

Award Number: W81XWH-10-1-0894

TITLE: Multicenter Clinical Trial of Keratin Biomaterial for
Peripheral Nerve Regeneration

PRINCIPAL INVESTIGATOR: Zhongyu (John) Li, MD, PhD

CONTRACTING ORGANIZATION:
Wake Forest University Health Sciences
Winston-Salem, North Carolina 27157

REPORT DATE: December 2015

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT:

X Approved for public release; distribution unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.					
1. REPORT DATE : December 2015		2. REPORT TYPE Final		3. DATES COVERED 15Sep2010 - 14Sep2015	
4. TITLE AND SUBTITLE Multicenter Clinical Trial of Keratin Biomaterial for Peripheral Nerve Regeneration				5a. CONTRACT NUMBER W81XWH-10-1-0894	
				5b. GRANT NUMBER OR090621	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Li, Zhongyu (John), MD, PhD; Koman, L. Andrew, MD; Van Dyke, Mark E., PhD; Smith, Beth P., PhD E-Mail: zli@wakehealth.edu				5d. PROJECT NUMBER W912SQ0122N652	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Wake Forest University Health Sciences Medical Center Boulevard Winston-Salem, NC 27157				8. PERFORMING ORGANIZATION REPORT NUMBER 33-150040-00000-101-13224	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
13. SUPPLEMENTARY NOTES					
14. ABSTRACT Common injuries sustained by war fighters in Iraq and Afghanistan result from blast and shrapnel wounds which cause extensive damage to skin, bones, and nerves. The management of damaged peripheral nerves is challenging for patients and surgeons. Peripheral nerve transection injuries may fail to regenerate even when managed surgically. These injuries are associated with long term disability and impaired function. Nerve guidance conduits have been developed for use in surgery to bridge the gap between transected nerve ends and to support nerve regeneration. A team of scientist and clinicians at Wake Forest School of Medicine has developed a keratin biomaterial hydrogel that can be used as luminal filler in nerve guidance conduits to facilitate nerve regeneration. Studies in mice, rabbits, and nonhuman primates have established the feasibility of this keratin hydrogel to promote nerve regeneration. A Phase I prospective, randomized trial was designed to compare nerve regeneration in patients sustaining peripheral nerve injuries treated either with keratin hydrogel and a nerve conduit or with a nerve conduit alone. The clinical trial was to be initiated as soon as the FDA provided an IND for the keratin biomaterial hydrogel. However, due to delays in the FDA approval process, the clinical study was not initiated. However, a fate and distribution study demonstrated that C ¹⁴ labeled keratin hydrogel remained in high concentrations at the site where it was implanted intramuscularly.					
15. SUBJECT TERMS Peripheral nerve transection, nerve conduit, nerve repair, keratin biomaterial hydrogel, clinical trial					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT U	b. ABSTRACT U	c. THIS PAGE U			19b. TELEPHONE NUMBER (include area code)
			UU	17	

Table of Contents

	<u>Page</u>
Introduction.....	4
Body.....	4
Key Research Accomplishments.....	5
Reportable Outcomes.....	9
Conclusion.....	10
References.....	11
Appendices.....	NA

INTRODUCTION

While body armor, advanced resuscitation techniques, rapid transportation of wounded soldiers, and damage control surgery have made major contributions to saving lives on the battlefield, more can be done to improve the outcomes for soldiers who sustain extremity injuries such as traumatic nerve transections. Extremity trauma with nerve injury can be associated with long term functional limitations and impairments (Rosen 2000; Ruijs 2005). Damaged peripheral nerves may fail to regenerate in patients even when they are managed by surgical intervention. The use of sensory nerve autografts for nerve repair is considered to be the “gold standard”; however, this procedure is associated with donor site morbidity and the possibility that there may be insufficient donor nerve available for extensive repairs involving several nerves (Lohmeyer 2009).

To avoid the issues associated with the use of autografts, nerve guidance conduits have been developed to bridge the gap between the transected nerve ends and to support nerve regeneration (Taras 2008). A team of scientists and clinicians at Wake Forest School of Medicine has developed a keratin biomaterial hydrogel that can be used as luminal filler in nerve guidance conduits in order to facilitate nerve regeneration. Preliminary studies in mouse, rat, rabbit and non-human primate models have established the feasibility and clinical relevance of using a keratin biomaterial filler to promote nerve regeneration (Apel 2008, Hill 2011, Lin 2012, Sierpinski 2008, Pace 2013, 2014).

The objective of the proposed clinical trial was to conduct a combined Phase I prospective, randomized, two center trial that would follow patients treated for traumatic peripheral nerve transections. Patients would have been randomized to one of two treatment groups in order to compare nerve regeneration following two methods of nerve repair. One group of patients would have undergone nerve repair using the keratin hydrogel as filler for a commercially available nerve conduit, and the other group would have undergone nerve repair using nerve conduit alone. This trial also was designed to document the safety of the keratin hydrogel. The specific aim of the clinical trial was to determine the safety and efficacy of keratin hydrogel biomaterial as lumenal filler in nerve conduits used to manage traumatic peripheral nerve transection.

