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13. SUPPLEMENTARY NOTES

14. ABSTRACT **Intraperitoneal seeding of ovarian cancer is common, difficult to treat, and intraperitoneal (i.p.) chemotherapy is being investigated ovarian cancer clinical trials. We propose to identify new drug combinations that improve the activity of cisplatin + taxane therapy against i.p. ovarian cancer using novel, patient-derived, i.p. ovarian cancer xenograft models (PDX). Fenretinide (4-HPR) is a cytotoxic retinoid that has been shown to be cytotoxic for and enhance cisplatin activity in ovarian cancer cell lines. Our phase II study in ovarian cancer of a poorly bioavailable 4-HPR capsule formulation showed that women achieving higher 4-HPR plasma levels had a higher event-free survival. We have since developed novel intravenous and oral formulations of 4-HPR (the latter 4-HPR/LXS) that reliably achieve drug plasma levels of >10 to 50 µM, have achieved multiple, sustained, complete responses in Phase I trials of relapsed neuroblastoma and T-cell lymphomas, and our 4-HPR/LXS oral powder formulation + ketoconazole (as a P450 metabolism inhibitor to increase 4-HPR plasma levels) is currently being tested in a Phase I/II ovarian cancer trial. EpHA2 is a cell surface antigen expressed on ovarian cancers. MM-310 is a novel liposomal formulation of docetaxel that is targeted to ovarian cancer using an EpHA2 antibody that is expected to enhance taxane activity against ovarian cancer.**

15. SUBJECT TERMS

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Background: Intraperitoneal seeding of ovarian cancer is common, difficult to treat, and intraperitoneal (i.p.) chemotherapy is being investigated ovarian cancer clinical trials. We propose to identify new drug combinations that improve the activity of cisplatin + taxane therapy against i.p. ovarian cancer using novel, patient-derived, i.p. ovarian cancer xenograft models (PDX). Fenretinide (4-HPR) is a cytotoxic retinoid that has been shown to be cytotoxic for and enhance cisplatin activity in ovarian cancer cell lines. Our phase II study in ovarian cancer of a poorly bioavailable 4-HPR capsule formulation showed that women achieving higher 4-HPR plasma levels had a higher event-free survival. We have since developed novel intravenous and oral formulations of 4-HPR (the latter 4-HPR/LXS) that reliably achieve drug plasma levels of >10 to 50 μM, have achieved multiple, sustained, complete responses in Phase I trials of relapsed neuroblastoma and T-cell lymphomas, and our 4-HPR/LXS oral powder formulation + ketoconazole (as a P450 metabolism inhibitor to increase 4-HPR plasma levels) is currently being tested in a Phase I/II ovarian cancer trial. EpHA2 is a cell surface antigen expressed on ovarian cancers. MM-310 is a novel liposomal formulation of docetaxel that is targeted to ovarian cancer using an EpHA2 antibody that is expected to enhance taxane activity against ovarian cancer.

Hypothesis: We hypothesize that combining *systemic* 4-HPR + *systemic or intraperitoneal* paclitaxel or docetaxel with intravenous or *i*ntraperitoneal cisplatin will be tolerable and highly active against ovarian cancer. We also hypothesize that docetaxel (targeted to ovarian cancer by EPhA2 in the MM-310 formulation) either alone or in combination with 4-HPR, will be highly active against ovarian cancer.

Specific Aims: All Aims will test activity of novel drug combinations against patient-derived intraperitoneal ovarian cancer xenografts. **Aim 1** will test 4-HPR/ketoconazole + paclitaxel; **Aim 2** will test 4-HPR/ketoconazole + EphA2-targeted docetaxel (MM-310) vs. untargeted liposomal docetaxel; **Aim 3** will compare the antitumor activity of the most active taxane combination (from Aims 1 and 2) in combination with cisplatin.

Study Design: We will explant ascites from our twelve intraperitoneal (i.p.) ovarian PDX models and test 4-HPR + paclitaxel for synergistic cytotoxicity using our *in vitro* DIMSCAN cytotoxicity assay. We will determine the activity and tolerability of 4-HPR-based drug combinations (see Specific Aims) in three i.p. patient-derived xenograft (PDX) models of intraperitoneal ovarian cancer. As a comparator to previously published ovarian cancer preclinical models we will assess the most active 4-HPR + taxane drug combination together with cisplatin in three PDX i.p. and two PDX subcutaneous xenograft models.

