Abbreviated Title: KS Imaging

CC Protocol Number: 01-C-0158 F

Version Date: May 21, 2018

NCT Number: NCT00026793

Title of Study: **Protocol to Assess Vascularity in Kaposi's Sarcoma Lesions Utilizing Non-Invasive Imaging Techniques**

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PRÉCIS

Background:

Kaposi's sarcoma is a highly vascular tumor. As such, it may provide a good model for the study of angiogenesis-based therapy in cancer. However, there are no standardized techniques now available to assess the effects of anti-angiogenesis therapy on blood flow in KS tissues. The present protocol is written to allow us to explore and gain experience with four promising techniques to examine tumor vasculature and structure in cutaneous KS lesions: a) laser Doppler imaging; b) multi-spectral imaging; c) infrared thermal imaging; and d) optical coherence tomography.

Objectives:

The main objective is to assess, in preliminary fashion, non-invasive methods for studying tumor vascularity, structure, and vascular changes in patients with Kaposi's sarcoma using four different imaging techniques.

Eligibility:

Patients 18 of more years of age with biopsy-proven cutaneous Kaposi's sarcoma involving the skin or mucosa are eligible. They must be willing and able to give informed consent.

Design:

This will be a preliminary study to explore these techniques in Kaposi's sarcoma. Selected Kaposi's sarcoma lesions of patients will be assessed using laser Doppler imaging, multi-spectral imaging, infrared thermal imaging, and optical coherence tomography at entry and then additional timepoints for up to 4 years. Lesions will also be assessed by conventional measurement and photographs with conventional cameras. In selected patients in which there are Kaposi's sarcoma lesions on the arm, the effects of stopping venous flow for up to 10 seconds will be assessed on the measurements. A complete blood count will be done the day of the measurements. The results of the imaging techniques will be compared with each other, and with conventional tumor assessments. Changes over time will be assessed.

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1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary Objectives

1.1.1.1 To assess in preliminary fashion non-invasive methods for studying tumor vascularity, structure, and vascular changes utilizing four different non-invasive imaging techniques

1.1.2 Secondary Objectives

1.1.2.1 Cross correlate these techniques to each other and to conventional assessment in order to relate the quantitative measurements to the biological process of angiogenesis in a preliminary manner

1.1.2.2 Assess the response of these techniques to therapies for Kaposi's sarcoma such as anti-angiogenesis therapy

1.2 BACKGROUND AND RATIONALE

Kaposi's sarcoma is a highly vascular tumor. Histologically, the lesions show hyperproliferation of vascular endothelial cells of both vascular and spindle cell origin. There is evidence that pro-angiogenic factor-driven hyperproliferation of endothelial-derived spindle cells is important at all stages of the disease (1,2). Spindle cells produce and respond to pro-angiogenic factors such as basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF) (1,3). Other angiogenic molecules such as oncostatin M, which is produced by activated lymphoid cells and has autocrine growth properties for cells derived from AIDS-KS, are also involved(4,5). Cells exposed to oncostatin M develop spindle morphology, increase proliferation in soft agar, and increase secretion of IL-6 (5), and induction of bFGF (6). Oncostatin induces increased expression of a subunit component of the IL-6 receptor, and cells can be responsive to IL-6(7). Since AIDS-KS cells express high levels of IL-6, it is likely that these interactions are involved in an IL-6 autocrine growth loop (7,8).

A novel herpesvirus called Kaposi's sarcoma-associated herpesvirus (KSHV) or herpesvirus-8 (HHV-8) was discovered in 1994 (9). Essentially all patients with KS are infected with this virus and KSHV/HHV-8 appears to represent an essential factor in the pathogenesis of KS(10-15). KSHV/HHV-8 is present in the flat endothelial cells lining the vascular spaces of KS lesions as well as in typical KS spindle cells (12). KSHV/HHV-8 can induce the production of a number of virally encoded mimics of human cytokines and other factors involved in KS pathogenesis (16,17). KSHV encodes for viral homologues to human IL-6, macrophage inhibitory protein (MIP), and interferon regulatory factor. Also, the constitutively active KSHV/HHV-8-encoded G-protein coupled receptor (KSHV-GPCR), expressed on infected cells(18), upregulates production of VEGF and other angiogenic factors (19). In addition, there is some evidence that the KDR receptor for VEGF is upregulated in cells infected with KSHV, providing a basis for the paracrine effects of this angiogenic factor in KS (20). The KSHV-GPCR can also cause oncogenic transformation in transfected cells (21). Moreover, chemokines, such as