BODY

On June 8, 2010, a pre-IDE package describing the keratin hydrogel was submitted to the FDA for review. An independent IRB organization (Copernicus Group Independent Review Board, Research Triangle Park, NC) granted conditional approval for the proposed clinical trials using keratin hydrogel in October 2010 with final approval to be granted as soon as the FDA provided an IDE designation for the keratin hydrogel. Feedback from the FDA in October 2010 indicated that their main concern was whether the keratin hydrogel should be classified as a device or a biologic. A request for designation (RFD) was submitted to the FDA in May 2011; in July 2011, the FDA designated the keratin hydrogel as a “therapeutic biological product.” The product was assigned to the Center for Drug Evaluation and Research (CDER) as the lead agency for premarket review and regulation. However, the FDA remained uncertain about how to coordinate our request for the nerve application for the keratin hydrogel given that there was a co-pending application for a keratin product for use in burn patients. Therefore, the FDA scheduled a meeting for March 2012 to finalize recommendations on the designation of the keratin hydrogel. In May 2012, Dr. VanDyke and Dr. Burnett (KeraNetics) met at the FDA with

representatives from the Center for Drug Evaluation Research (CDER), the Center for Biologics Evaluation Research (CBER) and the Center for Devices and Radiological Health (CDRH) to clarify the designation of the hydrogel. As a result of this meeting, steps required for an IND for the keratin hydrogel were discussed including the requirement for purity and potency assays and a fate and distribution study (FDS). Plans were made to complete these tests because we believed that they would be required regardless of the IND or IDE designation provided by the FDA.

A revised statement of work (SOW) and budget were submitted to CDMRP to transfer the funds necessary to complete the FDA-required testing. The request was approved in July 2014. Conversations with Toxikon, a contract research organization were initiated to develop a protocol to complete the FDS. The purpose of the study was to determine the fate and distribution of C14 labeled keratin hydrogel following intramuscular implantation of the hydrogel in Sprague Dawley rats. Approximately 0.08 ml of the labeled hydrogel was implanted intramuscularly in the quadriceps muscle. The animal care and use protocol developed at Toxikon included eight rats to perform a preliminary FDS. Perkin Elmer was designated as the company to prepare the radio-labeled keratin hydrogel.

During this time, KeraNetics filed two additional patents describing a keratin hydrogel that differed enough from the original product to argue for a new request for designation application for the new product to submit to the FDA to request an IDE as a device.

In June 2014, keratin hydrogel (the new product) was shipped to Perkin Elmer in order to label the hydrogel with C¹⁴ for the fate and distribution study. In February 2015, eight rats were implanted with the C¹⁴ keratin. Animals were humanely euthanized at days 1, 3, 5, 7, 10, 15, 21, and 30 days after surgery. The specimens were frozen in a dry ice hexane bath and were prepared for analysis by quantitative whole body autoradiography techniques.

No abnormalities were noted during daily clinical observations of the rats. A marked low level of radioactivity was detected in all tissues on Day 3. This finding was most likely attributed to the irregularity in the amount of test article that was implanted during the surgical procedure and the escape of the keratin hydrogel into the whole fatty inguinal tissue.

Whole radiography indicated that the C¹⁴ keratin hydrogel-derived radioactivity C max in tissues ranged from 342nCi/g in the kidney cortex (Day 1) to 0.117nCi/g in the lens (Day 3). The majority of peak tissue concentrations were observed on Day 1 after dosing, except for the whole brain, brain stem, cerebellum, cerebrum, medulla oblongata, seminal vesicle, whole spinal cord, testes, urinary bladder wall, and white fat at Day 5. The peak radioactivity concentrations occurred at Day 7 in the cerebrospinal fluid and olfactory lobe.

There was rapid absorption phase at all-time points based on the majority of peak tissue radioactivity concentrations observed the first day after implantation of the test material. The pattern of radioactivity distribution after the single intramuscular dose of keratin hydrogel was consistent with the uptake of the test article primarily by the urinary tract and gastro-intestinal tract involved in the metabolism and excretion of the C¹⁴ keratin hydrogel. In addition, the endocrine and secretory tissues demonstrated preferential uptake of the test article.

At the site of implantation in the quadriceps muscle, the concentration of the keratose nerve formulation remained between 1033 n Ci/g and 2697 n Ci/g at all-time points. This finding demonstrated that the radiolabeled test article tended to remain in high concentrations at the site where it was implanted intramuscularly. The concentration of labeled keratin hydrogel in the muscle was 4 to 10 times more concentrated than in all other tissues combined at each experimental time point.

KeraNetics will use the preliminary fate and distribution study to design a larger fate and distribution study. The pilot FDS supported KeraNetics belief that the keratin hydrogel should be designated as a device since the majority of the radioactivity remained at the site of implantation even 30 days after the material was implanted. The radioactivity that was observed throughout the body was probably C¹⁴ not attached to the keratin hydrogel protein. Specifically, the radioactivity located in the brain was probably unattached C14 since the molecular weight of the keratin hydrogel would prevent it from crossing the blood brain barrier.

KeraNetics will pursue their work with the FDA to obtain an IDE for the keratin hydrogel, in which case the clinical trial using keratin hydrogel for peripheral nerve repair can be completed. If the FDA decides that the keratin hydrogel is a drug or biologic agent and requires an IND, KeraNetics has a low level of interest in pursuing the clinical trials necessary to obtain FDA approval of this product for supporting the repair of peripheral nerve injuries.

The final amended report from Toxikon dated September 29, 2015 is included as an attachment. A summary of the report is included on page 4 of this document.