Innovation: Few ovarian cancer preclinical studies employ i.p. xenograft models and fewer yet employ i.p. PDX models. Our novel 4-HPR formulation (especially when combined with ketoconazole) allows higher 4-HPR exposures to be obtained in mice and in patients. No previous studies have examined 4-HPR at high exposures combined with taxanes. The MM-310 EphA2-targeted docetaxel is a recently developed novel taxane formulation that has not previously been tested in ovarian cancer.

Impact: Fenretinide (4-HPR) has shown preclinical and clinical signals of activity in ovarian cancer. Thus, novel 4-HPR-based drug combinations provide a promising novel approach to treating recurrent ovarian cancer. As the applicants are the FDA IND sponsors of 4-HPR INDs, and our GYN oncology co-investigator is the study chair of the ongoing South Plains Oncology Consortium (SPOC) (www.SPONC.org) phase II study of 4-HPR/LXS + ketoconazole, success in the proposed preclinical studies could enable us to rapidly develop and undertake novel phase I clinical trials in SPOC. The drug sponsor of MM-310 (Merrimack Pharmaceuticals) is enthusiastic about our proposed use of MM-310 and would likely provide clinical-grade MM-310 for early phase trials if the preclinical studies proposed in this application support carrying out such clinical trials. As William Beaumont Army Hospital (Ft Bliss, TX) is a member of SPOC, military service members, retired military, and military family members would have rapid access to this novel therapeutic approach to recurrent ovarian cancer.

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INTRODUCTION: We propose to identify new drug combinations that improve the activity of cisplatin + taxane therapy against i.p. ovarian cancer using novel, patient-derived, i.p. ovarian cancer xenograft models (PDX). Fenretinide (4-HPR) is a cytotoxic retinoid that has been shown to be cytotoxic for and enhance cisplatin activity in ovarian cancer cell lines. Our phase II study in ovarian cancer of a poorly bioavailable 4-HPR capsule formulation showed that women achieving higher 4-HPR plasma levels had a higher event-free survival. We have since developed novel intravenous and oral formulations of 4-HPR (the latter 4-HPR/LXS) that reliably achieve drug plasma levels of >10 to 50 μM, have achieved multiple, sustained, complete responses in Phase I trials of relapsed neuroblastoma and T-cell lymphomas, and our 4-HPR/LXS oral powder formulation + ketoconazole (as a P450 metabolism inhibitor to increase 4-HPR plasma levels) is currently being tested in a Phase I/II ovarian cancer trial.

EpHA2 is a cell surface antigen expressed on ovarian cancers. MM-310 is a novel liposomal formulation of docetaxel that is targeted to ovarian cancer using an EpHA2 antibody that is expected to enhance taxane activity against ovarian cancer. **Aim 1** will test 4-HPR/ketoconazole + paclitaxel; **Aim 2** will test 4-HPR/ketoconazole + EphA2-targeted docetaxel (MM-310) vs. untargeted liposomal docetaxel; **Aim 3** will compare the antitumor activity of the most active taxane combination (from Aims 1 and 2) in combination with cisplatin.

1. **KEYWORDS:** ovarian cancer cisplatin taxanes fenretinide EpHA2 antibody patient-derived xenografts

2. **OVERALL PROJECT SUMMARY:**

   Major Task 1 (Specific Aim 1)
   Determine if fenretinide (4-HPR), administered as 4-HPR/LXS oral powder with concurrent ketoconazole as a P450 4-HPR metabolism inhibitor to increase 4-HPR exposure, enhances the activity of paclitaxel against intraperitoneally-seeded ovarian cancer patient-derived, xenografts (PDX).

   Subtask 1: Submit documents on planned mouse experiments for ACURO approvals, which include local IRB and IACUC approval that has already been obtained.
   Receive HRPO/ACURO approval before initiating relevant experiments.

   This goal has been fully accomplished; all animal experiments for this project have been approved.

   Carry out experiments testing paclitaxel +/- 4-HPR/LXS against intraperitoneal ovarian cancer patient-derived xenografts from 3 patients

   Considerable effort was expended in evaluating ovarian cancer patient-derived xenografts (PDXs) as intraperitoneal (IP) tumors. While we were able to obtain growth of IP tumors form most PDX models in our panel, some of these models rapidly invaded out of the peritoneum, a behavior not observed in the patients from which the tumors were obtained. For those models in which growth was confined (as expected) to the peritoneum we found that quantifying tumor response was exceedingly difficult. Consultation with other

investigators using ovarian cancer PDXs confirmed our impression that quantifying response in IP models was likely going to limit the chief goal of the project, which was to evaluate activity of novel drug combinations. Thus, we determined it was necessary to focus our efforts on testing ovarian cancer PDX models as subcutaneous xenografts.

