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May 9, 2019

Tamara Syrek Jensen
Director, Coverage and Analysis Group
Centers for Medicare and Medicaid
Services Mail Stop S3-02-01
7500 Security Boulevard
Baltimore, Maryland 21244-1850

RE: Request for Reconsideration of Medicare
National Coverage Determinations Manual, § 270.3,
Autologous Blood-Derived Products for Chronic Non-Healing Wounds

Dear Ms. Syrek Jensen:

This is a formal request to reopen and revise Section 270.3 of the Medicare National Coverage Determinations Manual, which addresses Autologous Blood-Derived Products for Chronic Non-Healing Wounds. Autologous Platelet-Rich Plasma (PRP) is the prevalent blood-derived therapeutic product used for treating chronic non-healing wounds. This letter, and the accompanying attachments, set out the body of clinical evidence to support the conclusion that the use of Aurix (the proprietary formulation of autologous PRP manufactured by Nuo Therapeutics, Inc.) for chronic, non-healing wounds including pressure ulcers, diabetic foot ulcers, and venous ulcers compared to usual wound care, significantly and reliably improves the rate of complete healing, speed and progress to healing, and quality of life in the Medicare population. Based on our discussions with you and the staff at Coverage and Analysis Group (CAG) over the past six (6) months, this request is specific to use of Aurix for the treatment of chronic, non-healing diabetic foot ulcers. As you are aware, CAG established a Coverage with Evidence Development (CED) program in 2012 upon conclusion of a request for reconsideration initiated via a request letter dated October 4, 2011. The decision memo issued upon conclusion of the reconsideration was issued effective August 2, 2012. We respectfully request that the entirety of the October 4, 2011 (including the various supporting attachments) reconsideration request be referenced by CAG as necessary during its review of the request made here as of today's date. This letter will focus largely on the clinical evidence and data generated during the conduct of the CED program since the issuance of the CED decision memo in August 2012.

Introduction

Autologous blood products, and, specifically PRP, have been used widely for the treatment of chronic non-healing wounds. The benefits of autologous blood products have been the subject of research published in peer-reviewed medical journals since 1985.

In this submission, we request that CMS update its current National Coverage Determination to authorize Medicare coverage for the use of Aurix to treat diabetic foot ulcers (DFUs) when conventional treatments have been tried for at least 30 days and failed to reduce the wound size or induce an adequate wound healing trajectory.

The information and data discussed in this request amply demonstrate that Aurix offers a healing benefit for individuals with DFUs when compared with conventional treatments. The advantages of Aurix treatment include improved healing, faster healing time, reduced infection and pain, improved net health outcome, and reduced overall cost. This combination of increased effectiveness and efficiency establishes the need to update the current NCD to allow for the use of Aurix for the treatment of DFUs in clinically appropriate settings.

This request focuses on the use of Aurix for the treatment of chronic, non-healing DFUs, describes the progress in this field since 2012, and is a specific request for Medicare coverage. It includes:

- Exhibit A: Clinical study under CED entitled "*A Pragmatic Randomized Controlled Trial Conducted Under Medicare's Coverage with Evidence Development (CED) Paradigm Demonstrates Aurix Gel is an Effective Intervention for Chronic Diabetic Foot Ulcers" –* accepted for publication and scheduled for print publication in September 2019 issue of *Advances in Skin and Wound Care.*

- Exhibit B: Letter from Indian Health Service (Oklahoma Region) Director, Office of Chief Medical Officer and referenced report.

- Exhibit C: Published abstract from *Journal of British Surgery* 2015 entitled "*Platelet rich plasma (PRP) is an adjunct for the accelerated closure of high-risk diabetic foot wounds*".

- Exhibit D: Entirety of October 4, 2011 reconsideration request including all attachments.

I. Regulatory Background

1. FDA Clearance

As a preliminary procedural matter, this request to update Section 270.3 to cover Aurix is limited to certain clinical indications that have been reviewed and cleared for use by the Food and Drug Administration. In September 2007, the FDA granted Section 510(k) clearance for the AutoloGel™ System; in its notice, the FDA approved the following indications for use:

> 510(k) Number: BK060007
> Device Name: AutoloGel System
> Indications for Use:
> The AutoloGel™ System is intended to be used at point-of-care for the safe and rapid preparation of platelet-rich plasma (PRP) gel from a small sample of a patient's own blood. Under the supervision of a healthcare professional, the PRP gel produced by the AutoloGel™ System is suitable for exuding wounds, such as leg ulcers, pressure ulcers, and diabetic ulcers and for the management of mechanically or surgically-debrided wounds.
>
> *Note: Aurix is now the tradename for the product previously known as AutoloGel. This reconsideration request is specific to the treatment of DFUs.*

2. Previous CMS Consideration of Autologous Blood Derived Products for Chronic Wounds

CMS' previous considerations of Section 270.3 occurred during 2007/2008 with the final decision memo issued in March 2008 and, as mentioned above, in 2012 with the final decision memo issued in August 2012. In 2008, CMS determined that PRP would remain a non-covered item under Medicare. Its decision was based on the finding that the evidence available at that time was suggestive but not adequate to conclude that autologous PRP is reasonable and necessary for the treatment of chronic non-healing, cutaneous wounds, or for the treatment of acute surgical wounds when the autologous PRP is applied directly to the closed incision, or for dehiscent wounds.[4] In 2012, CMS established coverage under CED when the patient was "enrolled in a clinical research study that that addresses the following questions using validated and reliable methods of evaluation". The question necessary to be answered was defined as:

"Prospectively, do Medicare beneficiaries that have chronic non-healing diabetic, pressure, and/or venous wounds who receive well-defined optimal usual care along with PRP therapy, experience clinically significant health outcomes compared to patients who receive well-defined optimal usual care for chronic non-healing diabetic, pressure and/or venous ulcers as indicated by addressing at least one of the following:

a. complete wound healing
b. ability to return to previous function and resumption of normal activities; or
c. reduction of wound size or healing trajectory, which results in the patient's ability to return to previous function and resumption of normal activities?"

II. Aurix: A Therapy to Address the Chronic Wound Problem

Aurix is an autologous biodynamic hydrogel derived from the patient's own blood (more specifically, the plasma fraction containing the substantial majority of platelets within the plasma fraction) containing proteins that regulate tissue growth for wound healing. When Aurix is activated, the platelets release a multitude of cytokines, growth factors, and chemokines to act on the cell receptors to facilitate cellular growth and migration and the fibrinogen in the plasma converts to a fibrin matrix scaffold on which the cells can adhere. This biological system is integral to normal wound healing.

To help understand this complex biological system, ***Attachment A*** in the October 2011 submission (**Exhibit D**) provides a detailed description of the scientific mechanism of wound healing and the role of these proteins in facilitating wound healing. ***Attachment B*** of the October 2011 submission (**Exhibit D**) describes the importance of specific formulations and standardization of Aurix to achieve consistent wound healing outcomes.

III. Clinical Evidence That Demonstrates the Effectiveness of PRP

Exhibit A: Clinical study under CED entitled **"***A Pragmatic Randomized Controlled Trial Conducted Under Medicare's Coverage with Evidence Development (CED) Paradigm Demonstrates Aurix Gel is an Effective Intervention for Chronic Diabetic Foot Ulcers***"**

The above study has been accepted for publication in *Advances in Skin and Wound Care* and Nuo has been informed that the article is scheduled for inclusion in the September 2011 issue. The content of the article has been the subject of multiple discussions between Nuo and CAG staff over the past several months. Nuo references the article here for CMS benefit and as an included exhibit to the formal request but we do not believe further discussion is necessary at this time.

Exhibits B and C: Letter from Indian Health Service (Oklahoma Region) Director, Office of Chief Medical Officer and referenced report (**Ex. B**) and published abstract *from* Journal of British Surgery *2015 entitled "*Platelet rich plasma (PRP) is an adjunct for the accelerated closure of high-risk diabetic foot wounds*"* (**Ex. C**).

Nuo believes that CMS is unlikely to have seen the data contained in these two exhibits previously. It is provided as a supplement to this reconsideration request as further third party evidence of the healing benefits of Aurix in chronic (and often complex) wounds including diabetic foot ulcers.

Summary

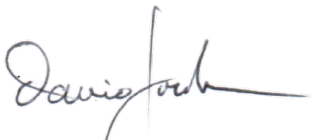
Since the 2012 CMS final decision memo establishing coverage under CED for blood-derived products for the treatment of chronic wounds, Nuo Therapeutics has operated in good faith to execute to the fullest extent possible the agreed protocols for data collection. Over the many discussions with CAG staff, CMS is aware of the insurmountable challenges to fulfillment of the data collection objectives. But, yet also aware of Nuo's strongly held opinion that the data generated for DFUs is robust in every sense and highly supportive of Aurix's effectiveness as a wound healing treatment option.

The use of Aurix for the treatment of non-healing DFUs can facilitate healing, improve healing rates, reverse a non-healing trajectory to a healing trajectory, shorten the time to healing, reduce length of stay, reduce excess use of expensive treatment modalities, has minimal to no adverse events, improves the net health benefit to the patient and their family, and is cost effective.

Based on meeting these evidence criteria and having the documented outcomes described, we respectfully request that CMS amend its current coverage under the CED decision and provide coverage for the use of Aurix, specifically for the treatment of diabetic foot ulcers.

We welcome the opportunity to further discuss with you any of the evidence supporting Medicare coverage for Aurix or to answer any questions. For convenience, please contact myself at (832) 236-9060 or djorden@nuot.com or Nuo's counsel, Jayson Slotnik, at (202) 253-8780, or jayson@healthpolicystrategiesllc.com.

Sincerely,



David Jorden
Chief Executive Officer
Nuo Therapeutics, Inc.

Exhibit A

A Pragmatic Randomized Controlled Trial Conducted Under Medicare's Coverage with Evidence Development (CED) Paradigm Demonstrates Aurix Gel is an Effective Intervention for Chronic Diabetic Foot Ulcers.

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Word Count: 11,758

Key Words: Coverage with Evidence Development, diabetic foot ulcers, Medicare, pragmatic trial, platelet rich plasma, Aurix

Abstract

Objective: Autologous platelet-rich plasma (PRP) products can significantly vary with respect to platelet concentration, the presence of additional cellularity and the use of additives. Therefore, the utility of each formulation for treating chronic wounds needs to be established.

Approach: Under a Medicare Coverage with Evidence Development (CED) paradigm, the effectiveness of up to 12 weeks of treatment with the Aurix® hematogel for healing diabetic foot ulcers was evaluated against usual and customary care including any wound modality in 129 patients. This pragmatic randomized controlled trial was conducted in 28 clinical sites and an inclusive design allowed participation of difficult health risks, co-morbidities (e.g. peripheral arterial disease, smoking), and any wound severity (Wagner 1-4).

Results: Kaplan-Meier analysis showed significant (log Rank P=0.0476) time to heal advantage with 45% of wounds healing with Aurix therapy as compared to 30.2% with usual and customary care (OR 2.1796 CI 1.0579-4.4906, p=0.034). A higher percentage of healing was observed for Aurix across all wound severities (Wagner grade 1-4). Subgroup analysis revealed a significant healing advantage for Aurix when treating wounds accompanied by peripheral arterial disease (PAD), (OR 2.2123, CI 1.0699-4.5742, p-=0.0319) and a demonstrated advantage for smokers.

Innovation: A unique study with few inclusion/exclusion criteria examining an autologous platelet rich plasma product used in the real world outpatient wound setting within standard protocols on patients commonly seen but normally excluded from RCT's.

Conclusion: This first CED study in wound care demonstrates the effectiveness of Aurix for treating DFUs in Medicare beneficiaries.

Short Abstract:

Under a Medicare Coverage with Evidence Development (CED) paradigm, the effectiveness of up to 12 weeks of treatment with the Aurix® hematogel for healing all severities of DFUs was evaluated against usual and customary care including any wound modality in 129 patients including smokers or those with PAD.

Introduction

The impact of non-healing diabetic foot ulcers on health economics.

The incidence of diabetes approached 10% of the US population (2017 estimate), and an additional 84 million people have pre-diabetes with only 10% being aware of their condition. Diabetic Foot Ulcers (DFUs) are common in the diabetic population with a 25% lifetime risk of developing a foot ulcer. Between 10-15% of DFUs do not heal and account for approximately 20% of the hospital admissions for diabetic patients. Despite aggressive treatment of DFUs, 25% of non-healing ulcers require amputations that are associated with a post-amputation 5-year survival rate of approximately 50%.

The United States Food and Drug Administration (FDA) has evaluated a wide range of drugs and medical devices including many advanced healing technologies that are now available on the US market for the treatment of chronic DFUs. However, health care providers still encounter significant challenges in the treatment of DFUs to achieve healing for many patients suffering with chronic diabetic ulcers. The observed variances of health status and comorbidities of diabetic patients contribute to the disparate response to clinical interventions and widely variable healing outcomes of DFUs.

Effectiveness studies for extrapolating health outcomes to the broader population.

A number of approved therapies are available for treating non-healing DFUs. Nonetheless, it is important to consider that determination of "safe and effective' by the FDA relies on assessments of traditional clinical study data. These clinical study data may not adequately allow extrapolation to the broader population of DFU patients, as studies conducted for FDA marketing approval of drugs or devices are based on inclusion/exclusion criteria that significantly limit the tested population[1]. While these tightly controlled studies are valuable tools for an initial evaluation of safety and efficacy, their design may not lead to the collection of data that can serve as a robust predictor of a therapy's clinical effectiveness across broader patient populations[2].

Three of the four randomized controlled chronic DFU studies we identified studied a patient population that includes only Wagner grade 1 & 2 ulcers[3,4,5]. Patients with common health risks such as smoking or comorbidities such as peripheral arterial disease (PAD) were also excluded from participation. A more recently published randomized controlled study targeting a broader patient population compared a platelet-based therapy to good standard of care for the treatment of hard-to-heal ulcers[6], defined as those having a wound area that does not decrease by more than 50% or increase by more than 25% within a 4 week run in period. A mix of ulcer sizes was evaluated within the study with a majority (74%) having an area of ≥ 1cm^2 and a mean ulcer size of 2.4 cm^2. This notwithstanding, the authors suggested a weakness of the study is that the patient population may not be representative of populations typically seen within a wound clinic. Presented in the study predominantly are data on superficial DFUs (87%) with an additional 10% of ulcers down to tendon and 3% down to bone. Comorbidities that commonly present in the diabetic patient such as PAD were also excluded from participation. While these data support a healing advantage of the platelet-based therapy as compared to accepted standard of care, the restrictive nature of patient enrollment in this traditional randomized controlled trial (RCT) must be carefully considered before generalizing results to the broader population of chronic DFU patients.

In an effort to overcome the limitations around patient enrollment that is typical of such traditional RCTs, the Centers for Medicare & Medicaid Services (CMS) has established the Coverage with Evidence

Development (CED) paradigm. Under CED, Medicare will provide reimbursement coverage to promising new or existing therapies and services while data are collected to better understand their impact on the Medicare population[7]. CED encourages the design of studies with inclusive patient enrollment and treatment protocols that more closely reflect every day practice to provide health outcomes data that more accurately represent "real world" care of the broader population of Medicare beneficiaries, and thereby better inform future coverage decisions. To date 23 CED programs have either completed or are ongoing and presented here is the first clinical study conducted under CED addressing the effectiveness of wound healing modalities, the National Coverage Decision on Autologous Blood-Derived Products for Chronic Non-Healing Wounds (NCD 270.3)[8]. To provide context, presented in **Table 1** are important study design elements and operational aspects of a traditional RCT as compared to the pragmatic RCT conducted to evaluate "effectiveness" under CED.

Autologous blood derived products for wound care

Autologous blood derived products for healing of chronic wounds have garnered attention of health care providers for many years based on the platelet serving as the first hematological response to cutaneous wounding[9,10]. Although it is well established through efforts such as the platelet proteome project that platelets release a full complement of biomolecules regulating processes critical for tissue regeneration[11,12], the use and effectiveness of platelet-based therapies in wound care, among other fields, has driven a warranted debate[13,14].

This debate is partially due to the use of many different medical device technologies to obtain preparations of platelets from a sample of patient blood[15]. The majority of these technologies have been cleared by the FDA with a specific orthopedic indication: to produce a platelet rich plasma (PRP) concentrate to be mixed with bone graft material prior to an orthopedic surgical procedure[16] and were not developed or approved for use in wound care.

The platelet concentration and the presence of accompanying white blood cells will differ between the devices and meaningfully impact the relative concentrations of cytokines, chemokines and growth factors within platelet rich plasma samples[17]. Furthermore, it has been demonstrated that PRP containing lower concentrations of platelets can promote proliferation and differentiation responses important for healing of soft and hard tissues, and PRP containing higher concentrations of platelets can have opposite effects [18,19,20,21]. Therefore, to achieve the most meaningful health outcomes using autologous blood therapies, it is important to match the choice of technology with the intended indication.

With these differences noted, recent clinical studies have supported benefit of platelet-based therapies as productive interventions for treatment of chronic wounds[22,23,24,25,26,27]. A recent systematic review of the chronic DFU literature also was published in which the authors conclude "that the topical application of PPR for DFUs results in statistically superior healing rates and lower complication rates compared to controls"[28]. Expanding on our understanding of the value of autologous blood therapies for treating chronic ulcers, the CED program implemented by CMS as defined within NCD 270.3 provides the framework under which data can be collected to evaluate the merit of different autologous blood therapies for treating non-healing ulcers, specifically in the population of Medicare beneficiaries.

FDA indicated Aurix Therapy for exuding wounds (including chronic ulcers)

Aurix Therapy, a biodynamic hematogel, is a proprietary formulation containing a physiologic concentration of platelets and pharmaceutical grade reagents. Calcium chloride and thrombin reagents activate platelet receptors and convert plasma fibrinogen into a fibrin gel that can serve as a reservoir of growth factors and other biomolecules released by platelets. The addition of ascorbic acid required for collagen synthesis also acts as a scavenger of damaging free radicals and controls consistency of the fibrin gel. It also may provide additional benefits fostering collagen deposition and preventing tissue deterioration in cases where the wound bed has been depleted of this essential co-factor.

The Aurix gel is produced using the Aurix System at the patient point-of-care, and based on a double-blinded-randomized controlled trial[3], the Aurix System has been cleared for use by the FDA, specifically for exuding wounds[29]. In this pivotal trial, 81% of the most common size DFUs (≤ 7cm^2) healed on average in 6 weeks with Aurix treatment versus 42% of the wounds treated with saline hydrogel as a control. Previously published studies addressing the effectiveness of Aurix include prospective observational studies of a before-and-after design in which DFUs were subjected to several treatments and were followed to 50% reduction in area or complete wound healing[30]. Some of these studies had extensive run-in periods for the majority of wounds, including Wagner grade 3 or 4 DFUs in ischemic patients[31,32] that deteriorated over several months immediately prior to receiving Aurix Therapy. Thus the body of peer-reviewed evidence supporting Aurix as a treatment for chronic ulcers includes both comparison and observational studies evaluating healing outcomes of 390 chronic wounds treated in sites of care including outpatient wound centers, VA hospitals and Long Term Acute Care facilities [30,31,32,33,34].

Clinical Problem Addressed:

As a complement to this body of evidence, presented here is the first multicenter, pragmatic, randomized, controlled study conducted under CED to evaluate the "on label" use of the Aurix System to heal chronic DFUs exclusively in the population of Medicare beneficiaries. The aim of the study is to evaluate healing outcomes associated with the "real world" use of Aurix Therapy when administered by providers within the typical environments at participating sites of care.

Materials and Methods

General design considerations

This pragmatic, randomized, controlled, multicenter clinical trial was conducted under CMS' CED program, specifically National Coverage Decision (NCD) 270.3, implemented to collect data on the benefit of autologous blood-based therapies for treatment of chronic DFUs in the Medicare population. The present study was approved by CMS to evaluate the "on-label" use of the Aurix System + UCC as compared to UCC only to treat chronic DFUs in an intended total study population of 760 patients in up to 100 sites in the United States. The Aurix + UCC and UCC only groups were randomized at a ratio of 1:1 at a total of twenty-eight investigative sites contributing to the data collection. The study was conducted with Institutional Review Board (IRB) oversight and informed consent was obtained prior to any study related procedures. Patient participation was limited to Medicare beneficiaries with at least one non-healing diabetic foot ulcer.

In standard clinical practice, differences in the patient demographics, provider skill sets and approaches to UCC within different wound centers are variables that can influence treatment outcomes. To provide for the appropriate collection of data to evaluate the Aurix System as it is used in actual clinical practice, the training of investigators was limited to instruction in the International Conference on Harmonisation Good Clinical Practice (ICH GCP), to the CED protocol and specific steps for the production and application of Aurix Therapy. Furthermore, unlike conventional RCTs, on-site clinical study coordinators or research staff were not used for this effectiveness study and therefore recruitment and documentation of data were the responsibility of investigators and available support staff. The Sponsor's clinical specialists and clinical affairs teams were made available upon request to provide support for the appropriate preparation and application of Aurix and to clarify any questions related to the study protocol.

Subjects receiving Aurix Therapy were to be treated on average twice a week for the first 2 weeks, and then, once a week thereafter while under active treatment, but actual frequency of treatment was determined by the treating clinician. In the UCC only arm of this study, investigators were instructed to use any treatment modality or combination of treatment modalities available so long as the treating clinician and patient considered it to be in the best interest to heal the chronic ulcer; for example, hyperbaric oxygen therapy (HBOT) for Wagner grade 3-5 DFUs. Additional care in the Aurix + UCC group was restricted as discussed below under "Treatment visits".

Wound closure was defined as complete epithelialization in the absence of drainage and without the need for wound dressings. The primary endpoint was complete wound healing after 12 weeks of treatment for those subjects healed and treated with Aurix + UCC versus UCC only. Additional analysis includes the proportion of healed DFUs in the treatment period. Immediately prior to randomization and again at the end of the 13-week study period, Quality of Life data were collected using the Quality of Life with Chronic Wounds short-form instrument (W-QOL)[35]. Correlation of changes in W-QOL scores and changes in wound trajectory will be presented in a subsequent publication.

Source data were gathered from centers utilizing the NetHealth Wound Doc EMR (Pittsburgh, PA). In this case, de-identified data were captured by ITS Integrations (Columbia, SC) via direct electronic transfer of records from the EMR to the 3rd party data-base. Of the 28 participating wound centers, eight did not utilize the Wound Docs EMR and case report forms were utilized for data capture. Independent

statisticians from Amarex Clinical Research (Germantown, MD) and PharmaData Associates (Piscataway, NJ) developed the statistical and data analysis plan.

Study eligibility

Prior to any study-related procedures, the Investigator obtained IRB approved, informed consent from the subject. Patients were able to withdraw from the study at any time. Study participants were 18 years or older with Medicare as primary insurance coverage, having type I or type II diabetes with a Wagner grade 1-5 DFU located on the dorsal, plantar, medial, or lateral aspect of the foot or heel that was at least 1 month old with debrided ulcer sized between 0.5 cm^2 and 50 cm^2, and a demonstrated off-loading regimen. Study exclusion criteria were developed with consideration of the Aurix System's FDA approved labeling and included potential sensitivity to Aurix components (calcium chloride, bovine thrombin, ascorbic acid), patients on chemotherapeutic agents or malignancy in the wound area, serum albumin of less than 2.5 g/dL, platelet count of less than 100 X 10^9/L and hemoglobin of less than 10.5 g/dL. As determined by the provider, patients must have demonstrated inadequate progress toward healing following active treatment with UCC at the investigative site for a minimum of 2 weeks immediately prior to screening. Adequate venous access for the periodic blood draw for Aurix administration also was required.

Participation of patients was precluded when there was a presence of another wound that could interfere with treatment of the index ulcer. Patients were required to self-report using a validated Wound Quality of Life Form and therefore could not be cognitively impaired. Clinically infected DFUs must have been treated (per the IDSA Guidelines or other algorithm) before the subject was randomized to a treatment arm. In this case, antibiotics were administered for at least 48 hours with the continued use of traditional wound care until the infection exhibited clinical signs of antibiotics response. When a DFU was treated for infection and clinically responded, randomization could proceed following thorough cleansing of the wound-bed. The comprehensive list of inclusion/exclusion criteria are presented in **Table 2.** After meeting all study inclusion/exclusion criteria and signing the informed consent, the subject was able to be immediately randomized for study participation. The full list of study activities is presented in **Table 3**.

Randomization

Subjects that continued to meet all the inclusion and had none of the exclusion criteria after screening completion were randomized to one of the two treatment groups. Randomization and treatment could occur on the same day as the screening visit or at any time within 7 days of the successful completion of screening. In the event randomization did not take place within the allowed 7-day window, a 30-day waiting period was required before patients could be re-screened for participation. Eligible patients were randomly assigned to receive Aurix + UCC or UCC only using a 1:1 randomization ratio. Randomization codes were generated electronically by Amarex Clinical Research (Germantown, MD) using mixed blocks of size 2 and 4. For this open-label study, randomization certificates including the randomization codes and treatment assignment group were distributed to providers using the Amarex Clinical Research WebView platform.

Procedures

Aurix preparation and application

The Aurix System is FDA cleared and its labeled use for treating chronic DFUs was evaluated in this study. The Aurix System is comprised of a small, purpose-built, portable centrifuge for separating platelets and plasma from other blood constituents, a reagent kit providing pharmaceutical grade additives used to create a fibrin gel containing activated platelets, and the wound dressing kit supplying the complement of accessories required for venous access as well as for preparing and applying the bioactive Aurix gel. Using the Aurix System, venipuncture was performed to obtain 5-20 cc of blood. The patient blood sample then was centrifuged for approximately 1 minute to produce a platelet/plasma fraction that was harvested directly into a mixing chamber. Pharmaceutical grade reagents were sequentially introduced into the mixing chamber and then gently inverted, typically for 15 to 30 seconds, to produce a gel with appropriate consistency for application. The Aurix gel was immediately applied to the wound bed and a barrier cream was placed on intact skin surrounding the wound. A non-adhering contact dressing layer was placed over the Aurix gel and the wound was covered with a non-absorbent dressing. An absorbent layer was then secured over the wound to absorb any wound exudate.

Patient assessments

Prior to any initial treatment, patients were required to fill out the W-QOL short form to provide a baseline wound specific Quality of Life assessment. Regardless of the randomized assignment, basic good standard-of-care practices were required as part of UCC for both study groups. Investigators were instructed to follow the Standard of Care Considerations for Chronic Cutaneous Ulcers as described in the 2006 FDA Guidance and were provided information pertaining to appropriate debridement, offloading, maintenance of a moist wound environment, management of infection, wound cleansing and nutritional support including blood glucose control. At each treatment visit, investigators were to record vital signs, conduct symptom-guided physical exams as necessary, image the wound by digital photography, and assess wound infection as well as needs for debridement and moisture management. Wound measurements (length, width, depth) were to be performed at each visit. To ensure a standardized approach for obtaining wound measurements, investigators were instructed to use the method of initially establishing a "clock face" over the wound bed where 12:00 is toward the patient's head, 6:00 toward the feet and with 3:00 and 9:00 equidistant between 12:00 and 6:00. The length and width of the wound were to be always considered from 12:00 to 6:00 and from 3:00 to 9:00, respectively. The standardization of wound measurement across investigative sites using this technique was intended to remove subjectivity associated with establishing greatest length and width measurements. Subject compliance was assessed and additional patient education about the protocol was provided when needed. Offloading method(s) and concomitant medications (antibiotics only) were documented. Investigators were asked to document treatment-emergent adverse events (TEAEs).

Treatment visits

Following wound assessments, either Aurix + UCC or UCC only were to be administered. In the case of the UCC only group, patients were treated with therapies that the provider and patient determined were in the best interest for healing the wound. All patients received Standard of Care that could include the use of semi-occlusive dressings such as Tegaderm or hydrocolloid dressings such as Triact plain with or without an absorbent dressing such as an ABD pad. For the UCC only group, the use of chemically impregnated dressings such as Triact Ag or Iodoflex was allowed. Standard of Care alone or in combination with advanced wound care such as hyperbaric oxygen (HBO), negative pressure (NP), cellular and tissue products (CTP) such as skin substitutes and amniotic membrane, as well as any other healing

modality, with exception of Aurix Therapy, were permissible in the UCC only arm of this study. Investigators were encouraged to schedule UCC visits in accordance with the treatment regimens as they typically are prescribed at the clinical site. For example, while patient treatments within the UCC only group generally were scheduled to occur on a weekly basis, the use of other therapies such as daily treatment with hyperbaric oxygen was allowed. Furthermore, a continuum of care or treatment algorithms such as the daily delivery of hyperbaric oxygen along with periodic application of a skin substitute or other advanced dressings was allowed. All wound care provided in the UCC only group was to be documented.

All patients randomized to the Aurix + UCC group received Standard of Care and Aurix Therapy. While Standard of Care included the use of semi-occlusive and hydrocolloid dressings as described above, it is important to note that unlike the UCC only arm of the study, the use of materials containing any active ingredients was prohibited (e.g., methylene blue, gentian violet, zinc oxide, silver, hydrogen peroxide, acetic acid, or iodine) in the Aurix + UCC arm. It was intended that patients receive two Aurix applications in each of the first two weeks of treatment followed by one application every week thereafter. Given the pragmatic nature of this study and intent to gather data on Aurix as it may be used in clinical practice, if patients were either unable or unwilling to make 2 treatment visits within each of the first two weeks, a single treatment for each week during the 12-week treatment period was acceptable. Furthermore, understanding that a single treatment modality may not be sufficient and considering the range of wound severities and comorbidities, to bring all DFUs to complete closure in a cost effective manner, the present study was initially powered with intent to collect data to assess the benefit of Aurix Therapy both as a "stand alone" therapy and also when used at the discretion of the patient and provider as part of a continuum of care or defined treatment algorithm. Therefore, additional advanced wound care was allowed in the Aurix + UCC treatment group if the provider and patient determined it be in the best interest in healing the patient's wound. The types and frequency of wound care used in the UCC only as well as for the use of concomitant care in the Aurix + UCC group are listed in **Table 4** and discussed below under "**Concomitant care**".

End of treatment and post-treatment visits

Wound closure was assessed at each patient visit. Week 13 of the study protocol was the scheduled End of Treatment visit. Patients with index wounds that did not close after completing 12 weeks of Aurix treatment were to return to the clinic for the week 13 visit to document healing status. Activities at the End of Treatment visit were to include all assessments as previously conducted for each weekly treatment visit. During the End of Treatment visit, patients also were to complete an end of study W-QOL for comparison to the W-QOL form completed as a baseline measurement prior to the first study treatment. If the index ulcer closed prior to week 13, the treatment visit where closure was observed and documented was considered the End of Treatment visit. When the determination of complete closure was made, a follow-up visit was scheduled for approximately 2 weeks later to confirm closure. If closure was not confirmed at this follow-up visit, patients was to continue with the same treatment assignment for the duration of the planned 13-week study period. In cases where ulcers did not achieve complete healing but at least 50% area reduction was demonstrated after 12 weeks of treatment, treatment could continue for up to 20 weeks at the discretion of the patient and provider. Data for treatment beyond 12 weeks were collected for separate analysis as a tertiary endpoint.

Results

Site Participation

The enrollment of 760 patients was planned to provide statistical power for detailed subgroup analysis of the effectiveness of treatments dependent on ulcer severity, the relative effectiveness of commonly used advanced treatment modalities in the UCC group and effectiveness of treatments for patients with various health risks and comorbidities. However, due to many unanticipated challenges to enrollment (detailed in the **Discussion** section), the Sponsor agreed with a request by CMS to analyze the existing data set. Presented here are analyses of the available data for the Intent-To-Treat (ITT) population that randomized 66 patients to Aurix + UCC and 63 patients to the UCC only arm of the study. The ITT population includes all patients randomized to the study and who returned to receive at least one treatment and post-baseline ulcer measurement. Twenty-eight facilities across the United States participated in this study representing both the physician's office and outpatient wound center treatment settings that are designated by CMS as POS-11 and POS-22 sites of service, respectively. Represented within the pool of 28 investigative sites were 2 physician's offices and 26 outpatient wound centers. The disparity in the number of participating POS-22 and POS-11 sites of service primarily was due to reimbursement hurdles addressed in the **Discussion** section. In alignment with the intent to evaluate the effectiveness of Aurix Therapy as it is generally used in clinical practice, sites representing diverse urban and rural geographies were selected independent of investigator and wound center staff previous clinical research experience. Designated study coordinators were present in only 2 of the 28 clinical sites, based on those sites having active research programs. The remaining 26 sites relied on the clinical support staff and investigators to enroll subjects and document health outcomes data.

Patient demographics and baseline characteristics

Characteristics of the study population are reported in **Table 5** and include Age, Race, Sex and Health Status comprising both health risks and comorbidities. The mean age was similar for patients randomized to the UCC only and Aurix + UCC study groups: 66.9 and 64.7 years old, respectively. Sex and Race were also balanced between treatment groups. The study enrolled predominately male subjects, 77.8% in the UCC only group and 77.3% in the Aurix + UCC study group. The enrolled population was predominately white, 81.8% of subjects in the UCC only group and 90.5% of subjects in the Aurix + UCC group. Additionally, represented in this study were Black, Asian and Other; however, no category exceeded 7.6% in either study arm.

One or more health risks and comorbidities including smoking, peripheral arterial disease, immunosuppression, renal failure, arthritis or transplant affected 73.6% of the enrolled DFU subjects. A history of smoking, present in 46% of the UCC only subjects and 57% of the Aurix + UCC group, as well as peripheral arterial disease, present in 47.6% of the UCC only subjects and 39.4% of Aurix + UCC subjects, were most prevalent within the study population as compared to the other health risk/comorbidities each having prevalence of 3-12% of the study population. Immunosuppression, renal failure, arthritis and previous transplant were represented in both study groups each with prevalence ranging from 3 to 12%.

Wound size and severity

Average wound area prior to the first study treatment was 4.1cm^2 for Aurix + UCC and 5.6cm^2 for UCC only treatment groups. The distribution of smaller (<1cm^2), intermediate (>1cm^2 to ≤7cm^2) and larger

(>7cm^2) ulcers was approximately the same for the treatment groups (**Table 6**). Smaller ulcers accounted for approximately 29%, intermediate-sized ulcers for approximately 53% and larger DFUs for approximately 18% of wounds in each of the treatment groups. All Wagner grade wound severities were allowed in this pragmatic study. The majority of wounds within each of the treatment groups were of intermediate severity including 48.5% Wagner grade 2 and 44.4% Wagner grade 3 ulcers (**Table 7)**.

Concomitant care

Table 4 displays the different wound care treatment options utilized in the two study groups and the associated healing rates. In the Aurix + UCC group, 51 of 66 (77.3%) subjects received only Standard of Care as compared to 29 of 63 (46.0%) subjects in the UCC only group. The percent healed for the Aurix + UCC and UCC only groups that received only standard of care were 51.0% and 27.6%, respectively. In combination with advanced care, healing for the Aurix subgroup was 40% compared to 32.4% in the UCC only subgroup. Advanced care was utilized more frequently in the UCC only group with 34 of 63 (54%) subjects receiving the additional care as compared 15 of 66 (22.7%) in the Aurix treatment group. Interestingly, while HBO was the predominant form of advanced care used in the Aurix subgroup (13 of 15 subjects), CTPs were the predominant form of advanced care used in the UCC only subgroup (22 of 29 subjects). The utilization of different combinations of advanced care was more frequent in the UCC subgroup (11 of 34 subjects) as compared to the Aurix subgroup (3 of 15 subjects).

Analysis of effectiveness

Primary endpoint

In this study, 48.5% of subjects treated with Aurix healed within the 13-week study period, compared to 30.2% of subjects treated with UCC only **(Tables 8 and 8a,** p = .034**)** and a greater percentage of ulcers healed in the Aurix treatment group for all Wagner categories (**Table 9)**. Kaplan-Meier analysis of the time to heal for Aurix + UCC and UCC only over the 13-week study period was performed censoring those cases where healing was not observed within the 13-week time period. **Figure 1** shows a separation of the survival curves at approximately 6 weeks and the log-rank test p-value of p=0.0476 indicating a statistically significant time to heal advantage for Aurix.

Treatment up to 20 weeks

Subject's having wounds that did not heal after 12 weeks of Aurix treatment but with at least a 50% reduction in wound area were allowed to continue Aurix treatment for up to 20 weeks. The group with extended treatment included 8 patients, 2 of which healed within the 20-week period. The small number of subjects in the extended treatment group does not provide sufficient data for additional analysis.

Smoking and PAD status

Despite numerical imbalances in smoking and PAD noted in the baseline characteristics (**Table 5**), negative bias was not inserted on the UCC group. The percentage of subjects with PAD in the UCC group that healed was 30% (**Table 10a**), the same as the overall healing rate for the entire UCC only group, 30.2% (**Table 8**). Furthermore, when stratified by baseline PAD status (**Table 10a**), 53.9% of subjects with PAD in the Aurix arm healed compared to 30% in the UCC only group (p=0.0319, OR 2.2 95% CI 1.0696-4.5654). Cox regression comparing time to healing with PAD status as a covariate (**Table 11**) also shows that Aurix provides a significant (p=0.0486) healing advantage (HR 1.8 95% CI 1.004-3.135). Similarly, despite a health risk of smoking in 57.6 % of subjects in the Aurix + UCC group as compared to 46% in the UCC only

group, smoking did not confer negative bias on the Aurix + UCC group. In this case, healing of the Aurix + UCC group is 65.8% compared to healing of 34.5% in the UCC only group. The CMH analysis stratified by smoking shows a favorable trend (p=0.0657, OR 1.9869 CI 0.951-4.15) in the Aurix + UCC group (**Tables 10b and 11**).

Concomitant antibiotics and healing

Healing in both the Aurix + UCC and UCC only groups in the presence and absence of concomitant antibiotic use is presented in **Table 12**. Eighty-five (66%) of the subjects in this study did not receive one or more weeks of antibiotic treatment during the 13-week study period while 44 (34%) of subjects did. The use of antibiotics correlated with a decrease in healing for both treatment groups. In the absence of antibiotics, 55% of subjects healed in the Aurix + UCC group and 35.6% of subjects healed in the UCC only group. When antibiotics were administered, the healing rate dropped to 38.5% in the Aurix + UCC group and 16.7% in the UCC only group. When comparing proportion healed stratified by antibiotic use during the 13-week study period, the odds of healing remains higher in the Aurix + UCC group compared with UCC only group (p=0.0193, OR 2.4368 95%, CI 1.154-5.1457) after controlling for the effect of antibiotic use. Cox regression analysis comparing time to healing between Aurix + UCC and UCC only with antibiotic use as a covariate shows that subjects without antibiotic use healed faster than subjects with antibiotic use, (p=0.0114, HR 2.266,). After adjusting for antibiotic use, the Aurix + UCC over UCC only hazard ratio of healing is 1.861 (CI 1.053-3.290 p=0.0325), indicating time to achieving healing is significant sooner for the Aurix + UCC patients than for the UCC only patients.

Assessment of safety

In this pragmatic study, on-site clinical monitors were not used to facilitate the documentation of TEAEs and the Investigators were instructed to capture TEAEs within the EMR or case report form during the patient encounter. All of the spontaneously recorded TEAEs are presented in **Table 13**. TEAEs include seven serious adverse events (SAE), none of which were judged to be treatment related. The SAEs included two amputations in the UCC only treatment group. No amputations were documented for the Aurix + UCC treatment group. Only one of the TEAEs was suspected to be related to the Treatment. Specifically, a subject in the UCC only treatment group developed a new ulcer resulting from the placement of a total contact cast.

Durability of healing

Assessment of wound healing durability was limited to a 2-week wound healing confirmation visit. However, the inability to provide patients with transportation, copay reimbursement and/or other incentive under Medicare rules contributed to limited numbers of patients returning for the 2-week follow-up. Documented 2-week healing confirmation visits included 20 subjects from the Aurix + UCC group (2 wounds re-opened) and 5 subjects from the UCC only group (one wound re-opened). Based on the small number of subjects that returned after initial healing, intended analysis could not be completed.

Discussion

This landmark study is the first of its kind in wound care conducted under the Medicare Coverage with Evidence Development (CED) program. The results of the study indicate that Aurix Therapy provides advantages for healing chronic diabetic foot wounds, specifically when used to treat the Medicare population. Furthermore, this CED study experience provides useful insight for developing operational strategies for CED programs both within and outside the field of wound care.

Study operations and enrollment

This pragmatic effectiveness study was intended to randomize 760 chronic DFU patients. The study design was intended to gather health outcomes data for Aurix Therapy as typically used for chronic wounds. Thus, the inclusion/exclusion criteria listed in **Table 2** are intentionally minimal, based on the FDA approved labeling for the Aurix System, and to provide access for the broader population of Medicare beneficiaries as they present with chronic DFUs. The Schedule of Study Events (**Table 3**) was designed to minimally impact the day-to-day delivery of care that can vary depending on the clinical site.

Data elements required for analysis of study endpoints were established for consistency with those most commonly documented for patient encounters within the Wound Doc (NetHealth) Electronic Medical Record (EMR), the most commonly implemented EMR for wound care. This simplified the direct transfer of source data to the study database and obviated the need for case-report forms in a majority of investigative sites. Considering the study design was inclusive and data collection mechanisms allowed for relatively seamless implementation in a large pool of investigative sites, the expectation was that patient enrollment would be significantly more efficient than what traditionally has been experienced for the conventional RCT[1]. This was unfortunately not the case in practice as many unique aspects of the Coverage with Evidence Development paradigm (**Table 1**) proved to present unanticipated hurdles to anticipated study enrollment.

Conventional RCTs generally rely on the sponsor to provide resources for most if not all of study treatments, for the transportation of patients to and from treatment facilities and for the costs of time and effort that providers and staff use to perform study-related activities. In contrast, CMS policy under CED is that Medicare should reimburse for study treatments, physician's fees, facility fees and other claims directly related to patient care. Because treatments and services are covered under CED, study sponsors, investigative sites and patients must follow Medicare rules. Under those rules, sponsors are prohibited from making payments to patients, providers or institutions affiliated with the investigative sites for any activities within the normal scope of patient care.

In the case of the present CED study, this proved to have important repercussions on patient participation, provider engagement and study site retention. The vast majority of study candidates with or without secondary insurance, other than certain Medicaid plans, were responsible for a standard 20% Medicare Part B copay. This included all treatments administered throughout the 13-week study duration. As a majority of secondary insurance payors would not cover treatment under CED, the Sponsor pursued and obtained an advisory opinion from the Office of the Inspector General (OIG) of the Department of Health and Human Services allowing investigative sites to waive copay requirements pursuant to stipulated provisions. These activities failed to have the intended effect. Over the 3 ½ year study enrollment period and despite an estimated 800 insurance verifications after clinical eligibility screening, many patients were unable to access this program and certain demographics are under-represented (**Table 5**) within the 129

subject ITT population. In addition to this patient consideration, the requirements of CED had unexpected consequences on provider engagement and study site retention.

CED was first established in 2006 and CMS' commitment to and rules of CED are well established[7]. Nonetheless, CED has yet to be widely implemented and it was the Sponsor's experience that stakeholders did not have an equal and shared understanding of the program. While on a yearly basis CMS updated published mandates for payments to be made for products and services provided under the CED program, in certain cases, Medicare Administrative Contractors (MACs) appeared to continually exercise discretion with respect to actual payment to facilities and providers under this wound care CED. This was especially problematic for reimbursement in the physician's office setting and resulted in this site of service being significantly under-represented in this CED effort. Adding complexity, infrastructures used by certain facilities and providers to submit claim information required modification by IT staff to handle claim elements that were needed to identify CED related activity. The end result of these compounding factors was long payment cycles or the complete absence of payment for either or both the physician and facility.

Unsurprisingly, significant attrition of investigators and clinical sites was experienced. Over the course of this CED program, 48 investigative sites were either trained or completed site initiation and were open for enrollment. Immediately prior to the decision to open this CED study for data analysis, the number of actively participating sites was three (3). The Sponsor's experiences with secondary insurance payment and claims denials by the MACs may be unique in the CED experience in that the use of autologous blood products for wound care is associated with a long-standing historic non-coverage decision by CMS. Nonetheless, our experience with CED may be instructive to future CED programs that may be well-served by a sponsor's thorough consideration of mechanisms for coding and claim submission at potential investigative sites, as well as secondary insurance payor mix within the reimbursement landscape.

Effectiveness

As previously discussed, at the suggestion of CMS and compelled by slow enrollment, this pragmatic study was opened for analysis prior to enrollment of the intended 760 subjects. While the current 129 patient data-set does not support many of the planned subgroup analysis, the data set did allow for a meaningful analysis of the primary and some secondary endpoints, and provides compelling effectiveness data.

A novel feature of this pragmatic study is that it contained very few limitations to enrollment in the inclusion and exclusion criteria. To the Sponsor's knowledge, this is the first randomized controlled study in wound care evaluating a patient population with health risks and comorbidities including smoking, peripheral artery disease, immunosuppression, renal failure, arthritis and previous organ transplant (**Table 5**); conditions that frequently present in wound treatment facilities across the United States.

