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13. SUPPLEMENTARY NOTES

14. ABSTRACT

This report covers the second year of this three-year project. During the indicated period of performance, we have focused our activities on all three specific aims. With regards to Aim #1 (to develop a clinical low-cost and high-throughput device for production and administration of HP propane contrast agent for ultimate research use in volunteers), we have continued working on testing the robustness of the portable propane hyperpolarizer device. With respect to Aim #2 (To develop a safe method for HP propane gas administration and utilization, and test the purity and safety of the HP contrast agent produced by our device) we have employed previously developed approaches (during Y1) to continue work on testing the quality of the produced gas in the context of reaction completeness and the levels of polarization. While the progress of working on all aims of this project has been substantially impacted by the COVID-19 pandemic, this year we made some progress towards Aim #3 (To assess the feasibility of MRI using HP propane gas for *in vivo* functional imaging of normal lungs and in a bleomycin-induced COPD model in sheep)—specifically, we have completed the installation of 0.35 T clinical MRI scanner, performed personnel training, and installed ^{129}Xe torso imaging coil and performed MRI scanner upgrade with ^{129}Xe capability at WSU. Moreover, the partnering site (SIUC) has completed installation of a portable MRI scanner and has demonstrated its utility for hyperpolarized proton MRI.

15. SUBJECT TERMS
low-field MRI; pulmonary imaging, high-throughput; low cost

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1. Introduction

We are developing a low-cost and high-throughput propane hyperpolarization and 3D sub-second imaging technology that can be potentially employed for functional MRI imaging of lungs, and that can be deployed to remote areas without specialized infrastructure (e.g. cryogens). The other essential component, the FDA-approved 0.35 T MRI scanner, is already commercially available. Thus, the integrated imaging platform (0.35 T MRI scanner and propane hyperpolarizer) will enable high-throughput population screening and monitoring response to treatment for a wide range of lung diseases. The end result of the project will be the development of a clinical propane hyperpolarizer, *in vivo* validation of the hyperpolarizer and contrast agent administration system, and commercialization. The next step will be an FDA-approved clinical trial. We focus our research effort on the high-risk critical challenges that must be solved to enable clinical implementation of hyperpolarized gases for pulmonary imaging.

Here, we propose taking the next significant step to develop and test a hyperpolarized propane production technology device under the Specific Aims described below:

Specifically, the research efforts during Year 2 have focused on all three Specific Aims as described in the Statement of Work (Appendix 1). The work under Aim #3 (substantially delayed due to covid-19 pandemic) has resumed as discussed below.

Aim 1. Develop a clinical low-cost and high-throughput biomedical device for production of hyperpolarized propane contrast agent: the future propane hyperpolarizer will be developed to enable its Good Manufacturing Practices (GMP), mass-production, and robust use.

Our efforts have focused on studying the robustness of polarizer operation with respect to its preparation (including catalyst packaging with inert copper media), chemical conversion, level of polarization, and percentage of active ingredient (Rh) deposition on the surfaces of titania catalyst supports.

We have also investigated the feasibility of butane gas hyperpolarization using butane and butadiene hydrogenation precursors.

Aim 2. Develop a safe method for HP propane gas administration and utilization, and test the purity and safety of the HP contrast agent produced by our device.

The activities during Year 2 of the project primarily focused on using a new approach for safe gas administration. We have also performed extensive quantitative testing of propylene impurities in produced HP propane gas batches.

Aim 3. Assess the feasibility of MRI using HP propane gas for *in vivo* functional imaging of normal lungs and in a bleomycin-induced COPD model in sheep. We will compare the effectiveness of hyperpolarized propane MRI to that using a more established contrast agent (hyperpolarized ^{129}Xe gas—created using a polarizer we developed in our previous DOD-funded work), and also to the standards of care: computed tomography and spirometry. Furthermore, we will also investigate the effectiveness of hyperpolarized propane gas MRI to monitor the progression of bleomycin-induced lung injury in the sheep animal model.

No progress was made towards this Aim during Y1 of the project due to compounding effects of the pandemic, because the 0.35 T MRI scanner (proposed for this work) could not be installed during Year 1. In Year 2, we have worked with the Health safety committee at Wayne State University to facilitate the installation visit of two vendors engineers to perform installation or MRI scanner and its upgrade to enable ^{129}Xe scanning. We have also performed personnel training, which was largely performed remotely. The installation process took approximately 2 months. This is a critical milestone that will allows us to make progress in the future. The partnering PI SIUC site has successfully completed the acquisition and installation of portable low-field MRI scanner, and started its utilization in hyperpolarized MRI research.

2. Keywords

Low-field MRI, lung imaging, molecular imaging, functional imaging; propane; xenon-129; NMR; MRI; hyperpolarization.

3. ACCOMPLISHMENTS

Please refer to Appendix 1 for the statement of work (SOW) of the entire project. The following sections describe the specific areas/tasks of the project performed during Year 2 of this project. As detailed in the corresponding section below, our research progress has been delayed substantially due to compounding challenges of covid-19 pandemic.

Aim 1. To develop a clinical low-cost and high-throughput device for production and administration of HP propane contrast agent for ultimate research use in volunteers.

Subtask #1-3: Optimize propane hyperpolarizer performance

Systematic studies of the new reactor. A new portable and handheld propane polarizer design was reported last year. Moreover, we have developed approaches for quantitative characterization of a given propane hyperpolarizer with respect to: 1) level of residual (unreacted) propylene and 2) level of proton polarization. Specifically, we have developed an automated protocol for data analysis to measure propane polarization and residual propylene fraction using a 1.4 T bench-top NMR spectrometer (see last year report). We have determined that the presence of a small excess of parahydrogen in the propylene:parahydrogen mixture (by ~5%) does not lead to any substantial loss of polarization or changes in relaxation properties – we have performed over 200 hyperpolarization runs with propane alone in a systematic manner using over 10 reactor modules filled with ~200 mg of 1% Rh/TiO_2 and ~6 g of inert copper metal. Based on the results of our studies, we have concluded that 1) 100% chemical conversion of propylene is achieved even under conditions of high flow rates (over 10 standard liters per minute); 2) we achieve 1% propane polarization robustly and reproducibly with our standard preparation procedure; 3) the reactors maintain their performance (without any noticeable decrease of performance) for preparing tens of liters of HP propane gas (corresponding to over 20 doses of HP propane gas – based on the results so far, we anticipate that a reactor could be reused for at least 100 cycles). The limited longevity studies revealed that reactor performance may deteriorate (detected as decrease of propane polarization level and reduction of chemical conversion), but we were not able yet to make any quantitative measurements yet. These results were presented at two conferences and one workshop and are being prepared for publication.

