Items	**Recommendation**	**Our Response with Section/line number or reason for not reporting**
Study design	1. For each experiment provide brief details of study design including: a. The groups being compared, including control groups. If no control group has been used, the rationale should be stated. b. The experimental unit (e.g. a single animal, litter, or cage of animals)	1. Full details of in vivo Therapeutic efficacy Studies Design (a and b): All in vivo work has been performed at an IACUC approved laboratory and in accordance with ARRIVE guidelines for animal welfare. Animal studies were approved by the Institutional Animal Care and Use Committees of the Harry S. Truman Memorial Veterans Hospital and the University of Missouri and were performed in accordance with the Guide for the Care and Use of Laboratory Animals under an IACUC approved protocol number 8767. We have over three decades of experience in conducting hypothesis driven cancer research with *in vivo* models using tumor bearing SCID mice to minimize discomfort and adverse effects in study animals (both control and treated animals). Here are a few representative publications where we have outlined similar in vivo investigations which have been accepted by the global scientific peers: (1) Ravi Shukla, Nripen Chanda, and Kattesh V. Katti et al: ^{198}AuNP-EGCg for prostate cancer therapy: Proceedings of the National Academy of Sciences Jul 2012, 109 (31) 12426-12431; DOI: 10.1073/pnas.1121174109. (2) Nripen Chanda, Vijaya Kattumuri, Kattesh V. Katti, et al: Bombesin functionalized gold nanoparticles show in vitro and in vivo cancer receptor specificity: Proceedings of the National Academy of Sciences May 2010, 107 (19) 8760-8765; DOI: 10.1073/pnas.1002143107. (3) Nripen Chanda, Para Kan, Kattesh V. Katti, et al; Radioactive gold nanoparticles in cancer therapy: therapeutic efficacy studies of GA-198AuNP nanoconstruct in prostate tumor–bearing mice: Nanomedicine: Nanotechnology, Biology and Medicine, Volume 6, Issue 2, 2010, Pages 201-209, ISSN 1549-9634, https://doi.org/10.1016/j.nano.2009.11.001. **Brief Description of In vivo Investigations:** Male SCID mice (4 – 5 weeks of age; Taconic Farms, Hudson, NY) were housed in a temperature and humidity-controlled pathogen-free barrier facility. **NOTE:** Although male mice have been selected in our investigations, it is important to note that this is a xenograft model, thus murine gender is not anticipated to

		significantly influence tumor biology. Importantly, as we are studying prostate cancer, the use of male mice only is appropriate. **Description of animal procurement, housing, and grouping:** Animals were maintained on a 12 h light-dark cycle and had access to sterilized standard chow and water *ad libidum*. Animals were allowed to acclimate for 7–10 days prior to initiation of work. Human prostate cancer cell line PC-3 was obtained from the American Type Culture Collection (ATCC; Manassas, VA), and cultured according to ATCC recommendations by the University of Missouri Cell and Immunobiology Core facility. Mice received ear tag identifiers under inhalational anesthesia (isoflurane/oxygen) followed by unilateral, subcutaneous hind flank inoculations of 10×10^6 PC-3 cells suspended in 0.1 mL of sterile Dulbecco's phosphate buffered saline (DPBS) and Matrigel® (2:1, *v*:*v*). Solid tumors were allowed to develop over a period of 3 weeks, and animals were randomized (Day 0) into control and treatment groups (n = 7) having no significant difference in tumor volumes ($p = 0.64$; Student's t-test) or body weights ($p = 0.17$). Tumor volumes were estimated from caliper measurements using the formula V = length x width x depth. On Day 8, animals in the treatment group received intraperitoneal administrations of MGF-AuNP agent in DPBS (100 μL) while under inhalational anesthesia in doses as outlined in the following section. Similarly, control animals received 100 μL of saline intraperitoneally. No significant difference ($p = 0.93$) in tumor volume or body weight ($p = 0.21$) was noted between the groups. Tumor volumes, body weights and health status were then determined twice each week. At the end of the study (Day 42), mice were euthanized by cervical dislocation, and blood sample was collected by cardiac puncture. Samples of spleen, liver, tumor, and blood were harvested, weighed and submitted to the University of Missouri Neutron Activation Analysis (NAA) facility at the University of Missouri Research reactor (MURR) for the accurate quantification of gold in various tissues by NAA analysis.