KEY RESEARCH ACCOMPLISHMENTS

- Pre-IDE package submitted to the FDA, June 8, 2010.
- September 28, 2010: Submission of the clinical protocol to the Copernicus Group, an independent IRB located in Research Triangle Park, North Carolina. Due to the conflict of interest policies at the Wake Forest School of Medicine, Dr. Li was asked to send the protocol to an independent review board.
- October 13, 2010: The Copernicus Group granted conditional approval of both Phase 0 and Phase I/II protocols pending the assignment of an IDE number or confirmation of 510K justification acceptance from the FDA.
- October 18, 2010: FDA feedback provided to Dr. Li and Dr. Van Dyke indicated that the FDA's main concern was whether the keratin hydrogel should be classified as a device or a biologic. The FDA suggested that a meeting between the FDA and Drs. Li and Van Dyke should take place; however, the primary reviewer at the FDA was unable to identify a date for a meeting.
- A Request for Designation (RFD) was submitted to the FDA in May 3, 2011. Based on this submission date, the FDA had until July 9, 2011 to reply to the request.
- July 7, 2011: The FDA designated the keratin hydrogel biomaterial as a "therapeutic biological product." The product has been assigned to the Center for Drug Evaluation and Research (CDER) as the lead agency for premarket review and regulation based on the keratin hydrogel's primary mode of action.
- On July 18, 2011, there was a conference call with Miriam Darnell, PhD, Science Officer for Grants Management and the investigators at Wake Forest (Zhongyu Li, MD, PhD, Mark Van Dyke, PhD, and Beth Paterson Smith, PhD). During the call, the submission of the pre-IND package to the FDA and the scheduling of a pre-IND meeting with the FDA were discussed. In addition, the expected request by the FDA for a fate and distribution study of the keratin hydrogel was discussed. Because funding will be required to complete these studies, possible funding sources were discussed.
- July 25, 2011: Dr. Darnell sent an email regarding the possibility of re-budgeting the grant funding to cover the estimated \$250,000 required for the fate and distribution studies in an

animal model. Dr. Darnell requested a written statement describing the anticipated animal study, the requirement for the study, the study timeline, and any other pertinent information. These issues also need to be discussed with Ms. Susan Dellinger, the USAMRAA Grants Officer who has the final authority on issues of statement of work and budgets.

- July 25, 2011: Dr. Van Dyke responded to Dr. Darnell's email to provide information that a contract research organization (CRO) had been contacted regarding a quote to cover the costs of the fate and distribution study. Dr. Van Dyke also outlined the reasons why he requested a representative from CDMRP be present at the pre-IND meeting with the FDA.
- July 26, 2011: Dr. Van Dyke sent an email to Dr. Darnell describing the fate and distribution studies including the four to six month period needed to complete the study at a cost of approximately \$250,000.
- August 1, 2011: An email was sent to Brian Garland, Administrative Coordinator of the Human Research Protection Office at USAMRMC containing the June 23, 2011 Clinical Trial Quarterly Technical Progress Report to provide him with the status of our progress on the clinical trial.
- August 8, 2011: The request for a pre-IND meeting with the FDA was submitted.
- On August 19, 2011, Dr. Darnell sent an email to Christopher Baker, CIV USA MEDCOM USAMRAA regarding the request for re-budgeting to cover the costs of preclinical animal studies to determine the fate and distribution of the keratin hydrogel. On August 23, 2011, Mr. Baker requested a revised budget and statement of work for consideration.
- Beginning August 23, 2011, we worked with our Office of Research to develop the re-budgeting plan and statement of work required to complete the keratin hydrogel fate and distribution studies.
- August 31, 2011: The FDA sent a letter providing the date for the pre-investigational new drug application of KeraGenics Nerve. The meeting was scheduled for November 8, 2011 from 12:00-1:00 p.m. in Silver Spring, Maryland. Miriam Darnell, PhD the Science Officer for Grants Management and LTC(P) Leggit, the director of CDMRP agreed to attend this meeting.
- October 7, 2011: The Type B meeting package for KeraNetic's KeraGenics™ Nerve (PIND No. 113077) was sent to Ms. Daughterty at the FDA.
- October 31, 2011: The attorneys at Hogan Lovells received a telephone call from the FDA cancelling the FDA meeting scheduled for November 8, 2011. This meeting cancellation occurred because the FDA was uncertain about how to coordinate our request for the nerve application for the keratin hydrogel given that there was a co-pending application for a keratin product for use in burn patients. The FDA determined a path for the burn device and is now working on the designation of the nerve application to be used in our clinical trial to study nerve regeneration. An internal FDA meeting was scheduled for January 8, 2012. After this meeting, feedback is expected regarding our request for designation of the keratin hydrogel for use in nerves.
- March 9, 2012: Dr. Van Dyke emailed Dr. Darnell to update her on the conversations he had with the FDA. The FDA is involved in internal discussions regarding the designation of the keratin biomaterial hydrogel. The FDA has scheduled a meeting for March 26, 2012 to finalize recommendations on the designation of the keratin biomaterial.
- May 1, 2012: Dr. Van Dyke and Dr. Luke Burnett (KeraNetics) met at the FDA with representatives from the Center for Drug Evaluation Research (CDER), the Center for Biologics Evaluation Research (CBER), and the Center for Devices and Radiological Health

(CDRH) to clarify the designation of the keratin hydrogel. During this meeting, the parties agreed on the next steps required for an IND package for the keratin biomaterial hydrogel.