interleukin 8 (IL-8) and growth-related protein-alpha can activate KSHV-GPCR over constitutive levels in vitro, suggesting that endogenous chemokines may be evolved in KS pathogenesis, in part through KSHV-related pathways (22).

The study of KS as an angiogenic tumor model may lead to insights for regarding KS as well as other tumors dependent on angiogenesis for growth. Our group has demonstrated clinical responses in KS to two putative antiangiogenesis-based therapies: thalidomide and interleukin-12 (23,24). An important yet difficult component of such research, however, is the assessment for evidence of changes in tumor vascularity in patients undergoing such therapy. To this end, development of non-invasive measurements of tumor vascularity is warranted. The present protocol will utilize four different non-invasive imaging techniques to quantify vascularity and blood flow in KS lesions on the skin: a) Laser Doppler imaging; b) multi-spectral imaging; c) infrared thermal imaging; and d) optical coherence tomography.

Laser Doppler imaging of blood flow in the microvasculature utilizes a low power laser beam in a raster pattern over skin or other tissue surfaces (25). It is anticipated that this will be done with a MoorLDI-2λ scan (Moore Instruments, Inc., Wilmington, DE) using wavelengths of 685-690 nm and 780 nm (Section **10** Appendix A). The instrument for this procedure is very similar to an FDA approved scan manufactured by the same company that uses one wavelength (6332.8 nm) and creates a color-coded image of blood flow in the microvasculature. This technique has been found useful for the study of burns, irritant and allergic responses, and for the assessment of psoriasis treatments. The imaging system gives a map of RBCs motion in the capillary bed with a resolution of 100 microns per pixel. The scanning procedure is estimated to take a maximum of 3 minutes per lesion.

Multi spectral imaging uses infrared reflectance spectroscopy principles applied for wavelengths at the isosbectic absorbance of oxy- and deoxy- hemoglobin (800nm) and at wavelength at which the absorbance of deoxy/oxy is large (e.g., several fold at 700nm) (Section **10** Appendix A). In this way the total blood volume and the oxygenation can be measured (26). This technique has been utilized in the research setting for a variety of clinical applications including investigations to distinguish benign skin lesions from malignant melanoma, assessment of the depth and viability of burn wounds (27-29). The lesion will be illuminated, and a charge coupled device (CCD) digital camera created for this purpose will be utilized to create an image of the lesion and surrounding tissue. The camera is expected to be low voltage and interfaced by cable to a personal computer. The camera is expected to be more than 5 cm from the patient, and it is not necessary for any of the equipment to touch the patient. It is estimated that this procedure will take approximately 2 minutes per lesion.

Infrared thermal imaging utilizes a digital infrared camera to map the temperature on the skin (Section **10** Appendix A). The concept is that areas with higher blood vessel density show higher temperature in the thermal map. The procedure has been used in clinical research to investigate organ blood perfusion and tissue viability (30,31). These cameras utilize 12 to 13 volts (with an AC adapter) and will generally be held about 50 cm from the patient.

Spectral Domain Optical Coherence Tomography (OCT) uses the principles of light interference (32) and allows 3D imaging of tissue structures. The system was built in house and is very similar to FDA approved devices for eye imaging, where even stricter ANSI standards apply. One example of an FDA approved OCT system for eye imaging is the Stratus OCT system developed by Zeiss (33). OCT skin imaging uses the same principles as OCT eye imaging and has been applied to different skin conditions already (34). The system described here uses a low coherent light source (Super luminescence diode (SLD) from Goodrich/SUI) with 1310nm central wavelength and 130nm spectral width for illumination of the lesion. The power of the light source is <6mW on the sample, which is below FDA approved standards for non-ionizing optical skin imaging. Before illumination, light is being split into two paths, one illuminating the sample in one spot and the other reflecting off a mirror. After reflecting from the skin, light is being combined again with the beam from the mirror and imaged in an InGaAs line scan camera. The acquired data is Fourier transformed and yields to one depth scan of the tissue with <5μm depth resolution over 2mm. In order to acquire a full 3D data set, the light beam is being raster scanned over the lesion by an x-y scanner. Acquisition of a 10mm x 10mm x 2.5mm volume is anticipated to take less than 1 minute.

