Microscopic Fluorescence-guided Vestibular Schwannoma Resection Using Fluorescein Sodium and YELLOW 560

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Clinical Investigation Plan

Microscopic Fluorescence-guided Vestibular Schwannoma, Meningioma, Head and Neck Paraganglioma, or Head and Neck Schwannoma Resection using Fluorescein Sodium and YELLOW 560

Mayo Clinic, Rochester, Minnesota, USA

Version 1.3

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1.0 Introduction

1.1. Statement

The study will be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312) and International Conference on Harmonization guidelines, applicable government regulations and institutional (Mayo Clinic) research policies and procedures*.*

1.2 Definitions

Term	**Description**
YE560	YELLOW 560 fluorescence module (available for KINEVO 900)
CIP	Clinical Investigation Plan
CRF	Case Report Form
AEF	Adverse Event Form
Device Manufacturer	Carl-Zeiss Meditec AG (Jena, Germany)
FDA	Food and Drug Administration
IDE	Investigational Device Exemption
IRB	Institutional Review Board
OR	Operating Room
Sponsor	Carl Zeiss Meditec, Inc. (Dublin, CA)
VS	Vestibular Schwannoma
GTR	Gross Total Resection
HNP	Head and Neck Paraganglioma
HNS	Head and Neck Schwannoma

1.3. Purpose

The purpose of this study is:

- To evaluate the benefit of using intravenous fluorescein sodium (FS) and YELLOW 560 nm microscope filter (YE560) during surgery for vestibular schwannomas (VS) menigioma, head and neck paraganglioma (HNP), or head and neck schwannoma (HNS)
- To determine the optimum FS dose and timing to be used with the YE560 device

VSs are benign, slow-growing neoplasms of the myelin-containing Schwann cells of the vestibular division of the vestibulocochlear nerve. These tumors are in close proximity to the facial nerve and cochlear division of the vestibulocochlear nerve. These nerves allow for facial movement and hearing, respectively. Management of these indolent tumors has shifted to more conservative means of minimizing growth and maintaining facial function and hearing. Management of VSs includes observation with serial MRI exams, stereotactic radiosurgery, and microsurgery.

Similarly, HNP, HNS and skull base meningiomas are benign but infiltrative tumors that grow adjacent to important neurovascular structures including, the cranial nerves. These tumors can be observed, treated with radiosurgery, or microsurgery based on size, symptoms, and patient preference.

When microsurgical resection of VS, schwannomas, meningiomas, or paragangliomas is performed, distinguishing the tumor from healthy nerve tissue is performed via direct visualization under light microscopy and tissue manipulation. Often, these tumors are intimately associated with the surrounding nerves, and can be difficult to distinguish under the microscope. In many cases intraoperative cranial nerve monitoring allow for the use of a stimulation probe to help make this distinction.

The hypothesis is that FS and YE560 may be used as a supplementary visualization aid to better distinguish the tumor from healthy nerve tissue and secondarily improve conservation of facial and hearing nerve function and attainment of gross total resection (GTR). Use of intravenous FS has FDA approval for ophthalmologic applications and its safety profile is well documented (Yanuzzi 1986). There have been studies demonstrating uptake of FS by VS tissue in vitro (Perez 2018). Additionally, the feasibility and utility of intraoperative fluorescence from FS intravenous administration and use of YE560 have been demonstrated with peripheral schwannomas in vivo (Pedro 2019). The YE560 fluorescence module available for the KINEVO 900 will be used to visualize fluorescein-stained tissue in yellow light. FS will be administered upon initial visualization of the VS. Utility of FS and YE560 will be assessed via surgeon survey, correlation with biopsy of fluorescent VS tissue, and color image analysis of healthy and VS tissue using the YE560.

A total of 90 consenting patients, either male or female, clinically indicated for resection of a suspected VS will be enrolled into the study. No exclusions will be made for recurrent VS following stereotactic radiosurgery or microsurgical resection or VS associated with neurofibromatosis type 2 (NF-2).

1.4. Application

The document applies to the YE560 Fluorescence option available for KINEVO 900 supplied for the intended clinical investigation and protocol at the specified hospital site. The Clinical Investigation Plan (CIP; or Sponsor Protocol) must be agreed to by the Sponsor and Clinical Investigators involved in the study.

1.5. Responsibility and Authority

Mayo Clinic Institutional Review Board (IRB) for Human Research must grant approval of the study, and record their determination of the study's risk profile as a non-significant or significant risk medical device study.

Clinical investigators, research collaborators, and Carl Zeiss Meditec, Inc staff involved in the study are responsible for implementing the described procedures.

2. Details of the clinical Investigation

2.1. Principal Investigator

Matthew L. Carlson, MD

Professor of Otorhinolarynology and Neurosurgery

Mayo Clinic - Rochester

200 First Street SW

Rochester, MN 55905, USA

2.1.1. Co-Investigators

Colin L. W. Driscoll, MD

Brian A. Neff, MD

Stephen A. Chan, MD

Michael J. Link, MD

Jamie J. Van Gompel, MD

Cynthia Chweya

Mayo Clinic - Rochester

200 First Street SW

Rochester, MN 55905, USA

2.2. Institution where investigation will be conducted

Mayo Clinic

Rochester, Minnesota, USA

2.3. Analysis of the Study Data

The Study results will be analyzed by:

• Clinical Investigator at Mayo Clinic (Rochester, MN, USA)

2.4. Sponsor of the Clinical Investigation

Carl Zeiss Meditec, Inc.

5160 Hacienda Dr,

Dublin, CA 94568

Data and Quality Management

2.5. Database Management

Clinical Investigators shall be responsible for day-to-day database management, keeping a secure record of all patients recruited for the purposes of the study and details of the procedure performed. All data generated from this clinical study will comply with Federal Drug and Food Administration (FDA) compliant guidelines.

