplatin concentration in tumor tissue. (Figure 6)

An SKOV3 luciferase transfected cell line is now being used so that light signals via luminescence can more accurately measure tumor size and response to treatment. Fluorescent-labeled nanoparticles have been used in biodistribution studies (Figure 7).

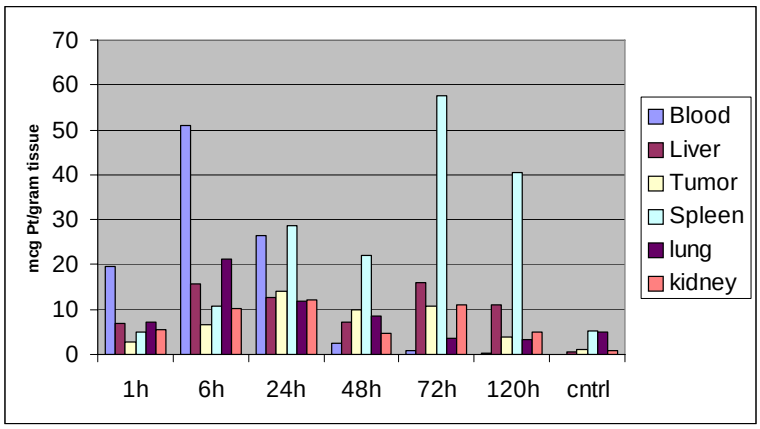
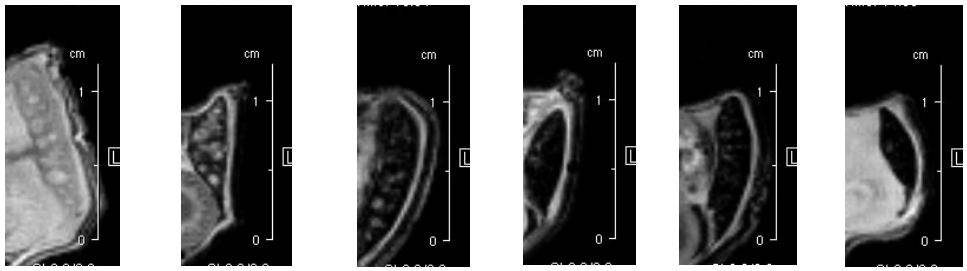


Fig. 4. Graphic illustration of cisplatin levels in tissue and blood for non-targeted nanoparticles. SKOV3 tumor-bearing mice were injected on Day 28 with untargeted cisplatin-containing nanoparticles. Mice were sacrificed at timepoints 1-, 6-, 24-, 48-, 72-, and 120-hour intervals.

MRI images below (Figure 5), correlate with tissue levels in Figure 4.

A.