OCT uses light which penetrated the skin and reflects again, without being scattered multiple times and without being absorbed. OCT has been applied to tumor imaging before [3] and results showed that the tumor area can be well separated from the surrounding tissue. However, the signal coming back from the tumor is usually much weaker due to generally higher absorption compared to the non-tumor skin. We therefore hypothesize that it will be possible to clearly define tumor boundaries over a depth range of approximately 2mm, and we will evaluate if the system is capable to depth resolve the lesion itself. A 3D structural image of a KS lesion will lead to better evaluation of treatment outcome in a non-invasive way.

There are a number of technical details of each modality, and complicating factors that should be taken in to account at the time of measurements. One is the effect of the environment. Laser Doppler imaging and multispectral imaging involve the use of active devices in which light from the devices penetrates the tissue and the resulting signal is detected. Thus, there is relatively little environmental influence on the outcome. As noted above, we plan to use a MoorLDI-2λ scan (Moore Instruments, Inc., Wilmington, DE) for the laser Doppler imaging. There is extensive experience in the use of a related one-wavelength device for studies on the skin.

Spectral imaging uses several infrared sources tuned to those wavelengths at which the absorptions of oxy- and deoxy-hemoglobin are relatively high compared to those of other analytes such as water and lipid. Moreover, the choice of these wavelengths is such that one of them is more absorptive for deoxy (670nm) than the other one that has an isosbectic point (800nm). Therefore, one can estimate the oxygenation and the total blood volume based on the relative absorbencies. However, there are complicating factors. The effects of light scattering makes the quantitation difficult. Our collaborators have extensive experience in photon migration theory that offers explanations of the separate effects of scattering from absorption. Experience has already been generated and reported for *in-vivo* imaging of pig heart as a collaboration with Laboratory of Cardiac

Energetics at NHLB (35,36). This technique can be affected by the patient's hemoglobin, and to study this we plan to measure a blood hemoglobin the day the measurements are made.

For thermal imaging, an infrared camera (8-12micron) will be used. By contrast to laser Doppler and spectral imaging, this technique is passive and is more sensitive to the environment. Therefore the outcomes are sensitive to the environment. The ambient temperature can be a confounding source of background "noise" which can lead to difficulty in accurate interpretation of the data. Dr. K. Zamani at The Walter Reed Army Research Institute has developed a device to cool the ambient temperature to 18 C as a means of standardizing the environment. Since the airflow inside the room could also affect the data, precise calibration is needed for this method. By discussing these issues on an ongoing basis with Dr. Zamani, we hope to gain insights as to how best to control for these potential drawbacks. Also, expertise will be inputted through ongoing discussions with Drs. Alex Gorbach and Ed Oldfield, who are using infrared camera in the intra-operative setting. Other potential confounding issues include the metabolic and physiologic aspects of the patient, such as pulse rate. However, in other studies this is being investigated by dynamic measurements using techniques to stop and release the blood flow at the site. The rationale for dynamic measurements is based on the fact that the rate of changes is only associated with blood, somehow similar to pulse oximetry.

The main reason for trying different technologies is that all the techniques have the potential to be of use, none are quantitative in the absolute sense, and they measure slightly different parameters that assess different aspects of blood flow. Using cross correlation methods (correlating the results of each technique to the other techniques and the clinical observations) one can relate the quantitative measurements to each other and to the biological process of angiogenesis.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Biopsy proven Kaposi's sarcoma involving the skin or mucosa

2.1.2 Age ≥ 18 years

2.2 EXCLUSION CRITERIA

2.2.1 Unable or unwilling to give informed consent

2.3 RESEARCH ELIGIBILITY EVALUATION

2.3.1 Designation of KS lesions to be studied.

2.4 PATIENT REGISTRATION

2.4.1 On-study

Authorized staff must register an eligible candidate with NCI Central Registration Office (CRO) within 24 hours of signing consent. A registration Eligibility Checklist from the web site (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) must be completed and faxed to 301-480-0757. Verification of Registration will be forwarded electronically via e-mail to the research team. A recorder is available during non-working hours.

