**Assessing the Impact of Pioglitazone on Squamous Cell Skin Cancer Incidence in High Risk Patients**

**Principal Investigator – Alice P Pentland, MD**
**Department of Dermatology Box 697**
**University of Rochester Medical Center**
**Rochester NY 14642**

## 1. PURPOSE OF THE STUDY AND BACKGROUND

### 1.1. Purpose of the study

This study is intended to determine if treatment with the oral agent pioglitazone (also called Actos, a drug approved to treat diabetes) can decrease the number of new squamous cell cancers in people who have a high risk of developing skin cancer. This patient population includes, but is not limited to those with immunosuppression secondary to organ transplantation.

Specifically, the purposes of this pilot study are to collect preliminary data in order to plan for a larger study, the objective of which will be to assess whether pioglitazone will:

- Decrease the number of new skin cancers patients develop while taking the drug compared to their stable baseline
- Determine if skin cancer formation reoccurs when stopping drug
- Determine if the study drug affects the phenotype of the skin cancers occurring while on treatment (i.e., well differentiated vs. poorly differentiated tumors)
- Change the expression of markers influenced by PPARγ or AKR1C3 (apoptosis, proliferation) in tumors removed from treated patients.

### 1.2. Background

Squamous cell carcinoma (SCC) is the second most common form of skin cancer, with a steadily increasing annual incidence of over 250,000 cases in the United States(Miller *et al.*, 2010; Schmults *et al.*, 2013). The mortality rate of these cancers remains low (~1 %), however in certain populations such as organ transplant recipients, rates of metastasis and mortality are considerably higher(Mullen *et al.*, 2006). When patients develop metastatic disease, up to 50 % will have a recurrence with a mortality rate of up to 85 %(Goh *et al.*, 2010). Beyond mortality and high morbidity, treating non-melanoma skin cancers is costly, accounting for 4.5 % of all Medicare cancer costs, a figure that increased 41 % between 1992 and 1995(Rogers *et al.*, 2010). Clearly, therapies that can prevent or stop the progression of SCC are sorely needed. Recent work demonstrated that selective inhibition of prostaglandin E synthesis using Celebrex resulted in a 40 – 50% decrease in squamous cell cancers over a 6 month period(Elmets *et al.*, 2010). However, the utility of Celebrex and other NSAIDs is decreased because of the significantly increased risk of heart attacks(Schmults *et al.*, 2013).

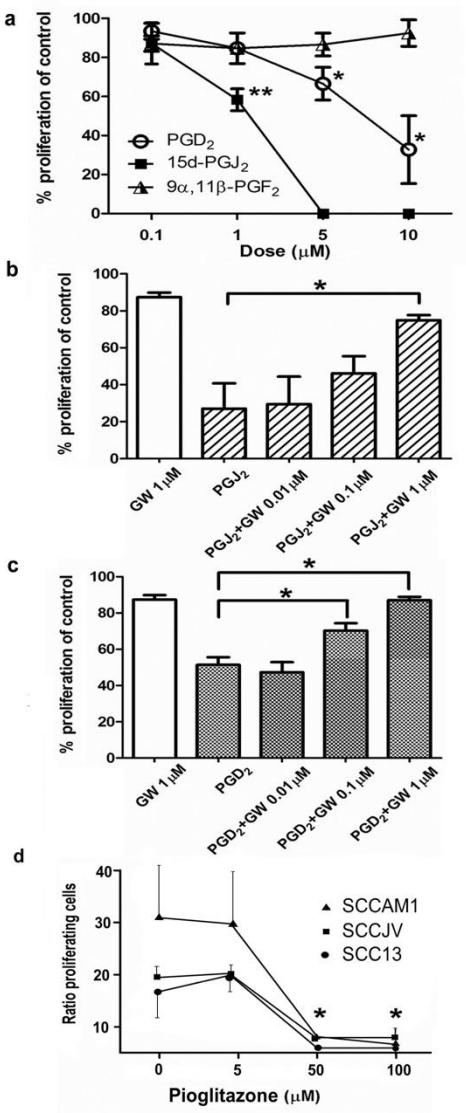



**Figure 1, above. $PGD_2$ and 15d-$PGJ_2$ attenuated SCC cell proliferation in a PPARγ-dependent manner.** (**a**) SCC cells were treated with the indicated concentration of $PGD_2$, 15d-$PGJ_2$, 9α,11β-$PGF_2$ or DMSO for 24 hours followed by cell proliferation assessment by Click-iT assay. Data presented is the average of 3 experiments (±SEM), *P<0.005, **P=0.012. To study the involvement of PPARγ activation in the anti-proliferative effects mediated by 15d-$PGJ_2$ and by its precursor $PGD_2$, SCC cells were treated with 1.5μM 15d-$PGJ_2$ (**b**) or 7μM $PGD_2$ (**c**) in the presence of the indicated concentration of the PPARγ specific inhibitor GW9662 (GW). Cell proliferation was assessed by Click-iT assay. n=3 *P<0.05. (**d**) The effects of Pioglitazone on SCC proliferation. Each SCC cell line was treated for 24h as indicated and cell proliferation was assessed by Click-iT assay.(n=3)

Aldo-Keto Reductase 1C3 (AKR1C3) is another prostaglandin-synthesizing enzyme that could be a target for SCC prevention(Matsuura *et al.*, 1998). AKR1C3 belongs to a large superfamily of NAD(P)(H)-dependent AKR enzymes which metabolize various substrates including prostaglandins and sex hormones (Jez and Penning, 2001). In humans, the AKR1C subfamily consists of 4 enzymes termed AKR1C1, AKR1C2, AKR1C3 and AKR1C4 which despite their extensive structural overlap, exhibit different substrate binding preferences (Penning *et al.*, 2000). Among these enzymes, AKR1C3 expression has been associated with cancer risk.