2.6. Security of Patient Data

Patients will be assigned unique identifiers codes upon enrollment. Digital images and videos captured from surgery will be saved on a department drive in a folder with limited access. Electronic data will be stored in Rave Electronic Data Capture (Medidata, New York, NY). Digital images, raw procedure data, photos, macroscopic images, video, other imaging data (e.g. MRI, CT), medical history or histopathology data used in publication will not contain any information that identifies individual subjects.

2.7. Verification of Source Data

Digital images that are collected are saved in jpeg format for images and mpeg-2 format for videos as a procedure result, which is associated with a unique session ID number. Images and videos may be exported from the microscope and saved in various common image file formats (e.g. jpg, bmp, tif). Source data can be verified by viewing images in the original saved format (Procedure Result) via the instrument software.

2.8. Data Storage

Data will be collected from surgeon surveys and the medical record and recorded electronically in Rave Electronic Data Capture (Medidata, New York, NY), a product in compliance with FDA 21 CFR Part 11. Only authorized research staff will have access to the electronic data. Paper copies of informed consent forms and completed surgeon surveys will be stored in a binder kept by the study coordinator.

2.9. Data Retention

The principal investigator will retain clinical research data for 3 years after completion and closure of the study.

3. Overview of the clinical Investigation

3.1. Objectives

Primary objective of this initial feasibility study is to evaluate the benefit of using YE560 to visualize fluorescein sodium (FS) during surgery of VS, Meningioma, HNP, or HNS, determined from surgeon assessment.

Secondary objectives are:

• To evaluate the optimum FS dose and timing to be used with the YE560 device as determined by reported performance on surgeon surveys

• Quantify tissue differentiation of fluorescent VS and adjacent nerves using RGB image analysis

• To report clinical outcomes, including GTR rates, postoperative hearing outcomes, and postoperative facial nerve function

3.2. Literature Review and Rationale

In VS cases where intervention is recommended, stereotactic radiosurgery and microsurgical resection offer two therapeutic options. Comparatively, microsurgical resection carries the advantage of offering the highest chance of a cure.The goal of surgery remains to resect the tumor while maintaining the integrity of the adjacent, and sometimes intimately adherent cochlear and facial nerves. Thus, gross total resection cannot always be achieved. Nakatomi and colleagues described the superior 15-year recurrence-free survival rates in patients receiving gross total resection (56%) versus subtotal resection (8%).[1] Intraoperatively, maximum resection of the tumor while preserving facial function and hearing relies on the ability to distinguish normal nerve tissue and tumor. Additional means of distinguishing these tissues could facilitate greater rates of gross total resection and preservation of cranial nerve function.

Intravenous FS has been used in ophthalmologic and neurosurgical applications to differentially cause fluorescence in tissues intraoperatively. This technique was reported in 1948 by Moore et al[2] and more recently by Koc and Toda in obtaining gross total resection of high grade gliomas[3,4]. Fluorescein angiography has been routinely used for retinal imaging for vascular conditions and neoplasms since first described in 1961.[5]

As previously mentioned, two recent studies have demonstrated the application of intravenous FS to VSs. Perez et al have reported in vitro preferential uptake of FS in VS cells compared to native Schwann cells.[6] Additionally, Pedro et al demonstrated feasibility of YE560 and intravenous FS in visualizing benign peripheral nerve sheath tumors—an identical histological entity to VS—located in the extremities.[7] In our proposed study, the fluorescence light will be visualized with a surgical microscope (Kinevo 900) with YE560 fluorescence module. The device has optimized filter and optics; therefore, the concentration of fluorescein sodium per patient used in this study is expected to be lower than in earlier studies that do not use the fluorescence module.

Intraoperative fluorescence imaging has been utilized in the resection of HNP and meningiomas. Indocyaningreen fluorescence angiography has been used to assess post-embolization vascularity of paraganglioma to estimate bleeding risk.[8] FS has been utilized with the YE560 to distinguish meningioma and hyperostosis from surrounding brain and healthy bone.[9]

FS is FDA approved for ophthalmologic applications. The standard dose for retinal imaging in adults is a 500 mg bolus into a peripheral vein (7.1 mg/kg in a 70 kg patient).[10] The safety profile of intravenous FS is well-described in the ophthalmology literature. Nausea and vomiting are the most common side effect reported, but 87% of surveyed ophthalmologists reported rates less than 5%. Urticaria is estimated to occur at a rate of 1 in 82 patients. Severe adverse reactions, including respiratory reactions (1:3,800), cardiac events (1:5300), and tonic-clonic seizure (1:13.900) are rare and estimated to occur cumulatively in 1 in 1900 patients.[11]