Catalyst concentration. So far, we have performed the bulk of our studies with 1% Rh/TiO_2 catalyst. Because Rh is a relatively expensive metal, we have investigated the feasibility of performing propane hyperpolarization with lower Rh nanoparticle loading. Specifically, we have studied the performance of 1%, 0.1% and 0.01% Rh loading. Propane polarization was similar in all three cases, but the chemical conversion efficiency was markedly decreased for 0.01% loading—clearly indicating that we have reached the limit of catalytic conversion activity for this material. Therefore, we conclude that 0.1-1% catalyst load would be a good choice for biomedical applications.

Catalyst concentration. Previously, we have performed studies to investigate the limits of T_1 and T_S (singlet) relaxation of hyperpolarized propane, efforts that clearly indicated that per-deuteration of propylene substrate does not improve the relaxation properties of HP propane gas (for reference, T_S is ~3 seconds at clinically relevant conditions of 1 atm). Therefore, we have looked into feasibility of butane hyperpolarization as a potential alternative to propane hyperpolarization. We have found that butadiene substrate is “toxic” to our catalyst and resulted in quick reactor poisoning (observed as loss of hydrogenation activity and levels of polarization). Butene-1 and butene-2 substrates have also been investigated. Remarkably, both precursors lead to production of HP butane, seen as HP resonances of methyl and methylene protons. We hypothesized that butane-2 undergoes the process of isomerization on our catalyst, leading to the same intermediate—we have confirmed this hypothesis by preparing a 3-D-propylene (i.e., mono-deuterated in position 3) precursor and its hydrogenation studies that revealed that pairwise parahydrogen addition follows 1,2- and 2,3- routes (studies are in progress to investigate the feasibility of 1,3-addition). Systematic relaxation studies of HP butane indeed revealed longer (by ~20%) T_1 and T_S for this larger (compared to propane) molecule. This gain in relaxation constants is welcomed in the context of biomedical applications because the HP state can be retained longer. Polarization levels of HP butanes were similar to that of propane. However, one translational disadvantage of HP butane was quickly realized – the maximum partial butene gas pressure was approximately 3 times lower than that of propene, resulting in undesirable gas condensation in our apparatus. While this may not necessarily be a show-stopper for clinical translation of HP butane, low precursor pressure limits the propelling capacity of the content for the hyperpolarization process. These results are being prepared for publication in three separate manuscripts.

The efforts in Y3 (for Aim 1) will focus on development of good manufacturing practices and the preparation of a bill of materials for our propane hyperpolarizer.

Specific Aim 2 - To develop a safe method for HP propane gas administration and utilization, and test the purity and safety of the HP contrast agent produced by our device.

Subtask #2-2: Assess purity of produced HP propane gas by detecting the excipients

As described above (our progress under Aim 1), we have developed an automated spectroscopic protocol to detect residual propylene in produced gas mixtures. Using this approach and ~5% parahydrogen excess in the gas mixture, we have been able to consistently achieve complete precursor conversion: no residual propylene was detected in the produced propane gas.

In Y3, elemental analysis for excipients (Rh, Ti, Si, Al, Cu) using our polarizer design will be performed. We will also work on confirming the capturing of used propane gas by the carbon filters to mitigate any residual flammability of the HP propane gas contrast agent.

Specific Aim 3 - To assess the feasibility of MRI using HP propane gas for *in vivo* functional imaging of normal lungs and in a bleomycin-induced COPD model in sheep.

0.35 T clinical MRI scanner installation at WSU site. As reported previously, in Year 1 of the project, we could not make any progress towards Aim 3 due to covid-19 pandemic restrictions because the MRI scanner installation was incomplete due to travel restrictions in the US and other countries. With the third scheduling attempt and in coordination with WSU health and safety committee, we were able to schedule the installation of the MRI scanner. Two engineers based in India and Indonesia traveled to WSU to complete the scanner installation, with total duration of their stay exceeding three months.

The 0.35 MRI scanner was installed without any substantial challenges (besides covid-related limitations on the WSU campus). Following scanner installation, the WSU team (consisting of the PI, a PD fellow and two PhD students, see Figure 1(left)) was trained to use this MRI scanner for proton imaging. One week of rigorous training covering the MRI fundamentals was supplied by the vendor in person and also remotely (to mitigate covid-related issues). This 0.35 T MRI scanner fully met the vendor's specification and the accreditation requirements in the US for clinical utilization. Following scanner installation, the trainees took turns for their hands-on experience with this MRI scanner. **Figure 2** shows an example of a practice session to record high-resolution images of squash by the trainees.

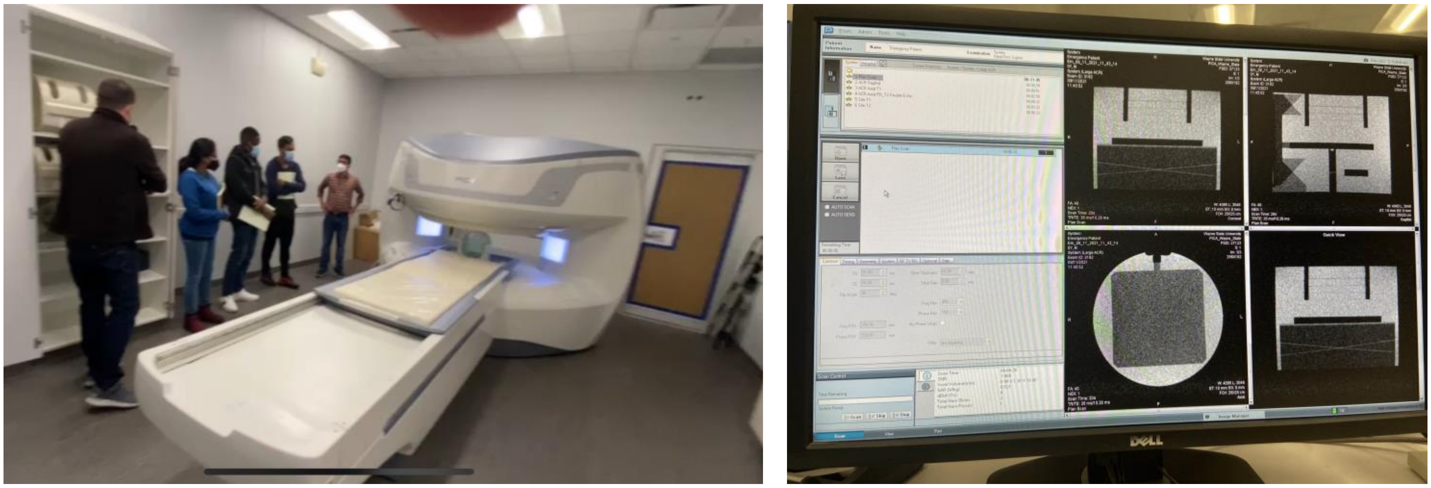

Figure 1. (**left**) Screenshot of the video recording of the MRI scanner training session at the WSU site. (**right**) Regular clinical quality assurance of the MRI phantom for the installed 0.35 T MRI scanner.