- a. The subcutaneous injection trial (Phase 0) was eliminated from the trial design. The FDA determined that the Phase 0 trial was unnecessary.
 - b. Preparation of the keratin hydrogel for use in the clinical trial was discussed. The nerve conduits will be prefilled with keratin; these prefilled conduits will be lyophilized and packaged for terminal sterilization. The use of the prefilled conduits will allow the surgeon to rehydrate the conduit a few minutes prior to implantation. The FDA agreed that this preparation was appropriate because they prefer terminal sterilization of products.
 - c. The FDA agreed on the following purity and potency assays to be completed before beginning the Phase I clinical trial: 1) analytical tests to determine purity (size exclusion chromatography for molecular weight, amino acids analysis, ELISA for protein identification, and gel rheology) and 2) a cell adhesion assay using a rat Schwann cell line to determine the potency of the hydrogel.
 - d. The FDA discussed their preferred experimental design for preclinical animal testing. FDA agreed to review the preclinical data from KeraNetics.
 - e. The FDA agreed on the design of the fate and distribution study. Labeled keratin gel will be placed inside nerve conduits. The ends of the conduits will be closed, and the conduits will be implanted in rat muscle. The rats will be followed to determine the fate and distribution of the labeled keratin biomaterial hydrogel. Depending on the outcomes of this study, additional pharmacokinetic studies may be warranted. The FDA will review the results of the fate and distribution study and will determine if any additional studies will be required.
- May 31, 2012: A revised SOW and budget to reflect the extra funds needed to complete the testing required by the FDA were developed. Wake Forest agreed to provide funding up to the difference of \$107,244 between the total costs of the required studies (\$363,244) and the \$256,000 available from the CDMRP. A letter confirming this arrangement between CDMRP and Wake Forest School of Medicine was sent to Dr. Darnell. In addition, documents were provided to document the breakdown of costs, the timeline for performance of preclinical work for the FDA, and the cost sharing information provided by KeraNetics.
 - June 29, 2012: A request was submitted to CDMRP requesting additional funding to perform the purity and potency assays and the fate and distribution studies on the keratin hydrogel
 - July 22, 2013: A conference call was made to Miriam R. Darnell, PhD by Zhongyu Li, MD, PhD, L. Andrew Koman, MD and Beth Paterson Smith, PhD. Drs. Koman, Li, and Smith expressed their concerns regarding the designation of the keratin hydrogel as a drug versus a device. These concerns are related to the time it has taken the FDA to determine the designation of the keratin hydrogel based on the FDA's response to the keratin used for burn applications versus keratin used for nerve regeneration. Therefore, Drs. Li, Koman, and Smith asked for assistance from Dr. Darnell's group for moving the FDA process forward in order to identify a pathway for designation of the keratin hydrogel for use in the proposed nerve studies.
 - July 25, 2013: Dr. Darnell sent an email to Drs. Li, Koman, and Smith with information that she had a discussion with a regulatory expert at another agency at USAMRMC about the proposed study and the FDA regulatory pathway. He was given documents and will provide

his assessment on the information. Dr. Darnell also requested documentation from Drs. Li, Koman, and Smith to support the continued relevance of repairing a two cm nerve gap.

- August 1, 2013: An email was sent to Dr. Darnell with an attachment containing a summary of the recent literature on nerve repairs and information regarding the question about the relevance of repairing a two cm nerve gap.
- August 6, 2013: An email was sent to Dr. Darnell that included several points regarding FDA designation from Mark Van Dyke, PhD.
- Further work on the clinical trial cannot be performed until clarification regarding the designation of the keratin hydrogel is provided to Dr. Li and his research team by the FDA.
- October 2, 2013: Dr. Darnell sent an email to Drs. Li, Koman, and B. Smith that included a summary of the discussion that occurred during a review of Dr. Li's clinical trial by the Tri-Service Chairs. The first part of the document included a summary of the research study and a description of the various hurdles that KeraNetics and Dr. Li have encountered working with the FDA. The document also included specific directives from the Tri-Service Chairs that need to be addressed with a plan for a course of action to resolve the issues with the FDA. The summary also included information from Dr. Robert Miller at the Division of Regulated Activities and Compliance. Dr. Darnell also included the recommendations that she sent back to the Tri-Service Chairs regarding potential actions by Dr. Li and KeraNetics to move the project forward. Based on this document, Dr. Li and his team are expected to provide a response and a solution to move the project forward.
- October 16, 2013. Conference call with the Toxikon Corporation, Dr. Beth Smith, and Dr. Luke Burnett, the chief science officer at KeraNetics: Toxikon is the contract research organization that will be performing the keratin hydrogel fate and distributions studies. Toxikon indicated that the radiolabelling of the keratin would be performed outside Toxikon. The representatives from Toxikon agreed to set up a conference call so that Toxikon, Dr. Burnett, and Dr. Smith could discuss the radiolabelling process and the cost of both the radiolabelling and fate and distribution studies.
- October 21, 2013: Dr. Darnell participated in a conference call with Drs. Li, Koman, and B. Smith: During this conference call, it was decided that preclinical work further characterizing the keratin hydrogel is required regardless of whether the FDA designates the keratin hydrogel as a drug or a device. It was decided that based on the recommendations provided by the Tri-Service Chairs, Dr. Miller, and Dr. Darnell, Dr. Li and his team would submit: 1) a comprehensive revised statement of work including a description of the preclinical work requested by the FDA and 2) a revised budget to cover the costs of the revised statement of work. In addition, they will provide documentation regarding the availability of keratin hydrogel and documents supporting the partnership between Dr. Li at Wake Forest and KeraNetics. Dr. Darnell explained that the revised statement of work and budget should be submitted to Ayi Ayayi, the USAMRAA Contract Specialist assigned to this project. Mr. Ayayi has the authority to approve changes in the statement of work and the budget for Dr. Li's award.

In addition, it was agreed that the revised statement of work would include information about a proof of concept clinical trial that would take place after the FDA is provided with the appropriate preclinical data. This proof of concept trial would be dependent on the understanding that the FDA may request changes in the proposed trial based on the outcomes of the fate and distribution studies. Dr. Darnell stressed that the revised statement of work and budget must be forwarded to Mr. Ayayi as soon as possible.

