BRIEFING PACKAGE

NX DEVELOPMENT CORP

5-aminolevulinic acid hydrochloride (5-ALA HCl)

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5-ALA	5-aminolevulinic acid
5-ALA HCl	5-aminolevulinic acid hydrochloride
^{18}F-FET	^{18}F-fluoroethyl-L-tyrosine
$A_{e,ur}$	amount excreted in urine
AA	anaplastic astrocytoma
ADR	adverse drug reaction
AE	adverse event
ALT	alanine aminotransferase
AO	anaplastic oligodendroglioma
AUC	area under the plasma concentration time curve
BA	bioavailability
bw	body weight
CI	confidence internal
C_{max}	mean peak plasma concentration
CNS	central nervous system
CT	computed tomography
DF	dispersion factor
ECG	electrocardiogram
EMA	European Medicines Agency
EOR	extent of resection
EU	European Union
FAS	full analysis set
FDA	Food and Drug Administration
FET-PET	^{18}F-fluoroethyltyrosine-positron emission tomography
FGS	fluorescence guided surgery
FL group	fluorescence-guided resection group
FL	fluorescent light
FN	false negative
FP	false positive
GBM	glioblastoma multiforme
Gd	gadolinium
GeoMean	geometric mean
GGT	gamma-glutamyl transferase
HGG	high-grade glioma
iMRI	intraoperative magnetic resonance imaging
i.v.	intravenous

KPS	Karnofsky Performance Scale
LGG	low-grade glioma
MET	metastasis
MRI	magnetic resonance imaging
NDA	New Drug Application
NNT	number needed to treat
NPV	negative predictive value
NXDC	NX Development Corporation
OS	overall survival
PFS	progression free survival
PK	pharmacokinetics
p.o.	per os; by mouth or orally
PpIX	protoporphyrin IX
PPV	positive predictive value
PSUR	Periodic Safety Update Report
PT	preferred term
QTc	QT corrected
SAE	serious adverse event
SD	standard deviation
SOC	system organ class
$t_{1/2}$	plasma concentration half-life time
TEAE	treatment-emergent adverse event
T_{max}	time to reach C_{max}
TN	true negative
TP	true positive
U.S.	United States
WHO	World Health Organization
WL	white light
WL group	conventional resection group

1 EXECUTIVE SUMMARY

1.1 Glioma

A glioma is a type of primary tumor that mainly originates from the supportive glial cells in the brain. According to the American Brain Tumor Association, more than 79,000 new cases of primary brain tumors are diagnosed each year in the United States (U.S)., and close to 25% are gliomas (over 19,000). Gliomas can be nonmalignant (astrocytomas, oligodendrogliomas, and ependymomas) or malignant (glioblastomas [GBM], anaplastic oligodendrogliomas, and anaplastic ependymomas) based on their pathology. Approximately 80% of gliomas are malignant. There are few known risk factors for glioma, and these include prior exposure to ionizing radiation and certain genetic conditions. The initial signs and symptoms of glioma may include headache, nausea and/or vomiting, confusion, seizures, memory loss, vision loss or blurred/double vision, difficulty with speech or language, one-sided weakness, difficulty with walking or balance, and personality changes. The symptoms may vary based on the part of the brain in which the glioma is present and may be causing mass effect.

Patients who present with these symptoms are initially tested using magnetic resonance imaging (MRI) to evaluate whether a brain tumor is present and to characterize the tumor by imaging features. A conclusive diagnosis of glioma and the staging of the glioma are obtained following biopsy of the tumor and histopathological examination. Gliomas are graded based on the World Health Organization (WHO) classification with grade I tumors being the slowest growing and grade IV carrying the worst prognosis. Low-grade gliomas (LGG) are mainly WHO grade II tumors and include diffuse astrocytomas and oligodendrogliomas. High-grade gliomas (HGG) include WHO grade III (anaplastic astrocytomas, anaplastic oligodendrogliomas, and anaplastic ependymomas) and grade IV astrocytomas, also known as GBM. WHO grade III malignant gliomas are characterized by actively, reproducing abnormal cells that invade or infiltrate the adjacent normal brain tissue. The majority of WHO grade II tumors transform to a high-grade tumor with time. WHO grade III anaplastic astrocytomas, furthermore, can recur as WHO grade IV GBM tumors. GBM tumors are characterized by abnormal cells that reproduce rapidly, form new blood vessels to maintain growth, and have areas of necrosis present within the tumor.