Sample size	2. a. Specify the exact number of experimental units allocated to each group,	**Details of Sample Size with descriptions for a and b**: As outlined above, SCID mice were randomly divided into four groups (n=7/group) with no significant difference in tumor volumes. On day zero, mice were administered intraperitoneal injection of MGF-AuNP agent in DPBS (100 μL) (or saline for the control group) as follows:

	and the total number in each experiment. Also indicate the total number of animals used. b. Explain how the sample size was decided. Provide details of any a priori sample size calculation, if done.	Group 1- saline treated; Group 2- MGF-AuNPs treated (0.5 mg/kg bw); Group 3- MGF-AuNPs treated (1.0 mg/kg bw); Group 4- MGF-AuNPs treated (1.5 mg/kg bw). The fifth group (n=7) was kept as control group (no tumor and no treatment) and served as a control for the evaluation of complete blood count (CBC) values and body weight measurements. Animals were subjected to vaporizer 5% isoflurane and sacrificed at the end of study (Day 42). The above-mentioned sample size is based on consultations with our biostatistician who confirmed that a n=7 in different treatment and control groups, as elaborated above, would provide scientifically credible statistical significance to our preclinical data.
Inclusion and exclusion criteria	3. a. Describe any criteria used for including and excluding animals (or experimental units) during the experiment, and data points during the analysis. Specify if these criteria were established a priori. If no criteria were set, state this explicitly. b. For each experimental group, report any animals, experimental units or data points not included in the analysis and explain why. If there were no exclusions, state so. c. For each analysis, report the exact value of n in each experimental group.	**Inclusion criteria: On the choice of the animal model, we have used** Male SCID mice in our investigations. **NOTE:** Although male mice have been selected in our investigations, it is important to note that this is a xenograft model, thus murine gender is not anticipated to significantly influence tumor biology. Importantly, as we are studying prostate cancer, the use of male mice only is appropriate. **Experimental and Control Groups:** SCID mice were randomly divided into four groups (n=7/group) with no significant difference in tumor volumes. On day zero, mice were administered intraperitoneal injection of MGF-AuNP agent in DPBS (100 μL) (or saline for the control group) as follows: Group 1- saline treated; Group 2- MGF-AuNPs treated (0.5 mg/kg bw); Group 3- MGF-AuNPs treated (1.0 mg/kg bw); Group 4- MGF-AuNPs treated (1.5 mg/kg bw). The fifth group (n=7) was kept as control group (no tumor and no treatment) and served as a control for the evaluation of complete blood count (CBC) values and body weight measurements. Animals were subjected to vaporizer 5% isoflurane and sacrificed at the end of study (Day 42). There were no exclusions in our investigations. Exclusion criteria: N/A

Randomization	4a. State whether randomization was used to allocate experimental units to control and treatment groups. If done, provide the method used to generate the randomization sequence. b. Describe the strategy used to minimize potential confounders such as the order of treatments and measurements, or animal/cage location. If confounders were not controlled, state this explicitly.	Mice received ear tag identifiers under inhalational anesthesia (isoflurane/oxygen) followed by unilateral, subcutaneous hind flank inoculations of 10×10^6 PC-3 cells suspended in 0.1 mL of sterile Dulbecco's phosphate buffered saline (DPBS) and Matrigel® (2:1, *v*:*v*). Solid tumors were allowed to develop over a period of 3 weeks, and animals were randomized (Day 0) into control and treatment groups (n = 7) having no significant difference in tumor volumes ($p = 0.64$; Student's t-test) or body weights ($p = 0.17$). Tumor volumes were estimated from caliper measurements using the formula V = length x width x depth. On Day 8, animals in the treatment group received intraperitoneal administrations of MGF-AuNP agent in DPBS (100μL) while under inhalational anesthesia in doses as outlined in the following section. Similarly, control animals received 100μL of saline intraperitoneally. No significant difference ($p = 0.93$) in tumor volume or body weight ($p = 0.21$) was noted between the groups. Tumor volumes, body weights and health status were then determined twice each week. All animal facilities at the Harry S. Truman Memorial Veterans Hospital and the University of Missouri, Columbia, Missouri, were visited daily by the veterinarian care staff, inspected by the institutional animal care and use committee members throughout the investigation. Mice were examined daily, and removed from the study if unresponsive to supportive care, moribund, if weight loss is >20% body weight or if tumor size > 5 cm^3 with poor body condition (hunched posture and loss of >20% body weight, or easily palpated exoskeleton) or with lassitude with written protocols to euthanatize such animals to minimize animal discomfort.