The large average starting wound area and range of wound sizes enrolled in this study are notable (**Table 6**). The inclusion/exclusion criteria in this study were not intended to force equal enrollment of all wound severities and a substantial majority of wounds enrolled for both the UCC and Aurix + UCC groups were Wagner grade 2 and 3 involving ligament, tendon, deep fascia or bone (**Table 7**). While the numbers of wounds these Wagner categories are similar between the Aurix + UCC and UCC treatment groups, there is bias against the Aurix + UCC group that enrolled 10% more of these severe ulcers as compared to the UCC only group (**Table 7**).

We believe that the preponderance of severe wounds enrolled in this study is greater than the distribution seen in most wound centers. A critical requirement for enrollment was that ulcers must have been considered hard-to-heal (chronic ulcers that failed previous treatment at the investigative sites), thus one possible explanation for the prevalence of severe wounds is that the study provided investigators a treatment opportunity for DFUs that previously could not be effectively accessed with other healing modalities. Nonetheless, the present study addresses a mix of wound sizes, severities and comorbidities that is problematic for healing and that is not addressed in other published RCTs. For example, the recently published RCT evaluating autologous LeucoPatch® PRP technology excluded common comorbidities, and larger more severe wounds with the majority (90% for standard of care and 84% for the treatment group) of hard-to-heal wounds identified as superficial (no tendon or bone involvement)[6]. It is noteworthy to point out that in the present study, although the wound distribution is contributed mostly by Wagner grade 2 and 3 ulcers, all the Wagner grade 1-4 wound severities are represented in each of the Aurix + UCC and UCC only treatment groups (**Table 9**).

Considering that the range of comorbidities and severity of wounds evaluated in this study is a first for an RCT in wound care, the healing benefit (p=0.034, OR 2.1796 95% CI 1.0579-4.4906) observed for Aurix **(Table 8b)** and Kaplan Meier time to heal (log rank p value=0.0476) presented in **Figure 1** are notable. This is especially true considering a recently published RCT showing that another autologous blood technology achieved a 34% healing rate for a majority of superficial wounds over a 20 week study period and in the absence of serious comorbidities [6]. As compared to that study, while a majority of wounds in the present study were larger and more severe including Wagner grade 2 and 3 categories (in excess of 90%) involving tendon and bone (**Table 9**), the higher overall healing rate observed for the Aurix + UCC treatment group of 48.5% was achieved within a 13-week study period. The observation that the Kaplan-Meier curves for Aurix + UCC and UCC only separate at about 6 weeks and do not re-cross and that the Aurix treatment arm has an approximate 2-fold healing advantage at the end of the 13-week study period is striking considering that published double-blinded RCT data show early and continuous separation of healing curves when Aurix was compared to saline hydrogel[3]. The previous RCT was limited to Wagner grade 1 and 2 wounds with tight control over comorbidities and no concomitant care. We suggest that the relatively and more frequent use of advanced care within the UCC only arm of this study contributed trial noise that may have included early initial healing responses within the initial weeks of treatment that cannot adequately be explored with the number of patients available for this analysis. This notwithstanding, Kaplan-Meier analysis supports that Aurix Therapy provides a significant time to heal benefit as compared to UCC including one or more advanced treatment modalities.

In addition to achieving the study's primary endpoint, an important and unexpected finding is that while smoking or a baseline diagnosis of PAD (**Table 10**) did not meaningfully impact the overall healing rate of 30.2% observed for the UCC only group **(Table 8**), numerically higher healing rates in these 2 subgroups were observed for both PAD subjects (53.8%) and smokers (65.8%) treated with Aurix (**Table 10**) and contributed to the significantly improved overall healing rate seen in the primary endpoint. Furthermore, the increased healing observed for PAD subjects was significant, (p=0.0486) (**Table 11**). Though unexpected, this observation may not be surprising considering that smoking is a well-established risk factor for developing PAD and as both PAD and smoking can profoundly decrease blood flow to the extremities. We hypothesize that the improved healing of smokers and subjects with PAD may lie in the fact that the activation of platelets and topical application of the resulting bioactive hematogel serves to bypass the lack of perfusion and delivers to the wound bed a natural complement of growth factors and

other biomolecules that facilitate tissue regeneration. In this case, while bypassing the lack of perfusion may facilitate wound healing, one might expect that the durability of wound healing could be compromised as the underlying effects of smoking and PAD on the circulation would remain. Future studies exploring the long-term durability of wound healing specifically in the population of smokers and PAD patients would be of value.

Given the wide range of DFU severities and underlying comorbidities that often are seen at wound centers, efficient and cost-effective wound healing may sometimes require different healing modalities within a continuum of care. That understood, in this study advanced care was allowed in both treatment arms, and analysis was conducted for treatments performed both in the presence and absence of such care. Presented in **Table 4**, the overall healing rate for the Aurix + UCC group with and without advanced therapies was 48.5% and this is approximately the same as the percentage healed when Aurix was used only with Standard of Care (51%). Interestingly, when advanced care was added, the percentage healed in the Aurix + UCC group declined to 40%. However, care should be taken when interpreting this result as the limited sample size (15 of 66 patients) is not sufficient for robust statistical testing. In contrast, the majority of patients (34 of 63) in the UCC only group were provided with one or more forms of advanced care and the observed healing rate for this group was 32.4%, higher than the 27.6% observed for UCC only in the absence of advanced care, but substantially lower than the 51% healing observed for Aurix when delivered with standard of care only. Related to this point, Aurix may not only provide a healing benefit, but may also provide additional economic benefit by reducing the need for expensive advanced care options. For example, in this study 54% of patients in the UCC only arm received advanced care as compared to 22.7% of patients in the Aurix arm. Furthermore, while 11 of 34 (32%) of patients in the UCC only group received combinations of advanced care, only 3 of 15 (20%) patients received combinations of advanced care in the Aurix + UCC group.

Critically, this study evaluated healing of wounds including larger and severe wounds, in the presence of difficult comorbidities. Significant healing benefit is established in terms of proportion healed (**Table 8**), time to healing (**Figure 1**) as well as for healing wounds of patients diagnosed with PAD (**Table 11**). Aurix confers a unique healing benefit not provided by other modalities. This view is consistent with the notion that Aurix Therapy bypasses a lack of perfusion to provide a bioactive gel containing platelets that, as the first line of defense for wound healing, provide the appropriate representation of growth factors such as endothelial cell growth factor (VEGF), epidermal growth factor (EGF), transforming growth factor beta (TGF-B) and other biomolecules known to participate in biological activities important for the regeneration of cutaneous tissue[36,37,38].

Limitations

There are some limitations to this study. The open-label design prohibits blinding of the treating clinicians and subjects which can introduce unintentional bias in treatment and carry through. While the inclusion/exclusion criteria are not as restrictive as most published studies and RCT's, they do prohibit inclusion of all patients normally seen in an outpatient wound setting. The determination of durability past 2 weeks could not be studied adequately as the mechanics of CED requiring a patient co-pay made it unlikely that patients would return to the clinic after wound closure for an observational assessment. This would be an important subject for future study, as wound re-opening is common in patients with PAD and diabetes.

Innovation

Aurix is a unique autologous hematogel prepared from platelet rich plasma containing a near physiologic platelet concentration and formulated with ascorbic acid, thrombin and calcium chloride. As compared to traditional randomized controlled trials carried out in wound care, the limited inclusion/exclusion criteria in this study allowed enrollment of patients having a range of severe comorbidities and Wagner grade DFUs. Investigators retained autonomy to employ treatments according to their standard protocols including advanced therapies such as cellular and tissue based products and negative pressure. The results of this pragmatic study are more applicable to the typical wound care setting population of Medicare patients with diabetic foot ulcers.

Conclusion:

This publication is the first pragmatic randomized controlled CED study in wound care. Based on our experience, the CMS CED paradigm can be a promising tool for establishing the effectiveness of therapies and informing robust coverage decisions. However, its implementation requires that CMS, sponsors, investigators and even potential patients understand the operational influences that differ from traditional clinical studies. Specifically, our experience presented here highlights the need to fully understand the reimbursement landscape and mechanisms including, but not limited to, the payor mix and the methods that planned investigative sites may utilize for claim submission.

While many hurdles to enrollment were encountered over the course of this CED study, analyses of a 129 patient ITT data-set support the effectiveness of Aurix Therapy when used to treat chronic DFUs in the Medicare population. In this pragmatic study, Aurix was evaluated when used alone or as part of a continuum of care dependent on treatment decisions made by patients and providers in clinical practice. Results of this pragmatic randomized controlled CED study in wound care support that Aurix Therapy, alone or in combination with other advanced therapies improves healing of chronic DFUs of all severities, even in the presence of serious comorbidities, in the Medicare population as compared to Usual and Customary Care (UCC) as provided in an outpatient wound center. Results should not be used to support other platelet rich plasma products due to the unique formulation, unenriched platelet count, and the incorporation of ascorbic acid and thrombin to make Aurix.

Key Findings:

- This unique design studied a “real world” Medicare-aged, diabetic patient population including those with chronic DFU’s of all Wagner grades 1-4, and severe comorbidities including peripheral arterial disease and smoking.
- The Aurix hematogel system was used with standard of care and in conjunction with advanced therapies, and compared to usual and customary care that could include the use of advanced therapies.
- 48.5% of subjects treated with Aurix healed within the 12- week study period compared to 30.2% of subjects treated with UCC only (p=0.34). 53.9% of subjects with PAD in the Aurix arm healed compared to 30% in the UCC only group. 65.8% of subjects who smoked in the Aurix arm healed compared to 34.5% in the UCC only group. The use of expensive advanced therapies such as Cell and Tissue Products was markedly decreased in the Aurix arm.
- Operational considerations involving the Coverage with Evidence Development paradigm cause considerable challenges to patient enrollment despite the limited exclusion criteria.

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Table 1. Comparison of the Conventional RCT for FDA and Pragmatic RCT under CMS CED

Study Activity	Conventional RCT	Pragmatic RCT
General Operations		
Responsible for Conduct of Study	Sponsor Responsible	Sponsor Responsible
IRB Oversight	Yes	Yes
Informed Consent	Yes	Yes
Investigator Research Background	Experienced	Most Naïve Some experienced
Data Collection and Monitoring	Sponsor Responsible	Sponsor Responsible
Designated Study Coordinator	Yes	No
Study Design		
Protocol Development	Sponsor/FDA	Sponsor/CMS
Inclusion Exclusion Criteria	Narrow Population (limited)	Diverse Population (all-comers)
Multicenter	Not Always	Yes
Financial Aspects		
Cost of Therapy	Sponsor Responsibility	Medicare Reimbursement
Physician Fee	Sponsor Responsibility	Medicare Reimbursement
Facility Fees	Sponsor Responsibility	Medicare Reimbursement
Patient Copay	None	20% Standard Medicare Rules
Patient Incentives	Sponsor Responsibility	Not Allowed per Medicare Rules
Research Payment to Providers	Sponsor Responsibility	Not Allowed per Medicare Rules
Research Payment to Hospital/Facility	Sponsor Responsibility	Not Allowed per Medicare Rules
Regulatory Oversight	FDA	CMS (and FDA if use is off-label)

Presented in **Table 1** are noteworthy comparisons between Conventional Safety & Efficacy studies and Pragmatic Effectiveness studies. Differences in investigator research experience and access to designated study coordinators can present challenges to enrollment, protocol adherence and data collection. The Conventional RCT is exclusive, targeting highly-defined less-representative patient populations thereby limiting prospects for patient enrollment. While the pragmatic study targets enrollment of all-comers, Medicare rules preventing the sponsor from providing patient, provider and hospital incentives under impact enrollment and patient compliance. This is further impacted by the requirement most patients will have to contribute a 20% copay for medical care.

Table 2. Inclusion and Exclusion Criteria

Inclusion Criteria
• Medicare eligible • ≥18 years of age • Type I or II diabetes requiring medical treatment as determined by the physician • The largest non-healing wound, (Index Ulcer) is a Wagner 1-5 DFU that is located on the dorsal, plantar, medial, or lateral aspect of the foot or heel (including all toe surfaces) • The largest ulcer will be selected as the Index Ulcer for study. There must be at least 4 cm between the Index Ulcer and other ulcers • Debrided ulcer size between 0.5 cm2 and 50 cm2 • Subject has received UCC care for ≥ 2 weeks at treating wound clinic • Demonstrated adequate offloading regimen • Duration ≥ 1 month at first visit • Subject must be willing to comply with the Protocol
Exclusion Criteria
• Subjects known to be sensitive to Aurix components (calcium chloride, thrombin, ascorbic acid) and/or materials of bovine origin • Presence of another wound that is concurrently treated and might interfere with treatment of the index wound by Aurix • Ulcer not of DFU pathophysiology (e.g., venous, vasculitic, radiation, rheumatoid, collagen vascular disease, pressure, or arterial etiology) • Patients on chemotherapeutic agents or any malignancy in the wound area • Subjects who are cognitively impaired • Serum albumin of less than 2.5 g/dL • Plasma Platelet count of less than 100 x 109/L • Hemoglobin of less than 10.5 g/dL • Subject has inadequate venous access for repeated blood draw required for Aurix administration.

The inclusion and exclusion criteria presented in **Table 2** were developed with consideration of the Aurix System's FDA approved product labeling. The inclusion/exclusion criteria are intended to provide the possibility of participation to all-comers independent of most co-morbidities and different health status.

Table 3. Schedule of Study Events

PROCEDURES	SCREENING (up to 1 week)	RANDOMIZE (WK 1, V1)	TREATMENT (WK 2-12)*	END OF TREATMENT	HEALING CONFIRMATION
Informed Consent[1]	X				
Inclusion/Exclusion	X	X			
Vital Signs[2]	X	X	X	X	
Screening Labs[3]	X				
Medical History	X				
Physical Exam[4]	X	As needed	As needed	As needed	
Wound History	X				
Vascular Assessment[5]	X				
Concomitant Antibiotics	X	X	X	X	X
Demographics	X				
Wound Assessment[6]	X	X	X	X	X
Infection Assessment[7]	X	X	X	X	X
Off-loading Assessment	X	X	X	X	
Subject Education[8]	X	X	X	X	X
Subject Compliance			X	X	X
Randomization[9]		X			
Aurix + UCC		X	X		
UCC only	X	X	X		
Assess Adverse Events		X	X	X	X
W-QOL		X		X	
Assess Wound Closure			X	X	
Confirm Wound Closure					X

1. Informed Consent must be obtained prior to any study related procedures.
2. Vital signs include blood pressure, pulse, respiratory rate and temperature.
3. Laboratory results for Albumin, Hgb and platelets within the previous 60 days are permissible.
4. Physical exam required at screening, and as needed for remainder of study.
5. Document any vascular assessment that takes place at this visit as well as any assessment that has happened in the past 12 months.
6. To include digital photography, wound measurements and wound bed preparation assessment. Refer to Appendix 2 - Standard Digital Photography and Appendix 3 - Wound Measurement Instructions.
7. Refer to protocol section 7.1.2 (screening), section 7.1.4 (treatment) and Appendix 4 - Infectious Diseases Society of America (IDSA) Guidelines for Diabetic Foot Infections.
8. Refer to Appendix 5 - Subject Education.
9. Randomization to Aurix + UCC or UCC only. Randomization may occur on screening.

Note: If wound closure is not confirmed at week 12 for subjects treated with Aurix but with improved healing, treatment may continue for a maximum of 20 weeks from WK1 at the discretion of the patient and treating clinician.

Table 4: Concomitant Care in the Aurix + UCC and UCC Only Study Groups

Wound Care under UCC	Aurix N (%)	Healed n (%)	UCC N (%)	Healed n (%)
Total Patients	N=66	32 (48.5)	N=63	19 (30.2)
Received Standard of Care Only	51 (77.3)	26 (51.0)	29 (46.0)	8 (27.6)
Received Advanced Therapies	15 (22.7)	6 (40.0)	34 (54.0)	11 (32.4)
Hyperbaric Oxygen (HBO) Only	10 (15.2)	3 (30.0)	8 (12.7)	4 (50)
Negative Pressure (NP) Only	2 (3.0)	1 (50.0)	2 (3.2)	0 (0)
Cell and Tissue Products (CTP) Only	0 (N/A)	N/A	12 (19.0)	4 (33.3)
HBO & NP	2 (3.0)	2 (100)	1 (1.6)	0 (0)
HBO & CTP	0 (N/A)	N/A	4 (6.3)	2 (50)
HBO & NP & CTP	1 (1.5)	0 (0)	1 (1.6)	1 (100)
NP & CTP	0 (N/A)	N/A	5 (7.9)	0 (0)
Not Recorded	0 (N/A)	N/A	1 (1.6)	0 (0)

Usual and Customary Care includes good wound care and allows additional concomitant care. Summarized in Table 4 is the use of wound care that treating clinicians and patients deemed to be in the best interest of healing. The care utilized were Standard of Care dressings only or Standard of Care dressings with one or more advanced concomitant care options including Hyperbaric Oxygen (HBO), Negative Pressure (NP) and Cell and Tissue Products (CTPs) such as skin substitutes and amniotic membranes.

Standard of Care includes the use of semi-occlusive dressings such as Tegaderm or a hydrocolloid dressing such as Triact plain with or without an absorbent outer dressing such as an ABD pad.

The use of chemically impregnated dressings such as Triact Ag was allowed in the UCC only group and was prohibited in the Aurix + UCC arm as described under "**Treatment visits**".

TABLE 5: Patient Demographics for the UCC Only and Aurix + UCC Study Groups

Characteristics	UCC Only (n=63)	Aurix + UCC (n=66)
Mean Age	66.9	64.7
Sex, n (%)		
Male	49(77.8)	51(77.3)
Female	14(22.2)	15(22.7)
Race, n (%)		
White	54(81.8)	57(90.5)
Black	5(7.6)	4(6.3)
Asian	2(3.0)	0(0.0)
Other	5 (7.6)	2(3.2)
Health Risks/Comorbidities, n (%)		
Smoking	29(46.0)	38(57.6)
Peripheral Artery Disease	30(47.6)	26(39.4)
Immunosuppressed	4(6.3)	4(6.1)
Renal Failure	7(11.1)	8(12.1)
Arthritis	3(4.8)	2(3.0)
Transplant Recipient	2(3.2)	5(7.6)
None	19(30.2)	15(22.7)

Presented in **Table 5** are the patient characteristics Age, Sex, Race and Health status including both Health Risks and Comorbidities. These data include the number (n) and corresponding % patients within each study group. Note that while sums of n, % values for Sex and Race will correlate 1:1 with the study group population, patients frequently have multiple Health Risks/Comorbidities. In this case the sum of n, % values do not correlate 1:1 with the study group population but do correlate with each occurrence for the particular health risk/comorbidity observed.

Table 6: Distribution of Ulcer Sizes Across the Aurix + UCC and UCC Only Groups

Area Parameter	Aurix + UCC	UCC only
Avg Wd Area	4.1 cm^2	5.6 cm^2
Wd Area Range**	0.4cm^2-39cm^2	0.04cm^2-46.2cm^2 ******
Wd(n) ≤1 cm^2	N=19 (28.8%)	N=18 (28.6%)
Wd(n) >1cm^2 to ≤7cm^2	N=35 (53.0%)	N=34 (54.0%)
Wd(n) >7cm^2	N=12 (18.2%)	N=11 (17.4%)

Listed in **Table 6** are Wound (Wd) parameters including the average wound area (Avg Wd Area), the wound area range (Wd Area Range), and the number of wounds (Wd(n)) within the designated wound size groupings. The total number of patients (N) and corresponding percentage (%) within each wound size grouping is presented for the Aurix + UCC and UCC only study treatment arms.

****The 0.4cm^2 and 0.04cm^2 ulcer area presented for the Aurix + UCC and UCC only groups are below the 0.5cm^2 minimum area threshold specified by the study inclusion/exclusion criteria but are still considered for analysis within the Intent to Treat population.**

Table 7: Distribution of Subjects in Each Wound Severity Category

	Wagner 1		Wagner 2		Wagner 3		Wagner 4	
Treatment	**Total Patients**	**%**	**Total Patients**	**%**	**Total Patients**	**%**	**Total Patients**	**%**
Aurix (N=66)	3	4.5	32	48.5	27	40.9	4	6.1
UCC (N=63)	7	11.1	19	30.2	31	49.2	6	9.5

A total of 66 patients were enrolled in the Aurix + UCC treatment group and 63 patients in the UCC treatment group. The numbers of patients enrolled within each Wagner grade wound severity category are listed under **Total Patients**. The percentage (**%**) that each value represents within the total patient population of the Aurix + UCC or UCC only study groups is also listed.

Table 8: Cochran-Mantel-Haenszel Test Comparing the Proportion of Patients Healed Between the Aurix + UCC and UCC Only Treatment Groups Within the 13 Week Study Period and Without Stratification

a. Healing Status of the ITT Population

Treatment	Healed n, (%)	Not Healed n, (%)	Total
Aurix + UCC	32 (48.5)	34 (51.5)	66
UCC only	19 (30.2)	44 (69.8)	63
Total	51	78	129

b. Cochran-Mantel-Haenszel Statistics

Odds Ratio (Aurix/UCC)	95% Confidence Limits		P Value
2.1796	1.0579	4.4906	0.034

Table 9: Total Wounds and Wounds Healed in Each Wagner Grade Severity Category

	Wagner 1		Wagner 2		Wagner 3		Wagner 4		Totals	
Treatment	**N, n**	**%**	**N, n**	**%**	**N, n**	**%**	**N, n**	**%**	**NT, nT**	**%**
Aurix + UCC	3, 1	33.3	32, 16	50.0	27, 12	44.4	4, 3	75.0	66, 32	48.5
UCC Only	7, 2	28.6	19, 5	26.3	31, 9	29.0	6, 3	50.0	63, 19	30.2

Listed in **Table 9** are the number of patients enrolled (**N**), the number of healed patients (**n**) and the **%** healing for wounds in each Wagner severity category treated with Aurix + UCC or UCC only. Values listed under **Totals** include the total population of patients (**NT**), the total healed patients (**nT**) and the corresponding **%** healed for the entire Aurix + UCC and UCC only treatment groups.

Table 10: Cochran-Mantel-Haenszel Test Comparing the Proportion of Patients Healed Between the Aurix + UCC and UCC Only Treatment Groups Within the 13-week Study Period and Stratified by Peripheral Artery Disease (PAD) or Smoking

a. Analysis of the ITT Population Healing Stratified by PAD Status

Population with PAD

Treatment	Healed n (%)	Not Healed n (%)	Total
Aurix + UCC	14 (53.85)	12 (46.15)	26
UCC only	9 (30.00)	21 (70.00)	30
Total	23	33	56

Population without PAD

Treatment	Healed n (%)	Not Healed n (%)	Total
Aurix + UCC	18 (45.0)	22 (55.0)	40
UCC only	10 (30.3)	23 (69.7)	33
Total	28	45	73

Odds Ratio (Aurix/UCC)	95% Confidence Limits		P Value
2.2123	1.0699	4.5742	0.0319

b. Analysis of the ITT Population Healing Stratified by Smoking Status

Population that Smoke

Treatment	Healed n (%)	Not Healed n (%)	Total
Aurix + UCC	25 (65.8)	13 (34.2)	38
UCC only	10 (34.5)	19 (65.5)	29
Total	35	32	67

Population that Do Not Smoke

Treatment	Healed n (%)	Not Healed n (%)	Total
Aurix + UCC	7 (25.0)	21 (75.0)	28
UCC only	9 (26.5)	25 (73.5)	34
Total	16	46	62

Odds Ratio (Aurix/UCC)	95% Confidence Limits		P Value

1.9869	0.9510	4.1513	0.0657

Table 11: Cox Regression Comparing Time to Healing between Aurix + UCC and UCC only Treatment Groups within the 13 Week Study Period and with PAD or Smoking as a Covariate

Covariate	Hazard Ratio (Aurix/UCC)	95% Confidence Limits		P Value
PAD	1.774	1.004	3.135	0.0486
Smoking	1.666	0.942	2.945	0.0792

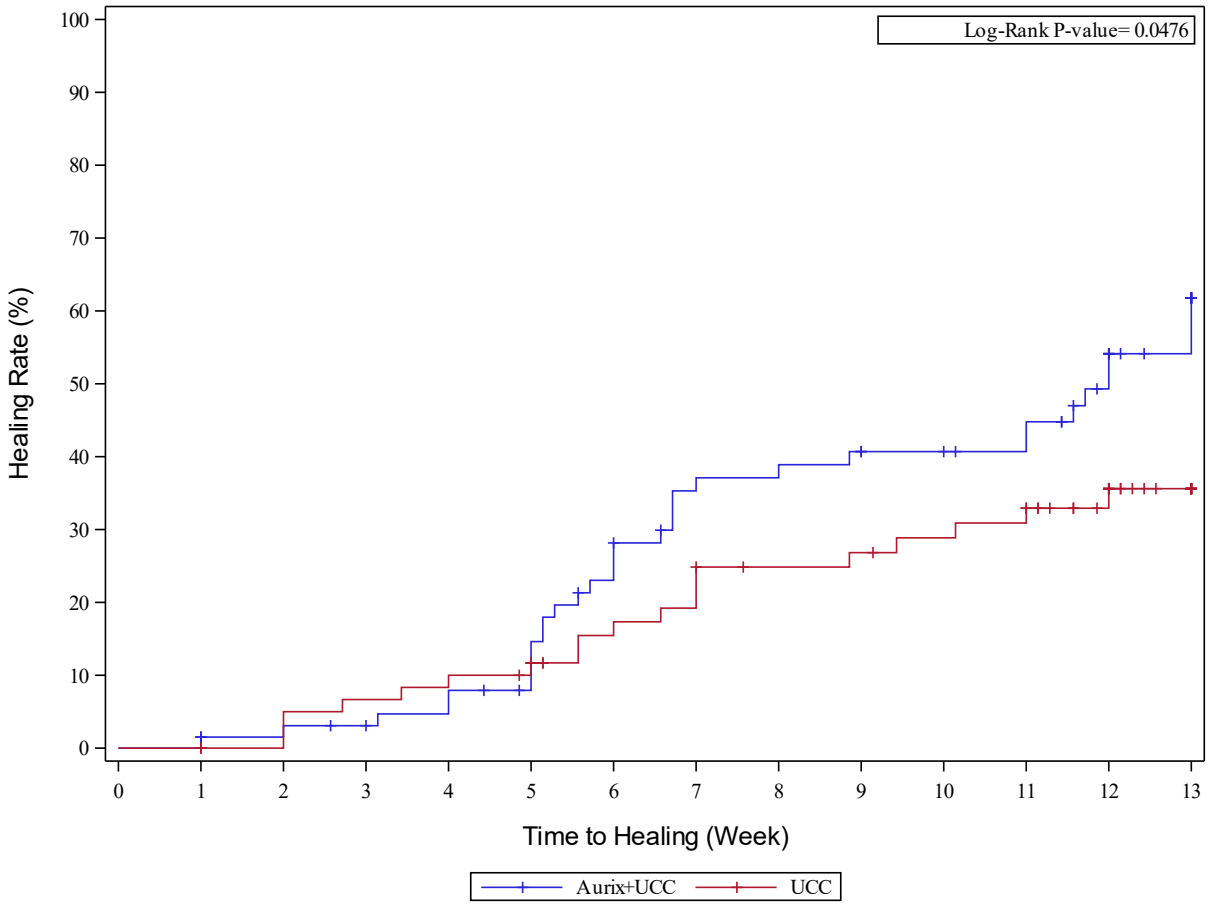
Table 12. Frequency of Use and Healing for Concomitant Antibiotics

	No Antibiotic Treatment			Antibiotic Treatment		
	Healed			Healed		
Treatment Group	Yes n (%)	No n (%)	Total	Yes n (%)	No n (%)	Total
Aurix + UCC	22 (55.0)	18 (45.0)	40	10 (38.5)	16 (61.5)	26
UCC Only	16 (35.6)	29 (64.4)	45	3 (16.7)	15 (83.3)	18
Total	38	47	85	13	31	44

Table 13: Spontaneously Documented Treatment Emergent Adverse Events

Description	N	Serious (Yes/No)	Related to Index ulcer	Treatment Related	Treatment Group
Upper respiratory infection	2	No	No	No	Aurix
Hospitalization, MRSA	1	Yes	No	No	Aurix
Hospitalization, pneumonia	1	Yes	No	No	Aurix
Localized infection	3	No	Yes	No	Aurix
Developed new ulcer	2	No	No	No	Aurix
Osteomyelitis	1	No	No	No	Aurix
Confusion/brain bleed	1	Yes	No	No	Aurix
Cellulites	1	No	Yes	No	UCC
Hospitalization, hypoglycemia	1	Yes	No	No	UCC
New Ulcer	1	No	Yes	Yes	UCC
Hospitalization, amputation	2	Yes	Yes	No	UCC
Hospitalization, nausea/vomiting	1	Yes	No	No	UCC

Figure 1: Kaplan-Meier and Log-Rank Test Comparing Time to Healing Between the Aurix + UCC and UCC Only Treatment Groups



The percentage of healed patients (Healing Rate %) versus the Time to Healing (Week) is plotted for the Aurix + UCC and UCC only treatment groups. The Kaplan-Meier plot and log rank test compares time to healing between Aurix + UCC and the UCC only Treatment groups. Healing is considered for analysis if achieved within 13 weeks after initial treatment (patients who were randomized, received study treatment and provided at least 1 post-baseline ulcer measurement). The time to heal advantage for Aurix is statistically significant with the log rank p-value of 0.0476.

Exhibit B

RECEIVED OCT 3 1 2005

DEPARTMENT OF HEALTH & HUMAN SERVICES **Public Health Service**

Oklahoma City Area Indian Health Service
Five Corporate Plaza
3625 NW 56th Street
Oklahoma City OK 73112-4519

Date: October 13, 2005

To Whom It May Concern,

In June, 2004, The Indian Health Services (IHS) in the Oklahoma City Service Area collaborated with Cytomedix, Inc., a biotechnology company from Rockville, Maryland specializing in wound care, to launch a ***Chronic Wound Management Education Initiative*** at several sites throughout the IHS Oklahoma City region. Our objective was to improve the clinical outcomes of wound care patients while achieving optimal cost-containment in this growing specialty area.

Our IHS team of physicians and nurses, along with a certified wound care specialist from Cytomedix, directed a comprehensive program of wound care services, which included standard and advanced wound treatment. The AutoloGel System, which utilizes the patients own platelets in conjunction with pharmaceutical reagents, was the treatment alternative for patients with full-thickness and complicated wounds that failed to progress or deteriorated with prior treatment(s).

One hundred and three (103) patients with venous, diabetic and pressure ulcers; surgical, dehiscence and other wound types were evaluated. In this cohort, 46 patients presented with wounds that met the criteria to be treated with the AutoloGel System, 35 of which completed therapy. During the course of the year, 91% (42/46) of the wounds healed in an average of 9.8 weeks. This is in contrast to the continued non-healing with an average of 23 weeks treatment during standard care. The results of the entire study are enclosed in the attached paper.

Based upon these results and the evaluations of our clinical team, IHS has determined that the use of the AutoloGel System in a comprehensive wound care program is essential for providing improved clinical outcomes and cost effectiveness for the IHS System.

Sincerely,

Gloria J Holder

Gloria J. Holder, Director, CHS
Office of the Chief Medical Officer
Oklahoma City Area Indian Service
5 Corp Plaza; 3625 NW 56th St.
Oklahoma City, Okla 73112

A Prospective Cohort Analysis of Indian Health Services Chronic Wound Patients Treated With the AutoloGel™ System

Objective

To evaluate the wound healing outcomes in chronic wound patients treated with autologous platelet-rich plasma gel derived from the AutoloGel™ System in the Oklahoma City Service Area of the Indian Health Service. The healing outcomes using this advanced therapy are compared with the outcomes using traditional, standard of care practices.

Background

The Oklahoma City Area Indian Health Services (IHS) and Cytomedix, Inc. cooperated in the development of a Chronic Wound Management Educational Initiative (Initiative) program. Five Indian Health Service sites of the Oklahoma City Service Area participated in the Chronic Wound Management Initiative.

The Initiative's objective was to demonstrate whether patients receiving wound care within the IHS direct-care system would experience a reduction in amputations and a resulting reduction in total wound management costs if:

- physicians and clinicians had training and mentoring at the point-of-care and
- the overall management of chronic wounds included the use of appropriate modalities, timely referrals and use of additional advanced therapies such as the AutoloGel™ wound treatment process.

The analysis component of the Initiative was conducted on IHS-approved data elements collected during the period of August 1, 2004, to July 31, 2005. The goal of the analysis was to identify opportunities to expand or improve the wound care provided by IHS facilities and to evaluate the impact of using the AutoloGel™ to treat the wounds.

IHS and Cytomedix management agreed that all data generated by the Initiative would be the property of IHS. It was also agreed that Cytomedix, Inc., would be specifically required to:

- adhere to HIPPA regulations regarding patient confidentiality and
- limit its use of the data for patient / wound management and for reimbursement purposes.

AutoloGel Treatment Method and Plan

IHS physicians and nurses worked with a Cytomedix wound-care specialist to provide quality care of the patient wounds participating in the IHS Initiative. Patients presenting with chronic wounds were initially treated using standard of care practices. The treatment for the wounds was predominantly gauze dressings with topical agents; a more complete list of types of standard care is provided in Table 4.

Patient and wound data was entered into a Wound Data Registry. The Registry was used to identify current wound treatment modalities for each patient prior to patient participation in the Initiative and to document the progress of patients who were referred into the program.

Patients with the following types of wounds were treated:

- Diabetic wounds
- Venous stasis
- Ischemic
- Pressure ulcers
- Surgical dehiscence
- Other wounds that resist healing

Once the IHS medical staff determined that patient wounds were not responding to standard of care practices, the patient wounds were treated with an advanced therapy, autologous, platelet-rich plasma gel prepared with the AutoloGel System™. The AutoloGel System consists of a centrifuge, a single-use treatment kit and reagents. A small amount of blood is drawn from each patient and the blood is centrifuged to separate the platelet-rich plasma (PRP) and the red blood cells. The PRP was drawn into an applicator system, activated, and the resulting gel (AutoloGel™) was applied topically to the wound. The AutoloGel contains the patient's multiple growth factors and fibrin matrix scaffold.

AutoloGel Patient Selection

Criteria used for wound selection:

- Any wound presenting for treatment that was full-thickness and has been present for more than 4 weeks, was infected, or probed to bone or
- Full-thickness wounds that had not healed by 50% in 4 weeks or completely healed in 8 weeks or
- Any wound that had worsened since first being treated or
- Any wound that had exposed bone, tendon, ligament, or joint or
- Any wound with abscess, osteomyelitis, or necrotic tissue in the surrounding tissue or the wound or
- Wound in compromised patients or
- Wounds with significant ischemia.

Data Collection Plan

Five IHS sites and facilities participated in the Registry, utilized standard approved consent forms for release of medical information and made photographic records of wound progress.

- Patient / wound data was collected as treatments were provided.
- Originals of all patient / wound assessment forms were available for inclusion into the patient medical record.
- Participating IHS facilities were allowed to collect data consistent with their internal reporting and data compilation practices and procedures.

Patient data was collected weekly and entered into the Wound Registry to:

- identify current treatment modalities for wounds prior to the patients/wound being selected for the Initiative,
- document the progress of patients with wounds that were referred into the program and
- allow for analysis and evaluation.

Wound data was tabulated, depicted and analyzed using the following systems:

- Cytomedix Wound Data Registry
- Photography
- Fylling Formula© Wound Data Analysis System
- Clinical Case Studies

Data Elements-

- Key data elements were outlined as age, sex, race, past medical/surgical history and wound treatments, measurements and management elements.
- Photo documentation of wound progression was obtained.
- Forms used were as follows:
    - Patient Demographics – age, sex (Wound History Form)
    - Past Medical/Surgical History – co-morbid conditions were identified (Wound History Form)
    - Past Wound Treatments/Measurements (Wound History Form and Wound Description Form)
    - Current Wound Treatments/Measurements (Wound Evaluation and Treatment Form)

Data Analysis

The goal of analysis was to evaluate the impact of using AutoloGel™ to treat the wounds and to identify opportunities to expand or improve the chronic wound care provided by IHS facilities.

The approved data elements for analysis were:

- All Wound Patients/AutoloGel Treated Patients/Site (Table 1)
- Patient Demographics – age, sex (Table 2)
- Wound Types, Co-Morbid Conditions, Wound Causes (Table 3)
- Previous Treatments Per Wound Type (Table 4)
- Healing or Treatment Time Previous vs. AutoloGel™ (Table 5)
- Outcomes Per Wound Type (Table 6)

For purpose of data collection and analysis, patients with three visits or less were excluded from analysis due to lack of measurable outcomes. Similarly, patients lost to follow-up for conditions such as, non-wound related hospitalizations, relocation to a new area, and death were also not included in the analysis.

Results

One hundred three (103) patients were seen for wound management in the IHS sites participating in the Initiative from August 1, 2004 to July 31, 2005. Of these:

- The lesser severe wounds on fifty-nine (59) patients did not meet the criteria for treatment with AutoloGel thus they were treated with standard of care practices.
- Forty four (44) patients' wounds met the criteria and were treated with AutoloGel following initial treatment with standard of care practices.
    - Of the total AutoloGel treated patient population, five (5) were treated only one time with AutoloGel and did not return for subsequent visits; therefore were not included in the analysis.
    - Four (4) patients treated with AutoloGel were lost to follow-up due to the following reasons and, thus, were not included in the analysis:
        - 2 – wound related surgical procedures, skin graft and toe amputation due to osteomyelitis
        - 1 – non wound related hospitalization
        - 1 – patient left the IHS health care system
- The final cohort that was analyzed included thirty five (35) patients with 46 wounds that met the criteria and were treated with the AutoloGel System (34% of the patients); see Table 1.
- The majority of AutoloGel patients were of 51-60 years of age with the next, most prevalent group being 61 years of age or older.
- Ninety-one percent (91% = 42/46) of the wounds treated with the AutoloGel System healed in an average of 9.8 weeks.

The following types of wounds presented:

venous ulcers (26%)
surgical dehiscence (24%)
diabetic foot ulcers (20%)
insect bites (9%)
pressure ulcers (4%)
collagen vascular disease wounds (4%)
trauma (2%)
other (11%).

The majority of the wounds had other co-morbid conditions with infection being the most common. (Table 3)

Patients received treatments with a variety of standard of care practices prior to treatment with AutoloGel. The length of time to heal with the AutoloGel System was much shorter than this period of previous wound duration without healing especially for venous ulcers and collagen vascular disease wounds; see figure below.

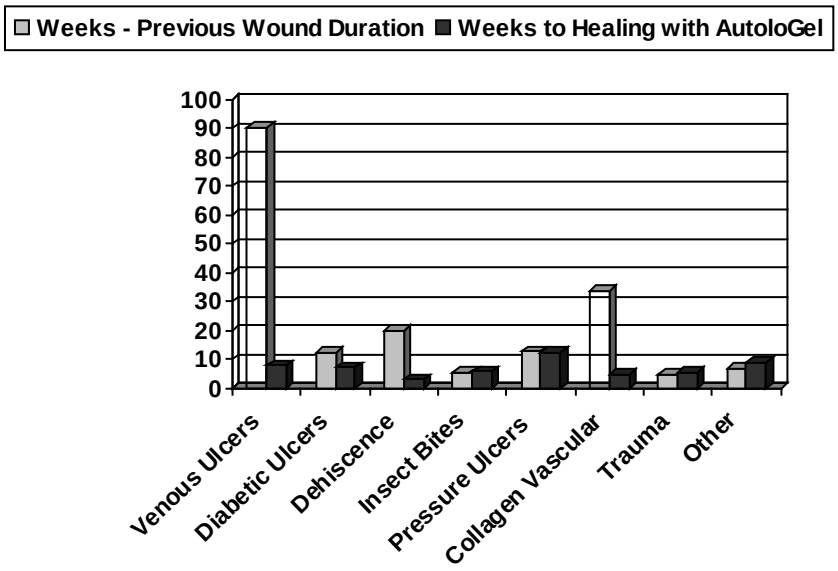


Table 4 provides the number of wounds without healing after the various standard of care practices used with each patient. Once treated with PRP derived from the AutoloGel System, ninety-one percent (91% = 42/46) of the wounds healed in an average of 9.8 weeks, Table 6. In comparison when treated with standard of care practices, these same wounds had an average of 23.5 weeks without healing, Table 5.

One hundred percent (100%) of dehiscence wounds (11), pressure ulcers (2), insect bites (4) and collagen vascular disease wounds (2) treated with AutoloGel healed within 18 weeks of AutoloGel treatment. One diabetic wound of a total of nine diabetic wounds treated with AutoloGel did not heal; refer to Table 6. Two (2) of 12 venous ulcer wounds and one (1) Other wound of five treated with AutoloGel continued under palliative treatment and had not healed by July 31, 2005.

Conclusions

Chronic wounds that had not responded satisfactorily to standard of care practices at five Indian Health Service sites were healed when treated with PRP derived from the AutoloGel System, except for three wounds which continued under palliative AutoloGel treatment. Chronic wounds treated with AutoloGel had shorter healing time and increased healing incidence when compared to previous standard of care practices. This analysis of the IHS cohort indicates that the use of the AutoloGel System may be an effective treatment for healing of venous ulcers, dehiscence wounds, diabetic ulcers and other chronic wounds that have not responded to standard of care practices.

Table 1: AutoloGel™ Patients / Treatments per Site

IHS Sites	1	2	3	Other	Total
Patients seen for wound management	57	19	25	2	103
Patients receiving AutoloGel™	19	9	5	2	35
Percentage of AutoloGel patients	33%	47%	20%	*	34%
Average number of treatments for healed patients **	2.8	5.4	2.7	*	3.7

Table 2: AutoloGel™ Treated Wounds – Age of Patient

AutoloGel™ Treated Patients (35)	Age Group 20-30	31-40	41-50	51-60	61 and up	Total
Male	1	4	5	4	5	19
Female	2	1	2	7	4	16
Total	3	5	7	11	8	35

Table 3: AutoloGel™ Treated Wounds – Wound Types, Co morbid Conditions, and Wound Causes

No. of Patients	35
No. of Wounds	46

Wound Types	**n**	**%**
Venous ulcer	12	26%
Diabetic wound	9	20%
Dehiscence wound	11	24%
Other	5	11%
Insect Bites	4	9%
Pressure Ulcer	2	4%
Collagen Vascular Disease	2	4%
Trauma	1	2%
Total Wounds	**46**	**100%**

Wound types (n)		**Co morbid Conditions (n)**		**Wound Causes (n)**	
Venous ulcer	12	Infection	12	Injury	6
		Spinal cord injury	2	Appeared gradually	8
Diabetes	9	Infection	8	Appeared gradually	7
		Diabetes with neuropathy	1	Surgical procedure	2
		Peripheral arterial disease	1	Other	1
Dehiscence	11	Infection	8	Surgical procedure	10
		Diabetes with neuropathy	1	Appeared gradually	1
Other	5	Infection	3	Appeared gradually	2
		Other	1	Injury	2
Insect Bites	4	Infection	2	Injury	1
				Other	3
Pressure Ulcer	2	Infection	1	Appeared gradually	2
		Spinal cord injury	1		
Collagen Vascular Disease	2	Infection	1	Appeared gradually	2
Trauma	1	Infection	1	Other	1

Table 4: AutoloGel™ Treated Wounds – Previous Wound Treatments without Healing

		Number of Wounds and Types of Previous Treatment							
Wound types	**n**	**Soaks**	**Unna Boot**	**Gauze Drsg**	**Hyrocolloid Drsg**	**Topical Ointment**	**Flap**	**VAC**	**Other- List**
Venous ulcer	12		7	12	2	12			
Diabetes wound	9			9		9			
Dehiscence	11			11		11		3	Surgery
Other	5			5		5			Dakins
Insect Bites	4			4		4			
Pressure Ulcer	2			2		2	1		
Collagen Vascular Disease	2	1		2	1	2			
Trauma	1			1		1			

Table 5: AutoloGel™ Treated Wounds - Outcomes

	Venous Ulcers	Diabetes	Dehiscence	Insect Bites	Pressure Ulcers	Collagen Vascular Disease	Trauma	Other	All Wounds
Wounds (n)	12	9	11	4	2	2	1	5	46
Total Time of Previous Treatment per Wound Type (Weeks)	90.2	12.4	20.2	5.8	13	34.0	5.0	7.2	23.5
Average Time (Weeks) to Heal with AutoloGel <u>for Healed Wounds</u>	8	11.3	18	6	12.5	8	5.6	9.2	9.8

Table 6: AutoloGel™ Treated Wounds as of July 31, 2005

AutoloGel Treated Wounds	Venous Ulcers		Diabetes		Dehiscence		Trauma			
n	12		9		11		1			
	n	%	n	%	n	%	N	%		
Healed	10	71%	8	89%	11	100%	1	100%		
Length of time to healing (Wks)	8		11.3		18		5.6			
Non-Healed			1							
Length of time to non-healing (Wks)			3	11%						
In Treatment (Wks)	2	16%								
Length of time in tx (Wks)	32									

AutoloGel Treated Wounds	Insect Bites		Pressure Ulcers		Collagen Vascular Disease		Other		All Wounds	
n	4		2		2		5		46	
	n	%	n	%	n	%	n	%	n	%
Healed	4	100%	2	100%	2	100%	4	80%	42	91%
Length of time to healing (Wks)	6		12.5		8		9.2		9.8	
Non-Healed									1	2%
Length of time to non-healing (Wks)										
In Treatment							1	20%	3	6%
Length of time in tx							9			

Exhibit C - see last page

Aneurysms/Mesenteric ischaemia

A1

AAA screening in patients undergoing lower limb peripheral duplex sonography: a retrospective and prospective prevalence study

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The prevalence of Abdominal Aortic Aneurysm (AAA) in selected high risk populations is suggested to be higher than within the National Screening Programme. Patients examined for peripheral arterial disease (PAD) at the vascular laboratory in our unit are routinely screened for AAA. The aim of this study was to study the prevalence of AAA in this population.