AKR1C3 is overexpressed in various cancers originating from non-steroidogenic tissues such as kidney (Azzarello *et al.*, 2009), central nervous system (Park *et al.*, 2010), pancreas, gastrointestinal tract and lung (Chang *et al.*, 2013; Miller *et al.*, 2012). AKR1C3 can activate carcinogens (Lan *et al.*, 2004), modulate oxidative stress (Birtwistle *et al.*, 2009), and its polymorphisms are associated with risk for childhood leukemia (Liu *et al.*, 2008) and bladder cancer (Figueroa *et al.*, 2008). Collectively, these observations suggest a tumor promoting function for AKR1C3 (Azzarello *et al.*, 2009; Miller *et al.*, 2012). Unlike other AKR1C members, AKR1C3 can also synthesize $PGF_{2\alpha}$ from $PGH_2$, an arachidonic acid derivative synthesized by cyclooxygenase (Komoto *et al.*, 2006). Its role in prostaglandin metabolism also includes the conversion of $PGD_2$, a pro-inflammatory lipid mediator, to 9α,11β-$PGF_2$ (Byrns *et al.*, 2010; Komoto *et al.*, 2006; Matsuura *et al.*, 1998). Thus, via its involvement in PG metabolism and its downstream effects on PPARγ, AKR1C3 may regulate biological and pathological responses in hormone-independent tissues.

Our recent work investigated AKR1C3 expression in human non-melanoma skin cancers, revealing overexpression in 7 human squamous cell carcinomas (SCC) examined. Effects of AKR1C3 expression were then evaluated using 3 SCC cell lines (Fig. 1). AKR1C3 was detected in all SCC cell lines and its expression was upregulated in response to its substrate, $PGD_2$. Treatment of the SCCs with $PGD_2$ and its dehydration metabolite, 15d-$PGJ_2$,decreased SCC proliferation, indicating that the PPARγ receptor was the key effector of this profound inhibitory response. **SCC treatment with the PPARγ**

**agonist pioglitazone was then tested, showing a profound capacity to inhibit SCC proliferation, using concentrations achieved in patients on drug treatment (50 – 100 μM)**(Christensen *et al.*, 2005). This work suggests that AKR1C3 over-expression within the tumor microenvironment may protect SCC cells from the anti-proliferative effects of $PGD_2$ and that PPARγ agonists such as pioglitazone may be a useful treatment adjunct for those at high risk of SCC. There are no currently available approved selective inhibitors of AKR1C3. However, Pioglitazone is a currently approved oral treatment for diabetes that can be safely administered to non-diabetics(Bell *et al.*, 2012). We propose to learn in this clinical trial if its use can be helpful in patients who develop multiple SCCs each year. The protocol outlined here is intended to examine this possibility as well as address the mechanisms by which it exerts its effects.

## 2. STUDY DESIGN

### 2.1. Overview

This is a single center open label feasibility pilot project based at the University of Rochester. Patients followed in the University of Rochester Dermatology Clinic who have had between 2 to 6 squamous cell cancers treated in the past year, without contraindication for the use of Pioglitazone, and are on a stable drug treatment regimen will be offered participation. The study has a cross-over design, so patients will be enrolled and randomized in one arm of two treatment protocols: 1) six months of usual care while documenting and characterizing any new tumors that occur followed by 6 months of Pioglitazone treatment plus usual care for the next 6 months or 2) six months of Pioglitazone treatment followed by 6 months off treatment receiving usual care (see protocol diagram, Section 5, below). The second group will offer the opportunity to assess whether there is any persistent beneficial effect after pioglitazone treatment ends, while tumors from the first group that occur during the initial 6 months of usual care will be characterized by study parameters for comparison to tumors arising while on treatment. At the end of the one year treatment period, the number of new tumors that patients develop while taking Pioglitazone will be counted and compared with the number that patients developed during the 6 month period they were not receiving treatment as well as the numbers that occurred during the 6 months after treatment (washout effect). This information will be used as the basis for a larger multicenter study.

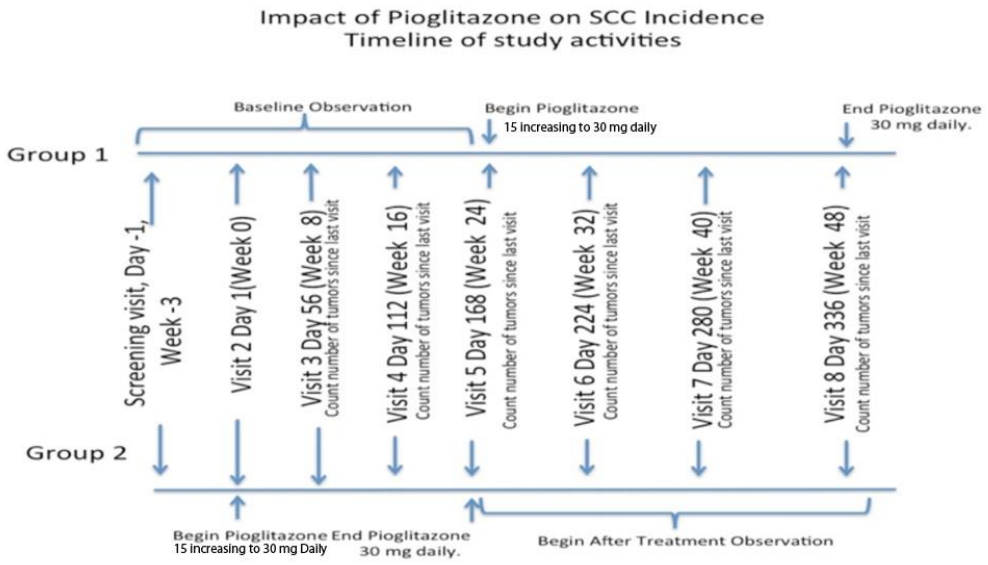



Fig 2. Schematic for the Proposed Study (study visit day are +/- 7 or 14 days)

### 2.2. **Rationale for Study Design**

An open label study with a crossover design was selected because this is a feasibility pilot project funded by the Department of Dermatology, and we require a study design that can yield useful statistical information for sample size for a subsequent multi-center trial with the number of subjects we are likely able to recruit here at Rochester. We calculate that 40 patients will be needed to provide the sample size required to determine a statistically significant drug effect for a larger study. We also expect that it may not be possible to identify and enroll more high risk patients at Rochester in a reasonable period of time given the incidence of multiple SCC and the eligibility criteria. It is possible this design may not give a long enough period of time on drug to substantially decrease the onset of new tumors, however we believe it will be sufficient since 2 other drugs that affect the frequency of SCC in susceptible patients do produce substantial effects in this time frame (Celebrex, Soriatane)(Elmets *et al.*, 2010; Kadakia *et al.*, 2012). A one year long study will permit completion of a feasibility pilot in a reasonable period to allow a larger, definitive study to be done. We hypothesize that Pioglitazone will have the capacity to reduce incidence of SCC by >50%, as >90% decrease in growth is seen in tissue culture of human SCC using levels of pioglitazone available in human serum (see preliminary data, above). This is also the effect size seen with Celebrex treatment, a drug that can also selectively modulate prostaglandin synthesis.