0.35 T clinical MRI scanner upgrade for ^{129}Xe capability. The standard configuration of this MRI scanner (as with any clinical MRI scanner) allows for scanning protons. The scanner purchase also included a scanner upgrade to be able to perform ^{129}Xe scanning natively using the scanner environment. The vendor has delivered a ^{129}Xe detection torso RF coil, and also installed a ^{129}Xe transmit coil inside the MRI scanner. In this sense, the ^{129}Xe capability is designed and built for our typical clinical workflow (instead of using a custom-made single transmit and receive coil). While the upgrade has been successful overall, we were not able to properly calibrate the ^{129}Xe transmit RF coil (in a straightforward manner), which we will continue working on with the vendor on developing the advanced approaches for RF coil calibration. This is not a trivial task since ^{129}Xe does not ordinarily have sufficient sensitivity in the non-hyperpolarized state. Nevertheless, we remain optimistic that we will be able to develop a good approach for pulse calibration.

Having this scanner installed at WSU clears a substantial roadblock for the overall progress of this project. We anticipate the activities in Year 3 will focus heavily on phantom imaging, and we will likely be requesting the no-cost extension to allow for sufficient amount of time for in vivo studies.

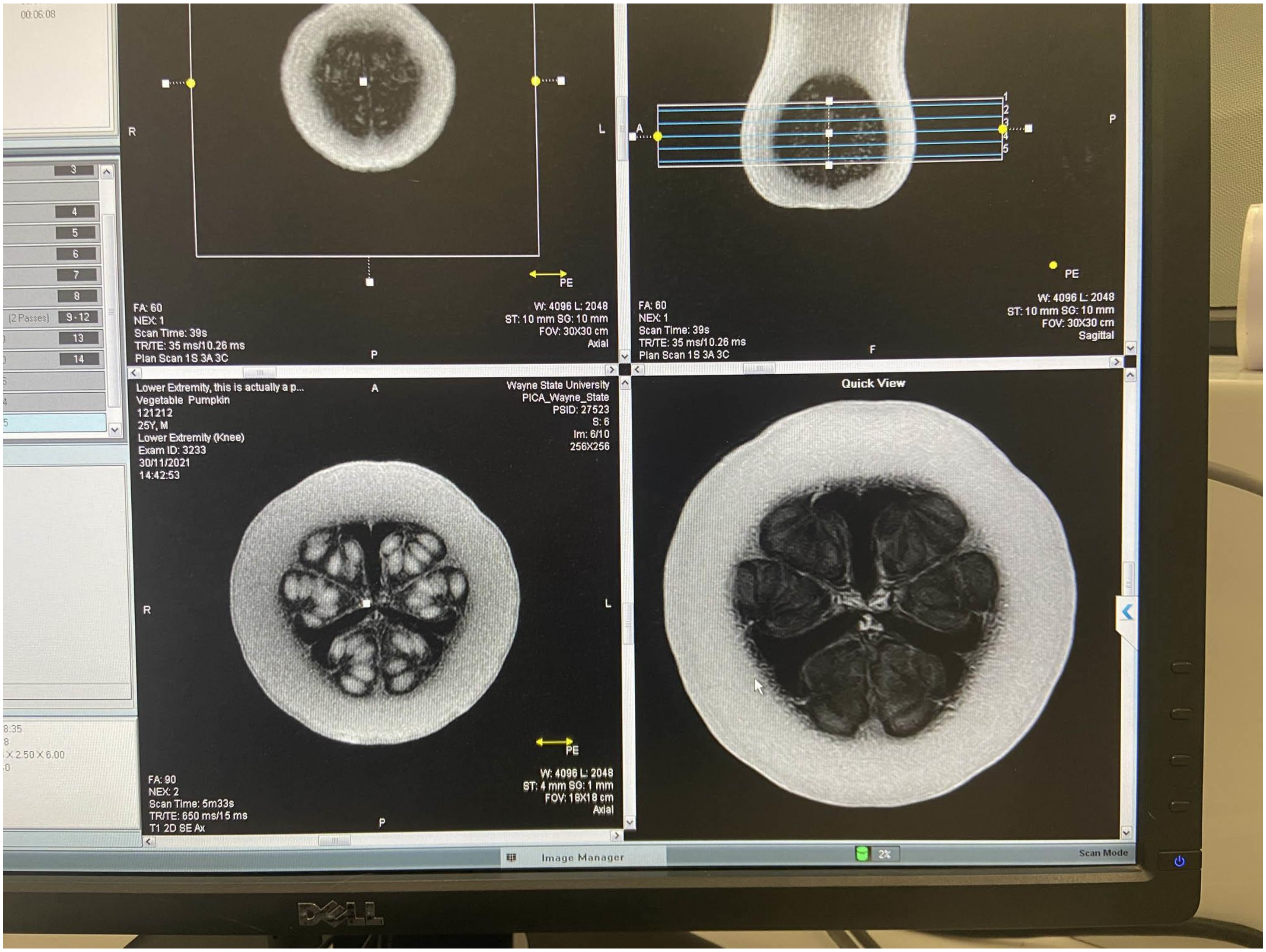

Figure 2. Photograph from an imaging session involving high-resolution multi-slice imaging of a squash.

Portable MRI scanner installation at SIUC. MRI can provide exquisite high-resolution images of anatomical features, function, and pathology of soft tissues in the body, without ionizing radiation. However, the increasingly stronger magnets used in most clinical scanners (~1–7 T) are expensive, bulky, and immobile, and the scans can be time-consuming and confining for many patients. Moreover, these stronger magnetic fields (along with concomitantly higher RF resonance frequencies) can present safety concerns in some circumstances, as well as contraindications for a number of patients and health conditions. Low-field (LF) MRI has gained renewed interest because it can potentially obviate all of these limitations. However, signal strength, detection sensitivity, and contrast limitations present ongoing challenges to many LF MRI technologies and their applications.

