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KANSAS JOURNAL of MEDICINE

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PREFACE

Frontiers Edition
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This is the first year that Frontiers: University of Kansas Clinical and Translational Science Institute has had the privilege of having an entire edition of the Kansas Journal of Medicine – a terrific academic, open-access publication – dedicated solely to Frontiers research. We are also excited to have this Frontiers-focused issue published to coincide with our annual Frontiers Research Day, which aims to highlight the translational research being done throughout the region and to facilitate interdisciplinary collaborations.

Every abstract, commentary and paper in this volume was facilitated by Frontiers and truly showcases the breadth of support the CTSI provides across the translational research spectrum. Contributors in this issue range from pre-doctoral TL1 trainee scholars to administrative staff to senior investigators from not only the University of Kansas Medical Center, but also from the University of Kansas – Lawrence, the University of Kansas School of Medicine – Wichita, the University of Missouri – Kansas City and Children's Mercy - Kansas City.

Frontiers is part of a national network of medical research institutions working together to speed the research process from scientific discovery to patient care. We are supported by a five-year, $25 million grant from the National Center for Advancing Translational Sciences (NCATS) of the National Institutes of Health (NIH). Frontiers provides support to investigators and their teams across the spectrum of translational research, from animal health studies to community-based and population health outcomes research. We would like to express our appreciation to Dr. James Kallail for making this Frontiers' issue of the Kansas Journal of Medicine possible.

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A Randomized, Double-Blinded, Placebo-Controlled, Cross Over Study Evaluating the Efficacy and Safety of Timolol Ophthalmic Solution as an Acute Treatment of Migraine

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ABSTRACT

Introduction. Daily oral beta-adrenoreceptor antagonist has been shown to be effective in preventing migraine headaches. Timolol 0.5% ophthalmic solution is a non-selective beta-adrenoreceptor antagonist, where the primary use is for glaucoma. There have been case reports that timolol is effective in aborting or improving an acute migraine headache. The objective of this study was to assess the efficacy (decrease of ≥ 50% in pain scale at 120 minutes) of timolol 0.5% ophthalmic solution compared to placebo in acute treatment of migraine headache.

Methods. We performed a randomized, double-blind, crossover, placebo-controlled, study. Study entry criteria required subjects to have one to eight migraine episodes per month. The primary outcome was comparison of the change in a visual analog pain scale (VAS) at 120 minutes after taking the study medication. Study subjects were given a pain scale with a range of 1 (no pain) to 10 (most severe pain) to complete after onset of migraine but before administration of study drops and 120 minutes after administration of study drops. Improvement was defined as a ≥ 50% decrease in pain scale.

Results. Nineteen subjects completed the study and were used for analysis. The primary outcome changes in pain scale, 120 minutes after dose, showed a similar decrease for placebo and drug with a slightly wider 95% CI for placebo. Six subjects in each arm experienced a ≥ 50% decrease in pain scale.

Conclusion. These results support that timolol 0.5% ophthalmic solution is not an efficacious treatment for acute migraine headache.
Kans J Med 2020;13(Suppl 2):2-5.

INTRODUCTION

Migraine is a common, disabling neurological disorder, affecting 10 – 12% of the U.S. population; 18% women and 6% men.[1] The treatment of migraine headache includes preventive therapies and abortive therapies. There are several preventive therapies available with the goal to decrease the overall frequency and intensity of migraine attacks. The abortive medications are used during acute migraine episode. There are several methods that are used for migraine abortive therapy, such as triptans and NSAIDs.

Despite the various migraine abortive therapies available, there is an ongoing need for novel therapies. Beta-blocker medications have been used for migraine prevention for decades. However, their use for migraine abortion has not been well-identified.

Oral beta-blockers are a class of medications frequently used to control blood pressure, angina, and heart irregularities. Certain oral beta-blockers, such as propranolol, are used on a daily basis to prevent migraines. Both topical and oral timolol can be used daily prophylactically to decrease the frequency of migraine.[2-5] However, propranolol and timolol tablets have not been shown to be effective as an acute treatment to stop attacks of migraine because of their longer onset of action.

In this study, we tested the use of topical beta-blocker eye drops for acute treatment of migraine headache. It was proposed that beta-blocker eye drops, since they are quickly absorbed unlike tablets, can be beneficial and efficacious in the treatment of headache abortion. A prior case series of seven cases reported a substantial benefit of beta blocker eye drops in acute migraine in all patients.[6] The case series was published with four editorials in the same journal recommending that further studies be done in the form of randomized-controlled trials.[7-10] We now report a randomized, placebo-controlled, blinded-controlled trial to test the use of timolol for acute migraine headache.

METHODS

Trial Design. We performed a randomized, double-blind, crossover, placebo-controlled, study containing a screening visit, a double-blind treatment phase, and an end-of-study visit. The University of Kansas Medical Center was the only site as part of the study that enrolled patients from April 2017 to February 2018.

Standard Protocol Approvals, Registrations, and Patient Consents. The trial was approved by the Institutional Review Board at the University of Kansas Medical Center. Written informed consent was obtained by all participants, in accordance with the Declaration of Helsinki and the principles of Good Clinical Practice. This study was registered at clinicaltrials.gov (NCT03836664).

A total of 26 subjects were enrolled in a randomized, double-blind fashion. Participants ranged in age from 22 - 64 years; 25 female and one male. Subjects enrolled had a diagnosis of migraine with or without aura according to ICHD-2 criteria for at least one year prior to informed consent. Frequency of migraine episode was between one to eight migraines per month for inclusion. Women of childbearing potential had to complete a negative pregnancy test and practice effective birth control methods throughout the duration of the study.

Patients were excluded if they were not able to distinguish between migraines and headaches; had headaches at a frequency greater than 15 days per month; used chronic opioid therapy for headaches; had a history or signs and symptoms of ischemic cardiac syndromes; a history of glaucoma and/or current treatment with prescription eye drops; history of naso-lacrimal duct obstruction or surgery; treatment for any severe ophthalmic disease; physical problems or coordination difficulty or eye avoidance sensitivity that would preclude proper

installation of eye drops; history of uncontrolled asthma, COPD, diabetes mellitus, hyperthyroidism, or reversible airway disease; history of clinically symptomatic bradycardia, congestive heart failure, or hypotension; and a history of drug or alcohol abuse as defined by DSM-IV criteria.

Baseline characteristics include the location, symptom, and character of migraines experienced by enrolled subjects. Frontal was the most common location migraines were experienced at 42% of study population. The different characters associated with migraines experienced by the study population were throbbing, pulsating, sharp, stabbing, and pressure. Symptoms associated with migraines were also recorded. Symptoms recorded were photophobia, nausea, phonophobia, and visual.

Outcomes and Measures. Baseline characteristics included gender, age, and ethnicity. Migraine history was also collected including frequency, location, character, and associated symptoms. Subject-reported pain scales, satisfaction questionnaires, adverse events, and concomitant medications were assessed at visit two and visit three.

The primary end point of the study is to compare differences in headache severity at 120 minutes between study medication and placebo. Study subjects were given a visual analog pain scale (VAS) with a range of 0 (no pain) to 10 (most severe pain) to complete at two different time points: at onset of migraine and before administration of study drops, and at 120 minutes after administration of study drops. To account for the crossover design, the mean difference in the change in headache severity following placebo compared to timolol for each patient along with its 95% confidence interval was calculated.

The secondary end points were to assess subject satisfaction with treatment, proportion of subjects with at least a 50% decreased in pain severity at two hours, and safety. Patients were given a Treatment Satisfaction Scale to be completed 120 minutes after administration of study drops. The Treatment Satisfaction Scale contained five items: not satisfied at all, somewhat not satisfied, neutral, somewhat satisfied, and very satisfied. Adverse events were recorded at two timepoints throughout the study for safety.

Randomization and Blinding. Patients were randomly assigned to timolol 0.5% solution or placebo based on their subject number at the baseline visit. Subjects were assigned study IDs in the numerical order they were enrolled. Consistent with the design of the crossover study, patients that did not receive timolol 0.5% solution at the baseline visit were given timolol at visit two and vice versa. The investigator, study staff, and subjects were blinded to treatment assignment.

Sample Size and Calculation. A total of 26 subjects were enrolled in the study. Twenty subjects were needed to have an 80% chance of detecting a one-point difference in the change in pain scores between the two arms, at an alpha of 0.05.

RESULTS

Twenty-eight patients were screened for eligibility; 26 were enrolled. Reasons for screen failure included headache frequency greater than 15 episodes per month and not experiencing a migraine within a month after being enrolled. One patient dropped out of the study and six patients did not complete the study. Nineteen total participants completed the study and were used for analysis.

Out of the 26 subjects enrolled, participants were nearly exclusively female (96%) and an average age of 41 years (range 21 - 63). Participants averaged three migraine episodes per month with a range of one to eight episodes per month. The most common character of migraines was throbbing (96%) and nausea (77%) was the most common symptom (Table 1).

Table 1. Baseline characteristics of migraines.

Symptom	**Number**	**%**
Photophobia	25	96
Nausea	20	77
Phonophobia	13	50
Visual	2	8
Location	**Number**	**%**
Frontal	11	42
Occipital	8	31
Holocephalic	8	31
Temporal	4	15
Character	**Number**	**%**
Throbbing	25	96
Pulsating	6	23
Sharp	4	15
Stabbing	3	12
Pressure	1	4

Table 2 shows the raw data. Pain reductions were not significantly different following placebo or timolol treatment. Table 3 shows the mean difference of differences in change in pain, 0.1 (SD 2.6) VAS points favoring placebo, 95% CI -1.1 to 1.3. Similarly, there were no significant differences in the number of patients reporting 50% or more reduction in pain severity (six for each treatment) or for satisfaction with treatment (timolol = 3, placebo = 6).

One adverse event was experienced throughout the study, which was a mild stinging/burning sensation in the eye shortly after administration of eye drops. Each was graded mild, related, and all subjects recovered. Four of 19 study completers experienced this adverse event.

Table 2. Summary of subject responses while using timolol and placebo.

Drug						
Subject number	Pain scale before drug	Pain scale 120 min. after drug	Delta	Percent decrease	≥ 50%[a]	Satisfied[a]
M001	7	3	4	57	1	0
M003	3	0	3	100	1	1
M004	9	8	1	11	0	0
M006	8	6	2	25	0	0
M007	8	8	0	0	0	0
M009	8	8	0	0	0	0
M010	4	6	-2	-50	0	0
M011	6	6	0	0	0	0
M012	8	3	5	63	1	0
M013	9	4	5	56	1	0
M014	10	4	6	60	1	1
M016	9	7	2	22	0	0
M017	7	7	0	0	0	0
M019	7	6	1	14	0	0
M021	8	5	3	38	0	0
M022	5	4	1	20	0	0
M023	8	4	4	50	1	1
M024	7	5	2	29	0	0
M025	7	6	1	14	0	0
Placebo						
M001	7	3	4	57	1	1
M003	2	2	0	0	0	0
M004	7	4	3	43	0	0
M006	9	3	6	67	1	1
M007	7	4	3	43	0	1
M009	8	8	0	0	0	0
M010	5	7	-2	-40	0	0
M011	5	4	1	20	0	0
M012	7	1	6	86	1	1
M013	9	5	4	44	0	0
M014	8	9	-1	-13	0	0
M016	9	4	5	56	1	1
M017	6	7	-1	-17	0	0
M019	6	7	-1	-17	0	0
M021	7	5	2	29	0	0
M022	6	6	0	0	0	0
M023	4	2	2	50	1	0
M024	7	2	5	71	1	1
M025	7	7	0	0	0	0

[a] ≥ 50% decrease and satisfaction coded 0 = no, 1 = yes.

Table 3. Change in VAS.*

Timolol			**Placebo**		
Onset	Two hours	Delta	Onset	Two hours	Delta
7.3 (1.7)	5.3 (2.0)	2 (2.1)	6.63 (1.7)	4.7 (2.2)	1.9 (2.5)

*Mean (SD)

DISCUSSION

Migraine and other benign recurrent headache disorders are a major public health problem, particularly among reproductive-aged women. They are associated with substantial personal suffering, disability, and societal expense. The impact of migraine is substantial because of its high prevalence, accompanying significant disability, and risk for other comorbidities. Data from the National Ambulatory Medical Care Survey (NAMCS), and the National Hospital Ambulatory Medical Care Survey (NHAMCS) indicate that headache is among the top 20 reasons for outpatient medical visits and among the top five reasons for emergency department visits. Several preventive and abortive medications are available for the treatment of migraine headaches. Despite of various treatments available, there seem to be an ongoing need for novel therapies.

Certain beta-blockers medications like propranolol and timolol have been used as a preventive therapy for migraine headaches. Their safety and efficacy in the prevention of migraines has been well established. In this study we used topical beta-blocker eye drops for acute treatment of migraine attack. Topical and local application beta-blocker eye drops are directly absorbed through the ophthalmic and nasolacrimal mucosa and enters the blood stream within minutes.

Timolol is a non-selective beta-adrenoreceptor antagonist. Oral timolol (20 - 30 mg daily) has been studied in three randomized controlled trials and have been found to reduce headache frequency by more than 50% when compared to placebo.[3-5] It has been approved by FDA for prophylactic use in migraine patients and had Level A evidence to support this indication. The prophylactic benefit of beta-blockers in migraine treatment is not completely understood. It may be related to the effect of beta-blockers on central autonomic vascular tone center, which in turn modulate the cerebrovascular reactivity to sensory stimulation.[11-12] Propranolol, a beta-adrenergic blocker modulates serotoninergic transmission, regulates periaqueductal pathway activation and prevents central sensitization, normalizes neuronal excitability in the CNS, and blocks cortical spreading depression. Topical ocular beta blockers when used daily have been reported to prevent migraine attacks.[13-14]

The specific aims in this study were to 1) assess the efficacy (headache freedom at 120 minutes) of timolol 0.5% ophthalmic solution compared to placebo in acute treatment of migraine headache; and 2) assess the safety and tolerability of timolol 0.5% ophthalmic solution in treatment of acute migraine headache.

The results of this randomized-controlled trial support that timolol 0.5% ophthalmic solution is not an efficacious treatment for acute migraine headache in this group of patients.

Our results are similar to a recent report by Cossack et al.[15] They reported a randomized-controlled study of timolol in acute migraine. Ten patients participated that reported one to eight migraine attacks per month. In this series, the average percentage of headaches with a severity of none or mild at 120 minutes was 57% with placebo compared with 78% with timolol. This was not statistically significant (p = 0.26). In an exit survey, four patients found timolol highly effective compared to placebo and one patient reported placebo highly effective compared to timolol. The other five subjects did not report major differences between the two.

Therefore, while there is a good rationale for the use of topical ophthalmic beta-blocker medication for acute migraine, and prior case reports suggested such a benefit, we now have two randomized-controlled trials that did not demonstrate a marked effect of the drug compared to placebo. This is not to say that physicians may encounter individual patients who could report a beneficial effect of beta blocker eyedrops for acute migraine headaches. However, we could not demonstrate in a randomized-controlled trial the superiority of beta blocker eyedrops over placebo. Also, perhaps other formulations of beta blocker medications might be effective for treating acute migraine such as sublingual or intranasal. Finally, perhaps trials with a larger number of subjects with more power to detect a difference might show different results. Such a trial would require an external sponsor, either a pharmaceutical industry or the NIH.

FUNDING SUPPORT

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Development of Silica-Immobilized Vaccines for Improving Thermo-Tolerance and Shelf-Life

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ABSTRACT

Introduction. It is estimated that 50% of vaccines produced annually are wasted because effectivity is dependent on protein structure and heat exposure disrupts the intermolecular interactions that maintain this structure. Since 90% of vaccines require a temperature-controlled supply chain, it is necessary to create a cold chain system to minimize vaccine waste. We have developed a more sustainable technology via the adsorption of Invasion Plasmid Antigen D (IpaD) onto mesoporous silica gels, improving the thermal stability of protein-based therapeutics.

Methods. The solution depletion method using UV-Vis was utilized to study the adsorption of IpaD onto silica gels. The silica-IpaD complex is heated above the denaturing temperature of the protein and then the IpaD is removed using N,N-Dimethyldodecylamine N-oxide (LDAO) and their secondary structure is tested using circular dichroism (CD).

Results. Pore diameter, pore volume and surface area were characterized for seven different silica gels. Silica gels designated as 6389, 6378, and 6375 had an adsorption percentage above 95% at pore volumes of 2.2, 2.8 and 3.8 cm^3 mg-1, respectively. CD analyses confirmed that the adsorbed IpaD after the heat treatment displayed a similar "W" shape CD signal as the native IpaD, indicating the conservation of α-helices. In contrast, the unprotected IpaD after being exposed to high temperature shows a flat CD signal, demonstrating the loss of secondary structure.

Conclusion. We have successfully increased the thermo-tolerance for IpaD using mesoporous silica and continue to further optimize mesoporous silica's physiochemical properties to improve adsorption and desorption yields. ***Kans J Med 2020;13(Suppl 2):6-9.***

INTRODUCTION

Temperature-sensitive substances composed of proteins, enzymes, and antibodies are inherently unstable at ambient temperature because they lose their secondary structure and their activity over time. This is a particularly sensitive issue for protein-based medications, such as vaccines, as their storage and handling usually requires continuous cold chain transportation (2 to 8°C) to maintain their functional properties. Cold chain logistics are expensive and prone to disruptions, which often leads to rapid loss of protein potency and deactivation, creating serious concerns for global public health.[1] Although there have been several efforts to develop successful approaches for enhancing the long-term thermal-stability of proteins and vaccines, including pegylation,[2] addition of excipients,[3,4] biomineralization[5] and nanopatches.[6] These methods are unable to provide satisfactory thermal stability, while ensuring the delivery in a biologically active form at the point of care, particularly in developing countries. Thus, the development of innovative and cost-effective strategies capable of protecting thermo-sensitive substances would eliminate the need for an uninterruptible cold chain and help to solve one of the most important issues associated with vaccine wastage. Such advancement can help to ensure that proper treatment is delivered safely and efficiently to the patient, from vaccine production to the patient bedside.

Silica gels have demonstrated the ability to retain protein structure and functionality.[7,8] Some of the most relevant advantages of silicas in protein adsorption include: high thermal stability, useful porosity range for proteins, simple chemistry, mild pH, tunable surface chemistry, and encapsulated biomolecular samples that retain high activity.

Shigellosis is a gastrointestinal disease that causes over a million deaths annually,[9] especially in developing countries and among children.[10] *Shigella flexneri*, the bacteria that is responsible for causing Shigellosis, is spread through the fecal-oral route and only a very low dose is needed for causing an infection.[11] *S. flexneri* is acid-tolerant, and once it reaches the large intestines, it is transcytosed through M cells.[9,12] At this point, the bacteria induces apoptosis in macrophages and invades epithelial cells using a type-III secretion system (TTSS).[13] Invasion plasmid antigen D (IpaD) has a crucial role in TTSS, as it controls the secretion of IpaB and IpaC. All three Ipa proteins must be released for epithelial cell invasion.[14]

Currently, there is no existing vaccine for shigellosis[15] despite extensive attempts to formulate a vaccine against this bacteria.[16,17,18] This is because previously developed vaccines have shown high reactogenicity in human trials.[19] IpaD is a promising target for shigellosis vaccine development since it has already been demonstrated that IpaD antibodies are present in serum from infected patients.[20,21]

Circular dichroism (CD) and Fourier transform infrared (FTIR) have proven that IpaD is predominantly an alpha-helical protein.[13,22] IpaD has a high thermal stability with denaturing occurring above 80°C. Approximately 13% of IpaD is made up of serine amino acids,[23] which are able to form hydrogen bonds at the surface of the silica as shown in Figure 1. Therefore, it is possible to encapsulate IpaD within mesoporous silica and prevent protein denaturation.

METHODS

Adsorption. Silica gel particles were crushed and sifted to a particle size of 90 - 150 μm. Then 0.7 mL of IpaD in 1xPBS buffer at 1.5 mg/mL was added to 30 mg of silica. The solution was mixed on the ThermoMixer at 950 rpm and 25°C for 20 hours. The samples were centrifuged at 14,800 rpm for four minutes at ambient temperature to separate the silica-IpaD complex from the supernatant.

The solution depletion method was utilized to determine the amount of IpaD transferred from the solution into the silica, such that the supernatant concentration indirectly determined the proteins adsorbed. The concentration of the supernatant was measured at 280 nm with an IpaD extinction coefficient of 9.48 $M^{-1}cm^{-1}$ using the Nanodrop 2000 UV-Vis spectrophotometer. The supernatant concentration was used along with the original concentration of IpaD to quantify the adsorption process in Equation 1:

Percent adsorption = (1-(Supernatant IpaD concentration)/(Inital IpaD concentration))*100

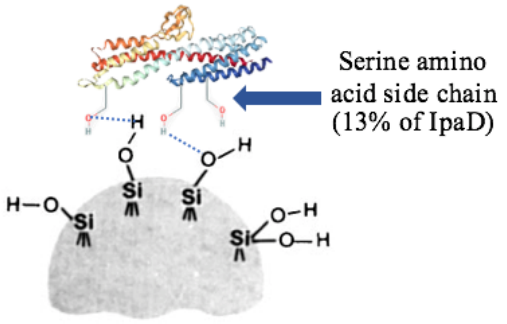

Figure 1. Hydrogen bonds between IpaD and mesoporous silica.