Between April 2010 and August 2012 1347 patients aged over 18 were referred to the vascular laboratory with suspected PAD. From January 2013 to July 2014, 234 patients aged over 65 were prospectively screened for AAA as part of their peripheral arterial duplex.

Within the retrospective cohort, no AAA were identified in patients aged under 64 years. Among 750 patients aged 65 and over, 154 were excluded as they had an aortic scan. The aorta was not visualised in 129/596 patients. 30 (5.0%) AAA were identified (29/497 male, 1/99 female). The aortic diameter was: 3.0-3.9 cm, n = 17/30; 4.0-4.4 cm, n = 3/30; 4.5-5.4 cm, n = 9/30; over 5.5 cm, n = 1/30.

In the prospective cohort, 26 (11.1%) AAA were identified (17/148 males, 9/86 females). The aortic diameter was: 3.0-3.9 cm, n = 17/26; 4.0-4.4 cm, n = 5/30; 4.5-5.4 cm, n = 2/26; over 5.5 cm, n = 2/26. The aorta was not visualised in 11 patients. The prevalence of co-morbidities in AAA patients was high.

This study confirmed a high prevalence of AAA among patients referred to the vascular laboratory, but they comprise a high risk cohort of patients for operative intervention. The clinical and cost-effectiveness of this approach is unproven.

A2

Abdominal aortic aneurysm is associated with visceral, but not subcutaneous, adiposity

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Modifiable risk factors are useful targets for conservative therapy in cardiovascular disease. Although abdominal aortic aneurysm (AAA) is clearly associated with smoking and hypertension, the role of obesity is less clear. We set out to investigate markers of obesity and fat distribution in AAA.

We evaluated anthropometric indices (body mass index, BMI and waist-to-hip ratio, WHR) in 1076 patients (538 with AAA) from a single UK centre. In a sub-population of AAA patients (n = 32) we studied three-dimensional AAA geometry, via multi-level polygonal segmentation (AAA Analyser v5.2) and semi-automated calculation of visceral-to-superficial adipose ratio (V:S; Analyze 11.0) on computed tomograpghy (CT) imaging. Associations were evaluated using multilevel linear regression (SPSS 20.0). Data are presented as median, interquartile range.

Anthropometric measures of abdominal, but not generalised adiposity were associated with the presence of AAA (AAA vs. controls: WHR 0.97 vs. 0.93; $p = 0.004$; BMI, 27.3 vs. 27.5kgm^{-2}; $p > 0.05$), in covariate-adjusted analysis. In the study sub-population, 87.5% had predominantly visceral (V:S >0.40) adipose distributions (0.74, 0.47-1.03), with high areas of abdominal visceral fat (157.0, 108.5-233.9cm^2; >110cm^2 associated with elevated cardiovascular risk). Participants with visceral-type distributions had larger aneurysms measured by anteroposterior diameter (6.70 vs. 5.55cm, $p = 0.03$) and volume (226.41 vs. 168.76cm^3, $p = 0.007$). There was no association with intraluminal thrombus volume ($p = 0.36$).

Increased WHR was specifically associated with the presence of AAA in our study population. AAA patients had high levels of visceral fat, which correlated with AAA size. Reduction in WHR may help reduce AAA development and progression.

A3

Functional significance of the novel CRAC channel inhibitor, JPIII in abdominal aortic aneurysm vascular smooth muscle cells

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Vascular Smooth Muscle Cells (VSMC) respond to abdominal aortic aneurysm (AAA) formation by reverting to their synthetic phenotype and proliferating. This leads to telomere shortening, replicative senescence and apoptosis. Calcium (Ca^{2+}) entry through the Ca^{2+} release activated Ca^{2+} channel (CRAC) controls these cellular functions in VSMCs. We investigated if pharmacological blockade of the CRAC channel with JPIII modulates AAA VSMC function.

We isolated and cultured VSMC from the aneurysms of patients undergoing open surgery and studied them in culture using an IncuCyte FLR time lapse microscope. JPIII was tested for potency in healthy VSMC. We studied proliferation (cell counting and WST-1 assay) and 24 hour cell migration into a linear wound driven by platelet derived growth factor (PDGF) in addition to apoptosis in response to staurosporine. In all experiments, JPIII was compared to dimethylsulfoxide (DMSO) and a negative control. JPIII was also compared to murine VSMC expressing a pore-dead mutant of the CRAC channel (dnO1). A $p < 0.05$ was taken to be statistically significant.

Seven day AAA VSMC proliferation was reduced in response to JPIII compared to DMSO in both the WST-1 and cell counting experiments ($p < 0.01$). Migration into a linear wound was also inhibited by JPIII compared to DMSO ($p < 0.05$). Further, JPIII conferred protection to the cells from staurosporine induced apoptosis compared to DMSO treated cells. These functional effects of JPIII were recapitulated in the mutant dnO1 mouse VSMCs.

Blockade of CRAC channels by JPIII can modulate AAA VSMC function *in-vitro* and warrants further *in-vivo* study.

A4

The role of Thrombin-Activatable Fibrinolysis Inhibitor in Human Abdominal Aortic Aneurysms

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Patients with Abdominal Aortic Aneurysms (AAA) form dense clots which are resistant to fibrinolysis. Using the elastase infusion model, mice deficient in Thrombin-Activatable Fibrinolysis Inhibitor (TAFI) develop large AAA that are prone to rupture. The aim of this study is to characterise the role of TAFI in human AAA.

250 AAA patients and 210 controls were recruited between 2008-2013. Plasma levels of intact TAFI, TAFI activation peptide (TAFI-AP) and activated/inactivated TAFI (TAFIa/ai) were measured using ELISAs. TAFI activity was analysed by measuring lysis in the presence and absence of potato-carboxypeptidase inhibitor (TAFI-retardation time). TAFI Thr325Ile (rs1926447) genotype was determined using real-time PCR. Results are expressed as mean ± standard error.

Levels of TAFIa/ai and TAFI-AP were higher in patients than controls (27.1 ± 1.4 µg/ml vs 18.2 ± 1.0 µg/ml, $p < 0.001$ and 372.3 ± 16.2ng/ml vs 280.0 ± 11.7ng/ml, $p < 0.001$). TAFIa/ai levels were positively correlated with TAFI-AP ($r = 0.3$, $p < 0.001$). TAFI-retardation time was lower in patients than controls (44.6 ± 0.9 mins vs 51.6 ± 1.1 mins, $p < 0.001$). Intact TAFI levels were not different between patients and controls (13.5 ± 0.2 µg/ml vs

13.3 ± 0.3 μg/ml, p = 0.7). There was no difference in Thr325Ile genotype distribution between patients and controls, although possession of an isoleucine allele was associated with higher TAFI-retardation time (50.0 ± 1.1mins vs 45.7 ± 1.0mins, p = 0.004) and lower intact TAFI levels (13.0 ± 0.2 μg/ml vs 13.8 ± 0.3, p < 0.05).
The increase in TAFIa/ai and TAFI-AP, and decrease in TAFI activity suggest increased TAFI turnover in patients with AAA. Whether these changes reflect cause or effect is unknown. Prospective studies are required to fully elucidate the role of TAFI and fibrinolysis in AAA pathogenesis.

A5

Transthoracic Echo as a Predictor of Long-Term Survival after Aortic Aneurysm Surgery

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Transthoracic echocardiography (TTE) is routinely requested as part of the clinical assessment of patients awaiting endovascular repair (EVR) of abdominal (AAA). However, the long-term prognostic value of this imaging modality is unclear. The aim of this study was to assess pre operative echocardiographic predictors for all-cause mortality in a group of patients undergoing EVR in order identify those patients that are likely to suffer the poorest long-term survival after EVR.
Two hundred and seventy three patients who underwent EVR for abdominal aneurysms between 2008 and 2010 had a TTE prior to their surgery. Patients were prospectively analyzed, with a retrospective review of long-term all-cause mortality as the primary outcome measure to the end of 2013 undertaken.
273 patients underwent elective AAA during the study period. There was 1 death within 30 days (0.4%), and 78 deaths in total over the entire study period (28.6%) from all-causes. Age, left ventricular ejection fraction (LVEF), Echo tubular ascending aortic diameter and the presence of mitral regurgitation (MR) were the strongest predictors of all-cause mortality. This was converted to a risk score that divided the patients into three distinct tertiles. The highest risk tertile demonstrated mortality of >50% at 2 years post surgery.
TTE provides important long-term prognostic information in patients undergoing EVR, over and above conventional risk factors in this high-risk patient group. This study has shown that there is an identifiably high-risk cohort in this patient group who suffer particularly poor longer-term survival after EVR. It is important we target this cohort in particular for pre-operative optimisation and appropriate intensive post-operative follow-up and risk factor management.

A6

Vascular Dysfunction and Thrombogenicity in Abdominal Aortic Aneurysm Disease

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To determine whether systemic vascular and endothelial dysfunction and enhanced thrombogenesis contributes to the pathogenesis of abdominal aortic aneurysm (AAA) disease.
Twelve ex-smokers with an AAA >40 mm and 12 age- and sex-matched ex-smoker healthy controls underwent (i) bilateral forearm venous occlusion plethysmography during intra-arterial acetylcholine, bradykinin and sodium nitroprusside to assess endothelium-dependent and independent vasomotor function, and (ii) a Badimon chamber study to assess *ex vivo* thrombus formation. Both groups demonstrated a dose-dependent increase in forearm blood flow in response to all 3 vasodilators, although increases in blood flow were reduced in patients with AAAs (P < 0.05 for all versus controls). Patients with AAAs also showed greater *ex vivo* thrombus formation (P < 0.05).
Patients with AAAs have impaired endothelium-dependent and -independent vasomotor function, as well as increased thrombogenicity, independent of previous smoking status. This suggests that the pathogenesis of AAA disease is, in part, mediated by systemic vascular dysfunction leading to an increased propensity to reduced vasodilatation and increased thrombus formation.

A7

Positron Emission Tomography and Magnetic Resonance Imaging of Cellular Inflammation in Patients with Abdominal Aortic Aneurysms

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To compare 18F-flurodeoxyglucose (18F-FDG) positron emission tomography and computed tomography (PET-CT) with ultrasmall superparamagnetic particles of iron oxide (USPIO)-enhanced magnetic resonance imaging (MRI) in patients with abdominal aortic aneurysm (AAA).
Fifteen patients with asymptomatic AAA with diameter 46 ± 7 mm underwent PET-CT with 18F-FDG, and T2*-weighted MRI before and 24 h after administration of USPIO. The PET-CT and MRI data were then co-registered. Standardised uptake values (SUV) were calculated to measure 18F-FDG activity, and USPIO uptake was determined using change in R2*. Comparisons between the techniques were made using a quadrant analysis and a voxel-by-voxel evaluation. When all areas of the aneurysm were evaluated, there was a modest correlation between the SUV on PET-CT and the change in R2* on USPIO-enhanced MRI (n = 70,345 voxels; r = 0.30; p < 0.0001). Whilst regions of increased 18F-FDG and USPIO uptake co-localised on occasion, this was infrequent (kappa statistic 0.074; 95% CI, 0.026 - 0.122). 18F-FDG activity was commonly focused in the shoulder region where as USPIO uptake was more apparent in the main body of the aneurysm. Maximum SUV was lower in patients with mural USPIO uptake. Both 18F-FDG PET-CT and USPIO-MRI uptake identify vascular inflammation associated with AAA. Whilst they demonstrate a modest correlation, there are distinct differences in the pattern and distribution of uptake suggesting a differential detection of macrophage glycolytic and phagocytic activity respectively.

A8

Long-term survival after elective infrarenal abdominal aortic aneurysm repair 1969-2011

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Improved critical care, preoperative optimization and the advent of endovascular surgery (EVR) have dramatically reduced 30-day mortality for elective abdominal aortic aneurysm (AAA) repair. However, it remains unknown whether this has translated into improvements in life expectancy and 5-year survival after surgery. The aim of this study was to quantify how 5-year survival after elective AAA repair has changed over time.
A systematic literature search was performed conforming to PRISMA standards. Only studies analysing elective infrarenal (IR) AAA were included. A meta-analysis of all English language literature quoting long-term survival was conducted. 1794 papers were reviewed with 36 studies identified as providing suitable data for analysis. These studies consisted of 60 study arms and a total of 107,814 patients published between 1980 and 2013, with data covering the period 1969 to 2011.
The pooled estimate for the overall 5-year survival after elective IR AAA was 69%, with meta-regression showing there was no significant improvement in 5-year survival between 1969 and 2011 (−0.001, 95% CI -0.014 to 0.012).
Meta-regression also demonstrated that patients with larger aneurysms at the time of surgery were likely to suffer poorer 5-year survival (−0.058, 95% CI −.0.095 to −0.021, I^2 = 85%).
This study demonstrates that 5-year survival after elective infrarenal AAA repair remains poor, and there has been no measurable improvement in this despite over 50 years of advances medical care. More work is urgently required to address the cardiovascular risk that causes this shortfall in the long-term survival of aneurysm surgery survivors.

A9

Management, follow-up and outcomes of patients with type B intramural haematoma of the aorta

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Aortic intramural haematoma type B (IMH-B) is part of a spectrum of conditions known as acute aortic syndrome. It involves the descending aorta and is managed medically. The aim of this study was to review our practice in the management of patients with IMH-B.

In this retrospective study from 2006 to 2014, 31 patients with IMH-B were identified from the hospital database. Prospectively data was collected on these patients. Management, follow up and long term survival was assessed.

The mean age at presentation was 60.4y (31- 90). There was no gender difference (16/15 M/F). The majority of patients (90%) presented with symptoms of aortic disease (chest/interscapular pain). 28 patients were managed medically (28 of 31; 90%). 3 received stent grafts (TEVAR). Early mortality was 12.9% (4 patients). 4 late deaths from unrelated causes. At follow up (range 8-36 months), 20 medically managed patients (20 of 28; 71.4%) and all 3 patients who received stent grafts were alive.

In the group managed medically, 16 (85%) demonstrated resolution or no change on of IMH-B on follow up CT, 3 progressed to type B dissection (11%) and 1 (4%) developed aortic aneurysm; all were managed conservatively. All 3 patients treated with TEVAR showed complete resolution on a follow up CT scan.

IMH-B patients have been managed medically with satisfactory outcomes. 4 patients (15%) of IMH-B however progressed on follow up scan. Although small in numbers, patients who received TEVAR had very good outcomes with no post-operative complications, and complete disease resolution.

A10

Defining an approach to mesenteric ischaemia with clinical variables

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Patients presenting with abdominal symptoms (acute and chronic) are increasingly investigated with contrast enhanced CT. Emergency laparotomy without imaging is now rare. Mesenteric vessel atherosclerosis is frequently identified by contrast imaging; defining the importance of this is challenging.

The aim of the present work was to explore practical variables to help guide clinical decision making.

Cases referred to a regional vascular service over a four-year period (November 2010 to August 2014) were reviewed. Index cases were defined as those undergoing invasive intervention. Variables of interest included mode of presentation, inflammatory and CT findings.

There were 69 procedures performed for mesenteric ischaemia. Revascularisation strategies included angioplasty/stent, hybrid procedures, superior mesenteric thromboembolectomy and bypass. The 42 emergency patients underwent significantly more bowel resections (18) and formations of stoma (9) than the 27 elective (3 and 1 respectively) [$p < 0.05$ CHI squared test]. 28 patients presented with an elevated WCC which was associated with a significantly greater rate of bowel resection and stoma formation [$p < 0.05$]. In this series, contrast CT failed to reliably identify gall bladder and small bowel infarction.

Complications and reintervention rates are high in this group of patients. Evidence of a systemic inflammatory response mandates laparotomy irrespective of radiological appearances and revascularisation strategy.

A11

Orai1 is a viable drug target in abdominal aortic aneurysm

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Orai1 is a calcium ($Ca2^+$) permeable ion channel in the vasculature, activated in response to depletion of $Ca2^+$ stores (store operated calcium entry, SOCE). SOCE is reliant on the store sensor STIM1 and can modulate VSMC function, itself dysregulated in AAA disease. We set out to determine if SOCE was functionally expressed in human AAA VSMCs and if it could be inhibited by the SOCE inhibitor Synta66.

Patients undergoing open AAA repair were invited to donate aortic wall which was used to explant VSMC. RNA was extracted from VSMC using a TRIreagent protocol and rtPCR for Orai1/STIM1 performed relative to ßActin. VSMC were seeded into 96 well plates and used for calcium imaging experiments using a ratiometric $Ca2^+$ indicator dye (Fura-2AM) and a Flexstation plate reader. SOCE was induced by thapsigargin (TG) or platelet derived growth factor (PDGF) and Synta66 compared to vehicle control (dimethylsulfoxide). A $p < 0.05$ was taken to be statistically significant.

Orai1 and STIM1 mRNA were detectable in the explanted VSMC. SOCE was detectable in response to pharmacological store depletion with TG or extracellular application of PDGF. Taken together these data suggest Orai1 is functionally expressed in AAA VSMC. Pre-treatment of the cells for 15minutes with the potent Orai1 blocker, S66 at 5 μM reduced these $Ca2^+$ entry signals compared to the vehicle control ($p < 0.001$).

Orai1 is functionally expressed in VSMC from AAA patients and can be blocked by our pharmacological inhibitor Synta66. This drug deserves further investigation as a novel therapy for AAA disease.

C1

Conservative management of patients not undergoing carotid surgery does not carry disproportionate mortality or further neurological event rates

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Early carotid surgery incurs significant reduction of further ischaemic events in patients with symptomatic carotid artery stenosis. There is limited current information about patients with symptomatic carotid artery disease turned down for surgery. Data on patients presenting to a regional vascular unit with symptomatic stenosis not undergoing surgery is presented.

Clinical imaging databases were interrogated between January 2011 and April 2012. Data of all patients undergoing a carotid duplex with stenosis grade >50% were retrieved. Patient management was referenced against the joint stroke and vascular MDT database. The MDT decisions for patients not undergoing carotid surgery were evaluated.

74 patients undergoing carotid duplex with stenosis >50% and neurological event attributable to carotid disease did not undergo carotid surgery. Evidence of an MDT discussion was found for 73 patients. The reason for non-surgical management at MDT was determined in 66 patients with 5 patients failing to have a reason recorded, and one mortality prior to the MDT. The active surgical turndown rate was 50% (cardiac comorbidity (24/66), delay in presentation (3/66), terminal neoplasm (2/66), cognitive impairment (3/66), other neurology (1/66). Turndown due to change in clinical decision following second imaging modality was 19/66(29%). A further 14/66(21%) patients actively declined surgery. The overall 30-day mortality was 1.4%, there were no further 30-day neurological events, 2.7% events at one year, and overall one-year mortality was 11%.

Conservative management of patients not undergoing carotid surgery did not carry disproportionate mortality or further neurological event rates.

C2

Management of patients with asymptomatic carotid stenosis: What is current practice? Results from a national survey

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Despite evidence from the ACAS and ACST-1 trial, the variation in management of asymptomatic carotid stenosis appeared to vary between vascular surgeons.

Following a pilot survey amongst members of the South West Vascular Surgeons, a revised online survey was redrafted and distributed via email to members of the Vascular Society.
Email contact was available for 396 Vascular Society members. 156 members completed the survey, resulting in a response rate of 39.4%. 70.5% of respondents stated they performed carotid endarterectomies (CEA) in asymptomatic patients.
Of those who performed asymptomatic CEAs, 88.4% would not intervene in patients with a 50-59% carotid stenosis (as classified by NASCET measurements on duplex); whereas in patients with >90% carotid stenosis, 80.2% would offer a CEA to a surgically fit 50 year old male compared with only 20.9% who would offer a CEA to a surgically fit 76 year old female. 85% would offer a CEA in a male with asymptomatic >70% stenosis with a complete contralateral occlusion compared with 74% in females. 78.4% and 31% would not offer a CEA to patients found to have a unilateral or bilateral stenosis respectively, prior to coronary artery bypass grafting. Majority stated that plaque morphology did not affect their decision. 23% respondents stated they were participating in the ACST-2 trial.
Our results confirm considerable variation in the management of asymptomatic carotid stenosis. Contribution to trials in this area should be encouraged, to hasten definitive advice for the management of asymptomatic carotid stenosis.

C3
Patient Co-operative General Anaesthesia for Symptomatic Carotid Endarterectomy

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Carotid endarterectomy (CEA) is the treatment of choice for symptomatic carotid artery stenosis. The GALA study showed no difference in stroke or mortality outcome between general (GA) and local anaesthetic (LA). Patient co-operative anaesthesia (PCGA) offers a third option, offering the benefits of both LA and GA, but has not been adequately assessed for CEA. The objective was to evaluate feasibility and efficacy PCGA for symptomatic CEA.
A prospective cohort study of consecutive patients with symptomatic stenosis undergoing CEA over a four year period. Target controlled infusion (TCI) remifentanil was titrated so the patient could obey commands yet had good analgesia. TCI propofol was commenced and LA applied to the airway prior to intubation. TCI propofol was reduced at skin incision with and neurological status assessed (blinking and squeezing a plastic toy). Technical success was defined as 'having a fully co-operative patient at carotid clamping' and selective shunting was performed.
Overall 143 symptomatic CEA were performed. The median age was 71 years (IQR 62-77) and 99/143 (69%) were men. Thirty four (24%) patients presented with amaurosis fugax, 72 (50%) TIA, 36 (25%) stroke and 1 cerebral hypo-perfusion. The median time from index event to surgery was 13 days (IQR 9-21). A fully co-operative GA was obtained in 109/121(90%) patients with 18/109(16.5%) requiring a shunt. Conversely 12/121 (10%) patients failed to wake up or required conversion to GA. The thirty day stroke and mortality rates were 1/112 (0.9%) respectively.
PCGA is safe anaesthetic technique for CEA in acute symptomatic patients.

E1
A 14-year experience of thoracic endovascular aortic repair and relevance of the bovine aortic arch anatomical variant

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Bovine aortic arch (BA) anatomical variants are associated with an increased incidence of thoracic aortic disease but whether this anatomical variant affects outcomes after intervention remains unknown. We compare outcomes following thoracic endovascular aortic repair (TEVAR) in patients with BA and those with a normal aortic arch configuration (NA).
A database of 346 patients who underwent TEVAR between 2000 and 2014 was analysed. Computerised tomography angiograms were reconstructed to assess the morphology of the aorta. Outcomes were analysed to include in-patient mortality, stroke, paraplegia, endoleak, revascularisation of the left subclavian artery (LSA), and the length of aorta covered.
Bovine aortic arch variants (BA) were identified in 49pts (14%). Indication for TEVAR was aneurysmal disease in 57% of NA and 49% of BA groups with the remaining patients treated for aortic dissection. There were no significant differences in mortality (BA: 5%, NA: 7%), stroke (BA: 8%, NA: 7%), endoleak (BA: 8%, NA: 6%) or LSA revascularization rate (BA: 8%, NA: 10%). There was a trend for a higher incidence of paraplegia in patients with a BA (10%) compared to NA (4%) patients, despite a similar length of aortic coverage by the stent graft (BA: 30cm, NA: 29cm).
The bovine aortic arch variant is common in patients with thoracic aortic disease and may be associated with higher rates of paraplegia following TEVAR. We suggest routine revascularization of the LSA prior to TEVAR in patients with a bovine aortic arch.

E2
A systematic review and meta-analysis of endovascular popliteal aneurysm repair using the Hemobahn or Viabahn stent-graft

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Endovascular repair is now more common than open repair for suitable aortic aneurysms, but surgical bypass is the current gold standard in the management of popliteal aneurysms. With recent technological advances, there is a need to better characterise the results of endovascular repair for popliteal aneurysms. To perform an evidence-synthesis study to assess outcomes of endovascular popliteal aneurysm repair (EVPAR) using the Hemobahn or Viabahn stent-graft.
A systematic review was conducted conforming to PRISMA standards. Primary and secondary patency rates were reported as primary outcomes. Secondary outcomes included re-intervention rates, endoleak, and limb salvage.
14 studies reported outcomes for 514 popliteal aneurysms. There was considerable heterogeneity in reporting standards between studies. Pooled primary and secondary patency were 69.4% (95% CI 63.3%-76.2%) and 77.4% (95% CI 70.1%-85.3%) respectively at 5 years. Five studies compared open surgical repair to EVPAR and no difference in primary patency was found on evidence synthesis (HR 1.30, 95% CI 0.79-12.14, p =0.189).
EVPAR provides an effective alternative to open repair in the treatment of popliteal aneurysms. Further studies are required to optimise both patient selection and follow-up protocols.

E3
1059
Haemodynamic Evidence that Large Iliac Arteries Increase the Risk of Limb Migration following Endovascular Aneurysm Repair

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The distal fixation force of a Zenith limb extension is approximately 9.5N. Our aim was to determine whether the haemodynamic distraction force acting against this was greater in limb extensions that subsequently developed migration and to identify morphological features associated with this increased risk.
Computer models of 43 iliac limb extensions were constructed from the first postoperative CT scan (ScanIP software). Distraction force was obtained by Computational Fluid Dynamic analysis. All blood flow simulations were based on peak systole using patients' preoperative blood pressures. Migration of the distal seal zone was measured on the first and last CT scan using a central luminal line technique. Migration was defined as >4mm movement between imaging episodes.

Four of the 43 limb extensions underwent migration. Median imaging interval was 26 months (range 4-72mths). Distraction force was significantly higher in the migration group versus the no migration group (Median 2.9N, range 2.7-6.3N vs 1.6N, range 0.4-3.8N, p = 0.003, MWU).
Cross-sectional area of the distal limb extension was significantly larger in the migration group (Median 183mm^2, range 123-380mm^2 vs 95mm^2, range 25-254mm^2, p = 0.018, MWU).
This in-silico study shows that proximal migration of iliac limb components may occur in the presence of distraction forces smaller than the reported fixation force. Limb extensions exposed to greater distraction force are more likely to undergo migration and the size of the distal seal zone is significantly associated with this.
These results indicate that care should be taken when planning stent-graft deployment in large, ectatic iliac arteries.

E4
Incidence and management of iliac limb stenoses/occlusions after endovascular aneurysm repair (EVAR) in a high volume endovascular centre

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Secondary re-intervention after endovascular aortic aneurysm repair (EVAR) is common. Future attempts to reduce re-intervention rates require knowledge of the natural history of EVAR-related complications. We aimed to describe the incidence and subsequent management of iliac limb stenosis/occlusion detected after EVAR in our practice.
We retrospectively interrogated clinical records for 431 consecutive elective infrarenal EVAR procedures performed between April 2010 and August 2013. Hospital imaging records and clinical notes were used to obtain information regarding diagnosis of limb complication and management.
36 patients (42 limbs) developed a 50-74% stenosis. 9 (21%) of these cases were actively treated. Of the 33 (79%) patients managed conservatively, 4 (13%) progressed to higher grade stenosis or totally occluded. 79% of patients with 50-74% stenosis were diagnosed within 1 year of EVAR. 15 patients (16 limbs) had 75-99% stenosis. 13 (87%) of these patients were diagnosed with a de-novo 75-99% stenosis. 100% were treated. 87% of patients with a 75-99% stenosis were diagnosed within 1 year of EVAR. 12 patients (12 limbs) had occluded. 92% were identified de-novo and only the symptomatic cases (33%) were treated.
Moderately severe (50-74%) iliac limb stenosis occurs in 8% of patients following infra-renal EVAR. Conservative management of these cases is safe, with only 13% of untreated cases progressing to a greater stenosis or occlusion. High-grade stenosis (75-99%) or occlusion only occurs in 6% of patients, and is most often found de-novo. Centres performing EVAR should have a pre-defined strategy for the management limb stenoses detected during surveillance.

E5
Initial Experience with an 'Off-the-Shelf' Branched Thoracoabdominal Stent Graft

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The Zenith t-Branch is an off-the-shelf device allowing a total endovascular alternative to open and visceral hybrid reconstruction of thoracoabdominal aneurysms (TAAA). We describe our early experience with this device.
A consecutive series of patients who had repair of TAAAs between January 2013 and July 2014 using the t-Branch device were studied. Outcome measures included aneurysm exclusion, 30 day mortality, renal failure, organ / spinal cord ischaemia and target vessel patency.
Thirteen patients (7 male, median age 76 years (range 67-80) had repair of elective (n = 8), emergent (n = 3) and ruptured (n = 2) TAAAs. These included Crawford classification Type 1 (n = 1), Type 2 (n = 4), Type 3 (n = 5) and Type 4 (n = 3) aneurysms. Median operating time was 5h 24min (range 2h 54min - 9h 45min). Branches were cannulated via the right (9 cases) axillary artery, left axillary artery (1 case) or the ascending aorta via a mini-sternotomy (2 cases) or right thoracotomy (1 case). In 4 cases, a single stage procedure was carried out; a 2 stage approach was used for 9 cases. In all cases, the aneurysm was successfully excluded. Forty-five of 50 patent target vessels were successfully stented. There was one death in a patient with a ruptured aneurysm, two patients required temporary dialysis for acute kidney injury and 3 patients developed transient limb weakness.
The Zenith t-Branch is an effective solution for repair of thoracoabdominal pathologies and may be particularly useful for patients in whom the delay associated with the use of custom made devices would negatively impact prognosis.

E6
Optimising the imaging surveillance of thoracic aortic aneurysms

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Yearly imaging surveillance is recommended by most clinicians for patients who are known to have a thoracic aortic aneurysm (TAA), but this practice is not evidence based. This study aimed to determine the rate of TAA expansion to identify optimal surveillance intervals for individuals based on initial maximum diameter.
Morphological data submitted to the M2S database were obtained for 900 consecutive patients that underwent CT surveillance for TAA. Annualised growth rates based on diameter at presentation and time to an intervention threshold of 55m was calculated. The number of patients that would have achieved threshold undetected was determined based on simulated imaging intervals of 6 months, 1, 2 and 3 years.
The median aortic expansion rate was between 1-2mm per year, but an exponential increase in expansion rate at sizes approaching 45mm was noted. No patients with a starting diameter of 30-39mm and only 5% of those with a TAA of 40-44mm achieved threshold size within 2 years, but 25% of those with a starting size of 45-49mm did. At 1 year 36% of those with a starting diameter of 50-54mm had expanded beyond 55mm.
Based on a threshold of 55mm for intervention, most patients with a maximal aortic diameter of <45mm could undergo surveillance every 2 years. Those with a diameter of >50mm should be optimised for repair if this is clinically appropriate.

E7
Atherosclerotic plaque analysis may help to predict outcome following lower limb endovascular intervention

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Atherosclerotic plaque analysis using CT angiogram has been found to be accurate and reproducible in the coronary and carotid arteries. The aim of our study was to assess the utility of this technique in predicting outcome following lower limb endovascular interventions.
We retrospectively analysed pre-procedural CT angiograms in 50 patients who had undergone Femoro-popliteal (F-P) angioplasty (+/−stenting). Plaque analysis was performed using TeraRecon® workstation by two observers blinded to the long term outcome. The section of artery undergoing angioplasty was subdivided into volumes of soft (−100-100HU) fibro-calcific(101-300HU) or calcified(300-1000HU) plaque according to Hounsfield Units scale. The end points were vessel patency, binary restenosis rate and amputation free survival (AFS) at 12 months using Kaplan Meier analysis.
The technical success rate was 98%, with 48% of patients receiving F-P stents. The AFS was 90%, primary patency 84%, assisted primary patency 88% and binary restenosis 44% all at 1 year. A significantly greater total volume of calcified plaque (1.1(0.01-3.2) cm^3 vs 0.11(0-1.86) cm^3, P < 0.001) was found in

patients developing restenosis (>50%) compared to those who did not. Total calcified plaque volume was significantly lower in patients remaining free from SFA occlusions or re-interventions (0.21(0.01-2.6) cm^3 vs 1.3(0-3.2)cm^3, P = 0.007) and in patients surviving 2 years without major amputations (0.34(0.01-3) cm^3 vs 1.4(0-3.2) cm^3, P0.038).
The burden of calcified plaque, but not soft or fibro-calcific plaque is related to restenosis, re-intervention and AFS. CT plaque analysis may form an important non invasive tool for risk stratification in patients undergoing endovascular procedures.

E8
Distal fixation forces of different endovascular limb extensions in an experimental porcine model

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Secure fixation is essential for successful endovascular repair. The aim of this experimental study was to assess the effect of iliac limb stent-graft design and distal diameter upon the fixation force at the distal seal zone.
Freshly prepared porcine aortas were pressurised to 100mmHg using normal saline warmed to 37°C. Iliac limb extensions were deployed at pre-marked sealing zones based on 10% oversizing of the stent-graft. Four different Zenith (Cook Medical Ltd, Bloomington, USA) limb extensions were investigated: a 16mm Flex (TFLE), 16 and 20mm Spiral-Z (ZSLE) limbs and a 20mm Low Profile (ZALL). Cephalad force was applied to each limb extension using a tensile tester and the peak force required to cause 20mm of migration was recorded. Each limb extension was tested three times in one aorta and peak force was compared between devices and distal diameter.
The mean peak force required to initiate migration in the 16mm TFLE limb extension was 2.32N (±0.56N) versus 2.52N (SD ± 0.45N) for the same sized ZSLE device (p = 0.548, MWU). The mean peak force required to cause migration in the 20mm ZALL limb extension was significantly lower than for the 20mm ZSLE device (0.71N ± 0.32N versus 1.91N ± 0.27N, p = 0.036, MWU). There was no significant difference in peak force between the 16mm and 20mm ZSLE device (p = 0.250, MWU).
Distal fixation force was not significantly affected by distal diameter of the limb extension. The ZALL nitinol frame device required approximately three times less force to initiate migration as compared to the other two stainless steel based devices which may make it more susceptible to migration.

P1
Standardising the Management of Aortic and Peripheral Vascular Graft Infection (VGI): The Northwest London Hospitals VGI Management Algorithm

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Background: A paucity of evidence surrounding diagnosis and management of vascular graft infection (VGI) means that most patients are managed on an *ad hoc*, individual basis. Despite calls for management similar to orthopaedic prosthetic infections, under specialist multidisciplinary team (MDT) guidance there are no standardised pathways for VGI management.
Methods: We identified all patients managed for VGI, trust-wide, over a six year period. Paper and electronic records were used to extract biochemical and microbiological results, antimicrobial therapy and surgical intervention characteristics as well as outcomes from diagnosis to present. An expert panel reviewed our experience and the literature to produce a standardised pathway for the diagnosis and treatment of VGI, which can be audited to assess its clinical effectiveness.
Results: Fourteen aortic and 17 peripheral VGI were identified. Median age was 80 (52-91) years. Median time from diagnosis to present/death was 16 months. Mortality rates were 50% (7) for aortic and 18% (3) for peripheral VGI. Microbial cultures were positive in 68% (21) of cases. Imaging was suggestive of VGI in 93% aortic and 53% peripheral VGI. Proposed criteria for diagnosis of VGI were met in 94% of cases. Length and selection of antimicrobial treatment and surgical intervention was heterogeneous between individuals.
Discussion: We developed a standardised diagnostic and management algorithm for VGI to promote early suspicion of VGI and timely clinical, microbiological and radiological investigation. Diagnosis will initiate MDT management of our patients with appropriate medical and surgical intervention based on investigation results or expert agreed empirical treatment strategies.

P2
Distal Bypass versus Angioplasty for Infra-popliteal disease in patients with Critical Limb Ischaemia

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The management of critical limb ischaemia (CLI) in patients with distal arterial disease remains a major challenge. There is limited high-quality evidence to support treatment choices in this area with both bypass surgery and endovascular intervention shown to have good outcomes. Our aim was to compare outcomes in patients undergoing distal bypass surgery with those undergoing distal angioplasty for CLI.
We compared consecutive patients undergoing distal bypass (n = 120) to a matched cohort undergoing distal angioplasty (n = 120) for CLI (Rutherford 4-6) at a single institution. These patients were enrolled in a graft/angioplasty duplex surveillance programme. The end points were primary, primary assisted and secondary vessel patency and amputation free survival (AFS) at 12 months using Kaplan Meier analysis.
Comparing bypass surgery and angioplasty, target vessels included the anterior tibial (32% and 40%), peroneal (21% and 18%), posterior tibial (18% and 24%), tibio-peroneal trunk (18% and 18%) or dorsalis pedis artery (11% bypass only). Primary patency (77% vs 71% P = 0.04) and assisted primary patency (83% vs 76% P = 0.03) at 1 year were significantly better after distal bypass than endovascular intervention. However secondary patency (83% vs 78% P = 0.05) and AFS (76% vs 78%, P = 0.8) were similar. Re-intervention rate was significantly higher following bypass surgery than endovascular intervention (38% vs 18% p < 0.001).
Both infra-popliteal bypass and endovascular intervention have been shown to be effective with good medium-term outcomes in patients with CLI. Distal bypass surgery has been shown to have better overall outcomes, however close surveillance and re-intervention is needed.

P3
HTATIP2 and MAPK3 signalling: Potential targets for enhancing the angiogenic activity of autologous cells from patients with critical limb ischaemia

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Impaired angiogenic activity and early cell death limit the clinical efficacy of autologous cell therapies in patients with critical limb ischaemia (CLI). We have previously identified TIE2 expressing monocytes (TEMs) as a novel candidate for angiogenic cell therapy in CLI patients. Here we (i) compare the angiogenic capacity of TEMs isolated from CLI patients/controls and; (ii) investigate the mechanisms responsible for any functional impairment.
Circulating TEMs were isolated from CLI patients and age-matched controls by FACS and their angiogenic potential compared using the matrigel tubule assay. Differential gene expression between TEMs from CLI patients and controls (n = 6/group) was identified by Agilent Whole Human Genome Oligo Microarray with functional gene analysis (angiogenesis, inflammation, senescence). Expression of genes of interest (selected on biological plausibility, fold-change and significance) was validated by qPCR.
Tubule formation (area and length, P < 0.05) and TIE2 receptor phosphorylation was reduced in CLI TEMs compared with controls. Microarray identified 1098 genes that were differentially expressed (fold change >3-fold, P < 0.05) between control and CLI TEMs. Of 22 genes selected for confirmation by qPCR, increased expression of human HIV-1 TAT interactive protein 2

(HTATIP2) and mitogen-activated protein kinase 3 (MAPK3) was detected in CLI TEMs.
CLI TEMs have impaired angiogenic activity, which is associated with HTATIP2 and MAPK3 overexpression. HTATIP2 expression impairs angiogenesis through secretion of angiogenic inhibitors and modulation of angiogenesis-associated gene expression, while MAPK3 dependent signalling mediates biological functions such as cell survival. Ex-vivo modulation of HTATIP2 and MAPK3 in TEMs may enhance their angiogenic activity prior to clinical use.

P4
Statin therapy in lower limb peripheral arterial disease: Systematic review and meta-analysis

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To investigate and analyse the existing evidence supporting statin therapy in patients with lower limb atherosclerotic arterial disease.
A systematic search of electronic information sources was undertaken to identify studies comparing cardiovascular outcomes in patients with lower limb peripheral arterial disease treated with a statin and those not receiving a statin. Estimates were combined applying fixed- or random-effects models.
Twelve observational cohort studies and two randomised trials reporting 19,368 patients were selected. Statin therapy was associated with reduced all-cause mortality (odds ratio 0.60, 95% confidence interval 0.46 - 0.78) and incidence of stroke (odds ratio 0.77, 95% confidence interval 0.67 - 0.89). A trend towards improved cardiovascular mortality (odds ratio 0.62, 95% confidence interval 0.35 - 1.11), myocardial infarction (odds ratio 0.62, 95% confidence interval 0.38 - 1.01), and the composite of death/myocardial infarction/stroke (odds ratio 0.91, 95% confidence interval 0.81 - 1.03), was identified. Meta-analyses of studies performing adjustments showed decreased all-cause mortality in statin users (hazard ratio 0.77, 95% confidence interval 0.68 - 0.86).
Evidence supporting statins' protective role in patients with lower limb peripheral arterial disease is insufficient. Statin therapy seems to be effective in reducing all-cause mortality and the incidence cerebrovascular events in patients diagnosed with peripheral arterial disease.

P5
Smoking cessation advice and best medical therapy (BMT) in the outpatient setting; how well are Vascular Surgeons managing peripheral arterial disease (PAD) for their elective cases?

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There are several factors to consider when providing optimal care for patients with PAD. BMT comprises of smoking cessation, antiplatelet-agent use, cholesterol reduction and exercise. The desired standard is for 100% of patients to be on BMT prior to their operation and this project investigates current practice in the Outpatient setting.
Retrospective data collection between January - June 2014. Data was collected from computerised hospital patient records and analysed in Microsoft Excel.
Between January - June 2014, 107 patients underwent elective vascular procedures in a single vascular-centre (AAA n = 21, Carotid endarterectomy n = 23, Femoral endarterectomy n = 12, Vascular bypasses n = 43, Revision surgery n = 8). Smoking status was documented for 34 patients preoperatively: n = 23 smokers and n = 11 non-smokers. Of the patients that were identified as smokers; 26%(n = 6) had smoking cessation advice documented pre-operatively; 26%(n = 6) reduced their smoking, and post operatively 43% (n = 10) continued to smoke whereas 30% (n = 7) had stopped. Pre-operatively 79% (n = 85) were on an antiplatelet and operatively this had significantly increase to 96% (n = 103); p = 0.01. Additionally 80% (n = 85) were on a statin pre-operatively and 92% (n = 98) post-operatively.
From the results obtained it is clear that there is poor documentation in the outpatient setting, particularly with regards to smoking status. This makes it difficult to truly assess how well BMT guidelines are being followed. However with a significant increase in BMT post-operatively, it shows that pre-operative management needs to be improved. We propose the introduction of parallel smoking cessation clinics and mandatory documentation sticker to be used in outpatients to improve the departments' performance.

P6
Factors Predicting Severe Crural Arterial Disease In Patients With Diabetes Mellitus

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Diabetes mellitus (DM) is frequently associated with crural artery disease (CAD) that often precludes revascularisation. This study aimed to identify factors that predicted severe CAD in patients with DM.
Consecutive patients presenting to the diabetic foot (DFU) clinic were identified. Those patients who had undergone lower limb angiography as part of their assessment/treatment were included (n = 115). Demographic data was collated and the pre-intervention angiograms were assessed using the Bollinger scoring method. Patients were determined as having mild / severe CAD.
Significant differences were seen in the median Bollinger score of patients for each of the three crural vessels: Posterior tibial 26 (4-30), anterior tibial 13 (0-28) and peroneal artery 2 ((0-13) ($p < 0.05$ Mann Whitney U test). Patients with severe CAD were significantly older (79.5 vs. 73 years; p = 0.013), were more likely to have diabetic retinopathy (52% vs. 30%; p = 0.038) with no difference in duration of DM. Furthermore they were prescribed significantly fewer anti-hypertensive drugs (1 vs. 2; p = 0.029), and specifically were less likely to be prescribed an ACE inhibitor (41% vs. 61%; p = 0.041) with no difference between groups in renal function and statin use.
Differences in the distribution of CAD occur which affects revascularisation strategies. Earlier identification of patients who will ultimately develop severe CAD is key to slowing the disease process and ultimately reducing the risk of major limb amputation. The links between retinopathy and specifically the effect of ACE inhibitors require more in-depth investigation in what will continue to be a significant challenge for vascular surgeons.

P7
From the bench to bedside: $CD16^+$ monocyte cell therapy to salvage the ischaemic leg

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$CD16^+$ monocytes mediate revascularization during tissue remodeling and are mobilised in patients with critical limb ischaemia (CLI). These cells may therefore be a novel, potent candidate for cell therapy in "no option" CLI patients. Our objectives were to:

1. determine the angiogenic activity of these cells;
2. isolate these cells under GMP conditions for clinical use.

Pre-clinical: $CD16^+$ and $CD16^-$ populations were selectively isolated (immunobeads) from CLI patients (n = 7) and their angiogenic activity measured using HUVEC proliferation and tubule formation assays. These cells were migrated towards conditioned media obtained from cultured ischaemic and healthy muscle. In-vivo angiogenic activity was measured by delivering the 2 populations into ischaemic limbs of nude athymic mice (n = 15).
Clinical: $CD16^+$ monocytes were isolated in our GMP cell therapy unit using clinical grade reagents and the CliniMACS system (n = 3 full scale runs). Cell sterility (Bact/ALERT), viability and purity were measured.