3.3. References

1. Nakatomi H, Jacob JT, Carlson, ML, Tanaka S, Tanaka M, Saito N, Lohse CM, Driscoll CLW, Link MJ. Long-term risk of recurrence and regrowth after gross-total and subtotal resection of sporadic vestibular schwannoma. J Neurosurg. 2017. 1-7.
2. Moore GE, Peyton WT, Lyle A, French MD, Walker WW. The clinical use of fluorescein in neurosurgery; the localization of brain tumors. 1948. J Neurosurg. 5(4): 392-8.
3. Koc K, Anik I, Cabuk B, Ceylan S. Fluorescein sodium –guided surgery in glioblastoma multiforma: a prospective evaluation. Br J Neurosurg. 2008. 22(1):99-103.
4. Toda M. Intraoperative navigation and fluorescence imaging in malignant glioma surgery. Keo J Med. 2008. 57(3)155-161.
5. Novotny HR, Alvis DL. A method of photographing fluorescence in circulating blood in the human retina. Circulation 1961; 24(1):82-86.
6. Perez, ER, Bracho O, Ein L, Szczupak M, Monje PV, Fernandez-Valle C, Alshaiji A, Ivan M, Morcos J, Liu XZ, Hoffer M, Eshraghi A, Angeli S, Telischi F, Dinh CT. Fluorescent Detection of merlin-deficient schwann cells and primary human vestibular schwannoma cells using sodium fluorescein. Otol and Neurotolog. 2018. 39: 1053-59.
7. Pedro MT, Eissler A, Schmidberger J, Kratzer W, Wirtz CR, Antoniadis G, Koenig RW. Sodium fluorescein-guided surgery in peripheral nerve sheath tumors:first experience in 10 cases of schwannoma. World Neurosurg. 2019. E1-E9.
8. Siedeck V, Waggershauser T, Berghaus A, Matthias C. Intraoperative monitoring of intraarterial paraganglioma embolization by indocyaningreen fluorescence angiography. Eur Arch Otorhinolarnygol (2009) 266: 1449-1454.
9. Akcakaya MO, Goker B, Kasimcan MO, Hamamamcioglu MK, Kiris T. Use of Sodium Fluorescein in Meningioma Surgery Performed Under the YELLOW-560 nm Surgical Microscope filter: Feasibility and preliminary results. World Neurosurgery. 2017. 107: 966-973.
10. "Fluorescein drug summary." Prescribers' Digital reference. Downloaded 5/20/19. <https://www.pdr.net/drug-summary/Fluorescite-fluorescein-1002>
11. Yannuzzi LA, Rohrer KT, Tindel LJ, Sobel RS, Costanza MA, Shields W, Zhang E. Fluorescein Angiography Complication Survey. Ophthalmology 1986. 93(5):611-17

3.4. Hypothesis

12. Given prior evidence of preferential uptake of FS in VS and meningiomas due to disrupted blood brain barrier, we hypothesize that use of intravenous FS will allow for visual differentiation of tumor and surrounding structures and nerves using YE560.
13. The optimal dosing for FS has not been established for this application. Pedro et al utilized 0.5-1.0 mg/kg body weight to visualize peripheral nerve sheath tumors with YE560. The authors found saturation of all tissues with 3-4 mg/kg dose within 18 seconds of administration. The blood-brain barrier may play a modulating role. Schebesch et al (2013) used 3-4 mg/kg to visualize malignant gliomas and observed fluorescence after 20-30 minutes. We suspect that the optimal dose will be 3-4 mg/kg, and peak fluorescence will take a similar amount of time. However, we will initially utilize 1.0 mg/kg SF administered at the time of first visualizing the tumor to enable subsequent doses (up to a maximum of 500 mg or 7 mg/kg). This will allow us

to determine the minimum effective dose and observe the time to peak fluorescence. The optimal dosing strategy will be finalized after the first 10 cases.

14. We hypothesize that the surgeons will be satisfied with the use of SF and YE560, as reflected in responses on the Surgeon Survey. Specifically, we hypothesize that greater than 75% of surgeons would choose to use SF and YE560 at least in the majority of future VS, menigoma, HNP, or HNS resections based on responses from the Surgeon Survey. Secondarily, we hypothesize that surgeons will rate the correlation of fluorescence with visual assessment with white light microscopy and electrostimulation to be "good" or "excellent" for over 75% of the cases.
15. Because the yellow fluorescence is primarily comprised of red and green components, we hypothesize that RGB component analysis of intraoperative images will yield significantly higher red and green values in the VS compared to the facial and cochlear nerves. Similar analysis may be conducted between the tumor and surround nerves or structures of interest for meningiomas, HNP, and HNS.

3.5. Design and Proposed Analysis

16. The feasibility study will involve 90 patients, focusing on intra-operative visualization of fluorescein using YE560 in VS, menigioma, HNP, or HNS, inclusive of recurrent tumors, previously radiated tumors, and patients with neurofibromatosis type 2. There will be an allowance to enroll up to 95 patients to meet the goal of 90 subjects if subjects are withdrawn from the study due to a different pathologic entity diagnosed on biopsy of the tumor. Establishing the optimal dose and timing for FS will be a part of the feasibility study. Flexibility will be allowed with the dose and timing to achieve optimal visualization condition based on surgeon feedback with a maximum cumulative dose of 500 mg (standard dosage for ophthalmology angiography). The study will be largely descriptive, documenting the macroscopic appearance of FS and describing surgeon observations with regards to timing of peak fluorescence, correlation with electrostimulation, and utility in differentiating normal tissue from tumor.
17. Images containing the tumor and facial and cochlear nerves (or other relevant structures of interest in the case of meningioma, HNP, or HNS) will be captured with the microscope with the YE560 filter by the surgeon and analyzed; the YE560 filter will then be turned off and another identical image will be captured under white light microscopy for the separate survey validation analysis. For the RGB component image analysis, the fluorescence of the tumor will be measured in comparison to the facial nerve and cochlear nerve using ImageJ image analysis software to quantify the red, green, and blue components of the images. Inferential statistics will be used to compare the red, green, and blue components of regions of interest from the VS and the adjacent facial or cochlear nerve.
18. For the survey validation analysis, we will validate the question "How well did fluorescence correlate with your visual assessment of the tumor and nerve tissue under normal microscopy without the Yellow 560 filter?" The neurosurgeons and neurotologists will be presented with 30-60 second videos of the VS and facial and/or cochlear nerves, with and without YE560 active. The surgeons will respond to the above question with choices of "no correlation," "some

correlation," "good correlation," and "excellent correlation." Other questions from the surgeon survey will not be validated due to the scope of the feasibility study.