Despite improvements in the diagnosis and treatment of malignant gliomas, these brain tumors remain universally lethal. The 5-year relative survival rates for glioma vary by age. The 5-year survival rates for low grade diffuse astrocytoma range from 21% for patients 55-64 years to 65% for patients 20-44 years; the rates for anaplastic astrocytoma range from 10% for patients 55-64 years to 49% for patients 20-44 years; and the rates for GBM range from 4% for patients 55-64 years to17% for patients 20-44 years (American Cancer Society 2017). The majority of

patients with malignant glioma do not live past 18 months (GBM) or 36 months (anaplastic astrocytoma) from diagnosis. Current treatments for malignant glioma are inadequate and focus on palliative management rather than a cure. The current standard of care for malignant gliomas worldwide is surgery followed by external beam radiation with concurrent and adjuvant chemotherapy (temozolomide). Almost all malignant gliomas are resistant to therapy and will recur after treatment. Independent prognostic factors of malignant glioma include age, Karnofsky Performance Scale (KPS) score, tumor necrosis on MRI, genomics of the tumor (e.g., IDH1/2 mutation, chromosomal 1p/19q co-deletion), methylation status of the DNA repair enzyme O-5-methylguanine-DNA methyltransferase, extent of tumor resection, and response to chemotherapy and radiation (Walid 2008, Lacroix et al. 2001).

For patients with LGGs, surgery is required to establish the initial diagnosis and to plan appropriate treatment. Maximum safe resection is the first treatment. Patients typically undergo fractionated external beam radiation therapy and/or chemotherapy at the time of recurrence and malignant progression. Patients with malignant glioma most commonly undergo maximal surgical resection of the tumor followed by external beam radiation therapy with concurrent and adjuvant chemotherapy.

1.2 Surgical Resection

The goal of surgical resection is to remove as much of the tumor as possible without affecting the areas of the brain considered "eloquent" (those parts of the brain that control motor and sensory function or neurocognitive functions such as speech). Surgical resection of gliomas is challenging due to the difficulty in visualizing the tumor and its margins. Gliomas have no distinct borders and infiltrate into normal brain. MRI neuronavigation permits localization of the tumor for resection purposes. However, preoperative MRI images are used for neuronavigation and do not take into account brain shift that occurs in patients during surgery as a result of change in head position, drainage of cerebral spinal fluid, and the tumor resection (Zhang et al. 2015). The use of intraoperative MRI (iMRI) can improve surgical accuracy; however, the cost of this technology is prohibitive in most centers. Also, in those centers with iMRI, the location of the device is outside of the operating room thus interfering with the flow of surgery. In determining the extent of tumor resection, neurosurgeons rely on the information provided by neuronavigation techniques as well as their understanding of neuroanatomy, the location of the tumor with regard to eloquent areas of the brain, and their surgical skill. During surgery, methods for direct and accurate visualization of the tumor for localization, with a sufficiently high resolution for microsurgery, would be of considerable value and would provide real time information to the neurosurgeon for guidance surgery. Presently such real time imaging methods are not available to surgeons in the U.S.

Tumor resection is intended to facilitate the success of adjuvant treatments with chemotherapy and radiotherapy, and therefore, increase progression free survival (PFS) or overall survival (OS). Several investigations have concluded that complete gross total tumor resection has a greater benefit on patient survival than subtotal resection, and a recent meta-analysis found that in over 41,117 newly diagnosed GBM patients, gross total resection substantially improved OS and compared to subtotal resection (Brown et al. 2016). The extent of resection (EOR) for any patient depends on several factors including tumor location (eloquent areas), tumor size, patient condition, surgical skill, and the ability to visualize tumor margins. As a result, an effect of EOR on patient survival is not consistently observed. Patient survival is also impacted by the patient's age and Karnofsky Performance Scale (KPS) score, tumor necrosis on MRI, genomics of the tumor (e.g., IDH1/2 mutation, chromosomal 1p/19q co-deletion), methylation status of the DNA repair enzyme O-5-methylguanine-DNA methyltransferase, and response to chemotherapy and radiation (Walid 2008, Lacroix et al. 2001).