- November 4, 2013. Conference call with Toxikon, Dr. Burnett, and Dr. B. Smith: Representatives from Toxikon indicated that Perkin Elmer would be responsible for radiolabeling the keratin hydrogel. Before talking to Perkin Elmer, the representatives from Toxikon asked for additional information from Dr. Burnett and Dr. B. Smith regarding the properties of the keratin hydrogel, *i.e.* how it would be used clinically, and the most appropriate animal model to use for the fate and distribution studies. Rats were suggested as the animal model. Toxikon requested that Dr. Li send them nerve conduits from Integra that they could use to fill with the radiolabelled keratin hydrogel. Toxikon also asked questions about the clinically relevant dose of keratin hydrogel that would be used for the implantation of the conduits and the time required for the keratin to degrade. Toxikon requested that this additional data be provided to them before they would be able to finalize the fate and distribution study protocol. Dr. Burnett and Dr. Smith agreed to provide this information at a follow-up teleconference. In addition, they agreed to provide the nerve conduits.
- November 20, 2013: Follow-up teleconference with Toxikon, Dr. Burnett, and Dr. Smith: Thomas L. Smith, PhD was asked to participate in the conference call because of his experience in several animal model studies using keratin hydrogel-filled conduits for peripheral nerve repair. Perkin Elmer is ready to discuss the process for radiolabelling the keratin once information about the dosage of keratin is determined. There also was a discussion about the best way to implant the keratin hydrogel. It was decided that the keratin filled conduits should be buried between two muscles in a fascial plane in order to replicate the clinical scenario. Questions remained regarding the expected amount of time required for the keratin hydrogel to degrade. Based on previous nerve repair studies using conduits and keratin hydrogel in nonhuman primates, it is known that the keratin is gone one year after peripheral nerve repair using a conduit filled with keratin hydrogel. However, information on the presence of hydrogel in the conduits at shorter periods after nerve repair is not available. Toxikon proposed a pilot study to follow animals and collect samples at 1, 3, 7, 14, 21, and 28 days after implantation of the conduits in order to obtain preliminary information on keratin hydrogel degradation rates. The dose of keratin to be used will be estimated by measuring a piece of silastic tubing with the same diameter and length as the Integra nerve conduits and determining the volume of the conduit.
- November 22, 2013: Discussion with Perkin Elmer, Toxikon, Dr. Burnett, Dr. B. Smith, and Dr. T. Smith regarding radiolabelling of the keratin hydrogel: The estimated volume of keratin to be implanted was calculated to be 15 mg. Dr. Burnett described the keratin hydrogel as an extracted family of proteins with multiple reactive sites on the molecule. Keratin is soluble in water but not in salts or organic solvents. Perkin Elmer suggested implanting two conduits in each rat to ensure that there would be sufficient radiolabelled material for successful completion of the fate and distribution studies. They will acetylate the keratin hydrogel using C14 as the label. They will label a test batch of keratin hydrogel and discuss the results with Dr. Li and Dr. Burnett.
- December 2, 2013: Discussions with Luke Burnett, Dr. Li, and Dr. B. Smith regarding fate and distribution studies and revised budget. Once the scope of work and price quote from Toxikon is finalized, the revised statement of work and budget will be submitted to Mr. Ayayi. We will also send Mr. Ayayi the letter of support from KeraNetics.
- December 31, 2013: Letter provided by KeraNetics in support of the revised statement of work and budget to be submitted to Mr. Ayayi (Contract Specialist). The letter described

KeraNetics commitment to provide the FDA with the information required for a clinical safety study of the keratin hydrogel.

- January 13, 2014: Beth P. Smith, PhD and Deanna Sizemore (Research Administrative Coordinator, Department of Orthopaedic Surgery) met with Paula Means, Assistant Dean and Institutional Officer, Office of Research, Wake Forest University Health Sciences. Dean Means assisted Dr. Li in the preparation of the revised statement of work and budget and also provided assurances that Wake Forest is committed to moving Dr. Li's grant forward.
- January 23, 2014: Request for a no-cost extension, revised statement of work, project timeline, and budget for the award submitted to Ayi J. Ayayi. Deanna Sizemore sent an email copy of the correspondence that was sent to Mr. Ayayi.
- March 13, 2014: Dr. Smith sent an email to Mr. Garland with an update explaining the submission of the revised documents to Mr. Ayayi on January 23, 2104.
- March 13, 2014: Dr. Darnell sent an email to Drs. Li, B. Smith, and Koman describing a meeting involving Dr. Darnell, Dr. Milutinovich (PRDRP Program Manager), Ms. Susan Dellinger (Grant Officer), and Mr. Ayayi (Contract Specialist). The email described the concerns discussed during the meeting regarding the revised budget and SOW submitted to Mr. Ayayi on January 23, 2014. Dr. Darnell requested a written response to her email by close of business March 18, 2014.
- March 18, 2014: A written response to Dr. Darnell's email of March 13, 2014 was sent to Dr. Darnell and Mr. Ayayi which included a revised timeline and the email from Dr. Luke Burnett, the chief scientific officer at KeraNetics describing the accelerated process they put in place in order to be responsive to moving the study forward.
- March 21, 2014: Conference call with Dr. Darnell, Dr. Milutinovich, Dr. Burnett, and Dr. B. Smith: Dr. Burnett explained the new strategy for getting an FDA ruling on the keratin hydrogel developed by KeraNetics through consultation with their attorneys. The plan is to abandon the current FDA submission that designated the keratin hydrogel as a biologic. The new plan involves submitting a new application to the CDRH for a new product. This new product is the material that is produced in the validated manufacturing facility at KeraNetics. This product differs from the product produced by Dr. Mark Van Dyke. Dr. Burnett filed two patents in 2012 based on the differences in the two products. These subtle differences are enough to argue that a new application can be submitted to the FDA in August/September 2014. The possible risks with this new strategy were discussed. Dr. Darnell asked Dr. Burnett to provide her a document describing the new FDA plan. Dr. Darnell also stressed that the animal testing for fate and distribution should begin as soon as possible. Dr. Burnett stated that KeraNetics is committed to accelerating their timeline in order to be responsive to Dr. Li's project. The plan is to initiate a safety study as soon as the FDA responds to the new request. The safety study will use the keratin produced by KeraNetics. Assuming a positive safety study, a clinical study to determine the efficacy of the keratin hydrogel in promoting nerve repair will be necessary. A new source of funding will be sought to fund the efficacy study.
- March 24, 2014: Dr. Burnett sent a letter to Dr. Darnell outlining KeraNetics' new regulatory strategy for getting FDA approval for the keratin nerve guidance conduit filler to be tested under contract award number W81XWH-10-1-0894.
- April 21, 2014: Dr. Darnell sent Dr. Li an email acknowledging the annual report had been reviewed and accepted.