3.6. Methodology

3.6.1. Investigational Type

19. The current investigation is a feasibility study, to assess the usability of the YE560 option for visualization of FS during VS resection.

20. *3.6.2. Patients*

21. A total of 90 consenting patients, either male or female, clinically indicated for surgical resection of suspected VS, meningioma,HNP, HNS or recurrent VS, menigioma, HNP, or HNSwill be part of the feasibility study phase. Patients with neurofibromatosis type 2 and those receiving prior radiation therapy will not be excluded from the study.

3.6.3. Timeframe

22. The estimated timeframe for enrollment is approximately 18 months (from January 2019 – June 2021).

3.6.4. Recruitment Process

23. Patients under the care of the Clinical Investigators who are clinically indicated for surgical resection as a suspected VS, meningioma, or head and neck paraganglioma or recurrent VS, meningioma, HNP, or HNSwill be considered eligible for participation in the study. Each potential participant will be assessed against the inclusion and exclusion criteria. Potential patients shall be provided with plain language information about the study and will have the opportunity to ask questions of the study investigators. Informed consent shall be obtained by the Principal Investigator or Co-Investigators from eligible patients wishing to participate. Any patient that chooses not to participate in the study will undergo their indicated surgery with standard techniques, and their health care will not be compromised in any way. Patients shall be free to withdraw from the study at any time.

3.6.5. Inclusion Criteria

Inclusion criteria shall be:

- Patient with a suspected VS, menigioma, HNP or HNS
- Recurrent VS, menigioma, HNP, or HNS with prior microsurgical resection or radiation therapy
- Clinical indication for microsurgical resection

3.6.6. Exclusion Criteria

Exclusion criteria shall be:

- Children (patients less than 18 years of age)
- History of allergy to FS
- History of renal failure
- Pregnant women
- Those with inability to give informed consent
- Prisoners and inmates

3.6.7. Point of Enrollment

Potential subjects will be provided with plain language information about the research on the informed consent form, prior to signing the consent form indicating their willingness to participate. The point of

enrollment shall be upon clinical evaluation and consent for surgery. The patient shall be considered as part of the study population once they have undergone the YE560 visualization during the indicated microsurgery with confirmatory histopathology. Patients will be withdrawn from the study population if their final histopathology is not consistent with vestibular schwannoma; this is expected to occur in less than 5% of subjects. Clinical data, intraoperative images, survey results and postoperative clinical data will still be maintained for these patients for potential case presentation or evaluation of bias.

3.6.8. Primary Endpoint

Primary endpoint of this study will be an assessment of the utility of FS and YE560 via survey of the operating surgeons.

24. The primary outcome will be response to the question "Based on the use and performance of FS in this case alone, how likely are you to use FS in future cases (excluding the research trial) involving this tumor?" We hypothesize that > 75% of responses will be "Plan to use in the majority of cases" or "Plan to use in all cases."
25. This primary outcome will be supported by responses assessing correlation of FS with electrostimulation and visual assessment using light microscopy using Likert-style ratings. The responses to the latter correlation will be validated via presentation of fluorescence and white light microscopy images to the surgeons.

3.6.9. Secondary Endpoints

The first secondary endpoint is the differential measurement of red, green, and blue levels in VS tissue compared to the facial nerve and cochlear nerve using FS and YE560. ImageJ software will be used to analyze representative images capture from microsurgery. The operating surgeon will delineate regions of interest for the VS, facial nerve, and cochlear nerve. Separate similar analysis will be performed for other tumor types.

The second secondary endpoint will include gross total resection rate, postoperative facial nerve function, and postoperative hearing status. Facial nerve function will be assessed by clinical evaluation at 3 month postoperative visits as well as intraoperative supramaximal stimulation and minimum stimulation levels as routinely performed in surgery.

3.6.10. Primary safety endpoints

As FS is approved for the intravenous injection for ophthalmological applications and the safety profiles are well characterized and considered low risk. As described above the most common side effects are nausea and vomiting. This is side effect is commonly experienced with resection of vestibular schwannoma due to transection of the vestibular nerve and resulting vertigo. Thus, this side effect will not be tracked or reported. Other adverse effects of SF—urticaria, anaphylaxis, seizure, cardiac events, respiratory compromise—will be tracked and reported up to 3 months after surgery. The relevance of the adverse event to the administration of SF will be determined by the principal investigator.

3.6.11. Microscope imaging during YE560 fluorescence use

1. Standard methods of anesthesia as appropriate for open craniotomy or otologic surgery will be used.

2. Surgeons will gain access to the lesion of interest through a craniotomy or translabyrinthine approach.

3. At the time of tumor exposure, patients will be given intravenous FS. The timing and dose may vary. Maximum cumulative dose will not exceed 500 mg.

- Initial dosing protocol will be 1 mg/kg intravenous FS.
    - If insufficient fluorescence is obtained or washout occurs, additional doses of 1 mg/kg may be administered
- Based on the experience of the first 3 cases, modifications to the dosing protocol may be made.
    - A higher, single dose may be administered if there is insufficient fluorescence or premature washout. Additional doses may be given during surgery.
    - If saturation of normal tissue with fluorescence occurs, the dose may be decreased or the FS may be administered at an earlier time point (opening of dura, anesthesia induction, etc.) during subsequent cases
- Continued adjustments may be made to the dosing and timing, with a goal of standardizing the dose after 10 cases

4. Once the VS has been surgically exposed and FS has been administered, the YE560 fluorescence module shall be used to visualize fluorescence in the tissue. Images will be captured prior to tumor dissection, after exposure of the intracapsular tumor contents, and after maximum resection. A pair of images—one using the YE560 and one with white light microscopy—will be captured during each event.