Washing. The unbound proteins in the supernatant were extracted by removing the supernatant from the silica-IpaD particles. A sample containing 0.6 mL of 1xPBS was added to the silica to remove any unattached IpaD trapped within the silica particles. The vials were mixed briefly to loosen the unbound proteins then centrifuged at 14,800 rpm for four minutes, and the wash supernatant was extracted. The washing method was repeated twice, with the exception that 1xPBS was not added back to the sample vial on the third wash. The supernatant of each wash was combined into a single vial, and the concentration was measured.

Heating. The samples were then placed on the ThermoMixer at 95°C for two hours at 950 rpm. The samples were allowed to cool down to room temperature for at least 30 minutes.

Desorption. To detach the proteins from the silica gel, 0.7 mL of 10% LDAO was added to each sample. The samples were then mixed at 950 rpm and 25°C for 20 hours. Finally, the samples were centrifuged at 14,800 rpm for four minutes and the percent IpaD desorption was calculated using the equation:

Percent desorption= ((Mass of desorbed IpaD)/(Mass of IpaD on silica after wash))*100

Circular Dichroism. A Jasco J-1500 Circular Dichroism Spectrophotometer instrument was used to evaluate the secondary structure of chiral protein samples at a temperature of 15°C over a wavelength range of 190 - 260 nm. Before CD measurements were performed, the samples were diluted to 0.4 mg/mL in 10% LDAO which is within the required concentration range of 0.2 - 0.5 mg/mL. The nitrogen flowrate was set at 30 SCFH. A 1 cm pathlength quartz cuvettes was cleaned using 2% Hellmanex II solution to remove any remaining biological specimen from previous runs. Then the cuvettes were thoroughly washed with milliQ water before purging with nitrogen to remove remaining moisture. The cuvettes were filled with 250 µL of sample and placed in the cuvette holder in the instrument. The raw data from CD was reported in Q (mdeg). This was converted to molar ellipticity to account for the concentration of the sample and properties of IpaD using the equation:

$$\theta=(Q\cdot 100\cdot MW)/(C\cdot l\cdot n\cdot 1000)$$

MW represents the molecular weight of the protein sample and n represents the number of amino acids. For IpaD, these values are 38825.29 Da and 352, respectively. *C* is the concentration of the sample in mg/mL, and it is found at 280 nm using UV-Vis absorbance spectrophotometry. The length (*l*) of the quartz cuvette is 1 cm for the J-1500 instrument.

RESULTS

Physiochemical Characterization. The physicochemical properties of seven mesoporous silica gel materials were characterized to generate a "tool box" with a range of physicochemical properties. The material selection criteria for the immobilization of IpaD were correlated between the physico-chemical properties of the support (pore sizes, volumes and shapes, and surface functional groups) and with those of the protein. The physico-chemical properties of selected mesoporous silica gel materials used as supports for the protein thermal-stabilization studies are provided in Table 1. *The identification of the specific silica gels (6360, 6369, 6765, 6389, 6378 and 6395) are confidential and a patent application is being prepared for filing.*

Table 1. Physicochemical characterization of selected mesoporous silica gel materials.

CEBC code	Average pore diameter (nm)	Accessible pore volume (cm^3/g)	Accessible surface area (m^2/g)
6339	17.7	1.41	319.3
6360	3.9	0.07	39.0
6369	8.1	0.30	125.6
6378	36.3	2.87	286.1
6389	24.2	2.18	370.7
6395	30.2	3.78	300.0
6765	15.0	1.28	332.0

Effect of silica pore volume on IpaD percent adsorption. After improving the protocol to adsorb IpaD onto various silica gel materials, the material properties with the most impact on the adsorption efficiency of IpaD were investigated. Our results show that the successful adsorption of IpaD protein is strongly influenced by the pore volume of the silica gel material (Figure 2). We define accessible pore volumes as the pores with diameters ≥ 5 nm.

IpaD secondary structure. To assess whether the adsorption into silica-based supports protects the protein against high temperatures, the IpaD adsorbed onto the silica gels was heated to 95°C for 2.5 h, desorbed from the support, and then analyzed by CD. Non-adsorbed (native) IpaD which was not heated was used as control. The CD analyses demonstrate that the adsorbed IpaD after the heat treatment (and desorption step) displays a similar "W" shape CD signal as the native unheated IpaD.

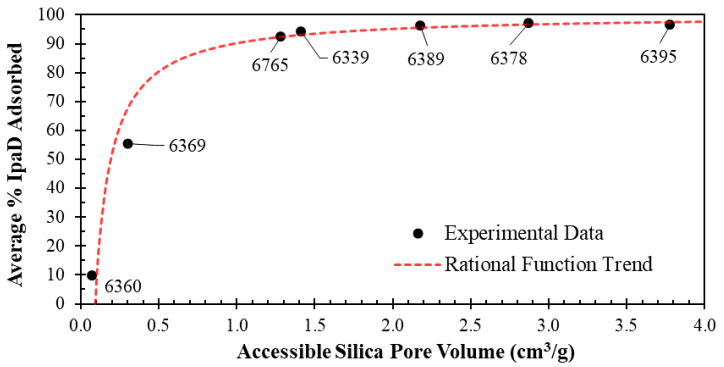

Figure 2. Average percent of IpaD adsorbed to mesoporous silica gel materials as a function of accessible pore volumes (cm^3/g).

DISCUSSION

Effect of silica pore volume on IpaD adsorption. The adsorption of IpaD onto various mesoporous silicas has been hypothesized to have similar surface chemistry for all silicas tested. The adsorption process is governed by electrostatic interactions and hydrogen bonding between the hydroxyls on the silica surface and the properties of the amino acid side chains on IpaD.[23] The most abundant amino acid in IpaD is serine, which makes up 13% of the structure. This creates the potential for a high level of hydrogen bonding between serine residues and the silica surface. As shown in Figure 2, there is a strong correlation between the pore volume of the silica gel to the percent of IpaD adsorbed. For example, silica gel 6765 has a pore volume of 1.29 cm^3/g and adsorbs an average of 92.2% IpaD, while silica gel 6360 has a pore volume of 0.20 cm^3/g and adsorbs an average of 9.7% IpaD. According to Figure 2, silicas with a pore volume greater than 1 cm^3/g are expected to adsorb more than 90% IpaD. The pore diameter of the silica gels followed a similar trend to the pore volume. Therefore, silicas with larger pore volumes and pore diameters tend to adsorb more IpaD. This indicates that the proteins are not only attaching to the surface of the silica, but also being adsorbed into the interior of the larger silica pores. IpaD's thermostability while attached to silica during heating further supports the idea that IpaD is confined within the pores during adsorption.

IpaD secondary structure. The purpose of this study was to demonstrate that silica gels improve the thermostability of IpaD. It is evident from Figure 3 that the presence of silica during heating enables IpaD to maintain its α-helical structure as shown by the characteristic "W" curve. The IpaD in solution completely denatures during heating, and the IpaD heated on silica has a small decrease in molar ellipticity from that of the native IpaD. We hypothesize that this decrease in molar ellipticity will not reduce IpaD's efficacy in a vaccine.

The source of the loss in structure for the IpaD heated on silica is most likely due to variables other than heating. It has been commonly reported that proteins lose some of their conformational structure upon adsorption to a solid surface.[24,25,26,27] Another possible alternative for this loss in structure is the effects of LDAO on IpaD. This zwitterionic detergent is used at a high concentration to remove IpaD from the silica in these studies. One problem is that LDAO increases the high tension (HT) voltage due to the high concentration (10% LDAO). During CD experimentation, it has been observed that the HT voltage is above the recommended value of 600 V at wavelengths below 200 nm. This indicates that the sample is absorbing too much light, which means it is overly concentrated. This effect creates noise in the CD wavelength range of 190 - 200 nm, which eliminates the expected Cotton effect peak at 195 nm that is seen in the native IpaD sample. Furthermore, the drop in molar ellipticity in Figure 3 is potentially due to the buffer concentration interference with the CD signal for the protein. While it is difficult to isolate the effect of LDAO on the CD signal from the physical effect of LDAO interactions with IpaD, further studies have indicated that LDAO is partially denaturing IpaD during the desorption process.

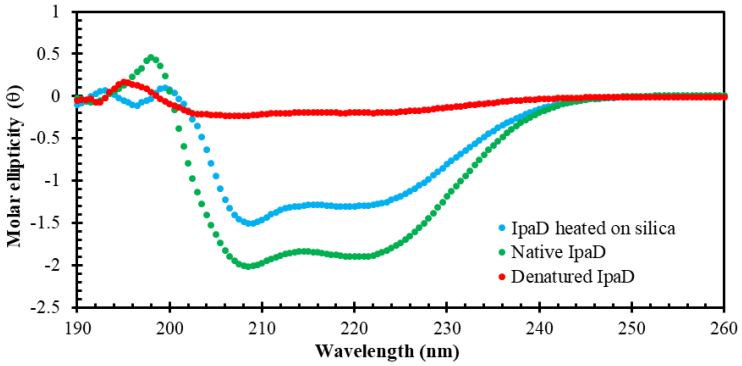

Figure 3. CD spectra for IpaD heated to 95°C for 150 minutes attached to silica gel 6339, native unheated IpaD, and IpaD denatured in PBS solution at 95°C for two hours. The blue curve is the average of 10 samples, and the green curve is the average of two samples.

CONCLUSION

This study has successfully characterized the physiochemical properties of different silica gels using the BJH method. With these physiochemical properties, it was possible to determine the relationship between pore volume and percent adsorption. In general, as pore volume increases, percent adsorption also increases. The most significant result is that IpaD adsorbed in mesoporous silica has a higher thermal stability than unadsorbed IpaD. This was confirmed by the characteristic W shape observed by CD, indicating the presence of alpha helices even after heat treatment.

Future research will explore alternative desorbing agents that are not as harsh as LDAO to reduce the small loss of protein conformational structure during desorption. The next steps will include the design of a device where a vaccine encapsulated in silica can be mixed with the desorbing agent for removing the bound protein and made ready for administration to a patient.

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Methotrexate Polyglutamation in a Myasthenia Gravis Clinical Trial

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ABSTRACT

Introduction. Methotrexate (MTX) is an immunosuppressive and anti-inflammatory drug used to treat rheumatoid arthritis (RA) and other autoimmune conditions. MTX is transported into cells, where glutamate moieties are added and is retained as methotrexate polyglutamates (MTXPGs). In the RA literature, it has been reported that the degree of polyglutamation correlates with the anti-inflammatory effect of MTX in RA. There are no prior studies evaluating the relationship between MTXPGs and myasthenia gravis (MG) outcome measures. The objective of this study was to assess the correlation between methotrexate (MTX) polyglutamates (MTXPGs) with Myasthenia Gravis (MG) outcome measures.

Methods. An analysis was done of blood drawn from patients enrolled in the 12-month randomized, placebo-controlled study of MTX in MG study. Red blood cell MTXPGs were measured via ultra-performance liquid chromatography and tandem mass spectrometry. MTXPG was correlated to MG outcome measures using Spearman Correlation Coefficient. A two-group t-test was used to determine the difference in MTXPG based on clinical outcome responder definitions.

Results. Twenty-one polyglutamate samples were analyzed of subjects on MTX while eight samples were analyzed from subjects on placebo. Pentaglutamate had the strongest correlation with the MG-ADL (0.99), while tetraglutamate had the strongest correlation with the QMG (0.54). Triglutamate had the strongest correlation with MGC (0.76).

Conclusion. There were variable correlations between $MTXPG_{1\text{-}5}$ and MG outcomes (rho range: 0.08 to 0.99). There are strong correlations between MTXPG and the MG-ADL, QMG, and MGC. Long chain methotrexate polyglutamates correlate better with MG outcomes. ***Kans J Med 2020;13(Suppl 2):10-13.***

INTRODUCTION

Methotrexate (MTX) is a disease-modifying antirheumatic drug (DMARD) used in the management of rheumatoid arthritis (RA). Methotrexate is a folate analogue, used in treatment of cancers and autoimmune diseases. Given orally or subcutaneously, MTX is transported into cells, where additional glutamate moieties are added and is retained as methotrexate polyglutamates (MTXPGs). Prior studies suggest the degree of polyglutamation correlates with the anti-inflammatory effect of MTX in RA. There are no prior studies evaluating the relationship between MTXPGs and myasthenia gravis (MG) outcome measures.

Although the full molecular anti-inflammatory mechanism of MTX is not clearly elucidated, it is known that MTX acts as a folate antagonist. Once intracellular, MTX is bioactivated to the polyglutamated form of methotrexate (MTXglun) by folylpolyglutamyl synthase (FPGS), which promotes cellular retention and inhibition of several enzymes.[1] No or low glutamation leads to the efflux of MTX by the ATP-binding cassette (ABC) family of transporters. FPGS and ABCG2 are of particular interest as folate deprivation has been associated with increased expression of FPGS and decreased expression of ABCG2,[2] suggesting a cellular response to low folate with an increase in polyglutamation and decrease in folate export to promote retention of folate within the cell. Additionally, upregulation of ABCG2 protein expression has been associated with MTX resistance in cancer cells. Therefore, allelic variation in these genes resulting in increased or decreased activity may be associated with either increased or decreased MTXglun. This entire process is also likely dependent upon the folate status of the patient, reflected by the polyglutamation of folate itself, and the relative concentrations of the two groups of mutually antagonistic compounds.

As serum MTX concentrations have been notoriously unreliably associated with MTX clinical outcomes,[3-5] the search for more stable biomarkers of diseases response to MTX have been ongoing. An association between RBC MTXglun and effectiveness of MTX in RA has been reported.[6-7] Higher levels of "long chain MTXglun" (defined as MTXglu3 or greater) were associated with improved effectiveness of MTX in RA. There has also been reported variability of MTXglun concentrations and patterns associated with MTX dosing route and dose of administration in Juvenile Idiopathic Arthritis patients prescribed this medication.[8] Since RBC folate concentrations are established during erythropoiesis and represent the average folate status over the preceding 120 days,[9] by extension, MTX concentrations in RBCs are a surrogate biomarker of average drug exposure over a similar period of time. Furthermore, methotrexate polyglutamates in RBCs are considered to be representative of intracellular MTX levels in target tissues, are more stable than serum levels of MTX, and may potentially predict response to the drug.[6-7, 10-11]

Methotrexate may also cause hepatotoxicity,[12] probably as a result of accumulation in the liver as polyglutamates[13] and can cause increases in serum alaninine aminotransferace (ALT) and aspartate aminotranseferase (AST).[14] Renal impairment is a significant contributing factor to the occurrence of toxicities in other organ system, particularly hematopoietic and gastrointestinal systems. Whether methotrexate produces some degree of renal insufficiency has not been clearly established. Headache, dizziness, vertigo, mood changes, seizures, ataxia and cognitive impairment have been infrequently described.[15]

We performed a randomized-controlled trial of oral methotrexate in myasthenia gravis involving 50 subjects at 19 sites.[16] Myasthenia gravis is an autoimmune disease with antibodies derived against the acetylcholine receptor in muscle at the neuromuscular junction.[17] Prednisone is the first line immunosuppressive therapy for MG.[18] We studied if methotrexate would be of benefit to MG patients on prednisone. In this study, we failed to show MTX subjects were on a lower prednisone dose at the end of twelve months, which was our primary endpoint. Secondary endpoints such as the Quantitative Myasthenia Gravis (QMG) and Myasthenia Gravis - Activities of Daily Living (MG-ADL) showed trends toward greater improvement in MTX subjects but did not meet statistical significance in the pre-determined intent-to-treat analysis. When alternative intent-to-treat analyses were done post-hoc, QMG and MG-ADL did meet significance. We also performed polyglutamation blood studies on participants at the conclusion of the 12-month study.

METHODS

Trial Design. Analysis was done on blood drawn from patients enrolled in the 12-month randomized, placebo-controlled study of MTX in MG. Red blood cell MTXPGs were measured via ultra-performance liquid chromatography and tandem mass spectrometry.[19] MTXPG was correlated to MG outcome measures using Spearman Correlation Coefficient.

Standard Protocol Approvals, Registrations, and Patient Consents. The trial was approved by the Institutional Review Board at the University of Kansas Medical Center. Written informed consent was obtained by all participants, in accordance with the Declaration of Helsinki and the principles of Good Clinical Practice.

Outcomes and Measures. Monoglutamate (1), diglutamate (2), triglutamate (3), tetraglutamate (4), and pentaglutamate (5) were analyzed for correlation to the MG-ADL scale, the QMG scale, and the Myasthenia Gravis Composite (QMC) scale, all standard MG research outcome measures.

Statistics. A total of 33 polyglutamate samples were collected in the study and 21 samples were used from subjects who were on active MTX for analysis. The differences of the outcome measures between the baseline and end of study visit was calculated. A two-group t-test was used to determine the difference in MTXPG based on clinical outcome responder definitions. We correlated $MTXPG_{1\text{-}5}$ to MG outcome measures using Spearman Correlation Coefficient.

RESULTS

A total of 33 polyglutamate samples were collected in the study. Twenty-five samples were from subjects on MTX and eight samples were from subjects on placebo. Twenty-one samples were used for analysis (Figure 1). The median age of subjects was 64 ± 11.3 and the male/female ratio was 16:5.

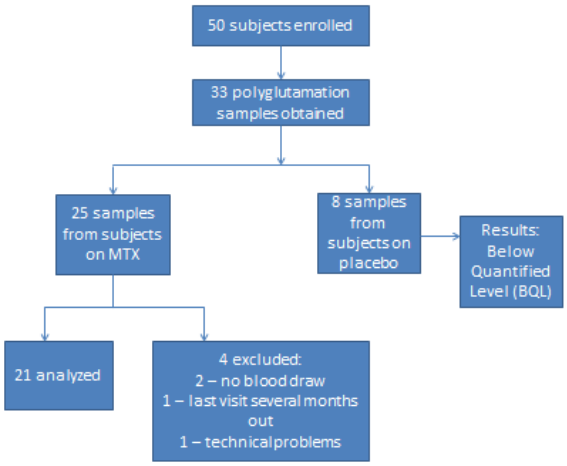

Figure 1. Study design.

The results of the degree of polyglutamation for each sample are shown in Figure 2. There were variable correlations between $MTXPG_{1\text{-}5}$ and MG outcomes with a rho range: 0.08 to 0.99 (Table 1). Strong correlations were seen between $MTXPG_{2,4,5}$ and MGADL, $MTXPG_{4,5}$ with QMG and $MTXPG_{3}$ with MGC. Both coefficients lie between -1 and +1. The more the correlation coefficient comes closer to -1 or 1, the more there is a correlation between two variables. Our interpretation of strength of correlation in this study is as follows: < 0.15 = very weak, 0.15 – 0.25 = weak, 0.25 – 0.40 = moderate, 0.40 – 0.75 = strong, >0.75 = very strong (Table 1).

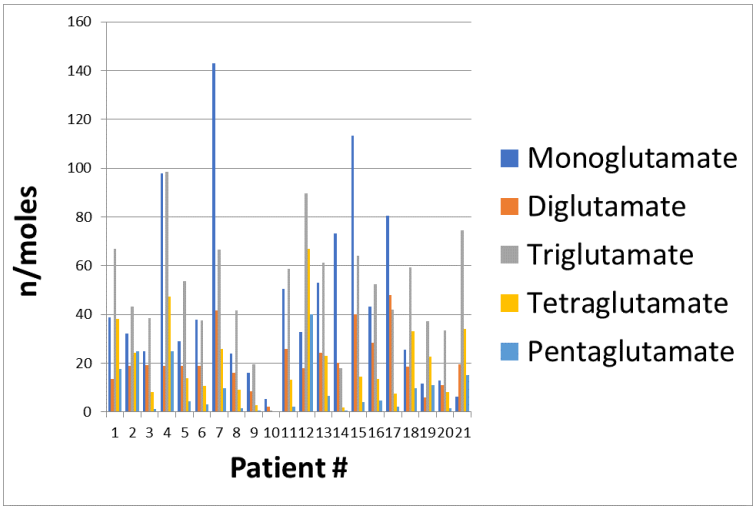

Figure 2. Degree of methotrexate polyglutamation in MG subjects.

Table 1. Correlation of methotrexate polyglutamation and MG.

Polyglutamates*	Correlation with MG outcomes (rho)			
	MG-ADL	QMG	MGC	Prednisone dose
Monoglutamates	0.20	0.08	0.08	0.17
Diglutamates	**0.60**	0.02	0.39	0.14
Triglutamates	0.38	0.19	**0.76**	0.36
Tetraglutamates	**0.71**	**0.54**	0.30	0.30
Pentaglutamates	**0.99**	**0.53**	0.15	-0.18

MG-ADL = Myasthenia Gravis Activities of Daily Living; QMG = Quantitative Myasthenia Gravis; MGC = Myasthenia Gravis Composite
*Bolded are strong and very strong correlations.