$CD16^+$ monocytes induced significantly greater endothelial proliferation ($P < 0.05$) and tubule formation (length $P = 0.015$ and area $P = 0.012$); and showed increased migration towards ischaemic muscle ($P < 0.02$) compared with $CD16^-$ monocytes from the same patients. Delivery of CD16+ monocytes into ischaemic hindlimbs resulted in 100% limb salvage vs 40% with $CD16^-$ monocytes.
We obtained sterile $CD16^+$ monocytes, with >90% cell viability and >70% purity following GMP-grade isolation.
$CD16^+$ monocytes are highly angiogenic and preferentially migrate towards ischaemic muscle, suggesting that they may be a superior candidate for cell therapy in CLI. We have successfully isolated these cells under GMP conditions in readiness for a 'first in man' study.

P8
Diabetes mellitus exaggerates ischaemia induced inflammation and subsequent tissue damage

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Foot ulceration is a common and challenging complication of diabetes. There is increasing recognition of diabetes as a pro-inflammatory condition and evidence that abnormal innate-immune system activation through excessive toll-like receptor4 (TLR4) activation contributes to abnormal chronic inflammation and impairment of wound healing. We aim to study the effect of TLR4 expression, signaling and activation on human dermal fibroblasts in simulated diabetic-ischaemic conditions.
Fibroblasts cultured at 5.5mM were exposed to glucose concentrations from 0mM to 25mM. Identical samples were placed within a hypoxic chamber. Migration was assessed by scratch wound assay and proliferation by crystal violet assay. The effects of a TLR4 neutralising-antibody and antagonist on fibroblast migration were assessed in the 25mM glucose groups.
Hypoxia led to an increase in TLR4 protein expression. This effect was significantly increased ($p < 0.05$) in very high glucose concentrations (25mM), and resulted in increased apoptosis and IL-6 release. Hypoxia resulted in impaired fibroblast migration, particularly at high glucose concentrations ($p < 0.05$). High glucose alone resulted in increased fibroblast proliferation (<0.0001), however when conducted in hypoxia, proliferation became significantly impaired ($p < 0.01$). Inhibition with a TLR4 neutralising-antibody and antagonist ameliorated the effects of high glucose and ischaemia ($p < 0.05$).
Hypoxia stimulates an up-regulation of TLR4 protein expression and this effect is exaggerated by hyperglycaemia. This results in an increase in cellular apoptosis and IL-6 release. The migration of fibroblasts in hypoxia was also disproportionately impaired in the very high glucose treatment groups. TLR4 inhibition resulted in significantly improved fibroblast migration, reduced apoptosis and IL-6 release under these conditions.

P9
A 10-year population-based study of ischaemic peripheral arterial events: implications for primary prevention

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There are no reliable population-based data on the risk factors, incidence, or long-term outcome of critical limb ischaemia (CLI), acute limb ischaemia (ALI) and acute visceral ischaemia (AVI). These data are required to inform health service planning, monitor effectiveness of prevention and enable risk-prediction. We report incidence and outcome for all acute peripheral events in a population of 92,728 in Oxfordshire (2002-2012). Prevalence of key risk factors and levels of primary and secondary prevention were assessed.
510 acute events occurred in 386 patients requiring 803 interventions. Incidence increased steeply with age: 59.3% of events occurred in those aged >75yrs; 26.3% in those >85. Acute events were associated with hypertension (age-adjusted OR = 4.2, 95%CI 3.3-5.2, $p < 0.001$), smoking (2.7, 2.1-3.4, $p < 0.001$), and diabetes, particularly for CLI (7.5, 5.6-10.0, $p < 0.001$). Diabetes and smoking were associated with occurrence at younger ages (OR/10yr age-reduction: 1.4,1.1-1.7,$p = 0.002$; 1.6,1.3-2.0,$p < 0.001$ respectively). 73.5% of patients had diagnosed prior cardiovascular disease and 96.8% had vascular risk factors, yet only 54.4% were on an antiplatelet and 44.6% on a statin. Despite 69.7% taking antihypertensives, 42.9% recorded BPs >140/90 during 5-years prior to event. 88 (23.6%) events were cardio-embolic; of those with known AF (70.5%) only 14.1% were on warfarin despite 82.3% having CHADSVASC scores >2 without contraindications. 30-day and 5-year survival were lowest for AVI (28.2%;24.4%) compared to ALI (75.3%;55.9%) and CLI (92.6%;70.8%) ($p < 0.001$). Pre-morbid cardiac failure and renal dysfunction were independently predictive of 1-year mortality and diabetes of 1 and 5-year limb loss.
Most incident patients with incident peripheral arterial events have known vascular disease in other territories and multiple risk factors, but there remains considerable scope for improving prevention.

P10
Influence of revascularisation modality on level of lower limb amputation in England

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Patients requiring an above knee amputation (AKA) have poorer outcomes but it is not known whether attempted revascularisation influences level of amputation. We determined the effect of revascularisation modality on the level of amputation.
Hospital episode statistics were interrogated to determine the number of major amputation and revascularisation procedures performed in England between 1st April 2003- 31st March 2009. Demographics (age, sex, level of deprivation, treatment location), co-morbidities (diabetes, hypertension, hypercholesterolaemia, coronary heart disease, ischaemic cerebrovascular disease and smoking) and revascularisation modality (endovascular/surgical) were extracted. Multi-variate analysis determined the odds ratios of an AKA in relation to revascularisation attempts (if any).
There were 25 312 major amputations in the six year period of which 7544 (29.4%) were linked to a revascularisation attempt. Level of amputation was significantly influenced by revascularisation attempt. Compared to patients not linked to a revascularisation, those requiring endovascular treatment were less likely to undergo an AKA (OR .82; 95% CI .75-.90). Surgical (OR 1.16; 1.07-1.25) and combined endovascular/surgical treatment (OR 1.24; 1.09-1.40) had the opposite effect. Men (.64; .55-.74) and diabetics (.44; .550.74) were less likely to undergo an AKA whereas patients with coronary (1.28; 1.10-1.47) or cerebrovascular (1.90; 1.33-2.71) disease were more likely to have the procedure. Age, deprivation, hypertension, hypercholesterolaemia, smoking and geographical location did not influence the level of amputation.
When a major leg amputation is necessary, the risk of this being carried out above the knee is lowest after endovascular revascularisation attempts and highest after combined endovascular and surgical treatment.

P11
^{18}F-FDG For In Vivo Tracking Of Late Outgrowth Endothelial Cells

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Endothelial progenitor cells (EPC) hold theoretical promise as regenerative therapy in cardiovascular disease but progress has been hampered by a lack of consensus on how to identify them. Although not an EPC, the late outgrowth endothelial cell (EOC) arises from one. This cell is cultured from peripheral blood and can form capillary-like structures in vitro and perfusing vessels in vivo. In order to further characterise the role of these cells in vascular repair, the aim of this study was to develop a method designed to permit in vivo cell-tracking using ^{18}F-FDG and positron emission tomography (PET).
To test model feasibility, varying numbers of EOCs were incubated in fixed concentrations of ^{18}F-FDG and scanned in a clinical PET/CT system. Fixed numbers of cells were then exposed to ^{18}F-FDG in varying conditions to ascertain how to maximise labelling efficacy. Uptake was assayed by gamma

scintigraphy. The effects of ^{18}F-FDG on cell viability and function were assayed using trypan blue exclusion and tubule formation.
Labelling increasing numbers of EOCs resulted in an increase in PET detectable radioactivity. Labelling efficacy was observed to be time and concentration dependent and highest when cells were incubated in a glucose poor medium. However, significant quantities of ^{18}F-FDG (up to 36% of the initial internalised amount) were noted to leak from labelled cells. ^{18}F-FDG had no discernable effect on cell viability and tubule formation.
It is possible to label EOCs with ^{18}F-FDG to permit cell tracking with PET/CT but the technique may be hampered by leakage of ^{18}F-FDG.

V1
Do elastic bandages perform better than inelastic (non-stretch) bandages in achieving and maintaining compression in the lower limb?

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Compression is often used in venous disease management. Recently, Mosti (2012) showed that negative ankle-calf pressure gradients enhance calf muscle pump function (CMPF). Further, ankle sub-bandage pressures (SBP) of 30-40mmHg are considered optimum. This study compares pressure profiles for inelastic (Panelast®: P) and elastic (Setopress®: S) bandages in respect of these aims.
18 volunteers randomised into two equal groups (P&S) had a below-knee bandage applied by a consultant or trainee. Lower and upper pressure sensors were placed 1 cm above the medial and lateral malleoli (LM, LL) and 10 cm below the tibial tuberosity in the same coronal plane (UM, UL). SBP was measured (all sensors) at 0 and 120min (subjects performed normal activities between measurements).
Median SBP (0min) for P was >30mmHg at all points (LM 57, LL 55, UM 33, UL 38) and higher than in S (LM 27, LL 49, UM 37, UL 36; $p < 0.001$ for LM, LL). The fall (0-120min) in SBP was greater in P compared to S: LM: 22 v 0, $p = 0.006$; LL: 25 v 1, $p = 0.004$; UL: 18 v 7, $p = 0.013$). The median ankle-calf pressure gradients (ACPG, 120min) were 17.5mmHg (P) versus an inverse gradient (-6.5mmHg) in S, $p < 0.008$.
P provides high initial pressures that reduced to appropriate levels by 120min. Conversely, S achieved and maintained appropriate ankle pressures at 0 and 120min. Although the ACPG for P adheres to conventional aims S, from recent work, should enhance CMPF. Further studies are justified to examine the clinical efficacy of elastic bandages.

V2
Comparison of microbubble presence in the right heart during mechanochemical and radiofrequency ablation for varicose veins

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Mechanochemical ablation (MOCA) is a novel technique for ablation of varicose veins utilising liquid sclerosant and mechanical vein wall irritation. Ultrasound Guided Foam Sclerotherapy (UGFS) has been reported to have caused 13 strokes, as well as transient neurological symptoms including visual disturbances (1.4%) and hemiparesis (2%). Air emboli have been implicated as a cause and microbubbles in the heart during UGFS have been demonstrated. There is only one report of stroke after endothermal treatment in the literature. This study investigated the presence of microbubbles during varicose vein ablation by MOCA and radiofrequency ablation (RFA).
Ethical approval was obtained to recruit patients undergoing Great Saphenous Vein ablation by MOCA or RFA. Participants' hearts were assessed using transthoracic echocardiogram for microbubble presence during treatment. Offline blinded image quantification was performed using International Consensus Criteria grading guidelines. Patients were assessed for neurological symptoms immediately and 30 minutes post procedure.
From 32 recruited patients, 28 data sets were analysed. 11 underwent MOCA and 17 underwent RFA. There were no neurological complications. In total 39% (11/28) of patients had Grade 1 or 2 microbubbles detected. Thirty-six percent (4/11) of MOCA patients and 29% (5/17) of RFA patients had microbubbles with no significant difference between the groups ($p = 0.8065$).
A comparable prevalence of microbubbles between MOCA and RFA both of which are lower than that previously reported for UGFS suggests that MOCA does not confer the same risk of neurological events as UGFS for treatment of varicose veins.

V3
Compression regimes after endovenous ablation for superficial venous insufficiency - a survey of members of the Vascular Society of Great Britain and Ireland

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The optimal compression regime following Ultrasound Guided Foam Sclerotherapy (UGFS), Radiofrequency ablation (RFA) and Endovenous Laser Ablation (EVLA) for varicose veins is not known. The aim of this study was to document current practice in Great Britain and Ireland.
A postal questionnaire was sent to 348 consultant members of the Vascular Society of Great Britain and Ireland (VSGBI).
Valid replies were received from 41% (n = 141) surgeons representing at least 68 (61%) vascular surgery units in Great Britain and Ireland. UGFS was provided by more surgeons (74%), than RFA (70%) or EVLA (32%), but significantly fewer patients were treated by UGFS (median 30 (range 2-500) per year, than by endothermal treatment (median 50 (range 2-300) - $P = 0.019$.
All surgeons prescribed compression following UGFS and endothermal ablation: following UGFS for a median of 7 days (range 2 days to 3 months) and after endothermal ablation for 10 days (range 2 days to 6 weeks) - $P = 0.298$. Seven different combinations of bandages, pads and compression stockings were reported following UGFS. Where bandages and stockings were used sequentially, the change was performed after 5 days (1-14). Following endothermal ablation 4 different combinations were reported - the majority (71%) used bandages, followed by compression stockings after a period of 2 days (range 1-14).
Compression regimes after treatments for varicose veins vary significantly among members of the VSGBI. Research to identify the optimum regime is warranted.

V4
Long Term Results of Transjugular Coil Embolisation for Pelvic Vein Reflux - Results of the Abolition of Venous Reflux at 8 Years

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Pelvic vein reflux (PVR) is associated with leg varicose veins in 20% of women who have had previous vaginal delivery. Pelvic vein embolisation (PVE) with coils has shown to be successful treatment in the short term. The objective of this study was to ascertain the long-term outcome of PVE for PVR.
Patients undergoing PVE between 2005 - 2007 for PVR were invited for transvaginal duplex scanning (TVS) in 2013. TVS results were compared from pre-embolisation, 6 weeks post-embolisation and long-term follow-up.
28 females aged 40 to 75 years (mean 53.5) attended. Parity before PVE ranged 1 - 5 children (mean 2.8). The mean between PVE and follow-up scan was 8 years. Pre-procedure reflux patterns are shown in Figure 1.
Initial six weeks outcomes: 25 women had complete/virtual elimination of all PVR. Three women had persistent reflux in at least one vein post embolisation.
8 year outcomes: 11 women had complete elimination of PVR: 7 had elimination of all truncal reflux but had minor reflux and vulval veins, six had minor reflux in one pelvic vein. 4 had significant reflux, all of which were internal iliac veins. One of these had a baby 1 year post PVE and one had coils removed at a gynaecological operation. Pattern of reflux in long term shown in Figure 2.
PVE is a durable technique for the abolition of PVR, particularly in the ovarian veins.

V5

Varicose vein surgery and pharmacological thromboprophylaxis: Providing a waypoint in an evidence wasteland

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NICE CG92 recommends thromboprophylaxis (TP) for patients at risk of Hospital Associated Thrombosis (HAT). Not all patients are at the same risk of HAT with limited evidence regarding day case varicose vein (VV) surgery. Since 2009 we have applied root cause analysis (RCA) to all cases of thrombosis allowing determination of the incidence and risk factors. We have used these data to inform local practice for TP in situations where there is no explicit guidance. Patients with HAT occurring within 90 days of GA day case VV surgery were identified September 2009-April 2014. Non-fatal cases were identified through the outpatient VTE service, the inpatient anticoagulation and radiology services; fatal cases from death certificates and post mortem records.

9 cases of HAT (4 DVT and 5 PE [1 fatal]; age range 22-78) were identified out of 955 procedures resulting in an incidence of symptomatic HAT of 0.94%. Analysis of identifiable risk factors found that 1 had none; 3 had 1; 2 had 2; 2 had 3 and 1 had 4. 7 had surgery >60 minutes, 5 BMI >30, 3 previous history of phlebitis, 1 age >60, and 1 on oestrogen-containing pill. 7 received TEDS, 8 had 'stat' doses of LMWH, none had extended pharmacological TP.

The incidence of HAT in our practice is lower than reported elsewhere, but at a level where other surgical specialties offer extended TP. RCA can be used to inform practice when there is limited published evidence and has led to restructuring of our TP protocol.

V6

Multi-sequence non-contrast MRI characterisation of experimental venous thrombi predicts susceptibility to lysis and is feasible in man

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Non-contrast MRI using magnetisation transfer rate (MTR), apparent diffusion coefficient (ADC) and T1 mapping can characterise different aspects of organisation in a resolving venous thrombus. We now investigate whether multi-sequence thrombus imaging (MSTI) can identify thrombi suitable for lysis in an experimental model, and whether it may be translated to man.

Magnetisation transfer, diffusion weighted images and T1 relaxation times were measured at days 2, 4, 7, 10, 14, 21 and 28 after venous thrombus induction in mice (n = 8/gp). Tissue plasminogen activator (10mg/kg) was administered through tail vein injection immediately after imaging at each time point and mice scanned 24hrs later to evaluate the effect of lysis. Murine imaging sequences were combined and optimised to image the pelvic veins in man using healthy volunteers in order to produce a clinically useable imaging card. MSTI sequences were validated using phantoms before application to patients with iliofemoral deep vein thrombosis (DVT) undergoing lysis.

ROC curve analysis shows that the combination of MTR smaller than 2,900 (%/cm3), ADC larger than 0.93($\times 10^{-3}$ mm^2/s) and T1 shorter than 784ms has a sensitivity of 88% and specificity of 97% to identify thrombi amenable to lysis. MSTI is feasible in man, with optimisation leading to successful characterisation of iliofemoral DVT in under 25mins.

Non-contrast MR imaging, using a combination of MTR, ADC and T1 mapping, accurately identifies experimental venous thrombi susceptible to lysis. These MSTI sequences can also be readily translated to man where may find utility in characterising the age and structure of thrombus.

V7

Randomised trial of EVLA versus surgery for superficial venous insufficiency: 5 year results

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NICE recommends endothermal ablation as first line treatment for superficial venous insufficiency, however there is a paucity of long-term outcome data. We present the 5 year outcomes of a large RCT of Endovenous Laser Ablation (EVLA) versus surgery for superficial venous insufficiency.

Patients with primary, symptomatic, unilateral superficial venous insufficiency, due to isolated saphenofemoral junction incompetence, and great saphenous vein reflux were randomised to receive EVLA or surgery. Outcomes included: Venous Clinical Severity Score (VCSS), Disease specific Quality of Life (QoL) using Aberdeen Varicose Vein Questionnaire (AVVQ), Generic QoL using SF-36 and EQ-5D and requirement for secondary procedures.

212 patients (76%) of 280 initially randomised were assessed at 5 years. Follow-up was similar between both groups (EVLA n = 108 surgery n = 104, P = 0.477). At 5 years reported VCSS was significantly lower in the EVLA group (EVLA 0 (0-1) vs Surgery 1 (1-2) P = .025), however no intergroup difference was reported between EVLA and surgery as measured by AVVQ (3.401 (.172-7.206) vs 4.623 (1.623-10.294) P = 0.074), EQ-5D (1.000 (.772-1.000) vs 1.000 (.760-1.000) P = 0.158) nor any domains within the SF-36; aside from Social Functioning, whereby EVLA reported higher (better) QoL scores (87.5 (50.0-100.0) vs (50.0 (50.0-100.0) P = 0.003). No difference in requirement for secondary procedures was detected between either group (P = 0.749).

The long-term durability of EVLA strengthens its utilisation as the primary treatment option for superficial venous insufficiency.

V8

Incidence of deep venous thrombosis (DVTs) after differing varicose veins procedures

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The aim of this study was to compare the post-operative incidence of DVT following different varicose vein procedures, including: long and short saphenous ligation and stripping; radiofrequency ablation; endovenous laser therapy; foam sclerotherapy; and treatment of unilateral and bilateral recurrent varicose veins.

Hospital Episode Statistics (HES) data was analysed for all varicose vein procedures performed between 2003 and 2013, and all readmissions for DVT in the same patients at 30 days, 90 days, and one year. Comparison of the incidence of DVTs between procedures was performed using a Chi-squared test.

Between 2003-2013 261169 varicose vein procedures were performed. There were 686 DVTs recorded at 30 days (0.26% incidence), 884 at 90 days (0.34% incidence), and 1246 at one year (0.48% incidence). DVT incidence for different procedure was between 0.15-0.35% at 30 days, 0.26-0.65% at 90 days, and 0.46-0.65% at one year. At 30 days there was a significantly lower incidence of DVTs for foam sclerotherapy procedures (χ^2 (6) = 16.4, p = 0.01, standardised residual = -3.49). There was no difference in DVT incidence between procedures at 90 days (χ^2 (6) = 9.8, p = 0.13) or one year (χ^2 (6) = 9.28, p = 0.16).

Patients undergoing varicose vein procedures have a small but very definite increased incidence of DVT compared to the general population. Foam sclerotherapy was observed to have a lower incidence of DVT at 30 days, but this effect did not persist at 90 days or one year. There was no other significant difference in the incidence of DVTs between open and minimally invasive treatments.

V9
Pilot trial of neuromuscular stimulation in the management of chronic venous disease

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In chronic venous disease the calf muscle pump is impaired. We investigated the effect of bilateral leg neuromuscular stimulation (NMES) on subjects with chronic venous disease.
40 subjects were recruited from 4 groups: healthy, superficial insufficiency, deep insufficiency, and deep obstruction. Haemodynamic venous measurements were taken from the right femoral vein with ultrasound; laser doppler fluximetry from the left hand and foot. Devices were then worn for 4-6 hours per day, for 6 weeks. Haemodynamic measurements were repeated at week 6. Quality of life questionnaires were taken at week 0, 6 and 8.
The mean age was 48.7, BMI 28.6kg/m^2, and maximum calf circumference 39.0 cm. 24 subjects were men. NMES increased femoral vein PV, TAMV and volume flow by 57.7%, 24%, 37.1% at 20 minutes (all $p < 0.05$), which was enhanced at week 6 (PV and TAMV $p < 0.05$). Mean increases in arm and leg temperature were 0.9'C (2.7%, $p < 0.01$) and 0.3'C (1.3%, $p < 0.05$). Mean increases in arm and leg fluximetry were 71% and 203.9% (both $p < 0.0001$). Leg swelling was reduced by mean 329.7 cm^2 ($p = 0.01$). Changes compared to baseline quality of life were not significant.
NMES improves venous haemodynamic parameters in chronic venous disease, which is enhanced by regular use. The immediate effect is attenuated by deep venous disease. NMES reduces leg oedema, improves blood supply to the skin of the foot, and has a systemic effect.

V10
Elective Abdominal Aortic Aneurysm Repair in England and USA: The Impact of EVAR on Population Mortality

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Procedural mortality is of paramount importance for patients undergoing elective abdominal aortic aneurysm (AAA) repair. International comparative analysis can contextualise national results and highlight modifiable factors associated with the best outcomes. This study compared the use of endovascular repair (EVAR) and in-hospital mortality for elective AAA repair in England and the USA.
The English Hospital Episode Statistics and the USA Nationwide Inpatient Sample were interrogated for elective AAA repair from 2005-2010. In-hospital mortality and the use of EVAR were analysed separately for each healthcare system, after within-country risk-adjustment for age, gender, year, and co-morbidity index.
The study included 21,272 patients with AAA in England, of whom 86.61% were male with median (IQR) age 74 (69-79) years. There were 196,113 AAA in the USA, of whom 76.14% were male, with median (IQR) age 73 (67-78) years. In-hospital mortality was greater in England (4.09% vs 1.96%, $p < 0{\cdot}001$) and EVAR less common (37.33% vs 64.36%, $p < 0{\cdot}001$). These observations persisted in age/gender-matched comparison. In both countries, lower mortality and greater use of EVAR were seen in centres performing greater numbers of AAA repairs/annum. In England, lower mortality and greater use of EVAR were seen in teaching hospitals with larger bed-capacity.
In-hospital survival and the uptake of EVAR are lower in England than the USA. In both countries, mortality was lowest in high-caseload centres performing a greater proportion of cases with endovascular repair. These common factors suggest strategies for improving outcomes for patients requiring elective AAA repair.

W1
Mortality from ruptured abdominal aortic aneurysm is associated with hospital structures and processes

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There is significant variation in the mortality rates of patients with a ruptured abdominal aortic aneurysm (rAAA) admitted to hospital in England. This study sought to investigate whether modifiable differences in trust structures and processes were associated with differences in patient outcome.
Patients diagnosed with rAAA between 2005-2010 were extracted from the Hospital Episode Statistics. After risk adjustment trusts were categorised as high and low mortality outliers. Trust level structure and process variables were compared between categories, and tested for association with risk adjusted 90 day mortality and non-corrective treatment (palliation) rate using binary logistic regression models.
There were 9,877 patients admitted to 153 English NHS Trusts with rAAAs during the study period. The overall combined (operative and non-operative) mortality rate was 67.4% (palliation rate 41.3%). 7 (4.6%) trusts were high mortality and 15 (9.8%) trusts were low mortality outliers. Low mortality outliers utilised significantly greater resources per bed (doctors [0.922 versus 0.513, $p < 0.0001$], consultant doctors [0.316 versus 0.168, $p < 0.0001$], nurses [2.341 versus 1.770, $p = 0.0002$], critical care beds [0.045 versus 0.019, $p < 0.0001$], operating theatres [0.027 versus 0.019, $p = 0.0017$] and performed more fluoroscopies [12.6 versus 9.2, $p = 0.0458$] than high mortality outlier trusts. On multivariate analysis, greater levels of consultants, nurses, fluoroscopies, teaching status and weekday admission were independent predictors of lower mortality and a lower rate of palliation.
The variability in rAAA outcome in English NHS trusts is associated with modifiable hospital resources. Such information should be used to inform any proposed Quality Improvement Programme surrounding rAAA.

W2
Mortality from ruptured abdominal aortic aneurysms: clinical lessons from a comparison of outcomes in England and the USA

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The outcome of patients with ruptured abdominal aortic aneurysm (rAAA) varies by country. Study of practice differences might allow the formulation of pathways to improve care.
We compared data from the Hospital Episode Statistics for England and the Nationwide Inpatient Sample for the USA for patients admitted to hospital with rAAA from 2005-2010. Primary outcomes were in-hospital mortality, mortality after intervention, and the rate of intervention for rAAA. In-hospital mortality and intervention rates were analysed by conditional regression, after adjustment for age, sex, year, and comorbidity index.
The study included 11,799 patients with rAAA in England and 23,838 patients with rAAA in the USA. In-hospital mortality was lower in the USA than in England (53.05% [95%CI 51.26-54.85] vs 65.90%; $p < 0.0001$). Intervention was offered to a greater proportion of cases in the USA than in England (19,174 [80.43%] vs 6897 [58.45%]; $p < 0.0001$) and endovascular repair was more common in the USA than in England (4003 [20.88%] vs 589 [8.54%]; $p < 0.0001$). Post intervention mortality was similar in both countries (41.77% for England and 41.65% for USA). These observations persisted in age-matched and sex-matched comparisons. In both countries, reduced mortality was associated with increased use of endovascular repair, increased hospital caseload (volume) for rAAA, higher hospital bed capacity, hospitals with teaching status, and admission on a weekday.
In-hospital survival from rAAA, intervention rates, and uptake of endovascular repair are lower in England than the USA. In England and the USA, the lowest mortality was seen in teaching hospitals with larger bed capacities, treating a greater proportion of cases with endovascular repair. These common factors suggest strategies for improving outcomes for patients with rAAA.

W3
Does type of anaesthesia influence postoperative mortality in acute limb ischaemia?

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Thrombembolectomy is one of the most frequently performed vascular surgical procedures.
The choice of anaesthesia is influenced by the length of procedure, ease of access to operative site, comorbidities, patient preference and heparin therapy.
The aim of this study was to determine the impact of general vs. locoregional anaesthesia on mortality, in a regional vascular centre.
The paper represents a retrospective analysis of a prospectively collected patient data base covering September 2009 to December 2013. 208 consecutive patients who underwent upper or lower limb thrombembolectomy were included in the study.
The relation between postoperative deaths, anaesthesia, ASA grade and age was tested using Chi Square.
125 patients (60%) had thrombembolectomy under locoregional anaesthesia vs. 83 patients (40%) who had the procedure under general anaesthesia. 84 (67.2%) of the first group and 60 (72·28%) of the second group were ASA 3/4. The 30 days mortality in the first group was 11.2%, vs. 7.22% in second group. The mortality for ASA 3 was higher for the first group (n = 69), 14.49 % vs. 4% (n = 50) (P = 0.036, RR 3.62), and similar for ASA 4.
The mortality in the first group for patients >60 yr. old was 12.28% vs. 8.88% (P < 0.0001, RR 1.38).
In this non-randomised study general anaesthesia appeared to have a lower mortality even in high risk patients. Further studies are needed to confirm these results.

W4

Day of the week and in-hospital mortality after emergency vascular surgery

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Recent publications/media campaigns have focussed on hospital mortality for patients undergoing weekend operations, such that major service reconfiguration with 7/7 working for the NHS has been proposed. This study examines in-hospital emergency mortality after vascular surgery with respect to operative day of week.
A prospectively collected database was interrogated for 2965 emergency vascular operations from 1988-2013. Mortality was compared for surgery carried out on weekdays and weekends. Type of surgery was categorised into AAA, amputation, embolectomy, revision/re-do surgery, infra-inguinal and miscellaneous.
On average, 494 operations were performed each weekday compared to 249 operations each weekend day. Overall mortality for emergency surgery was 679/2965 (23%). Mortality for surgery performed on weekdays was 22% (538/2468) vs 28% at weekends (141/497, P = 0.002, Chi square). Risk ratios for weekend mortality for the six operation types were not significantly different from 1.0. There was variation in case mix, with 21% of emergency AAA being done at weekends compared to 10% of infra-inguinal surgery. Overall mortality risk from the 3 procedures done most commonly at weekends was 25% (443/1776) compared to 20% (236/1191) for the 3 less commonly performed procedures (P = 0.0013, Chi square).
Fewer emergency vascular operations are performed at weekends with higher operative mortality. The mortality risk from any particular operation type was no higher at weekends than on weekdays but there was a higher mortality case mix at weekends. The data suggests that it is case selection rather than lack of 7/7 working practice contributing to increased weekend mortality.

W5

High volume, better outcome? The impact of centralisation of vascular services on the emergency workload for an arterial hub - a 2 year experience

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Guy's & St. Thomas' Vascular Unit (South East Thames Arterial Hub)

The justification for centralising vascular services was to improve outcomes for elective surgery. A side-effect of centralisation is the burden of the emergency admissions to the arterial hub, which is difficult to quantify prior to centralisation.
Prospectively collected data for emergency admissions to a vascular hub serving a population of 3.5 million for two years after centralisation (2012-2014) were examined.
There were 1852 emergency admissions (median age 73 years; range 4mths-100yrs; 63% men). An average of 18 admissions each week - a 79% increase above that predicted.
876 (47%) were transferred from other hospitals, of which 769 (88%) were from local networked hospitals and 107 (12%) were tertiary referrals. The overall inpatient mortality for the whole cohort was 11%, with a median length of stay (LOS) of 9 days (0-293). 1221 patients (66%) had at least one operation, with an operative mortality of 9%.
The predominant reasons for admission were: Acute on chronic critical limb ischaemia 33% (median LOS 14 days); Aortic emergencies 17% (9 days); Acute limb ischaemia 11% (7 days); Diabetic foot sepsis 9% (15 days); Carotid pathology 5% (4 days) and deep vein thrombosis 4% (5 days). The overall readmission rate was 8%.
Centralisation of elective vascular services is associated with a significant increase in the emergency workload. Investment in staff and infrastructure is required in order to deliver a safe, high quality service with good patient outcomes. We would advise units intending similar change to plan for more emergency resources than historic activity would suggest.

W6

Hybrid revascularisation procedures: The way forward ?

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Hybrid revascularisation (HR) procedures are increasingly commonplace in vascular centres across the UK. We report our experience with these novel procedures.
A prospectively maintained database was analysed to identify all patients who underwent HR between November 2011 and June 2014. Outcome measures evaluated included technical success, symptom relief, patency and re-intervention rates. All patients were followed up with a duplex surveillance programme.
One hundred and twelve patients [84 male, mean age 72 (47-93) years] were identified. Indications for treatment were identified as critical limb ischaemia (65%) and short distance claudication (35%); with multi-level disease unsuitable for open surgery or angioplasty in isolation. Pre-operative computed tomography (CT) scans were evaluated and lesions classified as per the Trans-Atlantic Inter-Society Consensus (TASC) stages [A & B 19 patients (17%), C & D 93 patients (83%)]. Procedures performed included common femoral artery endarterectomy (FEA) + aorto/iliac stenting (51%) and FEA + infra-inguinal angioplasty (49%).
Technical success rate was 96%, symptomatic improvement occurred in 94% at discharge and 1-year limb salvage rate was 98%. Kaplan Meier analysis showed 1-year amputation free survival 89%, primary, primary assisted and secondary patency rate of 72%, 79% and 81% respectively. 12 patients required re-interventions [7 endovascular (3 iliac, 4 SFA) and 5 surgical operations].
HR for complex multilevel arterial disease provides acceptable technical success, symptomatic improvement and limb salvage rates. It is a viable alternative to standard surgical or endovascular techniques in this group of high-risk patients with advanced peripheral vascular disease.

W7

Re-admissions in Vascular and Endovascular Surgery: Pilot Study

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Readmissions have a significant impact upon patient outcomes and vascular services with financial penalties for unplanned readmission within 30 days of discharge in NHS England. The primary aim of this study was to determine

the readmission rate in a large teaching-hospital vascular service and assess the applicability of a scoring system, previously validated in the USA to UK practice. The secondary aim was to assess the direct financial impact of these readmissions.
Patients readmitted within 30 days of discharge following major vascular or endovascular procedures were identified retrospectively from a maintained electronic database over an eight month period. Venous interventions were excluded. Factors predictive for readmission were recorded and risk scores calculated using a direct comparison of the scoring tool. The costs for each admission were calculated from service-line reporting.
There were 29 (4.5%) unplanned 30-day readmissions out of the 650 patients who underwent major vascular interventions over the study period. 18 patients (62%) had a high predictive readmission risk score (>8pts), 10 patients (34%) had a moderate risk score (4-7pts) with 1 patient (3%) scoring low risk (0-3pts). The mean score for these patients was 8.5 (high risk).
Overall cost of readmissions was £127,116 (£684-£10,394 mean = £4383).
This validated risk scoring tool for predicting vascular readmissions can be applied directly to our UK patient cohort. A multi-centre study is planned in order to further assess the validity of the scoring system and to compare readmission rates across the UK.

W8

Variability in Clinical Coding Implication for Health Outcomes Research using Hospital Episode Statistics

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National Health outcomes research, which is based on the Hospital Episode Statistics database (HES), relies on the assumption that variability in clinical coding is uniform across centres. We tested this assumption by comparing the accuracy and variability of HES data with clinical notes from three vascular centres over a three year period.
Procedure number, demographic and co-morbidity data were extracted for all patients undergoing above knee amputations between 2006 and 2009. 90% was set as the standard for accuracy, sensitivity and specificity for the following co-morbidities: diabetes, hypertension, coronary heart disease (CHD), hypercholesterolaemia, cerebrovascular disease (CVD) and smoking.
HES either under or over reported the number of procedures by 8% in individual centres (Overall: 178 hospital records, 191 HES records). Coding of hypertension and CHD was both accurate (>90%, varied between centres by 24% and 13% respectively) and sensitive (>90%, varied by 48% and 37% respectively). Coding of diabetes, hypercholesterolaemia, CVD and smoking had below the 90% standard accuracy and sensitivity.
National health outcomes research should not assume the inaccuracy inherent in HES data is uniformly spread. Such research should take into account variability in clinical coding as it potentially explains poorer outcomes in some hospitals.

W9

Utilisation of prosthetic limbs following major leg amputation - who benefits?

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To review the use of prosthetic limbs to regain functional mobility following major limb amputation.
This retrospective cohort study investigated patients who underwent lower limb amputations between January 2011 and October 2013. Limb fitting data, mortality rates and comorbidities were analysed.
81 vascular patients (72% male; median age 70) underwent amputations (52 below knee amputations (BKA) and 29 above knee amputations (AKA)). 40 (49%) had diabetes mellitus (33 BKA, 7 AKA). 18 (22%) had end stage renal failure (17 BKA 1 AKA). The 30 day, 6 month and 12 month mortality was 13.4%, 29% and 33% respectively for BKAs and 20.6%, 31% and 31% respectively for AKAs.
42 (52%) patients were referred for limb prosthesis. Data about prosthetic limb usage was available for a subgroup of 28 of these patients. From this subgroup 19 (67%) received a prosthesis (13 BKA, 6 AKA). 15 (54%) of these patients were able to achieve safe mobilisation (10 BKA, 5 AKA). Only 2 patients (7%) achieved more than a low level of activity.
Patients received a prosthetic limb a median of 80 (BKA) and 120 (AKA) days postoperatively. Neither age, sex, diabetes, renal function nor level of amputation significantly affected ability to mobilise with a prosthesis.
Mortality rates were low beyond 6 months. There was no significant difference in mortality or prosthesis usage between AKA and BKA within the first year. When prosthetic limb usage is possible patients rarely achieve more than a low level of activity.

W10

Measuring Ankle Brachial Pressure Index: A comparison of two oscillometric devices with handheld Doppler

D Moore[1], B Cooper[2], J Brittenden[1]

[1]Medical School, University of Aberdeen [2]NHS Grampian

Oscillometric devices may represent a simpler faster method to measure the Ankle Brachial Index (ABI) than the gold standard hand held Doppler which may be time consuming and requires trained operators. We aimed to compare the ABI measurements obtained using two oscillometric devices against hand-held Doppler in new patients referred with suspected Peripheral Arterial Disease (PAD).
The ABI of 79 patients (158 legs) referred to a tertiary vascular unit was assessed using the BOSO and WatchBP oscillometric ABI devices. Two consecutive measurements were taken for each device. These were compared to the ABI measured by a blinded vascular technician using the hand-held Doppler method as recommended by NICE.
In total, 47% of patients (74 legs) had an ABI <0.9 measured by hand-held Doppler, yet the oscillometry devices obtained values >0.9 in 1 in 5 of these patients (BOSO 20% (n = 14), WatchBP 22% (n = 15)). The BOSO device had an 80.3% sensitivity, and the WatchBP ABI device a 77.0% sensitivity to detect an ABI less than 0.9. ABI measurements from the oscillometric devices were consistently higher than Doppler determined values. The mean differences between Doppler and mean oscillometry measurements were 0.12 ± 0.11 for the BOSO device, and 0.15 ± 0.14 for WatchBP. The correlation between hand held Doppler & BOSO devices were 0.82 and WatchBP device 0.73, $p < 0.01$. In diabetic patients (n = 14), correlation was stronger between Doppler and oscillometric measurements (BOSO 0.86, Watch BP 0.81, $p < 0.01$).
The results suggest that ABI values obtained via oscillometric devices should be used and interpreted with caution.

W11

Mechanochemical Ablation (MOCA) Increases Endothelial Damage and Penetration of Damage into the Media layer of the Vein Wall Compared to Sclerotherapy Alone - a Study Using Histology and Immunohistochemistry

CT Lee[1], JM Li[1], MS Whiteley[2]

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Mechanochemical Ablation (MOCA) is a non-thermal non-tumescent ablative treatment for truncal vein reflux. It has been suggested that MOCA causes mechanical damage of the endothelium, enhancing the effect of sclerotherapy on the endothelial layer. The aim of this study is to investigate whether MOCA affects the endothelial layer only of if there is a deeper effect in the media layer.
Extra fascial great saphenous vein (GSV) harvested at varicose vein surgery was treated extra-corporally with nothing (control), 3% sodium tetradecyl sulphate (STS) or MOCA with 3% STS. The veins were fixed and examined histologically with haematoxylin and eosin (H&E) and immunohistochemical antibodies against CD 31 (endothelial marker) and alpha-actin (smooth-muscle marker).
H&E showed damage in the intimal layer only with 3% STS, whereas treatment with MOCA and 3% STS showed damage within the media suggestive of a shearing force. Immunohistochemistry showed 50% reduction of endothelial

cells (CD 31) and 30% reduction of media smooth-muscle cells (alpha-actin) for the innermost 150 μm, and less than 20% reduction to a depth of 500 μm. Adding MOCA significantly increased the damage profile; 60% reduction of endothelial cells and 40% reduction of smooth-muscle cells to a depth of 200 μm and over 20% reduction to a depth of 500 μm.
MOCA enhances sclerotherapy damage to the vein wall in both the endothelial and media layers. The effect in the media appears to be via mechanical shearing of the layers allowing ingression of sclerosant deeper into the media.

W12
Meta-analysis of the effects of statins on perioperative outcomes in non-cardiac vascular and endovascular surgery

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To investigate the role of peri-operative statin therapy in non-cardiac vascular and endovascular surgery.
A systematic search of electronic information sources was undertaken to identify studies comparing outcomes after non-cardiac surgical or endovascular arterial reconstruction in patients receiving a statin and those not taking a statin in the peri-operative or peri-interventional period. The Cochrane collaboration's tool and Newcastle-Ottawa scale were used to assess the methodological quality and risk of bias of the selected studies. Random effects models were applied to calculate pooled outcome data.
Four randomised controlled trials and 20 observational cohort or case-control studies were selected for analysis. The number of patients enrolled in the randomised and observational studies was 675 and 22,861, respectively. Statin therapy was associated with a significantly lower risk of all-cause mortality (odds ratio 0.54, 95% confidence interval 0.38-0.78), myocardial infarction (odds ratio 0.62, 95% confidence interval 0.45-0.87), stroke (odds ratio 0.51, 95% confidence interval 0.39-0.67), and the composite of myocardial infarction/stroke/death (odds ratio 0.45, 95% confidence interval 0.29-0.70). No significant differences in cardiovascular mortality (odds ratio 0.82, 95% confidence interval 0.41-1.63) and the incidence of kidney injury (odds ratio 0.90, 95% confidence interval 0.58-1.39) between the groups were identified.
High level evidence supporting statins' protective effects in non-cardiac vascular and endovascular surgery is insufficient. Statin therapy seems to be beneficial in improving operative and interventional outcomes, and might be considered as part of the optimization strategy for prevention of adverse cardio- and cerebro-vascular events and mortality.

W13
Necrotizing Soft Tissue Infections in Intravenous Drug Users - A Vascular Surgical Emergency?

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[1]Department of Vascular Surgery, Ninewells Hospital and Medical School, Dundee; [2]Department of Infectious Diseases, Ninewells Hospital and Medical School

To assess the short and long-term outcomes of Necrotizing Soft Tissue Infection (NSTI) in Intravenous Drug Users (IVDU) admitted to a regional vascular centre.
Retrospective analysis of all IVDUs with NSTI admitted between January 2009-November 2013 to the regional vascular surgical unit. Clinical outcome measures were duration between admission and surgery, duration of hospital stay, duration of ITU/HDU stay, post-operative complications, in-hospital mortality and one-year mortality rate.
13 patients were identified (median age 38yrs (range 30-42yrs), male:female ratio of 10:3). Overall median duration between admission and surgery was 17 hours (range 4-195hrs). This was significantly less when admission was direct to vascular surgery (median 5.5hrs, range 4-7hrs) compared to other specialties (median 36 hrs, range 16-195 hrs). Six patients required HDU/ITU care with a median stay of 7.5days (range 2-12 days). Five patients (39%) required amputation of which only one patient (20%) had a prosthetic limb fitted. Six patients required simultaneous ligation of the femoral arteries, of which only two required limb amputation. There was one in-hospital death (8%). One patient died whilst awaiting limb fitting 4 months post discharge, resulting in a one year mortality of 15%.
There is significant delay in intervention possibly as a result of delayed diagnosis if the patient is not directly admitted to vascular surgery. These patients have high rates or morbidity and require significant critical care input. Early diagnosis and referral to vascular surgery is crucial in the management of these patients.

W14
Occupational Radiation Exposure during FEVAR: A Stage-By-Stage Analysis

M Li[1], C Riga[1,2], S Cheung[1], N Burfitt[2], R Thomas[2], M Jenkins[2], N Cheshire[1,2], M Hamady[2], C Bicknell[1,2]

[1]Department of Surgery and Cancer, Imperial College London, London; [2]Imperial Vascular Unit, Imperial College Healthcare NHS Trust, London

Occupational radiation exposure(OE) is an important safety issue, but often overlooked. Robotic technology can potentially reduce OE during endovascular intervention through remote device manipulation. We aimed to measure OE during FEVAR and identify potential targets for robotic technology applications.
Radiation exposure data was collected prospectively during 12 consecutive FEVAR cases over a 4-month period at a tertiary referral centre, using digital over-the-lead dosimeters. OE was measured according to pre-defined procedural stages; total patient exposure(PE) was also measured. The robot was used for renal cannulations only. Cannulation technique(robotic/manual), operator positioning, c-arm angulation and procedure/fluoroscopy times were noted. Non-parametric tests were used for comparisons.
Median OE per case was 0.36mSv [IQR (0.19 - 0.62)] compared with 2.28mSv(1.49 - 3.49) for PE. OE readings per stage were: stent alignment[0.03 (0.03 - 0.24)], renal cannulation[0.03 (0.03 - 0.03)], visceral cannulation[0.05 (0.03 - 0.24)], completion[0.07 (0.03 - 0.15)]. OE during visceral cannulation was highest($p = 0.02$), and lowest during renal cannulation($p = 0.004$). For renal cannulations, there were no significant differences in procedure/fluoroscopy times or OE between manual and robotic techniques, although the robot was used for anatomically challenging targets. Dosimeter readings were significantly affected by operator positioning, with the highest doses observed in the cephalad position[0.08 (0.03 - 0.27)] during manual visceral vessel manipulation via the axillary/brachial approach and in acute C-arm angulations($p = 0.005$).
OE for FEVAR is considerable and higher than reported figures for standard EVAR. With increasing case-load and complexity, a significant cumulative OE is likely. Radiation awareness with staff education is therefore essential. Robotic use including future developments should be targeting high-risk intra-procedural stages.

