Fakultät für Medizin

Klinikum rechts der Isar Technische Universität München

INVIVO

Comparison of Fluorescein-**IN**tra-**VI**tal microscopy **V**ersus conventional frozen section diagnosis for intra**O**perative histopathological evaluation (**INVIVO**)

Vergleich der Fluorescein-INtra-VItal-Mikroskopie mit der konventionellen Gefrierschnittdiagnose für die intraoperative histopathologische Beurteilung (INVIVO)

Phase II Clinical Trial

Investigational Medicinal Product:

Fluorescein sodium (Alcon®)

Study Code: INV-GEM-0200-I

EudraCT Number: 2019-004512-58

First Patient First Visit: 30.11.2020 **– Last Patient Last Visit:** 17.06.2022

Termination of Clinical Trial: 17.06.2022 (LPLV)

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Version: 1.0, Date: 15.06.2023

Synopsis

1.	**Sponsor:** Technische Universität München (TUM), Fakultät für Medizin Ismaninger Straße 22, D- 81675 München, Germany **Sponsor Delegated Person (SDP), Leiter Klinische Prüfung (LKP):** Prof. Dr. med. Jens Gempt
2.	**Name of Finished Product:** Fluorescein (Alcon®) product approved in Germany to be used according to clinical routine at the clinical trial sites
3.	**Name of Active Ingredient:** Fluorescein sodium
4.	**Individual Study Table:** (only required for submissions) NAP
5.	**Study Title:** Comparison of Fluorescein-INtra-VItal microscopy Versus conventional frozen section diagnosis for intraOperative histopathological evaluation (INVIVO)
	Study Design: This is an investigator-initiated, prospective, multi-center, single arm, off-label, phase II, non-inferiority clinical trial. All patients will receive Fluorescein 20-40 min prior to tumor resection and will have both in-vivo microscopic histological analysis and intraoperative frozen section histological analysis. Accuracy of in-vivo and frozen section will be calculated using the final histological result.
	Study (Protocol) Code Number: INV-GEM-0200-I
	Eudra-CT Number: 2019-004512-58
6.	**Principal Investigators (PI):** # 1: Prof. Dr. med. Jens Gempt # 2: Prof. Dr. med. Karl Schebesch # 3: Dr. med. Frederik Enders
7.	**Clinical Trial Sites:** The clinical trial was planned and conducted as a multi-centre clinical trial. **# 1**: Technische Universität München, Klinik und Poliklinik für Neurochirurgie Ismaninger Str. 22 D- 81675 München, Germany **# 2**: Universitätsklinikum Regensburg Franz-Josef-Strauß-Allee 11 D-93053 Regensburg, Germany **# 3**: Universitätsklinikum Mannheim Theodor-Kutzer-Ufer 1-3 D-68167 Mannheim, Germany
8.	**Publication**:
9.	**Study period**