2.4.2 Off-study

Authorized staff must notify Central Registration Office (CRO) when a subject is taken off-study. An off-study form from the web site (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) main page must be completed and faxed to 301-480-0757.

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

Patient volunteers will be scheduled to undergo initial evaluation and then sequential non-invasive imaging. We plan to target imaging at approximately 3-month intervals for up to 4 years. In addition, patients may be assessed at other points when a clinical assessment indicates a change in the status of KS lesions according to standard KS evaluation. Measurements may also be made at more frequent intervals to assess the short-term variation in results or at the point that therapy changes. It is worth stressing that this study is exploratory in nature and that we may make measurements at more or less frequent intervals, depending on what we learn during the study. On certain days, we may not do all four measurements because of unavailability of one or the other machines, etc. Also, as described in section 8.3, this study is of no therapeutic benefit to the patient, and we will schedule evaluations around patient preferences when possible. In particular, assessments will in general be scheduled to take place when patients are being seen for other treatment or protocol-related matters to reduce inconvenience to the patient.

For each of the techniques, the probes (or camera) will be placed directly over the lesion or area of skin to be studied, pointing towards the lesion (see Section 10 Appendix A). In addition, comparisons may be made with normal tissue (usually surrounding skin or skin on the opposite side of the body). It is expected that we will gain experience during the study of the first few patients on the optimal distance from the skin to place the probes. For the laser Doppler imaging, it i.e. expected that the optimal distance will be between 5 and 100 cm from the patient. For the multispectral imaging and the thermal imaging, it is anticipated that the optimal distance will be more than 5 cm. The set-up for each measurement is about five minutes or less. It is estimated that the laser Doppler imaging measurements will take about 3 minutes/lesion, that the multispectral imaging will take about 2 minutes/lesion, that the thermal imaging will take about 1 minute per lesion, and that the optical coherence tomography will take less than 2 minutes per lesion.

3.2 ON-STUDY EVALUATION

3.2.1 Documentation of specific prior and/or ongoing KS therapy

3.2.1.1 Dates of initiation and cessation, as available

3.2.1.2 Documentation to the extent possible of recent changes in the lesions studied and changes with current KS therapy (if applicable)

3.2.2 If known to be HIV seropositive, documentation of HIV therapy at time of study entry

3.2.3 Measurement of KS lesions concurrently with non-invasive imaging techniques. Only lesions imaged need be measured. Additional lesions of interest may also be measured.

3.2.4 Conventional photographs of the lesions studied

3.2.5 Non-invasive blood flow assessments to coincide within 7 days of clinical lesion measurement described in 3.2.1. From 1 to 10 lesions may be assessed by investigator discretion. In general, lesions measured will be at least 0.2 cm in diameter. Also, lesions will generally be selected to be representative of the patients disease. However, as we gather information early in the study, lesions may be selected that are of particular interest based on the preliminary findings. In addition, up to 3 control areas of normal skin may be assessed using the same techniques for comparison. (Note: On selected patients, one or more additional sets of measurements will be made on the same day to assess the reproducibility of the measurements over a short time period.) In each case, the devices do not touch the patient and generally do not come closer than 5 cm to the skin.

3.2.5.1 Laser Doppler imaging.

3.2.5.2 Multi spectral imaging

3.2.5.3 Near infra red thermal imaging

3.2.5.4 Optical coherence tomography (also called spectral domain optical coherence tomography)

3.2.6 In selected patients in which there are KS lesions on the arm, a blood-pressure cuff will be inflated to greater than venous pressure for up to ten seconds and the released. The effects of this maneuver on laser Doppler imaging will be assessed.