DISCUSSION

We found that the therapeutic effect of MTXPGs correlates with MG clinical outcome measures used in our prospective randomized trial. The therapeutic effects of MTX in RA depend on its conversion to MTXPGs, so measuring intracellular MTXPGs has been proposed as an objective method to guide MTX therapy in various disease conditions. Most of the information on MTXPG is from rheumatoid arthritis literature. Methotrexate helps stabilize rheumatoid arthritis conditions. Long chain MTXPGs stay in the cell longer than short chain MTXPG; therefore, long chain MTXPG keeps MTX in active form longer inside the cell, hence possibly allowing MTX to work longer. Rheumatoid arthritis literature showed higher concentrations of RBC long chain MTXPGs were associated with increased therapeutic response to MTX. However, literature is limited in other conditions.

This is the first study studying MTXPGs in Myasthenia Gravis and correlating it with outcomes in a randomized-controlled trial. We showed a wide inter-patient variability of RBC MTXPG concentrations in patients receiving similar dose of MTX therapy, which is similar to the rheumatoid arthritis literature. Also similar to the rheumatoid literature, the long chain polyglutamates (tetra and penta) correlated well with outcomes. In this case outcomes used were QMG, MG-ADL, and MGC. Prior studies showed no statistical significance of the MTXPGs levels with side effects; however, this analysis was not performed in our study. Of note, none of the subjects who were part of this sub-study developed liver toxicity.

Although the phase II Methotrexate in MG trial was negative (did not show significant reduction in the prednisone dose under the curve), the polyglutamate analysis showed strong correlations with MG outcome measures. This supported our other findings that secondary outcomes showed trends in improvement when patients were on methotrexate versus placebo.

As a result of this analysis, we believe that polyglutamation may be a useful personalized medicine biomarker to predict clinical improvement in MG when patients are on methotrexate. However, further studies should be done using this tool in myasthenia gravis.

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Keywords: myasthenia gravis, methotrexate, polyglutamate

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KANSAS JOURNAL of MEDICINE

Development of GCRC and CTSA Programs at the University of Kansas Medical Center: A Personal 10-year Perspective

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Early Beginnings

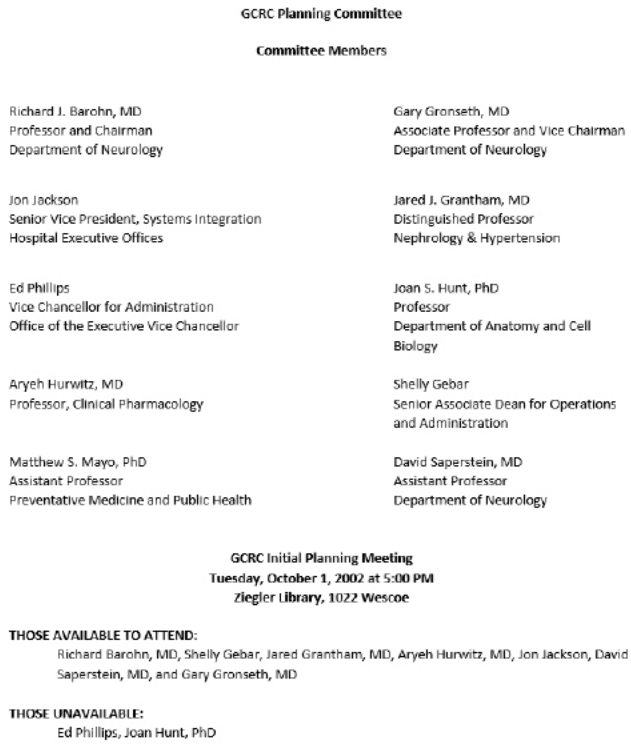
Drs. Thomas DuBose (Chair of Medicine) and Richard Barohn (Chair of Neurology) had arrived to KUMC in 2000 - 2001 from the University of Texas system where General Clinical Research Center's (GCRC) were an important infrastructure component for researchers. Dr. DuBose had called one meeting in 2001 to discuss the possibility of a GCRC at KUMC. Dr. Barohn and others attended but there were no minutes recorded from that meeting. Dr. DuBose left KUMC shortly thereafter (to be Chair of Medicine at Wake Forest Medical Center). After several months Dr. Barohn asked the executive dean, Dr. Barbara Atkinson, if he could restart the initiative and was given permission to form the GCRC planning committee. It should be noted KUMC had a GCRC once before for one funding cycle in the early 1970s. Drs. Jared Grantham and Areyh Hurwitz, who were on the GCRC Planning Committee, were at KUMC during that time.

The Process Begins: Initial Goals. We began the process of initiating a GCRC for The University of Kansas Medical Center (KUMC) campus in 2002. At that time we established four goals:

- Provide clinical investigators from the School of Medicine, School of Nursing and School of Allied Health (as it was then called) with a modern, state of the art facility in which clinical research could be conducted.
- Enhance multidisciplinary research across departments and the three schools.
- Enable and train junior faculty and trainees to become more involved in clinical research.
- Apply for federal funding to support the GCRC.

GCRC Planning Committee. A GCRC planning committee was formed in 2002 and Figure 1 shows the initial members and the commencement of the first official meeting.

This committee met for two years in order to develop the infrastructure needs for the GCRC. Minutes were taken by Dr. Barohn at all committee meetings and prepared by Debbie Jursch, Dr. Barohn's administrative assistant in the Department of Neurology (Appendix A, B, C, D, E, F, G, H, I; available at journals.ku.edu/kjm). As seen in these minutes, Dr. Barohn added a number of additional members quickly, including representation by the Schools of Allied Health (Dr. John Ferraro) and Nursing (Dr. Marge Bott). The committee was active, collegial and lively, but there was some suspicion that we would not be able to pull this off. As we made progress this skepticism disappeared. As I was new to KUMC and had been focused primarily on neurology issues, through this committee I developed close friendships with colleagues across the campus in all these schools including Marge Bott, John Ferraro, Matt Mayo, and Jared Grantham.



GCRC Planning Committee

Committee Members

Richard J. Barohn, MD
Professor and Chairman
Department of Neurology

Gary Gronseth, MD
Associate Professor and Vice Chairman
Department of Neurology

Jon Jackson
Senior Vice President, Systems Integration
Hospital Executive Offices

Jared J. Grantham, MD
Distinguished Professor
Nephrology & Hypertension

Ed Phillips
Vice Chancellor for Administration
Office of the Executive Vice Chancellor

Joan S. Hunt, PhD
Professor
Department of Anatomy and Cell Biology

Aryeh Hurwitz, MD
Professor, Clinical Pharmacology

Shelly Gebar
Senior Associate Dean for Operations and Administration

Matthew S. Mayo, PhD
Assistant Professor
Preventative Medicine and Public Health

David Saperstein, MD
Assistant Professor
Department of Neurology

GCRC Initial Planning Meeting
Tuesday, October 1, 2002 at 5:00 PM
Ziegler Library, 1022 Wescoe

THOSE AVAILABLE TO ATTEND:
Richard Barohn, MD, Shelly Gebar, Jared Grantham, MD, Aryeh Hurwitz, MD, Jon Jackson, David Saperstein, MD, and Gary Gronseth, MD

THOSE UNAVAILABLE:
Ed Phillips, Joan Hunt, PhD

Figure 1. Initial members and commencement of the first GCRC planning committee.

The space in Delp Pavilion was identified (G040 suite of rooms) that had previously been space for the Parkinson's program in the Department of Neurology. The reason the Parkinson's space became available was because the Neurology department had relocated to The Landon Center on Aging. An old driving simulation lab that was no longer used (also in Neurology) in Delp, across the hall from G040, was closed, as was an adjacent ophthalmology office, and these, plus the abandoned radiation therapy space in Delp (G037 suite of rooms), was to become the administrative and nursing offices for GCRC staff. A state-of-the-art metabolic kitchen was installed in one of the G037 rooms through funding by Dr. Joan Hunt, Vice Chancellor of Research. Dr. Deborah Sullivan, Department of Nutrition in the School of Allied Health (as it was then called) directed the metabolic kitchen. There was a lot of discussion on how to organize the clinical GCRC space and we looked at many possible floor plans provided by KUMC architect Steve Smallwood. Originally, the GCRC clinic space had only one bathroom. Dr. Aryeh Hurwitz lobbied the planning committee successfully for two bathrooms, one for the staff and one for patients providing specimen samples and his suggestion was adopted. Many of us remember Dr. Hurwitz request fondly and of course he was correct. Dr. Hurwitz died too young from a cardiac arrest in October 2005 but he did get to see the new GCRC open with two bathrooms. Remodeling began in June 2004 and was completed in October 2004. The GCRC officially opened its doors in January 2005 (Figure 2). At the same time, we disbanded the GCRC planning committee as its function was completed.

Figure 2. GCRC in 2005.

Foundation of the GCRC Advisory Committee

Drs. Barbara Atkinson and Richard Barohn announced the formation of the (GCRC Advisory Committee) GAC in the summer of 2004 and notice went out to all clinical investigators on the KU Medical Center campus to indicate that research applications could be submitted in order to use the new GCRC space and resources. Dr. Barohn asked Dr. John Ferraro (Associate Dean of Research, School of Health Professions) to chair the committee of approximately 20 faculty and GCRC staff (the GAC began reviewing protocols monthly in September 2004). The GAC was a group that took their role seriously when they gave feedback to faculty applying to use the GCRC. When Dr. Patricia Kluding (who was an assistant professor in Physical Therapy) applied to use the GCRC she received intense criticism for her proposal. She rose to the occasion and fixed her protocol. We immediately appointed her to be a member of the GAC so she could be part of the process of reviewing future protocols in order to assist those investigators. She has been a key leader in our clinical research infrastructure ever since.

Initial Internal Funding, Staffing and Organization of the GCRC

Two primary sources provided the initial funding for the KU Medical Center GCRC since its inception. The majority of the funds were supplied through the Executive Vice Chancellor/Vice Chancellor of Research office at KUMC. Dr. Barbara Atkinson was the Executive Vice Chancellor (EVC) in addition to the Executive Dean of the School of Medicine and Dr. Joan Hunt was the VCR. Funds were provided for renovation of space, office supplies and computers, and most significantly nursing, administrative and biostatistical personnel. In addition, the University of Kansas Hospital generously provided all initial equipment and medical supplies in the clinical research areas, including a metabolic cart, electrocardiogram, freezers, and centrifuges. All of this financial support was provided in anticipation of an NIH grant submission. Mr. Jon Jackson, one of the KU Hospital vice presidents, was the primary champion to develop GCRC. In addition, some funds were obtained from the Kansas City Area Life Sciences Institute that was used to hire research nurses. Dr. Barohn received a planning grant from the Research Institute to prepare the GCRC application. Dr. Barohn invited Judy Otey, RN, from the GCRC at the University of Texas Southwestern in Dallas to be the Chief Nurse of the GCRC. Dr. Barohn had worked in the GCRC at UTSW and he was introduced to Ms. Otey as a potential candidate to be the nurse and GCRC leader in Kansas. He and Ms. Otey hired Suzie Schmidt to be the administrative director, Nicole Ladisech to be the administrative assistant and Mary Reed, RN, to be a clinical research nurse (Figure 3).

Figure 3. Initial GCRC leadership and staff (January 2005 Grand Opening). Left to right: Nicole Ladisech, Suzie Schmidt, Richard J. Barohn, Judy Otey, Mary Reed.

Shortly after construction in the initial Delp GCRC space began, KU Hospital announced they would be closing the old mechanics machine shop (located in Delp in the rear of the old radiation therapy room, Delp G036). Dr. Barohn obtained this space from KU Hospital, and funds for remodeling, so that it would become the initial exercise physiology laboratory in the GCRC. The exercise physiology space was large and well equipped and used by all three schools. It was a major factor in recruiting Dr. Jeffrey Burns to the Department of Neurology to begin the Alzheimer's Disease program which was to have an exercise and metabolic focus. The initial organization of the GCRC is displayed in Figure 4. Dr. Barohn has recorded parts of the development of the GCRC KU study at the Merrill Retreat sponsored by KU-Lawrence in 2016.[1]

GCRC NIH Grant

Simultaneous with the development of a functioning GCRC, a grant application was prepared. We notified the NIH/NCRR of the development of our GCRC at KU Medical Center and they had scheduled us to submit our proposal in 2006. The KU Medical Center GCRC NIH grant application was submitted on June 1, 2006 and was funded in the spring of 2007 for three years at approximately $3 million dollars/year. It was the last new GCRC application funded by the NIH. The NIH officials informed Dr. Barohn that the Clinical and Translational Science Award (CTSA) was being initiated, and that this would replace the GCRC program. They advised Dr. Barohn to submit the GCRC application, but said "if funded it would only be for three years and after that time KUMC would have to obtain a CTSA grant." They also told Dr. Barohn that there would be an opportunity to apply for a CTSA planning grant.

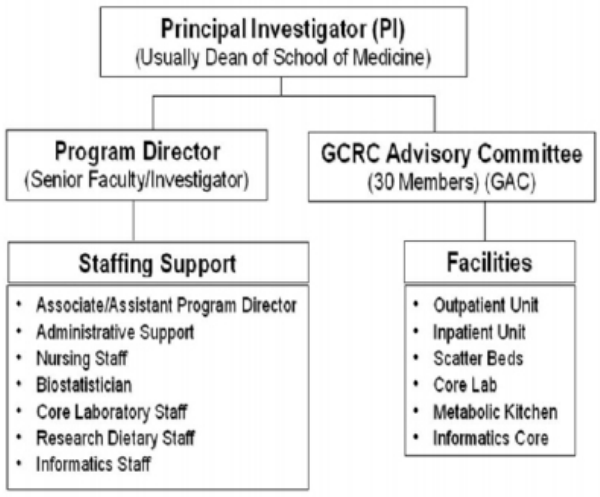


Figure 4. Initial organization of the GCRC.

Announcement of the Clinical and Translational Science Award Program

In September 2005, the NIH released an RFA announcement for institutional Clinical and Translational Science Awards (CTSA). The stated purposed for the CTSA project was to forge a transformative and integrative academic home for clinical and translational science. Dr. Zerhouni, the NIH Director, stipulated that these new homes in academic health centers would be a center, department, or institute. These clinical research units were to encompass all components of clinical research, including education, career development, and regulatory components for clinical research infrastructure.[2] These new clinical research units were to promote multi-disciplinary research teams, create an incubator for innovative research tools, and catalyze the application of new knowledge to clinical practice. These clinical research units were to also provide degree-granting capabilities in clinical research that will lead a trainee to either a masters or a doctorate degree.

The clinical research infrastructure provided by GCRCs were to be incorporated into the larger CTSA awards. By doing so, this would give academic health centers greater flexibility in modeling clinical research infrastructure space for the future. In this new model, there were potentially fewer restrictions on collaborations with industry in developing clinical research programs at academic health centers. There was no restriction on number of industry sponsored studies or in the amount of funds that a clinical research center can receive from industry. Under GCRC rules this was not the case.

Planning Process for the CTSA at KUMC and the Kansas City Region

Shortly after the RFA for the new CTSA awards, a planning process was initiated at KU Medical Center to develop our university's response to this new program.[3] The various planning committees and subcommittees were established in October 2005 (Figure 5) and began meeting regularly.

CTSA COMMITTEE	CHAIR(S)	PURPOSE
Planning Steering Committee	Barbara Atkinson, MD Richard Barohn, MD	Direct and oversee entire planning process
Governance Planning Sub-Committee	Richard Barohn, MD Barbara Atkinson, MD	Develop and propose overall governance and structure of HICR
Grant Writing Planning Sub-Committee	Richard Barohn, MD Lauren Aaronson, PhD, RN	Write the full NIH CTSA application
Education Planning	Ed Ellerbeck, MD, MPH	Develop K-12 and T-32 training programs for the full CTSA application; propose mechanisms to coordinate and incorporate existing training programs (e.g., K30, other T32s)
Clinical Research Resources Planning	Richard Barohn, MD	Expand current GCRC to include additional service resources for investigators (e.g., protocol development, peer review assistance, pilot grant program); create new Clinical Research Resource Center (CRRC)
Clinical Pilots Planning Sub-Committee	John Ferraro, PhD Ted Knous, PhD	Create policies & procedures for support of clinical & pilot studies in priority areas within the CRRC
Biostatistics & Informatics Planning	Matt Mayo, PhD	Design expanded infrastructure and procedures for biostatistician and informatics support
Regulatory Planning	Jim Voogt, PhD John Finley, JD	Develop mechanisms to support investigators with regulatory requirements
Novel Methods & Translational Technologies Planning	Curt Hagedorn, MD Paul Terranova, PhD	Coordinate use of technologies applicable to clinical research and propose new methodologies
Health Disparities Research Planning	Patricia Thomas, MD Kirby Randolph, PhD	Design Health Disparities Research Center with resources and services to integrate disparities issues in all clinical research
Community & Participant Planning	Joshua Freeman, MD Lauren Aaronson, PhD, RN	Create policies and procedures for provider and study participant involvement in the HICR and mechanisms for regular communication
Regional Academic Institution Planning	Jim Voogt, PhD	Develop formal partnership agreements with current partners and explore inclusion of other institutions from the KC area
Private Sector Commercialization Planning	Scott Weir, PhD	Identify opportunities for clinical & translational research with private industry

Figure 5. CTSA planning committees and sub-committees.

The timeline was that applications had to be submitted to the NIH by March 27, 2006. At that time, the NIH was accepting two types of applications. The first was a planning grant for $150,000 that would allow academic health centers time and some resources to further develop a global CTSA application. The second type of grant was a full CTSA application that could be as large as $6 million dollars per year if pediatric clinical research was involved; up to $4 million dollars without clinical pediatric research. In addition to the $6 million dollar per year CTSA award, all existing K30, T32, and GCRC grants were to be rolled in the CTSA application. By doing so, a full CTSA application would become one of the largest institutional research grants that an academic health center could receive. The stated goal of the NIH was to fund 60 small CTSA planning grants and five to seven full CTSA grants in the first round of applications. Their ultimate goal was to fund 60 full CTSA awards throughout the United States by 2012.

As our planning committees met, we recognized there would be a number of weaknesses and strengths for KU Medical Center CTSA application. The weaknesses included:

- No current GCRC NIH funding
- No clinical T32 training grants
- Relatively small number of clinical mentors and mentors with current clinical R1 grants

The strength of KU Medical Center CTSA application included:

- The existing NIH funded K30 program and the Masters of Science and Clinical Research Program
- The current existing GCRC infrastructure (before it was funded)
- The Research Institute at KU Medical Center
- The multidisciplinary and collaborative research efforts between the School of Medicine, School of Nursing and School of Allied Health (which became the School of Health Professions)
- A strong bioinformatics center
- Strong ties with programs at the University of Kansas-Lawrence Campus, particularly drug development and the Lifespan Institute
- Potential strong ties with partners in the community, other regional academic centers and in private industry

As a result of the CTSA planning process, it was decided to submit a CTSA planning grant before the March 2006 deadline.

Lauren Aaronson, Ph.D., RN, came to KUMC School of Nursing in 1989 as the Associate Dean for Research. In 2004 she went to the NIH in Bethesda as a senior advisor to the director of the National Institute of Nursing Research (NINR) as part of an interagency personal agreement between NIH and KUMC to join the new Roadmap for Research Initiative. One of her duties was to help craft components of the emerging CTSA plan and research announcement. Drs. Barohn and Burns attended the NIH event to announce the CTSA program in the Fall of 2005 and they met Dr. Aaronson. Dr. Aaronson's time at the NIH ended in December 2005 and she returned to KUMC. She immediately joined Dr. Barohn to begin the CTSA Planning Grant application. Dr. Barohn took the lead in preparing the GCRC grant and Dr. Aaronson took the lead in preparing the CTSA Planning Grant, but they worked on each application intensely. Both were submitted within four months of each other. They remained research grant 'partners' for the next 10 years, when Dr. Aaronson retired.

Concept of a Heartland Institute for Clinical Research

Our CTSA planning grant application outlined the concept of a new Heartland Institute for Clinical Research (HICR) as a new integrated home for clinical and translational research, both at KU Medical Center and in the region. We anticipated that the HICR would consist of many centers, and each center would have a number of cores or programs (Figures 6 and 7).[3]

The education center would house the existing Masters of Clinical Research and K30 programs and new additional K12 and T32 career development awards that can provide significant salary support and thus release time so that young investigators can pursue training in clinical research. The education center also encompassed developmental programs, including a recently initiated Introduction to Clinical Research course that was launched in the fall of 2006 semester, as well as a proposed Research Coordinator Training Program that was under development.

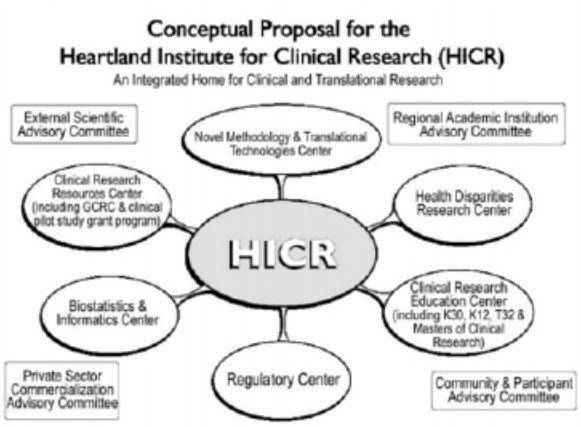


Figure 6. Early conception of HICR, 2006.