We evaluated ovarian cancer PDXs from 3 patients: TX-OV-075x, TX-OV-121x, and TX-OV-225x. All 3 of these PDX models generated consistent engraftment and growth and were deemed suitable for the proposed studies. Figures 1 and 2 show the evaluation of paclitaxel (as Abraxane) combined with carboplatin in TX-OV-075x and TX-OV-225x. Interestingly, in 075x carboplatin is not effective as a single agent or when combined with Abraxane, demonstrating that 075x provides a true platinum-refractory ovarian cancer PDX model. By contrast in the 225x model carboplatin and Abraxane are both active and the combination shows greater activity than either single agent.

Figure 1

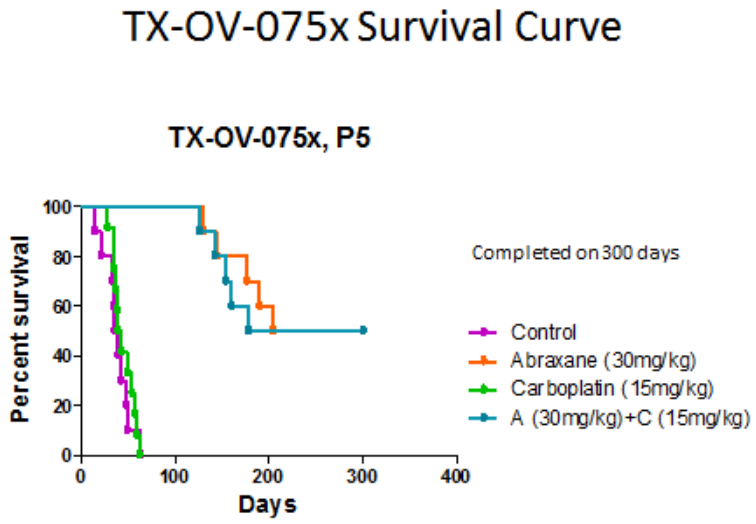
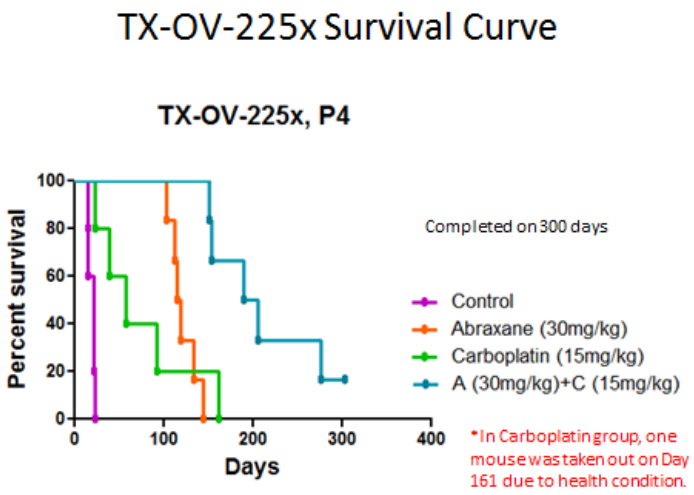


Figure 2



We assessed 4-HPR + carboplatin and 4-HPR + taxanes in a number of ovarian cancer cell lines. We found that addition of 4-HPR to either of those agents did not provide a high amount of cytotoxicity to warrant xenograft testing. Because of this we have focused our efforts on studying docetaxel in the novel EPhA2 antibody-targeted formulation (MM-310) compared to the untargeted liposomal formulation of docetaxel, both as a single agent and in combination with carboplatin. As shown in Figure 3, MM-310, the EPhA2 antibody targeted formulation of docetaxel was more effective than the non-targeted formulation in the 075x model, a very encouraging observation given the platinum-refractory nature of this model. However, as shown in Figure 4 in some models MM-310 was not more effective than the untargeted formulation.