1.3 5-ALA HCl (5-ALA)

5-aminolevuolinic acid hydrochloride (5-ALA HCl), referred to throughout this document as 5-ALA, is an imaging agent to facilitate the real time detection and visualization of malignant tissue during glioma surgery. The benefits of 5-ALA are that it provides real time, high resolution delineation of malignant tissue, is well tolerated by patients, is appropriate for use in patients with primary or recurrent brain tumors, can be used with existing standard operating neurosurgical microscopes without interrupting surgery, and results in greater surgeon confidence to identify tumor tissue. The recommended dose is 20 mg 5-ALA HCl per kilogram body weight (bw). The efficacious dose was determined by examining the fluorescence of doses of 0.2, 2, and 20 mg/kg bw in a dose-range finding clinical study. Visual and spectrometric fluorescence assessment showed that 20 mg/kg elicited the strongest fluorescence in tumor core and margins, which correlated with cell density. In vitro studies have indicated that higher doses do not result in useful increases in tissue fluorescence.

5-ALA is provided from the hospital or neurosurgical pharmacy to surgeons who have been successfully trained in the use of the intraoperative imaging agent. It is supplied as a single use vial of lyophilized powder. The powder is reconstituted in 50 mL drinking water. The solution is administered orally 3 hours before anesthesia prior to glioma resection surgery, under the supervision of a healthcare professional.

5-ALA is a naturally occurring metabolite in the heme biosynthesis pathway. For imaging, 5-ALA functions as a nonfluorescent prodrug, which is metabolized intracellularly in tumor cells to form the fluorescent molecule protoporphyrin IX (PpIX). Following ingestion, 5-ALA is

absorbed, crosses the blood-brain barrier, and is converted into PpIX within tumor cells. During glioma surgery, PpIX, which has selectively accumulated in malignant brain tissue, can be stimulated by excitation with blue light (wavelength range from 375 nm to 410 nm, and for observation of red emission from the tissue in the upper wavelength from 620 to 710 nm). PpIX has a unique excitation/emission spectrum that results in emission of a red-violet light that is visible to the neurosurgeon. The preferential accumulation of PpIX allows for identification of glioma tissue for removal, as determined by the neurosurgeon. This includes both HGG and areas of anaplastic foci from which malignant progression may occur in LGGs. This imaging agent has been used over the last decade by numerous surgeons throughout the world in more than 50,000 brain cancer cases in the European Union (EU) and in 9 other countries.

In patients who have taken 5-ALA, fluorescence-guided surgery (FGS) of their glioma is accomplished through the use of a standard surgical microscope using a low band pass filter placed in front of the standard white light source. The accessory kit consists of the add-on low and high band pass observation filters needed to visualize fluorescence of PpIX. These filters allow the passage of blue wavelength projected to the surgical field to visualize the fluorescence of PpIX in the excitation wavelength range from 375 nm to 410 nm, and for observation of red emission from the tissue in the upper wavelength from 620 to 710 nm. The accessory kit has excitation and emission filters with slightly overlapping transmissions integrated into its optical configuration. The filters are designed to transmit red porphyrin fluorescence, as well as a fraction of backscattered blue excitation light necessary for distinguishing nonfluorescing tissue, thus, allowing for the differentiation of normal brain tissue (appearing blue in the surgical field) from malignant tumor tissue (appearing red and pink in the surgical field) during glioma surgery.

The underlying technology of fluorescent excitation and fluorescence detection of PpIX has been consistent since the inception of the early clinical trials through the approval of 5-ALA (under the marketed name “Gliolan”) by the European Medicines Agency (EMA) and subsequent regulatory authorities in the rest of the world. 5-ALA has been approved for the visualization of malignant tissue during surgery for malignant glioma in the EU since 2007 and was subsequently approved in South Korea Taiwan, Japan, Israel, Australia Ukraine, Kuwait, Hong Kong, and New Zealand.