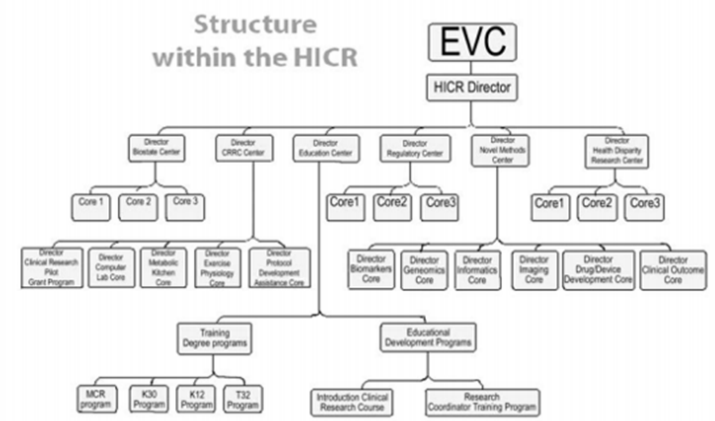


Figure 7. Early structure of HICR, 2006.

The current GCRC would be incorporated into a new Clinical Research Resource Center (CRRC). The CRRC would also include a Clinical Research Pilot Grant Program where a junior investigator can apply for up to $25,000 seed funds for a new clinical research project. The CRRC would also contain a new protocol development/assistance core to aid a new investigator in preparing a grant submission. Within the Novel Methodology and Translational Technologies Center, core technologies to facilitate translational research would be housed. An investigator would be able to apply to the Novel Methodology and Translational Technologies Center for access to these individual cores for their research protocols. For example, if a pilot project required brain imaging at the Hoglund Brain Imaging Center, the investigator would apply to the Novel Methodology and Translational Technologies Center for funding and intellectual support. In addition, we planned to establish four important committees that would bring in partners from the external scientific community, the regional academic centers, the private sector, and lay community organizations and institutions.

Plans to Create a Regional Translational Clinical Research Center

In the early CTSA planning stages in 2006, Dr. Barohn and KUMC leadership believed that including other partners in the program would raise the level of research in the Kansas City region. As Dr. Barohn was a graduate of the UMKC School of Medicine, this seemed natural and he still had ties to UMKC, Truman Medical Center, Children's Mercy Hospital and St. Luke's Medical Center. He asked for meetings with various leaders of these institutions to explain the new CTSA program and how obtaining a CTSA award could benefit researchers at all of these institutions.

Initially in these meetings, there was no immediate buy-in to the plan. Over a series of meetings, the cross-town hesitancy to collaborate softened and by the time the CTSA planning grant was submitted the concept of a regional enterprise was a component of the future HICR (Figure 8).

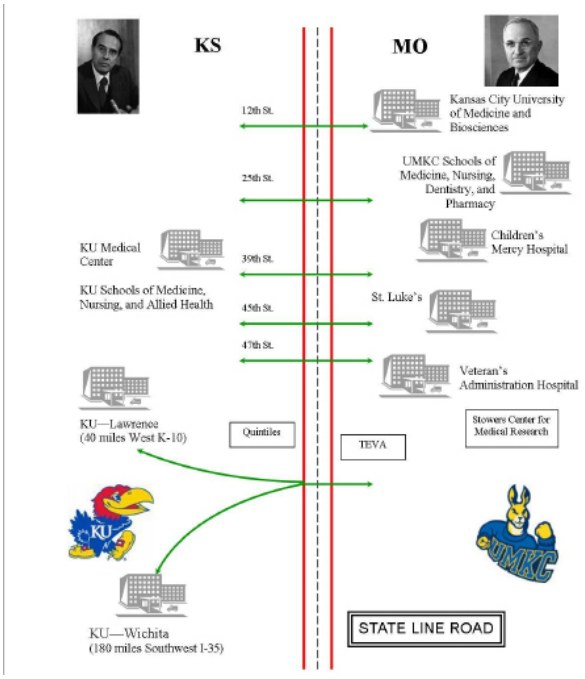


Figure 8. Regional partnership plan for HICR, 2006.

After the planning grant was submitted in March 2006, Dr. Barohn continued to have meetings with leaders in Missouri, to determine who could be included from these institutions in the Fall CTSA submission. Dr. Barohn found welcome partners in the School of Medicine, including Dr. Gary Salzman who was a classmate of Dr. Barohn's. He also met frequently with leaders of Children's Mercy Hospital research unit, one of which was Dr. Ralph Kauffman. Steve Leeder, Pharm.D., Ph.D., at CMH also became involved. Dr. Barohn was introduced to Dr. John Spertus at St. Luke's who ultimately would become a leader in the CTSA program.

The Kansas City VA Medical Center was initially included as a partner as well. And of course, all KU Faculty on the Lawrence campus and in the Wichita School of Medicine were all partners. The concept was that if an institution was a partner in the HICR, then all the faculty of that institution would have access to the components of the institute.

The name of the institute was ultimately changed to the HICTR-Heartland Institute for Clinical Translational Research. Views were split on how favorable or unfavorable the acronym HICTR was perceived. Dr. Atkinson often expressed her unfavorable view. But the acronym HICR was used for the CTSA planning grant submission in 2006, and HICTR for the full grant submission.

Both the GCRC and CTSA planning grants were funded in 2007. The planning grant was small, providing $150,000 direct funds for one year compared to GCRC grant ($3 million dollars/year for 3 years.)

For 12 months the HICTR grant writing team prepared the large paper application that was submitted in 2008. The Office of Grants and Research in the SON and Dr. Marge Bott (Associate Dean for Research, SON) and her staff played a critical role in the grant preparation and assembly, along with the GCRC staff. Dr. Atkinson had announced the official launch of the HICTR several months before the grant submission, with Drs. Barohn and Atkinson as director and co-director, respectively. An event was held at Union Station to celebrate the launch of the HICTR and was well attended by leaders from all partner institutions.

We received our score in February 2009. It was not good - 311. In fact, it was a terrible score. I recall Dr. Aaronson calling me with the score when I was away in Dallas and she said "Rick, are you sitting down? If not, you need to." But we believed we could fix the critiques. In the Fall of 2009, we resubmitted. In February 2010, we got our score, which was much better, but still not fundable – 31. The NIH had moved to a two-digit grading system and a perfect score was 10. In the critiques it was clear a major problem was our informatics program. We assembled an External Advisory Board led by Dr. Claire Pomeroy (Dean of the School of Medicine, University of California, Davis) and other leaders from institutions with CTSA grants. They gave us a clear idea of how we could improve the application. We began a search for an informatics leader. We recruited Dr. Russ Waitman to fill this role. Dr. Waitman arrived in 2010 and rewrote the informatics section as he simultaneously set up a system to extract medical data from the Epic electronic medical record for research using the I2b2 software and a new program code he and his team wrote. He called the program HERON. HERON quickly became a highly useful research tool for faculty.

The CTSA application was submitted for a third time by Drs. Barohn and Aaronson and their team in the fall of 2010. In February 2011, we received our score while Drs. Barohn and Aaronson were in Dr. Atkinson's EVC office. At the beginning of their meeting, no score had been posted. Forty-five minutes later Dr. Atkinson checked again. The score was 14. The three professors yelled "Fourteen!" and as I recall there was a three-person hug and some jumping up and down.

Two other major changes occurred in our CTSA program. In addition to hiring external consultants, we had internal brainstorming sessions to see if there was a better name than HICTR for our program. C.J. Janovy, our director of communications at the time, came up with the name Frontiers. It seemed perfect and although I hated to give up HICTR, I was clearly in the minority and since then we are Frontiers. Both regional and nationally, we are Frontiers. We made this name change after we were funded in 2011.

The other major change was the Johnson County Education Research Triangle (JCERT) initiative and the move to Fairway. Dr. Atkinson and her leadership team, with the help of local community leaders, accomplished the amazing fete of getting a sales tax for research passed by the voters of Johnson County in November 2008, in the middle of a severe recession. This sales tax provided funds in perpetuity for KUMC research at two office buildings (purchased by the Hall Foundation and donated to KUMC) in what is now our

Fairway campus. The tax also provides funds for the KU Edwards Campus and to the K-State Olathe campus. The KUMC Fairway funds paid for renovations of the buildings and on-going maintenance and operations. A major focus of the Fairway campus was to create a phase 1 trial unit for the cancer institute on the second floor. We moved the GCRC to the third floor and called it the Clinical Translational Science Unit (CTSU). This is what we called the unit when we submitted the CTSA grant in 2010 and we moved into the new unit just before the grant submission. The new CTSU was a major expansion. We now had 23 research rooms to see research participants, and a large infusion suite with eight bays, a large exercise lab, a large metabolic kitchen, and three extended stay rooms with beds for patients who needed extended stays for PK/PD studies. In this new expanded CTSU, Dr. Jeffrey Burns became the director in 2011. Dr. Burns was recruited to KUMC because we were opening a GCRC in 2006 and we built an exercise unit in large part for his research needs as noted above. Therefore, this was another example of a junior faculty member using the GCRC who eventually moved into a leadership role in Frontiers.

In July 2011, Frontiers was funded at $4 million dollars a year for five years. In the fall of 2010 Drs. Russ Swerdlow and Jeffrey Burns were funded for an NIH Alzheimer's Disease Center. Also, in 2011 Dr. Roy Jenson and his team were funded for a NIH Cancer Center. All three centers were funded within 12 months. The year 1905 has been referred to as Einstein's "Annus mirabilis" because he had four groundbreaking papers published that year and he went from obscurity to worldwide recognition.[4] While probably not as scientifically and culturally significant as Einstein's 1905 breakthroughs, the 12 months in 2010 and 2011 was KUMCs "annus mirabilis" and pulled KUMC research from obscurity to national relevance.

FUNDING SUPPORT

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Keywords: GCRC, CTSA, Frontiers

CTSU Open House
February 16, 2012
8 months after CTSA Award was funded

a.) Lauren Aaronson, Ph.D., RN
Richard Barohn, MD
Matt Mayo, Ph.D.

b.) Jo Denton, RN
(Patient Subject Advocate)
Judy Otey, RN

c.) Jeff Burns, MD
William Brooks, Ph.D.
John Ferraro, Ph.D.

d.) Lauren Aaronson, Ph.D., RN
Marge Bott, Ph.D., RN

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Navigating the NIH Public Access Policy for Peer-Reviewed Manuscripts - Why and How to get a PMCID Number

Robin Liston, MPH, Richard J. Barohn, M.D.
University of Kansas Medical Center, Frontiers: University of Kansas Clinical and Translational Science Institute

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BACKGROUND

In 2008, the National Institutes of Health (NIH) enacted the Public Access Policy requiring all NIH-funded investigators to submit accepted, peer-reviewed manuscripts to the National Library of Medicine's digital repository PubMed Central. The purpose of the policy is to "ensure the public has access to the published results of NIH-funded research." (Accessed Jan. 14, 2020 at https://www.nih.gov/health-information/nih-clinical-research-trials-you/what-is-nih-public-access-policy). Historically, the burden of cost to access manuscripts was on readers; however, the policy shifted the responsibility to researchers. Publishing a manuscript costs on average between $1,500 - $3,000, but these costs can be included in grant proposal budgets.

The NIH's National Library of Medicine (NLM), established in 1836, originated with a few books in the office of the Surgeon General of the Army. Over the next century the NLM amassed millions of publications related to medicine, public health and the allied sciences for use by physicians and health professionals throughout the United States. The computer and technology era enhanced the reach of literature and produced numerous NIH online resources including the National Center for Biotechnology Information, MEDLINE, ClinicalTrials.gov, PubMed and PubMed Central (PMC). The NLM continues to maintain an immense amount of medical publications and biomedical clinical research manuscripts, primarily in online databases.

Why it is Important to Comply with the NIH Public Access Policy

Medical and public health researchers have a shared goal of developing innovative strategies to improve population health and of utmost importance is translating the effective discoveries to individuals. One approach to accelerate research findings to improve population health is to ensure the scientific and lay community has access to published research outcomes in a timely manner.

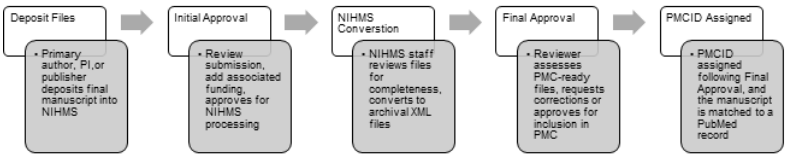
While translating effective strategies to improve population health is important to investigators, it is also imperative researchers comply with the NIH Public Access Policy to avoid interruption to scientific discovery. Any NIH Institute or Center can withhold future funding from the primary author of a manuscript and the principal investigator of acknowledged funding support, including non-competitive renewals and new grant proposals in non-compliance with the policy.

What Non-Compliance to the NIH Public Access Policy Means

Manuscripts are required to be publicly available no later than 12 months following original publication date depending on the embargo period of the publisher; however, manuscripts are non-compliant if the PubMed Central Identification (PMCID) number has not been acquired 90 days after the original publication date. Steps to acquire a PMCID are provided below. Embargo periods for each journal in PMC can be found in the "Free Access" column on the PMC Journal List. The exact release date for each article under embargo is displayed in PMC search results, on the table of contents for the issue, or in the corresponding PubMed record. To obtain access to an article prior to its availability in PMC, individuals must contact the respective journal publisher directly.

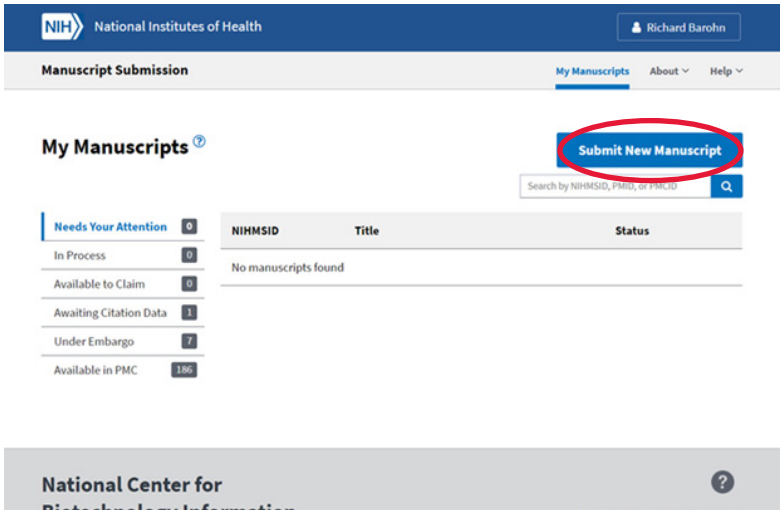
NATIONAL INSTITUTE OF HEALTH MANUSCRIPT SUBMISSION (NIHMS) PROCESS OVERVIEW

Step-by-Step Guide to Move Manuscripts through the NIH's Databases to Acquire a PubMed Central Identification (PMCID) Number



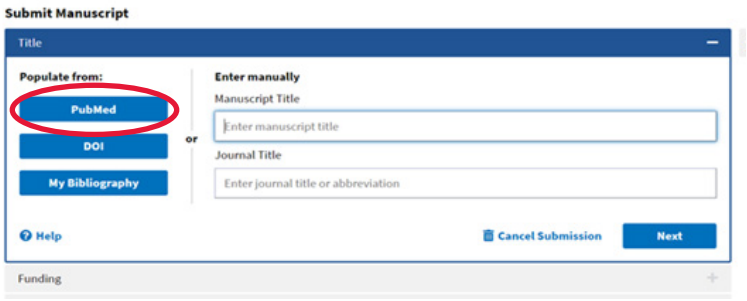
Step 1: Deposit Files into the NIHMS

1. Select Submit New Manuscript - Primary Author or Principal Investigator (PI; or a delegate assigned in eRA Commons) of funding mechanism that supported the research can process manuscripts through the NIHMS database. The individual that initiates the process will need to complete all remaining steps. Delegates cannot serve as a Reviewer for the Initial or Final Approvals.
   a. This process should be initiated immediately following official publication. Some journals may deposit the paper into the NIHMS for authors, but have a 12-month timeframe for submission, which is not within the 90 day compliance window.

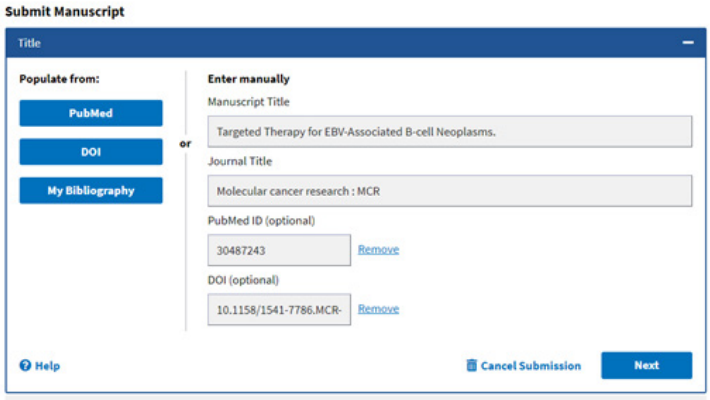


2. Select submission method. Recommend using PubMed, as most journals submit abstracts to PubMed upon publication. Enter in the PubMed Identification (PMID) number or manuscript title in the PubMed search field.
   a. PubMed is an online, free database comprised of over 30 million published biomedical abstracts and citations. PubMed's aim is to support retrieval of abstracts and citations to improve population health. PubMed was created by and is sustained by the National Center for Biotechnology Information (NCBI) of the NLM.

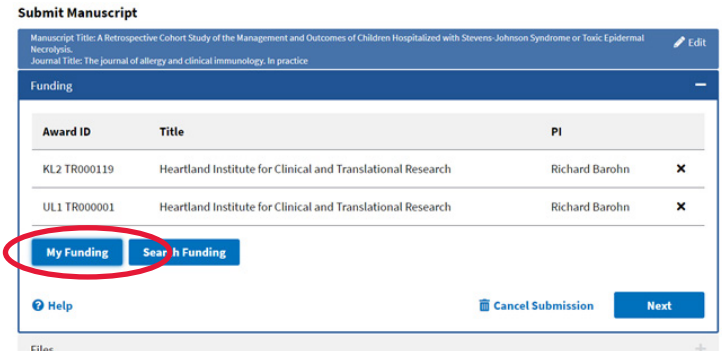
b. Many journals provide abstracts and citations to NCBI, which then populates PubMed. While some links to full text articles are provided, PubMed is not a full-text repository. PMC was developed to be a full-text repository for biomedical and life science literature to ensure the public has free access to research outcomes. The NIH Manuscript Submission System (NIHMS) was developed to "facilitate the submission of peer-reviewed manuscripts for inclusion in PMC in support of the NIH Public Access Policy" (Accessed Jan. 6, 2020 at https://www.nihms.nih.gov/db/sub.cgi?page=overview).



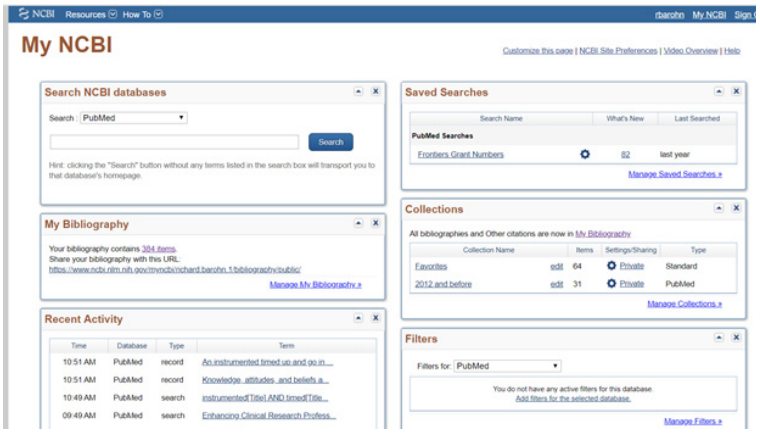
- Manuscript data will populate into the NIHMS. Select Next.



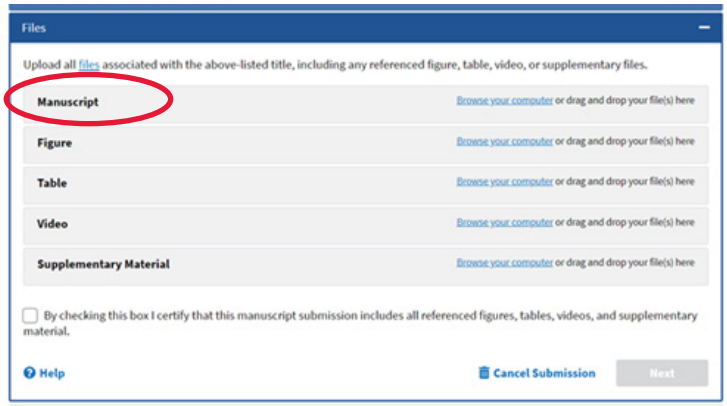
3. Select "My Funding" to open a list of grant awards. Choose the "Award ID(s)" associated with the manuscript. Select Next.