3.2.7 CBC obtained on the day of measurement may be obtained if not done within the previous 7 days

3.3 HANDLING OF RESEARCH SPECIMENS

3.3.1 It is not anticipated that any pathological research specimens will be generated on this study. The only laboratory tests are complete blood counts, and the blood for these will be handled according to the procedures of the NIH Laboratory of Clinical Pathology.

3.4 CONCURRENT THERAPIES

3.4.1 None restricted

3.5 OFF STUDY CRITERIA

3.5.1 Voluntary patient withdrawal

3.5.2 Investigator discretion

4 SUPPORTIVE CARE

Appropriate care will be given after phlebotomy.

5 DATA COLLECTION AND EVALUATION

5.1 STUDY MONITOR

Dr. Robert Yarchoan

5.2 RECORD KEEPING

Clinical data described above will be entered into the 4th Dimension and/or the CCR C3D database systems. In addition, specific electronic records will be kept of the information gathered by the techniques.

Data may be presented at meetings and reported in the literature as derived from a pilot study. In these instances, patient identifiers will not be used.

5.3 TOXICITY CRITERIA

This is a study of non-invasive imaging techniques, not a therapeutic trial, and given the nature of the imaging techniques no adverse events are expected. In the unlikely event that an adverse event occurs during or are attributable to the non-invasive imaging, the following criteria will be used for grading such events. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40)

5.4 ADVERSE EVENT REPORTING

For this study, adverse events (AEs) occurring as a result of therapeutic interventions (whether investigational or not) and not attributable to the non-invasive imaging procedures will not be tracked or reported for this protocol; however, if they occurred as the result of a therapeutic protocol, they should be tracked and reported as required by the relevant treatment protocol under which the therapy was administered. Also, AEs occurring as part of the general medical care of the patient either at the NIH or outside the NIH, will not be reported. For this study, the following definitions of AEs (Section 7.1.1, 7.1.2, 7.1.3, and 7.1.4) therefore apply only to those attributable to the non-invasive imaging procedures.

For this study, AEs will include events reported by the patient, as well as clinically significant abnormal findings on physical examination or laboratory evaluation that are attributable to the non-invasive imaging procedures. A new illness, symptom, sign or clinically significant laboratory abnormality or worsening of a pre-existing condition or abnormality is considered an AE.

All AEs that are attributable to the non-invasive imaging procedures, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event.

End of study procedures: Data will be stored according to HHS, FDA regulations, and NIH Intramural Records Retention Schedule as applicable.

Loss or destruction of data: Should we become aware that a major breach in our plan to protect subject confidentiality and trial data has occurred, the IRB will be notified.

6 STATISTICAL CONSIDERATIONS

6.1 RACIAL/GENDER MAKE-UP

Subjects from both genders and all racial/ethnic groups are eligible for this study if they meet the eligibility criteria. However, because of the demographics of KS, it is anticipated that nearly all patients will be male.

6.2 SAMPLE SIZE

Up to 32 patients will be enrolled. Volunteers will be recruited from the population of NIH patients with Kaposi's sarcoma. This is an observational study, and there will be no treatment related conclusions based on the observations. The main purpose of the studies will be to enable us to gain experience in the technique and a sense of the variation of the results. The data generated here will provide a basis for additional studies in which these techniques are used in a more structured manner.

6.3 STATISTICAL ANALYSIS

This is a feasibility study to determine in a preliminary fashion whether these techniques are clinically useful. If the preliminary observational data appears to predict or correlate with clinical observations, the preliminary findings of this study will be used to structure a more formal assessment in a future protocol. If patients undergo biopsies of their KS lesions or on another protocol or as part of their standard care, we will also attempt to correlate the results of this study with the biopsy results. Descriptive and correlative statistics will primarily be used for data analysis.

7 SAFETY REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

7.1 DEFINITIONS

7.1.1 Adverse Event

Any untoward medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in research, whether or not considered related to the subject's participation in the research.