We have chosen tumor incidence as the primary outcome measure. Because patients with high tumor risk develop new tumors at a variable pace, some subjects may not have any new tumors during the period of the study. However, in aggregate we feel that a tumor endpoint is the best choice. This decision was made because of our recent experience studying the effects of Celebrex on skin cancer incidence. That study assessed pre-skin cancers (actinic keratoses) as the primary endpoint(Elmets *et al.*, 2010). However, when data was analyzed, it was found there was no significant change in precursor lesions, only in actual tumor numbers. Thus, we have elected to focus on a high risk population that will have high numbers of new tumors. These patients are also the target group that could derive the most benefit from treatment.

This study will also assess secondary endpoints. First, we will examine whether the ratio of well-differentiated to poorly differentiated SCC is influenced while subjects are on treatment. This endpoint is included because it is unknown whether there may be more signaling mediated by PPARγ in one tumor morphology vs. another. Second, we will determine whether patients on treatment might have different numbers of "borderline" lesions biopsied; precancerous lesions are often suspicious enough to biopsy. It may be that there will be fewer lesions in this category, as well as fewer squamous cell cancers. Third, we will test tumors that are excised after they are processed for routine diagnostic pathology to see if markers of proliferation or apoptosis that are influenced by PPARγ activity or the presence of AKR1C3 are altered. These endpoints would support the idea that any changes produced in tumor incidence while patients are on study drug are related to the mechanistic effect on PPARγ that is proposed.

### 2.3. **Rationale for Dosage**

Subjects will be treated with Pioglitazone 30 mg po daily when on treatment, following an initial 2 week period to assess tolerability, particularly in patients with stage 1 or stage 2 heart failure. Pioglitazone was selected because of our recent investigations showing its profound efficacy in decreasing proliferation in human SCC in vitro (see BACKGROUND). The oral dose selected results in serum levels of Pioglitazone that correspond to the in vitro dose tested in our studies(Christensen *et al.*, 2005). In addition to the drug itself acting as an agonist for PPARγ, there are 2 persistent metabolites that have similar agonist effects, increasing the possibility of tumor growth inhibition. Thus, this dosing regimen is likely to give optimal suppression of tumor growth. This is a standard dose (30 mg) taken by patients who are taking Pioglitazone for management of their diabetes. When given to non-diabetic patients with non-alcoholic liver disease or those with hepatitis C to prevent cirrhosis(Bell *et al.*, 2012), there were no untoward effects on glucose metabolism.

## 3. CHARACTERISTICS OF THE RESEARCH POPULATION

### 3.1. **Subject Characteristics**

a) **Number of Subjects:**

Forty subjects will be recruited to participate in this protocol. It is expected that we will have 1 screen failure for every 4 subjects consented (mostly due to onset of other complex disease unrelated to their skin). There are no other participating sites.

**b) Gender and Age of Subjects:**

Adult subjects of both genders will be recruited. However, high frequency of SCC is more common in men, and the balance of patients recruited to the study will reflect this. SCC is a disease of older individuals, usually 50 and up, unless they are transplant recipients or have a genodermatosis that predisposes them to multiple tumors. This disease is virtually unknown in pediatric patients, so patients < 18years will be excluded.

c) **Racial and Ethnic Origin:**

Squamous cell carcinoma in skin is induced by chronic exposure to ultraviolet light. Individuals who have dark skin pigmentation are highly resistant to this disease, so the study population will be largely Caucasian. Most Hispanic individuals are also too darkly pigmented to suffer from this condition because of the capacity of their skin to tan.

### 3.2. Inclusion and Exclusion Criteria

a) **Inclusion Criteria:**

- >18 years of age, male or female, state of health stable
- Able understand protocol and give consent
- Has had treatment of 2 – 6 squamous cell carcinomas of the skin during the year prior to enrollment, & pathology is available for verification
- Stable treatment regimen for their skin cancer problems in place for 1 year, with expectation to keep medications the same during study
- Able to keep study appointments & comply with protocol

b) **Exclusion Criteria:**

- Unwillingness or unable to complete informed consent process
- < 18 years of age
- Allergy to Pioglitazone
- Taking Rifampin, Trimethaprim, Celebrex or Gemfibrozil
- Pregnant or breastfeeding (Pregnancy Category C)
- History of heart failure NYHA Class III or Class IV
- Subjects with type 1 or type 2 diabetes
- Problems with pedal edema
- Liver disease (ETOH, viral hepatitis, drug-induced hepatitis) or elevated ALT, AST or total bilirubin
- Osteoporosis with high risk of fracture
- History of bladder cancer
- Recent change in chronic oral medications. Participants enrolled while on a systemic medication for their skin cancer must remain on treatment.

### 3.3. Vulnerable Subjects

We will not be recruiting vulnerable subjects.

### 3.4. Discussion of Subject Population

Patients with diseases that are likely to be made worse by Pioglitazone are excluded (heart failure, liver disease, osteoporosis or bladder cancer). Patients must have had a sufficient number of prior tumors that their increased risk of developing tumors in the future is clear.