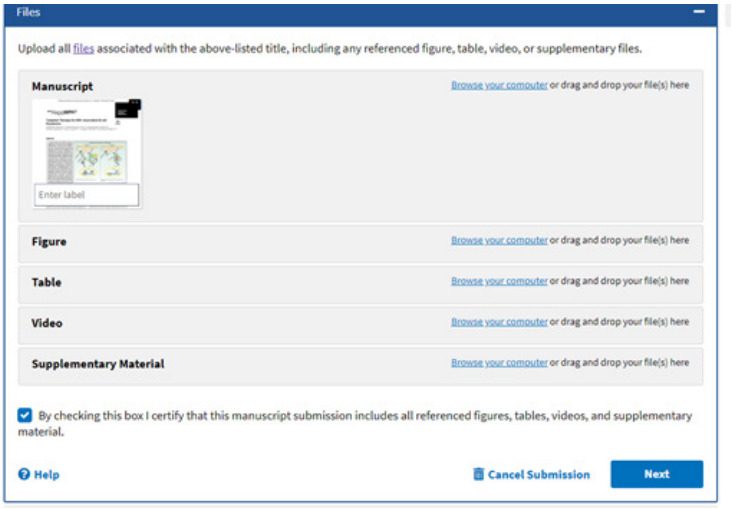


a. Selecting funding links this manuscript to the PI(s) of the funding mechanism. The citation will be added to the PIs "MyNCBI", the NCBI investigator-specific dashboard for researchers to manage and track their publications. NCBI maintains a series of databases containing biomedical and genomic data for use by scientists, contributing to the mission of the NIH to "uncover new knowledge".



4. Upload all files associated with the manuscript. The manuscript must be the final, published PDF. Naming convention: PILast-Name_et_al_year published_Journal name. Confirm that all figures, tables, videos and/or supplemental materials are included in the PDF. Some online publishers link to these items instead of including them with the manuscript. If not included, upload materials. Select Next.

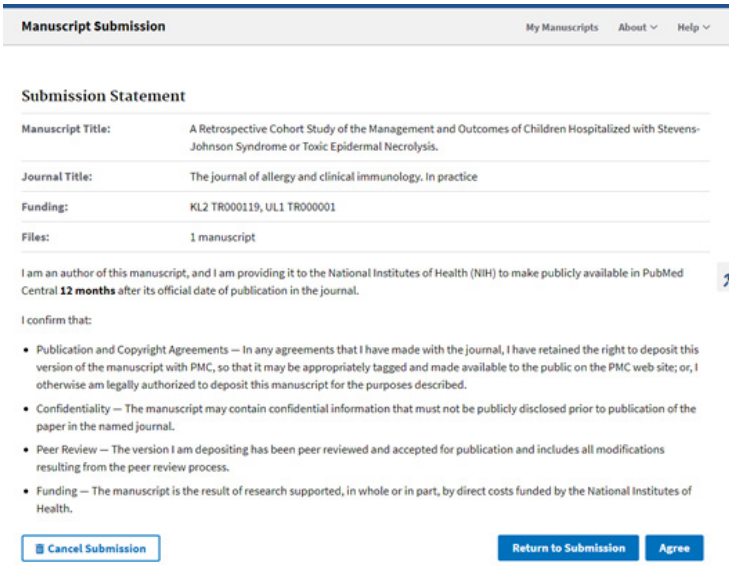




5. Assign Reviewer and Release Delay (Embargo)
   a. When an article is under an embargo it means that there is a delay, as specified by the publisher, between when the article is published and when its full version can be made freely available in PMC. The default embargo for each journal in PMC can be found under the "Free Access" column on the PMC Journal List. The exact release date for each article under embargo is displayed in PMC search results, on the table of contents for the issue, or in the corresponding PubMed record.

Step 2: Initial Approval

1. Review Submission Statement. Select Agree.



Step 3: NIHMS Conversion

The NIHMS conversion typically takes two-three weeks. NIHMS converts the deposited manuscript files into archival XML format. In this way, NIHMS makes papers publicly available in a format that ensures the permanent preservation of these research findings and makes the results of this research more readily accessible to the public, healthcare providers, educators, and the scientific community (Accessed Jan. 31, 2020 at https://www.nihms.nih.gov/about/publicaccess/).

Step 4: Final Approval

The individual that initiated the process in Step 1 will receive an email from nihms-help@ncbi.nlm.nih.gov with the subject line reading [nihms] Manuscript #1031896: Please review the PMC-ready Documents. *See Appendix A. Log into NIHMS to Review and Approve the submission for it to be rerouted to undergo conversion to PMC documents.

Step 5: PMCID Assigned

Once approved by the NIHMS the manuscript undergoes conversion to PMC documents. After file conversion the individual that initiated the process will receive an email from nihms-help@ncbi.nlm.nih.gov with the subject line: [nihms] Manuscript #1503085: Your manuscript is available in PMC. *See Appendix B. PI or primary author must again APPROVE the PMC-ready or final manuscript.

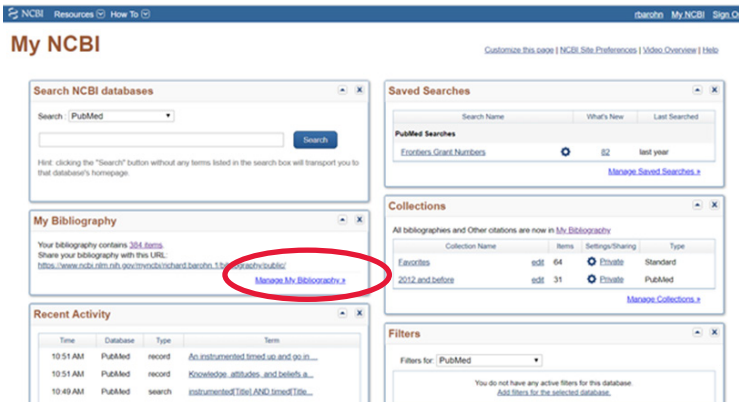
Note: PMCID is assigned when the following two criteria are met:

1. *Converted materials (i.e., the PMC-ready documents) have been approved by the Reviewer.*
2. *The manuscript has been matched to a PubMed record for one of the following: a final print publication date or an electronic publication date for a journal that is an electronic publication only or an electronic publication date for a journal where PubMed has not received the final print publication date within six months of the electronic publication date*

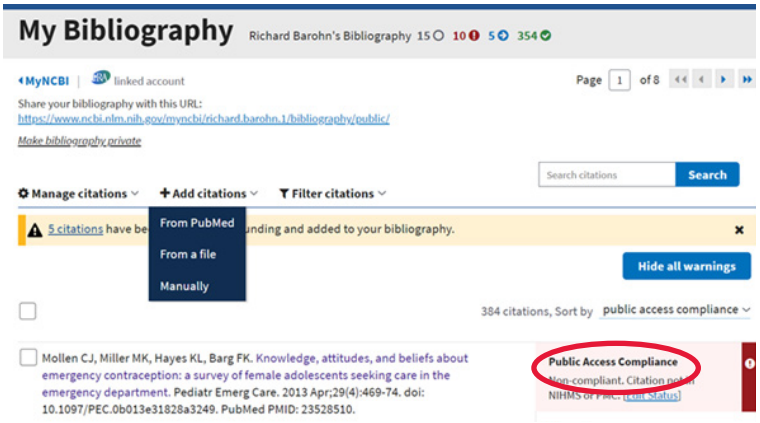
Steps for Processing Non-compliant Manuscripts Associated with Funding

The following three steps are provided to assist investigators with processing a non-compliant publication linked to their funding. Following completion of the three steps below, proceed to Step 1 above.

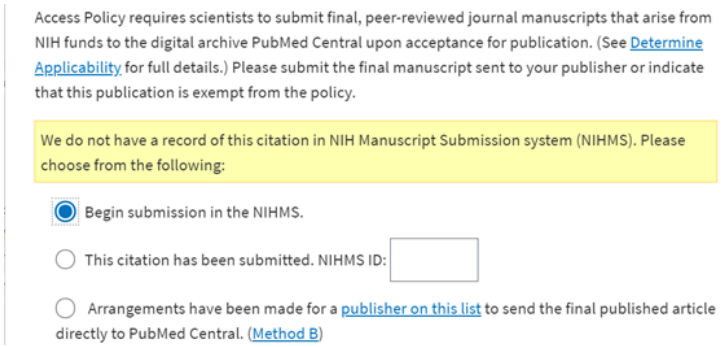
Step 1.i: Select Manage my Bibliography



Step 2.i: Select Edit Status



Step 3.i: Select Begin submission in the NIHMS. Save.


Access Policy requires scientists to submit final, peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication. (See Determine Applicability for full details.) Please submit the final manuscript sent to your publisher or indicate that this publication is exempt from the policy.

We do not have a record of this citation in NIH Manuscript Submission system (NIHMS). Please choose from the following:

- (●) Begin submission in the NIHMS.
- ( ) This citation has been submitted. NIHMS ID: [ ]
- ( ) Arrangements have been made for a publisher on this list to send the final published article directly to PubMed Central. (Method B)

Step 4.i: Follow Steps 1-5

Step 1: Deposit Files into the NIHMS
Step 2: Initial Approval
Step 3: NIHMS Conversion
Step 4: Final Approval
Step 5: PMCID Assigned

FUNDING SUPPORT

This work was supported by a CTSA grant from NCATS awarded to the University of Kansas for Frontiers: University of Kansas Clinical and Translational Science Institute (# UL1TR002366). The contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIH or NCATS.

Keywords: Public Access Policy, non-compliance, PMCID, NIHMS

APPENDIX A

From: <nihms-help@ncbi.nlm.nih.gov>
Date: June 3, 2019 at 5:04:46 AM PDT
To: <emailaddress>
Subject: [nihms] Manuscript #1031896: Please review the PMC-ready Documents

Dear Dr. X,

The PMC-ready version of your NIHMS manuscript (NIHMS1031896 - "A dyadic multiple mediation model of patient and spouse stressors predicting patient dietary and exercise adherence via depression symptoms and diabetes self-efficacy") is now available for your review.

Please proceed to the NIHMS system to review the PMC-ready documents. This is necessary to complete your manuscript submission to PubMed Central (PMC).

To sign in, go to https://www.nihms.nih.gov and choose the eRA sign-in route.

Your sign-in username is: This will be your eRA Commons user name

If you forgot your password, you can request a new one from eRA after going to the sign-in page.

Once signed in, you will be able to either approve the material for release to PubMed Central or request that corrections be made to the manuscript.

A step-by-step tutorial for completing the Final Approval is available at https://www.nihms.nih.gov/db/sub.cgi?page=stepbystep. (TIP: If you open a step-by-step tutorial in a different browser window, you can refer to it as you proceed with the process in the NIHMS system.)

PLEASE DO NOT REJECT THE PMC-READY DOCUMENTS FOR THE FOLLOWING REASONS:
- Not the final version: Public access policies often call for the submission of the peer-reviewed, accepted manuscript. To use the final published version, you need explicit permission from the publisher; please note, however, that due to copyright restrictions, the publisher can deny this request.
- PubMed link(s) missing from reference(s): These links are automatically generated and cannot be added manually by NIHMS staff.

If you have any questions, please contact the NIHMS Help Desk at https://www.nihms.nih.gov/db/sub.cgi?page=email for assistance.

Sincerely,

The NIHMS Help Desk

APPENDIX B

From: <nihms-help@ncbi.nlm.nih.gov>
Date: April 25, 2019 at 7:33:27 PM CDT
To: <emailaddress>
Subject: [nihms] Manuscript #1503085: Your manuscript is available in PMC

Dear Dr. X,

You are receiving this email because you are associated with Manuscript NIHMS1503085 ("Title of manuscript") as an author, PI, or other interested party in the NIHMS system. The manuscript has now been loaded into PubMed Central (PMC) and made available for public access:

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6309214

The submission process for this manuscript is now complete. As always, please feel free to contact the NIHMS Help Desk with any questions at https://www.nihms.nih.gov/db/sub.cgi?page=email.

Sincerely,

The NIHMS Help Desk

Step-by-Step Guide for Setting up a Company in Kansas and Missouri

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[2]Flux Tensor Corporation, Shift Pharmaceuticals, Entrepreneur in Residence, University of Kansas Medical Center
[3]University of Missouri-Kansas City Innovation Center
[4]University of Kansas Center for Technology Commercialization
[5]Frontiers: University of Kansas Clinical and Translational Science Institute, University of Kansas Medical Center

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Company Formation

The process of forming a company (startup) can be done online by individuals or with the assistance of an attorney. The first step to make your business legal is to decide what kind of business entity (legal structure) is best for you. This is an important step in entrepreneurship.

- The Kansas Business One Stop is the one-stop shop for planning, starting, operating and growing a business in Kansas.
  - Search for company name availability on the Kansas Business Center website
  - Create a KanAccess account to begin process of business registration online with the Kansas Secretary of State for corporations, LLCs and LPs
  - Follow links appropriate for type of business
- The Missouri Corporations Unit of the Secretary of State is responsible for the creation and maintenance filings for all domestic and out-of-state business entities doing business in Missouri.
  - Search for company name availability.
  - Follow links appropriate to type of business. For articles of incorporation, you can use a standard set of articles that outline the owners. File the articles and pay fees via credit card. If you want to pay through a company bank account, you must establish the bank account first with sufficient funds to support the registration process.
- Obtain a Federal Tax Identification Number
- Obtain a Certificate in Good Standing from Kansas or Missouri
- Establish a bank account
- Register for state taxes through the Department of Revenue and Department of Labor
- Obtain appropriate industry and professional licenses and registrations
- Check with the local city and county for local permits and registrations
- Acquire a Dun & Bradstreet D-U-N-S Number. A DUNS number is a unique identifier assigned to businesses, following a patented identity process identifies the company as being unique from any others. To expedite the process if applying for federal grants, calling is suggested.

Applying for Federal Grants

To apply for a Small Business Innovation Research (SBIR) or Small Business Technology Transfer (STTR) grant numerous accounts need to be established prior to submission.

What is the SBIR program? A highly competitive program that encourages domestic small businesses to engage in Federal Research/Research and Development (R/R&D) that has the potential for commercialization. Through a competitive awards-based program, SBIR enables small businesses to explore their technological potential and provides the incentive to profit from its commercialization (Accessed Jan. 30, 2020 at https://www.sbir.gov/about).

What is the STTR program? It expands funding opportunities in the federal innovation research and development arena. Central to the program is expansion of the public/private sector partnership to include the joint venture opportunities for small businesses and nonprofit research institutions (Accessed Jan. 30, 2020 at https://www.sbir.gov/about). The DUNS number is needed for all registrations so do it first. Also, all registrations must be completed prior to the application being submitted. Registration can take six weeks or more, so applicants should begin the registration process as soon as possible. The NIH Policy on Late Submission of Grant Applications states that failure to complete registrations in advance of a due date is not a valid reason for a late submission. The accounts are:

- System for Award Management (SAM) – Applicants must complete and maintain an active registration, **which requires renewal at least annually**. The renewal process may require as much time as the initial registration. SAM registration includes the assignment of a Commercial and Government Entity (CAGE) Code for domestic organizations which have not already been assigned a CAGE Code.
- SBA Company Registry – See "SF424(R&R) Other Project Information Component" for instructions on how to register and how to attach proof of registration to your grant application package. SBA Company registration is NOT required before SAM, Grants.gov or eRA Commons registration.
- eRA Commons - Organizations can register with the eRA Commons as they are working through their SAM or Grants.gov registration, but all registrations must be in place by time of submission. eRA Commons requires organizations to identify at least one Signing Official (SO) and at least one Program Director/Principal Investigator (PD/PI) account in order to submit an application.
- Grants.gov – Both a DUNS number and SAM registration are needed in order to complete the Grants.gov registration.

Notes: 1) Login to these websites periodically, as some will automatically expire passwords if the account is not accessed. 2) These websites may have incorrect or outdated instructions. If problems are encountered while following guidance, call the appropriate help desk.

Other Considerations

- Federal Contacting Assistance Programs are designed to help small businesses secure at least 23% of all federal contracting dollars annually.
- Explore the advantages of small, women, minority and veteran-owned businesses through the Associate of Procurement Technical Assistance Centers in Kansas and Missouri.

University of Kansas faculty and staff: If the company is going to use intellectual property owned by the University of Kansas, contact University of Kansas Center for Technology Commercialization (KUCTC) at kuctc@ku.edu. It is recommended that the company execute an Option or a License Agreement with KUCTC when an SBIR/STTR is funded. KUCTC staff will guide the company through the licensing process.

Resources

1. KCSourceLink provides an online startup guide that helps people start a business with information for Kansas and Missouri
2. Early-stage business set up checklist
3. Startup e-book
4. Start, Register and License a Business in Kansas

FUNDING SUPPORT

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Keywords: entrepreneurship, company, startup, SBIR/STTR, DUNS

KANSAS JOURNAL of MEDICINE

Dedicated to the Pipeline: KU Frontiers' Pursuit of Maintaining and Cultivating the Careers of Current and Future Physician-Scientists

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INTRODUCTION

The decline in the number of physician-scientists regionally and nationally represents a real and existential threat to the biomedical research community. The meager number of physicians substantially engaged in investigation is cause for concern, as the speed at which medical discovery translates to patient care depends on their commitment to conducting research. As a Clinical and Translation Science Association (CTSA)-funded institution, KU Frontiers is on the front lines of enriching the clinician-scientist and physician-scientist pipelines, by providing resources and robust training opportunities to junior, developing researchers. Through the KL2 Career Development Award and the TL1 Pre- and Postdoctoral Clinical and Translational Research Training Awards, KU Frontiers actively trains, educates and motivates the future leaders of medical advancement. The Frontiers training programs, along with the services and resources extended to trainees and scholars provides the structure and support needed to develop and foster physician-scientists, without whom medical innovation and discovery could be stunted and jeopardized.

Section 1: A Dwindling Physician-Scientist Population: What is the Problem?

Age is More Than a Number

The decline in the number of practicing physician-scientists has been well documented over the past 40 years. Data from the 1980s compared to that of 2014 indicates that the number of physicians dedicating substantial aspects of their professional time to research has declined from around 5% to 1.5%.[1] Further, the physician-scientist population is aging quickly, with 1.6 times more investigators over the age of 61 than under the age of 50 as of 2012.[2] The pipeline is aging and the number of physician-scientists between the ages of 31 and 50 has been declining.[2] This particular trend is alarming because of the unique and vital skillset physician-scientists bring to the realm of biomedical research. The ever-widening gap from bench to bedside dictates the need for physicians from a variety of disciplines who can use their expertise to navigate the world of discovery and clinical care. Historically, physician-scientists have been and need to be vital players in bridging the gap because of their ability to bring scientific innovation to the bedside of their patients quickly and efficiently to affect outcomes.

The Long Road Ahead

One of the most frequently noted barriers to becoming a physician-scientist is the training timeline. For those who desire to become a physician-scientist, the MD-PhD career path is a consideration. However, those interested in the MD-PhD report dissatisfaction with the length of the program, which can take an average of eight years to complete.[3] With the realities of a hefty time commitment and mounting student debt, the MD-PhD route has become unappealing with only 609 students entering an MD-PhD program in the U.S. in 2013.[4]

The combination of mounting debt and a daunting timeline leads those with an interest in research with few other choices for investigative exposure. Medical students experience very limited (if any) time or training opportunities to perform research, with the same limitations plaguing the physician-scientist pipeline throughout most residency programs. Hopeful physician-scientists may not have a true opportunity to perform research until fellowship, which only underscores the missed opportunities to engage physicians early in their training.

Mentorship and Role Models

There are nuances to mentorship that make significant differences in the experience and outcomes of the mentoring relationship. The needs of a physician-scientist are unique to that particular career path. To optimize the growth of a junior physician-scientist, it is helpful to have a physician-scientist mentor. However, those opportunities are limited not only by the small number of physician-scientists, but the aging physician-scientist population who will be or currently are looking toward retirement.

In addition to the small number of mentors available, there is a need for increased diversity in terms of fields of study and ethnic and racial backgrounds in the physician-scientist pipeline. Both women and minority groups such as African Americans and Hispanics are underrepresented in the physician-scientist pipeline. Women make up 42% of MD-PhD graduates, yet recent data indicates that women make up only 22% of NIH grant recipients with the dual degrees.[5] The disparity between white and non-whites (African American, Hispanic or Native American) is even more striking than that of men compared to women with only 5% of those combined minority groups receiving NIH funding as physician-scientists.[5]

Section 2: Combatting the Problem

Stage-Specific Exposure and Training

With the rising age of the physician-scientist population, it is important to expose those young in their medical training to research. KU Frontiers is committed to fostering trainees and scholars at the predoctoral, postdoctoral and junior faculty stages in their careers via the TL1 Research Training Program (pre- and postdoctoral tracks) and the KL2 Mentored Career Development Program (junior faculty). Each specific track offers a condensed (1 to 2-year), immersive research experience, with clearly delineated milestones for the

trainees and scholars to meet on their way to carving out a career as both a clinician and a scientist. With a primary concern of those interested in a career as a physician-scientist being the lengthy training time[2] the TL1 and KL2 programs address that concern by protecting trainee's time from clinical duties and allowing them to focus on an intensive research experience to acquire the skills that their clinical programs didn't offer enough of or at all. At the conclusion of both the postdoctoral TL1 program and the KL2 program, the expectation is for participants to have used their research projects and experience to apply for extramural funding to demarcate the beginning of their independent research careers.