Blinding	5. Describe who was aware of the group allocation at the different stages of the experiment (during the allocation, the conduct of the experiment, the outcome assessment, and the data analysis).	All experiments of MGF-AuNPs involving animals were approved by the Institutional Animal Care and Use Committees (IACUC, protocol number 8767) of the Harry S. Truman Memorial Veterans Hospital and the University of Missouri were performed according to the Guide for the Care and Use of Laboratory Animals. All animal facilities at the Harry S. Truman Memorial Veterans Hospital and the University of Missouri, Columbia, Missouri, were visited daily by the veterinarian care staff, inspected by the institutional animal care and use committee members throughout the investigation**.**

		The animal modeling staffs and veterinarians, who conducted our animal experiments, have over 20 years of experience in all aspects of pharmaceutical testing through placebo-controlled, blinded pre clinal investigations in tumor bearing mice. Such data has formed the basis for seeking approval for Phase 1 trials of various drugs discovered by us in the past. In the current investigation, as reported in our manuscript, our staff have exercised due care and caution to perform blinded experiments in prostate tumor bearing xenografts in SCID mice with our nanomedicine agents MGF-AuNP with saline in controls.
Outcome measures	6 a. Clearly define all outcome measures assessed (e.g. cell death, molecular markers, or behavioral changes). b. For hypothesis-testing studies, specify the primary outcome measure, i.e. the outcome measure that was used to determine the sample size.	**Outcome Measures:** Morbidity: As accepted by the institutional Animal Care and Use Committees (IACUC, protocol number 8767) of the Harry S. Truman Memorial Veterans Hospital and the University of Missouri Guide for the Care and Use of Laboratory Animals, we defined morbidity to include any animal where one of the following conditions exists: the tumor volume exceeds 5 cm^3, ulceration of the overlying skin of the tumor is observed, ulceration of the tumor itself is observed, body weight-loss of more than 20% is noted, and/or significant illness/depression (whether or not related to the experimental protocol) is observed. Animals exhibiting any signs of morbidity, as defined, will be sacrificed immediately to minimize and alleviate any unnecessary pain and suffering. Mortality was evaluated by measuring any differences in the total survival times between groups as a function of study termination time. These studies required 7 mice per experimental group to provide meaningful statistical results based on the expectation of accurately detecting a 20% difference in experimental tumor groups. **Hypothesis validation and expected primary outcome:** The overall hypothesis was to validate the antitumor characteristics of the experimental nanomedicine agent. The following groups of control and tumor bearing mice were administered intraperitoneal injections of MGF-AuNP agent in DPBS (100 μL) (or saline for the control group) as follows: Group 1- saline treated; Group 2- MGF-AuNPs treated (0.5 mg/kg bw); Group 3- MGF-AuNPs treated (1.0 mg/kg bw);

		Group 4- MGF-AuNPs treated (1.5 mg/kg bw). The fifth group (n=7) was kept as control group (no tumor and no treatment) and served as a control for the evaluation of complete blood count (CBC) values and body weight measurements. Animals were subjected to vaporizer 5% isoflurane and sacrificed at the end of study (Day 42). 50-80% reduction in tumor volumes in the treated groups, as compared to the control group, with minimal/no adverse toxic side effects, (Groups 2-4) was the expected outcome of this hypothesis driven investigation. Throughout the study, the animals were monitored for their tumor volume (groups 1-4), body weight and overall health (group 1-5). Mice were examined daily, and removed from the study if unresponsive to supportive care, moribund, if weight loss is >20% body weight or if tumor size > 5 cm^3 with poor body condition (hunched posture and loss of >20% body weight, or easily palpated exoskeleton) or with lassitude with written protocols to euthanatize such animals to minimize animal discomfort. At the end of the study (day 42) animals were subjected to vaporizer 5% isoflurane and before being sacrificed and following samples were collected from group 1-4, blood, tissues (spleen, liver, and tumor). Blood samples from all groups were used for complete blood count (CBC) values. The tissues (spleen, liver, tumor and blood) were submitted to the University of Missouri Neutron Activation Analysis (NAA) facility at the University of Missouri Research reactor (MURR) for the accurate quantification of gold in various tissues by NAA analysis.