## 4. SUBJECT IDENTIFICATION, RECRUITMENT AND CONSENT

### 4.1. Method Of Subject Identification And Recruitment

Patients receiving care at the University of Rochester Department of Dermatology for their squamous cell cancer who meet study inclusion criteria will be asked by Dermatologic surgeon Dr. Sherrif Ibrahim about their interest in study participation. Subjects will be given the consent

form to examine and the contact number for the study coordinator, JoAnne VanBuskirk, who is approved to enroll subjects. Dr. Ibrahim will have no involvement in the consenting process for individuals he sees as a patient. It will be made clear to the potential subjects that their participation is voluntary and their care in the Department of Dermatology will not be affected by their decision to participate in this study.

To further enhance enrollment, a "Dear Colleague" Letter may be sent to Dermatologists and Mohs surgeons in the Rochester area who work at sites other than Red Creek, or refer patients to the University of Rochester Department of Dermatology in order to recruit subjects. For those faculty employed full-time at the University of Rochester, who see potential study subjects in our Dermatology practice, a prescreening process will also be conducted by study coordinator JoAnne VanBuskirk. She will review the EPIC records of potential subjects coming for appointments to see if they meet eligibility criteria. If a patient coming to a visit meets study criteria, a bright pink reminder sheet titled "Inclusion-Exclusion Criteria Pioglitazone Pink Sheet" outlining the key aspects of the study and its inclusion and exclusion criteria will be placed with the intake form. This visual reminder to employed faculty that the patient they are about to see may be eligible to participate is intended to facilitate their asking the patient about their interest in the study. Faculty will then be able to let the patient know about the study and ask if the patient has an interest in being contacted by the study coordinator. If the potential subject shows interest, the study coordinator will contact them by phone to further explain the study. Interested subjects will be mailed the consent form and a date arranged for them to come to the office to formally review the consent document and give their consent. Again, it will be made clear to the potential subjects that their participation is voluntary and their care in the Department of Dermatology will not be affected by their decision to participate in this study.

### 4.2. Process of Consent

Dr. Pentland or the coordinator will meet with interested subjects in a private room. The consent form will be read in its entirety by the subject. Time allotted to consenting will be dependent on the subjects' needs, questions, and comprehension of the information in the consent form. Each subject will be encouraged to read the consent form on his/her own and ask any specific questions.

At the time of consent, subjects will be offered the option to provide permission to be contacted in the future regarding this and other dermatologic studies. Those who provide permission for future contact will be added to a list of high risk squamous cell skin cancer patients that are interested in participating in clinical research.

### Subject Capacity

We will not be recruiting subjects incapable of giving informed consent. The subject's capacity to comprehend the information in the consent form will be assessed by asking questions about the content and encouraging the subject to describe the study procedures.

### Documentation of Consent

The participant's consent will be documented by signing the consent form by both the study subject and the person obtaining consent.

## 5. METHODS AND STUDY PROCEDURES

A schedule of study visits is provided in Appendix 1. A description of the study procedures are also listed in appendix 1.

Outlined below are the assessments that will be performed to evaluate efficacy and those to evaluate safety. Tests and interventions which are considered experimental and performed exclusively for research purposes have been identified and differentiated from that which would occur regardless of the research (i.e., standard of care).

## 5.1. **Efficacy Assessments**

Administration of Pioglitazone to prevent/decrease the aggressive phenotype of skin cancer is experimental. NB- obtaining biopsy of lesions suspected of skin cancer is NOT experimental; it is required for usual patient care in these high risk patients. In order to know whether Pioglitazone is efficacious for preventing SCC in high risk individuals, we will count the number of skin tumors documented by biopsy/treatment that occur in our subjects before, during, or after Pioglitazone treatment. This assessment will take into account compliance, as described below. The tumor counts will be best interpreted in aggregate for the whole sample group, since there is variability in the frequency with which new tumors appear based on the predisposing condition. Since Pioglitazone has been on the market for some time without observation of a negative impact on skin or wound healing, the study focus will be determining if numbers of tumors decrease and whether their phenotype changes. The physician reading the pathology of the skin biopsies will be responsible for identifying the number of tumors that occur –i.e., some lesions thought to be tumors and therefore biopsied in clinical practice turn out to be hypertrophic actinic keratosis (not tumors). These would not be counted as skin cancers, since they are precancerous lesions.

## 5.2. **Safety Assessments**

The main safety issue associated with this trial is whether an adverse effect will occur in patients due to the administration of pioglitazone. Clinical endpoints to monitor for safety are: weight, pedal edema, dyspnea, heart failure, headache, myalgia, fracture, liver injury and vision changes related to macular edema, blood in the urine or other changes that are out of the ordinary. The study related blood monitoring is to ensure there are no preexisting conditions that make participants susceptible to drug-related side effects. For instance, individuals with preexisting liver disease may have hepatotoxicity when taking pioglitazone, hence they must be excluded from participation. However, since participants could develop new diseases while participating, blood will also be taken from subjects taking pioglitazone to screen for such concerns. At a study visit approximately 60 days after drug initiation, blood work will be repeated to detect changes suggesting drug related side effects. Monitoring of blood for glucose levels, altered liver function, or decreased Hemoglobin could indicate the patient should stop taking the study drug. Tests to be followed will be CBC and CMP at baseline, approximately 60 days after starting drug and at the end of treatment. Blood test results will be interpreted in the context of the clinical exam. The patient population being studied has co-morbidities that require them to regularly give blood to monitor their health as part of their routine care, so study related blood draws may not be necessary if these tests are obtained by their providers at appropriate intervals; it will not be necessary to repeat them. The risk of bladder cancer will be monitored by urinalysis. Taking Pioglitazone for > 1 year increases the risk of bladder cancer from 7 in 10,000 to 10 in 10,000. Female patients of child bearing age will have urine pregnancy testing at baseline and at subsequent study visits. **Stop Criteria:** Subjects will be

dropped from the protocol if clinical or laboratory assessment reveals grade II abnormalities as defined by Common terminology Criteria for adverse events V.3. Subjects will also be dropped from the protocol if they are unable to tolerate 30 mg Pioglitazone treatment when the dosage is increased to 30 mg daily after the initial 2 week treatment with 15 mg daily.

### 5.3. **Assessment of Subject Compliance**

The study coordinator will assess the subject's compliance with the protocol by counting the pills remaining in the prescription obtained for the study. The date the prescription was filled relative to the study visit date will also be noted.