	First patient first visit (FPFV): 30.11.2020; Last patient included: 13.06.2022, Last patient last visit (LPLV): 17.06.2022
	Approvals and Amendments **Approval:** Bundesinstitut für Arzneimittel und Medizinprodukte (BfArM): 25.06.2020 (No. 4044033); Ethics Committee (EC): 17.08.2020 (No. 75/20-Af) Clinical Study Protocol (CSP) Version (V) 1.0 from 27.01.2020 **Amendment 1**: EC: response to the deficiency letter from BfArM (dated 26.03.2020) and associated adjustment of the study protocol (Version 2.0_AM1 from 20.08.2020). Approval of AM1: EC: 21.09.2020, Submission of study protocol (Version 2.0_AM1 from 20.08.2020) to BfArM (information about already approved changes, but new study protocol version) During the course of the clinical trial BfArM/EC were informed of further changes (e.g. changes of PI site Mannheim). BfArM: cover letter 07.05.2021 EC: cover letter: 07.05.2021, Approval 10.06.2021.
10.	**Phase of development** Phase II
11.	**Objectives** **Primary Objective:** The primary objective of the clinical trial is to compare the significance of intravenously applied fluorescein as staining agent for assessment of brain tissue texture via in-vivo confocal microscopy with the conventional intraoperative histological frozen section analysis of identical brain tissue samples in the same patient. Both methods will be compared in terms of their accuracy using the standard of practice, the final pathological diagnosis (immunochemistry/molecular pathology). **Secondary Objectives:** - To assess the safety of the IMP as staining agent for in-vivo confocal microscopy by documentation of adverse events during surgery and until end of follow-up period. - Analysis of surgical time span: comparison of the time span to conduct the assessment of brain tissue texture with fluorescein intervention with the time span to conduct assessment of conventional intraoperative frozen section intervention.
12.	**Background/Methodology** Existing studies permit no valid conclusion that the use of intraoperative fluorescein-staining with in-vivo microscopy provides a sufficient diagnostic yield compared to conventional histopathology by frozen section evaluation in patients undergoing brain tumor resection. Fluorescein-staining combined with in-vivo microscopy of tissue to be resected would allow for a real-time feedback as to the histopathological entity. The time-consuming fixation and transport of tissue for histopathological work-up would be removed from the process of surgery, while facilitating a preliminary histopathological assessment. The aim of the clinical trial is to evaluate if Fluorescein-staining combined with in-vivo microscopy and evaluation by a neuropathologist is not inferior to routine frozen section analysis of brain tissue. By in-vivo microscopy, a reliable and time-saving instrument for the preliminary histopathology would be available. As a consequence, in future, a reduction of operative times would be possible, while maintaining a high diagnostic accuracy and atraumatic appliance of the novel method. Achieving a precise diagnosis during neurosurgical resection of intracranial tumors provides crucial information in real-time, possibly altering the course of surgery. Depending on the intraoperative in-vivo histopathological result, the surgeon might then adapt their surgical strategy and goals of resection without resorting to waiting for the frozen section analysis.

	Furthermore, this method can result in a further step towards telemedicine, as the intraoperatively acquired data can be made available online.
13.	**Sample size (planned/analysed):** **Planned:** To be assessed for eligibility (n): 255 To be assigned to the trial (n): 200 To be analyzed (n): 192 Sample size calculation is based on methods proposed by Lu et al. (Lu et.al 2003): Assuming that the maximum proportion of false-diagnoses of confocal microscopy is 2% and both diagnostic procedures have the same accuracy, N=192 patients will be needed to show non-inferiority of fluorescein-staining of brain tissue by confocal microscopy compared to the routine frozen section histopathology process in terms of accuracy when the non-inferiority margin Δ is 0.05, with a power of 90% and significance level of 5%. We aim to obtain a sample size of N=200 patients (with 8 additional patients) to ensure enough power in case of dropouts. **Included / analysed:** 210 patients were included in the clinical trial. Analysis was performed on three different sets of patients: FAS (n=203), PP (n=202), and SA (n=205).
14.	**Patient Population (Diagnosis):** Intracranial tumor; Patients with a diagnosed cerebral mass lesion scheduled for resection; ICD-10 classification R90.0
	Main criteria for inclusion • Age ≥18 years • Signed informed consent • Suspected intracranial tumor revealed by cranial magnetic resonance imaging (according to clinical routine) scheduled for resection with intraoperative frozen section evaluation **Main criteria for exclusion** • Known allergic or suspected allergic reactions to fluorescein sodium • Liver disease, CHILD B or C • Patients under medication with beta-blockers, digoxin, chinidin and probenecid as well as inhibitors of glucuronidation, such as immunosuppressants, when the medication must not be discontinued perioperatively • Patients with relevant congenital limitations of glucoronidation performance (e.g. Rotor syndrome, Gilbert-Meulengracht syndrome, Crigler-Najjar syndrome) • Patients with terminal renal failure requiring hemodialysis • Inability to provide informed consent • Pregnancy (incl. positive pregnancy test) • Women of childbearing age must be non-lactating and surgically sterile or using a highly effective method of birth control and have a negative pregnancy test. Acceptable methods of birth control with a low failure rate (i.e. less than 1% per year) when used consistently and correct are such as implants, injectables, combined oral contraceptives, hormonal intrauterine devices (IUDs), sexual abstinence or vasectomized partner.
15.	**Test product, dose and mode of administration** **Study treatment:** Fluorescein sodium (Alcon®) 10% Injection Solution, i.v. application as a staining agent (5mg/kg body weight). **Batch-No. (Ch.-B):**