7.1.2 Unexpected adverse reaction

An adverse event or suspected adverse reaction is considered "unexpected" if it is not consistent with the risk information described in the general investigational plan or elsewhere in the current application. "Unexpected", also refers to adverse events or suspected adverse reactions that are expected with similar research, but not specifically mentioned as occurring with this particular research.

7.1.3 Serious

An Unanticipated Problem or Protocol Deviation is serious if it meets the definition of a Serious Adverse Event or if it compromises the safety, welfare or rights of subjects or others.

7.1.4 Serious Adverse Event

An adverse event or suspected adverse reaction is considered serious if in the view of the investigator or the sponsor, it results in any of the following:

- Death,
- A life-threatening adverse drug experience
- Inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

7.1.5 Disability

A substantial disruption of a person's ability to conduct normal life functions.

7.1.6 Protocol Deviation (NIH Definition)

Any change, divergence, or departure from the IRB

7.1.7 Non-compliance (NIH Definition)

The failure to comply with applicable NIH Human Research Protections Program (HRPP) policies, IRB requirements, or regulatory requirements for the protection of human research subjects.

7.1.8 Unanticipated Problem

Any incident, experience, or outcome that:

- Is unexpected in terms of nature, severity, or frequency in relation to
  (a) the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents, and
  (b) the characteristics of the subject population being studied; **AND**
- Is related or possibly related to participation in the research; **AND**
- Places subjects or others at a g*reater risk of harm* (including physical, psychological, economic, or social harm) than was previously known or recognized.

7.2 NCI-IRB AND CLINICAL DIRECTOR REPORTING

7.2.1 NCI-IRB and NCI CD Expedited Reporting of Unanticipated Problems and Deaths

The Protocol PI will report in the NIH Problem Form to the NCI-IRB and NCI Clinical Director:

- All deaths that the study team becomes aware of, except deaths due to progressive disease
- All Protocol Deviations
- All Unanticipated Problems
- All non-compliance

Reports must be received within 7 days of PI awareness via iRIS.

7.2.2 NCI-IRB Requirements for PI Reporting at Continuing Review

The protocol PI will report to the NCI-IRB:

1. A summary of all protocol deviations in a tabular format to include the date the deviation occurred, a brief description of the deviation and any corrective action.
2. A summary of any instances of non-compliance
3. A tabular summary of the following adverse events:
   - All Grade 2 unexpected events that are possibly, probably or definitely related to the research;
   - All Grade 3 and 4 events that are possibly, probably or definitely related to the research;
   - All Grade 5 events that the study team becomes aware of regardless of attribution;
   - All Serious Events that are attributable to the non-invasive imaging procedures (i.e. that are possibly, probably or definitely related to the research.

NOTE: Grade 1 events are not required to be reported.

8 HUMAN SUBJECTS PROTECTIONS

8.1 RATIONALE FOR SUBJECT SELECTION

Subject selection will primarily be from patients with KS enrolled on other protocols in the HIV and AIDS Malignancy Branch. Other sources will be referrals from other institutes and physicians. Patients on other Branch protocols, or patients referred to the Branch who have KS will be queried regarding their interest in participating on this study.

8.2 PARTICIPATION OF CHILDREN

Children will not be allowed to participate because there are no benefits to the patient. Of note, KS in extraordinarily rare in North American children and it is unlikely that children with KS would be available for study consideration.

8.3 EVALUATION OF BENEFITS AND RISKS/DISCOMFORTS

There are no specific patient benefits. The risks of these non-invasive measures are minimal. The laser light used is low intensity (about that in a pointer used for making presentations). There is a theoretical possibility that patients could have vision impairment because of the infrared laser (at a wavelength not visible to humans) shining in their eyes during the laser Doppler imaging; however, the instrument is designed so this laser shines down and is colinear with a visible laser to prevent this possibility. The discomfort is primarily from phlebotomy and the time it takes to have the procedures done (about 3 minutes per lesion for laser Doppler imaging, 2 minutes per lesion for multispectral imaging, 1 minute per lesion for thermal imaging, and up to 2 minutes per lesion for optical coherence tomography), and these will be scheduled around patient preferences as much as possible. It is not anticipated that these techniques will make the Kaposi's sarcoma worsen.