### 5.4. **Data & Specimen Banking for Future Research Use**

This will not be done.

### 5.5. **Genetic/Genomic Research Activities**

No genetic/genomic research activities are planned.

### 5.6. **Costs to the Subject**

Subjects will be prescribed Pioglitazone, a drug that costs $12 per month. The cost of drug will be paid by the Department of Dermatology. Costs of blood tests specifically related to the study to monitor safety of drug administration are also covered by the department. The costs related to subject's cancer treatment (standard care) will be charged to insurance as is routine. There is only Department of Dermatology clinical grant support for this project for coverage of medication costs.

### 5.7. **Payment for Participation**

There is no payment for participation.

### 5.8. **Return of Individual Research Results**

Any side effects suspected to be related to the study drug will be reported to the subject at the time of their detection. Subjects who have significant side effects attributable to study drug will be dropped from the study, and the side effects reported to their physician. Since the study is not blinded, subjects will be directly aware of whether there is a detectable difference in the incidence of their skin cancers.

## 6. CONCOMITANT AND DISALLOWED MEDICATIONS

Subjects will be asked to continue their usual medications. Potential subjects with type 1 or type 2 diabetes will not be enrolled.

## 7. SUBJECT WITHDRAWALS

Subjects will be advised in the written informed consent provided that they have the right to withdraw from the study at any time without prejudice, as well as being notified of this by the study coordinator. Subjects may be withdrawn by the investigator without their consent for non-compliance, intercurrent illness, or pregnancy (Pioglitazone is pregnancy category C) for suspected drug related side effects. Stop criteria to be used are clinical or laboratory assessments revealing grade II abnormalities as defined by Common terminology Criteria for adverse event V.3. No additional study activities will be completed by the subject prior to withdrawal from the

study. Subjects withdrawn from the study will be replaced.

## 8. STUDY DRUG/DEVICE/BIOLOGIC ADMINISTRATION/ASSIGNMENT

Note: The package insert is included in appendix 2.

### 8.1. Study Drug/Device/Biologic

Pioglitazone is a currently FDA approved drug. A formal request to conduct the study with the product to obtain an IND is in process. Patients will be provided drug once they consent to participate and are found to be eligible.

### 8.2. Dosage of Study Drug/Biologic

Subjects will be treated with 15 mg Pioglitazone for the first 2 weeks. If tolerated, the dose will then be increased to 30 mg Pioglitazone daily while on protocol for drug treatment. Subjects unable to tolerate 30 mg Pioglitazone will be dropped from the study.

### 8.3. Subject Enrollment/Randomization

Subjects will be randomized to one of two study groups: initial observation or initial drug treatment followed by washout.

### 8.4. Accountability of Investigational Supplies

The study drug is ordered through the investigational drug service. The study drug will be dispensed to the Study Coordinator n transported to the Red Creek clinic site of Dr. Ibrahim. It will be stored in a locked cabinet at room temperature until the supply is given to the patient for use. Once patients give consent, the study drug will be dispensed when patients enter the active treatment arm of the protocol. On return appointment, patients will bring their study drug bottle with them. A pill count will be done at each study visit, and the date of drug dispensing will be noted. At the visit prior to completion of the active treatment arm, the exact number of pills required for the planned period until the next study visit will be dispensed to the patient. This will facilitate having patients stop taking drug at the same time as their last “on treatment” study visit .

### 8.5. Subject Withdrawal of Study Drug

If study subjects stop drug treatment (other than as indicated in the protocol) they will be dropped from the study. Compliance will be considered taking the drug appropriately >80% of the time.

### 8.6. Emergency Drug Disclosure

Not applicable

## 9. SAFETY AND REPORTABLE EVENTS

### 9.1. Adverse Event Definition

An adverse event is any symptom, sign, illness, or experience which develops or worsens during the course of the study, whether or not the event is considered related to study drug.

### 9.2. Serious Adverse Event

A serious adverse event is defined as any adverse medical experience that results in any of the following outcomes:

- death;
- is life-threatening;
- requires inpatient hospitalization or prolongation of existing hospitalization;
- results in persistent or significant disability/incapacity;
- is a congenital anomaly/birth defect; or
- requires medical or surgical intervention to prevent permanent impairment or damage.

9.3. **CBC, CMP will be monitored at baseline and at the study visit occurring 60 days after Drug treatment begins. Urinalysis will be checked for Blood at each study visit.**

At each subject visit the study staff will assess adverse events by recording all voluntary complaints of the subject and by assessment of clinical and laboratory features. At each study visit, the subject will be questioned directly regarding the occurrence of any adverse experience since his/her last visit.

All adverse events, whether observed by the Investigator, elicited from or volunteered by the subject, will be documented. Each adverse event will include a brief description of the experience, the date of onset, the date of resolution, the duration and type of experience, the severity, the relationship to taking Pioglitazone, contributing factors, and any action taken with respect to Pioglitazone.

Recording of adverse events will occur during the time that the patient is on study drug and for the next study visit after finishing drug. Follow up of adverse experiences will be until the event is resolved or stabilized or for 6 months.

### 9.4. Responsibilities for Reporting Serious Adverse Events

The Investigator will record all serious adverse experiences that occur during the study period in the appropriate source documents and/or AE log as applicable. The study period for reporting serious adverse events will be from the time of signing consent to final study visit.

As sponsor of the relevant IND, the PI is responsible for compliance with the FDCA (21 USC §§ 301 e. seq.) as well as the implementing regulations [Tittle 21 of the CRF]. These responsibilities include reporting any unexpected fatal or life-threatening suspected adverse reactions to the FDA/CDER no later than 7 calendar days after initial receipt of the information.

Additionally, the PI will comply with all federal regulations and RSRB policies regarding the reporting of adverse events.