Statistical methods	7 a. Provide details of the statistical methods used for each analysis, including software used. b. Describe any methods used to assess whether the data met the assumptions of the statistical approach, and what was done if the assumptions were not met.	Mortality was evaluated by measuring any differences in the total survival times between groups as a function of study termination time. These studies required 7 mice per experimental group to provide meaningful statistical results based on the expectation of accurately detecting a 20% difference in experimental tumor groups. All experimental data are described as mean±SEM. Statistical analysis was carried out using the one-way analysis of variances (ANOVA) using Graph Pad Prism software. $P<0.05$ was considered significant.
Experimental Animals	8 a. Provide species-appropriate details of the animals used, including species, strain and substrain, sex, age or developmental stage, and, if relevant, weight. b. Provide further relevant information on the provenance of animals, health/immune status, genetic modification status, genotype, and any previous procedures	**Species-appropriate details:** a. Male SCID mice (4 – 5 weeks of age; Taconic Farms, Hudson, NY) were housed in a temperature and humidity-controlled pathogen-free barrier facility. **NOTE:** Although male mice have been selected in our investigations, it is important to note that this is a xenograft model, thus murine gender is not anticipated to significantly influence tumor biology. Importantly, as we are studying prostate cancer, the use of male mice only is appropriate. Male SCID mice (4 – 5 weeks of age; Taconic Farms, Hudson, NY) weighing 20-25 g in weight were housed in a temperature and humidity-controlled pathogen-free barrier facility. b. **Description of animal procurement, housing, and grouping:** Animals were maintained on a 12 h light-dark cycle and had access to sterilized standard chow and water *ad libidum*. Animals were allowed to acclimate for 7–10 days prior to initiation of work. Human prostate cancer cell line PC-3 was obtained from the American Type Culture Collection (ATCC; Manassas, VA), and cultured according to ATCC recommendations by the University of Missouri Cell and Immunobiology Core facility. Mice received ear tag identifiers under inhalational anesthesia (isoflurane/oxygen) followed by unilateral, subcutaneous hind flank inoculations of 10×10^6 PC-3 cells suspended in 0.1 mL of sterile Dulbecco's phosphate buffered saline (DPBS) and Matrigel® (2:1, *v*:*v*). Solid tumors were allowed to develop over a period of 3 weeks, and animals were randomized (Day 0) into control and treatment groups (n = 7) having no significant difference in tumor volumes ($p = 0.64$; Student's t-test) or

		body weights (p = 0.17). Tumor volumes were estimated from caliper measurements using the formula V = length x width x depth. On Day 8, animals in the treatment group received intraperitoneal administrations of MGF-AuNP agent in DPBS (100 μL) while under inhalational anesthesia in doses as outlined in the following section. Similarly, control animals received 100 μL of saline intraperitoneally. No significant difference (p = 0.93) in tumor volume or body weight (p = 0.21) was noted between the groups. Tumor volumes, body weights and health status were then determined twice each week. At the end of the study (Day 42), mice were euthanized by cervical dislocation, and blood sample was collected by cardiac puncture. Samples of spleen, liver, tumor, and blood were harvested, weighed and submitted to the University of Missouri Neutron Activation Analysis (NAA) facility at the University of Missouri Research reactor (MURR) for the accurate quantification of gold in various tissues by NAA analysis.