8.4 RISK/BENEFITS ANALYSIS

Why risks to subjects are reasonable in relation to the anticipated benefits in relation to the importance of the knowledge that may reasonably be expected to results: the risks are minimal; non-invasive assessment of tumor vascularity may prove a valuable tool in the field of anti-angiogenic therapy for cancer.

8.5 CONSENT PROCESS AND DOCUMENTATION

All patients will read and sign the informed consent document prior to enrollment. Members of the protocol team will describe the protocol and insure patients do not feel coerced into participating. At the beginning of each session, patients will be informed by the research team of the plans for imaging that day (e.g. how many lesions and whether it is anticipated that repeat measurements will be taken).

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10 APPENDIX A: DESCRIPTION OF THE DEVICES

10.1 LASER DOPPLER IMAGING DEVICE

A Moor Instrument moorLDI-2λ-simultaneous two wavelength scan will be used. The scan images at 685-690 and 780 nm. This imager scans a low power laser beam (5mW, 800 micron diameter) in a raster pattern over the skin. There is no contact with the skin. The scanner is produced by Moor Instruments Ltd., 501 Silverside Rd., Suite 66, Wilmington, DE 19803. The scanner is held on a special stand (Moor MS2 stand) made specifically for such devices.

A very closely related instrument, the moorLDI-VR scanner is approved by the FDA by patient use. This scanner just utilizes one wavelength of light (633 nm). The device that will be used in the present study differs from the FDA-approved device in that it also assesses the lesions using a second wavelength and that the first wavelength is slightly longer. The other potential safety issue is that the 780 nm wavelength is in the infrared spectrum and would not visible. Thus patients and users would not necessarily blink if it shined into their eyes. However, both lights shine on the same spot so patients or users would blink upon seeing the 685 nm wavelength (which is visible). Also, the machine is constructed to minimize the chance of anyone (patient or user) looking into the laser light.

10.2 MULTISPECTRAL IMAGING

For multispectral imaging, a charge coupled device (CCD) handheld camera will be used. This uses CCD technology similar to that utilized in most digital cameras. The lesions will be illuminated uniformly by a white light held approximately 30 cm from the patients skin. Using optical filters, those wavelengths associated with oxyhemoglobin and deoxyhemoglobin absorption spectra will be selected and CCD images will be made. The camera is a cooled Princeton CCD Camera interfaced by cable with a personal computer with associated data analysis software. The camera is low voltage. The camera will not directly contact the patient and will be held 5 cm or more from the patient's skin (approximately 30 to 50).

10.3 THERMAL IMAGING

For thermal imaging, infrared-sensitive handheld cameras will be used. We plan to use a camera modified from the BioEar PRISM 2000 Thermal Metabolic Imaging System (10618 Rockley Rd., Houston, TX). The name of the camera is the ThermoVision™ Alert, FLIR Systems, USA. This camera is at ambient temperature. If it becomes available, we will also test a cooled camera modified from the ThermaCAM SC3000 (FLIR Systems, 16 Esquire rd., Boston, MA). Cooling has the potential of giving more accurate measurements with less background noise. If this camera is found to be superior and can be made available on a long term basis, we will switch over to its use.

These cameras use low voltage electricity (12- 13 volts) provided by an AC adapter. They do not touch the patient and will be held more than 5 cm from the patient's skin (approximately 50 cm).

10.4 SPECTRAL DOMAIN OPTICAL COHERENCE TOMOGRAPHY

For OCT imaging, a line scanning InGaAs camera (Goodrich/SUI) will be used, which captures the light, reflected form the skin. The camera is low voltage (<28V), 14bit, has 1024 pixels/line, and acquires 46000 lines / second, which allows for high speed data acquisition. The light source used is a 24mW superluminescence diode (SLD/DenseLight) with 1310nm central wavelength and 130nm spectral width at FWHM. The light beam scanning the skin is <6mW for the entire spectral width, which is well below FDA approved standards. A hand held probe, which contains the x-y scanner and a focusing lens, will be held approximately 4cm away from the lesion, therefore not touching the patient. Data will be stored and analyzed with a personal computer with associated data analysis software.