1.4 Nature of the Data Supporting the Safety and Efficacy of 5-ALA

The safety and efficacy of 5-ALA in the New Drug Application (NDA) 208630 relies on clinical data made available to NXDC by photonamic GmbH & Co. KG (“photonamic”), in cooperation with photonamic’s licensee medac GmbH, who sponsored the clinical studies to support the EU marketing authorization. In total, 6 medac-sponsored clinical studies conducted in Germany

have been completed in the development of 5-ALA. The studies that contributed data on the safety and/or efficacy of 20 mg/kg bw 5-ALA are summarized in Table 1. The clinical study populations include 548 healthy volunteers or subjects who received 20 mg/kg bw 5-ALA.

Table 1. Clinical Studies Supporting the Safety and/or Efficacy of 5-ALA

Study Number	Study Phase	Study Population	Number of Subjects/Patients Receiving 20 mg/kg bw 5-ALA	Safety and/or Efficacy Clinical Data
MC-ALS.20/BV (Study 20)	1	Healthy male subjects	21	Safety
MC-ALS.8-I/GLI (Study 8)	1/2	Malignant glioma	7	Safety
MC-ALS.28/GLI (Study 28)	2	Malignant glioma	36	Safety/efficacy
MC-ALS.30/GLI (Study 30)	2	Progressive/recurrent malignant glioma	40	Safety/efficacy
MC-ALS.3/GLI (Study 3)	3	Malignant glioma	201	Safety/efficacy
MC-ALS.32/GLI (Study 32)	3	Malignant glioma Progressive/recurrent malignant glioma	243	Safety

In addition to data from the clinical studies, the efficacy of 5-ALA is further supported by data from 12 peer-reviewed published literature on the use of 5-ALA to identify malignant glioma tissue in 377 patients. The safety of 5-ALA is further supported by data from 29 peer-reviewed publications on the use of 5-ALA to identify malignant glioma tissue and by postmarketing surveillance data.

1.5 Pharmacokinetics of 5-ALA

Study 8 and Study 20 demonstrated that the absorption of 5-ALA occurs rapidly with maximum plasma concentrations reached approximately 1 hour after oral administration of 20 mg/kg bw 5-ALA. In healthy volunteers (Study 20), an absolute bioavailability (BA) of the orally administered dose of 20 mg/kg bw 5-ALA is 100%, indicating 5-ALA is completely absorbed from the gastrointestinal tract. In Study 8, pharmacokinetic parameters were calculated following oral administration of 0.2, 2, and 20 mg/kg bw 5-ALA. Both the mean peak plasma concentration (C_{max}) and the area under the plasma concentration time curve (AUC_{inf}) of 5-ALA were dose proportional. Increased plasma levels of PpIX were observed after oral 5-ALA doses of 2 and 20 mg/kg bw (geometric mean AUC_{inf} of 255.80 and 779.90 mg x h/L, respectively). In this study, a positive dose-efficacy relationship between the dose levels and the extent and quality of fluorescence of the tumor core was detected. The highest dose of the investigative drug (20 mg/kg bw) was determined to be optimal for this purpose and has consistently been the benchmark for dosing in the clinical trials and is the approved dosing in the rest of world. The metabolic fate of exogenous 5-ALA is the same heme synthetic pathway as that of endogenously synthesized 5-ALA; the metabolites that are generated as a result of metabolism are PpIX and

heme. Approximately 30% of an orally administered dose of 20 mg/kg 5-ALA is excreted unchanged with urine within 12 hours (Study 20).