Experimental animals	9. For each experimental group, including controls, describe the procedures in enough detail to allow others to replicate them, including: a. What was done, how it was done and what was used. b. When and how often. c. Where (including detail of any acclimatization periods). d. Why (provide rationale for procedures).	**Full experimental details of all nnimal studies reported in our manuscript:** **Ethics Committee Approvals:** All in vivo work has been performed at an IACUC approved laboratory and in accordance with ARRIVE guidelines for animal welfare. Animal studies were approved by the Institutional Animal Care and Use Committees of the Harry S. Truman Memorial Veterans Hospital and the University of Missouri and were performed in accordance with the Guide for the Care and Use of Laboratory Animals under an IACUC approved protocol number 8767. **NOTE:** We have over three decades of experience in conducting hypothesis driven cancer research with *in vivo* models using tumor bearing SCID mice to minimize discomfort and adverse effects in study animals (both control and treated animals). Here are a few representative publications where we have outlined similar in vivo investigations which have been accepted by the global scientific peers: (4) Ravi Shukla, Nripen Chanda, and Kattesh V. Katti et al: ^{198}AuNP-EGCg for prostate cancer therapy: Proceedings of the National Academy of Sciences Jul 2012, 109 (31) 12426-12431; DOI: 10.1073/pnas.1121174109

		(5) Nripen Chanda, Vijaya Kattumuri, Kattesh V. Katti, et al: Bombesin functionalized gold nanoparticles show in vitro and in vivo cancer receptor specificity: Proceedings of the National Academy of Sciences May 2010, 107 (19) 8760-8765; DOI: 10.1073/pnas.1002143107; (6) Nripen Chanda, Para Kan, Kattesh V. Katti, et al; Radioactive gold nanoparticles in cancer therapy: therapeutic efficacy studies of GA-198AuNP nanoconstruct in prostate tumor–bearing mice: Nanomedicine: Nanotechnology, Biology and Medicine, Volume 6, Issue 2, 2010, Pages 201-209, ISSN 1549-9634,; https://doi.org/10.1016/j.nano.2009.11.001. **Justification for the Use of Animals**:There are no in vitro tests that can be used to substitute for the complex tumor microenvironment occurring in vivo when testing experimental candidates for their effectiveness as prostate cancer therapy agents. Our investigations of therapeutic effectiveness studies, of a new nanomedicine agent, MGF-AuNP, in vivo using human prostate tumor xenografts in SCID mice are necessary. Therefore, the SCID mice model with prostate tumor xenografts, as described in our manuscript, represents the most widely accepted and the best model for pre-clinical evaluations of novel therapeutic strategies ultimately intended for use in treating human prostate tumor patients. All experiments of MGF-AuNPs involving animals were approved by the Institutional Animal Care and Use Committees (IACUC, protocol number 8767) of the Harry S. Truman Memorial Veterans Hospital and the University of Missouri were performed according to the Guide for the Care and Use of Laboratory Animals. We have used severely compromised immunodeficient (SCID) mice bearing a flank model of human prostate cancer derived from a subcutaneous implant of 10 million PC-3 cells for therapeutic efficacy and pharmacokinetic studies (from Taconic Farms, Hudson, New York) were used for the therapeutic study. The mice used in our investigations weighed 24-27 g. ***In vivo* bio-distribution study by Neutron Activation Analysis (NAA).** To assess the gold content in tissue in SCID mice (n=7). 1.5 mg/ kg bw MGF-AuNPs were

		administered in these mice for seven weeks, while control mice (n=7) did not receive any treatment with MGF-AuNPs. Tumor, spleen, liver and blood were harvested upon euthanization (vaporizer 5% isoflurane), put into chloridometer vials and dried for approximately 48 hours at 100-120°C. Dried tissue mass of approximately 0.5-1.0 g was placed into polyethylene vials (used for control of counting geometry). We estimated the amount of gold in various tissue samples as described previously. ***In vivo* therapeutic efficacy study.