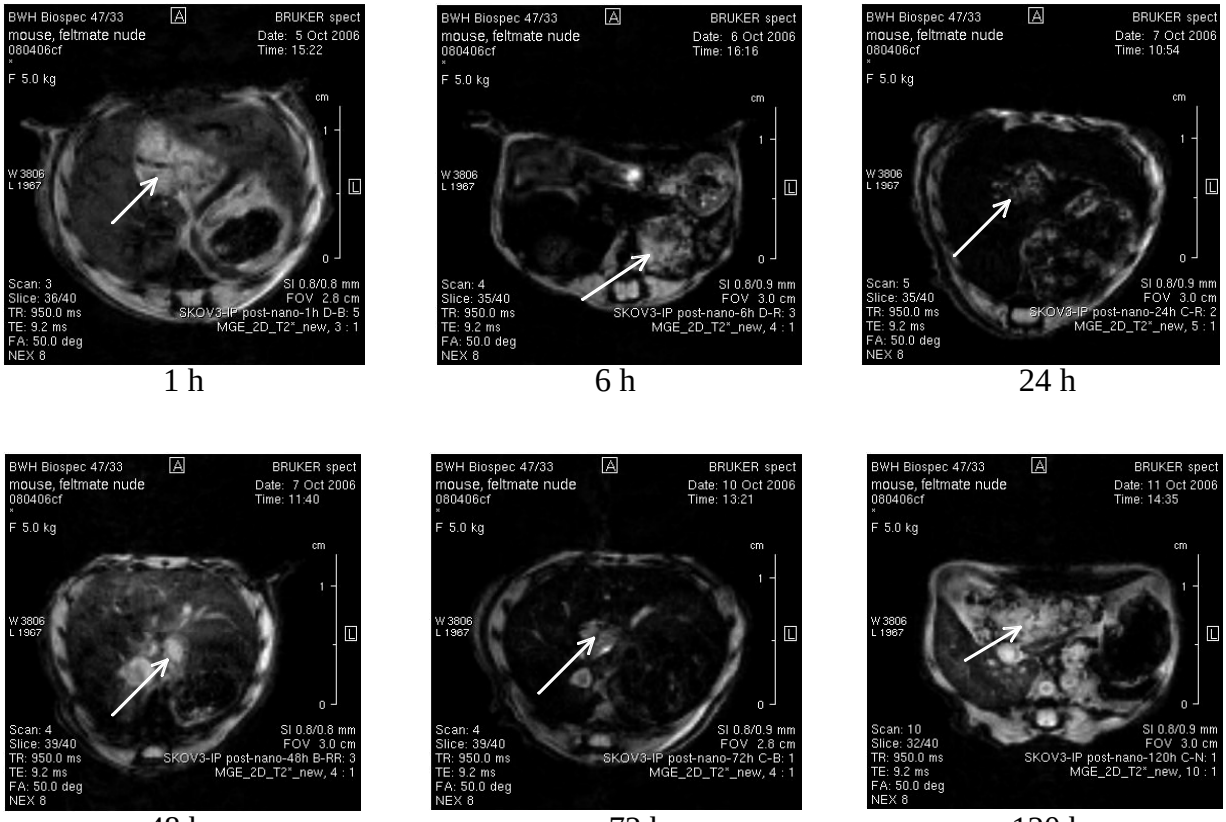
Spleen-2 / T2*



1 h 6 h 24 h 48 h 72 h 120 h

B.

Tumor / T2*



1 h 6 h 24 h

48 h 72 h 120 h

Figure 5. T2* -weighted MRI images of the (A) mouse spleen, and (B) tumor tissue (arrows) at different time points after MagNaGel injection.

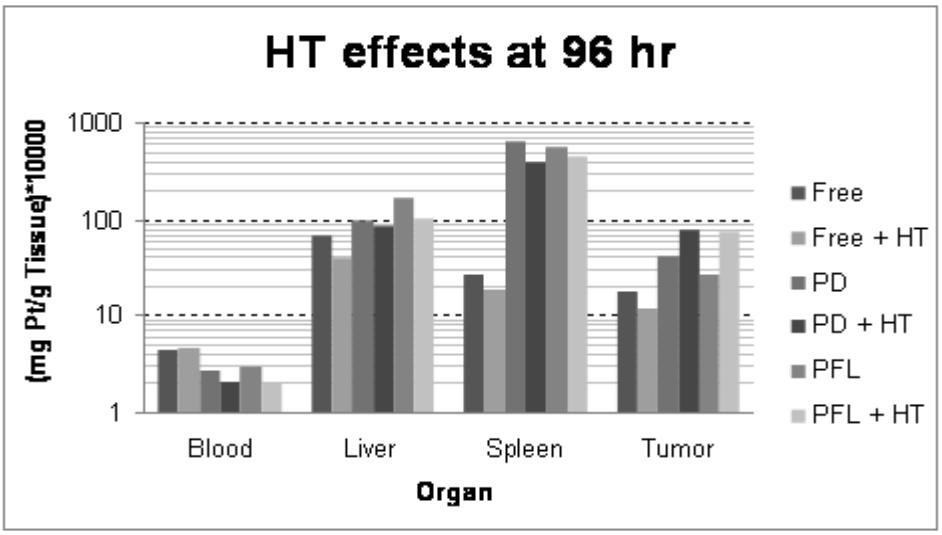
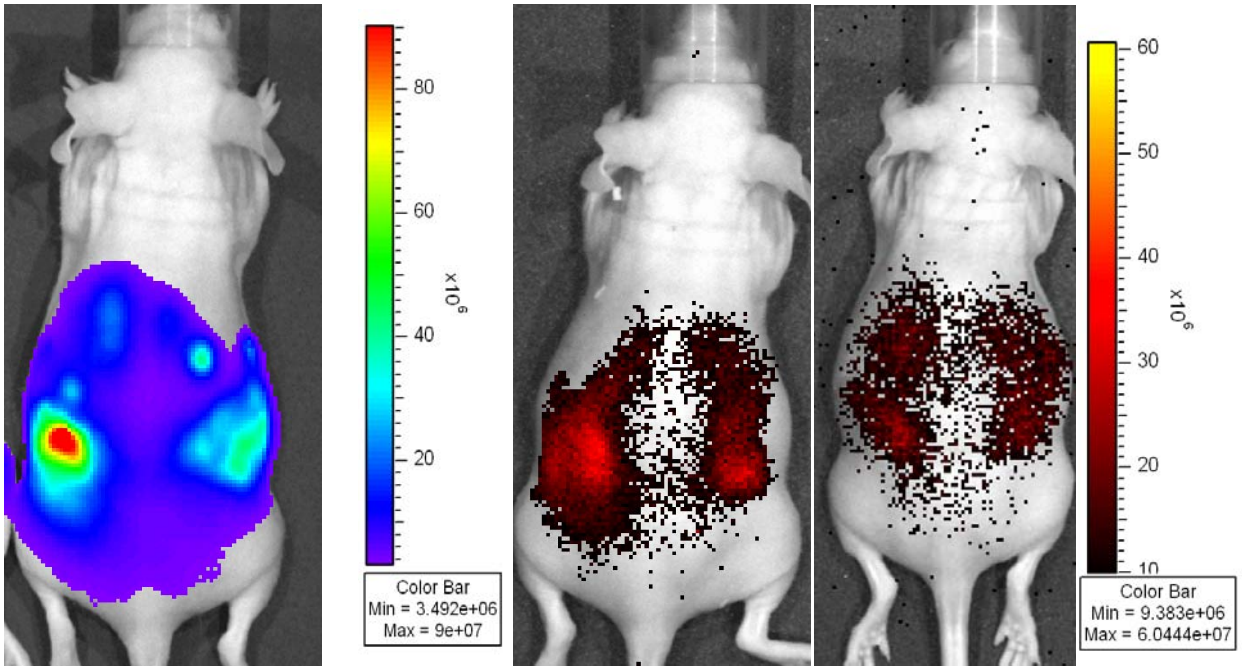