Figure 3

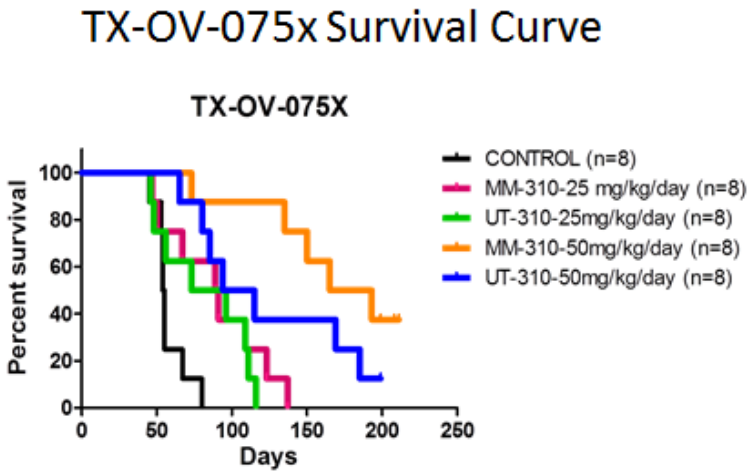
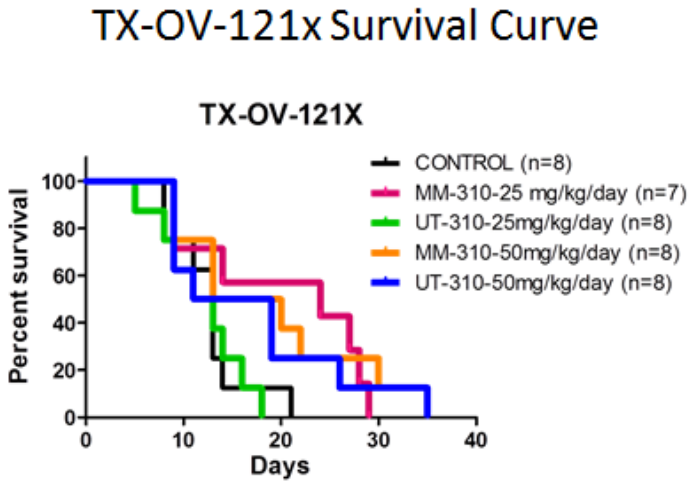


Figure 4



Using the commonly used A2780 cell line xenograft, to enable comparison with previous data from other investigators, we evaluated fenretinide (4-HPR) + ketoconazole (to increase 4-HPR exposures in mice) with and without MM-310. We observed a modest increase in MM-310 activity when combined with 4-HPR + ketoconazole (Figure 5).. These data are consistent with our cell line cytotoxicity data and caused us to re-think further pursuing drug combinations with 4-HPR> Also in discussions with the MM-310-sponsor it became clear that initial clinical trials would be of MM-310 with a platinum compound so we have focused our efforts on obtaining data to support an initial clinical trials.