One approach to mitigate the sensitivity and contrast limitations of conventional NMR/MRI is hyperpolarization, which can increase NMR/MRI detection sensitivity by orders of magnitude. Moreover, because the magnetization is endowed by a given hyperpolarization method (and not the magnet), hyperpolarized (HP) LF MRI can even be more sensitive than high-field MRI. Thus, hyperpolarization can be highly synergistic with LF MRI, particularly if the hyperpolarization technique is itself low-cost, rapid, and portable. One such approach is signal amplification by reversible exchange (SABRE). In SABRE, para-hydrogen (p-H_2) and molecular substrates are transiently bound to organometallic complexes (“catalysts”), allowing spin order to transfer spontaneously from p-H_2 to substrates when placed within an appropriate magnetic field.

With current DoD support, we have sited a new type of portable, point-of-care, low-field (64 mT) clinical MRI scanner (Hyperfine) in the partnering PI’s Lab at SIUC, **Figure 3**. This type of scanner has already been demonstrated elsewhere in clinical settings for bed-side imaging, including of brain injury and cerebral hemorrhage. Here we are investigating the potential for adapting this type of MRI scanner for use with HP substances. While we will report more of this effort next cycle, we are pleased to report here a brief summary of some of our initial efforts (which we have presented at this past ENC conference and are currently writing up for publication).

Figure 3. The portable, point-of-care 64 mT clinical MRI scanner (Hyperfine “Lucy”) in the Goodson Lab at SIUC. Scanner runs with a wireless (iPad), and DICOM images are stored on a cloud server.

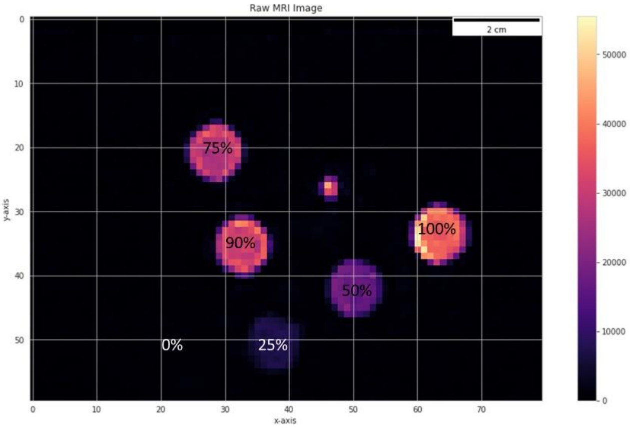

Figure 4. Demonstration of SABRE-enhanced ^{1}H low-field MRI. The image shows MRI signals from a central tube (smallest circular cross-section) containing a SABRE catalyst and 100 mM of pyrazine in deuterated methanol; outer circles are from thermally polarized water in falcon tubes with different concentrations of protonated water (100%, 90%, 75%, 50%, 10%, 0%) in deuterated water. Image was obtained with a fast spin echo (FSE) sequence under conditions of slow but continuous p-H_2 bubbling (<20 sccm).

In one study, pyrazine was successfully hyperpolarized via SABRE under continuous p-H_2 bubbling conditions and the HP agents were imaged using the 64 mT clinical MRI scanner (**Figure 4**). A 5 mm NMR tube was filled with a deuterated methanol solution containing the iridium-based SABRE catalyst and 100 mM of pyrazine. The sample was connected to a p-H_2 “bubbler” (~65 psi). Following confirmation of ^{1}H SABRE on a benchtop spectrometer, SABRE MRI was demonstrated with pyrazine following sample transfer to the 64 mT MRI and in situ imaging under continuous but reduced p-H_2 flow (20 sccm); low-field scanners suffer much less from susceptibility distortions, enabling imaging with gentle bubbling. The absence of signal in a control image using deuterated pyrazine (not shown) supports the conclusion that the p-H_2-enhanced MRI signal is coming from SABRE-hyperpolarized pyrazine (and not, say, from hyperpolarized o-H_2 or hydrides). The use of phantoms containing known and varying quantities of H versus D spins enabled the development of a simple approach to quantify the product of concentration and polarization from a given region of an image with good reliability. This approach allowed the ^{1}H SABRE enhancement in the central tube of the image to be estimated (~240-fold).

The current results demonstrate the ability to easily image and quantify SABRE hyperpolarization under in situ conditions of continuous p-H_2 delivery within the 64 mT MRI scanner. However, these conditions are non-ideal for two reasons: 1) 64 mT is non-optimal for ^{1}H SABRE (the field is too strong for efficient transfer of spin order); and 2) the current approach using standard sequences and parameters does not allow for "ultrafast" imaging of a bolus of an administered HP agent (thus missing out on one of the inherent advantages offered by hyperpolarization). Thus, ongoing / future efforts include optimization of the MRI sequences for faster SABRE imaging, as well as integration of the SABRE HP apparatus with a continuous recirculation setup to enable SABRE at optimal fields (e.g. ~6 mT) and rapid movement of HP liquids to the scanner, for extended periods of time. We also will investigate scaling up of the HP agent production to allow stopped-flow or continuous SABRE imaging of larger objects. Finally, we will also be working to adapt our approach to more biologically relevant target molecules / agents.

What opportunities for training and professional development has the project provided?

Students and other trainees attended conferences (virtually and in person) and presented results. For example, along with the PI and partnering PI, a number of students (including grad students Md Shahabuddin Alam, Ishani Senanayake, Tobi Gafar, Nadiya Iqbal, Shiraz Nantogma, Raduan Chowdhury, Clementinah Oladun, and Firoz Ahmed, undergrads Drew Brittin, Tony Petrilla, Maggie Pugh, and postdoc Nuwandi Ariyasingha, attended in person the 63rd ENC conference this past April (grad student Zahid Siraj attended virtually); all helped to present the posters listed in the products section (for most of them, it was their first in-person conference). The WSU team members attended selected sections of the ISMRM conference virtually. We can also mention that both our groups attended a recent all-day SABRE-simulations training seminar that was presented by Dr. Jacob Lindale, of the Warren Lab at Duke. Students at both sites are currently being trained in synthesis, catalysis, characterization, and NMR. Lastly, students at both sites received MRI training for the above-mentioned MRI scanners installed at each site.