	NAP (routine medication)
16.	**Duration of administration** One single intravenously application 20-40 minutes before tumor resection as a staining agent.
17.	**Background therapy:** Standard of care **Comparator: NAP**
	Blinding: NAP
18.	**Criteria for evaluation:** **Primary endpoint:** To show non-inferiority of fluorescein staining before in-vivo confocal microscopy to histological frozen section in terms of accuracy accessed using the gold standard, post-surgery white light microscopy with immunochemistry. **Secondary endpoints:** • Adverse events (AE) that have been recorded during surgery and until end of the follow-up period • Surgical time span to conduct the assessment of brain tissue texture with fluorescein sodium intervention and conventional frozen section are recorded with start and stop time, which are used to calculate duration. The cut point of 10 minutes will be used to classify the duration in "< 10 min" or "≥ 10 min".
	Efficacy: Accuracy of Convivo with fluorescein staining and histological frozen section accessed using the gold standard, post-surgery white light microscopy with immunochemistry. Accuracy is defined as the number of correctly classified over the total number of examined patients.
	Safety assessments The assessment of safety was based on the frequency of AE/SAE within the safety population (according to CTCAE Version 4.03), consisting of all patients who received at least one dose of IMP.
19.	**Statistical methods:** **Populations for analysis** All efficacy analyses are performed on the full analysis set (FAS), consisting of all patients who entered the clinical trial and received Fluorescein and had surgery. Patients with missing primary endpoint assessments are also excluded from this analysis set. The per-protocol set (PPS) consists of all patients in the FAS who have no major protocol deviations, as identified prior to database lock. The safety analysis set (SA) consists of all patients who received at least one dose of IMP. Adverse events are analyzed on the safety set. All other analyses are performed on the FAS. Analysis of the primary endpoint are additionally performed on the PPS, as it is more conservative in the non-inferiority setting. Missing values are not imputed in this clinical trial. **Statistical analysis** Description of the primary efficacy analysis:

	Primary endpoint of the clinical trial is to show non-inferiority of fluorescein staining before in-vivo confocal microscopy over histological frozen section in terms of accuracy accessed using the gold standard, post-surgery white light microscopy with immunochemistry. Accuracy is defined as the number of correctly classified over the total number of examined patients. The diagnostic accuracies of the individual methods used for preliminary intraoperative histopathological diagnosis (D1: fluorescein sodium with in-vivo microscopy versus D2: preliminary frozen section assessment) is calculated using the gold standard, the post-surgery white light microscopy with immunochemistry. The non-inferiority of D1 over D2 in terms of accuracy is tested using the following statistical hypotheses: H_0: Ac_{D1} - Ac_{D2} < - 0.05 vs. H_A: Ac_{D1} - Ac_{D2} ≥ - 0.05, where Ac_{D1} and Ac_{D2} denote the accuracy using diagnostic test D1 and D2 respectively. The non-inferiority margin Δ is set to 0.05. The two-sided 95% CI for AcD1 - AcD2 is calculated and non-inferiority is shown if the lower limit of that confidence interval is greater than or equal to – Δ. Non-inferiority needs to be shown both in FAS and PPS in order to have conclusive results. The PPS is the more conservative one in this setting. Missing data: Only the complete case analysis is performed. No data imputations are done. Secondary endpoint(s): All AEs are coded using MedDRA Version English 24.0. The absolute frequencies of events and the absolute and relative frequencies of subjects affected are reported by preferred term and system organ class for all SAEs and non-serious AEs. Further, the absolute and relative frequencies of SAEs are given by category of severity, relationship to IMP, relationship to device, and outcome. No relationship to the IMP or the device (confocal microscopy) was reported. The duration for the two procedures are summarized using descriptive statistics and compared using the Wilcoxon signed ranks test for related samples. The categorical duration (</≥ 10 min) is presented using absolute and relative frequencies and compared between procedures using the McNemar test for related categorical variables. All tests are two-sided with an exploratory significance level of 5%.
20.	**Summary - Conclusions:** **Patient demographics and patient disposition** In total 210 patients were included in the clinical trial. (FPFV: 30.11.2020; LPFV: 13.06.2022; LPLV: 17.06.2022). The full analysis set (FAS) consisted of 203 patients. Reasons for exclusion from the FAS were no surgery (n=4, 1.9%) and no frozen section results (n=3, 1.4%). Most patients in the FAS were recruited in Munich (n=117, 57.6%) and Regensburg (n=69, 34.0%). Mannheim study center recruited 17 FAS patients (8.4%). The per-protocol set consisted of 202 patients, one less than the FAS, excluded due to a major protocol deviation. The safety analysis set (SA) consisted of 205 patients. Reason for exclusion from the SA was no IP administration (n=5, 2.4%). From the 203 patients in the FAS, 201 completed the clinical trial. One (0.5%) died during the clinical trial and 1 (0.5%) was lost to follow-up. Summary tables 1 and 2 in the appendix give an overview. The FAS included 119/203 (58.6%) adult (18-64 years), 81/203 (39.9%) elderly (65-84 years), and 3/203 (1.5%) senior (85 years and older) patients. The median age was 61 years [range: 22 – 87]. The FAS included 112/203 (55.2%) female and 91/203 (44.8%) male patients. Distributions of relevant demographics at baseline are given in Table 3 (Appendix). **Medical history and comorbidities** Almost ¾ of the patients in the FAS had at least one concomitant disease (n=147, 72.4%). The median number of concomitant diseases per patient was 1 and ranged from 0 to 8. The number of patients in the FAS who had previous intracranial surgeries was 42/203 (20.0%). Patients with previous intracranial treatment were 47/203 (22.4%). A total of 33 patients in the FAS had a recurrence (15.7%). See also Table 4 of the Appendix.

	Compliance: Inclusion/Exclusion criteria There were no deviations from the inclusion/exclusion criteria. Investigational medicinal product (IMP): The mean dose of Fluorescein was 5mg/kg with a SD of 0.10mg/kg. Patient compliance to study treatment was not an issue, as patients were given the IMP shortly before surgery, and treatment administration was performed by the surgeon. A total of 205 patients received IMP. Table 5 of the appendix gives a summary of the IMP dosing on the FAS. Protocol Deviation (PD): A total of 109 PD were reported for this clinical trial, 5 of which were rated as major. For 3 patients frozen section was not available during surgery and for 2 patients there were technical issues with the Convivo microscopy. Most of the minor PD concerned Informed Consent (being on the day of surgery or more than 14 days before surgery) or incorrect order of frozen section and Convivo microscopy. A summary of all PD can be found in Table 6 of the appendix. **Safety Assessments (all patients included)** Annual Safety Reports have been provided to BfArM and EC for the following periods: DSUR 1: 25.06.2020 – 24.06.2021 DSUR 2: 25.06.2021 – 24.06.2022 Adverse Events and Serious Adverse Events were classified according to CTCAE V. 4.03 and coded according to MedDRA V. 24.0 English.
	Safety Results (AE, SAE, SUSAR) The number and severity of AEs and SAEs were consistent with the patient population and none of the SAEs were related to the IMP or the device. **Adverse Events (AE)** A total of 121 AEs were reported in 57/205 (28%) patients (see Table 7, Appendix). Overall, 63/121 (52%) AEs were deemed possibly related to the medical device (ADR, while 61/121 (50%) of the AEs were rated as possibly related to the IMP (AR). 75/121 (62%) AEs were rated Grade 1 (mild), 42/121 (35%) Grade 2 (moderate), 2/121 (2%) Grade 3 (severe), none Grade 4 (life-threatening), and 2/121 (2%) were rated Grade 5 (death). **Serious AE (SAE)** A total of 6 SAEs in 4/205 (2%) patients were recorded in the study database. Table 8 in the Appendix summarizes all SAEs using MedDRA System Organ Class and Preferred Terms. One patient experienced CNS ventriculitis and meningitis while 3 patients experienced one or more of the following nervous system disorders: brain oedema, cerebral haemorrhage, cerebral infarction, and epilepsy. The SAE severity was classified according to CTCAE. The severity and outcome of SAEs is summarized in tables 9 and 10 of the Appendix. One patient died during the clinical trial due to two serious adverse events. **Suspected Serious Adverse Reactions (SAR)** No SAR was reported in the clinical trial. **Suspected Unexpected Serious Adverse Reactions (SUSAR)** No SUSAR was reported in the clinical trial. **Non-serious Adverse Events (AE)**