- April 24, 2014: Email from Mr. Ayayi to Angela Horton requesting further clarification from Angela Horton (Office of Research, Wake Forest) regarding the revised budget and SOW for the requested no cost extension. The information he requested is required before the request can be sent to the GGO for signature/release.
- May 19, 2014: Email from Angela Horton to Mr. Ayayi providing the responses to questions outlined in Mr. Ayayi's April 24, 2014 email.
- June 13, 2014: Julie Hurt, PhD, Scientist, KeraNetics, informed Dr. Li that sterilized keratin hydrogel would be available to send to Toxikon for radiolabelling early in the week of June 23, 2014.
- June 30, 2014: Keratin hydrogel was shipped to Toxikon in order to label the hydrogel with C^{14} for use in a fate and distribution study. The raw material was sent to Perkin Elmer for radio-labelling.
- July 1, 2014: An email was sent to the PI, Zhongyu Li, MD, PhD stating that Modification P00002 was granted to extend the period of performance of the Award OR090621. In addition, the modification approved the incorporation of the revised SOW. The period of performance was extended until 14 September 2015.
- July 31, 2014: The test requisition form for the Single Dose Fate and Distribution Study in Rats using C^{14} Labeled Compound was finalized.
- August 12, 2014: Julie Hurt, PhD at KeraNetics reported that a small amount of keratin (50mg) had been successfully labeled with an estimated specific activity of 153 μCi . The labelling procedure requires the use of a more dilute solution. Therefore, the labeled keratin hydrogel must be concentrated and lyophilized to allow rehydration at a higher keratin concentration. An additional 100 mg of labeled keratin will be prepared to complete the testing protocol.
- August 14, 2014: Draft of the animal protocol for the fate and distribution study to be performed in rats evaluated by KeraNetics and TOXIKON.
- September 3, 2014: Additional keratin hydrogel was shipped to TOXIKON for radiolabelling with C^{14} .
- November 12, 2014: Toxikon began preparation of the IACUC protocol for the fate and distribution study
- November 13, 2014: Keratin powder (250 mg, 95% alpha Keratose, 5% gamma Keratose) was treated with C^{14} -acetic anhydride and subsequently lyophilized. The reaction produced approximately 200 mg of C^{14} -labeled keratin protein with a specific activity of 6.4 $\mu Ci/mg$. This material will be formulated at Toxikon Corporation for implantation into the quadriceps muscle of rats at a dose of 20mg/animal. Animals will be sacrificed at various time points for whole animal autoradiography in order to evaluate the time-dependent bioavailability of the labeled keratin throughout the animal.
- November 17, 2014: A description of the test article and implantation procedure was provided by KeraNetics to Toxikon for their development of the IACUC.
- December 11, 2014: Update sent to Brian Garland, Contractor – ERP International, LLC, and HRPO regarding the status of the IRB for the study protocol.
- January 21, 2015: Final study protocol sent from Toxikon to Beth Smith, PhD, Wake Forest School of Medicine and Julie Hurt, PhD, KeraNetics LLC
- January 28, 2015: The IACUC review included several questions regarding the protocol and requested more specific information regarding the target diseases the compound could

possibly treat or aid in treatment. This information will provide a statement of the potential value of the study in regards to human and animal health.

- January 28, 2015: Dr. Julie Hurt provided the requested information by the IACUC to Toxikon
- February 2, 2015: Dr. Beth Smith received a signed copy of the Toxikon protocol for the keratin fate and distribution study and from Toxikon.
- February 10, 2015: Email from Toxikon stating that the target date for starting the fate and distribution study at Toxikon is February 17, 2015 with formulation of the Keratin on that day. February 18, 2015 is the target date for implantation of the labeled Keratin. A back-up date of March 2, 2015 was also identified.
- February 12, 2015: Email from Dr. Darnell stating that the annual report was reviewed and accepted as written.
- February 17, 2015: The PRORP Steering Committee requested a status report on the clinical trial.
- February 23, 2015: Confirmation from Dr. Darnell that she received the requested status report.
- February 26, 2015: Email from Toxikon reporting that eight rats have been implanted with the keratin hydrogel and post dose procedures are ongoing. The company who will be analyzing the rats is inviCRO. The estimated date of their draft report of the whole body rat autoradiographs is June 1, 2015. At that time, Toxikon will obtain the data required to prepare the report of the study results. The calculated amount of hydrogel implanted ranged from 0.078 to 0.1 ml. A protocol deviation will be prepared noting the amount of test article was approximately 0.08 ml instead of 0.1 ml.
- March 5, 2015: Following discussions between Toxikon and inviCRO, inviCRO stated that they will provide images from the autoradioluminograms as soon as they are ready. Currently, it is time point Day 15 of the study. Since they did not need to euthanize a spare rat, Toxikon requested that KeraNetics direct what should be done with the spare rat.
- March 5, 2015: Dr. Hurt from KeraNetics requested that the spare rat should be euthanized at Day 21 to obtain a three-week post implantation data point.
- March 5, 2015: Toxikon agreed to add the extra time point on Day 21. Toxikon will need to write a Protocol Amendment/Deviation Report (PADR) for signature by Dr. Beth Smith at Wake Forest to add the 21 day time point.
- March 9, 2015: Dr. Hurt from KeraNetics confirmed her request for a Day 21 time point collection.
- March 10, 2015: Dr. Smith returned the signed PADR to Toxikon.
- March 20, 2015: Toxikon preparing to ship rat carcasses to inviCRO for autoradiography analysis. The last animal was euthanized today at time point Day 30.
- April 6, 2015: Sectioning of rat carcasses by inviCRO to start later in the week. The animals had no abnormalities detected by clinical observation throughout the duration of the study. This study is the longest implantation study performed by KeraNetics.
- May 13, 2015: Preliminary progress report sent from Toxikon to KeraNetics.
- June 1, 2015: Request to Toxikon from KeraNetics regarding the availability of updates on the fate and distribution data collection.
- June 30, 2015: The draft of the final report for the Fate and Distribution Study was sent to KeraNetics. Toxikon requested a review of this draft.