Both the KL2 and TL1 programs take an integrative approach to training, by bringing members of each program together to study and address the most critical components of becoming a successful researcher. As displayed in Table 1, Frontiers Scholar's Club addresses relevant and timely topics such as grant writing, career development and study design. The members of the TL1 and KL2 programs work together in these sessions to better understand each area and how they will utilize it to prepare themselves to become independent researchers. The integration of the groups allows the junior researchers to not only learn from mentors and senior scientists, but to learn from each other and accelerate their progress on their way to becoming independent investigators.

Table 1. 2019-2020 sampling of Frontiers Scholar's Club sessions.

Scholar's Club sessions	Topics	Speakers
Session 1	R01 Mock Grant Review	**Ed Ellerbeck, M.D., MPH**, KL2 PI, Chair of Population Health; **John Spertus, M.D., MPH**, KL2 PI, Endowed Chair in Metabolic and Vascular Disease Research; **Won Choi, Ph.D., MPH**, TL1 PI, Vice Chair of Education
Session 2	Tenure and Promotion	**Kim Richter, Ph.D., MPH**, Joy McCann Professor of Women I. Medicine and Science; **Laura Martin, Ph.D.**, Associate Professor and Associate Director of fMRI
Session 3	The Overlap of Between Research and Clinical Care: Addressing IRB and HIPPA Issues in Records-Based Research	**Karen Blackwell**, Director of Human Research Protections Program
Session 4	The Learning Health Systems Framework and the Implications for Research	**Tamara Winden, Ph.D.**, Chief Research Informatics Officer
Session 5	Manuscript Writing and Data Visualization	**John Spertus, M.D., MPH**, Endowed Chair in Metabolic and Vascular Disease Research
Session 6	Biostatistics, Epidemiology, Research Design (BERD): Understanding and Utilizing BERD Resources and Alternative Study Design	**Matt Mayo, Ph.D., MBA**, Founding Chair and Professor, Department of Biostatistics & Data Science

As an added benefit, the integration of the program also facilitates collaborative partnerships. In 2019, a member of the KL2 program and a postdoctoral TL1 trainee were able to leverage their expertise as a team and procure pilot funding on their first submission. The combination of stage-specific training and the integration of the KL2 scholars and TL1 trainees continues to provide great value in a considerably brief amount of time.

Over the lifetime of the training programs, the efforts appear to be paying off as 58% of KL2 graduates currently have NIH funding, with three leading multi-site NIH projects. With only one graduated cohort of postdoctoral graduates, results are still up for debate. However, two of the most recent TL1 graduates were awarded internal funding, with the other two graduates recently submitting for individual K awards. The trajectory of the programs is quite promising, as KU Frontiers remains steadfastly committed to providing immersive training experiences in a brief, manageable timeframe.

Adapt and Evolve

With the noted cost burden and time commitment associated with training as a physician-scientist, it is critical to think about innovative ways to cut down on both, while offering ample opportunities for physicians to get involved in research. For those who are not in a TL1 or KL2 program, there needs to be accessible options to pique their interest and encourage their ambition and curiosity. The Department of Population Health at Kansas University Medical Center (KUMC) recently created a four-week didactic training course for residents and fellows interested in obtaining the foundational knowledge of the clinical research process. Admittedly, the knowledge and experience obtained from the class alone is not enough to solidify a career as a physician-scientist. However, the course will set the foundation for a resident or fellow to begin to conduct their own research, while working with a faculty mentor to produce an abstract and submit a publication within a six-month period.

Those enrolled in the first iteration of the course (residents and fellows) were young in their careers and eager to learn and do more. By fostering that drive and innate curiosity with a brief, intensive, crash course in research methods (e.g., protocol design, basic biostatistics, regulatory issues, etc.), KUMC and Frontiers are doing their due diligence by training physicians and arming them with the basic skillsets to do their own research and carve out a career with investigation as an important element.

Recruitment to Enrich the Pipeline

The TL1 and KL2 programs spend several months each year recruiting the best and brightest from each of the Kansas and Missouri-based Frontiers affiliates, as well as nationally. The recruitment process is carefully and intentionally focused on enriching the pipeline of junior investigators, with an eye on both growth and diversification. Both programs have managed to attract female investigators at especially high rates, with 63% of women making up the KL2 program participants over its lifetime and 56% for pre- and postdoctoral TL1 programs, respectively. Admittedly, both the KL2 and TL1 programs have struggled with attracting underrepresented minorities to its programs. Tables 2 and 3 indicate the demographic makeup of each KL2 and TL1 graduating cohort. While women are well represented, African Americans and Latinos are not, making up only 7.5% of our awardees in the combined KL2 and TL1 graduated cohorts.

Table 2. TL1 graduate demographics.

Graduates by year	Male	Female	Non-URM	URM
2012	2	2	4	0
2013	3	1	4	0
2014	3	1	4	0
2015	1	3	2	2
2016	2	2	4	0
2017	0	0	0	0
2018	0	2	2	0
2019	3	3	5	1

50% female; 10.7% URM

Table 3. KL2 graduate demographics.

Graduates by year	Male	Female	Non-URM	URM
2014	1	5	6	0
2017	2	4	6	0

75% female; 0% URM

Both the TL1 and KL2 programs recognize the unique challenges of recruiting a diverse group of trainees into each program, particularly in Kansas and Missouri. Therefore, we refocused our approach to attracting diverse candidates in 2019 and re-engaged the Director of Diversity and Multicultural Affairs and the Office of Diversity and Inclusion to ensure the programs are reaching groups of students that historically have had limited opportunities in biomedical research. As a result, the 2019 award recipients for the pre- and postdoctoral TL1 programs represent the most diverse cohort that we have ever worked with, with 50% of those onboarded in July of 2019 being from an underrepresented minority group (Table 4).

Table 4. Current TL1 trainee demographics.

Current TL1 trainees	Male	Female	Non-URM	URM
2019	1	5	3	3

83.3% female; 50% URM

Two of our current predoctoral awardees were both members of the KUMC Pre-Admissions Program and the Pre-Matriculation Program, which are run out of the Office of Diversity and Inclusion. The goal of both programs is to recruit and prepare underrepresented minorities to succeed as medical students, and prepare them for a career as physicians and physician-scientists. Having programs in place to assist students in overcoming barriers and addressing any concerns from minority groups in particular, has proven successful in engaging women and others from underrepresented minority groups.

Physician-Scientist Advisors

Engaging the right advisor can make a significant difference in a junior investigator's career, especially when pursuing a career as a physician-scientist.[2] With full appreciation for such a challenge, KU Frontiers selected two accomplished, active, physician-scientists to lead the KL2 program and to act as advisors for the participants. Although the KL2 and TL1 awards are not limited to only physician-scientists, 50% of the training program participants are either studying to be physicians, while engaging in their research projects or are already physicians carving out careers as physician-scientists. With intentionality and care, the training programs have made a concerted effort to have a support structure in place for those pursuing the physician-scientist career path.

The experience of becoming a physician-scientist is a unique and arduous career choice, and, reportedly, can feel a little uncomfortable, based on individuals not fitting in with medical or research colleagues, wholly.[2] To temper the risk of experiencing those feelings, the training programs are structured as the ideal environment for those who are interested in the physician-scientist career path. KU Frontiers and its programs continue to be well-positioned to cultivate and train the next generation of physician-scientists and enrich the pipeline at a most critical time.

CONCLUSION

KU Frontiers and its training programs are fully dedicated to accelerating research to better reach patients in an innovative and efficient fashion. With an eye on medical advancement, KU Frontiers is well-positioned to assist in adding to the dwindling physician-scientist pipeline. Its investment in stage-specific training opportunities, recruitment, diversity, expediency, mentorship and advising sets the foundation for investigators locally and nationally to springboard their careers and enhance the likelihood of impactful medical discovery.

In particular, KU Frontiers and its TL1 and KL2 training programs continue to create an environment in which physician-scientists can flourish. It is the physician-scientist who is uniquely qualified to close the gap between making medical discoveries and providing innovative care to the patient's bedside based on those discoveries. With the declining number of physician-scientists posing a threat to the future of biomedical research, it is more important than ever to meet their needs and provide the right environment for them to grow into their research careers, and hopefully, grow in number. With a focus on innovation and steady growth, KU Frontiers and its training programs will continue providing intensive and expedited training experiences for those eager to carve out a path as clinicians and investigators.

FUNDING SUPPORT

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Keywords: physician-scientist, pipeline, training programs, mentorship

KANSAS JOURNAL of MEDICINE

Letter to the Editor: A Randomized, Double-Blinded, Placebo-Controlled, Cross Over Study Evaluating the Efficacy and Safety of Timolol Ophthalmic Solution as an Acute Treatment of Migraine

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I am an experienced and published researcher using timolol beta-blocker ophthalmic eyedrops for the successful treatment of acute migraines.[1-5] The above referenced paper contains many errors in study design and conduct.[6] The statistics, discussion, and conclusion are misleading.

Beta blockers are FDA approved and often effective for chronic migraine prevention by taking daily oral doses that maintain therapeutic blood levels. For acute migraine, oral beta blockers have not worked well because they take too long to achieve therapeutic blood levels.[1,2] There are three other ways beta blocker solutions can be used to quickly achieve therapeutic blood levels. The first is promptly and properly applied topical beta blocker eyedrops to normal eyes/eyelids/nasolacrimal ducts/nasal mucosa. Faster is sublingual beta blocker drops and the fastest is beta blocker nasal spray.[1,2] These last two preferred methods were not discussed in Aggarwal's paper.[6]

Ophthalmologists spend their careers listening to patients complain about the difficulties of using eye drops. Sublingual application has been studied and found effective for glaucoma control in a subgroup of drop-challenged patients.[7] Most acute migraine patients I have treated with timolol 0.5% eye drops prefer to take them sublingual for ease of application and efficacy rather than topical to the eyes.

Absorption of beta blockers and subsequent beta receptor blockade has been studied.[1,8] Of these three methods, nasal application has been shown to the be fastest and equivalent to intravenous beta blocker administration in a study of 80 human volunteers.[8] Until recently, no beta blocker nasal spray was commercially available. O'Brien Pharmacy (https://obrienrx.com/) now prepares a compounded nasal spray of timolol with Mucolux™ delivering 0.125 mg/0.1 ml spray. The nasal spray is shaken and one spray delivered into each nostril at first onset of migraine symptoms. Patients may also take their other acute migraine medications with the beta blocker nasal spray. If migraine headache persists a second set of one spray per nostril is repeated in 10-15 minutes. A maximum of 4 sprays per 24 hours is specified. An O'Brien pharmacist contacts the patient on receiving a prescription from a licensed physician and inquires about beta blocker contra-indication and instructs on use. The cost of the medication at this writing is $30 for a 10 ml bottle plus postage. All future research on using beta blockers for acute migraine should be done using nasal delivery. I have no financial interest in this product.

The Aggarwal study[6] has so many other deficiencies that for reasons of space I can only list them without much discussion: patients not beta blocker naïve were included; retrospective exclusion of them taints the already scant data; the patients were not instructed to take the eye drops as quickly as possible with migraine onset; instead they had to fill out a questionnaire about the migraine; the study does not state if or when a second set of eye drops were to be instilled (ideally 10 minutes after the first if migraine persists); it is not stated whether patients were allowed to take their usual acute migraine medications which they should have been; the migraine patients were recruited from a tertiary neurological referral center with a high number of refractory migraineurs. Most important, per their own analysis, the number of patients studied does not allow any reliable statistical validity which Aggarwal nevertheless erroneously claimed. Their discussion does not include consideration of the more effective and easier to use sublingual or nasal beta blocker administration; the discussion attributes findings and conclusions to another study[9] that are inaccurate and ignores optimistic statements of those authors about further study of beta blockers for acute migraine.[10]

Cossack et al.[9] reported that 4 of their 10 studied patients found the masked beta blocker eye drop a useful addition to their acute migraine therapy versus only 1 in favor of masked placebo. They did allow a second set of eye drops but no sooner than 30 minutes because of Institutional Review Board concerns. They did discuss that if used 10-15 minutes post first insertion it might have improved their results. They did not claim statistical significance of their study but determined that "*A future crossover study will require 86 patients to power a study with α≤0.05 and β≤0.2.*"[9,10] Cossack and Gratton conclude their discussion, "*We believe that, together, our work advances the notion that timolol drops are a safe effective, and already widely available abortive treatment in select migraineurs.*"[10] Neither the Cossack et al.[9] or the Aggarwal studies were large enough for statistical significance so it is a misrepresentation for Aggarwal et al.[6] to state "*we now have two randomized-controlled trials that would not demonstrate a marked effect of the drug compared to placebo.*"

I hope that either the pharmaceutical industry or grant funding will soon conduct a large, adequately-powered (N ≥ 86), placebo-controlled, cross-over study using the newly available nasal spray delivery as a novel, safe, relatively inexpensive treatment for acute migraines.

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Keywords: timolol, ophthalmic solution, migraines, letter

Abstracts from Frontiers Research Day
University of Kansas Medical Center, Kansas City, KS
March 4, 2020

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March 4, 2020

KANSAS JOURNAL of MEDICINE

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Frontiers Clinical and Translational Pilot Research Grant Recipient

Racial Disparities in Cesarean Delivery in Singleton Pregnancies

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ABSTRACT

Introduction: The past two decades have seen a revolution in the management of labor and delivery; rates of cesarean delivery (CD), medical induction, and electronic fetal monitoring have increased dramatically. CD occurs in one third of deliveries and is the most common inpatient surgery. The increase in CD has occurred despite concerns about maternal complications (infection, blood loss, hysterectomy, uterine rupture) and racial disparities. Since 1993, black and white women have gone from comparable CD rates to rates 40% higher among black women compared to white women.

Methods: This project has assembled a cohort of over 800,000 deliveries from the Cerner Corporation's Health Facts® database. Health Facts contains 63 million unique patients from over 600 U.S. healthcare facilities. Data include comprehensive clinical records from inpatient and outpatient encounters and includes data from pharmacy, laboratory, registration and billing data.

Results: CD was identified using a validated definition that incorporates International Classification of Disease (Version 9, Clinical Modification) codes for delivery outcome and relevant diagnosis-related groups and procedures. We examined 618,582 singleton deliveries to black and white women and identified 177,564 cesarean deliveries (28.7%).

Conclusions: The rate of cesarean was higher for black women overall and during the years 2000 - 2006 and 2011 - 2015 the CD rate for black women was significantly higher than for white women. Of note, during the period from 2007 - 2010 rates of cesarean were similar among black and white women. The rate of repeat cesarean was lower among black women, while the rate of induction of labor was higher.

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KANSAS JOURNAL *of* MEDICINE

Frontiers Predoctoral Trainee Scholar (TL1)

The Changing Health and Social Circumstances of Women Leaving Jails: A Three-Year Longitudinal Study

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ABSTRACT

Introduction: Ninety-five percent of all inmates are eventually released back into communities, with many being released without access to the most basic of necessities. Women in particular face complex health and social challenges – children, partners, strained employment opportunities, health problems – before incarceration that they must also face upon reentry. This poses a barrier to their successful reintegration back into society.

Methods: The present study analyzes data collected as part of the sexual health empowerment (SHE Project) health literacy intervention. Participants were recruited from three county jails in the greater Kansas City area. At baseline, participants completed a survey that assessed sociodemographic characteristics and social histories prior to incarceration. Women were followed annually after program completion to complete surveys to assess their long-term health and social circumstances. The present study is a secondary analysis of baseline and follow-up data.

Anticipated Results: We use Hobfoll's Conservation of Resources (COR) Theory to conceptualize the stress that justice-involved women may experience as a result of perceived or actual loss of resources. We hypothesize that "loss spirals", a term coined by Stevan Hobfoll, creates psychological stress that drives justice-involved women to assume risky sex and drug behaviors that will generate more resources and help to cope with the stress.

Significance of Impact: This study aims to define a succinct longitudinal timeline assessing biopsychosocial outcomes of women released from jail in order to improve prevention and intervention techniques for improvement in social and health circumstances of women leaving jail and their reduction in recidivism.

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KANSAS JOURNAL of MEDICINE

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Biostatistics, Epidemiology and Research Design (BERD) Trailblazer Award Recipient

Estimation and Construction of Confidence Intervals for the Cutoff-Points of Continuous Biomarkers Under the Euclidean Distance in 3D Settings

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ABSTRACT

Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal forms of cancer with a five-year survival rate ~7%. Thus, early detection could be key to reducing its mortality. Finding effective biomarkers is crucial to early detection. Pancreatic cancer biomarker studies typically consider more than two classes (i.e., recruited individuals that are either healthy, suffering from pancreatitis/cysts, or from aggressive PDAC). Conventional ROC curve analysis is the most popular tool to assess the discriminatory ability of biomarkers in distinguishing between two groups. For more than two groups, this approach is inadequate to determine decision cutoffs for decision-making purposes. Currently there is no accuracy-based statistical approach that is immune to the prevalence of the disease and is able to accommodate information from all three groups to optimally determine a decision rule for classification of patients. We propose a Euclidian distance-based approach as a method of estimating optimal cutoff values. We provide a full analysis framework when interest lies in decision-making and provide parametric and nonparametric approaches. Our approaches can easily be generalized into k-class problems when we are interested in distinguishing between four or more classes. We can extend our methods to consider the costs of incorrect decision making for each of the groups and weigh them accordingly. Additionally, combinations of biomarkers or relevant clinical information can also be accommodated straightforwardly to improve decision rules. We illustrate the usefulness of our approaches by analyzing a pancreatic cancer biomarker study conducted at the University of Texas MD Anderson Cancer Center.

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KANSAS JOURNAL of MEDICINE

CTSA Inter-Institutional Pilot Project Award Recipient

Humanized Mouse Models for Studying the Role of Immune Cells in Breast Cancer Progression

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ABSTRACT

Introduction: To study breast cancer progression, we developed the Mouse-Intraductal (MIND) model which involves intraductal injection of breast cancer cells into the mammary ducts of immunocompromised mice, where they display a course remarkably similar to the evolution of human breast cancer, with the formation of lesions in situ followed by invasion into the surrounding stroma. More recently, we are collecting peripheral blood from these same women and have been successful in humanizing mice with the patient's own hematopoietic stem cells (HSC).

Preliminary Data: We have achieved successful engraftment of peripheral blood-derived and bone marrow-derived human CD34+ cells into NSG (immunodeficient NOD-SCID Il2rg-/-), MISTRG (immunodeficient Rag2-/-Il2rg-/- transgene SIRPa expressing GM-CSF, IL-3, M-CSF, TPO) and MISTRG6 mice (plus IL-6) and observed recruitment of their progenitors to breast cancer lesions. Our preliminary studies demonstrate preferential myeloid development when using peripheral blood derived CD34+ cells, while both myeloid and lymphoid development are achieved when using bone marrow derived CD34+ cells. Furthermore, MISTRG6 mice show improved development of both human myeloid and lymphoid hematopoietic cells compared to MISTRG and NSG mice.

Impact and Future Aims: The major advantage of our model is the ability to observe progression of patient breast cancer and their associated immune cells in vivo. Analyzing patient samples eliminates the ability to track the progression of lesions and their associated immune cells in vivo. By maximizing these unique advantages, we hope our results will help guide individualized immunotherapy in future patient care.

Funding Support: This work was supported by a CTSA grant from NCATS awarded to the University of Kansas for Frontiers: University of Kansas Clinical and Translational Science Institute (#UL1TR002366). The contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIH or NCATS.

KANSAS JOURNAL of MEDICINE

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Frontiers Postdoctoral Trainee Scholar (TL1)

Evaluating the Adverse Effects of Hyperglycemia on the Airway Epithelium in Cystic Fibrosis and CF-Related Diabetes

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ABSTRACT

Introduction: Although causal linkage between hyperglycemia and decreased lung function in cystic fibrosis (CF) is unknown, it is thought to be related to excess inflammation and ion channel dysfunction.

Methods: We utilized complementary in vitro and in vivo approaches to assess the effects of hyperglycemia on the CF airway epithelium. In vitro: Primary CF bronchial epithelial (CFBE) cells at the air-liquid interface under hyperglycemic (HG; 255 mg/dL) or normoglycemic (NG; 100 mg/dL) conditions. Airway surface liquid (ASL) was measured by meniscus scanning and apical K+ (BK) ion channel current was measured in Ussing chambers. In vivo: In CF subjects with or without CF related diabetes (CFRD), glycemic control was measured using continuous glucose monitoring.

Results: Nasal epithelial cells were collected and analyzed by qPCR for the expression of RAGE, TGF-1, and LRRC26, the regulatory subunit of BK. Compared to normoglycemia, CFBE cells under hyperglycemia show significantly reduced ASL (68.4 ± 17.4 µL, NG vs. 51.5 ± 18.1 µL, HG; $p<0.05$), a significant decrease in BK activity (-10.6 ± 0.8 µA/cm2, NG vs. -1.6 ± 0.5 µA/cm2, HG), and a 69% increase in mRNA expression of RAGE ($p<0.05$). From our in vivo studies, CF patients with and without CFRD showed significant negative correlation between the mRNA expressions of TGF-1 and LRRC26 ($R2=0.73$, $p<0.05$).