## 10. RISK/BENEFIT ASSESSMENT

### 10.1. Potential Risks

Exacerbation of heart failure symptoms, fluid retention, pedal edema and weight gain are the primary risks of concern in this study. Similarly, fluid retention may cause anemia by diluting the blood. Liver problems have been reported in some patients receiving pioglitazone in the presence of active liver disease. Recent evidence links prolonged pioglitazone treatment (>1 year) in diabetics with increased risk of bladder cancer (from 7/10,000 to 10/10,000). Our subjects will be

treated for a maximum of 6 months. Additionally, by excluding subjects with a history of bladder malignancy we are following the FDA advice to not begin this medication in subjects with a past or present history of bladder cancer. Similarly, our subjects will not be on pioglitazone long enough to increase their risk of bone fractures. While subjects with diabetes have experienced macular edema and low blood sugar, these potential adverse effects are markedly less common in nondiabetics. Women with polycystic ovarian syndrome may have their ovulation cycles normalize.
As with other drugs, some patients may develop an allergic reaction to pioglitazone. Minor risks associated with pioglitazone treatment included headache, sinus infection, muscle pain, sore throat, tooth disorders and respiratory tract infections.
For full details, please see the attached package insert.

### 10.2. **Protection Against Risks**

Patients will be advised of possible drug side effects by study personnel and encouraged to contact the study coordinator if they suspect side effects are occurring. Risks will be minimized by 1) good case selection 2) education and 3) stopping drug treatment when significant side effects are identified. Appropriate screening of subjects for subclinical side effects of Pioglitazone will also minimize risk. Since the drug's side effects are not abrupt in their onset, the followup included in the study design should be effective. Should side effects be noticed, patients will be treated by study personnel and counseled, or referred to their provider.

### 10.3. **Potential Benefits to Subjects**

Subjects may benefit from reduced numbers of tumors if drug treatment works as expected.

### 10.4. **Alternatives to Participation**

No research alternatives are available to the subject, and they can pursue usual care.

## 11. CONFIDENTIALIAY OF DATA AND INFORMATION STORAGE

Confidentiality of protected health information and samples is ensured through the following measures:

- Only authorized persons will be granted access;
- Only authorized persons may enter and view study data;
- Passwords and system IDs will not be shared;
- Physical security of the workstations/files will be maintained;
- Adequate back-up plan is in effect;
- Staff trained on data entry system and importance of security procedures;
- Workstations with databases will not be left unattended while in use, and will be left locked and password protected.
- Only study team members will have access to subject identifiers. Samples and data will be labeled with unique, random codes.
- Codes will not be generated from existing identifiers, such as initials or enrollment date.
- A link will be maintained indefinitely between subject identifiers and the codes.
- The link will be stored on a password-protected computer, accessible only to study team members.

## 12. RESEARCH INFORMATION IN MEDICAL RECORDS

Blood test results will be in the patients' medical record for medical reasons unrelated to Pioglitazone treatment, as will results of biopsies as needed for normal patient care. No other study information will be in the medical record.

## 13. DATA ANALYSIS AND MONITORING

### 13.1. **Sample Size Determination**

This pilot study will be designed as a 2-by-2 crossover trial. We refer to Section 2.2 for a justification of the study design. Patients will be enrolled and randomized in one arm of two treatment protocols. Each protocol will include two treatments administered during two consecutive time periods:

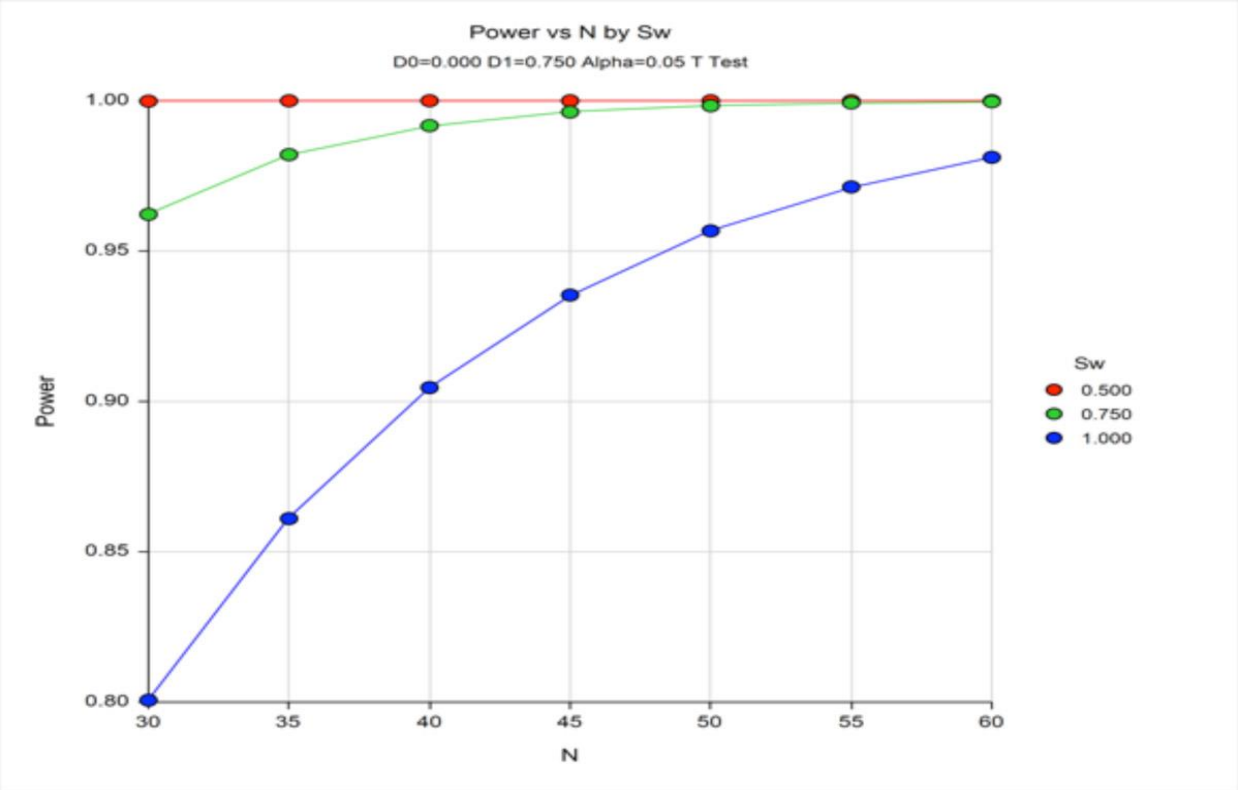
- *Treatment arm # 1:* Patients enrolled in the first arm will first receive six months of usual care (*Period 1*); next, they will be treated with Pioglitazone combined with usual care for the next 6 months (*Period 2*). Patients will take study drug until they come to their final "on Treatment" visit, which will occur 168 days +/- 14 days of their "on Treatment" date.