	A total of 115 non-serious AEs on 53/205 (26%) patients were reported during the clinical trial (see Appendix Tables 11 for a summary by MedDRA System Organ Class and Preferred Term).
	Efficacy Results **Primary Endpoint** Fluorescein staining before in-vivo confocal microscopy was tested for non-inferiority over histological frozen section in terms of accuracy accessed using the gold standard, post-surgery white light microscopy with immunochemistry, and using the non-inferiority margin of 0.05. The accuracy of Convivo intraoperative vs. final histopathological diagnosis was 0.8670 on the FAS (Table 12 of the appendix) and 0.0663 on the PPS (Table 13 of the appendix). The accuracy of frozen section intraoperatively vs. final histopathological diagnosis was 0.9064 on the FAS and 0.9059 on the PPS (Tables 14 and 15 of the appendix respectively). The difference between the Convivo and frozen section accuracies on the FAS was -0.0394 with a 95% CI (-0.1009, 0.0221). The difference between the Convivo and frozen section accuracies on the PPS was -0.0396 with a 95% CI (-0.1013, 0.0221). In both analysis sets the lower limit of the 95% CI lies below -0.05. Therefore, non-inferiority could not be shown (see also Table 16 of the appendix). **Secondary Endpoints** The analysis of AE is described in section Safety Results. The duration of assessment of brain tissue texture with Convivo microscopy and with conventional frozen section were calculated and given in table 17 of the appendix. The mean entire duration of surgery in the study was 189 minutes with a SD of 79 minutes. During this time the Convivo assessment took a median of 3 minutes with a range from 1 to 15 minutes, while the duration of the frozen section assessment took in median 27 minutes with a range from 10 to 110 minutes. The Wilcoxon signed ranks test for related samples comparing the duration of the two assessments was highly statistically significant ($p<0.001$). The cut point of 10 minutes was used to classify the duration in “< 10 min” or “≥ 10 min”. Only one of the 195 available time measurements for the Convivo procedure took more than 10 minutes while all of the 201 available time measurements for the frozen section procedure were longer than 10 minutes. The McNemar test for related categorical variables could not be performed due to the frequency of 0 in one of the groups. Additionally, the Karnofsky Index was recorded on Day 1 and between Day 4 and Day 10 post surgery. The median increased by 10 in the later measurement (see table 18 of the appendix).
	Overall Conclusion: In our study, the number and severity of AEs and SAEs were consistent with the patient population and none of the SAEs were related to the IMP or the device. For the primary endpoint, the accuracy of the Convivo assessment amounted to 87% versus 91% for the frozen section, which is a significant difference. The Convivo procedure lasted a median 3 minutes in contrast to 27 minutes for frozen section making the Convivo microscope a safe and easy to use instrument for the intraoperative microscopic assessment of cerebral tissue and tumors.