W15
Outcome analysis of ultra-distal bypasses in an era of pedal arch angioplasty

E Khalil, L Fiengo, T Parkinson, H Salim, H Mistry, D Valenti, RPS Gambhir, H Rashid

King's College Hospital, London

To assess outcomes of ultradistal bypasses to Dorsalis pedis or plantar artery in an era of aggressive pedal arch angioplasty.
A retrospective analysis was done of 51 ultra-distal bypasses performed over a 7-year period. Group A had bypass after pedal arch angioplasty and Group B had direct Ultradistal bypass. 30-day mortality, primary and assisted primary graft patency along with major amputation rate and overall survival at 1 year were assessed. Kaplan-Meier survival analyses and log-rank test were used as appropriate. (Graphpad Prism 6.0).
Group A had 26 and group B 25 patients with a median age of 72 (range 46-90) years and male to female ratio of 8:1. Co morbidities included diabetes mellitus (90%), hypertension (75%), end stage renal disease (30%), and ischaemic heart disease (30%). In group A, 10 patients needed a bypass within 1 month of angioplasty, 11 within 6 months and 5 at 1 year after angioplasty. With no 30 day mortality, primary and assisted primary patency rates at 1 year were 54% and 73% for group A and 68% and 84%, for group B ($p = 0.88$). 1 year overall

survival was 88% and 92% (p = 0.96). Major amputation rate in group A and B were 3,9% and 4% (p = 0.96).
The study showed that ultra-distal bypasses are still technically feasible with good outcomes at 1 year even after previous attempts at pedal arch angioplasty, in only 20% of patients pedal arch angioplasty was able to delay the need for a bypass beyond 6 months.

W16
Platelet rich plasma (PRP) is an adjunct for the accelerated closure of high risk diabetic foot wounds

T Martin, C Kyriakides, S Sarkar

Barts Health NHS Trust

To investigate the role of PRP in healing diabetic foot wounds in patients at high risk of limb loss.
Since September 2013, consecutive patients with diabetic foot sepsis, ulceration and gangrene judged to be at high risk of limb loss or with factors predicting slow wound healing were managed using the standard diabetic multi-disciplinary approach. In addition, autologous PRP gel and direct platelet injections were administered every 4-7 days. Serial volumetric measurements were undertaken using a 3D camera.
To date, 18 wounds have been treated in 15 patients.
5 had extensive tissue loss with exposed bone/tendon/cartilage; 3 were on renal replacement therapy; 4 had severe peripheral neuropathy; 4 with deep osteomyelitis. 1 had severe nutritional deficiency related to an eating disorder.
Wound volume at commencement of treatment averaged 8203.3 mm^3 (range 141.3 - 41 257.2mm^3)
At completion of PRP treatment mean wound volume was 2783.2 mm^3 (range 0.5 - 28809 mm^3)
The average reduction in volume of wound at completion of treatment was 75.5%.
Total number of days to >90% wound healing after commencing treatment was 17.9 days (range 6-40 days)
The average number of PRP treatments given was 4.11.
Post treatment surgical intervention rate was required in 2 patients who went on to a major amputation; the remainder were discharged without further intervention.
PRP is associated with rapid coverage of bone, tendon and fascia as well as wound closure. This contributes to limb salvage in cases where major amputation would be the traditional management.

Exhibit D

October 4, 2011

Louis B. Jacques, MD
Director, Coverage and Analysis Group
Centers for Medicare and Medicaid Services
Mail Stop S3-02-01
7500 Security Boulevard
Baltimore, Maryland 21244-1850

RE: Request for Reconsideration of Medicare
National Coverage Determinations Manual, § 270.3,
Autologous Blood Derived Products for Chronic Non-Healing Wounds

Dear Dr. Jacques:

This is a formal request to reopen and revise Section 270.3 of the Medicare National Coverage Determinations Manual, which addresses Autologous Blood-Derived Products for Chronic Non-Healing Wounds. Autologous Platelet-Rich Plasma (PRP) is the prevalent blood-derived therapeutic product used for treating chronic non-healing wounds. This letter, and the accompanying attachments and published articles, set out the body of clinical evidence to support the conclusion that the use of autologous PRP Gel for chronic, non-healing wounds including pressure ulcers, diabetic foot ulcers, and venous ulcers compared to usual wound care, significantly and reliably improves the rate of complete healing, speed and progress to healing, and quality of life in the Medicare population.

There is ample clinical evidence to support this conclusion. In a recently published systematic review of journal articles published in the past 10 years, many citations were identified and 21 randomized controlled trials and comparative studies were eligible for the systematic review.[1] *See* ***Attachment C***. Seventy five percent (75%) of the eligible articles are new since CMS's last reconsideration in 2008. A significant part of the attached case series observational data comes from a wound care registry of 285 wounds treated with autologous PRP Gel (AutoloGel™, Cytomedix, Inc). Within the registry, 45% of the wounds treated were from Medicare beneficiaries, and, upon comparison, their outcome performance was equivalent to the non-Medicare subset. [2] In addition, a recently published article documenting lack of wound healing

[1] Carter, MJ, Fylling, CP, Parnell, LKS. (2011) Use of Platelet Rich Plasma Gel on Wound Healing: A Systematic Review and Meta-Analysis. *www.eplasty.com*, *Open Access Journal of the Journal of Plastic Surgery.* September 15, 2011.

[2] de Leon J, Driver VR, Fylling CP, Carter MJ, Anderson C, Wilson J, et al. (2011) The clinical relevance of treating chronic wounds with an enhanced near-physiological concentration of platelet rich plasma (PRP) gel. *Advances in Skin and Wound Care, 24(8), 357-368.*

during a run-in period of care demonstrated that the application of autologous PRP Gel (AutoloGel™, Cytomedix, Inc) rapidly converted wounds with a non-healing trajectory to a rapidly healing trajectory.[3] This study provides valuable clinical insight since each patient's wound acted as its own control thus demonstrating the positive impact of PRP Gel to improve healing.
(I'm not sure this remains appropriate.)

Introduction

Autologous blood products, and in particular PRP, have been used widely for the treatment of chronic non-healing wounds. The benefits of autologous blood products have been the subject of research published in peer-reviewed medical journals since 1985.

In this submission, we request that CMS update its current National Coverage Determination to authorize Medicare coverage for the use of autologous PRP Gel to treat pressure ulcers, venous ulcers, and diabetic foot ulcers when conventional treatments have been tried for at least 30 days and failed to reduce the wound size or induce an adequate wound healing trajectory.

The information and data discussed in this request amply demonstrate that PRP Gel offers improved results for individuals with chronic wounds when compared with conventional treatments. The advantages of PRP Gel treatment include improved healing, faster healing time, reduced infection and pain, improved net health outcome, and reduced overall cost. This combination of increased effectiveness and efficiency establishes the need to update the current NCD to allow for the use of PRP Gel in clinically appropriate settings.

This request focuses on the use of PRP Gel for the treatment of chronic, non-healing wounds, describes the progress in this field since 2008, and is a specific request for Medicare coverage. It includes:

- Attachment A: The Science of Platelet Rich Plasma (PRP)
- Attachment B: A Standardized Formulation of PRP-Gel Provides Reproducible Efficacy
- Attachment C: Systematic Review of the Platelet Rich Plasma (PRP) Literature
- Attachment D: AutoloGel Platelet Rich Plasma (PRP) Case Series Observational Studies Outcomes – 2001 – 2011
- Attachment E: Platelet Rich Plasma (PRP) Net Health Benefit
- Attachment F: The AutoloGel™ Body of Evidence

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[3] Carter, M., Fylling, C., Li, W., De Leon, J., Driver, V., Serena, T., et al. (2011). A statistical analysis of a wound outcomes registry using run-in data: clinical impact of platelet rich plasma gel on healing trajectory. *Int Wound J.* doi: 10.1111/j.1742-481X.2011.00868.x

I. Regulatory Background

1. FDA Clearance

As a preliminary procedural matter, this request to update Section 270.3 to cover Autologous PRP Gel is limited to certain clinical indications that have been reviewed and cleared for use by the Food and Drug Administration. In September 2007, the FDA granted Section 510 (k) clearance for the AutoloGel™ System; in its notice, the FDA approved the following indications for use:

> 510(k) Number: BK060007
> Device Name: Autologel™ System
> Indications For Use:
> The AutoloGel™ System is intended to be used at point-of-care for the safe and rapid preparation of platelet-rich plasma (PRP) gel from a small sample of a patient's own blood. Under the supervision of a healthcare professional, the PRP gel produced by the AutoloGel™ System is suitable for exuding wounds, such as leg ulcers, *pressure ulcers, and diabetic ulcers* and for the management of mechanically or surgically-debrided wounds.
> (emphasis added).

2. Previous CMS Consideration of Autologous Blood Derived Products for Chronic Wounds

CMS's previous consideration of Section 270.3 occurred during 2007 and early 2008 with the final decision memo issued in March 2008. At that time, CMS determined that PRP would remain a non-covered item under Medicare. Its decision was based on the finding that the evidence available at that time was suggestive but not adequate to conclude that autologous PRP is reasonable and necessary for the treatment of chronic non-healing, cutaneous wounds, or for the treatment of acute surgical wounds when the autologous PRP is applied directly to the closed incision, or for dehiscent wounds.[4]

II. Epidemiology of Chronic Wounds

In a review of chronic wounds conducted in 2009, the Agency for Healthcare Research and Quality cited research noting that more than 2.8 million patients in the United States suffer from chronic wounds. The prevalence of chronic ulcers has been estimated to be 120 per 100,000 patients between the ages of 45 and 64 years, which increases to more than 800 per 100,000 patients over age 75.[5] Additional data compiled by the Medicare Evidence Development and

[4] The 2008 DecisionMemo is available at: http://www.cms.gov/medicare-coverage-database/details/nca-decision-memo.aspx?NCAId=208&ver=15&NcaName=Autologous+Blood+Derived+Products+for+Chronic+Non-Healing+Wounds&bc=BEAAAAAAIAAA&

[5] Agency for Healthcare Research and Quality, Technology Assessment: Negative Pressure Wound Therapy Devices (2009); available at: http://www.ahrq.gov/clinic/ta/negpresswtd/npwtd02.htm

Coverage Advisory Committee estimated that the cost of treating those wounds is approximately $30 billion per year. Between 15-20% of the Medicare-eligible population suffers from chronic wounds. Within this larger category, approximately 2.5 million patients are treated annually for pressure ulcers, at an annual cost of approximately $12 billion. 10% to 35% of the U.S. population has some type of venous disease, and lower extremity ulcers are reported in 1% to 22% of individuals over age 60.[5] In addition, approximately 15% of all diabetics suffer foot ulcers, which requires the amputation of a foot or limb in about 56,000 cases annually. Among patients over age 65, the majority never ambulate after surgery, and their overall survival rate after five years is less than 30%; for individuals who also have renal failure, that rate drops to less than 14%.[6] This data demonstrates that effective wound therapies are greatly needed.

III. Platelet Rich Plasma (PRP) Gel: A Therapy to Address the Chronic Wound Problem

Platelet rich plasma (PRP) gel is an autologous blood product containing proteins that regulate tissue growth for wound healing. When the PRP is activated, the platelets release cytokines, growth factors, and chemokines to act on the cell receptors to facilitate cellular growth and migration and the fibrinogen in the plasma converts to a fibrin matrix scaffold on which the cells can adhere. This biological system is integral to normal wound healing.

To help understand this complex biological system, ***Attachment A*** provides a detailed description of the scientific mechanism of wound healing and the role of these PRP proteins in facilitating wound healing. ***Attachment B*** describes the importance of specific formulations and standardization of PRP Gel to achieve consistent wound healing outcomes.

IV. Professional Standards for Reviewing Evidence of Chronic Wound Healing

The evaluation of PRP for use in treatment of chronic non-healing wounds must be grounded in accepted professional guidelines. The individual guidelines published by the Wound Healing Society for Pressure, Diabetic, Venous, and Arterial Insufficiency Ulcers, respectively, *each* state that a chronic wound should be converted into an acute wound in order to achieve definitive treatment. Consistent with the clinical overview above, these Guidelines state that: "wound bed preparation is defined as the management of the wound to accelerate endogenous healing or to facilitate the effectiveness of other therapeutic measures. The aim of wound bed preparation is to convert the molecular and cellular environment of a chronic wound to that of an acute healing wound."[7] Therefore, it is important to understand the role of growth factors and signal

[6] *See* Medicare Evidence Development and Coverage Advisory Committee Meeting Minutes (March 29, 2005; available at: https://www.cms.gov/medicare-coverage-database/details/medcac-meeting-details.aspx?&year=2005&MEDCACId=28&)

[7] JoAnne Whitney, et al., Guidelines for the Treatment of Pressure Ulcers, Wound Repair and Regeneration 14: 663-669, 670 (2006); David L. Steed, et al., Guidelines for the Treatment of Diabetic Ulcers, Wound Repair and Regeneration 14: 680–692, 685 (2006); Martin C. Robson, et al., Guidelines for the Treatment of Venous Ulcers, Wound Repair and Regeneration 14: 649–

molecules in the overall healing process regardless of the underlying wound pathophysiology. Platelets provide the growth factors and signal molecules present in the earliest stages of healing, helping to establish the molecular and cellular environment of an acute healing wound. As a result, bringing platelets to the wound site is essential to healing, and platelet dose and gel formulation are crucial considerations in the healing process.

A standard for evaluation of scientific data in this context has been summarized by the Food and Drug Administration in its guidance document, *Guidance for Industry: Chronic Cutaneous Ulcer and Burn Wounds — Developing Products for Treatment.* That guidance instructs that "because wounds differ in their pathophysiology, it is difficult to generalize results obtained from a trial conducted in subjects with one wound type to patients with another wound type. Therefore, separate clinical trials should be considered for each type of wound indication sought. *However, if a scientific rationale and clinical data support clinical activity of a product in more than one wound type, it may be possible for studies performed in one wound type to support another in establishing substantial evidence of efficacy and safety."*[8]

A recent Agency for Healthcare Research and Quality (AHRQ) assessment of comparative effectiveness methodological research (CER) stated:

> "Data from RCTs [randomized controlled trials] may be insufficient to address a review question about benefit for a number of reasons. RCTs may be inappropriate due to patient values or preferences; the intervention may be hazardous; or randomization may decrease benefit if the intervention effect depends in part on subjects' active participation based on their beliefs and preferences. RCTs may be unnecessary in interventions with obvious benefit, such as the treatment of susceptible organisms with penicillin or where the alternative to treatment of a new and otherwise fatal disease is a high likelihood of death. RCTs may be difficult to implement due to entrenched clinical practice or to active consumer pressure for access to a treatment, problems with recruitment when a drug is already marketed, the need for long-term follow up to detect either benefits or harms, or difficulty randomizing feasible intervention units. In situations where RCT data are impractical, infeasible, or incomplete, observational studies may provide valid and useful data to help address CER questions."[9]

662, 653 (2006); Harriet W. Hopf, et al., Guidelines for the Treatment of Arterial Insufficiency Ulcers, Wound Repair and Regeneration 14: 693–710, 701 (2006)

[8] FDA *Guidance for Industry: Chronic Cutaneous Ulcer and Burn Wounds — Developing Products for Treatment* at 2 (2006) (emphasis added). The full document is available at: www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm071324.pdf

[9] Norris S, Atkins D, Bruening W, et al. Selecting observational studies for comparing medical interventions. In: Agency for Healthcare Research and Quality. Methods Guide for Comparative Effectiveness Reviews [posted June 14, 2010]. Rockville, MD. Available at: http://www.ncbi.nlm.nih.gov/books/NBK47093/

Other reasons why RCTs may be insufficient to answer questions of benefits and harms in wound care relate to exclusion of minorities, vulnerable populations, generalization to "real-world" wound care populations, inappropriate endpoints, and inadequate follow-up time.[10,11,12,13,14,15] In other words, during the last few years, there has been an increasing recognition that an appropriate level of evidence to properly address treatments or interventions would have to include least one well-conducted RCT and one high-level observational study with subjects drawn from representative populations. Such studies typically include cohort, case-control, or comparative designs.

Turning again to AHRQ standards, the sufficient strength of evidence for coverage is described as follows:

> "At least 1 study with greatest design suitability and good execution; or at least 3 studies having moderate or better design suitability, fair or better execution, and consistent results."[16]

Within this definition, the term "greatest" in the context of design suitability means concurrent comparison groups and sufficient measures for other factors affecting outcome; the term "moderate" means non-concurrent comparison or no comparison groups and insufficient measures for other factors affecting outcomes.

When evidence-based medicine (EBM) is used to justify treatments or interventions in medicine, the level of evidence is the foundation for the development of clinical practice guidelines. As a result, there are two key concepts to understand: the *strength of the evidence*, which refers to the

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[10] Horn SD, Gassaway J. Practice-based evidence study design for comparative effectiveness research. Med Care 2007;45:S50-7.

[11] A EWMA patient outcome group document. Outcomes in controlled and comparative studies on non-healing wounds; recommendations to improve the quality of evidence in wound management. Journal Wound Care 2010;19239-268.

[12] Bagshaw SM, Bellomo R. The need to reform our assessment of evidence from clinical trials: A commentary. Philosophy Ethics Humanities Med 2008;3:23.

[13] Moffatt, CJ, Doherty DC, Smithdale R et al. Clinical Predictors of Leg Ulcer Healing. British J Derm. 2010;162:51-58

[14] Concato J, Shah N, Horwitz RI. Randomized, controlled trials, observational studies, and the hierarchy of research designs. New Engl J Med 2000;342:1887-92.

[15] Carter MJ, Fife CE, Walker D, Thomson B. Estimating the applicability of wound care randomized controlled trials to general wound-care populations by estimating the percentage of individuals excluded from a typical wound-care population in such trials. Adv Skin Wound Care. 2009;22:316-24.

[16] Hickam DH, Severance S, Feldstein A, et al. The Effect of Health Care Working Conditions on Patient Safety. Evidence Report/Technology Assessment Number 74. (Prepared by Oregon Health & Science University under Contract No. 290-97-0018.) AHRQ Publication No. 03-E Rockville, MD: Agency for Healthcare Research and Quality. April 2003.

quality, quantity, and consistency of the evidence in any body of studies[17] and *strength of the recommendations*, which describes the importance of a recommendation and is based on both the quality of the evidence and the magnitude of anticipated benefit or harm.[18] Study ratings typically follow a I, II, III format in which level I is higher than II; whereas, most recommendation schemes follow an A, B, C evidence-level format in which A is higher than B or where recommendations are provided in terms of strong/weak.[19]

The data presented here follow a systematic review approach, which uses a specific methodological approach to appraise the evidence and represents the highest level of a review of the evidence. In fact, several international organizations consider the systematic review to be class I evidence, including the Oxford Center for Evidence-based Medicine (OCEBM), SIGN, the National Health and Medical Research Council (Australia) when the review only includes RCTs, and NICE, when the systematic review includes meta-analysis. [17, 20]

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[17] West S, King V, Carey TS, et al. Systems to rate the strength of scientific evidence. Evidence Report/Technology Assessment No. 47 (Prepared by the Research Triangle Institute–University of North Carolina Evidence-based Practice Center under Contract No. 290-97-0011). AHRQ Publication No. 02-E016. Rockville, MD: Agency for Healthcare Research and Quality. April 2002.

[18] Shiffman RN, Shekelle P, Overhage JM, Slutsky J, Grimshaw J, Deshpande AM. Standardized reporting of clinical practice guidelines: a proposal from the Conference on Guideline Standardization. *Ann Intern Med*. 2003;139:493-8

[19] Carter MJ. Evidence-based medicine: an overview of key concepts. Ostomy Wound Manage 2010;56:68-85.

[20] Cytomedix respectfully submits that a systematic review differs from the methodology used in the 2008 reconsideration of Section 270.3 of the Medicare National Coverage Determinations Manual. At that time, CMS repeatedly used the phrase "insufficient evidence." Although describing general methodological review principles in its review and subsequent decision not to cover autologous platelet-rich plasma products (PRP) (*see* ***Appendix A***), CMS did not use a systematic review approach, and the Decision Memorandum did not describe the level of evidence specifically required for coverage, stating that "each coverage determination has its own unique methodological aspects." This is a critical omission. The review conducted by CMS in regard to PRP coverage cannot be regarded as a systematic review because it described no specific systematic review methodology, including bias and quality assessment. Rather, it should be considered to be a critical review, because it critically analyzed studies without rating the studies and assessing them a level of evidence. Critical reviews contain more bias than systematic reviews, in part because studies are not rated overall in a systematic fashion; instead, studies are critiqued according to investigators' opinions or beliefs, in part because of publication bias, (Yoshii , 2009) and lack of assessment of this important parameter. Furthermore, although CMS agreed to assess all studies submitted by Cytomedix as part of its assessment of PRP coverage, it did not do so, thus amplifying the publication bias issue. Several organizations have also provided definitions of evidence levels for supporting a treatment or intervention, based upon an evaluation of the benefits and harms, the most commonly used of which are GRADE, SIGN, and the OCEBM. Again, CMS used no systematic process for arriving at the conclusion that there was a "lack of evidence."

As a result, Cytomedix proposes that an adequate level of evidence according to the AHRQ definitions can be met with the current literature to support coverage of standardized autologous platelet-rich plasma gel for use in treating diabetic foot ulcers and pressure ulcers.

V. Clinical Evidence That Demonstrates the Effectiveness of PRP

As noted in the introduction, the body of clinical evidence since the time of the FDA's clearance of the AutoloGel™ System and CMS's 2008 reconsideration has grown substantially. This additional evidence is not simply additive to the evidence previously reviewed; it targets specific queries set out by CMS in its manuals and in other published guidance documents. That evidence, which is presented in detail, is summarized below.

Attachment C: Systematic Review of the Platelet Rich Plasma (PRP) Literature

An exhaustive review was conducted on the PRP literature published in the last 10 years. Only RCTs and comparative effectiveness studies were included in the review. Twenty one studies met the inclusion criteria. The wounds included in the systematic review include:

a) Chronic wounds
b) Acute wounds with PRP added to a surgical site prior to primary closure
c) Acute wounds with PRP added to a surgical site followed by secondary closure

The review includes:

a) Descriptions of the studies, types of wounds, and interventions used.
b) Detailed outcomes in each study.
c) Quality review of the studies: The score sheet documenting the SIGN grade based on the total score of external validity and internal validity (bias and confounding)
d) Quality assessment and summary of findings for studies comparing use of platelet-rich plasma treatments against standard care for chronic wounds (GRADE).
e) Quality assessment and summary of findings for studies comparing use of platelet-rich plasma treatments against standard care for acute wounds (GRADE).
f) Meta-analysis of PRP's impact on complete healing, reduction of infection, and reduction of pain.

This systematic review establishes that that PRP Gel results in improved wound healing, faster wound healing, reduced infection, and reduced pain.

Attachment D: AutoloGel Platelet Rich Plasma (PRP) Case Series Observational Studies Outcomes – 2001 – 2011

Because the AHRQ recommendations described above state that large observational studies are beneficial for comparative effectiveness research, the outcomes in 9 case series (observational studies) are documented. Data from 323 wounds are included in the analyses. In particular, the

largest wound registry (n = 285 wounds) documented the use of autologous PRP-Gel (AutoloGel™) and demonstrated that wounds in Medicare beneficiaries had the same healing progress as those wounds in non-Medicare beneficiaries, as shown in the following table.

	N	% wounds with volume reduction	Mean volume reduction % (cm^2)	Number of weeks to outcome	Number of treatments to outcome
Medicare	111[21]	89.9%	64.6%	2.2	2.8
Non-Medicare	135	91%	62.9%	2.3	2.8
		p = 0.75			

Similar results were identified in the Frykberg 65-wound registry.[22]

These observational studies document that PRP results in wound healing, faster wound healing progress compared to previous wound duration without healing, re-animation of stalled wounds into a positive wound-healing trajectory, reduction and closure of undermining and sinus tracts/tunneling in wounds, and growth of granulation tissue to prepare a wound for grafting. These types of outcomes were seen in Medicare patients, wounds of multiple etiologies, and wounds in patients with complex co-morbidities.

Attachment E: Platelet Rich Plasma (PRP) Net Health Benefit

Among CMS' concerns was whether or not a therapy contributes to the patient's quality of life or their net health benefit. The importance of a healed wound or progress toward healing is documented from the literature. The multiple net health benefit features described in the systematic review and observational studies are tabulated. In addition, a qualitative research survey was conducted with health professionals that had used autologous PRP-Gel (AutoloGel™) to treat patients' chronic wounds. Using AutoloGel™ to treat chronic wounds improved the net health outcome of the patients that were treated. The patients self-described their distinct positive changes in multiple areas of their lives due to the wound healing progress with AutoloGel™.

Cost effectiveness and improvement in a patient's quality of life are important net health benefits. AutoloGel™ was documented as being the most cost effective therapy for wound care over a 5 year period compared to standard of care and other advanced wound therapies as well as providing the best quality of life.[23] PRP Gel studies have also documented minimal adverse events from the use of PRP Gel. A recent safety study of 110 patients treated with multiple applications of AutoloGel™ has demonstrated no adverse events during the entire treatment

[21] 24% were under age 65, but eligible due to disability.

[22] Frykberg, R. G., Driver, V. R., Carman, D., Lucero, B., Borris-Hale, C., Fylling, C. P., et al. (2010). Chronic wounds treated with a physiologically relevant concentration of platelet-rich plasma gel: a prospective case series. *Ostomy Wound Manage, 56*(6), 36-44.

[23] Dougherty, E. J. (2008). An evidence-based model comparing the cost-effectiveness of platelet-rich plasma gel to alternative therapies for patients with nonhealing diabetic foot ulcers. *Adv Skin Wound Care, 21*(12), 568-575.

time.[24] As a result, there is strong evidence that autologous PRP Gel does improve the net health benefit of the patient.

V. The Body of Evidence Supports Coverage for Platelet Rich Plasma

The large body of published literature on the use of PRP for the treatment of wounds meets the FDA Guidance document recommendation, the Wound Healing Society Guidelines, and the AHRQ standard of evidence.

In addition, the evidence meets the rigorous standard developed by the Medicare Evidence Development and Coverage Advisory Committee (MEDCAC). At its meeting on March 29, 2005, which focused on the usual care of chronic wounds, they described areas that a therapy should meet to be considered for coverage. The following table summarizes how PRP Gel meets these criteria, which are explained in more detail in ***Attachments C, D, E, and F***:

MEDCAC Area	**PRP Gel Impact**
Complete healing	Several studies documented statistically significant healing versus control
Time to healing	Faster rate
Partial healing rate	Faster healing trajectory. Re-animating or "kick-starting" a wound after it has stalled
Recurrence	Less recurrence
Elimination of infection	Infection reduction or elimination
Amputation	Amputations reduced
Reduction of pain	Pain reduced
Resumption of normal activity	Return to life, school, activities of daily living, rehabilitation, family life

Other areas that MEDCAC did not address:

Salvage of exposed tendons	Granulation tissue covered the tendons so they were spared
Reduction of major surgeries	Wound healed or progressed so major surgeries were averted
Preparation for skin grafts	Granulation tissue grew quickly so skin grafts could be performed to definitively close the wound on a timely basis
Reduced need for NPWT	Professionals used PRP Gel instead of NPWT due to more tissue growth in a shorter period of time for less cost
Cost effectiveness	Reduced cost compared to existing wound therapies or standard of care due to healing efficacy

[24] AutoloGel™ Post Marketing Surveillance Study, *Data on file at the Company*

Adverse events	Minimal to none

In addition, the evidence in this request supports the use of PRP in the following targeted populations:

Medicare beneficiaries	While most of the studies included patients that were Medicare beneficiaries, specifically, the de Leon and Frykberg studies documented that Medicare beneficiaries had the same healing progress as non-Medicare beneficiaries.
Providers (facilities/physicians) in community practice rather than tertiary care specialty centers (universities, etc)	The data in the studies were predominantly from community settings: hospitals, outpatient clinics, long-term care, home care, physician's offices.

VI. Summary

Since the 2008 CMS non-coverage decision for blood products for the treatment of chronic wounds, extensive new literature has been published documenting the efficacy of autologous PRP Gel for the treatment of wounds. In addition, AHRQ broadened its evidence criteria to include large observational studies in addition to RCTs and comparative analysis studies. The published PRP literature includes all of the above.

The use of PRP Gel for the treatment of wounds can facilitate healing, improve healing rates, reverse a non-healing trajectory to a healing trajectory, shorten the time to healing, reduce length of stay, reduce excess use of expensive treatment modalities, has minimal to no adverse events, improves the net health benefit to the patient and their family, and is cost effective.

Based on meeting these evidence criteria and having the documented outcomes described, we respectfully request that CMS reverse its non-coverage decision and provide coverage for the use of PRP Gel, especially for the treatment of diabetic, venous, and pressure ulcers. We have attached a proposed revision to Section 270.3 that reflects the research and data presented in this submission. Alternatively, we believe that CMS can cover PRP gel through a National Coverage Determination with data collection as a condition of coverage; this would provide a practical means by which CMS can obtain the necessary data to evaluate the performance of PRP gel and to confirm the outcomes presented in this request.

We welcome the opportunity to meet with you to discuss any of the evidence supporting Medicare coverage for autologous PRP Gel or to answer any questions. For convenience, please contact Cytomedix' counsel, Robert Wanerman, at (202) 861-1885, or rwanerman@ebglaw.com.

Sincerely,

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Request for Reconsideration of Medicare
National Coverage Determinations Manual, § 270.3,
Autologous Blood Derived Products for Chronic Non-Healing Wounds

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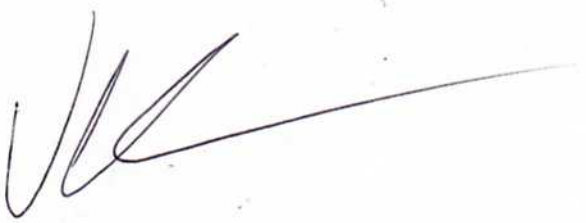

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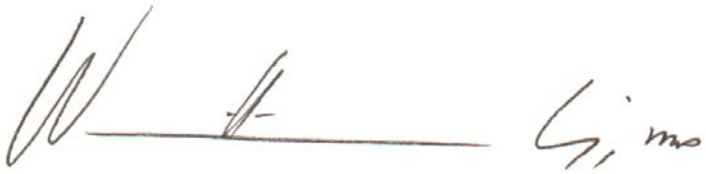

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APPENDIX

Proposed Amendment to Section 270.3

270.3 - Blood-Derived Products for Chronic Non-Healing Wounds - (Various Effective Dates Below)
(Rev. 83, Issued: 05-02-08, Effective: 03-19-08, Implementation: 06-02-08)

A. General

Wound healing is a dynamic, interactive process that involves multiple cells and proteins. There are three progressive stages of normal wound healing, and the typical wound healing duration is about 4 weeks. While cutaneous wounds are a disruption of the normal, anatomic structure and function of the skin, subcutaneous wounds involve tissue below the skin's surface. Wounds are categorized as either acute, in where the normal wound healing stages are not yet completed but it is presumed they will be, resulting in orderly and timely wound repair, or chronic, in where a wound has failed to progress through the normal wound healing stages and repair itself within a sufficient time period.

Platelet-rich plasma (PRP) is produced in an autologous or homologous manner. Autologous PRP is comprised of blood from the patient who will ultimately receive the PRP. Alternatively, homologous PRP is derived from blood from multiple donors.

Blood is donated by the patient and centrifuged to produce an autologous gel for treatment of chronic, non-healing cutaneous wounds that persists for 30 days or longer and fail to properly complete the healing process. Autologous blood derived products for chronic, non-healing wounds includes both: (1) platelet derived growth factor (PDGF) products, and (2) PRP.

The PRP is different from previous products in that it contains whole cells including white cells, red cells, plasma, platelets, fibrinogen, stem cells, macrophages, and fibroblasts.

The PRP is used by physicians in clinical settings in treating chronic, non-healing wounds, open, cutaneous wounds, soft tissue, and bone. Alternatively, PDGF does not contain cells and was previously marketed as a product to be used by patients at home.

B. Nationally Covered Indications

Effective [ ], PRP Gel produced by systems that have received clearance or approval by the Food and Drug Administration for the treatment of pressure ulcers, venous ulcers, or diabetic foot ulcers may be covered for those indications following a period of at least 30 days during which alternative covered treatments have been unsuccessful in reducing wound area or depth.

C. Nationally Non-Covered Indications

1. Effective December 28, 1992, the Centers for Medicare & Medicaid Services (CMS) issued a national non-coverage determination for platelet-derived wound-healing formulas intended to treat patients with chronic, non-healing wounds. This decision was based on a lack of sufficient published data to determine safety and efficacy, and a public health service technology assessment.

2. Effective July 23, 2004, upon reconsideration, the clinical effectiveness of autologous PDGF products continues to not be adequately proven in scientific literature. As the evidence is insufficient to conclude that autologous PDGF in a platelet-poor plasma is reasonable and necessary, it remains non-covered for treatment of chronic, non-healing cutaneous wounds. Also, the clinical evidence does not support a benefit in the application of autologous PRP for the treatment of chronic, non-healing, cutaneous wounds. Therefore, CMS determines it is not reasonable and necessary and is nationally non-covered.

3. Effective April 27, 2006, coverage for treatments utilizing becaplermin, a non-autologous growth factor for chronic, non-healing subcutaneous wounds, remains nationally non-covered under Part B based on section 1861(s)(2)(A) and (B) of the Social Security Act because this product is usually administered by the patient.
4. Effective March 19, 2008, upon reconsideration, the evidence is not adequate to conclude that autologous PRP is reasonable and necessary and remains non-covered for the treatment of chronic non-healing, cutaneous wounds. Additionally, upon reconsideration, the evidence is not adequate to conclude that autologous PRP is reasonable and necessary for the treatment of acute surgical wounds when the autologous PRP is applied directly to the closed incision, or for dehiscent wounds.
5. Effective [ ], upon reconsideration, the evidence is not adequate to conclude that autologous PRP is reasonable and necessary for the treatment of chronic non-healing wounds except as indicated in Subsection (B), above.

D. Other

In accordance with section 310.1 of the National Coverage Determinations Manual, the routine costs in Federally sponsored or approved clinical trials assessing the efficacy of autologous PRP in treating chronic, non-healing cutaneous wounds are covered by Medicare.

Attachment A:
The Science of Platelet Rich Plasma (PRP)

Introduction

The application of products derived from platelet rich plasma for dermal wound healing can be traced through an extensive body of literature dating to the late 1980s (Knighton 1986) and is supported by an understanding of fundamental principles in human biology. An immediate early event critical for wound healing is the influx of platelets to the site of wounding. Platelets bind to elements within damaged tissue and are activated to release a diversity of biomolecules from their alpha and dense granules. This provides signals essential not only for hemostasis but also for effective tissue regeneration.

While the molecules released by platelets have critical roles in the regeneration of both soft and hard tissues, autologous platelet rich plasma gel (PRP-Gel) meets a particular need in the treatment of chronic non-healing dermal wounds. The dermal application of PRP-Gel is intended to bypass the lack of local perfusion that is a hallmark of many chronic wounds and provides immediate biological signals that drive the formation of granulation tissue and ultimately healing.

This attachment presents an overview of the scientific rationale that underpins efficacy of PRP-Gel for wound healing, based on the published literature that accompanies this request. This discussion is not intended as a comprehensive review of PRP, but focuses on the information important for the current request to reconsider the language in Section 270.3 that would allow for Medicare coverage and reimbursement of a standard formulation of autologous PRP-Gel when used for the treatment of diabetic foot ulcers and pressure ulcers .

Summary of Wound Healing

Overview of Normal Wound Healing

Cutaneous wound healing can be divided into distinct but overlapping stages; the inflammatory phase, the proliferative phase, and remodeling (Singer 1999). Platelets are present in the inflammatory phase and release chemokines, growth factors, and cytokines in response to activation by elements within damaged tissue such as collagen fragments or endogenous thrombin (Anitua 2004 and Ueno 2011). The chemokines recruit populations of stem cells, fibroblasts, and leukocytes to the wound bed (Gillitzer 2001) while growth factors drive the proliferation and differentiation of the different cell types (Werner 2002). Cells within the wound bed respond to cytokines by differentiating and producing a new extracellular matrix that is the foundation for new tissue formation (Werner 2002). Neutrophils recruited to the wound bed in the inflammatory phase serve to “clean-up” the wound, which is accomplished through the release of free radicals (oxidative burst) that destroy bacteria (Kim 2008) and through the release of specific

proteases that remove debris, such as collagen fragments within damaged tissue (Mast 1996). As healing moves through the inflammatory phase, chemokines recruit macrophages which then become the prime orchestrator of cell expansion and continued synthesis of new tissue through the release additional cytokines, growth factors and chemokines (Eming 2007). Once the skin has closed, wounds progress through a remodeling phase in which type III collagen is replaced with the stronger type I collagen that is reorganized to provide tissue with increased tensile strength (Diegelmann 2004). In normal organized healing, wound closure should occur within 30 days with the inflammatory phase lasting from 2-5 days, the proliferative phase from 2 days to 3 weeks and remodeling continuing for several weeks to 2 years after wound closure.

Overview of Chronic Wounds

Chronic wounds such as diabetic foot and pressure ulcers are defined in the 2003 decision memorandum (CAG-00190N) as those that fail to heal within thirty days. Dysregulation of numerous cellular and biological responses contribute to the chronic wound phenotype. Chronic wounds have a decreased regenerative capacity that has been observed at several levels.

Chronic wounds have reduced levels of growth factors and concomitant decreases in cellular proliferation (Mast 1996). There is increased cellular senescence (Telgenhoff 2005), and there often is a lack of perfusion that can inhibit the delivery of nutrients and cells required for regeneration (Guo 2010). Chronic wounds frequently are described as “stuck in the inflammatory phase” harboring an environment that favors inflammation and tissue catabolism (Lobmann 2005, Mast 1996). They invariably contain a bioburden (Howell-Jones 2005) and while the bioburden may not always be readily clinically observable, it increases inflammatory responses by an increased presence of neutrophils (Fazli 2011, Diegelmann 2003). Beneficial inflammatory response and healing are uncoupled in the chronic wound, and pro-inflammatory mediators accumulate, such as neutrophil-derived interleukin-1 (IL-1) and tumor necrosis factor alpha (TNF-a) (Mast 1996, Barrientos 2008). These inflammatory peptides drive the expression of proteases and are suspected to contribute to the net tissue loss associated with chronic wounds (Eming 2007, Yager 1999, Lobmann 2005).

Also underlying the pathogenesis of chronic wounds, elevated concentrations of free radicals, products of the oxidative burst intended to destroy bacteria, accumulate and damage proteins, lipids, and carbohydrates in the tissue surrounding the chronic wound bed (Moseley 2004, James 2003). This is an important point and relevant to subsequent discussion regarding PRP-Gel formulations. In a normal wound healing process, ascorbic acid, a free radical scavenger, eliminates damaging free radicals from tissues (Padayatty 2003). In chronic wounds this activity is compromised leading to increased oxidative stress or free radical damage of tissue (Moseley 2004, James 2003). Considering that ascorbic acid is not produced by humans but must be supplied through diet, if the patient’s vasculature is compromised due to a wound, the delivery of nutrients will be compromised as well. As a result, local reserves of ascorbic acid may

rapidly be depleted within the high free radical environment of the chronic wound. The importance of this issue, although profound, is often overlooked. In addition to its role as a free-radical scavenger, ascorbic acid is an essential co-factor for proline hydroxylase (Murad 1981). This enzyme is an absolute requirement for appropriate synthesis and assembly of the collagen scaffold necessary for wound repair (Canty 2005). Consequently, observations of low ascorbic acid and poor wound healing are well documented, dating to the 18th century with observations of poor healing in sailors with scurvy and a subsequent volume of literature (Peterkofsky 1991).

In summary, the chronic wound results from dysregulation of numerous biological responses that produce an environment favoring deterioration. Considering the complexity of the pathogenesis of chronic wounds, it is reasonable to consider requirements for anti-infective/anti-inflammatory activity, as well as regenerative capacity to effectively address healing of chronic non-healing wounds.

The Efficacy of PRP-Gel Relates to Biological Activity Released by Platelets

Regenerative Capacity

Upon activation by collagen fragments, thromboxane A2, ADP, and/or thrombin, platelets release numerous different bioactive protein and non-protein molecules (Reed 2000, Nieswandt, 2003). It is known, through efforts such as the platelet proteome project, that more than 300 proteins are released by human platelets in response to thrombin activation (Coppinger, 2004). Considerable effort has gone into the characterization of proteins released by platelets and much is known about their specific roles as regulators of biological activity.

For example, PRP gels produced by the addition of thrombin contain growth factors and cytokines, such as vascular endothelial cell growth factor (VEGF), platelet derived growth factor (PDGF), epidermal growth factor (EGF), fibroblast growth factor (FGF) and transforming growth factor-beta (TGF-B) (Eppley 2004, Everts 2006). These proteins among others, are critical for organized wound healing and regulation of responses, such as vascularization, cell proliferation, cell differentiation, and deposition of new extracellular matrix (Barrientos 2008, Goldman 2004). In addition, thrombin activated platelets also release chemokines, such as Interleukin-8 (IL-8), stromal cell derived factor-1 (SDF-1), and platelet factor-4 (PF-4) (Chatterjee 2011, Gear 2003) that control the mobilization and migration of stem cells, fibroblasts, and leukocytes (Werner 2003 and Gillitzer 2001).

It is widely accepted that all of the above-mentioned proteins have critical roles in dermal wound healing and many have been or currently are in development as single molecule therapeutics. Interestingly, EGF, TGF-B, FGF, and VEGF have each failed in clinical trials as single protein therapeutics and PDGF (Regranex, a recombinant growth factor) has shown only modest clinical benefit (Braund 2007). This result is not surprising however, considering that chronic wounds are not the result of a single lesion

but result from a dysregulation of many biological responses regulated by a multitude of signaling molecules. Platelet rich plasma gel comprises the natural—endogenous—full complement of protein and non-protein signal molecules required for effective healing and it is likely that this contributes to the demonstrated efficacy of PRP-Gel for healing diabetic foot and pressure ulcers.

Anti-infective Activity

The management of chronic wounds most frequently involves oral or intravenous administration of antibiotics to fight infection. Even so, populations of bioburden in chronic wounds vary over time and these wounds invariably retain or become re-infected with some level of bacteria (Howell-Jones 2005). In addition to the regenerative capacity (cytokines, growth factors, and chemokines), potent anti-microbial effects have been demonstrated by platelet gels formed by the addition of thrombin to preparations of platelet rich plasma. In vitro studies revealed that PRP-Gel is bacteriocidal for *Staphylococcus aureus* (MSSA and MRSA), *Enterococcus faecalis*, and *E.coli* (Moojen 2007), and antimicrobial activity against *Staphylococcus aureus* was demonstrated in a rabbit model of osteomyelitis (Jia 2010). Further supporting these observations are additional in vitro evaluations of individual anti-microbial peptides that revealed direct anti-microbial activity against *C. neoformans*, *S. Aureus*, and *S. albicans* (Tang 2002, Bielecki 2007). These findings are supported by the retrospective analysis in a 2,259-patient study by Trowbridge et al in which the incidence of sternal wound infection dropped from 1.8% to 0% with the addition of thrombin activated platelet rich plasma gel (Trowbridge 2005).

Summary

PRP-Gel is intended to promote the growth of tissue via stimulation of cellular growth, the mobilization of cells, the initiation of angiogenesis, the development of a fibrin matrix on which cells can adhere, and PRP-Gel contains anti-infective properties to reduce infection in a wound. This combination of powerful autologous signal molecules, provided in a system for delivery to a non-healing wound, makes a PRP-Gel formulation an effective treatment for chronic non-healing wounds.

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Attachment B:
A Standardized Formulation of PRP-Gel Provides Reproducible Efficacy

Many Devices are Available for Producing PRP

A number of medical devices are commercially available for preparing autologous PRP (Roukis, 2006). However, the majority of these devices have not been cleared by FDA for use in chronic wound care; rather, they are intended for use in orthopedic procedures. These systems produce a sample of plasma containing a concentration of platelets, leukocytes and red cells. Traditionally, the PRP end products produced using these orthopedic devices have been mixed with any of various bone graft materials and used as a bone void filler or bone extender. The PRP- bone graft mixture is applied directly or after activation with thrombin, depending on clinical use requirements. More recently, PRP preparations obtained with the orthopedic devices have been evaluated for a number of different indications, based on the fact that platelets harbor peptides known to have roles in the regeneration of several different tissue types. In the case of wound care, the orthopedic devices have been used to prepare PRP in suspension, freeze thawed PRP and thrombin activated PRP-Gels. Discussed below, a significant volume of published literature supports efficacy of thrombin activated PRP gel.