Conclusions: In vitro data suggests hyperglycemia adversely affects airway hydration, in part, by impairing BK channel function. Reduction of LRRC26 expression in subjects with elevated TGF-1 and those with glycemic abnormalities point to BK as a target for therapeutic intervention in CFRD.

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KANSAS JOURNAL of MEDICINE

Frontiers Infrastructure Award Recipient

University of Kansas Medical Center Genomics Core

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ABSTRACT

The University of Kansas Medical Center – Genomics Core leveraged the Frontiers: KU Clinical and Translational Science Institute infrastructure award to purchase an Eppendorf MasterCycler Pro thermal cycler and Countess II Automated Cell Counter to support the single cell initiative of the Genomics Core at KUMC. The MasterCycler Pro and Countess II are used to support the 10X Genomics Chromium Controller for performing the full suite of 10X Genomics single cell applications which include Single Cell 3' Gene Expression, Single Cell 5' Expression and V(D)J Enrichment and Single Cell ATAC – Chromatin Profiling.

The Countess II Automated Cell Counter is used to ensure that adequate cell counts and high viability of submitted samples are present. The Countess also allows visualization of any cellular debris or cell clumping which will interfere with a successful 10X emulsification procedure required for a successful single cell library preparation. The MasterCycler Pro has been sequestered to exclusively support single cell library preparations. The MasterCycler performs the initial single strand synthesis which incorporates the 10X GEM barcode and Unique Molecular Identifier (UMI), second strand synthesis and Illumina adapter ligation to complete the 10X library preparation.

These instruments have proven critical to the successful execution of 16 single cell projects in support of seven different investigators. Frontiers' investment in the single cell initiative has had a positive impact on cutting edge single cell expression and epigenetic research performed at the University of Kansas Medical Center.

Funding Support: This work was supported by a CTSA grant from NCATS awarded to the University of Kansas for Frontiers: University of Kansas Clinical and Translational Science Institute (#UL1TR002366). The contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIH or NCATS.

KANSAS JOURNAL of MEDICINE

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Biostatistics, Epidemiology and Research Design (BERD) Trailblazer Award Recipient

Analysis of Mitochondrial Haplogroups with Alzheimer Disease Risk

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ABSTRACT

Introduction: Alzheimer's disease (AD) is the most common form of irreversible, progressive dementia that affects memory loss, cognitive capabilities, and behavior. Although, significant progress has been made to understand genetic and clinical risk factors associated with AD, our understanding of AD remains incomplete resulting in ineffective prevention or cure.

Methods: Several studies have indicated that the polymorphisms in the mitochondrial DNA (mtDNA) play an important role in the mechanism of AD. Therefore, in order to understand the association of the mitochondrial genetic alteration with AD, we carried out cross-sectional association analysis using the clinical and mitochondrial single nucleotide polymorphism (SNP) data. We used the data collected at University of Kansas Alzheimer's Disease Center (KU ADC) to carry out the analyses. We also used the data from Alzheimer's Disease Neuroimaging Initiative (ADNI) for the replication and validation analyses.

Results: The KU ADC data consists of 146 AD and 265 cognitively normal subjects, and ADNI data has 244 AD and 242 controls. For both datasets, the haplogroups information for each subject and APOE ε4 carrier status were also available.

Conclusions: We found that the rates of incidence of AD were statistically significantly different among the haplogroups, with differing strengths of association for different haplogroups. APOE ε4 was significantly associated with AD overall (p-value = 1.9×10-11) and the association varied by haplogroups (i.e., interaction). The results were consistent with the ADNI data. Our study has generated hypothesis and the data for further studies ahead to understand the etiology of AD.

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KANSAS JOURNAL *of* MEDICINE

Frontiers Infrastructure Award Recipient

Live Cell and Intravital Imaging Reveals Differences in Mitochondrial Redox State, Morphology and Number between Osteoblasts and Osteocytes

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ABSTRACT

Introduction: Age-related bone loss and associated fracture risk is an increasing concern worldwide. Oxidative stress causes mitochondrial damage and is associated with age-related degeneration in many cell systems, including bone cells.

Methods: To understand mitochondrial function in bone cells in situ, live-cell and intravital imaging of mitochondrial NADH and mitochondrial morphology was performed using transgenic mice expressing GFP in osteocytes as well as in MLO-Y4 osteocyte-like cells and OmGFP66 cells, which model osteoblast-to-osteocyte transition. NADH excitation and emission spectra in live osteocytes and osteoblasts matched well with pure NADH, with maximal multiphoton excitation at 740 - 760nm, resulting in blue NADH autofluorescence. With 760nm excitation, maximal NADH emission occurred at 460nm. Metabolic challenge was performed with carbonylcyanide-3-chlorophenylhydrazone and sodium cyanide, which convert NADH to its maximally oxidized or reduced states.

Results: This showed that osteoblasts had a lower resting redox state, with 40% of their NADH in a reduced state, compared to osteocytes with 75 - 80%. Live imaging using mitotracker Red-CMXRos showed that osteocytes have fewer mitochondria, lower mitochondrial area and % area, and increased solidity.

Conclusions: These data show that osteocytes in vivo under normoxic conditions have significant levels of mitochondrial NADH and show differences in mitochondrial number, morphology and function compared to osteoblasts. These live-cell imaging techniques can be used to determine changes in mitochondrial function during aging/cellular senescence and disease. These studies were supported by Frontiers equipment funding, including a Tokai-Hit CO2 incubation system, Leica 16x and 20x objectives and Imaris9.2 software which are available to other investigators through the UMKC Confocal Imaging Core.

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KANSAS JOURNAL of MEDICINE

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Frontiers Infrastructure Award Recipient

Targeting Prolactin Receptor Using FDA-Approved Drugs: A Molecular Docking Approach and Drug Binding Studies

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ABSTRACT

Introduction: Pancreatic cancer (PDAC) is the fourth leading cause of cancer-related deaths in the U.S. Gemcitabine remains the drug of choice for metastatic disease, but only little improvement is seen in survival rates. Hence, there is a dire need for novel therapeutics for PDAC. We found that PRLR is overexpressed in PDAC tissues and cell lines and knockdown of the receptor resulted in reduced tumor formation in mice. Hence, PRLR is a novel target for therapeutic interventions in PDAC.

Methods: As there is no small molecular inhibitor available for targeting the receptor, we developed a homology model of the intracellular domain of PRLR that encodes the JAK2 binding site, and then performed a virtual screening of small molecules using IDOCK and I-TASS-ER servers. Using Fragment-based drug design, we derived commercially available compounds for virtual screening. We selected several compounds that came out of the virtual screening and performed in vitro proliferation assays in the PDAC cells. We identified antipsychotic drugs including penfluridol that can inhibit the proliferation of PDAC cell lines. Moreover, pretreatment of these antipsychotic drugs inhibited PRL-induced STAT3 and ERK phosphorylation in PDAC cell lines. Further, we used cellular thermal shift assay and drug affinity binding assay to confirm the binding of Penfluridol and PRLR. We also used cell-free systems such as surface plasmon resonance (SPR) and magnetic relaxometry to confirm the drug binding. Moreover, Penfluridol treatment significantly decreased pancreatic tumor burden in animal models. In conclusion, we identified penfluridol is a potential drug candidate for targeting PRLR in PDAC.

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KANSAS JOURNAL of MEDICINE

Frontiers Dr. Lauren S. Aaronson Trailblazer Recipient

Neural Predictors of "Rapid Response" in Anorexia Nervosa

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ABSTRACT

Introduction: Anorexia nervosa (AN) is a serious condition associated with numerous medical complications, psychiatric comorbidity, and the highest mortality rate of any psychiatric disorder. Relapse rates remain high and for many, AN becomes an enduring condition. The only reliable prognostic indicator for AN is "rapid response" (i.e., weight gain over the first four weeks of treatment). Yet, there are no reliable predictors of rapid response at baseline. There is a pressing need to identify novel neuro-markers that confer early risk for poor treatment outcome.

Methods: Study goals were to systematically identify potential neuro-markers related to short-term AN outcomes by assessing brain activation associated with delayed discounting (capacity to delay immediate gratification), sensitivity to food reward (responses to rewarding foods and decisions to choose palatable foods), and threat sensitivity (heightened reactivity to potential danger or harm). The purpose of this study was to identify neural predictors of short-term treatment response in adolescents with AN. Participants were adolescent females with AN who entered their first course of eating-disorder treatment within the past six months.

Specific aims: 1) Test neural activation during tasks measuring delayed discounting, food reinforcement, and threat and 2) Identify prospective associations of task-performance and task-based neural activation with eating-disorder symptoms at one-month follow-up.

Conclusions: Differentiating neurobiological processes that contribute to AN chronicity may lead to the development of prognostic indicators at initial treatment presentation. These neuromarkers may improve clinical decision-making and allow providers to identify patients requiring more intensive treatment interventions to prevent chronicity and mortality.

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KANSAS JOURNAL of MEDICINE

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A High Throughput Assay for Quantitating β-catenin Signaling in the MLO-Y4 Osteocyte-like Cell Line

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ABSTRACT

Introduction: Osteocytes are the primary mechanosensory cell in bone and activate β-catenin signaling in response to mechanical loading of bone.

Methods: In order to understand the mechanism by which osteocytes activate β-catenin signaling, we have developed a stably transfected osteocyte cell line that expresses luciferase when β-catenin signaling occurs in the nucleus. To quantitate luciferase activity, we purchased a VICTOR Nivo Multimode Plate Reader (Perkin-Elmer) with the support of Frontiers funding. This instrument quantitatively measures luciferase activity (fluorescence and absorbance) in several plate formats, thus permitting high throughput screening of compounds that activate β-catenin signaling. We stably transfected the MLO-Y4 osteocyte-like cell line with the TOPflash vector pGL4.9[luc2P/TCF_LEFRE/Hygro] (Promega). Clone A3-F9 gave a dose response to increasing concentrations of Wnt3a (1-100ng/ml) and activation with 10mM LiCl gave the equivalent of a submaximal dose of Wnt3a (10ng/ml).

Results: We demonstrated that C2C12 myotubes, but not myoblasts, produce a factor that activates β-catenin signaling two-fold using these TOPflash-MLO-Y4 cells, but in combination with Wnt3a (10 ng/ml; 10-fold activation) produces a 20-30-fold increase in activity measured in our luciferase assay. We demonstrated that production of this factor by C2C12 myotubes is partially regulated by mTOR signaling. We are currently seeking to identify this factor using our TOPflash-MLO-Y4 cells.

Conclusions: The acquisition of the VICTOR Nivo Multiplate reader provides us the means to conduct a high throughput screening approach to identify this factor(s). This instrument is part of a Core in the UMKC School of Dentistry Department of Oral and Craniofacial Sciences and available to other investigators for their studies.

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KANSAS JOURNAL of MEDICINE

Exploratory Evaluation of an Online Educational Intervention for JUUL Use

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ABSTRACT

Introduction: Initiation of JUUL use by young adults is one of the most significant issues of concern within the debate on vaping. Despite the proliferation of products and the surge in prevalence, no studies have investigated individual-level interventions or prevention strategies for pod-mod use.

Methods: Participants (N = 947) were young adults (< 30 years old) recruited from Amazon's Mechanical Turk based on smoking (never, former, and current smokers) and JUUL use status (never and current users). In a pre-post design, participants completed baseline assessments, were presented with a brief JUUL-specific educational intervention and completed post-assessment measures. Primary outcomes were changes in JUUL knowledge, perceived harmfulness, intentions for future use, and motivation to change.

Results: Participants (Mean age = 26.1) were male (57%) and White (75%). Overall, the intervention increased JUUL-related knowledge, risk perceptions, commitment to quitting, and readiness to quit JUUL (all $p \leq 0.001$). Similarly, participants showed decreased interest in future JUUL use, interest in purchasing JUUL, and interest in future regular JUUL use (all $p \leq 0.001$). There were significant Time X Group interactions for JUUL-related knowledge ($p < 0.001$), with never JUUL/never smokers showing the greatest increase in product knowledge following the intervention. However, no other interaction effects were significant.

Discussion: The intervention was effective in increasing knowledge and risk perceptions while reducing intentions for future use. The intervention was most effective in increasing knowledge among non-users, suggesting that brief educational interventions may be useful tools for preventing pod-mod initiation while more intensive interventions may be necessary to alter attitudes and use behaviors among current users.

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KANSAS JOURNAL of MEDICINE

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American Indian College Students' Knowledge, Attitudes, Beliefs, and Behaviors Surrounding Alternative Tobacco Products

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ABSTRACT

Introduction: American Indians (AI) have the highest rates of smoking and smokeless tobacco (SLT) use among all major racial/ethnic groups. There is limited information about the use of alternative tobacco (AT) products such as electronic cigarettes. The objective of this pilot study is to understand the knowledge, attitudes, beliefs, and behaviors (KABB) surrounding AT product use among AI college students.

Methods: We conducted 19 focus groups with AI college students age 18 and older. Participants were stratified by AT use, cigarette use, and never users of tobacco products. Focus groups were audio-recorded and transcribed verbatim in preparation for coding and standard text analysis. Participants completed a short survey that collected information on demographics and KABB about tobacco products. Surveys were analyzed with frequencies and measures of central tendencies.

Results: Participants included 105 AI college students. Participant's mean age was 21 years and 50% were female. Focus groups ranged in size from two to nine. Related to e-cigarette use, participants cited flavors, discreteness, stress, and social aspects as factors for use. Survey results indicate that 35% of participants were current e-cigarette users, 30% were current smokers, and 9% were current SLT users.

Conclusion: This pilot study confirms that AI college students are following a similar trend to other racial/ethnic groups in terms of AT use. It is important that we obtain estimates of AT use and include AI in prevention and cessation efforts. This study will help develop appropriate prevention and cessation messaging for this population.

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KANSAS JOURNAL of MEDICINE

Frontiers Clinical and Translational Pilot Research Grant Recipient

Low Dose Daunorubicin Targets the Leukemia Stem Cell in AML

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ABSTRACT

Introduction: A proof of concept trial (NCT02914977) was designed to measure the ability of low dose DNR to inhibit pS552-β-catenin in LSCs of adult relapsed/refractory AML patients. A protocol amendment added a second cohort of patients with newly diagnosed AML undergoing standard chemotherapy with DNR and cytarabine (7+3 induction).

Methods: Treatment in each cohort consisted of DNR given at a dose of 6.75mg/m2/day x five days. Patients with relapsed/refractory AML had bone marrow aspiration pre-treatment prior to day 1, followed by DNR on days 1-5, and a post-treatment bone marrow aspiration on day 8. Patients with newly diagnosed AML were treated with conventional 7+3 chemotherapy.

Results: A sample from the day 14 bone marrow was collected. DNR was given on days 15 - 19. Bone marrow aspiration was performed with blood count recovery and samples sent for LSC marker assessment.

Discussion: In this study, we will determine the safety, pharmacokinetics, and pharmacodynamic effects of low dose DNR. The LSC population and phosphorylation status of β-catenin will be measured pre-and post-treatment. Two or more prior induction attempts are required for study entry for refractory patients; relapsed patients require only one prior induction. The trial completed its accrual goal of 18 patients. Low-dose DNR treatment was well-tolerated with no unexpected adverse events. In this pilot proof of concept trial, we will demonstrate the feasibility of serial bone marrow aspiration to measure biomarkers of LSC response.

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KANSAS JOURNAL of MEDICINE

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Frontiers Pilot and Collaborative Studies Funding Program Award Recipient

In Our Sacred Voice: An Exploration of Tribal and Community Leader Perceptions as Health Communicators of Disease Prevention among American Indians in the Plains

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ABSTRACT

Introduction: American Indians (AI) are disproportionately and significantly impacted by disease morbidity, mortality and poor behavioral health outcomes. Based on data from the National Vital Statistics Reports, the leading cause of death among AI is cancer (19%), followed by heart disease (18%). Regionally, health disparities among AIs reflect national statistics. Health communication targeted to address these health disparities exist; however, few evidence-based strategies integrate holistic approaches. The impact of health communication research and practice is evolving and continues to emerge as a core part of health promotion. Strategies that appeal and increase persuasiveness of health messages are more likely to penetrate existing cultural beliefs and positively influence health behavior outcomes. The objective of this study was to explore how tribal and elder leaders throughout the Plains viewed themselves as health communicators within their communities.

Methods: Members of the Center for American Indian Community Health conducted 39 in-depth interviews from December 2017 to December 2018 with members of federally recognized tribes living in reservation communities, as well as urban tribal communities across the region.

Results: Results from this sample show that these individuals do not see themselves as the "authority" health communicator within their tribe and community. The term health promoter was viewed as problematic in Native communities for cultural reasons.

Conclusion: Overarching themes included seeing themselves as communicators of holistic health, specific health prevention programs, access, targeted communication and health promoters as a collective unit. Collective social and cultural authority in tailored messages is perceived to bolster health communication and positively impact health outcome.

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KANSAS JOURNAL of MEDICINE

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Frontiers Pilot and Collaborative Studies Funding Program Award Recipient

Not About Us, Without Us - An Exploratory Study of Underserved and Minority Patients' Views of Cancer-Related Genetic Counseling and Testing Communication

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ABSTRACT

Introduction: Minority and underserved populations carry the largest cancer burden for the United States (ACS, 2016). Genetic counseling, testing and technologies show promise to help reduce cancer through personalized medicine opportunities however disproportions of genetic services, uptake and research about genetic testing among these populations exist (Ricker, et al., 2018). Inequitable cancer-related genetic and genomic testing (CGT) communication among minority and underserved populations and other barriers (e.g., genetic counselor bias) may contribute to CGT disparities. Exploring existing attitudes, perceptions and beliefs toward CGT among minority and underserved patient populations may bolster strategies to address communication inequities. The study aims were to explore an under-researched area of precision medicine, i.e., the communication of genetic risk factors among minority and underserved patient populations with high to moderate risk and disseminate and share findings with the community.

Methods: A Community Advisory Board comprised of researchers, cancer survivors and family members of cancer patients met from December 2018 - March 2019 to discuss research design and data collection. Six stratified focus group discussions (N = 53) were held March - May 2019 where a pre-focus group survey was administered.

Results: Survey results show most had not talked to their doctor/health care provider about genetic testing (87%). African Americans (AA) and Latinos expressed a lack of awareness and knowledge about CGT and the importance of cultural tailoring.

Conclusion: Separate themes that emerged among Latinos included immigration status and language as barriers to CGT. Among AA, participants were highly distrustful of medical personnel and expressed outrage about the lack of recommendation for CGT.

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KANSAS JOURNAL of MEDICINE

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Frontiers Clinical and Translational Pilot Research Grant Recipient

Changing how the Brain Responds When Making Decisions: Translating Neuroscience to Population Health

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ABSTRACT

Introduction: Engaging in healthy behaviors is not always rewarding (e.g., carrot vs. cake). Neural models of healthy behaviors focus on the balance between reward and regulation brain regions. This pilot study examines the engagement of reward and regulation regions during the evaluation of food and nonfood cues before and after a guided imagery exercise targeting positive associations with food and regulation. Our long-term goal is to understand the interaction between positive associations with healthy foods and thinking about future rewards may influence healthy decision-making. The objective of the current study is to empirically test the combined effects of positive affect and positive episodic future thinking on brain activation.

Methods: The project examined the effect of positive affect and episodic future thinking guided imagery on brain activation in regulation and reward regions. Participants included individuals with a body mass index greater than 25 (n = 20).

Results: Functional neuroimaging showed reduced activation in regulation-related brain regions (i.e., dorsomedial prefrontal cortex) to healthy and unhealthy food following guided imagery.

Conclusions: These results suggest more efficient regulation-related brain activation after positive episodic future thinking guided imagery.

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KANSAS JOURNAL of MEDICINE

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Engaging Patients and Defining Outcomes: A Minority Engagement Effort

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ABSTRACT

Introduction: The Establishing Meaningful Patient-centered Outcomes With Relevance for patients with Polycystic Kidney Disease (EMPOWER PKD) initiative aims to engage PKD stakeholders and patients to learn about health priorities, insurance issues, and patient engagement.

Methods: We utilized semi-structured focus groups. We developed and pre-piloted a guide that allowed for both conversational flow and consistency in questions among groups. We audio-recorded each group and transcribed the conversations verbatim. We performed an inductive thematic analysis. Two investigators completed all data coding independently and in duplicate. We compare the results of an ethnically diverse focus group to the other seven that mainly included Caucasian participants.

Results: 14 individuals participated in this part of the study. Of these, (64.2%) reported having PKD, and (14.2%) being caregivers. The mean age of participants was 48.8 (range 29 - 75) years. The group included 85.75% African American and 7.1% reported more than one race.

Conclusions: It was challenging to recruit this group and it required establishing a relation with community nephrologists and faith-based organizations leads. Multiple participants stated that they would not have participated if the group was not exclusively ethnically diverse as they fear that their opinion may be outnumbered by a Caucasian majority. Some discussed believing that PKD was a "white" disease. Many discussed hiding their diagnosis which affected their activation and engagement and came with unanticipated psychological impacts. Participants unanimously described lack of trust-worthy, easy to understand educational resources for minorities as a main barrier to engagement.