- *Treatment arm # 2:* Patients enrolled in the second arm will first receive six months of Pioglitazone treatment combined with usual care (*Period 1*); next, they will receive usual care alone during the following 6 months (*Period 2*). Patients will take study drug until they come to their final "on Treatment" visit, which will occur 168 days +/- 14 days of their "on Treatment" date.

A flow diagram describing the study design is presented in Section 2.

The primary endpoint will be the total number of skin tumors counted during each treatment period. Based on clinical evidence, we expect the total number of skin tumors to range between 0 and 3 per patient and per treatment period (or 0 to 6 per patient per year) and to average around 1.5. We would consider Pioglitazone to be clinically effective if it reduces tumor counts by 50% (that is, reduce the average number of tumors from 1.5 to 0.75 per patient and per treatment period). Previous work by this investigator has shown that such reductions in tumor numbers can occur when prostaglandin metabolism is affected by drug treatment (Elmets *et al.*, 2010).

A two-sided *t*-test achieves 96% power to detect a reduction in the number of skin tumors due to Pioglitazone when the total sample size of a 2-by-2 crossover design is 40 (20 patients per group). The calculations assumed that the actual mean difference is 0.75 (corresponding to a 50% reduction in the mean number of skin tumors due to treatment), the square root of the within mean square error is 1, and the significance level is 5%. The figure below displays the power of the study as a function of the sample size (N) for three values of the square root of the within mean square error (SW).




13.2. **Planned Statistical Analysis**

The main objective of the statistical analysis will be to estimate the effect of Pioglitazone on the occurrence of skin tumors when used in combination with usual care compared to usual care alone. The primary endpoint will be the total number of skin tumors counted during each treatment period. We will have two observations per patient, one for each treatment period of the 2-by-2 cross-over design (either baseline to 6 months, or 6 months to 12 months post-baseline). We will first compute the empirical mean (and its standard error), standard deviation and median for the number of skin tumors within each treatment arm and each treatment period. We will also construct histograms for tumor counts. We expect tumor counts to range between 0 and 6 per six months, such that parametric mixed analysis of variance (ANOVA) may not be the most appropriate approach to assess treatment effects. As an alternative, we will describe the number of skin tumors using log-linear Poisson regression models with random effects. The developed models will include treatment arm, period, and their interaction as covariates while adjusting for potential intra-subject dependencies (or between subject variation) using random intercepts. We will use this model to investigate the presence of washout effect (secondary aim).

In additional, exploratory analyses, we will assess if the phenotype of skin tumors changes during treatment with Pioglitazone. We will also determine if Pioglitazone changes the expression of markers influenced by PPARγ or AKR1C3 (apoptosis, proliferation) in tumors removed from treated patients. We will have an apoptosis and a proliferation score for every skin tumor that arises, both treated as continuous variables. The analyses will be conducted using mixed ANOVA modeling the apoptosis and the proliferation scores of each tumor as a function of treatment arm,

period, and their interaction. Random intercepts will also be included in the models to account for subject to subject variability. We will develop separate models for the apoptosis and for the proliferation scores. We will investigate modeling assumptions using plots of residuals and conditional residuals. In the event modeling assumptions appear violated, we will consider applying suitable transformations (e.g., logarithmic, square root) to normalize the data. As an alternative, we will consider performing statistical inference using a generalized estimating equations (GEE) approach.
All tests and construction of confidence intervals will be two-sided and conducted at the 5% significance level.

### 13.3. **Data and Safety Monitoring**

Drs. Pentland and Ibrahim will conduct continuous review of study data and patient safety. The PI and Study Coordinator are responsible for continuously monitoring the conduct of the trial and are the primary individuals charged with identification and reporting of all AE and SAE occurrences. The review will include the number of patients, significant Pioglitazone toxicities as described in the protocol, and responses observed. The Investigator will submit summaries of this data at least annually to the Data Safety Monitor (DSM; see below) for review.
Additionally, a copy of the completed RSRB Annual Progress report will be submitted to DSM.

The Data Safety Monitor will be assigned oversight of safety monitoring and study progress. The DSM will review will review accrual and adverse events at least annually and following study completion. The DSM, Dr. Leway Chen, is a board-certified and practicing cardiologist who has experience with clinical trials.

All serious adverse events that the PI classifies as related or possibly related to therapy will be submitted to DSM for determination as to whether further action is required within 48 hours.

## 14. INVESTIGATOR RESPONSIBILITIES

Adequate training and supervision of the study team will be ensured by the Principal Investigator as described below.

The PI will

- Provide copies of the protocol, consent and any other study related materials (including all other information relating to the preclinical and prior clinical experience) to all study personnel with delegated responsibilities for this study.
- Discuss all protocol materials and information with study staff to assure that they are adequately informed regarding Pioglitazone and conduct of the study.
- Assure that all study staff members are qualified by education, experience and training to perform their specific responsibilities.
- The Investigator will ensure appropriate documentation of compliance to the protocol, informed consent process and obtaining verbal or written consent on an ongoing basis, with written progress reporting quarterly.
- The Investigator (and study staff) will identify, report and manage potential conflicts of interest biannually.
- A delegation log to document activities that have been authorized by the Investigator to study staff and a training log to document necessary protocol training activities will be completed will be utilized as part of this process.

15. **REFERENCES**


Azzarello JT, Lin HK, Gherezghiher A, Zakharov V, Yu Z, Kropp BP*, et al.* (2009) Expression of AKR1C3 in renal cell carcinoma, papillary urothelial carcinoma, and Wilms' tumor. *Int J Clin Exp Pathol* 3:147-55.