5. With the YE560 turned on, images will be captured with the microscope. Video recording from the Pentero 900 and Kinevo 900 microscopes will be captured for then entirety of their use in the case.

6. The time from injection until to the point where the differential fluorescence is greatest will be recorded and documented in the surgeon's survey. Circulating nursing staff will record timestamps in collaboration with instruction from the operating surgeons.

7. The YE560 modulewill be applied during the aforementioned events and additionally at the discretion of the operating surgeons. Depending upon the number of potential regions identified for imaging and for possible biopsies, the YE560 fluorescence module is expected to add less than 10 minutes to the overall procedure.

8. At any time, the operating surgeon may turn off the YE560 module as dictated by the needs of surgery.

9. Following completion of tumor resection and prior to closing of the dura, supramaximal and minimum stimulation values will be obtained (if relevant), as is standard practice with vestibular schwannoma microsurgery at our institution. The process is as described in Schmitt et al. (2013). In brief, transcutaneous bipolar electrodes are used at the extratemporal main trunk of the facial nerve to stimulate the nerve at 100 msec pulses increasing from 0 mA until a plateau amplitude compound muscle action potential is obtained. A Prass probe is used to stimulate the facial nerve proximal to the tumor (at the brainstem if possible) at increasing current (starting at 0 mA) until a plateau of CMAP is obtained. The ratio of these amplitude responses is calculated and subtracted from 1 to calculate the supramaximal stimulation. The minimum stimulation is the minimum current level at which a reliable facian nerve response can be obtained with proximal stimulation of the facial nerve using the Prass probe.

11. Suspected lesions that are found to be a histopathological entity excluded from this study after microsurgical resection and biopsy will be withdrawn from the study.

12. Post–procedure data analysis will include analysis of surgeon survey responses and, red/green/blue component analysis using ImageJ software. Additional data (demographics, imaging characteristics, facial nerve and audiometric data) will be reviewed and recorded from preoperative and 3 month postoperative visits. Video recordings will be edited to 30-60 second videos following the case with

representative views of the VS, facial nerve and cochlear nerve with and without the YE560 active. These edited videos will be used for the aforementioned surgeon validation study rating the correlation of fluorescence with white light microscopy.
13. Upon completion of surgery, operating surgeons will complete the remainder of the paper survey (Appendix 3). The surveys will be collected, with responses logged in the Rave database. The paper surveys will be stored in a secure location in the Otolaryngology department.

3.7. Informed Consent Process

Patients under the care of the study investigators will be provided with the written informed consent form with plain language information about the research. The study will be explained to potential subjects, and they will be advised that participation is voluntary. Potential subjects will have the opportunity to ask questions of the study investigator before agreeing to participate and signing the consent form. Patients will not be coerced into the study. Any patient that chooses not to consent will undergo the standard microsurgical procedure and their health care will not be compromised in any way. Patients shall be free to withdraw from the study at any time.

3.8. Monitoring and Execution of the Trial

The Sponsor's Obligations are outlined in Appendix 1 of this document.
The Investigator's Agreement and Obligations are outlined in Appendix 2 of this document
The results, progress and execution of the feasibility study will be monitored as outlined in chapter 4.5.10 (Microscope imaging during YE560 fluorescence use).
Carl Zeiss Meditec, Inc. (or designee)
5160 Hacienda Drive
Dublin, CA 94568

The clinical investigation team will meet quarterly during active enrollment to review enrollment, current outcomes and any adverse events.

3.9. Record Keeping

Clinical investigators shall keep a record of subjects recruited in a secure database and/or spreadsheet, and study participation will be recorded in each patient's medical case notes stored by the hospital. Case report forms, images and other associated procedure data labelled with the patient's 3 digit code shall be provided to the Sponsor for analysis and back up. All required records will be stored by the Sponsor and Investigators for a period of 3 years after completion of the study.

4. Description of the Medical Device under Investigation

4.1. Manufacturer

Carl Zeiss Meditec AG
Goeschwitzer Strasse 51-52
07745 Jena/Germany

4.2. Item/Part Number

The microscope equipped with the YE560 fluorescence module for clinical evaluation comprises:

- **Surgical Microscope**

Name: Kinevo 900

- **Fluorescence module**

Name: YELLOW 560
REF: 303582-9205-000

• **Still Image and Video recording**

Name: Video Recording HD
REF: 303582-9700-000

• **Capturing device**
Name: High Definition Camera 3CCD NTSC
REF: 303581-9764-000

4.3. Intended Purpose of this Device

The Kinevo 900 with YE560 fluorescence option is a surgical microscope equipped with an visualization tool to detect fluorescence signals in a wave length range between 540nm to 690nm.

4.4. Clinical Indications & Contraindications for use in Study

The clinical indication for use of the YE560 fluorescence module in the proposed study is neurological pathology requiring open craniotomy or translabyrinthine otologic approach.
Use of the YE560 fluorescence option is contra-indicated in patients where the tumor resection bed is not directly surgically exposed and cannot be visualized with the surgical microscope.
A fluorescent contrast agent, FS will be administered intravenously within its labeled dose range for its approved use in ophthalmic applications to reveal fluorescence contrast during the use of YE560. Occasionally patients may experience adverse events. The fluorescein dye can rarely cause allergic reactions. Fluorescein dye is a FDA-approved medicine that is routinely used by ophthalmologists (eye doctors) to perform exams of the retina of the eye. Administration of Fluorescein is contraindicated in patients with:

• Known allergy or prior adverse reaction to Fluorescein
• Women who are pregnant or breastfeeding

4.5. Summary of Device Design

The Instruction for use covers basic set-up and use of the KINEVO 900 equipped with YE560 fluorescence option
The YE560 option is an accessory for intra-Figure 1 operative illumination of the surgical field with light of a wavelength between 460nm and 690nm.
The integrated fluorescence module YE560 serves to make fluorescent areas visible. It is designed for stimulus light penetrating the service in a wave length range from 460nm to 500nm and for visualizing the YE560 fluorescence light reflected by the service in a wave length range of 540nm to 690nm. Surgeons can switch between white light and fluorescence mode. Blue excitation light should be dimmed for optimum reproduction of the fluorescence light.
The YE560 lighting option can be operated from the hand grip or foot switch. The user manual explains how to set-up, operate and record images taken with the YE560 module.