In other news: In the last cycle, Nuwandi Ariyasingha (former PhD student at WSU), became a postdoc and successfully submitted an F32 application to NHLBI (this application was reported in our last-year progress report), which was funded. The partnering PI (Goodson) successfully matched ~10 CHEM 205/205H students with research opportunities on campus. Finally, we can add to this section a brief report on some awards that our team members have recently received this cycle:

* Travel Award to Md Shahabuddin Alam (PhD student, SIUC team), to present a poster: Characterization and Investigation of aMOF-Based(IrIMes@NU-1000) Catalyst for Hyperpolarization via Heterogeneous SABRE-SHEATH. 63rd ENC Conference, Orlando, Florida, April 24-29, 2022.

* Thomas C. Rumble University Graduate Fellowship Award to Shiraz Nantogma (PhD Student, WSU team) by the Department of Chemistry at Wayne State University, Detroit, MI. The fellowship provides a stipend of $20,000 for the academic year in addition to a tuition scholarship that provides payment of 7.5 – 10 credits for each of the fall and winter semesters at the effective tuition rate. The fellowship provides subsidized medical, vision and dental insurance.

How were the results disseminated to communities of interest?

Via oral and poster presentations (see below), as well as publications (see below).

4. IMPACT

What was the impact on the development of the principal discipline(s) of the project?

Nothing to Report.

What was the impact on other disciplines?

Nothing to Report.

What was the impact on technology transfer?

Nothing to Report.

What was the impact on society beyond science and technology?

Nothing to Report.

5. CHANGES / PROBLEMS

As with much of the world, we have been impacted by Covid-19 in a number of ways. First, the pandemic has limited (or in some cases outright eliminated) opportunities for outreach, career development for trainees, and personnel exchange; it has also caused us to postpone certain technologies: most importantly the 0.35 T MRI scanner (key instrument required for Aim #3) installation has been delayed. To mitigate some of these issues, we have continued our regular teleconferences (e.g., with Dr. Barlow from the UK). The main negative impact of the covid-19 pandemic during this reported period of performance was the lab re-opening phase of the initiating and partnering PI laboratories. As a result, a substantial amount of effort was focused on personnel training, and restarting our laboratories. Nevertheless, we look forward to catching up with research progress under this award.

6. PRODUCTS

Oral Presentations

1) BM Goodson. “Hyperpolarizing nuclear spins of atoms and molecules with light and parahydrogen.” Invited Chemistry Department Seminar, Missouri University of Science & Technology, 9/24/2021.
2) BM Goodson. “Putting a new ‘Spin’ on MRI” Invited evening lecture presented at the Illinois State Science and Humanities Symposium, Carbondale, IL, March 24, 2022.
3) BM Goodson. “‘Cutting-Edge’ Tools to Hyperpolarize NMR and MRI: From Lasers to SABRE” Invited Chemistry Department Seminar, North Carolina State University, Raleigh, NC, 4/5/2022.
4) Chekmenev, E. Y. Order-Unity ^{13}C Nuclear Polarization of [1-^{13}C]Pyruvate in Seconds. *2022 International Society for Magnetic Resonance in Medicine (ISMRM) & SMRT Virtual Conference & Exhibition*, May 9; 2022, London, UK.
5) Chekmenev, E. Y. SABRE-SHEATH hyperpolarization of pyruvate and other structurally similar biomolecules. *International Workshop on Hyperpolarization Methods in Magnetic Resonance*, May 27; 2022, Organized by a network of European NMR societies.

Conference Abstracts

1. Ariyasingha, N. M.; Samoilenko, A.; Birchall, J. R.; Kovtunov, K. V.; Kovtunova, L. M.; Bukhtiyarov, V. I.; Koptyug, I. V.; Qian, C.; Chekmenev, E. Y. In *Disposable and Handheld Clinical-scale HP Propane Hyperpolarizer*, 63rd Experimental NMR Conference, Orlando, FL, USA, April 24-29; Orlando, FL, USA, 2022.
2. Chekmenev, E. Y. In *Next-generation Equipment for Clinical-scale Parahydrogen Induced Polarization*, 63rd Experimental NMR Conference, Orlando, FL, USA, April 24-29; Orlando, FL, USA, 2022.
3. Chekmenev, E. Y.; Samoilenko, A.; Birchall, J. R.; Kovtunov, K. V.; Kovtunova, L. M.; Bukhtiyarov, V. I.; Koptyug, I. V.; Qian, C.; Goodson, B. M.; Ariyasingha, N. M. In *Disposable and Handheld Clinical-scale HP Propane Hyperpolarizer for Pulmonary MRI*, Joint Annual Meeting ISMRM-ESMRMB & ISMRT 31st Annual Meeting, London, UK (presented and attended virtually), May 07-12; London, UK (presented and attended virtually), 2022.
4. Chowdhury, R. H. C.; Birchall, J. R.; Nikolaou, P.; Barlow, M. J.; Shcherbakov, A.; Goodson, B. M.; Chekmenev, E. Y. In *Temperature-Ramped Batch-Mode Spin Exchange Optical Pumping of Xenon-129 using 3rd-generation Automated XeUS Hyperpolarizer*, 63rd Experimental NMR Conference, Orlando, FL, USA, April 24-29; Orlando, FL, USA, 2022.
5. Gafar, A. T.; Lu, H.; Basler, D.; Cocking, D.; Prince, M.; Molway, M. J.; Bales-Shaffer, L.; Alam, M. S.; Siraj, Z.; Petrilla, A. F., et al. In *Two-Orders-of-Magnitude Improvement in SEOP Production (P*N) of Hyperpolarized 131Xe and Measurement of 131Xe and 129Xe Spin-Dependent Neutron Scattering Lengths via Pseudomagnetic Precession*, 63rd Experimental NMR Conference, Orlando, FL, USA, April 24-29; Orlando, FL, USA, 2022.
6. Shahabuddin, A.; Brittin, D.; Iqbal, N.; Gafar, T.; Siraj, Z.; Petrilla, A.; Senanayake, I.; Pugh, M.; Poorman, M.; Sacolick, L., et al. In *Exploring SABRE-Enhanced Imaging with a Portable Clinical Low-field MRI Scanner*, 63rd Experimental NMR Conference, Orlando, FL, USA, April 24-29; Orlando, FL, USA, 2022.
7. Nantogma, S.; Adelabu, I.; Ariyasingha, N.; Samoilenko, A.; Chowdhury, R.; Kabir, M. S. H.; Goodson, B. M.; Chekmenev, E. Y. In *Next-generation Clinical-scale Instrumentation for Parahydrogen-Induced Polarization*, The Future of Molecular MR Workshop, Pasadena, CA, USA, July 23-26; Pasadena, CA, USA, 2022.