1.6 Efficacy of 5-ALA

The efficacy of 5-ALA as an imaging agent to facilitate the real time detection and visualization of malignant tissue during glioma surgery is supported by the predictive accuracy (positive predictive value [PPV]) of detecting malignant tissue and clinical usefulness of 5-ALA. Efficacy was demonstrated by the surgeon's ability to visualize malignant structural elements in real time during the fluorescent surgical procedure which was then confirmed by third party assessment of the histopathology of glioma, resulting in a PPV of 5-ALA. This allows the surgeon to identify tumor, distinguish tumor from normal brain, and locate the anatomy of the tumor relevant to eloquent regions of the brain during surgery. The PPVs calculated from the clinical study data were further supported by PPV calculated from data provided in peer-reviewed publications describing clinical studies of the efficacy of 5-ALA in the visualization of glioma. In addition, clinical usefulness, defined as the ability to aid surgeons in the real time identification of malignant tumor under fluorescent light that may otherwise be overlooked during conventional (standard white light) microsurgical resection, is supported by data from the clinical studies and data from peer-reviewed medical literature.

The pivotal clinical studies also included endpoints such as EOR as determined by postoperative MRI, PFS at 6 months, and OS, and these data were provided in the NDA for completeness. However, as with other medical imaging agents, 5-ALA is a tool that provides accurate information to the neurosurgeon regarding the location and presence of glioma tissue. Intended radicality of resection is typically determined by the neurosurgeon based on neuronavigation and his/her assessment of the glioma and its adjacent healthy brain tissue. PFS and OS are influenced by EOR, as well as response to treatment, and other factors and, therefore, these endpoints are not a measure of the performance of a medical imaging agent. The use of PPV and clinical usefulness as relevant efficacy endpoints for 5-ALA have been extensively discussed with the FDA review division.

1.6.1 Predictive Accuracy

In this NDA, PPV at the biopsy level (defined as the probability that fluorescent areas contain histologically confirmed tumor tissue) is presented as the primary efficacy endpoint. Other data contained in both the studies conducted in Europe and in published literature are presented in totality, such that the reader can draw conclusions based upon the robustness and reproducibility of the data. PPV data are available from Study 28 (Phase 2), Study 30 (Phase 2), and Study 3

(Phase 3), submitted to support the NDA, which represented 245 patients who received 20 mg/kg bw 5-ALA, including 36 patients with recurrent glioma. The biopsy-based PPV of 5-ALA-induced fluorescence, defined as percentage of biopsies from fluorescent areas that contain tumor cells, are summarized in Table 2. The PPVs for the 3 studies are 96.2%, 96.6%, and 97.8%, which strongly support that fluorescent tissue is malignant glioma tissue.

Table 2. Biopsy-based Estimates of Positive Predictive Value (Population: Full Analysis Set)

	Study 28	Study 30	Study 3
n/N (PPV)	178/185 (96.2%)	342/354 (96.6%)	312/319 (97.8%)
95% CI of PPV	92.4-98.5	94.2-98.2	95.5-99.1

n = number of fluorescent biopsies having tumor cells > 0%; N = number of fluorescent biopsies; PPV = positive predictive value (n/N*100)); CI = confidence interval.
Only patients who received 5-ALA are presented for Study 3.

Measurements of biopsy-based PPV are also available from 11 peer-reviewed publications (Coburger et al. 2014, Díez Valle et al. 2011, Ewelt et al. 2011, Hauser et al. 2016, Idoate et al. 2011, Lau et al. 2016, Panciani et al. 2012, Roberts et al. 2011, Stummer et al. 1998, Stummer et al. 2000, Valdés et al. 2010). Biopsy-based PPVs of typically greater than 90% for 5-ALA were commonly recorded or calculated.

In addition to PPV, traditionally, the predictive accuracy of a medical imaging agent may be assessed by:

- negative predictive value ([NPV], the probability that a subject does not have the disease when the test result is negative),
- sensitivity (the probability that a test result is positive when the subject has the disease), and
- specificity (the probability that a test result is negative when the subject does not have the disease).