5. Training and Experience Requirements for use of the Device

The Instruction for use covers basic set-up and use of the YE560 fluorescence option as well as recording function for capturing pictures. Training in the use of YE560 for all operating surgeons in this study will be provided by the Sponsor representative before use in surgery on enrolled subjects. A training log documenting personel and training dates will be kept by the study coordinator. All study cases will be assisted by a sponsor representative.

6. Description of Medical/Surgical Procedures involved in the use of the device

The YE560 fluorescence option will be used during open craniotomy procedures performed for the purpose of tumor resection and possible biopsy. Standard neurosurgical techniques shall be used throughout the procedure. The YE560 fluorescence will be used during the surgery, once the tumor is exposed. The YE560 fluorescence visualization option can be turned on/off during the tumor resection phase of the surgery. Images captured with the KINEVO 900 under YE560 light and simultaneously navigation pictures, representing the location of the biopsy during surgery will be linked to the biopsy results available after surgery. To obtain images, a fluorescent contrast agent is required. Fluorescein Sodium will be administered intravenously prior to imaging within its labelled dose range for its approved use in ophthalmic applications to reveal fluorescence contrast during the use of YE560.

7. Study Drug

7.1. Description

AK-FLUOR® (fluorescein injection, USP) 10% contains fluorescein sodium (equivalent to fluorescein 10% w/v). It is a sterile solution for use intravenously as a diagnostic aid. Its chemical name is spiro[isobenzofuran-1(3*H*), 9'-[9*H*]xanthene]-3-one, 3'6'-dihydroxy, disodium salt. The active ingredient is represented by the chemical structure (Figure 2):

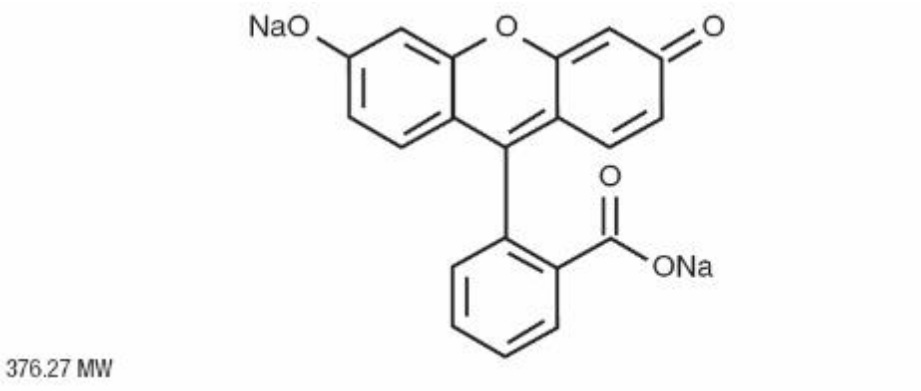

Figure 2 Fluorescein 10%

AK-Fluor® (fluorescein injection, USP) 10% is supplied as a sterile, unpreserved, single-use aqueous solution, that has a pH of 8.0 - 9.8 and an osmolality of 572-858 mOsm/kg. Active ingredient: fluorescein sodium Inactive Ingredients Sodium hydroxide and/or hydrochloric acid (to adjust pH), and water for injection.

7.2. Method for Assigning Subjects to Study Group

Patients under the care of the Clinical Investigators who are clinically indicated for surgical resection as a suspected VS or recurrent VS will be considered eligible for participation in the study. Investigators will determine if patients are eligible for the study during clinic appointments. Each potential participant will be assessed against the inclusion and exclusion criteria. Potential participants shall be provided with plain language information about the study on the IRB-approved consent form and will have the opportunity to ask questions of the study investigators. Informed consent shall be obtained by the Principal Investigator,Co-Investigators, or research coordinator from eligible patients wishing to participate. Any patient who chooses not to participate in the study will undergo their indicated microsurgery with standard techniques, and their health care will not be compromised in any way. Participants shall be free to withdraw from the study at any time. Participants will be withdrawn if their intraoperative biopsy yields an entity other than VS.

7.3. Preparation and Administration of Study Drug

The preparation and administration of fluorescein sodium is coordinated and executed by the anaesthesiologist responsible during the surgery according to dosage and administration recommended indicated by the product insert.

7.4. Subject Compliance and Adverse Event Monitoring

7.4.1. Adverse Event Monitoring

Adverse events (AEs) must be reported on the Adverse Event Form (AEF). Adverse events that will be monitored include urticaria, anaphylaxis, respiratory compromise, cardiac events, seizure, and death. These events will be monitored in the first 3 months after surgery. Complete clearance of SF is expected within 72 hours, and associated adverse events, if any, are anticipated within that timeframe. After discharge from the hospital, any reporting of adverse events will rely upon participants contacting the research team prior to the scheduled 3 month follow up. Nausea and vomiting will not be included as an adverse event as these symptoms are expected outcomes from surgery.