Peer-Reviewed Manuscripts, Dissertation & Book Chapters

1. Birchall, J. R.; Chowdhury, M. R. H.; Nikolaou, P.; Chekmenev, Y. A.; Shcherbakov, A.; Barlow, M. J.; Goodson, B. M.; Chekmenev, E. Y. Pilot Quality-Assurance Study of a Third-Generation Batch-Mode Clinical-Scale Automated Xenon-129 Hyperpolarizer. *Molecules* **2022,** *27* (4), 1327.
2. Burueva, D. B.; Kozinenko, V. P.; Sviyazov, S. V.; Kovtunova, L. M.; Bukhtiyarov, V. I.; Chekmenev, E. Y.; Salnikov, O. G.; Kovtunov, K. V.; Koptyug, I. V. Gas-Phase NMR of Hyperpolarized Propane with 1H-to-13C Polarization Transfer by PH-INEPT. *Applied Magnetic Resonance* **2022,** *53*, 653–669.

3. Chekmenev, E. Y.; Goodson, B. M.; Bukhtiyarov, V. I.; Koptyug, I. V. Bridging the Gap: From Homogeneous to Heterogeneous Parahydrogen-induced Hyperpolarization and Beyond. *ChemPhysChem* **2021,** *22* (8), 710-715.
4. Joalland, B.; Theis, T.; Appelt, S.; Chekmenev, E. Y. Background-Free Proton NMR Spectroscopy with Radiofrequency Amplification by Stimulated Emission Radiation. *Angewandte Chemie International Edition* **2021,** *60* (50), 26298-26302.
5. Lehmkuhl, S.; Fleischer, S.; Lohmann, L.; Rosen, M. S.; Chekmenev, E. Y.; Adams, A.; Theis, T.; Appelt, S. RASER MRI: Magnetic resonance images formed spontaneously exploiting cooperative nonlinear interaction. *Science Advances* **2022,** *8* (28), eabp8483.
6. Schmidt, A. B.; Bowers, C. R.; Buckenmaier, K.; Chekmenev, E. Y.; de Maissin, H.; Eills, J.; Ellermann, F.; Glöggler, S.; Gordon, J. W.; Knecht, S., et al. Instrumentation for Hydrogenative Parahydrogen-Based Hyperpolarization Techniques. *Analytical Chemistry* **2022,** *94* (1), 479–502.

Inventions, patent applications and licenses:

Nothing to Report.

Funding applied for based on work supported by this award

1. Ultrafast Functional Imaging of Lung Injury Using Clinical MRI Scanners
   PI: Chekmenev, Co-investigator (PI of a subcontract): Goodson
   Supporting agency: DOD CDMRP application, Army
   Performance period: 07/2023 to 06/2027
   Level of funding: $1,599,000
   Time Commitment: Principal Investigator: 12 calendar months; Mentor: 0 calendar months
   Project purpose: The goal of this project is to develop new instrumentation and approaches to enable detection of hyperpolarized 129Xe contrast agent on clinical MRI scanners.
   Overlap: None

2. Next-generation Lasers for Enabling Ultrafast Functional Pulmonary MRI
   PI: Chekmenev, Co-investigator (PI of a subcontract): Goodson
   Supporting agency: NIH NHLBI
   Performance period: 04/2023 to 03/2025
   Level of funding: $354,788
   Project purpose: The goal of this project is to test next-generation high-power laser technologies with the overarching aim of improving access to hyperpolarized 129Xe contrast agent in the clinical setting.
   Overlap: None

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Individuals who worked on the project

The following personnel worked on the project

Name:	Eduard Chekmenev
Project Role:	Initiating PI (Wayne State University)
Researcher Identifier (ORCID ID):	orcid.org/0000-0002-8745-8801
Nearest person month worked:	3
Contribution to Project:	Dr. Chekmenev was responsible for the overall progress of the project, performing some experiments with hyperpolarized propane and hyperpolarized xenon-129, developing RF coils, analyzing some of the data for the above-mentioned experiments, preparing the manuscripts.
Funding Support:	W81XWH-20-10576; NIH 1R21CA220137; NSF CHE-1904780, NIBIB 1R01EB029829, NHLBI 1R21HL154032

Name:	Nuwandi M. Ariyasingha
Project Role:	PhD / PD Fellow
Researcher Identifier (ORCID ID):	
Nearest person month worked:	5
Contribution to Project:	Nuwandi M. Ariyasingha worked on many of the experiments concerning hyperpolarized clinical-scale propane hyperpolarization. She also analyzed the data, prepared figures, and manuscripts. She was also trained to operate 0.35 T MRI scanner.
Funding Support:	W81XWH-20-10576, NHLBI 1R21HL154032, 1F32HL160108

Name:	Md Raduan Chowdhury
Project Role:	PhD student
Researcher Identifier (ORCID ID):	
Nearest person month worked:	3
Contribution to Project:	Raduan worked on the Xenon-129 hyperpolarizer to ensure that HP 129Xe would be available for the in vivo part of this proposal. He also worked on the parahydrogen component of this award by providing upgrade to our parahydrogen production facility. He was also trained to operate 0.35 T MRI scanner.
Funding Support:	Wayne State University teaching fellowship and NSF CHE-1904780

Name:	Shiraz Nantogma
Project Role:	PhD student
Researcher Identifier (ORCID ID):	
Nearest person month worked:	2
Contribution to Project:	Shiraz has been developing instrumentation for parahydrogen-based hyperpolarization. He has also produced

	parahydrogen for this project. He was also trained to operate 0.35 T MRI scanner.
Funding Support:	Wayne State University teaching fellowship and NSF CHE-1904780.

Name:	Clementinah Oladun
Project Role:	PhD student
Researcher Identifier (ORCID ID):	
Nearest person month worked:	1
Contribution to Project:	Clementinah worked on Xenon-129 hyperpolarizer to ensure that HP 129Xe would be available for the in vivo part of this proposal.
Funding Support:	Wayne State University teaching fellowship.