Bell LN, Wang J, Muralidharan S, Chalasani S, Fullenkamp AM, Wilson LA*, et al.* (2012) Relationship between adipose tissue insulin resistance and liver histology in nonalcoholic steatohepatitis: a pioglitazone versus vitamin E versus placebo for the treatment of nondiabetic patients with nonalcoholic steatohepatitis trial follow-up study. *Hepatology* 56:1311-8.

Birtwistle J, Hayden RE, Khanim FL, Green RM, Pearce C, Davies NJ*, et al.* (2009) The aldo-keto reductase AKR1C3 contributes to 7,12-dimethylbenz(a)anthracene-3,4-dihydrodiol mediated oxidative DNA damage in myeloid cells: implications for leukemogenesis. *Mutat Res* 662:67-74.

Byrns MC, Duan L, Lee SH, Blair IA, Penning TM (2010) Aldo-keto reductase 1C3 expression in MCF-7 cells reveals roles in steroid hormone and prostaglandin metabolism that may explain its over-expression in breast cancer. *J Steroid Biochem Mol Biol* 118:177-87.

Chang TS, Lin HK, Rogers KA, Brame LS, Yeh MM, Yang Q*, et al.* (2013) Expression of aldo-keto reductase family 1 member C3 (AKR1C3) in neuroendocrine tumors & adenocarcinomas of pancreas, gastrointestinal tract, and lung. *Int J Clin Exp Pathol* 6:2419-29.

Christensen ML, Meibohm B, Capparelli EV, Velasquez-Mieyer P, Burghen GA, Tamborlane WV (2005) Single- and multiple-dose pharmacokinetics of pioglitazone in adolescents with type 2 diabetes. *J Clin Pharmacol* 45:1137-44.

Elmets CA, Viner JL, Pentland AP, Cantrell W, Lin HY, Bailey H*, et al.* (2010) Chemoprevention of Nonmelanoma Skin Cancer With Celecoxib: A Randomized, Double-Blind, Placebo-Controlled Trial. *J Natl Cancer Inst* 102:1835-44.

Figueroa JD, Malats N, Garcia-Closas M, Real FX, Silverman D, Kogevinas M*, et al.* (2008) Bladder cancer risk and genetic variation in AKR1C3 and other metabolizing genes. *Carcinogenesis* 29:1955-62.

Goh A, Howle J, Veness Mj (2010) Managing patients with cutaneous squamous cell carcinoma metastatic to the axilla or groin lymph nodes. . *Aust J Exp Biol Med Sci* 51:113-7.

Jez JM, Penning TM (2001) The aldo-keto reductase (AKR) superfamily: an update. *Chem Biol Interact* 130-132:499-525.

Kadakia KC, Barton DL, Loprinzi CL, Sloan JA, Otley CC, Diekmann BB*, et al.* (2012) Randomized controlled trial of acitretin versus placebo in patients at high-risk for basal cell or squamous cell carcinoma of the skin (North Central Cancer Treatment Group Study 969251).

*Cancer* 118:2128-37.

Komoto J, Yamada T, Watanabe K, Woodward DF, Takusagawa F (2006) Prostaglandin F2alpha formation from prostaglandin H2 by prostaglandin F synthase (PGFS): crystal structure of PGFS containing bimatoprost. *Biochemistry* 45:1987-96.

Lan Q, Mumford JL, Shen M, Demarini DM, Bonner MR, He X*, et al.* (2004) Oxidative damage-related genes AKR1C3 and OGG1 modulate risks for lung cancer due to exposure to PAH-rich coal combustion emissions. *Carcinogenesis* 25:2177-81.

Liu CY, Hsu YH, Pan PC, Wu MT, Ho CK, Su L*, et al.* (2008) Maternal and offspring genetic variants of AKR1C3 and the risk of childhood leukemia. *Carcinogenesis* 29:984-90.

Matsuura K, Shiraishi H, Hara A, Sato K, Deyashiki Y, Ninomiya M*, et al.* (1998) Identification of a principal mRNA species for human 3alpha-hydroxysteroid dehydrogenase isoform (AKR1C3) that exhibits high prostaglandin D2 11-ketoreductase activity. *J Biochem* 124:940-6.

Miller SJ, Alam M, Andersen J, Berg D, Bichakjian CK, Bowen G*, et al.* (2010) Basal cell and squamous cell skin cancers. *J Natl Compr Canc Netw* 8:836-64.

Miller VL, Lin HK, Murugan P, Fan M, Penning TM, Brame LS*, et al.* (2012) Aldo-keto reductase family 1 member C3 (AKR1C3) is expressed in adenocarcinoma and squamous cell carcinoma but not small cell carcinoma. *Int J Clin Exp Pathol* 5:278-89.

Mullen JT, Feng L, Xing Y, Mansfield PF, Gershenwald JE, Lee JE*, et al.* (2006) Invasive Squamous Cell Carcinoma of the Skin: Defining a High-Risk Group. *Ann Surg Oncol*.

Park AL, Lin HK, Yang Q, Sing CW, Fan M, Mapstone TB*, et al.* (2010) Differential expression of type 2 3alpha/type 5 17beta-hydroxysteroid dehydrogenase (AKR1C3) in tumors of the central nervous system. *Int J Clin Exp Pathol* 3:743-54.

Penning TM, Burczynski ME, Jez JM, Hung CF, Lin HK, Ma H*, et al.* (2000) Human 3alpha-hydroxysteroid dehydrogenase isoforms (AKR1C1-AKR1C4) of the aldo-keto reductase superfamily: functional plasticity and tissue distribution reveals roles in the inactivation and formation of male and female sex hormones. *Biochem J* 351:67-77.

Rogers HW, Weinstock MA, Harris AR, Hinckley MR, Feldman SR, Fleischer AB*, et al.* (2010) Incidence estimate of nonmelanoma skin cancer in the United States, 2006. *Arch Dermatol* 146:283-7.

Schmults CD, Karia PS, Carter JB, Han J, Qureshi AA (2013) Factors predictive of recurrence and death from cutaneous squamous cell carcinoma: a 10-year, single-institution cohort study. *JAMA Dermatol* 149:541-7.