APPENDIX

Table 1: Populations for analysis

	N	*(%)*
Included in study	210	
Reason for exclusion from FAS:		
No surgery	4	
No frozen section results	3	
Included in FAS	203	
München, MRI (1)	117	(57.6%)
Regensburg (2)	69	(34.0%)
Mannheim (3)	17	(8.4%)
Reason for exclusion from PPS:		
Excluded from FAS	7	
Major protocol deviation	1	
Included in PPS	202	
Reason for exclusion from SA:		
No fluorescein administered	5	
Included in SA	205	

Table 2: Patient disposition (FAS)

	FAS (N = 203)	
Completed study (n, %)	201	(99.0%)
Total deaths (n, %)	1	(0.5%)
During study	1	(0.5%)
After withdrawal	0	
Withdrawn (n, %) ~		
Lost to follow-up	1	(0.5%)

~ For reasons other than death

Table 3: Demographics and Baseline Characteristics

	FAS (N = 203)	*PPS (N=202)*
Sex (n, %)		
Female	112 (55.2%)	112 (55.5%)
Male	91 (44.8%)	90 (44.6%)
Age group (n, %)		
Adults (18 - 64 years)	119 (58.6%)	118 (58.4%)
Elderly (65 – 84 years)	81 (39.9%)	81 (40.1%)
Senior (85 years and over)	3 (1.5%)	3 (1.5%)
Age (years)		
Mean	58.1	
Std	15.9	
Min	22	
Median	61	
Max	87	
Height (cm)		
Mean	170.5	
Std	9.3	
Min	150	
Median	170	
Max	193	
Weight (kg)		
Mean	75.9	
Std	17.4	
Min	40	
Median	73	
Max	150	

Table 4: Medical history and comorbidities

	FAS (N = 203)
Number of ongoing comorbidities (median [min – max])	1 [0 – 8]
Previous intracranial surgeries (n, %)	42 (20.0%)
Previous intracranial treatment (n, %)	47 (22.4%)
Recurrence (n, %)	33 (15.7%)

Table 5: Fluorescein dose (FAS)

	N	*Mean*	*Std*	*Min*	*Median*	*Max*
Fluorescein Dose (mg)	203	379.43	87.43	200	365	750
Fluorescein Dose (mg/kg)	203	5.00	0.10	4.29	5	5.42

Table 6: Protocol deviations (PD)

	All study participants (N = 210)
Minor PD (n, %)	104 (49.5%)
Pregnancy test	8 (3.8%)
Informed Consent on the day of surgery or more than 14 days before surgery	31 (14.8%)
Fluorescein time window deviation	14 (6.7%)
Fluorescein dose deviation	8 (3.8%)
Incorrect order of frozen section and Convivo microscopy	30 (14.3%)
Karnowsky Index time window deviation	13 (6.2%)
Major PD (n, %)	5 (2.4%)
Frozen section not available	3 (1.4%)
Technical issues with Convivo microscopy	2 (1.0%)

Table 7: Adverse events summary

	Exposed to Fluorescein N=205		
		Subjects affected	
	Events	*n*	*(%)*
All AEs	121	57	(28)
SAE	6	4	(2)
Non-SAE AEs	115	53	(26)

Table 8: SAEs.

	Exposed to Fluorescein N=205		
		Subjects affected	
System Organ Class Preferred Term	*Events*	*n*	*(%)*
Total SAE	6	4	(2)
Infections and infestations	2	1	(0)
CNS ventriculitis	1	1	(0)
Meningitis	1	1	(0)
Nervous system disorders	4	3	(1)
Brain oedema	1	1	(0)
Cerebral haemorrhage	1	1	(0)
Cerebral infarction	1	1	(0)
Epilepsy	1	1	(0)

Table 9: Severity of SAEs.

	Exposed to Fluorescein N=205	
		Subjects affected
System Organ Class Preferred Term	*Events*	*n (%)*
Total SAE	6	4 (2)
Severity		
Mild	0	0
Moderate	3	2 (1.0)
Severe	1	1 (0.5)
Life-threatening	0	0
Death	2	1 (0.5)

Table 10: Outcome of SAEs.

	Exposed to Fluorescein N=205	
		Subjects affected
System Organ Class Preferred Term	*Events*	*n (%)*
Total SAE	6	4 (2.0)
Outcome		
Not recovered/not resolved	0	0
Recovered/resolved	4	3 (1.5)
Recovering/resolving	0	0
Recovered/resolved with sequelae	0	0
Fatal	2	1 (0.5)
Unknown	0	0

Table 11: Non-SAE AEs.