- August 31, 2015: Toxikon requested comments from KeraNetics on the draft of the final report of the results from the fate and distribution study.
- August 31, 2015: KeraNetics requested that Toxikon move forward with preparing the final report. Toxikon reported that it would finalize the protocol. A signed PPDR describing consistency of test article was sent to Toxikon.
- September 1, 2015: Final report sent from Toxikon to KeraNetics.
- September 2, 2015: Julie Hurt from KeraNetics requested clarification from Toxikon regarding the specific activity of the keratin material prepared at Perkin Elmer.
- September 2, 2015: Stela Maura from Toxikon responded and agreed to prepare an amended report containing information on the specific activity of the keratin test product used in the fate and distribution study.
- September 23, 2015: Dr. Darnell requested a status update on the project. She requested that members of our team call her on September 24, 2015 for a quick status check. Dr. Beth Smith responded and said that she and Deanna Sizemore, Research Administrative Coordinator, Department of Orthopaedic Surgery would call her at 10:00 on the 24th. Dr. Darnell said that Dr. Melissa Green Parker, PRORP program manager will also participate in the call.
- September 24, 2015: During the conference call involving Dr. Darnell, Dr. Green Parker, Dr. Smith and Deanna Sizemore, Dr. Smith and Ms. Sizemore provided an update on the fate and distribution study performed for KeraNetics by Toxikon. Most of the labeled keratin hydrogel remained at the site of implantation in the quadriceps muscle of the eight rats included in the fate and distribution study. KeraNetics has requested further information from Toxikon regarding the specific activity of the labeled keratin hydrogel.

KeraNetics is working on another keratin product for use for the treatment of radioactive burns and will request an IDE for this product. If the burn product receives an IDE designation, this might provide information that could be submitted to the FDA to get an IDE for the keratin hydrogel formulation for use in peripheral nerve repairs. The IRB for the clinical trial was approved conditionally based on the FDA providing a product designation. Because it has remained unclear what the designation of the keratin hydrogel should be, the IRB could not be approved.

Dr. Smith and Ms. Sizemore confirmed that the pending quarterly reports and final report would be submitted by September 30, 2015.

Dr. Darnell provided details regarding the submission of these reports and requested that Mirlene Desir, CIV USARMY MEDCOM USAMRAA (US) be included to receive reports.

Dr. Darnell provided an email summary of the conference call to Drs. Smith and Green Parker.

Dr. Koman, Chair of Orthopaedic Surgery called Dr. Darnell to explain that it would not be possible to pursue a no-cost extension for the grant due to the issues involved with the FDA providing a product designation for the keratin hydrogel. Dr. Koman cannot predict how long it will take to clarify the designation so the timing to begin a clinical trial would be impossible to predict.

REPORTABLE OUTCOMES

Publications

Apel PJ, Garrett JP, Sierpinski P, Ma J, Atala A, Smith TL, et al. Peripheral nerve regeneration using a keratin-based scaffold: long-term functional and histological outcomes in a mouse model. *J Hand Surg Am* 2008 Nov;33(9):1541-7.

Hill P, Apel PJ, Barnwell J, Smith TL, Koman LA, Atala A, Van Dyke M. Repair of peripheral nerve defects in rabbits using keratin hydrogel scaffolds. *Tissue Eng Part A* 2011;17(11-12):1499-505

Lin YC, Ramadan M, Van Dyke, M, Kokai LE, Philips BJ, Rubin JP, Marra KG. Keratin gel filler for peripheral nerve repair in a rodent sciatic nerve injury model. *Plast Reconstr Surg* 2012;129:67-78.

Pace LA, Plate JF, Smith TL, Van Dyke ME. The effect of human hair keratin hydrogel on early cellular response to sciatic nerve injury in a rat model. *Biomaterials*. 2013 Aug;34(24):5907-14.

Pace L, Plate J, Mannava S, Barnwell J, Koman LA, Li Z, Smith T, Van Dyke M. A human hair Keratin hydrogel scaffold enhances median nerve regeneration In non-human primates: An electrophysiological and histological study. *Tissue Eng Part A*. 2014;507-17.

Sierpinski P, Garrett J, Ma J, Apel P, Klorig D, Smith T, et al. The use of keratin biomaterials derived from human hair for the promotion of rapid regeneration of peripheral nerves. *Biomaterials* 2008 Jan;29(1):118-28.

Presentations

Pace LA, Plate JF, Mannava S, Barnwell JC, Koman LA, Li Z, Smith TL, Van Dyke M. "Peripheral Nerve Repair in Non-human Primates" **Society of Military Orthopaedic Surgeons**: San Diego, CA 12/2011

Pace LA, Plate JF, Mannava S, Barnwell JC, Koman LA, Li Z, Smith TL, Van Dyke M. Peripheral nerve regeneration in non-human primates using a keratin biomaterial hydrogel. **Tissue Engineering and Regenerative Medicine International Society**; Houston, Texas. December 11-14, 2011.