The AutoloGel Experience

Specific to the healing of wounds, Cytomedix AutoloGel™ System is cleared by the FDA with indications for use specific to wounds. The specific wording is as follows;

> 510(k) Number: BK060007 – Sept 20, 2007
> Device Name: Autologel™ System
> Indications For Use:
> The AutoloGel™ System is intended to be used at point-of-care for the safe and rapid preparation of platelet-rich plasma (PRP) gel from a small sample of a patient's own blood. Under the supervision of a healthcare professional, the PRP gel produced by the AutoloGel™ System is suitable for exuding wounds, such as leg ulcers, pressure ulcers, and diabetic ulcers and for the management of mechanically or surgically-debrided wounds.

The AutoloGel System; Description of the Standardized Process and Components

Cytomedix AutoloGel™ System currently is the only device cleared by FDA for the production of a thrombin activated PRP-Gel for a wound indication. FDA clearance of the AutoloGel™ System includes the centrifuge device as well as reagents and methods necessary to reproducibly produce activated PRP-Gel from a sample of a patient's own blood. The methods and reagents used to obtain the therapeutic

AutoloGel™ formulation therefore are standardized as part of the FDA clearance and serves as an archetypal model for activated PRP-Gel.

Standardized Methods and Enhanced Formulation to Produce Active PRP-Gel

Detailed methods and all reagents necessary to provide a consistent and reproducible PRP-Gel formulation are included as part of the AutoloGel system. The AutoloGel System is FDA cleared for use on wounds. The procedures for use are as follows (See AutoloGel Instructions for Use in Exhibit 2 for detailed instructions):

Blood draw
Anti-Coagulant Citrate Dextrose Solution A (ACD-A), USP is mixed with whole blood during the initial venipuncture in a 1:10 ratio. 20 mL of whole blood is accessed.

Centrifugation
The centrifuge was custom designed and developed for the AutoloGel System. The centrifugation meets design input specifications for a short (<1 minute) spin time that preserves approximately 70% of the platelets in the plasma fraction. The high spin rate and short spin time maintains platelet integrity to produce PRP with minimal activation of platelets. The centrifuge accommodates up to 4 individual 5-mL tubes per spin providing the clinician flexibility to process a range of volumes to accommodate requirements for treating different wound sizes.

PRP Collection
The platelet rich plasma fraction is readily discerned from the red cell fraction and is harvested into one single syringe device. The syringe device also serves as a chamber for introduction and mixing of different formulation components discussed below.

Preparation of thrombin
Topical Thrombin is used to activate platelets and cleave fibrinogen to form the activated PRP-Gel. The bovine thrombin (USP, 5000 I.U. in a 5 mL vial) is resuspended in 5 ml of calcium chloride (USP 10%, 100 mg/mL in a 10 mL vial).

Addition of Ascorbic acid
Ascorbic acid is a free radical scavenger and is essential for collagen synthesis and assembly of collagen fibrils. Ascorbic acid (USP, 500 mg/mL, in a 50 mL vial) is added to PRP at a ratio of 1 mL ascorbic acid to 8 mL of PRP. The ascorbic acid-PRP solution is mixed by inversion.

Activation of PRP + Ascorbic Acid
Review of published trials indicates that activated PRP-Gel including AutoloGel is effective for healing chronic dermal wounds. Activated AutoloGel is formed by the addition of thrombin solution (1000 I.U/mL in calcium chloride solution) to PRP containing ascorbic acid. One ml of thrombin solution is introduced into 9 mL of PRP-

Ascorbic acid (as prepared above), mixed by inversion and expressed through a syringe device to form 10 mL of activated therapeutic PRP-Gel.

***Application of AutoloGel to Wounds*.**
Depending on features (size, location, tunneling, undermining) of the wound, AutoloGel can be directly expressed through a syringe or spray device onto the wound. Areas with undermining and tunneling are addressed first followed by application to the visible wound bed.

Dressings and coverings
A non-adherent contact layer is included in the wound dressing kit (Nterface©, Winthrop Laboratories, Dallas, TX). This is placed over the AutoloGel to maintain its position within the wound bed. The contact layer is covered with a transparent film dressing (e.g. Tegaderm©, 3M, St Paul, MN) to provide a vapor-permeable barrier. Absorbent dressings can be used outside of the transparent film dressing to capture wound exudate.

Certification training
Cytomedix requires that all clinicians and relevant staff participate in a competency certification training prior to using AutoloGel. The training program taught by wound care specialists covers 17 key steps in the selection and preparation of the wound, processing and the application of AutoloGel, documentation of wound status and universal precautions and disposal methods. The AutoloGel Certification Program includes a physical certificate and recording of name and contact information at Cytomedix corporate offices.

The Body of Evidence Supports Efficacy of Activated PRP-Gel

The FDA clearance of the AutoloGel™ system is unique in that it encompasses not only the centrifuge device for producing PRP, but also the kit, applicator, and reagents and methods for activation and ultimately the therapeutic PRP-Gel, AutoloGel™, that is applied directly to patient wounds.

The use of AutoloGel™ for treating chronic wounds is supported by a randomized controlled trial and additional data from a 285 wound registry, a 65 wound registry, a case series of spinal cord injured patients, and a powerful subset enabling comparison to healing modalities in the run-in period prior to AutoloGel™ treatment. Since publication of the randomized controlled trial with AutoloGel™, a significant body of chronic wound literature has emerged supporting the use of AutoloGel™ (See references in Exhibit 2) or similar thrombin activated PRP-Gels derived from PRP (Discussed in Attachments C and D).

It is important to point out that several forms of PRP have been evaluated for wound healing including PRP in the inactivated liquid form, freeze-thawed platelet concentrates and thrombin activated PRP-Gel. The body of published evidence strongly supports efficacy of activated PRP-Gel over other formulations. This finding is not surprising. In the case of autologous PRP-Gel, the proteins released by platelets are not subjected to extreme freeze-thaw temperatures that increase the chance for denaturing proteins with important biological activity. Furthermore, as compared to platelets in suspension, activated platelets release their regenerative capacity during formation of the PRP-Gel and provide the wound bed instant access to the proteins that drive healing. This point may be of particular importance when considering wounds associated with exudate that could rapidly dilute or wash out a topically applied therapeutic.

Summary

The evidence in this Formal Reconsideration Request documents the scientific role of PRP-Gel in healing a wound and also the healing and cost effectiveness along with the net health benefit. A standardized formulation that insures reproducibility is important for consistent outcomes.

Attachment C:
Systematic Review of the Platelet Rich Plasma (PRP) Literature

INTRODUCTION

Cytomedix requested a systematic review to assess randomized control trials (RCTs) and comparative non-RCT studies in wounds treated with autologous PRP Gel as compared to a control. The study data that was considered critical for this systematic review included those studies assessing (1) the impact of PRP and standard wound care on complete or partial wound healing; (2) time to complete or partial healing; (3) time to surgery; (4) healing trajectory, (5) velocity or rate; (6) reduction in wound area or volume; and (7) other healing information. Other items related to healing such as infections, pain levels, exudates, complications, adverse events and Quality of Life were also considered to be important in this review of PRP literature. No limitations on subject age, wound duration, or type of standard of care used were employed in order to find as many publications on PRP gel in cutaneous wounds as possible. RCT and non-RCT comparative studies were included in adherence with Agency for Healthcare Research and Quality (AHRQ) recommendations to insure comprehensive review while maintaining a sufficient strength of evidence.

I. METHODS

Selection criteria

For assessment of PRP treatment human intervention/treatment clinical trials, only randomized controlled trials (RCTs) and comparative studies (i.e., treatment/intervention groups compared with controls, or a group with run-in and treatment data that were compared) published in peer-reviewed journals (articles, brief articles, case studies, or letters) or presented at scientific meetings (abstracts) were considered for inclusion in this review, irrespective of the language in which they were written. The time span for the literature search was March 2001 through March 2011. Studies were eligible for inclusion if the participants had any kind of cutaneous ulcer or wound on the body (including dehisced wounds, open surgical wounds, acute, or chronic wounds, or spinal cord injury). Studies that included patients with mixed origin wounds, subsets of patients with different types of wound, and surgical wounds that were closed but had the addition of PRP to prevent complications that might convert the acute wound into a chronic wound were also eligible. In addition, to be eligible for inclusion, studies had to include platelet-rich plasma (PRP) treatment in the experimental groups or arms, whereas the treatment for the control arms or groups could be a placebo, or any conservative wound care or treatment. Non-inferiority trials in which one type of PRP treatment were compared against another were also eligible. Studies in which the experimental group received other treatments were also eligible provided that the control group also received the same treatment or care so that confounding was avoided and the systematic difference between the groups was only the primary intervention.

Those studies focusing on burns, dental or jaw treatment, bone fractures, orthopedic injection or plastic surgery were excluded because of the different healing characteristics of these wounds compared to cutaneous wounds. Studies that used homologous/allogenic PRP procedures, lysates, freezing, or freeze-dried techniques to produce PRP, or were considered to be “fibrin glue” were excluded due to the physiological differences of these products to autologous PRP gel. Studies that focused exclusively on safety (adverse events) or diagnostic aspects were also excluded.

For assessment of cost-effectiveness studies, the same rules were applied. Additionally, studies had to be of the cost-benefit or cost-utility design and focused on PRP with the same type of wound or ulcer as specified for clinical trial assessment.

Outcome measures

Studies eligible for inclusion had to report at least one wound-healing parameter as an outcome measure, or infection rates, incidence of infection, pain measures, exudation management, quality of life measures, or net health benefits. Examples of wound-healing parameters included complete wound healing (proportions in each group or percentages provided N for each group was reported; Kaplan-Meier, and Cox regressions); wound area reduction (mean or median, relative, absolute, or percentage); wound depth or volume reduction (same parameters as for area); healing rate (change in area or wound dimension per unit of time expressed in absolute terms or as a percentage), time to heal (mean or median, expressed in days or weeks), or comparison of clinical significant healing events, such as reaching a reduction of 50% or more in area using Kaplan-Meier or Cox regression. Outcomes could be unadjusted or adjusted for other covariates and factors, and compare baseline and final outcomes, or repeated measures. Follow-up for treatment/intervention trials had to be a minimum of 2 weeks.

Search strategy

The Cochrane Library, Scopus, Cinahl, and Pubmed databases were searched using combinations of terms (Table 1). The journals *Wounds*, *Worldwide Wounds* and the clinical trial database *clinicaltrials.gov* were hand searched using the same terms. Identified reviews and systematic reviews were also searched for additional references to RCTs and comparative studies that might have been missed. Narrative reviews, systematic reviews, and editorials were examined for references of potential trials. Several experts in the field were also consulted for their knowledge of RCTs. After initial selection of study abstracts that appeared to meet selection criteria, two reviewers, Marissa J. Carter (MJC) and Carelyn P. Fylling (CPF) reviewed each study in full to determine whether the study met the selection criteria and outcome measures.

The following data were recorded: numbers of citations for each search term entered (or combination of search terms); numbers of papers fully examined; numbers of papers eligible for review; and numbers of papers excluded with reasons. (Table 2) Duplicates were removed after confirming identical publication information. Papers suspected of containing the same results published elsewhere were reviewed to determine the originality of the results and which paper best met the selection criteria outlined above.

Initial quality assessment

The quality of each RCT or comparative study was assessed using a method reported by Downs and Black (Downs, 1998),[1] and modified by Carter, et al. (Carter, 2010).[2] The scoring sheet comprised 5 sections: reporting (quality of how the study data were reported), external validity (the generalizability of the study), internal validity (bias) (assessment of the potential for bias in the study), internal validity (confounding) (assessment of potential confounders that may have compromised the study), and power (assessment of the power of the study to discriminate the effect sizes of the outcomes). Modifications of the original method included replacement of the external validity module with an approach developed by Carter, et al. (Carter, 2009), which is based on the number of patients who would likely have been excluded from the study. The section is scored according to the category of the study: satisfactory: 3 points; problematic: 2 points; unsatisfactory: 1 point. Second, the power module was truncated with the following scoring scheme: reported sample size calculation (for RCTs): 1 point; reported more than 1 calculation: 2 points; no reporting of sample size calculation: 0 points; power reported for at least 1 clinically important effect (for comparative studies): 1 point; reported for all clinically important effect:: 2 points; no reporting of power for clinically important effects: 0 points. The total score possible was 29 points.

Bias was summarily reported using the SIGN grade methodology of Harbour and Miller (Harbour, 2001), which is defined as follows: ++ applies if all or most criteria from the checklist are fulfilled or where criteria are not fulfilled, the conclusions of the study or review are thought very unlikely to alter; + applies if some of the criteria from the checklist are fulfilled or where criteria are not fulfilled or are not adequately described, the conclusions of the study or review are thought unlikely to alter; – applies if few or no criteria from the checklist are fulfilled or where criteria are not fulfilled or are not adequately described, and the conclusions of the study or review are thought likely or very likely to alter. The assignment of grade to the bias of the study was accomplished by taking the total score of the external validity and internal validity (bias and confounding) sections of the quality assessment, and scoring as follows: 0 to 8 points (–); 9 to 12 points (+); 13 to 16 points (++). Scoring was carried out independently by MJC and CPF who then reconciled any discrepancies in subsequent discussion. Final grade assignments took into consideration serious flaws or inconsistencies, or other attributes that could decrease or increase initial grade assessment (GRADE, 2004).

Data extraction and analysis

Outcomes were categorized by type, and for each one the pretreatment and post-treatment numbers, median, or mean values (SD) were extracted where possible. To ensure that correct numbers were obtained, this process was performed by MJC and checked by CPF. No investigators were contacted for further clarification. The number needed to treat was calculated for studies reporting complete wound healing, and where protocol analyses were used, the data were updated to reflect an intent-to-treat analysis. Data were imported into software (Revman 5.0 Information Management, Nordic Cochrane Centre, Copenhagen, Denmark) to calculate 95% CIs and P values using fixed effects models where possible. The Mantel-Haenszel method was used with risk difference as the effect measure in the case of dichotomous events, and the inverse variance method was used with the weighted mean difference (WMD) as the effect measure in the case of continuous (interval) data. However, for dichotomous outcomes in which the Z test probability was .02 to .05, the odds ratio approach was also used to calculate the P value and 95% CIs.

Grading

After extraction of data and initial quality assessment were complete, important and critical outcomes were agreed upon using consensus and quality assessment and summary of findings for studies comparing use of platelet-rich plasma treatments against standard care were assessed using the GRADE system (GRADE, 2004; Guyatt, 2008; Guyatt, 2008) for each type of wound.

Meta-Analysis

Meta-analysis (statistical pooling) was carried out on those studies that had the following compatible outcomes and reasonable clinical homogeneity: (1) complete wound healing (chronic wounds and acute wounds, primary closure); (2) superficial infection; and (3) pain reduction. Results from RCTs were pooled separately from other comparative studies. For dichotomous events, a fixed effects model was employed that used the Mantel-Haenszel method with risk difference as the effect measure for easier interpretation. In the case of continuous (interval) data, a fixed effects model was also employed using the inverse variance method with the effect measure of WMD (weighted mean difference). Statistical heterogeneity was assessed using the I^2 (inconsistency) statistic, which indicates the percentage variation between studies that is a result of heterogeneity rather than chance (Higgins, 2002). If the I^2 (inconsistency) value was≥ 30%, meta-analysis was also conducted using a random effects model.

AHRQ Description of Sufficient Strength of Evidence for Coverage

In conducting this systematic review, we relied on standard definitions as developed by the Agency for Healthcare Quality and Research (AHRQ). AHRQ defines sufficient strength of evidence as:

> At least 1 study with greatest design suitability and good execution; or at least 3 studies having moderate or better design suitability, fair or better execution, and consistent results. *(Hickam DH, Severance S, Feldstein A, et al. The Effect of Health Care Working Conditions on Patient Safety. Evidence Report/Technology Assessment Number 74. (Prepared by Oregon Health & Science University under Contract No. 290-97-0018.) AHRQ Publication No. 03-E Rockville, MD: Agency for Healthcare Research and Quality. April 2003).*

AHRQ describes the study design suitability as follows:

> Greatest:
> Concurrent comparison groups and sufficient measures for other factors affecting outcome
>
> Moderate:
> Non-concurrent comparison or no comparison groups and insufficient measures for other factors affecting outcomes.
>
> *(Hickam DH, Severance S, Feldstein A, et al. The Effect of Health Care Working Conditions on Patient Safety. Evidence Report/Technology Assessment Number 74. (Prepared by Oregon Health & Science University under Contract No. 290-97-0018.) AHRQ Publication No. 03-E Rockville, MD: Agency for Healthcare Research and Quality. April 2003.)*

II. RESULTS

Search outcomes

- 8,567 citations reviewed (many duplicates) (Table 1)
- 65 studies described open wounds
- 44 studies excluded (Table 2)
    - Non-comparative designs
    - Case series or case studies
    - Narrative reviews
    - Ineligible PRP preparation
- 21 studies included
    - RCT's
    - Comparative Studies

Summary of Types of Studies

The literature describing the use of PRP to treat wound fell into 3 categories:

a) Open, chronic wounds
b) Acute surgical wounds where the open surgical wound was treated with PRP and then the surgical site was closed primarily.
c) Acute surgical wounds where the open surgical wounds was treated with PRP and allowed to close by secondary intention.

Abbreviations are listed below and at the bottom of each detailed table:

C –	*Control group*
CABG –	*Coronary artery bypass graft*
CI –	*Confidence interval*
CWH –	*Complete wound healing*
DFU –	*Diabetic foot ulcer*
E –	*Experimental group*
KM –	*Kaplan Meier*
NNH –	*Number needed to harm*
NNT –	*Number needed to treat (based on complete wound healing)*
NPWT –	*Negative pressure wound therapy*
ns –	*Not significant*
OR –	*Odds ratio*
PPP –	*Platelet Poor Plasma*
PR –	*Platelet Releasate*
PRP –	*Platelet rich plasma*
PU –	*Pressure ulcer*
RCT –	*Randomized controlled trial*
RD –	*Risk difference*
RR –	*Relative risk*
TKA –	*Total knee arthroplasty*
VU –	*Venous ulcer*
WMD –	*Weighted mean difference*
WRT –	*With respect to*

Both RCT's and comparative analysis were found in each category as follows:

- Chronic wounds:
  - 4 RCTs (2 RCTs graded –)
  - 3 comparative studies (1 under publication review; studies graded +)
- Acute wounds (primary closure):
  - 6 RCTs (3 graded ++, 3 graded –)
  - 5 comparative studies (graded +)
- Acute wounds (secondary closure):
  - 2 RCTs (graded +)
  - 1 comparative study (graded –)

In addition, 3 systematic reviews of the use of PRP were found and a cost-effectiveness study of the use of PRP Gel for treating chronic diabetic foot ulcers.

The following are judged critical or important under GRADE criteria based on clinical judgment because they affect time to heal, can cause complications, such as amputations or life-threatening situations, the quality of life, or cause large increases in cost:

- Complete wound healing
- Reduction in wound size/depth (e.g., 50%) for chronic wounds that have not changed in many months
- Wound healing problems, such as impairments or disturbances
- Wound exudation/drainage
- Wound infection (rates)
- Pain

Summary of Results in Each Category

The detailed results of all these parameters in all the articles and in each category are described in Tables 3 – 7. In summary, the results are as follows:

Chronic Wounds

- Quality of RCTs (4) was judged low (GRADE) with a 22% increase in complete wound healing. Meta-analysis showed significant and favorable outcomes for complete wound healing.
- One large comparative study (N = 26,599; GRADE: moderate) using previous generation Platelet Releasate (PR) product found an RR of complete wound healing of 1.38 for PR vs. controls
- A new study (N = 46) comparing wounds with extensive run-in versus treatment periods (GRADE: moderate) found significant reductions in area and depth with 2.5 to 3.5-fold decrease in time to reach 50% reduction in depth or area.
- Adverse events were consistently higher for controls compared to PRP groups.

Acute Surgical Wounds with Primary Closure

- Infection (1RCT, 3 comparative studies; GRADE: moderate): significantly increased infection in control groups versus PRP groups in 3/4 studies (sternal wounds and leg wounds from CABG)
- Wound-healing complications (1 RCT, 2 comparative studies; GRADE: low): significantly increased complications in control groups versus PRP groups in all studies (sternal wounds from CABG; inguinal surgical wounds)
- Exudation/drainage (1 RCT, 2 comparative studies; GRADE: moderate): significantly increased drainage in control groups versus PRP groups in all studies (various surgical wounds)

- Pain (4 RCTs, 1 comparative study; GRADE: very low): 2/5 studies report significantly reduced pain with PRP treatment.

Acute Surgical Wounds with Secondary Closure

- Time to heal (healing rates) (2 RCTs, 1 comparative study; GRADE: moderate): significantly increased healing rates in PRP groups versus control groups for all studies (surgical and traumatic wounds)
- Complete wound healing (1 RCT, 1 comparative study; GRADE: low): significantly increased complete wound healing in PRP groups versus control groups for all studies (surgical and traumatic wounds)
- Pain (1 RCT; GRADE: low): significantly reduced pain with PRP treatment compared to control treatment.

Meta Analysis

The results of the Meta Analysis in 3 categories are as follows:

Complete Wound Healing (Figure 1)
- 4 RCTs
- ITT populations in 3 and PP population in 1 due to treatment protocol violations
- Fixed effects, risk difference
- Outcome: complete wound healing.
- Favorable for complete wound healing with PRP

Infection (Figure 2)
- 1 RCT
- 1 comparative analysis study
- Outcome: reduction of infection
- Favorable for reduction of infection with PRP

Pain (Figure 3)
- 3 RCTs
- Outcome: reduction in pain
- Favorable for reduction of pain with PRP

Other PRP Systematic Reviews

- 3 systematic reviews found (2 published; 1abstract, to be published)
- Well-conducted reviews; reported meta-analytical results.
- One focused on DFUs and reported significant meta-analytical results (mostly older studies): “These findings indicate PRP as a treatment of choice for the topical care of wounds.” (Villela, 2010)

- One looked at chronic leg ulcers and with very positive meta-analytical results concluded "[T]here is scientific evidence regarding favorable outcomes of the use of PRP for the treatment of chronic wounds." (Villela, 2010)
- One studied efficacy/safety of PRP in oral/maxillofacial surgery, skin ulcers, and surgical wounds. Using some studies that were inappropriate in our opinion, they concluded "…treatment of skin ulcers with PRP increased the percentage of total recovery…" (Martinez-Zapata, 2009)

PRP Cost Effectiveness Study (Article not included in this systematic review) (Dougherty, 2008)

- Model had four health states:
  - Unhealed ulcer
  - Healed ulcer
  - Amputation
  - Deceased
- Hypothetical cohorts of 10,000 diabetic foot ulcer patients entered the model for a total of 200,000 observations:
  - Entered the treatment arms of the model, one at a time
  - Followed on a weekly basis (a cycle) for 5 years as they transitioned between health states
  - The cost of the wound treatment modality as well as physician care, hospitalizations, long term care, home health, rehabilitation, durable medical equipment, prosthesis, medications, etc were all included in the cost analysis.
- Data input was from published, peer reviewed journals
- Sensitivity analysis compared AutoloGel to alternative therapies that had conducted randomized controlled trials
- Outcome over 5 years:
  - Compared to the following:
    - Standard of Care
    - Tissue Engineered Grafts
      - Apligraf
      - Dermagraft
      - Orcel
    - Single Growth Factor
      - Regranex
    - Ultrasound
      - MIST Ultrasound
    - NPWT
      - VAC
  - PRP Gel (AutoloGel)
    - Was the most cost effective
    - Provided the best Quality of Life

Additional RCT Published After PRP Systematic Review Was Completed.

One month after the closing of the inclusion criteria timeframe for this PRP Systematic Review, another comparative analysis study of the use of platelet rich plasma (PRP) compared to using platelet poor plasma (PPP) to treat diabetic foot ulcers was published (Setta, 2011). Results indicated that the healing time for PRP was significantly faster than PPP. Mean healing time = PRP, 11.5 weeks (range 8-18 weeks) vs PPP, 17 weeks (range 14-20 weeks), (one-way ANOVA test and post hoc tests, $p < 0.005$). This additional study complements the positive outcomes depicted in the studies included in this Systematic Review.

SUMMARY

- The evidence presented in this new systematic review meets the definition of sufficient evidence of coverage (AHRQ) as follows:
  - For chronic wounds (all outcomes)
  - For acute wounds (primary closure): infection, wound-healing complications, drainage
  - For acute wounds (secondary closure): time to heal.
- The additional meta-analysis (this systematic review) and published systematic reviews also provide additional evidence that PRP has sufficient efficacy that it should be used in chronic wounds to "kickstart" healing. It may also be effective in certain acute wounds to prevent infection and wound complications, and accelerate healing.
- Data also suggest that PRP can be extremely cost-effective in certain chronic wounds.

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Table 1: Search Term Combinations for the PRP Systematic Review: Number of Citations in Each Category

Search Term	Single Term	CINAHL Single Term	Wounds	CINAHL Wound(s)	Chronic Wounds	Chronic Nonhealing Wounds	Open, Cutaneous Wounds	Cutaneous Wounds	Dehiscence	Dehisced	Surgical Wound	Diabetic Ulcer	Venous Ulcer	Pressure Ulcer	Sternal Wound	Total
PRP	257		148		10	1	0	1	0	0	77	6	1	2	1	**504**
PRP Gel	12	1602	8	88	3	1	0	1	1	0	20	4	1	1	1	**1743**
Platelet rich plasma	1546	2185	256	24	33	2	2	6	8	0	158	10	2	2	4	**4238**
Platelet rich plasma gel	141	24	26	17	9	1	1	3	0	0	32	1	2	1	3	**261**
Platelet gel	112	190	27	114	8	0	0	6	3	0	37	10	1	2	1	**511**
Autolo-gous growth factors	1184		93		11	1	0	0	0	0	20	4	1	6	0	**1320**
Total Per Cate-gory	**3252**	**4001**	**558**	**243**	**74**	**6**	**3**	**17**	**12**	**0**	**344**	**35**	**8**	**14**	**10**	**8577**

Table 2: Disposition of Studies During Initial Screening for the PRP Systematic Review

Potential Studies	
68 potentially eligible articles retrieved from lit search	
Accepted Studies	
20 eligible studies	
1 abstract RCT	
2 systematic reviews	
1 abstract systematic review	
Excluded Studies	
44 excluded due to following reasons	
Study (date)	**Reason excluded**
Akingboye (2010)	Narrative review
Alsousou (2009)	Narrative review
Anitua (2004)	Narrative review
Anitua (2007)	Narrative review
Arora (2009)	Narrative review
Balbo (2010)	Non-comparative study (case series)
Bernuzzi (2010)	Non-comparative study (case series)
Braund (2007)	Narrative review
Cervelli (2010)	Non-comparative study (case series)
Cervelli (2010)	Non-comparative study (case series)
Cervelli (2011)	Confounding wrt to other treatments
Crovetti (2004)	Non-comparative study (case series)
Dougherty (2008)	Cost-effectiveness
Englert (2006)	Insufficient reporting outcome data
Everts (2006)	Narrative review
Fanning (2007)	Not all wounds were cutaneous
Ficarelli (2008)	Narrative review

Frechette (2009)	Narrative review
Frykberg (2010)	Non-comparative study (case series)
Gottrup (2010)	Generic wound care research
Grant (2005)	Narrative review
Gunaydin (2008)	Outcome not in our eligibility list
Gurgen (2008)	Non-comparative study (case series)
Gurvich (2009)	Non-comparative study (case series)
Kakagia (2007)	Confounding wrt to other treatments
Klayman (2006)	Non-comparative study (single case study)
Lacci (2010)	Narrative review
Langer (2009)	Systematic review not covering PRP
McAleer (2006)	Case series
O'Connell (2008)	Non-comparative study (case series)
Peitramaggiori (2006)	Freeze-dried PTP
Roukis (2006)	Narrative review
Rozman (2007)	Narrative review
Scevola (2010)	Allogenic PRP
Schade (2008)	Non-comparative study (case series)
Senet (letter)	Letter discussing Senet (2003) RCT
Senet (2003) RCT	Frozen platelets
Smith (2009)	Narrative review
Soomekh (2011)	Narrative review
Stammers (2009)	Narrative review
Steenvoorde (2008)	Non-comparative study (case series)
van der hagen (2009)	Internal wounds
Whitlow (2008)	Survey barriers to PRP use
Yol (2008)	Animal study

Table 3: Description of studies: types of wounds and interventions used. Intervention group received all care described for control group unless otherwise stated.

Study	Design	N	Study Period	Wound Type	Control Group	Intervention Group
Almdahl	RCT	140	6 weeks	Leg wounds from long saphenous vein harvesting (CABG)	Standard closure (intracutaneous poliglecaprone)	Autologous PRP (GPS, Biomet Biologics; activated with autologous thrombin) sprayed prior to closure
Anitua	RCT	15	8 weeks	Cutaneous ulcers < 12 cm diameter, ≥ 4 weeks old	Moist saline gauze dressings and cleaning with normal saline; debridement and systemic antibiotics for infection	Autologous PRP (PRGF System, BTI Biotechnology Institute, Vitoria-Gasteiz, Spain) injected once in wound margins.
Buchwald	RCT	70	50 days	Leg wounds from long saphenous vein harvesting (CABG)	Standard closure	Autologous PRP (Angel; Dideco, Mirandola, Italy; activated with autologous thrombin) sprayed prior to closure
Carter	Comparative (run-in vs. treatment period)	46	≤ 86 days (run-in); ≤ 36 days (treatment)	DFUs, PUs, VUs, surgical, dehisced, & traumatic wounds, other types	Run-in period represented control group; moist wound care, dressing changes, debridement as required; compression or offloading per wound type; NPWT for some wounds	Autologous PRP gel treatment (AutoloGel, Cytomedix, Gaithersburg, MD, USA, bovine thrombin) applied to wound bed at least once.

Study	Design	N	Study Period	Wound Type	Control Group	Intervention Group
Driver	RCT	72	12 weeks	DFUs, 1A (U Texas), 0.5-20 cm^2, ≥ 4 weeks old	Cleaning, dressing changes, debridement as required; offloading; saline gel (Mölynycke Health Care, Norcross, GA, USA) applied after wound bed preparation biweekly for 12 weeks or until healed	Autologous PRP gel (AutoloGel, Cytomedix, Gaithersburg, MD, USA, bovine thrombin) applied after wound bed preparation biweekly for 12 weeks or until healed
Englert	RCT	30	~30 days	Sternal wounds (CABG)	Not reported	Autologous PRP (Magellan, Minneapolis, MN, USA,) "caulking bead" applied to sternum with cannula prior to closure
Everts	Prospective cohort (controls are consecutive patients who followed)	165	~1 week	Surgical wounds (TKA)	Wound drain, wound dressing, compression bandage.	Autologous PRP (Electa, Sorin Group, Mirandola, Italy; 85% activated with autologous thrombin, remaining activated with bovine thrombin) sprayed in back of knee cavity, posterior recess, gutters, etc.) and after deep closure injected on repaired extensor mechanism/prepatellar fat (no wound drain)
Friese	RCT	42	25 weeks (12 weeks for CWH)	DFUs, Wagner 1-3, > 0.7 cm^2, > 6 weeks old	Cleansing, debridement, dressing changes as needed & offloading	Autologous PRP (Harvest Technologies, Plymouth, MA, USA) every 2 weeks for 12 weeks

Study	Design	N	Study Period	Wound Type	Control Group	Intervention Group
Gardner	Retrospective comparison 61 PRP-treated wounds, 37 controls over same time period	98	~ 1 week	Surgical wounds (TKA)	Dressings and use of passive motion device after 24 hours	Autologous PRP (Medtronic Sequestra 1000 Autotransfusion System, Medtronic, Minneapolis, MN USA) injected into posterior recess, gutters, exposed femur/tibia surfaces, repaired extensor mechanism/ prepatellar fat (no wound drain)
Hom	Prospective comparison of treated wounds with contemporary own patient controls	8 pts 80 wds	6 months	PRP-treated skin punch wounds	Bactracin and semi-occlusive dressing	AutoloGel PRP gel (Magellan, Medtronic, Minneapolis, MN, USA; autologous thrombin-rich serum) plus white petrolatum ointment applied once or twice
Kazakos	RCT	59	3 weeks	Traumatic wounds	Cleansing, debridement, and dressings	Autologous PRP gel (PRP Fast system, Bioteck; bench centrifuge; autologous thrombin) applied before or after debridement, and then weekly
Khalafi	Retrospective analysis with propensity scoring (PRP/controls)	1,128	~ 1 week	Sternal and leg wounds (CABG)	Not reported.	Autologous PRP (GPS II, Biomet, Inc., Warsaw, IN, USA; activated with bovine thrombin) sprayed into sternal edges/subcutaneous tissue & graft harvest site

Study	Design	N	Study Period	Wound Type	Control Group	Intervention Group
Margolis	Retrospective cohort study with propensity scoring (PR/controls)	26,599	32 weeks	DFUs (neuropathic)	Standard treatment (moist wound care, debridement, offloading)	Autologous Platelet Releasate (Curative Health Services, Hauppauge, NY, USA) initiated within the first 12 weeks of care
Mazzucco	Prospective cohort with historical controls (dehiscent); cohort and controls (ulcers)	22 31	1 year	Dehiscent sternal wounds (CABG); necrotic skin ulcers	Daily topical washing/ cleaning, and antibiotic therapy as needed (dehiscent wounds); cleaning/dressing with hyaluronic acid/synthetic collagen gauze (ulcers)	Autologous PRP gel (ACD-A Vacutainer tubes, Becton Dickinson Labware, Franklin Lakes, NJ, and bench centrifuge; autologous thrombin) twice per week (dehiscent wounds) or once per week (ulcers) until healed.
Peerbooms	RCT	102	3 months	Surgical wounds (TKA)	Compression bandages and rehabilitation	Autologous PRP (GPS, Biomet, Inc., Warsaw, IN, USA) sprayed into knee cavity (synovium + cut edges of femur/tibia) and PPP sprayed into subcutaneous tissues; autologous thrombin
Saldala-macchia	RCT	14	5 weeks	DFUs Wagner 2/3 & ≥ 8 weeks old	Standard care	Autologous PRP gel application topically for 5 weeks, each week.

Study	Design	N	Study Period	Wound Type	Control Group	Intervention Group
Saratzis	Comparison 50 prospectively treated PRP-treated wounds with 50 controls over same time period	100	~ 30 days	Surgical wounds (inguinal)	Antibiotics, aspirin, clopidogrel, ambulation, and documentation of endograft integrity	Autologous PRP (Magellan, Minneapolis, MN, USA; not activated) injected subcutaneously and percutaneously
Spyridakis	RCT	52	30 days	Surgical wounds (pilonidal disease)	Standard dressings	Autologous PRP (GPS II system, Biomet, Inc., Warsaw, IN, USA; autologous thrombin) applied into the wound intra-operatively and before postoperatively day 4 and 12
Trowbridge	Retrospective comparison PRP-treated wounds with contemporary & historical controls	2,259	Not reported	Sternal wounds (cardiac surgery)	Standard care	Autologous PRP (CATS, Terumo Cardiovascular, Ann Arbor, MI, USA; Harvest Technologies, Plymouth, MA, USA; Angel, COBE Cardiovascular, Arvada, CO; bovine thrombin) sprayed to subcutaneous areas, as well as topical application
Vang	RCT	38	~ 3 weeks	Sternal wounds (CABG)	Standard dressings	Autologous PRP (Magellan, Minneapolis, MN, USA; bovine thrombin) sprayed into deep tissue and subcutaneous layers

Study	Design	N	Study Period	Wound Type	Control Group	Intervention Group
Yoo	RCT	52	~1 week	Surgical wound (thyroid)	Saline spray used instead of PRP (wound bed) and PPP (under skin incision); Penrose drain (5 minutes duration) and closed suction drain applied to surgical site after closure.	Autologous PRP (GPS, Biomet, Inc., Warsaw, IN, USA, autologous thrombin) sprayed into wound bed and PPP sprayed under skin incision

CABG: coronary artery bypass graft; CWH: complete wound healing; DFU: diabetic foot ulcer; NPWT: negative pressure wound therapy; PU: pressure ulcer; PPP: platelet-poor plasma; PRP: platelet-rich plasma RCT: randomized controlled trial; TKA: total knee arthroplasty; VU: venous ulcer.

Table 4. Detailed outcomes reported for the study period. Number needed to treat (NNT) was calculated based on complete wound healing information provided in the publication.

Study	Outcome Criteria	Outcomes	95% CI[1]	P value[1]	NNT
Chronic Wounds (RCTs)					
Anitua	1. Wound area reduction (%) 2. Complete wound healing 3. Adverse events	1. E: 72.9; C: 21.5 (8 weeks) (n=5; n=4)[3] 2. E: 1/8; C: 0/7 (8 weeks)[2] 3. E: 1/8; C: 3/7	1. 13.22 – 89.70	1. .008 2. ns 3. ns	
Driver	1. Complete wound healing 2. Complete wound healing 3. Complete wound healing 4. Time to heal (KM) (days) 5. Adverse events 6. Device related adverse events	1. E: 13/40; C: 9/32 (12 weeks)[2] 2. E: 13/19; C: 9/21 (PP1, 12 wks)[3] 3. E: 13/16; C: 8/19 (PP2, 12 wks)[3] 4. E: 42.9; C: 47.4 (PP2, 12 wks)[3] 5. E: 60/40; C: 62/32 6. E: 1; C:1	3. 0.10 – 0.68 4. -17.00 – 8.00	1. ns[4] 2. ns[4] 3. .036[4] 4. .018[4]	3

Study	Outcome Criteria	Outcomes	95% CI[1]	P value[1]	NNT
	7. Serious AEs 8. Device related serious AEs	7. E: 6/40; C: 17/32 8. E: 0; C:0			
Friese	1. Complete wound healing 2. Complete wound healing 3. Adverse events 4. Time to heal (weeks)	1. E: 11/21; C: 5/21 (12 weeks)[2] 2. E: 11/20; C: 5/21 (PP, 12 wks)[3] 3. E: 2/20; C: 9/21 4. E: 9.2; C:12.0	2. 1.03 – 14.87	1. ns 2. .05 3. .02[4]	
Saldala-macchia	1. Complete wound healing 2. Wound area reduction (%)	1. E: 2/7; C: 1/7 (5 weeks)[2] 2. E: 71.9; C: 9.2 (5 weeks)		1. ns 2. 0.039[4]	
Chronic Wounds (Comparative Designs; non-RCTs)					
Carter	1. Wound depth (%) 2. Wound area (%) 3. Mean time to reach 50% depth reduction (KM)	1. E: 65.9; C: 100[5] 2. E: 61.8; C: 100[5] 3. E: 22.3; C: 72.9 (days)[5]	1. 12.86 – 55.35[6] 2. 10.64 – 65.69[6] 3. -36.29 – -63.71	1. .00037[4,6] 2. .002[4,6] 3. .00034[4] 4. .028[4]	

Study	Outcome Criteria	Outcomes	95% CI[1]	P value[1]	NNT
	4. Mean time to reach 50% area reduction (KM)	4. E: 25.1; C: 66.0 (days)[5]	4. -41.92 – -66.66[4]		
Margolis	1. Complete wound healing	1. Propensity score quintiles: Grp 1: RR (healing with PR): 1.14 Grp 2: RR (healing with PR): 1.24 Grp 3: RR (healing with PR): 1.29 Grp 4: RR (healing with PR): 1.43 Grp 5: RR (healing with PR): 1.59 Overall: 1.38	 1.03 – 1.27[4] 1.16 – 1.34[4] 1.20 – 1.38[4] 1.33 – 1.52[4] 1.49 – 1.70[4] 1.33 – 1.42[4]		 19 12 11 7 6 11
Mazzucco	1. Time to heal (median; KM): Dehisced sternal wounds 2. Hospitalization time required to achieve complete healing: sternal 3. Time required before surgery (median; KM): necrotic skin ulcers	1. E: 3.5; C: 6.0 (weeks) 2. E: 31.5; C: 52.5 (days) 3. E: 15.0; C: 35.5 (weeks)		1. .0002[4] 2. <.0001[4] 3. <.0001[4]	

Study	Outcome Criteria	Outcomes	95% CI[1]	P value[1]	NNT
Acute Wounds Primary Closure (RCTs)					
Almdahl	1. Harvest site infection 2. Adverse events	1. E: 9/70; C: 8/70 (6 weeks) 2. None in either group		1. ns	
Buchwald	1. Normal wound healing 2. Abnormal wound healing 3. Wound healing impairment 4. Large-area hematomas 5. Postop pain level (mean)	1. E: 22/35; C: 16/35 (by day 50)[2] 2. E: 6/35; C: 5/35 (by day 50)[2] 3. E: 6/35; 11/35 (by day 50)[2] 4. E: 10/35; C: 21/35 (by day 50)[2] 5. E: 0.083; C: 0.11[4]	0.10 – 0.72	1. ns 2. ns 3. ns 4. .009 5. ns	
Englert	1. Postop chest pain (mean) 2. Postop leg pain (mean)	1. E: 1.47; C: 4.47 (day 1)[2] E: 1.40; C: 4.53 (day 3)[2] E: 0.53; C: 2.27 (day 30)[2] 2. E: 1.33; C: 3.06 (day 1)[2] E: 1.46; C: 2.80 (day 3)[2] E: 0.53; C: 2.33 (day 30)[2]		<. 001[4,7] < .001[4,5]	

Study	Outcome Criteria	Outcomes	95% CI[1]	P value[1]	NNT
Peerbooms	1. Wound closure 2. Pain at rest (median) 3. Pain, walking (median)	1. E: 4/50; C: 16/52 (2 weeks)[2] E: 4/36; C: 16/46 (2 weeks)[3] 2. E: 2; C: 2 (6 weeks)[2] 3. E: 2; C: 2 (6 weeks)[2]	-41 – -7 (%)[4]	1. ns[2] 2. .02[4] 3. ns[4] 4. ns[4]	
Vang	1. Sternum pain (yes) 2. Leg pain (yes)	1. E: 7/15; C: 7/15 (day 2)[3] E: 2/14; 4/15 (3 weeks)[3] 2. E: 0/15: C: 0/15 (day 2)[3] E: 3/14; C: 5/15 (3 weeks)[3]		1. ns[4] ns[4] 2. ns[4] ns[4]	
Yoo	1. Cumulative drainage (mean) 2. Pain (mean)	1. E: 44.9; C: 63.5 (mL; 24 hours)[2] 2. E: 2.1; C: 2.0 (12 hours)[3]		1. .039[4] 2. ns	
Acute Wounds Primary Closure (Comparative Designs; non-RCTs)					
Everts	1. Wound exudate leakage 2. Wound healing disturbances 3. Superficial infections 4. Hospital stay (mean days)	1. E: 2/85; C: 12/80 2. E: 0/85; C: 9/80 3. E: 0/85; C: 4/80 4. E: 6.4; C: 8.3		1. < .001[4] 2. .001[4] 3. <.05[4] 4. <.001[4]	8 9 20

Study	Outcome Criteria	Outcomes	95% CI[1]	P value[1]	NNT
Gardner	1. Mean intravenous narcotic use 2. Functional ROM at discharge (^{0}) 3. Hospital stay (mean days)	1. E: 17.0; C: 36.3 (mg/day) 2. E: 78.2; C: 71.9 3. E: 4.04; C: 5.29		1. .024[4] 2. .052[4] 3. .002[4]	
Khalafi	1. Chest infection 2. Chest drainage (notable) 3. Leg infection 4. Leg drainage (notable)	1. 0.074 (OR for E compared to C) 2. 0.042 (OR for E compared to C) 3. 0/560; 3/456 4. 0.120 (OR for E compared to C)	0.0032 – 1.753[4] 0.0085 – 0.210[4] 0.0714 – 0.200[4]	1. < .05[4] 2. < .001[4] 3. ns[4] 4. < .001[4]	
Saratzis	1. Postop complications (patient basis) 2. Postop complications (wound basis) 3. Hospital stay (post-op days)	1. E: 2/50; C: 9/50 (< 30 days) 2. E: 3/50; C: 12/50 (< 30 days) 3. E: 4.48; C: 6.14		1. .04[1] 2. .02[1] .026[4] 3. .001[4]	7 6

Study	Outcome Criteria	Outcomes	95% CI[1]	P value[1]	NNT
Trowbridge	1. Superficial infection 2. Deep sternal infection	1. E: 1/382; 17/948 2. E: 0/382; 14/948		1. < .05[4] 2. < .029[4]	65 68
Acute Wounds Secondary Closure (RCTs)					
Kazakos	1. Mean time required to heal to undergo reconstructive surgery 2. Surface area reduction (%) 3. Pain (VAS scale)	1. E: 21.3; C: 40.6 (days) 2. E: 12.2; C: 8.3 (1 week) E: 24.2; C: 16.0 (2 weeks) E: 36.4; C: 23.1 (3 weeks) 3. E: 57.6; C: 79.8 (3 weeks)		1. < .001[4] 2. .003[4] < .001[4] < .001[4] 3. < .001	
Spyridakis	1. Mean wound volume reduction 2. Time to complete wound healing 3. SF-36 scores (3 weeks) 4. Patient distress (days of medical service)	1. E: 11.6; C: 6.7 (mL; days 10-15) 2. E: 24; C: 30 (days) 3. E: 75; C: 62 4. E: 24; C: 30		1. < .01[4] 2. < .01[4] 3. < .03[4] 4. <.01[4]	

Study	Outcome Criteria	Outcomes	95% CI[1]	P value[1]	NNT
Acute Wounds Secondary Closure (Comparative Designs; non-RCTs)					
Hom	1. Percentage of closure 2. Full closure 3. Wound healing velocity	E: 81.1; C: 57.2 (day 17) E: 13/16; C: 7/16 (day 24) Summation of healing velocity at all time points between groups (detailed data not reported)	2.13 – 27.52	1. < .001[4,8] 2. .04[1] ns[4] 3. .001[4]	

[1]Values calculated using the Z test (fixed effects) when significant unless otherwise indicated (i.e., author values); [2]intention-to-treat (ITT) analysis; [3]per protocol (PP) analysis; [4]author values; [5]C represents wounds during a run-in period and E represents same wounds during treatment period; [6]multivariate repeated measures general linear model in which 95% CI is for mean difference; values for C represent area or depth at first pretreatment value and for E represent percent area or depth at last treatment time; [7]repeated measures ANOVA: multivariate for chest pain (Wilk's λ = 0.43) and univariate for leg pain.[8]repeated measures ANOVA, 42 days.