If adverse events occur, the first concern will be the safety and welfare of the patient. Appropriate medical intervention will be made. Any adverse events observed by the Investigator or reported by the patients will be recorded in the appropriate section of the patient's Case Report Form (CRF) as well as on an Adverse Event Form.

The Investigator must categorize each AE by degree of harm to the patient (mild, moderate, or serious), and the relationship to study device (not related, possibly, probably, or definitely) in the Adverse Event Form. Adverse events observed will be captured on the Adverse Event Follow-up Form and the CRF until such time the adverse event is reported as resolved, or for the course of the study if the event does not resolve.

A serious adverse event includes any prolong hospitalization, and any event that is life-threatening, results in permanent impairment of a bodily function or permanent damage to a body structure, or an event that necessitates medical or surgical intervention to preclude such permanent impairment or permanent damage.

Changes in any chronic condition or disease that are consistent with natural disease progression are not considered AEs and will not be recorded on an AE CRF.

Medical treatment related to any adverse events will not be paid for by the study, Mayo Clinic, or the Sponsor. Patients and their insurance will be billed for any treatments or encounters associated with an adverse event.

7.4.2. Adverse Event Reporting

Any serious adverse events, whether unanticipated or not, and whether or not ascribed to study device, will be communicated promptly to Carl Zeiss Meditec Inc and to the IRB. These reports must be confirmed in writing within 5 days of the occurrence.

7.5. Packaging

FS usually comes in a package of twelve vials. **Ak-fluor®** (fluorescein injection, USP) 10% is supplied in a single-use 5 mL glass vial with a gray bromobutyl serum siliconized stopper and orange flip-off cap. It contains a sterile, dark-reddish orange solution of fluorescein sodium.

7.6. Receiving, Storage, Dispensing and Return

7.6.1. Receiving of Drug Supplies

FS is currently available in the local pharmacy in the hospital. The supply during the course of this study will be coordinated as part of the regular drug supply in the OR

7.6.2. Storage

AK-Fluor® (fluorescein injection, USP) 10% will be temporarily storage at 2°- 25°C (36°- 77°F) in the sterile court for study cases in the OR.

7.6.3. Dispensing of Study Drug

The drug is currently available in the local pharmacy of the hospital. The Saint Mary's Hospital OR Pharmacy Managers will be informed of the need for drug in the OR during the IRB approval process. The OR pharmacy will dispense the drug to the anesthesiologist or certified nurse anesthetist participating in the case.

7.6.4. Return or Destruction of Study Drug

Fluorescein residual in a vial that was not used during the study procedure will be destructed.

8. Clinical Investigation and Documentation

8.1. Requirements before Starting the Clinical Investigation

- CIP written and signed
- Approval of ethics/IRB committee obtained

8.2. Training and Information for Clinical Investigators

The sponsor shall provide training for clinical investigators covering:

- YE560 fluorescence features available on the microscope
- YE560 module set up
- Turn on/off the YE560 module during surgery
- Contrast agents and clinical workflows
- Recording videos and capturing pictures during surgery
- Software operation
- Reviewing, managing and backing up procedure data (e.g. videos pictures)

8.3. Investigator and Sponsor File Maintained

- Clinical Investigation Plan
- CV for each clinical investigator
- Ethics committee approval and correspondence
- Correspondence with authorities required by national legislation
- Agreement between investigators and sponsor
- Insurance certificates
- Informed consent forms and information provided to subjects
- Case report forms
- Forms for reporting adverse events and adverse device effects
- Names and contact addresses of the monitor

8.4. Final Report

- Signed principal clinical investigator
- Available to all clinical investigators and the ethics committee
- Includes thorough identification of device
- Description of the methodology
- Design of clinical investigation
- Summary of the Clinical Investigation Plan and any deviations
- Data analysis with any statistical analyses (if applicable)
- Critical appraisal relating to aims of the investigation

APPENDIX 1

Sponsor's Obligations

Responsibilities of Sponsors

General responsibilities of Sponsors

Sponsors are responsible for providing investigators with the information they need to conduct the investigation properly.

Specific responsibility of Sponsors

1. *Obtaining agreements*. A Sponsor shall obtain from each participating Investigator a signed agreement that includes:

A statement of the Investigator's commitment to:

I. Conduct the investigation in accordance with the agreement, the investigational plan, this part and other applicable FDA regulations, and conditions of approval imposed by the reviewing IRB or FDA;

II. Supervise all testing of the device involving human subjects; and

III. Ensure that the requirements for obtaining informed consent are met (21CFR Part 50).

Sponsor Records

A Sponsor shall maintain the following accurate, complete, and current records relating to an investigation for a period of 3 years after completion of the study:

1. All correspondence with an Investigator, an IRB, or FDA, including required reports.
2. Records of shipment. Records of shipment shall include the name and address of the consignee, type and quantity of device, date of shipment, and serial number.
3. Signed Investigator agreements.
4. Records concerning adverse device effects (whether anticipated or unanticipated) and complaints.
5. Any other records that FDA requires to be maintained by regulation or by specific requirement for a category of investigation or a particular investigation.

Sponsor Reports

A Sponsor shall prepare and submit the following complete, accurate, and timely reports:

1. *Unanticipated adverse device effects.* A Sponsor who conducts an evaluation of an unanticipated adverse device effect shall report the results of such evaluation to FDA and to all reviewing IRBs and participating Investigators within 10 working days after the Sponsor first receives notice of the effect. Thereafter the Sponsor shall submit such additional reports concerning the effect as FDA requests.
2. *Withdrawal of IRB approval.* A Sponsor shall notify FDA and all reviewing IRBs and participating Investigators of any withdrawal of approval of an investigation or a part of an investigation by a reviewing IRB within 5 working days after receipt of the withdrawal of approval.
3. *Withdrawal of FDA approval.* A Sponsor shall notify all reviewing IRBs and participating Investigators of any withdrawal of FDA approval of the investigation, and shall do so within 5 working days after receipt of notice of the withdrawal of approval.
4. *Progress reports*. At regular intervals, and at least yearly, a Sponsor shall submit progress reports to all reviewing IRBs.