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KANSAS JOURNAL of MEDICINE

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Race-Related Stress and Food Decision Making in African American Women

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ABSTRACT

Introduction: The highest rates of obesity in the U.S. are among black women with a prevalence of 54.8%. Psychosocial factors, such as stress from racism, have been proposed as contributors to this disparity, but the mechanisms are unclear. The objective of this study is to understand the relationship between race-related stress and food choice among black women.

Methods: Forty-five participants were asked to come in fasted for two appointments, randomized to the order of stress exposure in a within subject design. Forty images for each condition were collected from two validated picture sets (International Affective Picture set and Socio Moral Image Database). The images were shown in random order and contained either racially charged scenes (e.g., Ku Klux Klan) or non-racially charged scenes (e.g., aggressive dog) matched on valence (pleasant vs. unpleasant) and arousal (physiologically stimulating). Food choice and food demand tasks were completed pre and post stress exposure. Participants were surveyed on their stress levels, past experiences of racism, and trauma.

Anticipated Results: It is expected that preferences for unhealthy foods will be greater after race related stress exposure compared to the other stress condition. We anticipate that those who have higher BMI's are more likely to be affected in their changes in eating behaviors in both stress exposure states, with a larger impact from the race related stress induction. Data collection is ongoing.

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Low Socioeconomic Status and Residential Distance of Less Than 10 Miles From a Frontier-State NCI-Designated Cancer Center is Associated with Worse Ovarian Cancer Survival

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ABSTRACT

Introdution: To examine the impact of residential distance and socioeconomic status on survival outcomes for patients receiving treatment for ovarian cancer at an NCI-CC.

Methods: Patients who were treated for ovarian cancer at a single institution from 2010 - 2015 were identified. Age at diagnosis, insurance status, and distance from the patient's home to the institution were abstracted. Clinical data including stage at diagnosis, surgical status, chemotherapy cycles, dates of diagnosis, recurrence, and death were obtained. Patients treated at other institutions and those with non-epithelial pathology were excluded. Patients were stratified into three groups based on distance from the institution. Overall survival (OS) and progression free survival (PFS) were generated by Kaplan Meier survival curves and Cox proportional hazard models using SAS v9.4.

Results: Two hundred and three patients were identified. Survival analysis based on distance demonstrated that patients who lived less than 10 miles from the institution have worse survival ($p = 0.0412$). Lower median income (< 56,179) regardless of distance to institution was also associated with worse survival, $p = 0.0283$. After adjusting for stage of disease, age at diagnosis, surgery status, chemotherapy resistance, income quartile and presence of comorbid conditions, distance < 10 miles from the institution was still a significant predictor of worse overall survival, HR 2.62 95% CI (1.333 - 5.142), $p = 0.0052$.

Conclusion: Among patients who received guideline-adherent care for ovarian cancer, lower median income and residential distance less than 10 miles to an NCI-CC was associated with worse overall survival.

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KANSAS JOURNAL of MEDICINE

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Understanding Race Bias in the Decision to Shoot with an Integrated Model of Decision Making

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ABSTRACT

Introduction: The shooting of unarmed citizens by police officers is a topic at the forefront of public awareness. The disproportionate rate at which unarmed Black citizens are shot is believed to reflect widespread racial bias on behalf of officers, which erodes public trust and reduces policing effectiveness.

Methods: We have developed an Attention-integrated Model-based Shooting Simulator (AiMSS) to gain a deeper understanding of the mechanisms underlying the decision to shoot. The AiMSS combines computational models of decision making, visual psychophysics and eye-tracking methods, and an immersive decision simulator to map the processes police officers use to decide to shoot.

Results: Results with the AiMSS reveal that policing scenario and suspect behavior accounted for the most variation in decisions. Process level measures show little evidence of an initial bias to shoot Black suspects. Instead, results reveal a diminished ability to distinguish objects held by Black suspects.

Conclusions: This work emphasizes the importance of contextual factors in the decision to shoot and highlights how past experimental studies on racial bias have neglected critical inputs into officer deadly force decisions.

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KANSAS JOURNAL of MEDICINE

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Frontiers Infrastructure Award Recipient

A Developing Shared Resource: Emerging Imaging Technologies and Applications Laboratory

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ABSTRACT

The Emerging Imaging Technologies and Applications Laboratory (EITAL) is a shared resource serving as a proof-of-concept hub for performing innovative and rigorous fundamental cancer biology research at subcellular to cellular length-scales in conjunction with the development and translation of advanced imaging technologies and applications. The scientific staff of EITAL are experts in developing and improving reagents, methods, and optical and electron microscopic imaging technologies, antibody development, screening, and characterization, immunohistochemistry, immunocytochemistry, correlative optical and electron microscopy, immunoelectron microscopy, workflow development, and experimental design and data analysis of imaging studies. EITAL promotes synergies between its imaging technology developers and University of Kansas Cancer Center (KUCC) cancer biology researchers. EITAL also provides research training and career development programs to ensure a talented and diverse workforce engaged in cellular tumor cancer biology imaging research. The collaboration between EITAL and the University of Kansas (KU) Microscopy and Analytical Imaging Research Resource Core Laboratory (MAI) imaging scientists, enables imaging research at the level of the whole animal, individual cells, as well as molecules inside the cells, and provides instrumentation and expertise for optical and electron microscopies, resulting in multi-scale/multi-dimensional correlative imaging research of living and fixed cells and tissues. The KUCC Support Grant (P30 CA168524) supports EITAL.

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KANSAS JOURNAL of MEDICINE

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Influence of Vision and Proprioception on Motor Control in ASD

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ABSTRACT

Introduction: Sensorimotor integration deficits are common in Autism Spectrum Disorders (ASD). Evidence exists for an over-reliance on both visual and proprioceptive feedback during motor control in ASD, suggesting deficits in the modulation of sensory feedback processing and inability to use the most reliable input. This study aims to test this hypothesis.

Methods: Forty persons with ASD (10 - 33 yrs) and 25 age-, sex- and nonverbal IQ-matched controls completed precision gripping tasks. They squeezed a force sensor with their index finger and thumb and tried to match their force output to a target force. Visual feedback was presented on a computer screen across low, medium, and high gain levels; the force output bar moved a greater distance per change in force at higher gains. A stationary bar represented the target force. 80Hz tendon vibration was applied at the wrist to create a proprioceptive illusion of muscle contraction. Force regularity (approximate entropy; ApEn) was examined.

Results: Preliminary results from 18 participants with ASD and 13 controls indicate a Group x Tendon Vibration x Visual Gain interaction for ApEn ($F = 1.559$, $p = 0.023$). Controls showed increased ApEn during 80Hz tendon vibration at low visual gain but decreased ApEn with vibration at high visual gain. Individuals with ASD show slight increases in ApEn with 80Hz vibration across visual conditions.

Conclusions: Early findings indicate that controls shift to a secondary source of sensory feedback (e.g., proprioception) when the primary source (e.g., vision) is degraded. Persons with ASD fail to dynamically recalibrate feedback processes across sensory systems when feedback conditions change.

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KANSAS JOURNAL of MEDICINE

Diversity Trailblazer Award Recipient

Health through Enhancing Awareness and Learning about Breast and Cervical Cancer Screening among African American Women (HEAL)

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ABSTRACT

Introduction: African American women experience higher incidence and mortality rates of cervical and breast cancer. The purpose of this study is to develop and implement a breast and cervical cancer screening intervention to increase awareness, uptake of screening, and follow-up among an African American female church-population in Sedgwick County, Kansas.

Methods: Academic researchers recruited local pastors (N = 11) to develop a faith taskforce. Using intervention mapping, we conducted three focus groups with the taskforce to assess barriers and facilitators to seeking breast and cervical screenings and multi-component strategies to improve education and awareness of breast and cervical cancer screening recommendations, screening rates, and follow-up among African American women.

Preliminary Results: Of the 11 faith taskforce pastors, three were females. The key identified themes focused on barriers and facilitators to implementation of church-based cervical/breast cancer interventions, such as time constraints, limited expertise in conducting faith-based interventions, and lack of awareness on burden of cancer in the community, awareness of screening recommendations, and support/accountability in cancer screening and follow-up. Participants also indicated that a mobile phone app to track screenings, follow-up appointments, and promote education about risk factors and a church health navigator to assist with app utilization and track screenings and follow-up appointments would need to be primary intervention components.

Conclusions: Findings from the taskforce focus groups highlight the need for multi-component church-based interventions that include a mHealth app and church health navigators to improve awareness and adherence to breast/cervical cancer screening recommendations and follow-up among African American female church populations.

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KANSAS JOURNAL of MEDICINE

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Frontiers Clinical and Translational Pilot Research Grant Recipient

RITUXimab IMmunogenicity in ANCA-associated Vasculitis (RITUXIMAV)

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ABSTRACT

Introduction: Rituximab (RTX) is an effective maintenance therapy in forms of ANCA-associated vasculitis (AAV), specifically granulomatosis with polyangiitis (GPA) and microscopic polyangiitis (MPA) (MAINRITSAN trial). Sixteen percent of patients will relapse despite RTX. Infliximab, an anti-chimeric antibody like RTX, is associated with a high rate of anti-drug antibody formation which effects its efficacy. The aims of this study are to a) evaluate the incidence of immunogenicity toward rituximab in AVV; and b) evaluate the relationship between anti-RTX antibodies and drug response. Herein we present the baseline data.

Methods: Inclusion criteria: a) meet either 1990 ACR and/or 2012 revised CHCC criteria for GPA or MPA; b) remission at entry; c) received Rituximab in last 12 months; d) plan to continue Rituximab; and e) no contraindications for receiving further RTX infusions.

Results: 30 participants (25 GPA and 5 MPA) were enrolled. ANCA by EIA was MPO positive in 14 (47%), PR3 positive in 13 (43%) and negative in 3 (10%). Mean age at enrollment was 62.5 years (IQR 46.5 - 67.5). 70% were female. The average immunoglobulin G (IgG) at baseline was 663 mg/dl (95% CI 566 - 760). 67% of participants had undetectable B-cells at baseline. Average time between the last Rituximab infusion and the first study Rituximab infusion was 34 weeks (95% CI 28 - 40 weeks).

Conclusions: At study baseline most of the participants were found to be B-cell depleted and mildly hypogammaglobulinemic. The average duration since the last Rituximab infusion at entry (8.5 months) was slightly longer than in the MAINRITSAN trial (six months).

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KANSAS JOURNAL of MEDICINE

Institute for Advancing Medical Innovation (IAMI) Trailblazer Award Recipient

Open-Label Pilot Study of Ranolazine for Cramps in Amyotrophic Lateral Sclerosis

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ABSTRACT

Objective: To determine safety and tolerability of two doses of ranolazine in patients with ALS and evaluate for preliminary evidence of drug-target engagement.

Background: Ranolazine, an FDA-approved drug for angina which inhibits the late Na+ current and intracellular Ca2+ accumulation, may be neuroprotective in ALS and reduce cramp frequency by reducing neuronal hyperexcitability.

Design/Methods: Open-label dose-ascending study of ranolazine with 12 participants in two sequential cohorts: 500 mg and 1,000 mg orally twice daily. Each has a two-week run-in period, four-week drug administration and six-week safety follow up. Primary outcome is the frequency of dose limiting toxicities and adverse events. Exploratory measures include cramp frequency and severity, fasciculation frequency (on ultrasound), cramp potential duration, ALS Functional Rating Scale-revised (ALSFRS-r), and forced vital capacity (FVC) compared from baseline to week six.

Results: The 10 currently enrolled patients (Cohort 1, n = 6; Cohort 2, n = 4) are mostly male (80%), and middle aged (median 53 years). There were no serious adverse events. One subject in cohort 2 discontinued the drug due to constipation. The most frequent drug-related adverse event was gastrointestinal (38%). Cramp frequency reduced by 44% (p = 0.01) and severity decreased by 36% (p = 0.002), with decreased awakening due to cramps and cramps interfering with daily activities or causing activity avoidance. Fasciculations, cramp potential duration, ALSFRS-r and FVC did not change.

Conclusion: Ranolazine was well tolerated in ALS up to 2,000 mg daily with gastrointestinal side effects being the most frequent. Ranolazine reduced cramp frequency and severity without significant effect on fasciculations, ALSFRS-r or FVC.

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KANSAS JOURNAL of MEDICINE

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Frontiers Infrastructure Award Recipient

Skin Carotenoid Measurement: A Biomarker for Dietary Fruit and Vegetable Intake

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ABSTRACT

Dietary intake of fruits and vegetables is consistently related to chronic disease risk. Unfortunately, measurement of fruit and vegetable intake is most often by self-report and thus, there are concerns about the validity of the data. The Veggie Meter (VM) uses Resonance Raman light scattering spectroscopy (RRS) to non-invasively measure skin carotenoid content. Skin carotenoids have recently been reported as a reproducible and valid biomarker for dietary intake of fruits and vegetables. In August of 2017, we purchased a VM and began using it in several studies. Due to demand, we were unable to accommodate all studies. Thus, in July 2018 Frontiers provided funds to purchase another VM. To date, we have used VM in seven completed pilot studies (six interventions and one observational study) and are using it in three ongoing studies. Specifically, the Frontiers-purchased machine is being used in two NIH-funded clinical trials. It is also used in community outreach events. The average age of individuals screened using this VM is 74.9 years and the VM score is 281.9 ± 70.1, indicating poor intake similar to the national average. Within 54 healthy older adults, VM scores correlated with intake of vegetables ($r2 = 0.2$, $p = 0.001$), fruits and vegetables ($r2 = 0.15$, $p < 0.01$), and four individual carotenoids ($r2 > 0.18$ & $p < 0.01$ for all) derived from self-reported 3-day food records. The VM is quick, non-invasive, and easy to use. It provides an objective measure of fruit and vegetable intake and will continue to be incorporated into our nutrition clinical trials and observational studies.

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KANSAS JOURNAL of MEDICINE

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Cognitive Function and Relationships with Intervention Dropout, Adherence and Weight Loss

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ABSTRACT

Objective: Greater cognitive function (CF) is associated with adherence to prescription medications, better program adherence and weight loss (WL) following bariatric surgery. The purpose of this study was to evaluate the association between baseline CF, intervention dropout, adherence and three-month WL.

Methods: One hundred and seven (Mean age = 40.9 yrs.), overweight/obese (BMI = 35.6 kg/m2) men (N = 17) and women (N = 90) completed a three-month WL intervention. Participants were asked to attend weekly behavioral sessions, comply with a reduced calorie diet and complete 100 min of moderate intensity physical activity (PA)/wk. CF tasks including Flanker (attention), Stroop (Executive control) and working memory, body weight and cardiovascular fitness (covariate) were assessed at baseline and three-months. Session attendance, adherence to PA and diet prescriptions and number of off-diet episodes were recorded weekly.

Results: Results indicated that attention was positively correlated with session attendance ($p = 0.016$), adherence to the diet ($p < 0.01$) and PA ($p = 0.023$). Executive control was positively correlated with WL ($p = 0.042$). Working memory (two tasks) was also positively correlated with WL ($p = 0.017$ and $p = 0.025$). Analysis of variance (ANOVA) indicated that baseline attention ($p = 0.012$) was positively related to WL and negatively associated with drop out ($p < 0.05$). Hierarchical linear regression showed executive control ($p = 0.036$, R2 = 0.054) and working memory ($p = 0.013$, R2 = 0.073 and $p = 0.017$, R2 = 0.068) were associated with WL when controlling for fitness.

Significance: These results suggest that stronger baseline attention is associated with completion of a three-month WL intervention. Executive control and working memory are associated with amount of WL achieved. Additional, larger and longer trials to assess the role of baseline CF on WL and evaluating the impact of interventions designed to improve CF on WL are indicated.

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KANSAS JOURNAL of MEDICINE

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Adolescent Mothers' Early Breastfeeding Experiences

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ABSTRACT

Introduction: Adolescent mothers are less likely to initiate and sustain exclusive or partial breastfeeding. About 70% of mothers under age 20 initiate breastfeeding, compared to 80% of 20 - 29-year-old mothers. Duration of breastfeeding to six months and one year by young mothers is 28% and 18%, compared to 48% and 28% in 20 - 29-year olds. Early breastfeeding experiences can adversely influence duration of breastfeeding. This study describes the early postpartum breastfeeding experiences among mothers 16 - 19 years of age.

Methods: This is a descriptive study within a larger pilot clinical trial. Four to eight weeks after giving birth, participants completed the Breastfeeding Experience Scale (BES), a 30-item measure which documents breastfeeding experiences, feeding patterns, and weaning. Severity of 18 early experiences are rated on a Likert type scale of 1 - 5 (1 = "not at all" to 5 = "unbearable"). Cronbach's alpha for the 18-item summated scale of the BES was 0.80. Descriptive statistics characterized experiences for this in-progress study.

Results: Thirty breastfeeding participants, ages 16 - 19 (X = 17.97, SD = 0.96) completed the BES. Twenty-one (70%) were breastfeeding at time of survey and 57% were doing so exclusively. The most frequently reported breastfeeding problems were engorgement and leaking which were rated mild to moderate in severity (X = 2.97, SD = 1.03). Nine mothers (30%) weaned between 14 and 30 days after birth; engorgement, latching difficulty, and drop in milk supply were most common reasons.

Conclusions: Most teenage mothers in this sample maintained breastfeeding past one month after birth and reported few problems.

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KANSAS JOURNAL of MEDICINE

Institute for Advancing Medical Innovation (IAMI) Trailblazer Award Recipient

A Therapeutic Trial Validating the Physiologically Based Pharmacokinetic Simulation of the Dose-Exposure Relationship of Metformin in Young Children with Insulin Resistance or Type 2 Diabetes Mellitus

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ABSTRACT

Introduction: Obesity affects ~14 million children/adolescents in the U.S. (8.9% of 2 - 5-year-olds; 17.5% of 6 - 11-year-olds) and increases the risk of developing type 2 diabetes (T2DM). Early metformin intervention may delay or halt progression to T2DM. However, clinical response to metformin in children with insulin resistance or T2DM is unpredictable. Additionally, no dosing recommendations exist for children < 10 years. Preliminary data show that clinical response to metformin may depend on the amount of metformin present in the body over time (systemic exposure), with maximum response observed at exposures = 30 mg/L*h. Modelling and simulations can help determine individualized doses to achieve this exposure, ideally achieving favorable response for every patient. This study aims to prospectively validate simulations of the metformin dose-exposure relationship in young children.

Methods: A 24-hour pharmacokinetic study (measuring systemic exposure) of 500 mg metformin in children (6 - 12 years) with IR. Pharmacokinetic simulations including individual parameters (e.g., sex, age, weight) were conducted for each subject using Simcyp, a physiologically-based pharmacokinetic simulator.

Results: Two out of 12 subjects have completed the research study: Subject 1: Female, 12 years, 158.6 cm, 70.2 kg, BMI: 97.44 percentile, HbA1C: 6.7%; simulation-recommended dose: 1750 mg. Subject 2: Male, 8 years, 134.9 cm, 36.5 kg. BMI 95.58 percentile, HbA1C: 6.5%; simulation-recommended dose: 1100 mg. Clinical pharmacokinetic data is not yet available.

Conclusion: Individualized doses of metformin may benefit young children with IR. Smaller/younger patients may require smaller doses of metformin than larger/older children; clinical pharmacokinetic data is needed to support simulation-guided individualized dosing.

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KANSAS JOURNAL of MEDICINE

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Targeting RNA-Binding Protein HuR in Human Liver Cancer

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ABSTRACT

Introduction: Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide and has one of the worst one-year survival rates of any cancers. Understanding molecular mechanisms leading to HCC and developing novel strategies for effective prevention and treatment are urgent medical needs. HCC arises exclusively on the background of chronic liver injury and inflammation. Thus, an attractive therapeutic approach is to target key genes that regulate both inflammation and oncogenic pathways in HCC. RNA-binding protein Hu Antigen R (HuR) is one such candidate that controls the translation of multiple genes involved in inflammation and oncogenic process. The objective of this study is to characterize HuR targets in human HCC and test HuR-targeted intervention for HCC treatment.

Methods: HuR knockout (HuR-KO) in HCC cell line MHCC97H cells was accomplished by CRISPR/Cas9 gene editing system. The transcriptome comparison identified 1,516 differentially-expressed genes between HuR-KO cells and wild type controls. The top pathways altered by HuR knockout were cell proliferation, hippo signaling, beta integrin, pluripotent stem cells, EGFR, WNT, and FAK-PI3K-Akt-mTOR. To test HuR-targeted intervention for HCC treatment in vivo, MHCC97H cells were subcutaneously injected into athymic nude mice to establish HCC xenografts. Mice were then treated with a novel synthetic HuR inhibitor KH-3 for four weeks.

Results and Conclusions: Strikingly, KH-3 treatment significantly inhibited the growth of HCC xenografts. Taken together, our study provides a foundation for understanding the role of HuR in HCC. The mechanistic insights elucidated from our study may pave the way for developing therapeutic strategies to specifically target HuR for HCC treatment.

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