Figure 5

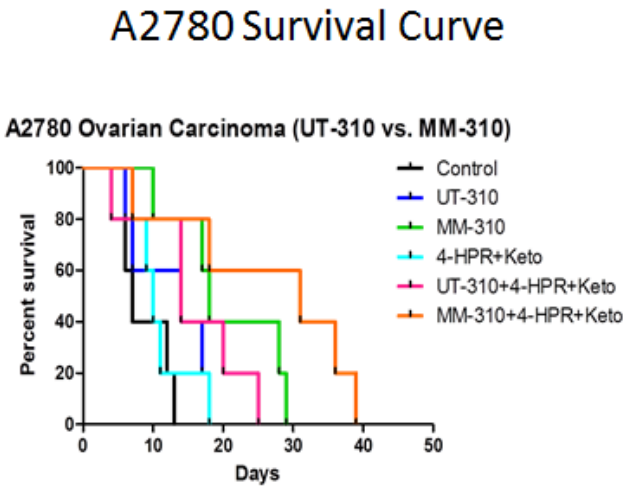


Figure 6

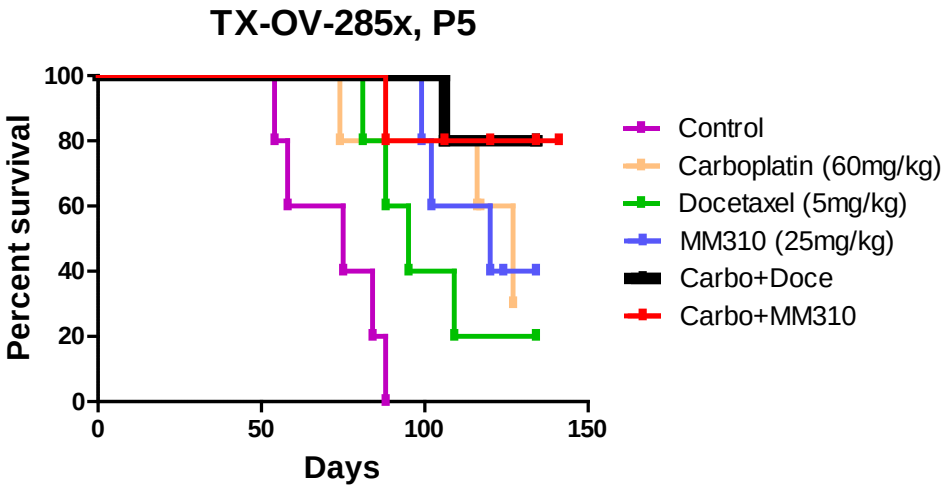
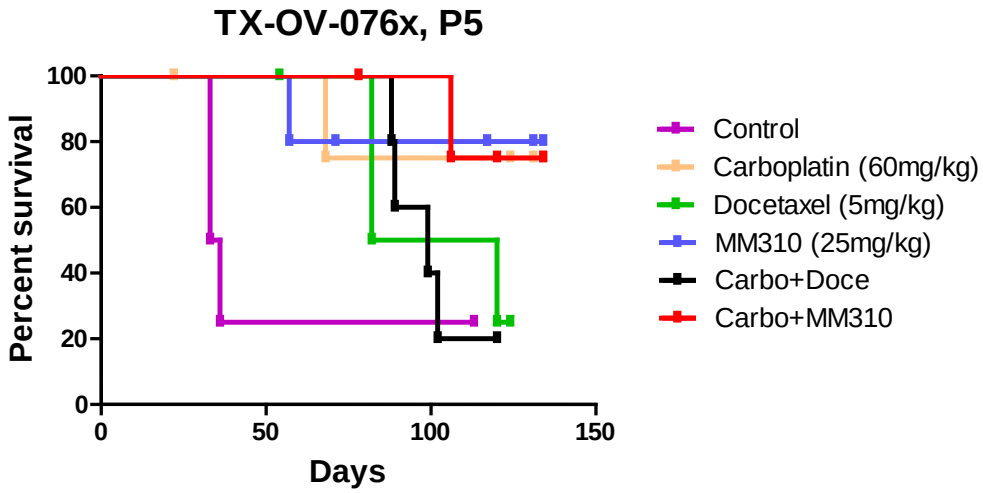


Figure 7



To better inform development of phase II clinical trials in ovarian cancer of MM-310 we used subcutaneous ovarian cancer PDXs to compare MM-301 + carboplatin to the standard clinical formulation of docetaxel + carboplatin.

As shown in Figure 6, testing the TX-OV-285x PDX both docetaxel + carboplatin and MM-310 + carboplatin showed superior activity (mouse event-free survival) compared to controls. However MM-310 + carboplatin was not superior to docetaxel + carboplatin.

As shown in Figure 7, using the TX-OV-076x PDX we found that MM-310 + carboplatin was superior to docetaxel + carboplatin.

We also evaluated combining MM-310 with fenretinide (4-HPR). As shown in Figure 8, fenretinide did enhance the activity of MM-310 in the TX-OV-076x ovarian PDX.

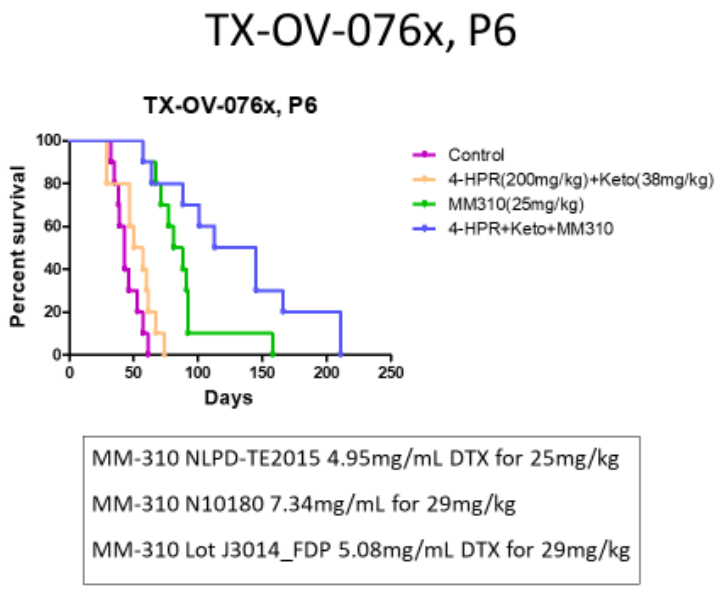


Figure 8.