APPENDIX 2

Investigator's Agreement and Obligations

Responsibilities of Investigators

General responsibilities of Investigators

An Investigator is responsible for ensuring that an investigation is conducted according to the signed agreement, the investigational plan and applicable FDA regulations, for protecting the rights, safety, and welfare of subjects under the Investigator's care, and for the control of devices under investigation. An Investigator also is responsible for ensuring that informed consent is obtained in accordance with 21 CFR part 50.

Study staff will meet on a quarterly basis to debrief and assess progress, with email contact in the interim as needed. Meeting outcomes will be documented and available to study staff.

Specific responsibilities of Investigators

1. *Awaiting approval.* An Investigator may determine whether potential subjects would be interested in participating in an investigation, but shall not request the written informed consent of any subject to participate, and shall not allow any subject to participate before obtaining IRB approval.
2. *Subject Qualification.* The Investigator is responsible for ensuring that all subjects entering the study conform to the patient selection criteria.
3. *Compliance.* An Investigator shall conduct an investigation in accordance with the signed agreement with the Sponsor, the investigational plan, all applicable FDA regulations, and any conditions of approval imposed by an IRB or FDA.

Investigator Records

A participating Investigator shall maintain the following accurate, complete, and current records relating to the Investigator's participation in an investigation for a period of 3 years after completion of the study:
1. All correspondence with another Investigator, an IRB, the Sponsor, or FDA, including required reports.
2. Records of each subject's case history and exposure to the device. Case histories include the case report forms and supporting data including, for example, signed and dated consent forms and medical records. Such records shall include:
a) Documents evidencing informed consent.
b) All relevant observations, including records concerning adverse device effects (whether anticipated or unanticipated), information and data on the condition of each subject upon entering, and during the course of, the investigation, including information about relevant previous medical history and the results of all diagnostic tests.
3. The protocol, with documents showing the dates have and reasons for each deviation from the protocol.
4. Any other records that FDA requires to be maintained by regulation or by specific requirement for a category of investigations or a particular investigation.

Investigator Reports

The Investigator shall prepare and submit the following complete, accurate, and timely reports:
1. *Unanticipated adverse device effects.* An Investigator shall submit to the Sponsor and to the reviewing IRB a report of any unanticipated adverse device effect occurring during an investigation as soon as possible, but in no event later than 10 working days after the Investigator first learns of the effect.
2. *Withdrawal of IRB approval.* An Investigator shall report to the Sponsor, within 5 working days, withdrawal of approval by the reviewing IRB of the Investigator's part of an investigation.
3. *Deviations from the investigational plan*. An Investigator shall document report to the Sponsor any deviation from the investigational plan.
4. *Informed consent.* If an Investigator enrolls a subject without obtaining informed consent, the Investigator shall report such use to the Sponsor and the reviewing IRB within 5 working days after the use occurs.
5. *Final report.* The Investigator shall complete a final report.
6. *Other.* The Investigator shall, upon request by a reviewing IRB, provide accurate, complete, and current

information about any aspect of the investigation.

APPENDIX 3

Surgeon Survey

MRN _____ **Date**_____

Survey Respondent's Specialty (circle one): Neurologic Surgery / Neurotology

Fluorescein Sodium (FS) Dose: _____ mg/kg IV

*For the following questions, please record the **time stamp** at which the event occurred (e.g. 9:30 am):*

1. Fluorescein Sodium Administered: _____ ; corresponding event (e.g. opening dura):_____
   a. If second dose given, time administered: _____, dose _____ mg/kg
   b. If third dose give, time administered:_____, dose_____mg/kg
2. Tumor first visualized: _____
3. Did the contrast between the tumor and normal tissue **change after initial visualization of the tumor?**

   No change in Contrast over Time / Increased Contrast over Time / Decreased Contrast over Time

4. If there was a change in contrast over time, **when did peak fluorescence** occur such that there was maximum contrast between the tumor and normal tissue? _____
5. Did the tissue become **saturated** with fluorescein such that there was no difference in the fluorescence of nerve and tumor? Yes / No
   a. If so, when did saturation occur? _____
6. When did the fluorescence diminish such that it was no longer useful _____ / Not Applicable

For the following questions, please circle one number in the boxes below.

7. How well did fluorescence **correlate with your visual assessment** of the tumor and nerve tissue under normal microscopy without the Yellow 560 filter?

No correlation	Some correlation	Good correlation	Excellent correlation
1	2	3	4

8. How well did fluorescence **correlate with electrostimulation** (e.g. tissues with high fluorescence do not stimulate, whereas nerve tissues with low fluorescence do stimulate)?

No correlation	Some correlation	Good correlation	Excellent correlation
1	2	3	4

9. **How helpful was the use of FS and Yellow 560 filter in….** (circle a rating for each row)

	Not helpful at all	Minimally helpful	Somewhat helpful	Very helpful	N/A
Distinguishing tumor from normal nerve	1	2	3	4	0
Preserving facial nerve function	1	2	3	4	0
Preserving hearing	1	2	3	4	0
Detecting residual tumor	1	2	3	4	0

10. Based on the use and performance of FS in this case alone, **how likely are you to use** FS in future cases involving this tumor type (excluding the research trial)?

Unsure	Plan to not use in any cases	Plan to use in select cases	Plan to use in the majority of cases	Plan to use in all cases
0	1	2	3	4

Comments for the research team: _____