System Organ Class *Preferred Term*	*Exposed to Fluorescein N=205* *Events*	*Subjects affected* *n*	*(%)*
Total non-SAE AEs	**115**	**53**	**(25)**
Eye disorders	**16**	**15**	**(7)**
Blindness unilateral	1	1	(0)
Eye swelling	8	8	(4)
Pupils unequal	1	1	(0)
Vision blurred	1	1	(0)
Visual impairment	5	5	(2)
Gastrointestinal disorders	**35**	**25**	**(12)**
Melaena	1	1	(0)
Nausea	22	22	(10)
Swollen tongue	1	1	(0)
**Vomiting*	*11*	*11*	*(5)*
General disorders and administration site conditions	**2**	**2**	**(1)**
Pain	1	1	(0)
Swelling	1	1	(0)
Infections and infestations	**4**	**4**	**(2)**
CNS ventriculitis	1	1	(0)
Infection	1	1	(0)
Post procedural infection	1	1	(0)
Urinary tract infection	1	1	(0)
Investigations	**3**	**3**	**(1)**
Blood pressure increased	1	1	(0)
Body temperature increased	1	1	(0)
Pupillary light reflex tests abnormal	1	1	(0)
Musculoskeletal and connective tissue disorders	**5**	**5**	**(2)**
Arthralgia	1	1	(0)
Back pain	2	2	(1)
Neck pain	1	1	(0)
Pain in extremity	1	1	(0)
Nervous system disorders	**45**	**35**	**(17)**
Aphasia	1	1	(0)
Brain oedema	1	1	(0)
Cerebral venous sinus thrombosis	3	3	(1)
Dizziness	7	7	(3)

System Organ Class *Preferred Term*	*Exposed to Fluorescein N=205* *Events*	*Subjects affected* *n (%)*
Facial paresis	4	4 (2)
**Headache*	*25*	*24 (11)*
Hemiparesis	2	2 (1)
Hypoaesthesia	2	1 (0)
Psychiatric disorders	**2**	**2 (1)**
Claustrophobia	1	1 (0)
Depressed mood	1	1 (0)
Surgical and medical procedures	**1**	**1 (0)**
Tumour excision	1	1 (0)
Vascular disorders	**2**	**2 (1)**
Hypertension	2	2 (1)

** Preferred terms with more than 5% frequency*

Table 12: Primary Endpoint: Accuracy of Convivo Intraoperative vs. Final Histopathological Diagnosis (FAS)

Actual frequency		*Final histological diagnosis*									
		Metas-tasis	*LGG*	*HGG*	*Menin-gioma*	*Schwan-noma*	*Ependy-moma*	*Reactive*	*Inflam-mation*	*other*	*Sum*
Convivo intraoperaive diagnosis	*Metas-tasis*	42	0	2	0	0	0	2	0	3	49
	LGG	0	5	1	0	0	0	0	0	3	9
	HGG	6	3	73	1	0	0	0	0	1	84
	Menin-gioma	0	0	1	44	1	0	0	0	0	46
	Schwan-noma	0	0	0	1	3	0	0	0	0	4
	Ependy-moma	0	0	0	0	0	2	0	0	0	2
	Reactive	0	0	0	0	0	0	1	0	0	1
	Inflam-mation	0	0	0	0	0	0	0	1	0	1
	other	1	1	0	0	0	0	0	0	5	7
	Sum	49	9	77	46	4	2	3	1	12	203

ACC = 176/203=0.8670

Table 13: Primary Endpoint: Accuracy of Convivo Intraoperative Histopathological Diagnosis (PPS)

Actual frequency		*Final histological diagnosis*									
		Metas-tasis	*LGG*	*HGG*	*Menin-gioma*	*Schwan-noma*	*Ependy-moma*	*Reactive*	*Inflam-mation*	*other*	*Sum*
Convivo intraoperaive diagnosis	*Metas-tasis*	42	0	2	0	0	0	2	0	3	49
	LGG	0	5	1	0	0	0	0	0	3	9
	HGG	6	3	72	1	0	0	0	0	1	83
	Menin-gioma	0	0	1	44	1	0	0	0	0	46
	Schwan-noma	0	0	0	1	3	0	0	0	0	4
	Ependy-moma	0	0	0	0	0	2	0	0	0	2
	Reactive	0	0	0	0	0	0	1	0	0	1
	Inflam-mation	0	0	0	0	0	0	0	1	0	1
	other	1	1	0	0	0	0	0	0	5	7
	Sum	49	9	76	46	4	2	3	1	12	202