Wound size reductions are reported as mean reductions unless otherwise stated; CIs are calculated for risk difference in dichotomous outcomes and for weighted mean difference in continuous outcomes unless otherwise stated. AE = adverse event; C = control group

(comparison); CI = confidence intervals; E = experimental group (PRP); KM = Kaplan-Meier; NNT = number needed to treat (based on complete wound healing); ns = not significant; OR: odds ratio; PRP = platelet rich plasma; PR=platelet releasate; RR = relative risk.

Table 5: Quality review of studies: Score sheet. SIGN grade was estimated using the general methodology of Harbour and Miller, assigning a grade based on the total score of External validity, Internal Validity (Bias and Confounding) as follows: 0-8 (–); 9-12 (+); 13-16 (++).

Study Quality Assessed	Downs and Black, Carter, Carter						Harbour and Miller	Author Comments
	Reporting	External Validity	Internal Validity (Bias)	Internal Validity (Confounding)	Power	Score (of 29)	SIGN	Reason for Upgrade or Downgrade
Chronic Wounds (RCTs)								
Anitua	9	1	3	3	0	16	–	
Driver	10	2	6	1	1	20	⁻	Downgrade - Efficacy analysis: drop-outs high; many treatment violations
Chronic Wounds (Comparative Designs; non-RCTs)								
Carter	8	3	5	3	1	20	+	
Mazzucco	10	3	5	2	2	22	+	
Margolis	8	3	3	4	0	18	+	
Acute Wounds Primary Closure (RCTs)								
Almdahl	11	3	7	5	1	27	++	
Buchwald	9	0	7	2	0	18	⁻	Downgrade- Not clear what treatment controls got
Englert	7	3	5	3	0	18	⁻	Downgrade- Not clear what treatment controls got
Peerbooms	9	2	7	3	1	22	++	Upgrade- ITT analysis showed better results than PP
Vang	10	2	4	1	2	19	–	
Yoo	9	2	6	5	0	22	++	
Acute Wounds Primary Closure (Comparative Designs; non-RCTs)								
Everts	8	3	5	2	0	18	+	

Study Quality Assessed	Downs and Black, Carter, Carter						Harbour and Miller	Author Comments
	Reporting	External Validity	Internal Validity (Bias)	Internal Validity (Confounding)	Power	Score (of 29)	SIGN	Reason for Upgrade or Downgrade
Gardner	5	3	4	2	0	14	+	
Khalafi	7	3	3	2	0	15	+	Upgrade- Large N, propensity scoring techniques used
Saratzis	10	2	6	4	0	22	+	
Trowbridge	9	3	4	4	0	20	+	
Acute Wounds Secondary Closure (RCTs)								
Kazakos	10	3	5	2	0	20	+	
Spyridakis	8	3	5	3	0	19	+	
Acute Wounds Secondary Closure (Comparative Designs; non-RCTs)								
Hom	10	2	4	2	0	18	–	

Friese citation was a RCT abstract and the Saldalamacchia citation was a research letter. Neither of these two citations could be scored.

Table 6. Quality assessment and summary of findings for studies comparing use of platelet-rich plasma treatments against standard care for chronic wounds.

Quality Assessment						Summary of Findings					
No of Studies	Design	Quality	Consistency	Directness	Other Modifying Factors	No of Patients		Effect			
						PRP	Controls	Relative (95% CI)	Absolute	Quality	Importance
Complete Wound Healing											
4	RCT	Serious limitations	Some inconsistency	No uncertainty	Small trials (power issues)	76	67	RD: 0.24 (0.07-0.40)[1]	22/100	Low	Critical
1	Compara-tive	No serious limitations	No inconsistency	No uncertainty	Large N, well-done analysis, evidence of better healing for severe wounds	6,252	20,347	RR: 1.38 (1.33-1.42)	9/100	Mod	Critical
Time to Heal (Days)											
1	RCT	Serious limitations	Some inconsistency	No uncertainty	Numerous protocol violations	19	21	WMD: -4.50 (-17.0 – 8.0)	-4.5 days	Low	Critical
1	Compara-tive	No serious limitations	No inconsistency	No uncertainty	Small N	10	12	—	-17.5 days	Low	Critical
Mean Time to Reach 50% Depth or Area Reduction											
1	Compara-tive	No serious limitations	No inconsistency	No uncertainty	Relatively small N	41 39	46 46	Depth: WMD: -50.6 (-37.56 – -63.64) Area: WMD: -40.9 (-26.19 –- 55.61)	Depth: 3.3-fold Area: 2.6-fold	Mod	Critical

Quality Assessment						Summary of Findings					
No of Studies	**Design**	**Quality**	**Consistency**	**Directness**	**Other Modifying Factors**	**No of Patients**		**Effect**			
Adverse Events											
3	RCT	Some limitations	No inconsistency	No uncertainty	Small aggregate N	63	74	—	NNH: 11	Low	Important

[1]Data from one RCT uses intermediate PP results (N = 19/21); mod: moderate; NNH: number needed to harm; RCT: randomized controlled trial; RD: risk difference; RR: relative risk; WMD: weighted mean difference

Table 7: Quality assessment and summary of findings for studies comparing use of platelet-rich plasma treatments against standard care for acute wounds.

Quality Assessment						Summary of Findings					
No of Studies	Design	Quality	Consistency	Directness	Other Modifying Factors	No of Patients		Effect			
						PRP	Controls	Relative (95% CI)	Absolute	Quality	Importance
Acute Wounds (Primary Closure)											
Complete Wound Healing											
1	RCT	No limitations	No inconsistency	No uncertainty	Short follow-up	50	52	RD: -0.23 (-0.37 – -0.08)	-23/100	Mod	Critical
Infection											
1	RCT	No limitations	No inconsistency	No uncertainty	Leg infection	70	70	RD: 0.01 (-0.09 – 0.12)	1.4/100	High	Important
1	Compara-tive	No serious limitations	No inconsistency	No uncertainty	Leg infection; large N	560	546	RD: -0.01 (-0.01 – 0)	-5.5/1000	Mod	Important
2	Compara-tive	No serious limitations	No inconsistency	No uncertainty	Superficial infection	467	1028	RD: -0.02 (-0.06 – 0.01)	-22/1000	Mod	Important
1	Compara-tive	No serious limitations	No inconsistency	No uncertainty	Chest infection; large N; propensity scoring	571	557	OR: 0.0743 (0.0032-1.7535)	—	Mod	Important
1	Compara-tive	No serious limitations	No inconsistency	No uncertainty	Chest infection	382	948	RD: -0.01 (-0.02 – -0.01)	14.8/1000	Mod	Important
Pain Reduction											
3	RCT	Some serious limitations	Inconsistency between studies	Some uncertainty (overall vs. chest pain)	Small N	62	61	WMD: -0.75 (-2.38 – 0.89)	-0.75/10	Very low	Important
1	RCT	No serious limitations	No inconsistency	Some uncertainty	PP analysis	32	41	—	No difference	Mod	Important

Quality Assessment						Summary of Findings					
No of Studies	Design	Quality	Consistency	Directness	Other Modifying Factors	No of Patients		Effect			
						PRP	Controls	Relative (95% CI)	Absolute	Quality	Importance
1	RCT	Serious limitations	No inconsistency	Some uncertainty	Dichot-omous outcomes	15	15	RD: 0 (-0.36 – 0.36)	No difference	Very low	Important
1	RCT	Serious limitations	No inconsistency	Some uncertainty	Time frame unknown	34	35	WMD: -0.03 (-0.33 – 0.28)	-0.03/10	Very low	Important
Wound Drainage/Exudation											
1	RCT	No serious limitations	No inconsistency	No uncertainty	Small N	26	26	WMD: 18.6 (36.92 – 0.28)	18.6 mL	Low	Important
1	Compara-tive	No serious limitations	No inconsistency	No uncertainty	Large N; propensity scoring	571	557	OR: 0.042	—	Mod	Important
1	Compara-tive	No serious limitations	No inconsistency	Some uncertainty		85	80	RD: -0.13 (-0.21 – -0.04)	-13/ 100	Mod	Important
Acute Wounds (Secondary Closure)											
Complete Wound Healing											
1	Compara-tive	Serious limitations	No inconsistency	No uncertainty	Small N	16	16	RD: 0.31 (-0.02 – 0.64)	31/100	Very low	Critical
Time to Complete Wound Healing											
1	RCT	No serious limitations	No inconsistency	No uncertainty	Small N	30	22	—	6 days	Low	Critical

Quality Assessment						Summary of Findings					
No of Studies	**Design**	**Quality**	**Consistency**	**Directness**	**Other Modifying Factors**	**No of Patients**		**Effect**			
Wound Volume Reduction											
1	RCT	No serious limitations	No inconsistency	No uncertainty	Small N	30	22	WMD: 4.9 (3.79 – 6.01)	4.9 mL	Mod	Important
Area Reduction (Quotient Method)											
1	RCT	No serious limitations	No inconsistency	No uncertainty	Small N	32	27	WMD: 0.13 (0.08 – 0.18)	0.13 cm^2	Mod	Important
Pain											
1	RCT	No serious limitations	Some inconsistency	No uncertainty	Small N	32	27	WMD: -22.2 (-31.16 – -13.28)	-22.2/100	Low	Important

[1]Data from one RCT uses intermediate PP results (N = 19/21); mod: moderate; OR: odds ratio; RCT: randomized controlled trial; RD: risk difference; RR: relative risk; WMD: weighted mean difference

Figure 1: Meta Analysis of Complete Wound Healing with Platelet Rich Plasma (PRP)

Study or Subgroup	PRP Events	PRP Total	Control Events	Control Total	Weight	Risk Difference M-H, Fixed, 95% CI
Anitua 2007	1	8	0	7	13.5%	0.13 [-0.17, 0.42]
Driver 2006	13	19	9	21	36.0%	0.26 [-0.04, 0.55]
Friese 2007	11	21	5	21	37.9%	0.29 [0.00, 0.57]
Saldalamacchia 2004	2	7	1	7	12.6%	0.14 [-0.28, 0.57]
Total (95% CI)		**55**		**56**	**100.0%**	**0.24 [0.07, 0.40]**
Total events	27		15			

Heterogeneity: $Chi^2 = 0.86$, $df = 3$ ($P = 0.84$); $I^2 = 0\%$

Test for overall effect: $Z = 2.77$ ($P = 0.006$)

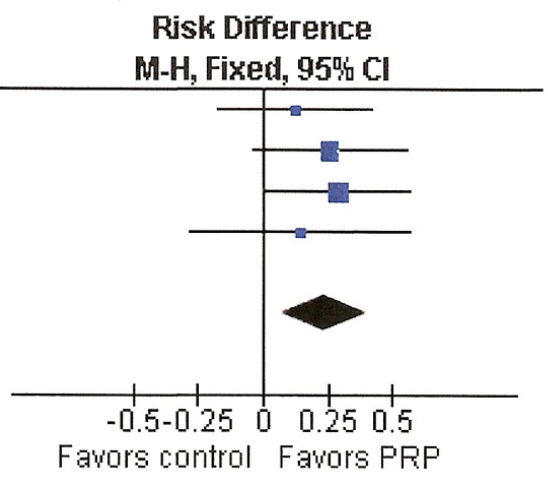


Figure 2: Meta Analysis of Infection Reduction with Platelet Rich Plasma (PRP)

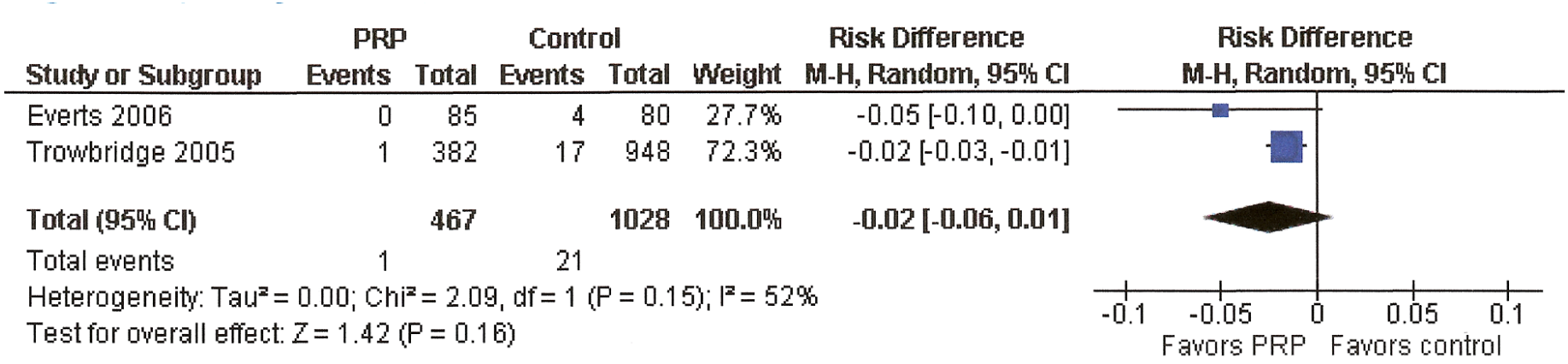
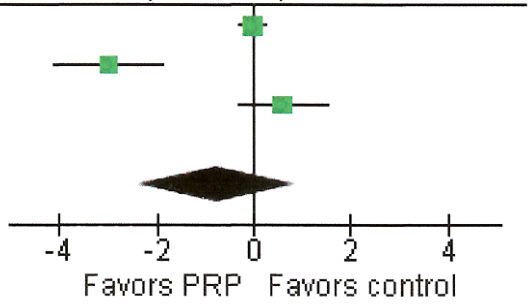


Figure 3: Meta Analysis of Pain Reduction with Platelet Rich Plasma (PRP)

	PRP			Control				Mean Difference	Mean Difference
Study or Subgroup	**Mean**	**SD**	**Total**	**Mean**	**SD**	**Total**	**Weight**	**IV, Random, 95% CI**	**IV, Random, 95% CI**
Buchwald 2008	0.083	0.69	35	0.11	0.59	34	36.0%	-0.03 [-0.33, 0.28]	
Englert 2005	1.47	0.83	15	4.47	2.06	15	31.1%	-3.00 [-4.12, -1.88]	
Yoo 2008	1.2	1.2	13	0.6	1.1	12	32.8%	0.60 [-0.30, 1.50]	
Total (95% CI)			**63**			**61**	**100.0%**	**-0.75 [-2.38, 0.89]**	

Heterogeneity: Tau² = 1.90; Chi² = 27.99, df = 2 (P < 0.00001); I² = 93%

Test for overall effect: Z = 0.90 (P = 0.37)



Attachment D:
Autologous Platelet Rich Plasma (PRP) Case Series Observational Studies Outcomes: 2001 – 2011

A recent Agency for Healthcare Research and Quality (AHRQ) assessment of Comparative Effectiveness Research (CER) explained that in several practical contexts, it is appropriate to consider data that is not produced in a randomized clinical trial. AHRQ explained its rationale as follows:

> Data from RCTs may be insufficient to address a review question about benefit for a number of reasons. RCTs may be inappropriate due to patient values or preferences; the intervention may be hazardous; or randomization may decrease benefit if the intervention effect depends in part on subjects' active participation based on their beliefs and preferences…. RCTs may be difficult to implement due to entrenched clinical practice … the need for long-term follow up to detect either benefits or harms, or difficulty randomizing feasible intervention units. In situations where RCT data are impractical, infeasible, or incomplete, **observational studies may provide valid and useful data to help address CER questions**." (*Norris S, Atkins D, Bruening W, et al. Selecting observational studies for comparing medical interventions. In: Agency for Healthcare Research and Quality. Methods Guide for Comparative Effectiveness Reviews [posted 2010]. Rockville, MD. Available at: http://www.ncbi.nlm.nih.gov/books/NBK47093/*

Based on this statement, the outcomes from the following Case Series Observational studies of the use of PRP for the treatment of wounds supplement the Systematic Review that contains RCT's and Comparative Analysis Studies (Attachment C):

Study PRP System Used	N	Wound Type	Patient Age: Mean Yrs (SD)	Previous Wound Duration: Mean Wks (SD)	Outcome Criteria	Outcomes
de Leon (2011) AutoloGel, Cytomedix, Inc, Gaithersburg MD	200 pts, 285 wds	All wound etiologies (N): Arterial-(included in other) Dehisced-24 Diabetic-41 Pressure-142 Sickle cell-(included in other) Surgical/ Trauma-38 Venous-32 Other-8	59.6 (16.65) (Range 19-96 years) 86% were Medicare bene-ficiaries	48.2	1. Area reduction (%) 2. Volume reduction (%) 3. Undermining reduction (%) n = 66 wds 4. Sinus tract/tunneling (ST/T) reduction (%) n = 28 wds	In 2.2 weeks with 2.8 AutoloGel treatments: 1. 86.3% wds responded with 47.5% reduction 2. 90.5% wds responded with 63.6% reduction In 1.8 weeks with 2.5 AutoloGel treatments: 3. 89.4% wds responded with 71.9% reduction In 1.8 weeks with 2.5 AutoloGel treatments: 4. 85.7% wds responded with 49.3% reduction

Study PRP System Used	N	Wound Type	Patient Age: Mean Yrs (SD)	Previous Wound Duration: Mean Wks (SD)	Outcome Criteria	Outcomes
					5. Area reduction per day (GLM Analysis – General Linear Modeling)	• % area reduction per day (rate of healing) model included area (3 levels: small, medium, and large), wound type (simplified: pressure ulcer, diabetic ulcer, venous ulcer, and other wound type), and the interaction (*) between the two factors (R^2 = 0.385, adjusted R^2 = 0.359. • Mean area reduction per day varied considerably by wound size with the largest reduction for large wounds (1.59cm^2/day), followed by medium (0.189 cm^2/day) and small (0.068cm^2/day) wounds after adjustment for wound type. • Differences between small and medium and small and large wounds were significant (p = 1.0 x 10^{-14}, and p ~1.0 x 10^{-250}, respectively). ***Overall, of the 285 wounds, only 10 failed to respond with a reduction in area, volume, undermining, or ST/T = 96.5% of the wounds responded. Additional detailed statistical analyses are described in the article.***

Study PRP System Used	N	Wound Type	Patient Age: Mean Yrs (SD)	Previous Wound Duration: Mean Wks (SD)	Outcome Criteria	Outcomes
de Leon: (2011) Sub-group analysis AutoloGel, Cytomedix, Inc, Gaithersburg MD	111 Medicare Wounds vs 135 Non-Medicare Wounds	All wound etiologies: Arterial Dehisced Diabetic Pressure Sickle cell Surgical/ trauma Venous	Medi-care= 69.7 Non-Medi-care= 49	Medicare= 51.4 Non-Medicare= 46.1	***Medicare Wounds:*** 1. Area reduction (%) 2. Volume reduction (%) 3. Undermining reduction (%) n = 19 wds 4. Sinus tract/tunneling reduction (%) n = 8 wds ***Non-Medicare Wounds:*** 1. Area reduction (%) 2. Volume reduction (%) 3. Undermining reduction (%) n = 40 wds 4. Sinus tract/tunneling reduction (%) n = 16 wds	***Medicare Wounds:*** In 2.2 weeks with 2.8 AutoloGel treatments: 1. 86.0% wds responded with 46.9% reduction 2. 89.9% wds responded with 64.6% reduction In 2.4 weeks with 3.2 AutoloGel treatments: 3. 82.6% wds responded with 65.6% reduction In 1.6 weeks with 2.4 AutoloGel treatments: 4. 88.9% wds responded with 61.6% reduction ***Non-Medicare Wounds:*** In 2.3 weeks with 2.8 AutoloGel treatments: 1. 86.5% wds responded with 48.0% reduction p = 1.0 (alpha w / Bonferroni = .0125) 2. 91.0% wds responded with 62.9% reduction p = 0.75 (alpha w/Bonferroni = .0125) In 2.1 weeks with 2.7 AutoloGel treatments: 3. 93.0% wds responded with 74.9% reduction p = 0.23 (alpha w/Bonferroni =0.0125) In 1.9 weeks with 2.5 AutoloGel treatments: 4. 84.2% wds responded with 43.1% reduction p = 1.0 (alpha w/Bonferroni = 0.0125) ***Medicare wounds had the same wound healing progress as the non-Medicare wounds.***

Study PRP System Used	N	Wound Type	Patient Age: Mean Yrs (SD)	Previous Wound Duration: Mean Wks (SD)	Outcome Criteria	Outcomes
de Leon: (2011) Sub-group analysis AutoloGel, Cytomedix, Inc, Gaithersburg MD	32 wds with albumin ≤ 2.5 and hemoglo-bin ≤ 10.5 (low labs) vs 33 wds with albumin > 2.5 and hemoglo-bin > 10.5 (higher labs)	All wound etiologies: Arterial Dehisced Diabetic Pressure Sickle cell Surgical/ trauma Venous	Low labs = 63.7 Higher labs = 57.8	Low labs= 61.5 Higher labs = 22.1	***Low labs:*** 1. Area reduction (%) 2. Volume reduction (%) 3. Undermining reduction (%) n = 8 wds 4. Sinus tract/tunneling reduction (%) n = 3 wds ***Higher labs:*** 1. Area reduction (%) 2. Volume reduction (%) 3. Undermining reduction (%) n = 0 wds 4. Sinus tract/tunneling reduction (%) n = 0 wds	***Low labs:*** In 1.3 weeks with 2.1 AutoloGel treatments: 1. 78.0% wds responded with 42.8% reduction 2. 87.8% wds responded with 58.5% reduction In 1.1 weeks with 2.1 AutoloGel treatments: 3. 80.0% wds responded with 70.4% reduction In 1.3 weeks with 2.8 AutoloGel treatments: 4. 75.0% wds responded with 27.4% reduction ***Higher labs:*** In 1.8 weeks with 2.8 AutoloGel treatments: 1. 86.8% wds responded with 44.6% reduction p = 0.23 (alpha w/Bonferroni = 0.025) 2. 94.7% wds responded with 65.4% reduction p = 0.20 (alpha w/Bonferroni = 0.20) 3. No wounds in this group 4. No wounds in this group ***Low lab wounds had the same wound healing progress as the higher lab wounds.***

Study PRP System Used	N	Wound Type	Patient Age: Mean Yrs (SD)	Previous Wound Duration: Mean Wks (SD)	Outcome Criteria	Outcomes
de Leon: (2011) Sub-group analysis AutoloGel, Cytomedix, Inc, Gaithersburg MD	55 wds from wound registry published in the Frykberg article (2010) vs 191 additional wds from wound registry included in De Leon article (2011)	All wound etiologies: Arterial Dehisced Diabetic Pressure Sickle cell Surgical/ trauma Venous Other	Fryk-berg group = 60.6 de Leon group = 59.3	Frykberg Group = 47.8 de Leon group = 48.4	***Frykberg group:*** 1. Area reduction (%) 2. Volume reduction (%) 3. Undermining reduction (%) n = 23 wds 4. Sinus tract/tunneling reduction (%) n=10 wds ***de Leon group:*** 1. Area reduction (%) 2. Volume reduction (%) 3. Undermining reduction (%) n = 36 wds 4. Sinus tract/tunneling reduction (%) n = 15 wds	***Frykberg group:*** In 2.8 weeks with 3.2 AutoloGel treatments: 1. 84.6% wds responded with 50.9% reduction 2. 89.2% wds responded with 62.0% reduction In 2.8 weeks with 3.2 AutoloGel treatments: 3. 100.0% wds responded with 77.8% reduction In 2.8 weeks with 3.2 AutoloGel treatments: 4. 100.0% wds responded with 45.8% reduction ***de Leon group:*** In 2.1 weeks with 2.7 AutoloGel treatments: 1. 86.6% wds responded with 46.5% reduction p = 0.65 (alpha w/Bonferroni = .0125) 2. 91.4% wds responded with 63.9% reduction p = 0.60 (alpha w/Bonferroni = .0125) In 2.3 weeks with 2.8 AutoloGel treatments: 3. 83.7% wds responded with 68.1% reduction p = 0.086 (alpha w/Bonferroni = 0.0125) In 1.7 weeks with 2.6 AutoloGel treatments: 4. 78.9% wds responded with 48.8% reduction p = 0.27 (alpha w/Bonferroni = 0.0125) ***The succeeding De Leon group had the same wound healing progress as the initial Frykberg group wounds.***

Study PRP System Used	N	Wound Type	Patient Age: Mean Yrs (SD)	Previous Wound Duration: Mean Wks (SD)	Outcome Criteria	Outcomes
de Leon: Per wound etiology AutoloGel, Cytomedix, Inc, Gaithersburg MD	2 pts, 2 wds	Arterial	51	20	1. Area reduction (%) 2. Volume reduction (%) 3. Undermining reduction (%) n = 1 wds 4. Sinus tract/tunneling reduction (%) n = 0 wds	In 2.9 weeks with 1.5 AutoloGel treatments: 1. 50.0% wds responded with 12.5% reduction 2. 100% wds responded with 44.9% reduction In 1.0 week with 1.0 AutoloGel treatments: 3. 100% wds responded with 100% reduction 4. No wounds in this group
de Leon: Per wound etiology AutoloGel, Cytomedix, Inc, Gaithersburg MD	21 pts, 24 wds	Dehisced	62.1	9	1. Area reduction (%) 2. Volume reduction (%) 3. Undermining reduction (%) n = 3 wds 4. Sinus tract/tunneling reduction (%) n = 2 wds	In 1.7 weeks with 2.2 AutoloGel treatments: 1. 66.7% wds responded with 53.0% reduction 2. 87.5% wds responded with 68.5% reduction In 1.6 week with 2.3 AutoloGel treatments 3. 100% wds responded with 48.4% reduction In 1.0 week with 1.5 AutoloGel treatments 4. 50.0% wds responded with 6.7% reduction
de Leon: Per wound etiology AutoloGel, Cytomedix, Inc, Gaithersburg MD	32 pts, 41 wds	Diabetic	65.5	37.9	1. Area reduction (%) 2. Volume reduction (%) 3. Undermining reduction (%) n = 3 wds 4. Sinus tract/tunneling reduction (%) n = 2 wds	In 3.4 weeks with 4.0 AutoloGel treatments: 1. 90.2% wds responded with 60.2% reduction 2. 87.9% wds responded with 74.0% reduction In 4.1 weeks with 3.8 AutoloGel treatments 3. 100% wds responded with 77.8% reduction In 2.5 weeks with 2.3 AutoloGel treatments 4. 100% wds responded with 80.0% reduction

Study PRP System Used	N	Wound Type	Patient Age: Mean Yrs (SD)	Previous Wound Duration: Mean Wks (SD)	Outcome Criteria	Outcomes
de Leon: Per wound etiology AutoloGel, Cytomedix, Inc, Gaithersburg MD	89 pts, 142 wds	Pressure	57	58.7	1. Area reduction (%) 2. Volume reduction (%) 3. Undermining reduction (%) n = 44 wds 4. Sinus tract/tunneling reduction (%) n = 17 wds	In 2.0 weeks with 2.6 AutoloGel treatments: 1. 88.0% wds responded with 46.8% reduction 2. 90.8% wds responded with 61.0% reduction In 2.3 weeks with 2.9 AutoloGel treatments 3. 90.9% wds responded with 66.5% reduction In 1.6 weeks with 2.4 AutoloGel treatments 4. 94.1% wds responded with 39.0% reduction
de Leon: Per wound etiology AutoloGel, Cytomedix, Inc, Gaithersburg MD	1 pt, 1 wd	Sickle cell	25	20.0	1. Area reduction (%) 2. Volume reduction (%) 3. Undermining reduction (%) n = 0 wds 4. Sinus tract/tunneling reduction (%) n = 0 wds	In 3.3 weeks with 3.0 AutoloGel treatments: 1. 100% wds responded with 65.2% reduction 2. 100% wds responded with 82.6% reduction 3. No wounds in this group 4. No wounds in this group
de Leon: Per wound etiology AutoloGel, Cytomedix, Inc, Gaithersburg MD	30 pts, 38 wds	Surgical/ trauma	55.3	18.5	1. Area reduction (%) 2. Volume reduction (%) 3. Undermining reduction (%) n = 12 wds 4. Sinus tract/tunneling reduction (%) n = 6 wds	In 1.8 weeks with 2.8 AutoloGel treatments: 1. 86.8% wds responded with 36.6% reduction 2. 89.5% wds responded with 63.3% reduction In 1.5 weeks with 2.5 AutoloGel treatments 3. 75% wds responded with 94.5% reduction In 2.2 weeks with 3.2 AutoloGel treatments 4. 66.7% wds responded with 78.0% reduction

Study PRP System Used	N	Wound Type	Patient Age: Mean Yrs (SD)	Previous Wound Duration: Mean Wks (SD)	Outcome Criteria	Outcomes
de Leon: Per wound etiology AutoloGel, Cytomedix, Inc, Gaithersburg MD	22 pts, 32 wds	Venous	71	70	1. Area reduction (%) 2. Volume reduction (%) 3. Undermining reduction (%) n=1 Wd 4. Sinus tract/tunneling reduction (%)	In 2.1 weeks with 2.4 AutoloGel treatments: 1. 87.5% wds responded with 40.2% reduction 2. 93.8% wds responded with 56.6% reduction In 1.0 week with 1.0 AutoloGel treatments 3. 100% wds responded with 100% reduction 4. No wounds in this group
Frykberg (2010) AutoloGel, Cytomedix, Inc, Gaithersburg MD	49 pts, 65 wds	All wound etiologies (N): Arterial-(included in other) Dehisced-5 Diabetic-14 Pressure-21 Sickle cell-(included in other) Surgical/ Trauma-6 Venous-16 Other-3	60.6 (14.7)	47.8 (Range 3-260)	1. Area reduction (%) 2. Volume reduction (%) 3. Undermining reduction (%) 4. Sinus tract/tunneling reduction (ST/T) (%)	In 2.8 weeks with 3.2 AutoloGel treatments: 1. 84.6% wds responded with 50.9% reduction 2. 89.2% wds responded with 62.0% reduction 3. 100% wds responded with 77.8% reduction 4. 100% wds responded with 45.8% reduction ***Overall, of the 65 wounds, only 2 failed to respond with a reduction in area, volume, undermining, or ST/T = 97% of the wounds responded.***
Frykberg: Per wound etiology AutoloGel, Cytomedix, Inc, Gaithersburg MD	2 pts, 2 wds	Arterial	51	20	1. Area reduction (%) 2. Volume reduction (%) 3. Undermining reduction (%) 4. Sinus tract/tunneling reduction (%)	In 2.8 weeks with 3.2 AutoloGel treatments: 1. 50.0% wds responded with 12.5% reduction 2. 100% wds responded with 44.9% reduction 3. 100% wds responded with 100% reduction 4. No wounds in this group

Study PRP System Used	N	Wound Type	Patient Age: Mean Yrs (SD)	Previous Wound Duration: Mean Wks (SD)	Outcome Criteria	Outcomes
Frykberg: Per wound etiology AutoloGel, Cytomedix, Inc, Gaithersburg MD	5 pts, 5 wds	Dehisced	58	11	1. Area reduction (%) 2. Volume reduction (%) 3. Undermining reduction (%) 4. Sinus tract/tunneling reduction (%)	In 2.8 weeks with 3.2 AutoloGel treatments: 1. 80.0% wds responded with 42.1% reduction 2. 80.0% wds responded with 67.6% reduction 3. 100% wds responded with 75.0% reduction 4. 100% wds responded with 6.7% reduction
Frykberg: Per wound etiology AutoloGel, Cytomedix, Inc, Gaithersburg MD	10 pts, 14 wds	Diabetic	62 (7.8)	44.7 (Range 8-260)	1. Area reduction (%) 2. Volume reduction (%) 3. Undermining reduction (%) 4. Sinus tract/tunneling reduction (%)	In 2.8 weeks with 3.2 AutoloGel treatments: 1. 85.7% wds responded with 67.2% reduction 2. 85.7% wds responded with 75.4% reduction 3. 100% wds responded with 77.3% reduction 4. 100% wds responded with 69.1% reduction
Frykberg: Per wound etiology AutoloGel, Cytomedix, Inc, Gaithersburg MD	17 pts, 21 wds	Pressure	52.9 (14.2)	48.3 (Range 3-126)	1. Area reduction (%) 2. Volume reduction (%) 3. Undermining reduction (%) 4. Sinus tract/tunneling reduction (%)	In 2.8 weeks with 3.2 AutoloGel treatments: 1. 76.2% wds responded with 49.0% reduction 2. 85.7% wds responded with 58.0% reduction 3. 100% wds responded with 67.7% reduction 4. 100% wds responded with 38.9% reduction
Frykberg: Per wound etiology AutoloGel, Cytomedix, Inc, Gaithersburg MD	1 pt, 1 wd	Sickle cell	25	20	1. Area reduction (%) 2. Volume reduction (%) 3. Undermining reduction (%) 4. Sinus tract/tunneling reduction (%)	In 2.8 weeks with 3.2 AutoloGel treatments: 1. 100% wds responded with 65.2% reduction 2. 100% wds responded with 82.6% reduction 3. No wounds in this group 4. No wounds in this group

Study PRP System Used	N	Wound Type	Patient Age: Mean Yrs (SD)	Previous Wound Duration: Mean Wks (SD)	Outcome Criteria	Outcomes
Frykberg: (2010) Per wound etiology AutoloGel, Cytomedix, Inc, Gaithersburg MD	4 pts, 6 wds	Surgical/ trauma	66.2	3.07	1. Area reduction (%) 2. Volume reduction (%) 3. Undermining reduction (%) 4. Sinus tract/tunneling reduction (%)	In 2.8 weeks with 3.2 AutoloGel treatments: 1. 100% wds responded with 41.7% reduction 2. 100% wds responded with 46.6% reduction 3. 100% wds responded with 90.2% reduction 4. 100% wds responded with 50.0% reduction
Frykberg: (2010 Per wound etiology AutoloGel, Cytomedix, Inc, Gaithersburg MD	11 pts, 16 wds	Venous	69.6 (15.2)	68 (8-260)	1. Area reduction (%) 2. Volume reduction (%) 3. Undermining reduction (%) 4. Sinus tract/tunneling reduction (%)	In 2.8 weeks with 3.2 AutoloGel treatments: 1. 93.8% wds responded with 47.7% reduction 2. 93.8% wds responded with 61.5% reduction 3. 100% wds responded with 100% reduction 4. No wounds in this group

Study PRP System Used	N	Wound Type	Patient Age: Mean Yrs (SD)	Previous Wound Duration: Mean Wks (SD)	Outcome Criteria	Outcomes
Frykberg: (2010) Sub-group analysis AutoloGel, Cytomedix, Inc, Gaithersburg MD	21 wds in pts ≥ 65 years of age vs 29 wds in pts < 65 years of age	All wound etiologies: Arterial Dehisced Diabetic Pressure Sickle cell Surgical/ trauma Venous	≥ 65 < 65	Not Available Not Available	***≥ 65 Yrs of Age Wounds:*** 1. Area reduction (%) 2. Volume reduction (%) 3. Undermining reduction (%) n = 6 Wds 4. Sinus tract/tunneling reduction (%) n = 1 wds ***<65 Yrs of Age Wounds:*** 1. Area reduction (%) 2. Volume reduction (%) 3. Undermining reduction (%) n = 12 wds 4. Sinus tract/tunneling reduction (%) n = 4 wds	***≥ 65 Yrs of Age Wounds:*** In 2.8 weeks with 3.2 AutoloGel treatments: 1. 46.0% reduction 2. 54.9% reduction 3. 93.4% reduction 4. 100% reduction ***< 65 Yrs of Age Wounds:*** In 2.8 weeks with 3.2 AutoloGel treatments: 1. 37.2% reduction 2. 50.6% reduction 3. 74.3% reduction 4. 18.3% reduction ***Wounds in patients ≥ 65 yrs of age had the better wound healing progress as wounds in patients <65 yrs of age.***
Gürgen (2008)	13 pts, 14 wds	Leg and foot ulcers	52.1 (range 35-76)	354 (8 – 1,092)	1. Healed 2. Reduction in ulcer size if not healed.	1. 50% healed 2. 35.7% reduced in size 14.2% unchanged

Study PRP System Used	N	Wound Type	Patient Age: Mean Yrs (SD)	Previous Wound Duration: Mean Wks (SD)	Outcome Criteria	Outcomes
Gurvich (2009) AutoloGel, Cytomedix, Inc, Gaithersburg MD	4 pts, 4 wds	A) Leg graft B) Trochan-ter Wd with sinus tracts C) Buttock ulcer D) Breast surgical wound	A) 62 B) 75 C) 88 D) 56	A) 6 mo B) 1 Yr C) 2 mo D) Not noted	Healing Outcome	A) PRP reactivated healing when stalled on NPWT. pd healed at 20 wks. B) PRP alternating with NPWT healed at 15 wks C) PRP post NPWT healed at 19th wk. D) PRP alternating with NPWT healed at 16 wks
Klayman (2006) SmartPrep Harvest Technologies, Plymouth MA	1 Pt, 1 wd	Nonhealing total knee replacement wound with exposed tendon	51	110 days	Wound healing progress to enable skin graft	NPWT used initially from Day 51 – 100 S/P surgery with minimal progress. PRP added along with NPWT. The PRP initiated the healing progress and caused enough granulation tissue formation that a skin graft occurred on Day 150.
McAleer (2006)	24 pts, 33 wds	Lower – extremity wounds: 3-venous 2-pressure ulcers 5-arterial 8-diabetic trauma 6-diabetic neuropathic	61.9 (range 25 – 91)	24	1. Wds Closed 2. ≥75% wound closure 3. 50 – 74% wound closure 4. 25 – 49% wound closure 5. No improvement	1. 20/33(61%) in 11.15 weeks 2. 3/33 (9%) 3. 3/33 (9%) 4. 2/33 (6%) 5. 5/33 (15%)

Study PRP System Used	N	Wound Type	Patient Age: Mean Yrs (SD)	Previous Wound Duration: Mean Wks (SD)	Outcome Criteria	Outcomes
Rappl (2011) AutoloGel, Cytomedix, Inc, Gaithersburg MD	20 pts, 20 wds	Spinal cord injured patients with pressure ulcers	49.2 (Range 27-75)	79.4 (Range 8-416)	1. Area reduction (%) 2. Volume reduction (%) 3. Undermining reduction (%) n = 4 wds 4. Sinus tract/tunneling reduction (%) n = 3 wds	In 3.4 weeks with 4 AutoloGel treatments: 1. 90.0% wds responded with 52.8% reduction 2. 90.0% wds responded with 67.3% reduction In 2.8 weeks with 4.0 AutoloGel treatments: 3. 75.0% wds responded with 47.0% reduction In 2.3 weeks 1.5 AutoloGel treatments: 4. 100% wds responded with 26.1% reduction
Schade (2008) Accelerate Platelet Concentra-ting System Exactech, Inc Gainesville, FL	13 wds	Soft tissue defects needing split thickness skin graft (STSG) in high risk patients with multiple co-morbidities.	70.5 ± 7.7 (range 42-80)	Not described in article	1. Time to ≥ 90% recipient site healing with STSG with PRP application under STSG	1. 16 ± 4.2 days
Sell (2011) SmartPrep Harvest Technologies, Plymouth MA	3 pts	Spinal cord injury patients with pressure ulcers	Pt 1=38 Pt 2=51 Pt 3=61	Not described	2. Reduction in wound size	The use of PRP therapy, involving a combination of sustained and immediate release of growth factors, appeared to stimulate acceleration of healing in 3 stalled pressure ulcers in hospitalized spinal cord injured patients.

Wound Healing Trajectory –
Comparing the de Leon Study (n = 285 Wounds) (2011) with the Published Literature on Wound Healing Trajectory

RCTs traditionally have focused on complete wound closure as an endpoint. To achieve this endpoint requires that wounds move in a timely, orderly fashion through the inflammatory and proliferative phases (reviewed in Attachment B). As wounds progress through these phases the biochemical and cellular milieu of the wound bed changes. Considering that wound healing does not result from a singular response but requires a progression of biological responses, it may be an unreasonable expectation that continued application of a single modality will provide the most effective path for healing. For example, negative pressure wound therapy is often used before surgical closure with a flap or application of a skin graft (Andros et al, 2006; Cipolla et al, 2008). Indeed, in clinical practice, a single modality is seldom used from initiation of treatment throughout the entire wound healing process. (Weir, MEDCAC, p 95). Therefore, treatment effectiveness should be determined by healing trajectories rather than wound closure (Robson 2000).

It would be useful to know if a therapy could reliably reduce a wound by a specified amount, especially if the wound was chronic. In diabetic foot ulcers, several studies have investigated percent reduction in wound size and confirmed that reduction is an early predictor of treatment outcome. One study concluded that protocols of care should be re-evaluated if a 50% reduction in wound size has not occurred within 4 weeks (Snyder et al, 2010). A similar conclusion was reached in an analysis of a cohort of 704 diabetic patients (Coerper, et al, 2009). In addition, earlier work from an RCT in which subjects had relatively few comorbidities showed a 53% reduction in wound area at 4 weeks led to 82% of wounds completely healed (Sheehan et al, 2003). In the de Leon study, a 60% reduction in wound area within 3.4 weeks was attained by 90% of the diabetic wounds following PRP-Gel therapy. This wound trajectory suggests that PRP-treated wounds would likely go on to complete healing. Of further interest, the wounds in the de Leon study were over 6 times larger in size (17.2 vs. 2.8 cm^2) compared to those in the cited study (Sheehan et al, 2003) and they had not responded to standard wound care for an average of 38 weeks prior to therapy. Similarly, a study on pressure ulcers in patients who were 60 to 70 years old, who had a good nutritional status at baseline and whose ulcers reduced at least 39% in size after 2 weeks, were found to heal much more expediently (van Rijwijk et al, 1994). The de Leon study results showed a 47% reduction in size within 2 weeks by 88% of pressure ulcers (clinical responders). Finally, several studies have laid the groundwork for predicting wound healing in venous ulcers at early healing stages, with a healing trajectory at 4 weeks having the best reliability (Phillips et al, 2000; van Rijwijk et al, 1993; Kantor et al, 2000). In the de Leon study, at 2 weeks, 87.5% of venous ulcers (clinical responders) were reduced an average of 40% in area, which is higher than that reported for compression alone in much smaller ulcers (Benigni et al, 2007).

Consistency in Outcomes

The above multiple datasets consistently demonstrate that using PRP Gel provides either complete healing, or re-animates a stalled wound so it starts the healing progress again. This progress toward healing occurs rapidly, as compared to other treatments that may take weeks, months, or years without progress. While complete healing is a goal, it is accepted among wound care professionals that multiple treatment modalities need to be used as the characteristics of the wound changes during the wound healing phases. Having PRP Gel as an advanced therapy to reverse the non-healing trend and start the healing trajectory in a positive direction is an asset and should be integral in the treatment of recalcitrant wounds. After

the wound is re-animated and positive progress is being made, it is possible to revert to standard wound care products to finish the last portion of the wound healing. In the de Leon study cited above, patients received initial treatments with AutoloGel to move wounds through the inflammatory phase. This was followed by standard wound care accompanied by patient monitoring to ensure continued progress. This treatment regimen produced wound healing trajectories that exceeded those reported in peer-reviewed literature and that are used as the basis for predictors of complete healing

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Study (date)	Reason excluded
Balbo (2010)	Homologous
Bernuzzi (2010)	Homologous
Cervelli (2010)	Confounding wrt to other treatments
Cervelli (2010)	Confounding wrt to other treatments
Cervelli (2011)	Confounding wrt to other treatments
Crovetti (2004)	Homologous, cryoprecipitate, lysate, confounding wrt to other treatments
O'Connell (2008)	Fibrin matrix, not PRP
Steenvoorde (2008)	Fibrin matrix, not PRP

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Attachment E:
Platelet Rich Plasma (PRP Gel) Net Health Benefit

In the March 19, 2008 CMS Decision Memo for Autologous Blood Derived Products for Chronic Non-Healing Wounds (CAG-00190R2), CMS indicated that it evaluates the relative magnitude of risks and benefits of an intervention being evaluated as well as the health outcomes, such as quality of life, functional status, duration of disability, morbidity, and mortality.