Name:	Firoz Ahmed
Project Role:	PhD student
Researcher Identifier (ORCID ID):	
Nearest person month worked:	1
Contribution to Project:	Firoz worked on Xenon-129 hyperpolarizer.
Funding Support:	Wayne State University teaching fellowship

Name:	Anna Samoilenko
Project Role:	Pharm D
Researcher Identifier (ORCID ID):	
Nearest person month worked:	3
Contribution to Project:	Anna contributed to this project by working on the development of the clinical-scale propane hyperpolarizer and its testing. She prepared reactors, collected and processed data, and prepared reports.
Funding Support:	NSF CHE-1904780, W81XWH-20-10576, NHLBI 1R21HL154032

Name:	Igor V. Koptyug
Project Role:	consultant
Researcher Identifier (ORCID ID):	https://orcid.org/0000-0003-3480-7649
Nearest person month worked:	1
Contribution to Project:	Wrote contents for presentations and manuscripts. Consulted in aspects of parahydrogen addition mechanisms.
Funding Support:	DOD W81XWH2010576

Name:	Larisa Kovtunova
Project Role:	consultant
Researcher Identifier (ORCID ID):	https://orcid.org/0000-0003-0922-6594
Nearest person month worked:	1
Contribution to Project:	Wrote contents for manuscripts. Consulted in aspects of heterogeneous catalyst preparation for propane hyperpolarization.
Funding Support:	DOD W81XWH2010576

Name:	Juri G. Gelovani
Project Role:	consultant
Researcher Identifier (ORCID ID):	https://orcid.org/0000-0002-8413-6161
Nearest person month worked:	1
Contribution to Project:	Wrote contents for manuscripts. Consulted in aspects of clinical translation of hyperpolarized inhalable gases.
Funding Support:	DOD W81XWH2010576

Name:	Anton Scherbakov
Project Role:	consultant
Researcher Identifier (ORCID ID):	
Nearest person month worked:	2
Contribution to Project:	Provided support for Xenon-129 hyperpolarizer.
Funding Support:	DOD W81XWH2010576

Name:	Boyd M. Goodson
Project Role:	Partnering PI (SIUC)
Researcher Identifier (ORCID ID):	orcid.org/0000-0001-6079-5077
Nearest person month worked:	1
Contribution to Project:	Prof. Goodson was responsible for the overall progress of the project at the partnering site, performing some experiments with hyperpolarized xenon-129, xenon-131, as well as imaging experiments on the new 64 mT scanner, analyzing some of the data for the above-mentioned experiments, contributed to efforts with hyperpolarizer development at the SIUC site, and preparing manuscripts and presentations.
Funding Support:	SIUC DOD W81XWH2010578 NSF-CHE-1905341 NSF DMR-2150489
Name:	Abdulbasit Tobi (“Tobi”) Gafar
Project Role:	PhD chemistry graduate student
Researcher Identifier (ORCID ID):	
Nearest person month worked:	2
Contribution to Project:	Participated in xenon-129 and xenon-131 spin-exchange optical pumping experiments, as well as imaging experiments on the new 64 mT scanner; contributed to efforts with hyperpolarizer development at the SIUC site. Processed and analyzed data, wrote up contents for presentations and manuscripts.
Funding Support:	SIUC / NMR Facility

Name:	Panayiotis “Peter” Nikolaou
Project Role:	consultant
Researcher Identifier (ORCID ID):	Contributed significantly to various aspects of hyperpolarizer design and assembly.

	Wrote contents for presentations and manuscripts.
Nearest person month worked:	1
Contribution to Project:	
Funding Support:	DOD W81XWH2010578

Name:	Zahid Siraj
Project Role:	PhD graduate student researcher
Researcher Identifier (ORCID ID):	
Nearest person month worked:	2
Contribution to Project:	Assisted with experiments involving hyperpolarizer assembly and testing, as well as imaging experiments on the new 64 mT MRI scanner.
Funding Support:	Teaching assistantship

Name:	Nadiya Iqbal
Project Role:	PhD graduate student researcher
Researcher Identifier (ORCID ID):	
Nearest person month worked:	2
Contribution to Project:	Assisted with, then led imaging experiments on the 64 mT MRI scanner.
Funding Support:	Teaching assistantship

Name:	Md Shahabuddin (“Alam”) Alam
Project Role:	PhD chemistry graduate student
Researcher Identifier (ORCID ID):	
Nearest person month worked:	2
Contribution to Project:	Participated in xenon-129 and xenon-131 spin-exchange optical pumping experiments, as well as imaging experiments on the new 64 mT MRI scanner; contributed to efforts with hyperpolarizer development at the SIUC site. Processed and analyzed data. Contributed to manuscripts and conference presentations.
Funding Support:	SIUC / NMR Facility

Name:	Anthony Petrilla
Project Role:	undergraduate student researcher
Researcher Identifier (ORCID ID):	
Nearest person month worked:	1
Contribution to Project:	undergraduate student researcher (since graduated, and is now a new PhD graduate researcher at SIUC
Funding Support:	DOD W81XWH2010578, NSF DMR-2150489, SIUC

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Yes
Title “*Next-generation ultrafast functional 3D pulmonary imaging*”
1F32HL160108-01 (PI=Ariyasingha, mentor=Chekmenev)
Performance period: 09/01/2021-08/31/2024
Supporting agency: NIH/NHLBI
Level of funding: ~total direct costs to support salary and training of Dr. Ariyasingha.
Time Commitment: Principal Investigator: 12 calendar months; Mentor: 0 calendar months
We propose developing next-generation ultrafast MRI to assess lung function using hyperpolarized propane gas as an inhalable contrast agent. The proposed low-cost and high-throughput technology for clinical-scale production of hyperpolarized propane can be employed for sub-second gas MRI, which we envision as a novel contrast agent for functional regional mapping of lung functions in a wide range of lung diseases. Here, we will study three contrast mechanisms in excised sheep lung model to demonstrate the robustness of this new quantitative 3D imaging method.
Aim 1: 3D MRI of lungs with deuterated HP propane. We will employ previously developed deuterated HP propane to acquire 3D MRI of excised sheep lungs. Deuterated HP propane has a directly detectable HP state and can be imaged directly on clinical MRI scanners. This aim will enable us to investigate the feasibility of this approach and to investigate the effect of background signal interference from the protons of tissue.
Aim 2: 3D MRI of lungs using HP propane. The advantage of HP propane versus deuterated HP propane is the possibility to employ specialized pulse sequences to filter the background signal of tissue protons. We will study the effectiveness of this filtering approach to obtain background-free images.
Aim 3: Creation of ultra-long-lived spin states (ULLS) in partially deuterated HP propane. We will investigate on the use of highly symmetric molecules to enable ultra-long lived spin states that can potentially lengthen the in vivo utility window to 1-2 minutes.
Overlap: Although this project is focused around propane, there is no overlap with the specific aims of the project. Moreover, this is a training award designed to develop the next-generation of researchers in the area of pulmonary imaging. The project additionally differs from this award in two ways. First, it emphasizes the contrast mechanism (rather than applications). Second, it focuses on mechanistic studies in excised lungs versus the animal model used under this award.