ACC = 175/202=0.8663

Table 14: Primary Endpoint: Accuracy of Frozen Section Intraoperative vs. Final Histopathological Diagnosis (FAS)

Actual frequency		*Final histological diagnosis*									
		Metas-tasis	*LGG*	*HGG*	*Menin-gioma*	*Schwan-noma*	*Ependy-moma*	*Reactive*	*Inflam-mation*	*other*	*Sum*
Frozen section intraoperaive diagnosis	*Metas-tasis*	41	0	1	1	0	0	0	0	0	43
	LGG	0	7	0	0	0	0	0	1	0	8
	HGG	0	0	72	0	0	0	0	0	0	72
	Menin-gioma	0	0	0	45	0	1	0	0	0	46
	Schwan-noma	0	0	0	0	4	0	0	0	0	4
	Ependy-moma	0	0	0	0	0	1	0	0	0	1
	Reactive	0	1	2	0	0	0	2	0	0	5
	Inflam-mation	0	0	0	0	0	0	0	0	0	0
	other	8	1	2	0	0	0	1	0	12	24
	Sum	49	9	77	46	4	2	3	1	12	203

ACC = 184/203=0.9064

Table 15: Primary Endpoint: Accuracy of Frozen Section Intraoperative vs. Final Histopathological Diagnosis (PPS)

Actual frequency		*Final histological diagnosis*									
		Metas-tasis	*LGG*	*HGG*	*Menin-gioma*	*Schwan-noma*	*Ependy-moma*	*Reactive*	*Inflam-mation*	*other*	*Sum*
Frozen section intraoperaive diagnosis	*Metas-tasis*	41	0	1	1	0	0	0	0	0	43
	LGG	0	7	0	0	0	0	0	1	0	8
	HGG	0	0	71	0	0	0	0	0	0	71
	Menin-gioma	0	0	0	45	0	1	0	0	0	46
	Schwan-noma	0	0	0	0	4	0	0	0	0	4
	Ependy-moma	0	0	0	0	0	1	0	0	0	1
	Reactive	0	1	2	0	0	0	2	0	0	5
	Inflam-mation	0	0	0	0	0	0	0	0	0	0
	other	8	1	2	0	0	0	1	0	12	24
	Sum	49	9	76	46	4	2	3	1	12	202

ACC = 183/202=0.9059

Table 16: Primary endpoint: Non-inferiority of Convivo over Frozen section in terms of accuracy with respect to the final histological diagnosis (FAS, PPS)

	Accuracy			*95% CI*		*Non-inferiority shown?*
	Convivo	*Frozen section*	*Difference*	*Lower*	*Upper*	
FAS	0.8670	0.9064	-0.0394	-0.1009	0.0221	No
PPS	0.8663	0.9059	-0.0396	-0.1013	0.0221	No

Table 17: Duration of procedures (FAS)

	N	*(%)*	*Mean*	*Std*	*Min*	*Median*	*Max*
Duration of surgery (minutes)	203		188.7	79.0	36	188	438
Duration INVIVO assessment (minutes)	195		2.9	1.6	1	3	15
< 10 min	194	(99.5)					
>= 10 min	1	(0.5)					
Duration histological assessment (minutes)	201		30.5	14.5	10	27	110
< 10 min	0						
>= 10 min	201	(100)					
*Difference in duration of assessments (minutes)**	193		27.5	14.5	7	25	106

** Wilcoxon signed ranks test for related samples p < 0.001*

Table 18: Secondary endpoint: Karnofsky Index

	N	*Mean*	*Std*	*Min*	*Median*	*Max*
Post OP Day 1	201	79.4	16.6	20	80	100
Post OP Day 4 – 10	200	85.3	12.7	30	90	100