A chronic wound plainly affects the life of the person suffering with it. Numerous articles have been written describing the impact on the quality of life not only on the patient, but also their family or care-givers. Wound care treatment modalities that can help a wound heal or progress toward healing can impact them positively.

1. Defining The Quality of Life Impact of Non-healing Wounds

There are well-accepted standards for assessing changes in quality of life in patients with chronic wounds, which have been applied in this analysis. For example, Baharestani (1994) describes five major themes expressed by elderly women who were caregivers to their husbands with pressure ulcers:

1) Difficulty caregiving, which was further delineated into the physical, emotional, safety, and financial realms.
2) Frailty of the caregiver
3) Limited socialization
4) Limited social support systems
5) Limited caregiving knowledge

Two minor recurring themes were:

1) Fear regarding the future
2) Symbolic meaning of the pressure ulcer.

Franks and Moffatt (2001) used the Nottingham health profile (NHP) to assess the quality of life of patients with venous ulceration. The quality of life dimensions that were evaluated include:

1) Energy
2) Bodily pain
3) Emotional status
4) Sleep
5) Social isolation
6) Physical mobility

Mean age of the responders was 74 years. The mean previous wound duration was 12 months. Thirty seven percent of the wounds healed in 12 weeks. When patients' wounds healed, the NHP scores reduced significantly in all dimensions, indicating

improvements in health status. The largest positive effect for these healed venous ulcer patients was in the area of bodily pain, sleep, and energy.

Walshe (1995) conducted a qualitative study documenting the experience of living with a venous leg ulcer from the patient's perspective. Thirteen patients, all elderly, half over 85 years of age who had their wounds from 4 months to 10 years were interviewed. Four major themes were identified:

1) Description of symptoms including pain, leakage, and smell
    a. Pain was the major feature identified—this experience of pain permeated the patients' descriptions and had a profound effect on their lives.
    b. Sleep disturbances caused by pain.
    c. The leakage and smell from the wound caused difficulties in maintaining dignity and outward appearance.
2) Description of treatments
    a. Patients wanted dressings to be comfortable
    b. They wanted dressings that would help the wound to feel better
    c. They questioned the efficacy of dressings to promote healing because they had not had good experiences with treatments working.
    d. They were concerned about alterations in regime. Quote: "The thing is, when you feel as though it is healing, they start to change the dressings again, mean it puts it right back."
    e. There was perceived inconsistency.
3) Restrictions caused by the ulceration
    a. There were significant restrictions in the people's lives
    b. Impaired mobility
    c. Becoming virtually housebound
    d. Difficulties managing own hygiene due to dressings and ulcer
    e. General appearance difficult to maintain. Quote: "I am not whole now – I have a hole."
    f. Living a restricted life has been described as a cause of suffering loss of self.
4) Perception of and coping with the ulceration
    a. A feature for most was the uncertainty of healing, the lack of a known timescale. This uncertainty was often mirrored by the nurses caring for them.
    b. Patients wanted healing to take place.
    c. If healing occurred, they anticipated a great boost to their morale and improvement in their standard of living as symptoms and restrictions were removed.
    d. "That uncomfortable and ineffective treatment is endured can only be explained by the desire for healing at all costs overwhelming the desire to be normal." (p. 1098)
    e. "If effective treatment promoting healing could be incorporated into a normal lifestyle this would enhance coping, and improve people's sense of personal control." (p. 1098)

Krasner (1998) also interviewed patients with venous ulcers: 14 patients, ages from 30 – 86, with ulcers present from 2 months to 7 years. Eight key themes regarding quality of life were documented:

1) Expecting pain with the ulcer
2) Feeling frustrated
    a. Slow healing rates
    b. Uncontrolled swelling
    c. Complications
    d. Development of infection
    e. Formation of a new ulcer, usually secondary to trauma
    f. Not being able to participate in normal life
    g. Inadequate healthcare providers
    h. Years of seeking out treatment after treatment to no avail
    i. Feeling blamed for the ulcer
3) Swelling = pain
4) Not standing
5) Interfering with the job
    a. Patients have had to make the difficult decision to either change vocations or retire due to their non-healing ulcer.
    b. This interference with their job can be a financial burden as well due to less income.
    c. These cause a major change in their quality of life
6) Starting the pain all over again: painful debridements
7) Having to make significant life changes
    a. Isolation because can't be as involved outside the home.
8) Finding satisfaction in new activities
    a. Patients describe getting involved in different activities that did not require as much standing, physical activity, or risk of injury

Participants in this study described the net effect of their painful venous ulcers as negatively impacting their quality of life.

"One of the major contributions that nurses make to their patients who require a long convalescence and rehabilitation is to reflect and document even the smallest increments of improvement….which offers hope and a sense of progress." (p.44)

The Quality of Life Impact of Healing a Chronic Wound

Franks et al (1999) describe 200 patients with venous ulceration with a duration of >2 months who participated in a randomized, prospective trial. Mean age was 69.6 years (range = 35 – 94) Using the Nottingham Health Profile (NHP), at the 24 week follow-up, it was found that patients with healed ulceration had improved in energy, pain, emotion, sleep, and mobility compared with those whose ulceration failed to heal ($p < .05$).

Franks et al (2003) also describe the quality of life impact of venous ulcers on 118 patients, mean age 78 (range 42.2 – 96.2), with wound duration of 12 months (range 0.75 – 360). They used the Medical Outcomes Short Form-36 questionnaire.

Compared to age-sex adjusted published normative scores, patients with leg ulcers had significantly lower mean scores (100 is best possible, 0 is worst possible) in the following domains:

1) Role-emotional (d = 20.7, p <.001)
2) Social functioning (d = 22.8, p <.001)
3) Role-functioning (d = 20.8, p <.001)
4) Role-physical (d = 20.7, p< .001)
5) Bodily pain (d = 12.3, p< .001)

Healed Ulcers:

Bodily pain improved in the 31 patients whose ulcers healed during that time (d = 14.6, p = .006; SRM = 0.60)

Non-healed Ulcers:

Pain did not improve in patients whose ulcers remained open (d = -2.1, p = 0.45)

Compared to patients whose ulcers did not heal, patients with healed ulcers experienced greater improvements in the following domains:

1) Bodily pain (d = 16.8, p = .003)
2) Mental health (d = 9.4, p = .013)
3) Role-physical (d = 19.7, p = .06)
4) Role-emotional (d = 17.2, p = .12)
5) Vitality (d = 9.0, p = .052)

II. The Impact of Autologous Platelet Rich Plasma (PRP) on Quality of Life

The only article found in the literature that specifically used a quality-of-life instrument to determine the impact of an unhealed versus a healed wound with the use of PRP evaluated the impact of the wound healing process and recovery in patients being operated on for pilonidal sinus disease (Spyridakis et al, 2009). Fifty two patients with pilonidal sinus disease underwent open excision and secondary closure of the surgical wound (n = 22) or additional local postoperative infusion of platelet-derived growth factors (n = 30). The researchers identified five results:

Results:

1) Wound healing rates were much higher in the platelet group (p < .01)
2) Complete healing of the surgical wound required 24 days for the platelet group while the respective time for the control group was more than 30 days (p <.01)
3) Patients in the platelet group returned to their normal activities at day 17 while control group patients managed to do so at day 25.

4) All patients completed the SF-36 questionnaire regarding quality of life 3 weeks after their surgery. The average score for the control patients was 62 ± 5.6 while the platelet group score was 75 ± 4.2 ($p<0.03$).
5) The psychological distress was higher in the control group than in the platelet group ($p<0.01$).

While this study specifically documents the positive impact of PRP on the quality of life, the articles cited above describe the positive influence of wounds that are in the progress of healing or are healed.

III. Net Health Outcomes Identified in the Studies in the PRP Systematic Review

The randomized controlled trials and comparative studies described in the PRP systematic review document consistent net health outcomes that can improve a patient's quality of life. The PRP groups demonstrated: (Table 1)

- Improved healing
- Shorter time to healing
- Less wound disturbances
- Less superficial and deep infections in sternal incisions
- Less wound infections
- Less narcotic pain medications
- Better cosmesis in the resulting scar
- Active healing trajectory after PRP compared to non-healing trajectory before PRP
- Less chest and leg pain
- Less blood transfusions
- Less bruising
- Less decreases in hemoglobin
- Less wound leakage/drainage
- Shorter length of stay
- Higher range of motion
- Less pain
- Faster healing in larger wounds
- Less wound recurrence
- Less surgical wound-related complications
- Return to work faster
- Reduced psychological stress
- Less need for medical services
- Less adverse events.

These outcomes can be extrapolated to the Medicare population. Table 2b documents the patients' age, as well as the previous wound duration in relation to the outcomes described in the randomized controlled trials and comparative studies described in the PRP systematic review. Considering the mean ages, standard deviation, and ranges,

the majority of the patients studied were Medicare beneficiaries. In addition, most of these studies were conducted in the community setting rather than university or tertiary care settings; as a result, the data can be easily extrapolated for evaluating the impact of PRP Gel treatment in everyday clinical settings.

IV. Net Health Outcomes Identified in the PRP Case Series Studies

Case series studies treating over 350 wounds with PRP in the community setting (Attachment D) complement the outcomes in the randomized, controlled trials and the comparative studies. In each of the studies, compared to the extensive previous wound duration without healing, the majority of the wounds treated with PRP either healed or had significant reduction in the wound size during the study period. Based on the previously described quality-of-life studies, this change in the healing trajectory is a significant factor in improving the quality of life for individual beneficiaries.

V. Net Health Outcomes of Patients Treated with AutoloGel PRP Gel

A qualitative survey was conducted of the health care providers that had used AutoloGel to treat their patient's wounds. They identified net health benefit outcomes that were verbalized to them by the patient, their family, their physician, or health care provider.

Methodology

Cytomedix has a registry of the patients and wounds that have been treated with AutoloGel (platelet rich plasma gel – PRP Gel) during clinical evaluations at sites. Included in the Registry are 200 patients with 285 wounds. Outcomes regarding these wounds have been documented and accepted for publication (De Leon, 2011).

The patient information in the registry is de-identified, so direct query of patients was not possible. Instead, Cytomedix conducted a qualitative survey of the health care professionals who treated the patients' wounds with AutoloGel to determine the net health benefit of using AutoloGel. Those patients known personally by the health care professionals, and could be contacted directly were interviewed by telephone. For both health care professionals and patients, an open ended survey interview was conducted.

Major themes queried were:

- Clinical Benefit
    - Was the goal of treatment accomplished?
    - What overall healing progress occurred?
- Utilization Benefit
    - Was there decreased utilization of health care services?
    - Was the patient able to be discharged to home or a lower acuity level?

- Patient Benefit
    - Did the patient have improved function, activity of daily living (ADL) changes?
    - Was there a change in the patient's psychological well-being?
- Patient/Family Comments
- Health Professional Comments

Results

The net health benefit outcomes for 86 (43%) of the 200 patients with 134 (47%) of the 285 wounds from 23 different health care facilities were documented.

1. Clinical Benefit: Progress During AutoloGel Treatment

Category: Was the goal of treatment achieved while the wound was treated with AutoloGel?

When starting AutoloGel on a patient, the Cytomedix Clinical Liaison identifies the health care provider's goal for the wound during the initial weeks of treatment. Upon completion of this 2 – 3 week clinical evaluation, it is determined by both clinicians whether the goal was achieved by using AutoloGel to treat the wound. The following are the goals and the number of patients' wounds that achieved those goals.

Goal	**Yes/No and number of responses**
Heal wound	Yes - 32
Decrease size of wound, progressing toward closure	Yes - 25
Cover bone, mesh, or tendon or attach flap	Yes -10
Decrease sinus tract, tunneling, undermining	Yes - 6
Improve granulation tissue to prepare for graft	Yes - 7
Decrease wound volume and allow use of less expensive dressings	Yes - 6
Decrease wound volume and eliminate NPWT	Yes - 7
Avoid amputation	Yes - 4

Category: Wound changes

Reduction of pain	14
Healing to the point of surgical closure	13
Reduction of exudate	7
Surgery was not needed due to healing	5
Reduction of smell	3
Reduction of infection potential	1

2. ***Utilization Benefit: What changes in health care delivery occurred because of AutoloGel use on the wound?***

Category: Decreased utilization of health care services

Enough progress occurred with AutoloGel so the patient progressed to less expensive wound treatments	21
Decreased length of stay	14
Avoided continued NPWT use	6
Less clinic visits	4

Category: Discharged to a lower acuity level

Site of AutoloGel Treatment	Discharged to Lower Acuity Site	No. of Patients
Long term acute care hospital	Home	26
Long term acute care hospital	Long term care	11
Long term acute care hospital	Skilled nursing facility	6

3. ***Patient Benefit: What changes in the patient's life occurred because of AutoloGel use on the wound?***

Category: Improved Function, Activity of Daily Living (ADL) Changes

Ability to participate in family life better	25
SCI Patient – went from bedrest to sitting	13
Able to return to work, school	11
Became ambulatory	7
Able to participate more with physical therapy without the NPWT pump	5
Dramatic improvement in daily function	4
Became able to participate in therapies,	3

out of bed, sitting up, ambulatory, independent in ADLs	
Wound closure sooner than with NPWT so patient able to have quicker reversal of diverting colostomy	1

4. Psychological Impact on the Patient

- Depression and attitude improved dramatically as wound began to show progress
- Depression lifted, spirits raised, wrote a poem and thank you note
- Depression made the patient look homeless and dejected. He became animated and talkative as he saw progress in the wound.
- His spirits became animated
- Spirits improved, not in danger of losing his job
- So much hope in seeing progress
- Although near end of life with multi-organ failure, several wounds had healed and the others were near healing. He was so happy that they were healing after no progress in 24 weeks.
- Excited that he responded so quickly after lengthy time with this ulcer.
- Greatly relieved that amputation avoided
- Depressed and uncommunicative, became animated and talkative, hopeful. Now there is an answer to heal these recurring sickle cell wounds.
- Demeanor improved as wound healed while pain decreased
- Decreased reliance on pain meds and fast wound improvement lightened her mood.
- Some relief that wounds were progressing again.
- Patient required long term care for medication and alcohol oversight due to mental health issues. These issues exacerbated on bedrest. hey decreased when he became more functional with the wound healing.
- Excited to get back to school.
- Excited to go home
- Greatly improved attitude, was considering giving up and “letting nature take its course” after many years of battling. It was hard to keep up the fight, but encouraged by wound progress with AutoloGel.
- Able to move back home after being in various facilities to treat the wound.
- Anxious to leave facility. Able to be discharged sooner.
- Patient was very agitated and anxious about VAC changes. Was more settled with AutoloGel dressings.
- Became psychotic due to long hospitalization and wound complications. Psychosis improved with wound progress.
- Able to achieve his major goal of returning home to family
- Wound healed. Patient had been hospitalized multiple times to try and heal the wound, and now it was healed. Relief!
- Happy to avoid further surgery

- Joy! Started walking normally. Back to gold. Like I have gotten my life back again.
- Hope for a full life now – travel
- Patient noted decrease in pain and hope for continued wound healing, trying to leave nursing home to get back to his wife who has Alzheimer's and save his foot.
- Very pleased to get his wound closed after 40 years open (since his stay in an iron lung), grafts would not take over tendon. Very relieved!
- Testimonial letters outline positive impact of AutoloGel treatment

5. Patient and Family Comments

- Mother was worried about infection from the non-healing wound, anticipating taking her home after seeing wound progress. Excited about that!
- Parents thrilled with progress, anxious to take son (paraplegic, 42 year old mentally impaired) home. He had a failed flap of 152-week duration.
- Was not able to go home for Christmas because her abdominal dehiscence wound was not responding on the VAC. AutoloGel applied and wound showed significant progress in 3 days so she was able to be home with her family for Christmas.
- I can now care for my grandson.
- I now have job security. I was afraid of losing my job as a cook because of my venous ulcers and all the standing. Now I'm not afraid anymore.
- 96% volume reduction closure in 3 treatments despite 40 week duration. So happy.
- With my wound healed, I am able to participate in normal activities like taking grandkids out to kid's things, able to take an anniversary trip. I drove all the way from Kansas City to Boston to get treated with AutoloGel. Glad that I did.
- Happy that did not have to have second leg amputated due to wound. 95% wound closure in 2 treatments.
- 260 weeks duration on amputation site wound. Healed with AutoloGel in 2 treatments.
- Had wound 20 weeks without healing, wound reduced 82.6% in 3 AutoloGel treatments, went on to heal completely.
- In hospital for 9 weeks with little progress, sinus tract closed completely and wound reduced 95.3% in 3 AutoloGel treatments within 10 days.
- Reduced pain with AutoloGel versus the pain of NPWT-4 patient's comments
- Very pleased to be able to go home instead of to a skilled nursing facility
- Patient able to transfer to skilled nursing facility closer to home that would not have accepted her if she had the VAC
- Able to return to the skilled nursing facility where she came from since she had no wound VAC
- Able to return home with home health rather than having to go to a skilled nursing facility

- Able to return home rather than going to a skilled nursing facility with NPWT
- Patient very pleased that no further surgery was needed and that she could return home
- Chronic ulcer and tunnel closure in wound that had made *no* progress with 6 weeks VAC therapy.
- Patient and family very happy he could return home without need for VAC, wife did dressing changes, initially 3 times per week.
- Patient and husband very happy she was able to return home without skilled nursing facility placement with VAC therapy.
- Run over by a tractor. Great progress. Looking forward to finally going home.
- Became ambulatory and able to participate in therapy, eventually discharged to home with no need for home care services because no pain medications were needed and the patient was functioning independently.
- Patient and care giver please he was able to discharge home without home health.
- Previous wound duration on Charcot foot was 72 weeks. Wound had 67% volume reduction in 3 AutoloGel treatments.
- A vasculitic ulcer in existence for 44 weeks had 83.3% volume reduction in 3 AutoloGel treatments. Pain reduced considerably.
- Already had one leg amputated. Saved remaining leg from amputation. Sobbed with relief.
- Wanted to be discharged because had new great grandchild and she wanted to take care of her. She was able to do that.
- After 104 weeks of duration, pressure ulcers on the ischium reduced 54.2% in area, trochanter reduced 81.3% in area and volume, plantar reduced 91.8% in area and 95.8% in volume in 2 weeks. This Medicare patient sad that he was being discharged to LTC where he couldn't keep using AutoloGel since it was the first time the wounds had responded.
- Medicare patient so excited because a wound that had been in place for 8 years reduced 99.8% on AutoloGel.
- Patient did not need surgery because his wound healed on AutoloGel.
- Patient went from morphine to no pain meds as AutoloGel closed the wound.
- Patient in a dying state, but family happy that the new pressure ulcer was healed.
- Attentive husband of 50 years was relieved to see the wound finally respond so his wife would not become more ill.
- Patient had sacral/ischial pressure ulcer for 104 weeks, thrilled to see 70.4% volume reduction in 3 AutoloGel treatments.
- Amazed that Achilles tendon was covered completely
- Able to see mother-in-law who was having surgery since he was no longer tied to twice a week clinic visits.
- Wife no longer needed to be as diligent as a caregiver and driving to the clinic twice a week. Able to visit mother in Tallahassee, gave wife her life back.
- Hope! His spirits rose, hoped to be able to participate in cardiac rehab program (post triple bypass) which the foot wound was preventing. This wound was life threatening because he couldn't do his cardiac rehab.

- Wound in place 36 weeks. Able to return to work 2 weeks after treatment completed and back to normal activities.
- Wound in place 28 weeks. Returned to work part time after first 2 weeks of AutoloGel treatment and full time after 4 weeks.
- Returned to work after 16 months
- Returned to enjoying his retirement wound free.
- Avoided another skin graft.
- Able to continue work unencumbered.
- Able to be discharged from LTC facility to home after a year due to the non-healing wound.

6. Health Professional Comments

- VAC healed in mm, AutoloGel heals in cm. When MD saw progress with AutoloGel on one wound, she stopped the VAC on another one of the patient's wounds and started AutoloGel with great results.
- AutoloGel possibly prevented a lung infection as it caused the flap to adhere so it covered the chest cavity. The sinus tract was also reduced.
- There is no way that the sinus tract that extended from the ischium to the trochanter would have closed without AutoloGel.
- You're healing the wound too fast.
- Phenomenal response to unusual wound: smooth skin around the wound even in a 77 year old.
- This is the first time that this vasculitic wound has responded to anything.
- I've used every advanced wound treatment modality with no results. When I use AutoloGel, boom, the wound takes off! It is amazing!!
- Much improved
- Trauma surgeon extremely pleased with granulation tissue. Recommended his hospital to allow AutoloGel use.
- Surgeon very impressed with wound progress with AutoloGel.
- Physician stated that the wound made 3 months progress in 1 month on AutoloGel.
- Physician stated that AutoloGel prevented further surgeries to close the patient's abdominal wound.
- Patient would not offload. Laid on back. Even though the wound looked parched and dry at first, the color improved and granulation tissue was growing in.
- Wound progress had been very slow with VAC, but mover faster to graft with AutoloGel and patient was discharged.
- Required led care in nursing home (her place of residence). Major benefit was decreased pain.
- Patient's wound was making progress on AutoloGel, but patient had to be transferred from the LTAC to the acute care hospital due to atrial fibrillation. Wound got worse while in the hospital. Patient was happy to get back to the LTAC so she could resume AutoloGel on the wound.

- Patient with large open abdominal wound hospitalized for 3 months on the VAC, with little progress. 42.2% volume reduction in 6 treatments in 3 weeks with AutoloGel.
- Amazed at response to AutoloGel
- Patient had been on the VAC a long time with little progress. Now able to be discharged.
- Fewer dressing changes a plus.
- AutoloGel saved the tendon and granulated over it so moist wound healing was used afterwards to closure.

Discussion

Consistent positive outcomes were described by the health care professionals from multiple sites when AutoloGel is used to treat the patient's wound. Being an open-ended interview, the clinicians did not answer each question category, but a preponderance of consistent themes were described.

Conclusion

Using AutoloGel to treat chronic wounds improves the net health outcome of the patients that are treated. Patients described distinct positive changes in multiple areas of their lives due to the wound healing progress with AutoloGel.

VI. Cost Effectiveness of PRP Gel (AutoloGel) for the Treatment of Wounds

Dougherty (2008) conducted a rigorous economic study documenting the cost of caring for diabetic foot ulcer patients over a 5-year period. While coverage is not based on cost, cost is important to the patient as a net health benefit. If a therapy is not covered, it denies access to the majority of patients. Only those patients that can pay out of pocket have access to the therapy. This 2-tier access is frustrating to the patient and provider, and especially true when the therapy has been proven to be healing effective *and* cost effective for the patient, provider, *and* payer.

Dougherty documented that AutoloGel PRP-Gel was the most cost effective therapy for treating diabetic wounds over a 5-year period in comparison to standard of care, tissue-engineered products, single growth factor product, and NPWT. This cost effectiveness would save Medicare money. In addition, Dougherty documented that AutoloGel PRP-Gel provided the best quality of life for the patients in comparison to the other listed therapies.

In addition, an acute care hospital has documented the cost effectiveness of using AutoloGel PRP-Gel for the treatment of pressure ulcers rather than using NPWT. In 17 complex patients that were treated with AutoloGel rather than NPWT, they saved over

$14,000, the wounds had greater area and volume reduction in a shorter time with less dressing changes, faster staff time to do the dressings, patients were able to participate more fully in therapies without the encumbrance on the NPWT pump, and there was less pain for the patient (Wilson, 2011).

In this era of cost containment for Medicare, quality of care for patients, and increased net health benefit for all concerned, PRP-Gel, as a covered wound therapy can help accomplish all these goals.

VII. Testimonials from Patients Regarding the Net Health Benefit of AutoloGel

Over the years, numerous testimonials from patients have been received by Cytomedix and its AutoloGel users. (Testimonials are at the end of this document). These testimonials demonstrate the benefit of AutoloGel for these patients with previously non-healing wounds. As one letter relates, “AutoloGel gave me my life back.”

VIII. Summary

- Nonhealing ulcers negatively affect the quality of life of patients
- Healing a wound can improve the quality of life for these patients
- PRP helps to heal wounds in a shorter period of time
- PRP helps to eliminate complications such as loss of tendon due to necrosis in an open, non-healing wound, infection from a wound staying open, and pain from nonhealing
- PRP has minimal adverse events
- The benefit of using PRP in open wounds outweighs the risk
- PRP can be cost effective for the patient and payer.

Table 1: PRP Systematic Review: Description of studies' adverse events and ultimate health outcome.

Study	Design	N	Study Period	Wound Type	Adverse Events	Net Health Outcomes
Almdahl	RCT	140	6 weeks	Leg wounds from long saphenous vein harvesting (CABG)	No adverse events in either group	6 weeks postop: The overall cosmetic result was nearly identical in the PRP and control group. Subgroup analysis showed that the top score "excellent" was borderline favorable for the PRP group, which is noteworthy by weakly suggesting a trend to better cosmesis in the PRP group.
Anitua	RCT	15	8 weeks	Cutaneous ulcers < 12 cm diameter, ≥ 4 weeks old	Ulcer bed infection: PRP = 1 Control =2, 1 serious enough for lab abnormalities Anemia: control = 1	Statistically significant differences were found between both groups (in favor of the PRP group) from the 2nd week post-treatment until the end of the study (8th week)

Study	Design	N	Study Period	Wound Type	Adverse Events	Net Health Outcomes
Buchwald	RCT	70	50 days	Leg wounds from long saphenous vein harvesting (CABG)	1 patient in each group had wound healing impairments during hospitalization in the form of suture dehiscences, increased secretion, and necroses. PRP: The 6 pts that developed abnormalities during hospitalization also developed wound healing disturbances in the follow-up. Control: The 6 pts who had shown normal wound healing course in the clinical setting develop wound healing impairments in the further postoperative course.	In the follow-up (51 ± 9 days) after surgery: -PRP: 17.6% (6/34), control: 31.4% (11/35) of the patients from the control group showed leg wound disturbances (p = .184) -PRP: 28/34 (82.3%), control:24/35 (68.6%) were satisfied with the wound healing and the resulting scar (except 3 in control group were not pleased with the scar.)
Carter	Compara-tive (run-in vs. treatment period)	46	≤ 86 days (run-in); ≤ 36 days (treat-ment)	DFUs, PUs, VUs, surgical, dehisced, & traumatic wounds, other types	Not described in the article	Comparing the outcomes during a run-in period of standard of care vs PRP treatment, significant clinical outcomes indicated previously nonresponsive wounds began actively healing in response to PRP therapy.

Study	Design	N	Study Period	Wound Type	Adverse Events	Net Health Outcomes
Driver	RCT	72	12 weeks	DFUs, 1A (U Texas), 0.5-20 cm^2, ≥ 4 weeks old	PRP: n = 60 (49%) AEs, control: n = 62 (51%) Serious AEs: PRP: n = 6 Control: n = 17	At 168 day follow-up (6 months), no statistically or clinically significant differences were noted between the PRP gel and control from baseline to endpoint laboratory shifts in hematology, clotting factors, and factor V tests. One PRP wound had reopened. All the rest remained closed in both groups.
Englert	RCT	30	~30 days	Sternal wounds (CABG)	Not described in article	PRP: Less chest and leg pain over time p < .001. Less bruising but not significant
Everts	Prospec-tive cohort (controls are consecu-tive patients who followed)	165	~1 week	Surgical wounds (TKA)	Not described in article	PRP: Less blood transfusions (p <.001); less decrease in hemoglobin immediately after surgery (p <.001 for day 1 and p < .01 for day 2); less wound leakage (p < .001); less superficial infections (p < .05); less wound healing disturbances (p = .001); and a shorter length of stay (p < .001). All these factors indicate that the PRP group had an improved recovery and were able to return home sooner than the control group.

Study	Design	N	Study Period	Wound Type	Adverse Events	Net Health Outcomes
Friese	RCT	42	25 weeks (12 weeks for CWH)	DFUs, Wagner 1-3, > 0.7 cm^2, > 6 weeks old	The frequency and severity of adverse events was significantly higher in the control group (9 vs. 2, p = .02), of which the most common was infection and any vascular complication.	PRP group had faster wound healing (p < .05). The rate of healing was faster, 9.2 vs 12.2 weeks.
Gardner	Retrospec-tive compari-son 61 PRP-treated wounds, 37 controls over same time period	98	~ 1 week	Surgical wounds (TKA)	Not described in article	PRP group had higher range of motion (78.2º vs 71.9º for control, p = .052) and were discharged 1 day earlier than the control group (4.04 vs. 5.29 days, p = .002); thus patients treated with PRP had greater range of motion in a shorter period of time.
Hom	Prospec-tive compari-son of treated wounds with contem-porary own patient controls	8 pts 80 wds	6 months	PRP-treated skin punch wounds	No serious adverse events	PRP: Over a 42 day period, increased wound closure (p < .02); epithelialization and granulation tissue formation appeared 3 days earlier than in controls, and increased endothelial cell proliferation (p < .04). These factors demonstrate faster wound healing with PRP.

Study	Design	N	Study Period	Wound Type	Adverse Events	Net Health Outcomes
Kazakos	RCT	59	3 weeks	Traumatic wounds	Not described in the article	PRP group had significantly less pain at weeks 2 and 3.
Khalafi	Retrospec-tive analysis with propensity scoring (PRP/ controls)	1,128	~ 1 week	Sternal and leg wounds (CABG)	No treatment-related adverse events	PRP reduced the odds of chest wound infection by 93%, chest drainage by 96%, and leg wound drainage by 88% for an improved recovery.
Margolis	Retrospec-tive cohort study with propensity scoring (PR/ controls)	26599	32 weeks	DFUs (neuropathic)	Not described in article	- Patients treated with PRP were more likely to heal than those patients not treated with PRP for all five propensity score strata. - The effect of PRP was greatest for those patients with larger wounds of higher grade. - Wound size and grade revealed a pronounced trend in the effectiveness of PRP across categories. - PRP is more likely to be used in more severe wounds and is more effective than standard care in these severe wounds.

Study	Design	N	Study Period	Wound Type	Adverse Events	Net Health Outcomes
Mazzucco	Prospec-tive cohort with historical controls (dehiscent) ; cohort and controls (ulcers)	22 31	1 year	Dehiscent sternal wounds (CABG); necrotic skin ulcers	No complication in the treatments groups.	Sternal wounds: During follow-up, neither recurrence or complication occurred in treatment group. Necrotic skin ulcers: Local recurrence did not occur in patients after surgery in treatment group. Almost all the patients in the treatment group reported significant pain relief thus bettering their quality of life.
Peerbooms	RCT	102	3 mos	Surgical wounds (TKA)	Superficial wound infections in 1 patient in each group. No deep infections seen.	After 3 months follow-up, no statistically significant differences between both groups regarding ROM, severity of complaints (WOMAC) and level of pain.

Study	Design	N	Study Period	Wound Type	Adverse Events	Net Health Outcomes
Saldala-macchia	RCT	14	5 weeks	DFUs Wagner 2/3 & ≥ 8 weeks old	PRP: No adverse events	A significant reduction of the wound area was observed in the PRP group, but not in the control group in a short period of observation. "Our results from a controlled study although obtained in a small group of patients, strongly support safety and effectiveness of platelet gel in addition to standard care as a means for accelerating the healing process in diabetic foot ulcerations."
Saratzis	Compari-son 50 prospec-tively treated PRP-treated wounds with 50 controls over same time period	100	~ 30 days	Surgical wounds (inguinal)	PRP: 1 pt developed a unilateral wound infection with lymphorrhea, 2 pts developed bi-lateral superficial infection. Controls: 12 pts developed a wound related complication.	Postop hospitalization significantly lower in PRP group (p = .001) Overall surgical wound-related complications lower in PRP group (p = .026) Complications in the control group were of greater extent and severity.

Study	Design	N	Study Period	Wound Type	Adverse Events	Net Health Outcomes
Spyridakis	RCT	52	30 days	Surgical wounds (pilonidal disease)	Not described in the article	During a 1 month follow-up, it was found that the PRP group had acceleration of the wound healing process which led to a shorter recovery period ($p < .01$) Complete wound healing in a shorter period of time ($p < .01$) Returned to work faster (day 17 postop vs. day 25 for controls) In addition, the level of psychological distress was higher in the control group as evidenced by the SF-36 quality of life questionnaire ($p < .001$) They were in need of medical services for a longer period of time (30 days vs 24 days).

Study	Design	N	Study Period	Wound Type	Adverse Events	Net Health Outcomes
Trowbridge	Retrospec-tive compari-son PRP-treated wounds with contem-porary & historical controls	2,259 PRP: 382 Con-trol: 948 HC (Hist-orical Con-trol): 929	Not reported	Sternal wounds (cardiac surgery)	Not described in the article	The incidence of superficial infection was significantly lower in the PRP group (0.3%) compared with the control (1.8%) and the historical control (HC) groups (1.5%) ($p < .05$). This was also found when comparing deep sternal wound infections between groups (PRP: 0.0% vs controls: 1.5% ($p < .029$) and PRP vs HC 1.7% ($p < .01$.) “The relative risk of platelet gel is low compared with the consequences of infections, especially those that involve the sternum.” (P 385.)
Vang	RCT	38	~ 3 weeks	Sternal wounds (CABG)	1 pt in each group experienced a leg infection at the EVH site after discharge.	At 3 weeks postop: PRP:11/15, control:10/15; reported no pain. PRP: 86%, controls: 73% were noted to have no bruising in the leg.

Study	Design	N	Study Period	Wound Type	Adverse Events	Net Health Outcomes
Yoo	RCT	52	~1 week	Surgical wound (thyroid)	Not described in article	PRP: Less drainage initial 24 hrs post-op, shorter LOS, & 92% of the patients discharged after 1 overnight stay. Controls: More drainage, longer LOS, and only 62% discharged after 1 overnight stay.

CABG: coronary artery bypass graft; CWH: complete wound healing; DFU: diabetic foot ulcer; NPWT: negative pressure wound therapy; PU: pressure ulcer; PPP: platelet-poor plasma; PRP: platelet-rich plasma; RCT: randomized controlled trial; TKA: total knee arthroplasty; VU: venous ulcer.

Table 2: PRP Systematic Review: Patient age and previous wound duration in comparison to the outcomes.

Study	Outcome Criteria	Patient Age: Mean Yrs (SD)	Previous Wound Duration: Mean Wks (SD)	Outcomes
Chronic Wounds (RCTs)				
Anitua	1. Wound area reduction (%) 2. Complete wound healing 3. Adverse events	E: 45 (20) C: 61 (16) Range: 20-80	> 4 weeks	1. E: 72.9; C: 21.5 (8 weeks) (n=5; n=4)[3] 2. E: 1/8; C: 1/7 (8 weeks)[2] 3. E: 1; C: 3
Driver	1. Complete wound healing 2. Complete wound healing 3. Complete wound healing 4. Time to heal (KM) 5. Adverse events 6. Serious AEs	E: 56.4 SD 10.2, Min 31, Max 75 C: 57.5 SD 9.1 Min 45 Max 86	≥ 4 weeks	1. E: 13/40; C: 9/32 (12 weeks)[2] 2. E: 13/19; C: 9/21 (PP1, 12 wks)[3] 3. E: 13/16; C: 8/19 (PP2, 12 wks)[3] 4. E: (PP2, 12 wks)[3] 5. E; 60; C: 66. 6. E: 6; C: 17
Friese	1. Complete wound healing 2. Complete wound healing 2. Adverse events	Not described	> 6 weeks	1. E: 11/21; C: 5/21 (12 weeks)[2] 2. E: 11/20; C: 5/21 (PP, 12 wks)[3] 2. E: 2; C: 9
Saldala-macchia	1. Complete wound healing	E: 61.1 (9.4) C: 58.1 (7.8)	E: 68 (61) C: 101 (164)	1. E: 2/7; C: 1/7 (5 weeks)[2]

Study	Outcome Criteria	Patient Age: Mean Yrs (SD)	Previous Wound Duration: Mean Wks (SD)	Outcomes
Chronic Wounds (Comparative Designs; non-RCTs)				
Carter	1. Wound depth (%) 2. Wound area (%) 3. Mean time to reach 50% depth reduction (KM) 4. Mean time to reach 50% area reduction (KM)	58.3 (25-89)	52.5 days	1. E: 65.9; C: 100[5] 2. E: 61.8; C: 100[5] 3. E: 22.3; C: 72.9 (days)[5] 4. E: 25.1; C: 66.0 (days)[5]
Margolis	1. Complete wound healing	Platelet releasate (PR): 63.1 (62.7-63.4) No PR: 64.2 (64-64.4)	PR:9.95 months (9.31-10.59), No PR: 9.1 months (8.8-9.5)	1. Propensity score quintiles: Grp 1: RR (healing with PR): 1.14 Grp 2: RR (healing with PR): 1.24 Grp 3: RR (healing with PR): 1.29 Grp 4: RR (healing with PR): 1.43 Grp 5: RR (healing with PR): 1.59 Overall: 1.38
Mazzucco	1. Time to heal (median; KM): Dehisced sternal wounds 2. Time required before surgery (median; KM): necrotic skin ulcers	Sternal Wds: E: 64±8 C: 66±5 Necrotic Wds: E: 61±18 C: 63±16	Not described in article	1. E: 6.0; C: 3.5 (weeks) 2. E: 35.5; C: 15.0 (weeks)

Study	Outcome Criteria	Patient Age: Mean Yrs (SD)	Previous Wound Duration: Mean Wks (SD)	Outcomes
Acute Wounds Primary Closure (RCTs)				
Almdahl	1. Harvest site infection 2. Adverse events	E: 64.8±8.6 C: 66 ±8.8	Treatment during surgery with primary closure	1. E: 9; C: 8 (6 weeks) 2. None in either group
Buchwald	1. Normal wound healing 2. Abnormal wound healing 3. Wound healing impairment 4. Large-area hematomas 5. Postop pain level (mean)	E:65.3 ± 8.2 C:66.6 ± 9.7	Treatment during surgery with primary closure	1. E: 22/35; C: 16/35 (by day 50)[2] 2. E: 6/35; C: 5/35 (by day 50)[2] 3. E: 6/35; 11/35 (by day 50)[2] 4. E: 10/35; C: 21/35 (by day 50)[2] 5. E: 0.083; C: 0.11 (by day 50)[4]
Englert	1. Postop chest pain (mean) 2. Postop leg pain (mean)	E: 61.87 (9.47) C: 68.47 (14.59)	Treatment during surgery with primary closure	1. E: 1.47; C: 4.47 (day 1)[2] E: 1.40; C: 4.53 (day 3)[2] E: 0.53; C: 2.27 (day 30)[2] 2. E: 1.33; C: 3.06 (day 1)[2] E: 1.46; C: 2.80 (day 3)[2] E: 0.53; C: 2.33 (day 30)[2]
Peerbooms	1. Wound closure 2. Pain at rest (median) 3. Pain, walking (median)	E: 77 (4.4) C: 78 (5.1)	Treatment during surgery with primary closure	1. E: 4/50; C: 16/52 (2 weeks)[2] E: 4/36; C: 16/46 (2 weeks)[3] 2. E: 2; C: 2 (6 weeks)[2] 3. E: 2; C: 2 (6 weeks)[2]

Study	Outcome Criteria	Patient Age: Mean Yrs (SD)	Previous Wound Duration: Mean Wks (SD)	Outcomes
Vang	1. Sternum pain (yes) 2. Leg pain (yes)	E: 63±11 C: 64± 9	Treatment during surgery with primary closure	1. E: 7/15; C: 7/15 (day 2)[3] E: 2/14; C: 4/15 (3 weeks)[3] 2. E: 0/15: C: 0/15 (day 2)[3] E: 3/14; C: 5/15 (3 weeks)[3]
Yoo	1. Cumulative drainage (mean) 2. Pain (mean) 3. Cumulative codeine days 4. Length of stay 5. Discharge postoperative day	E: 47.7±11 C:49.7 ±13.3	Treatment during surgery with primary closure	1. E: 44.9; C: 63.5 (mL; 24 hours)[2] 2. E: 1.2; C: 0.6 (24 hours)[3] 3. E: 110; C:193 4. E: 1.33; C: 1.12 5. E: 24/26 (92%); C: 16/26 (62%)

Study	Outcome Criteria	Patient Age: Mean Yrs (SD)	Previous Wound Duration: Mean Wks (SD)	Outcomes
Acute Wounds Primary Closure (Comparative Designs; non-RCTs)				
Everts	1. Wound exudate leakage 2. Wound healing disturbances 3. Superficial infections	E:69.4 ±9.1 C:67.4 ±9.9	Treatment during surgery with primary closure	1. E: 2/85; C: 12/80 2. E: 0/85; C: 9/80 3. E: 0/85; C: 4/80
Gardner	1. Mean intravenous narcotic use 2. Postoperative blood loss 3. Range of motion	E: 73.3 C: 72.9	Treatment during surgery with primary closure	1. E: 17.0; C: 36.3 (mg/day) 2. E: 2.7; C: 3.2 (g/dl) 3. E: 78.2º; C: 71.9º
Khalafi	1. Chest infection 2. Chest drainage (notable) 3. Leg infection 4. Leg drainage (notable)	E: 62.37 C: 61.40	Treatment during surgery with primary closure	1. 0.074 (OR for E compared to C) 2. 0.042 (OR for E compared to C) 3. 0/560; 3/456 4. 0.120 (OR for E compared to C)
Saratzis	1. Postop complications (patient basis) 2. Postop complications (wound basis)	71.4 ± 7.26	Treatment during surgery with primary closure	1. E: 2/50; 9/50 (< 30 days) 2. E: 3/50; 12/50 (< 30 days)

Study	Outcome Criteria	Patient Age: Mean Yrs (SD)	Previous Wound Duration: Mean Wks (SD)	Outcomes
Trowbridge	1. Superficial infection 2. Deep sternal infection	E: 64 (14) C: 64 (3) HC: 65 (13)	Treatment during surgery with primary closure also compared with historical controls (HC)	1. E: 1/382; C: 17/948: HC: 14/929 2. E: 0/382; C: 14/948: 3. HC: 16/929

Study	Outcome Criteria	Patient Age: Mean Yrs (SD)	Previous Wound Duration: Mean Wks (SD)	Outcomes
Acute Wounds Secondary Closure (RCTs)				
Kazakos	1. Mean time required to heal to then undergo reconstructive surgery 2. Surface area reduction (%) 3. Pain (VAS scale)	E: Males=36 (20-56), Females= 32 (23-47) C: Males=38 (19-52) Females= 31 (22-49)	Treatment during surgery with secondary closure	1. E: 21.3; C: 40.6 (days) 2. E: 12.2; C: 8.3 (1 week) E: 24.2; C: 16.0 (2 weeks) E: 36.4; C: 23.1 (3 weeks) 3. E: 57.6; C: 79.8 (3 weeks)
Spyridakis	1. Wound volume reduction 2. Time to complete wound healing 3. SF-36 scores (3 weeks)	16 - 38	Treatment during surgery with secondary closure	1. E: 11.6; C: 6.7 (mL; days 5-10) 2. E: 24; C: 30 (days) 3. E: 75; C: 62

Study	Outcome Criteria	Patient Age: Mean Yrs (SD)	Previous Wound Duration: Mean Wks (SD)	Outcomes
Acute Wounds Secondary Closure (Comparative Designs; non-RCTs)				
Hom	1. Percentage of closure 2. Full closure 3. Wound healing velocity	21-58	Treatment after punch biopsies with secondary closure	E: 81.1; C: 57.2 (day 17) E: 10/16; C: 5/16 (day 21) Summation of healing velocity at all time points between groups (data not reported)

[1]Values calculated using the Z test (fixed effects) when significant unless otherwise indicated (i.e., author values); [2]intention-to-treat (ITT) analysis; [3]per protocol (PP) analysis; [4]author values; [5]C represents wounds during a run-in period and E represents same wounds during treatment period; [6]multivariate repeated measures general linear model in which 95% CI is for mean difference; [7]repeated measures ANOVA: multivariate for chest pain (Wilk's $\lambda = 0.43$) and univariate for leg pain.[8]repeated measures ANOVA, 42 days.

Wound size reductions are reported as mean reductions unless otherwise stated; CIs are calculated for risk difference in dichotomous outcomes and for weighted mean difference in continuous outcomes unless otherwise stated. AE = adverse event; C = control group (comparison); CI = confidence intervals; E = experimental group (PRP); KM = Kaplan-Meier; NNT = number needed to treat (based on complete wound healing); ns = not significant; OR: odds ratio; PRP (PR) = platelet rich plasma; RR = relative risk.

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