** Antitumor efficacy of MGF-AuNPs was evaluated by developing prostate tumor model (in SCID male mice). The SCID male mice were subcutaneously inoculated with $10x10^6$ PC-3 cells (suspended in 0.1 mL of sterile DPBS and Matrigel® (2:1, v:v)) in the right hind flank under inhalation anesthesia (isoflurane/ oxygen). After inoculation, tumors were allowed to grow for 2-3 weeks, at which time the tumors were measured by digital caliper measurements and calculated as length x width x height. The mice were randomly divided into four groups (n=7/group) with no significant difference in tumor volume, randomization was generated using the standard = RAND() function in Microsoft Excel, and the day of randomization was considered the day zero of therapy study. On day zero, mice were given intraperitoneal injections as follows: Group 1: saline treated (100 μL); Group 2: MGF-AuNPs treated (0.5 mg/kg bw); Group 3: MGF-AuNPs treated (1.0 mg/kg bw) and Group 4: MGF-AuNPs treated (1.5 mg/kg bw)—all in 100 μL Dulbecco's PBS. Using this regimen, animals were treated twice per week until the end of the study (42 days). The animals were monitored for their tumor volume, body weight and health effects until they were sacrificed at the end of the study. The fifth group (n=7) was kept as control group (no tumor and no treatment) and served as a control for complete blood count (CBC) values and body weight measurements. Animals were sacrificed at the end of study. Measurement of tumor volumes were carried out twice each week until the end of the study (Day 42). Within two weeks (Day 14), tumor growth in the treated group (with MGF-AuNPs at 1.5 mg/kg bw), appeared to be slowing with respect to the controls. After 17 days of post administration (dose of MGF-AuNPs at 1.5 mg/kg bw), tumor volumes were two-fold lower ($p < 0.005$) for treated animals compared to controls. This significant therapeutic effect was maintained throughout our observational study. Tumor volumes for the control animals were fully six-seven-fold greater with

		respect to those for the MGF-AuNPs-treated group ($p < 0.0001$; 0.37 ± 0.05 *vs*. 0.06 ± 0.02 cm^3) groups—at three weeks, post administration of after MGF-AuNPs (1.5 mg/kg bw). These observations were indicative of >85% reduction in the overall tumor volume for the treated group. This profound therapeutic efficacy was observed throughout the 42 days long study. Tumors harvested from the treatment group consisted largely of necrotic tissue, indicating extensive death of tumor cells. The tissues (spleen, liver, tumor tissue and blood) were isolated from prostate tumor xenografts and were submitted to the University of Missouri Neutron Activation Analysis (NAA) facility at the University of Missouri Research reactor (MURR) for the accurate quantification of gold in various tissues by NAA analysis.
Results	10 For each experiment conducted, including independent replications, report: a. Summary/descriptive statistics for each experimental group, with a measure of variability where applicable (e.g. mean and SD, or median and range). b. If applicable, the effect size with a confidence interval	**Full description of Results/Summary (taken directly from our manuscript):** In our evaluations, unilateral solid tumors were allowed to grow for three weeks, and animals were randomized (denoted Day 0) into control and treatment groups (n = 7) with no significant differences in tumor volume. In vivo dosing involved administering on day 0 three doses of MGF-AuNP (0.5 mg/kg bw, 1.0 mg/kg bw and 1.5 mg/kg bw—in 100 μL Dulbecco's PBS) intraperitoneally, while the control SCID mice received only 100 μL Dulbecco's PBS/saline. Tumors were then measured twice each week until the end of the study (Day 42). Figure 16 shows results from the MGF-AuNPs-treated human prostate cancer bearing SCID mice. Within two weeks (Day 14), tumor growth in the treated animals started slowing down with respect to the control animals. Day 17, post administration of MGF-AuNPs (1.5 mg/kg bw), tumor volumes were two-fold lower ($p < 0.005$) for treated animals as compared to the control group. Three weeks, post administration of after MGF-AuNPs (1.5 mg/kg bw), tumor volumes for the control animals were fully six-fold greater with respect to those for the MGF-AuNPs-treated group ($p < 0.0001$; 0.37 ± 0.05 *vs*. 0.06 ± 0.02 cm^3)—suggesting >85% reduction in the overall tumor volume for the treated group. This significant therapeutic effect was maintained throughout the 42 days long study. Tumors harvested from the treatment group consisted largely of necrotic tissue, indicating extensive death of tumor cells.