Overlap: none

Personnel exchanges with collaborators:

While covid had greatly slowed the planned student exchange process, this cycle we were able to ramp that back up and enjoyed several visits. For example, SIUC team members (the PI, Md Shahabuddin Alam, Tobi Gafar, Drew Brittin, and Ishani Senanayake) visited the WSU team for training and experiments for nearly 1 week in the fall, and other trips are planned for the future (the SIUC team also took home a new SABRE hyperpolarizer built by the WSU team). Other hosting events by the WSU team in the past cycle included:

* SIUC Student Tobi Gafar: 2 additional long-term visits for over two months total;
* Henri de Maissin, PhD student, University of Freiburg, Germany: 2.5 months, 1 visit;
* Chris Nelson, PhD student, North Carolina State University: 2 weeks, 1 visit;
* Iuliia Mandzhieva, PhD student, North Carolina State University: 2 weeks, 2 visits;
* Dr. Andreas Schmidt, PhD: 1 visit 2 weeks — after that he was employed by WSU for 4 months;
* Prof. Alexei Ouriadov (with a trainee): The University of Western Ontario, Canada, 1 day, 1 visit;
* Prof. Dan Xiao: University of Windsor, Canada, 1 day, 1 visit;
* Dr. Jessica Ettegui: NHLBI, NIH, Bethesda, MD, 1 visit, 4 days.

We have also been interacting with collaborating scientists remotely.
Organization Name: University of Nottingham
Location of Organization: Nottingham, UK
Partner's contribution to the project:
In-kind support: Our collaborators provided their insights in the imaging aspects of hyperpolarized gases.
Collaboration: We have enjoyed regular (weekly to bi-weekly) teleconferences with the Nottingham team (lead PI: Dr. Michael J. Barlow).

8. SPECIAL REPORTING REQUIREMENTS

Collaborative Award

This is a collaborative award with two partnering PIs. Each PI submits a copy of the identical report with face page adjusted for each report.

9. APPENDICES

Appendix 1: Original Statement of work, Years 1-3

STATEMENT OF WORK – 07/03/2019
PROPOSED START DATE Apr. 01, 2020

Site 1: Wayne State University

Department of Chemistry
5101 Cass Ave
Detroit, MI 48202
PI: Dr. Chekmenev

Site 2: Southern Illinois University Carbondale
Neckers Building,
1245 Lincoln Dr
Carbondale, IL 62901
PI: Dr. Goodson

Specific Aim 1 – To develop a clinical low-cost and high-throughput device for production and administration of HP propane contrast agent for ultimate research use in	**Timeline**	**Site 1**	**Site 2**
Major Task 1	Months		
Subtask 1: Design of the clinical propane hyperpolarizer	1-6	X	X
Milestone # 1 clinical propane polarizer is designed	6	X	X
Subtask 2: Propane hyperpolarizer construction	7-12	X	
Milestone # 2 clinical propane hyperpolarizer is constructed	12	X	
Subtask 3: Optimize propane hyperpolarizer performance	13-18	X	X
Subtask 4: Development of Good Manufacturing Practices (GMP) of hyperpolarized propane production	19-24	X	X
Milestone # 3 HP propane production GMP is developed	24	X	X
Subtask 5: Create bill of materials for propane hyperpolarizer	25-30	X	
Specific Aim 2 - To develop a safe method for HP propane gas administration and utilization, and test the purity and safety of the HP contrast agent produced by our device			
Major Task 2			
Subtask 1: Address flammability of HP propane gas in the clinical hyperpolarizer by developing inhalation procedures, using carbon filter to capture exiting propane gas	4-18	X	X
Milestone # 4 Reduction of propane content in the exhaled gas mixture to ≤ 1% (i.e. significantly below LEL) in 3 air inhalation/exhalation cycles after the inhalation and MRI imaging of HP propane gas; propane content in the room less than 1000 ppm (i.e. below the mandated safety level).	16-18	X	X
Subtask 2: Assess purity of produced HP propane gas by detecting the excipients	1-15	X	X
Milestone # 5 No detectable compounds except propane (>98%), H_2 (<2%), and propylene (<0.1%) in the produced contrast agent	15	X	X

Specific Aim 3 - To assess the feasibility of MRI using HP propane gas for *in vivo* functional imaging of normal lungs and in a bleomycin-induced COPD model in sheep			
Major Task 3			
Subtask 1: Establish ventilation 3D ^{129}Xe MRI on a single sheep breath hold on 0.35 T using phantoms of hyperpolarized ^{129}Xe gas placed in Tedlar Bags	1-12	X	X
Milestone # 6 3D ^{129}Xe MRI imaging sequence / protocol is developed for 0.35 T MRI scanner	12	X	X
Subtask 2: Establish sub-second ventilation 3D HP propane MRI on 0.35 T using phantoms of hyperpolarized propane gas	1-12	X	
Milestone # 7 Sub-second 3D MRI imaging sequence / protocol is developed for 0.35 T MRI scanner to record MRI of propane gas ventilation	12	X	
Subtask 3: Submit documents for ACURO approval	13-16	X	X
Milestone # 8 ACURO approval obtained	18	X	X
Subtask 4: Test-retest study of HP ^{129}Xe and HP propane gas in healthy sheep [6 sheep X 4 test/re-test exams = 24 sheep scan sessions total]	19-33	X	X
Milestone # 9 Reproducibility and effectiveness of HP propane MRI to report on regional lung ventilation is demonstrated in sheep animal model	34	X	X
Subtask 3D: Bleomycin-induced lung injury detection using HP ^{129}Xe and HP propane gas in sheep [6 sheep X 2 time point exams = 12 sheep scan sessions total]	19-33	X	X
Milestone #10 Effectiveness OF HP propane MRI to detect lung injury is demonstrated in sheep animal model	34	X	X

Appendix 2: Abstracts Presented and Manuscripts Published and Accepted

7 conference abstracts and 6 published peer-reviewed publications are reported. All PDF files of 7 abstracts and 6 publications are provided below in the Appendix 2.