Finally, using the TX-OV-075x ovarian cancer PDX we compared combining docetaxel and carboplatin vs carboplatin + M-310 vs 4-HPR + MM-310 and we found that, as observed in the 075x PDX that carboblatin + MM-310 was the combination with the greatest activity.

Based on these studies we focused on working with Merricmack Pharmaceuticals to generate a definitive set of data demonstrating activity of MM-310 against ovarian cancer PDXs. Those data have been published, and support future clinical trials of MM-310 in ovarian cancer (1).

3. KEY RESEARCH ACCOMPLISHMENTS:

Identifying multiple new ovarian cancer PDXs that are platinum-refractory ovarian cancer PDX models that can be used for evaluating novel drug combinations.

Demonstration that in some ovarian cancer PDX models EPhA2-targeting of docetaxel improves activity. This was a key finding and the ovarian cancer data included in the initial publication describing MM-310 was supported by this grant (1). Those data support including ovarian cancer patients in future clinical trials using MM-310.

4. CONCLUSION:

We have identified appropriate PDX models that can be used for evaluating novel drug combinations and we have begun evaluating the novel drug combinations that are planned for study in this project. We have completed evaluating the potential for 4-HPR to enhance taxane + carboplatin regimens and determined that further studies of that combination are not warranted. We have obtained data that continue to indicate that in some ovarian cancers MM-310 is superior to the currently available clinical docetaxel formulation. Although our data support enhancing MM-310 activity can be done with fenretinide, the superior combination of drugs in our studies was MM-310 combined with carboplatin, which is what is recommended for initial clinical trials of drug combinations using MM-310 in ovarian cancer. Our preclinical data showing strong activity in ovarian cancer of MM-310 was published (1) and a *Nature Biomedical Engineering* News and Views article highlighted that publication (2).

5. PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS:

The following paper cited partial support by this grant:

Kamouri WS, Kirpotin DB, Huang ZR, Tipparaju SK, Noble CO, Hayes ME, Luus L, Kosharyev A, Kim J, Oliver K, Kornaga T, Oyama S, Askoxylakis V, Pien C, Kuesters G, Dummont N, Lugovskoy AA, Schihl S, Wilton JH, Geddie ML, Suchy J, Grabow S, Kohli N, Reynolds CP, Blaydes R, Zhou Y, Sawyer AJ, Marks JD, Drummond DC: Antitumour acivivity and tolerability of an EphA2-targeted nanotherapeutic in multiple mouse models. *Nature Biomedical Engineering* 3:264-280, 2019. PMID: 30952988

6. INVENTIONS, PATENTS AND LICENSES: Nothing to report

7. REPORTABLE OUTCOMES: Nothing to report.

8. OTHER ACHIEVEMENTS: Data characterizing ovarian cancer models that are responsive and non-responsive to carboplatin is valuable for the research community at large. These ovarian cancer PDX models are available to any investigators upon request.

9. REFERENCES:

1. Kamouri WS, Kirpotin DB, Huang ZR, Tipparaju SK, Noble CO, Hayes ME, Luus L, Kosharyev A, Kim J, Oliver K, Kornaga T, Oyama S, Askoxylakis V, Pien C, Kuesters G, Dummont N, Lugovskoy AA, Schihl S, Wilton JH, Geddie ML, Suchy J, Grabow S, Kohli N, Reynolds CP, Blaydes R, Zhou Y, Sawyer AJ, Marks JD, Drummond DC: Antitumour acivivity and tolerability of an EphA2-targeted nanotherapeutic in multiple mouse models. *Nature Biomedical Engineering* 3:264-280, 2019. PMID: 30952988
2. Moles E, Kavallaris M A potent targeted cancer nanotherapeutic. *Nat Biomed Eng.* 3:248-250, 2019. PMID: 30952987

10. APPENDICES:

1. Copy of the *Nature Biomedical Engineering* paper that was partially supported by this grant.

2. News and Views article discussing the *Nature Biomedical Engineering* paper supported in part by this grant.