Figure 6. Platinum concentration in tissue with and without hyperthermic conditions. Free: Platinum alone; HT: hyperthermic treatment; PD: untargeted particle; PFL: Her2-targeted particle.



A. Luminescent image

B. Fluorescent images: 2-hours and 96-hours after particle injection

Figure 7. IVIS images showing tumor luminescence of SKOV3 ovarian cancer in mouse model (A) and co-localization of nanoparticles with red fluorescent marker Anexa 780 at 2-hr and 96-hr intervals after injection.

Efficacy Studies

Kaplan Meier analysis was performed on groups of 10 mice/group. Treatment groups included: negative control, unloaded nanoparticles, cisplatin-alone, untargeted platin-loaded nanoparticles, Her2-targeted platinum-loaded nanoparticles. In addition, comparison was made between the intraperitoneal route of administration and intravenous administration in Her2-targeted platin loaded nanoparticles. The results show that there is not a significant difference between the particles, either targeted or untargeted, and cisplatin alone.

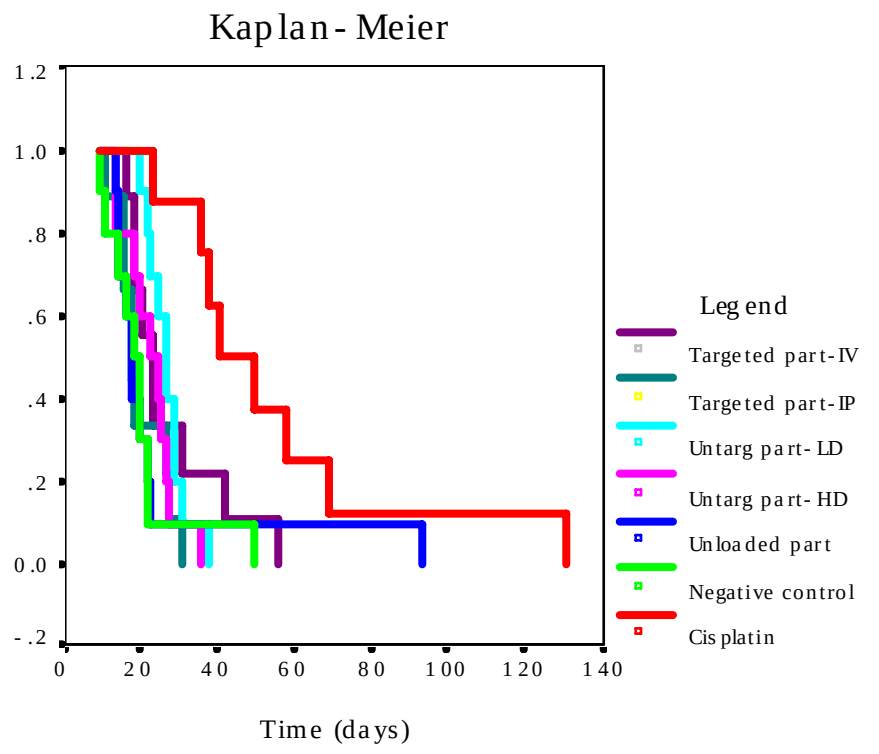


Figure 8. Kaplan-Meier Survival Analysis on mice injected with cisplatin alone, saline, unloaded particles without cisplat, loaded particles with cisplatin: HD high dose and LD low dose, targeted cisplatin-loaded particles via intraperitoneal (IP) route and intravenous (IV) route.