Smith T, Koman LA, Van Dyke M. Translation of biomaterial technologies from academic research to commercial use. **Clinical Orthopaedic Society Annual Meeting**. Charleston, SC. September 29-October 1, 2011

Pace LA, Hill P, Garrett J, Ma J, Apel P, Mannava S, Barnwell J, Smith B, Li Z, Koman LA, Smith T, Van Dyke M. Clinical translation of a keratin biomaterial hydrogel for nerve repair. **Society for Biomaterials Annual Meeting**. Orlando, FL. April 13-16, 2011

Pace LA, Plate JF, Mannava S, Barnwell JC, Koman LA, Li Z, Smith TL, Van Dyke M.
 “Clinical Translation of a Keratin Biomaterial Hydrogel for Nerve Repair” **North Carolina Tissue Engineering and Regenerative Medicine Society**: Raleigh, NC 11/2010

Barnwell J, Pace L, Li Z, Koman LA, Smith T, Van Dyke M. Peripheral nerve regeneration using keratin biomaterials: From bench to bedside. **Biomedical Engineering Society Annual Meeting**. Austin, TX. October 6-9, 2010

Posters

"A Keratin Biomaterial Hydrogel Improves Median Nerve Regeneration in a Non-Human Primate Model" **Gordon Research Conference in Neural Development**: Newport, RI 8/2012

"Clinical Translation of a Keratin Biomaterial Hydrogel for Nerve Repair" **Gordon Research Conference in Biomaterials and Tissue Engineering**: Plymouth, NH 8/2011; **Orthopaedic Research Society**: San Francisco, CA 2/2012

"Peripheral Nerve Regeneration in Non-Human Primates using a Keratin Biomaterial Hydrogel" **Western North Carolina Society for Neuroscience**: Winston-Salem, NC 11/2011; **North Carolina Tissue Engineering and Regenerative Medicine Society**: Winston-Salem, NC 11/2011

"Human Hair Keratin Hydrogel Enhances Peripheral Nerve Regeneration Following Conduit Repair" **Advanced Technology Applications for Combat Casualty Care**: St. Pete Beach, FL 8/2010; **Society for Neuroscience**: San Diego, CA 11/2010; **Tissue Engineering and Regenerative Medicine Society**: Orlando, FL 12/2010

"Cellular Interactions with a Human Hair Keratin Hydrogel Enhance Peripheral Nerve Regeneration" **Wake Forest Graduate School of Arts and Sciences Graduate Student Research Day**: Winston-Salem, NC 3/2010

CONCLUSIONS

KeraNetics will use the preliminary fate and distribution study to design a larger fate and distribution study. The pilot FDS supported KeraNetics belief that the keratin hydrogel should be designated as a device since the majority of the radioactivity remained at the site of implantation even 30 days after the material was implanted. The radioactivity that was observed throughout the body was probably C^{14} not attached to the keratin hydrogel protein. Specifically, the radioactivity located in the brain was probably unattached C^{14} since the molecular weight of the keratin hydrogel would prevent it from crossing the blood brain barrier.

KeraNetics will pursue their work with the FDA to obtain an IDE for the keratin hydrogel, in which case the clinical trial using keratin hydrogel for peripheral nerve repair can be completed. If the FDA decides that the keratin hydrogel is a drug or biologic agent and requires an IND, KeraNetics has a low level of interest in pursuing the clinical trials necessary to obtain FDA approval of this product for supporting the repair of peripheral nerve injuries.

REFERENCES

Apel PJ, Garrett JP, Sierpinski P, Ma J, Atala A, Smith TL, et al. Peripheral nerve regeneration using a keratin-based scaffold: long-term functional and histological outcomes in a mouse model. *J Hand Surg Am* 2008 Nov;33(9):1541-7.

Hill P, Apel PJ, Barnwell J, Smith TL, Koman LA, Atala A, Van Dyke M. Repair of peripheral nerve defects in rabbits using keratin hydrogel scaffolds. *Tissue Eng Part A* 2011;17(11-12):1499-505.

Lin YC, Ramadan M, Van Dyke, M, Kokai LE, Philips BJ, Rubin JP, Marra KG. Keratin gel filler for peripheral nerve repair in a rodent sciatic nerve injury model. *Plast Reconstr Surg* 2012;129:67-78.

Lohmeyer JA, Siemers F, Machens HG, Mailander P. The clinical use of artificial nerve conduits for digital nerve repair: a prospective cohort study and literature review. *J Reconstr Microsurg* 2009 Jan;25(1):55-61.

Pace LA, Plate JF, Mannava S, Barnwell JC, Koman LA, Li Z, Smith TL, Van Dyke M. A human hair keratin hydrogel enhances median nerve regeneration in non-human primates: An electrophysiological and histological study. Submitted to *Science Translational Medicine*.

Rosen B, Lundborg G. A model instrument for the documentation of outcome after nerve repair. *J Hand Surg Am* 2000 May;25(3):535-43.

Ruijs AC, Jaquet JB, Kalmijn S, Giele H, Hovius SE. Median and ulnar nerve injuries: a meta-analysis of predictor of motor and sensory recovery after modern microsurgical nerve repair. *Plast Reconstr Surg* 2005 Aug;116(2):484-94.

Sierpinski P, Garrett J, Ma J, Apel P, Klorig D, Smith T, et al. The use of keratin biomaterials derived from human hair for the promotion of rapid regeneration of peripheral nerves. *Biomaterials* 2008 Jan;29(1):118-28.

Taras JS, Jacoby SM. Repair of lacerated peripheral nerves with nerve conduits. *Tech Hand Up Extrem Surg* 2008 June;12(2):100-6.

APPENDICES

Non-GLP Report: 14-02748-N1
Single Dose Fate and Distribution Study in Rats Using C¹⁴ Labeled Compound

Final Amended Report Date: September 29, 2015
Toxikon Corporation
Bedford, MA