Key Research Accomplishments:

- Two orthotopic ovarian cancer mouse models have been developed. One without intraperitoneal tumor and one with both tumor and ascites
- MRI has been successfully utilized to document the development of tumor within our mouse model. It can also be utilized to follow nanoparticle distribution in various organs and tumor tissue.
- MRI images have been found to correlate with tissue and tumor cisplatin levels.
- IVIS Imaging system imaging complements MRI imaging and facilitates quantifying tumor via via bioluminescence signal using luciferase gene transfected tumor cells.

Reportable Outcomes:

- Data generated have been used for the application of an NIH R33 grant
- Data generated have been used for several smaller grants/funds. A Dana-Farber Friends grant was recently received in support of this work. Private funding sources are also being explored for further support of this research.
- Further research is underway in a collaborative effort with another group at Brigham and Women's Hospital utilizing other nanoparticles and different chemotherapeutic agents.

Conclusions:

Initial research has established a reliable orthotopic mouse model which can be consistently utilized in further research using nanoparticles. MRI has proved to be a valuable tool in documenting tumor development and following particle distribution in tumor and organs of interest. Studies utilizing these particular nanoparticles did not release effective cisplatin levels when compared with unincapsulated drug. Further work with particle shell characteristics for improved release and more specific targeting is ongoing. Hyperthermia may increase effective distribution and uptake of particles. Other nanoparticle shells are may be explored as with the use of other chemotherapeutic agents.

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2. Bibby DC, Talmadge JE, Dalal MK, Kurz SG, Chytil KM, Barry SE, Shand DG, Steiert M: Pharmacokinetics and biodistribution of RGD-targeted doxorubicin-loaded nanoparticles in tumor-bearing mice, Int J Pharm 2005, 293:281-290

Appendices:

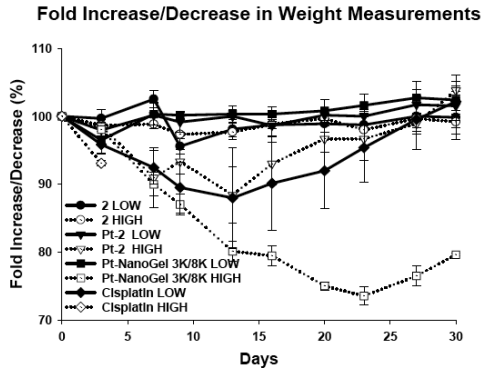

Fig. A1. Weight Measurements for mice dosed with formulations of MagNaGel (2) Pt-MagNaGel (Pt-2) a Pt-NanoGel formulation, and cisplatin, with each material dosed at a high (150 mcg Pt) and a low (50 mcg) dose. BALB/c mice, n=3 per group; tail vein injections were given on days 0, 2, 4, 7, and 9. The results showed that in the cisplatin high dose group all animals died within a day of the completion of their third dose. The low dose Pt-MagNaGel, Pt-2, was particularly well tolerated. Their diminished toxicity reflects an improved safety margin and possible enhancement of a therapeutic index.

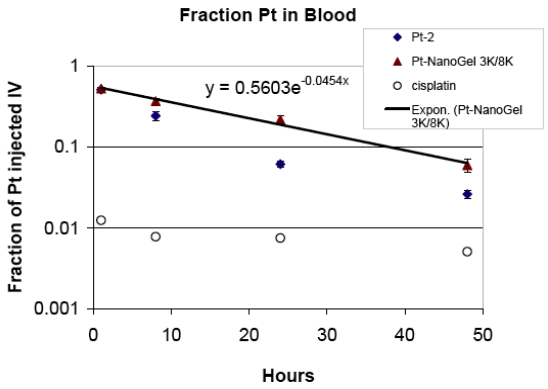

Fig. A2. Blood levels of Pt for two nanoparticle formulations and cisplatin. A single injection into the tail vein was given for each mouse at Hour 0. An exponential fit is shown for the Pt-NanoGel formulation. The results showed that nanoparticle formulations have extended circulation compared to cisplatin. Approximately half of the MagNaGel formulation remains in circulation after one hour. For the Pt-NanoGel formulation, the data from 1 to 24 hours (the beta phase, or elimination phase) is well-fit by an exponential decay with a 22 hour half life. This extended circulation time is promising for tumor accumulation in future studies using tumor bearing mouse models.