However, it is not appropriate to adapt traditional diagnostic accuracy measurements to biopsies obtained during brain surgery due to not being able to sample normal brain tissue and the visual bias of such studies. For example, the number of samples and the locations from which they are taken strongly influence the NPV since with infiltrating tumors, the prevalence of tumor cells is low and diminishes with distance away from the tumor bulk. If samples are taken immediately beyond the margins of the tumor, the NPV will be low. Since the number of detectable tumor cells in the infiltration zone will decrease markedly with the distance away from the tumor bulk, the NPV will increase if samples are collected further from the tumor site. With regard to specificity (the number of fluorescence-negative biopsies among all biopsies devoid of tumor

cells) and sensitivity (the number of fluorescing biopsies among all biopsies with positive identification of tumor cells), calculation of these values would require blinded tissue sampling, which is unachievable when using FGS. Such an approach would require that random biopsy sampling of normal and functional portions of the brain to be obtained, and this is not safe or feasible, nor ethical, in medical practice or in the setting of the 5-ALA FGS clinical trials because such sampling would contribute to morbidity. Also, if fluorescent tissue samples were only obtained from the center of the tumor, all would be confirmed as tumor cells, resulting in a sensitivity of 100% (specificity 0%), whereas taking samples far from the tumor in areas in which there is no fluorescence, the specificity would be 100% with a sensitivity of 0%.

1.6.2 Clinical Usefulness

The clinical usefulness of 5-ALA is demonstrated by its ability to aid surgeons in the real time identification of malignant tumor under fluorescent light that may otherwise be overlooked during conventional microsurgical resection. Studies 28 and 30 provide evidence that 5-ALA fluorescence enables surgeons to identify residual locations of tumor seen in real time under blue light, which were not identified under white light only. This is also supported with data from 4 peer-reviewed, published literature articles (Coburger et al. 2014, Hefti et al. 2008, Panciani et al. 2012, Stummer et al. 1998), where it was consistently shown that more tumor-containing areas were identifiable under blue light as compared to white. Studies were considered supportive of clinical usefulness if resection was performed under standard white light and after the surgeon deemed the resection complete, the light source was switched to blue-violet light to assess residual malignant tissue. Studies in which the surgeon intermittently switched from white to blue light were not included in this analysis, although such procedures highlight how 5-ALA is utilized by many trained neurosurgeons during resection, because such studies cannot quantify the ability of 5-ALA to detect malignant tissue that otherwise may have been overlooked during resection.

1.6.3 Identification of Anaplastic Foci in Low Grade Tumors

5-ALA is useful in identifying the presence of anaplastic foci in gliomas. LGGs, even those that present as nonenhancing masses on MRI, are genetically and pathologically heterogeneous. A standard MRI cannot always detect areas of anaplasia denoting focal malignant grade III or even grade IV lesions, which may not be sampled during biopsy. In close to 50% of cases of tumors that lack contrast enhancement and are characterized as LGGs on imaging, these tumors contain malignant, or anaplastic foci. Surgically, these tumors are not resected en bloc. Rather, they are decompressed carefully from within, with much of the tissue being removed by suction and not being available for histopathological assessment. Even when using the surgical microscope,

areas of anaplasia are rarely evident to the eye and are easily overlooked. Importantly, if such regions of anaplasia are not specifically identified pathologically, tumors will be under graded and necessary adjuvant treatments for malignant gliomas will be withheld, resulting in under treatment of patients. 5-ALA-derived tumor fluorescence allows proper identification of high-grade glioma tissue intraoperatively that are typically not observed by surgeon, not captured by biopsy, and thus not observed by the pathologist.

1.6.4 Extent of Resection, Progression-free Survival, and Overall Survival

In the pivotal efficacy trials in the NDA (Study 30 and Study 3) completeness of resection was evaluated as the percentage of patients without residual tumor on early postoperative MRI. In Study 3, with the help of 5-ALA supported fluorescence-guided resection, significantly more patients in the experimental arm became tumor-free than in the control group. It must however be emphasized that fluorescing tissue cannot always be removed completely if eloquent areas are involved. In Study 28, in 69.7% of patients, residual tumor/fluorescence was left behind to avoid potential harm to functional areas. In 65.2 % of these patients, residual contrast enhancement in early postoperative MRI could be detected. This was much higher than in the 5-ALA-arm of Study 3 because of different inclusion criteria (resectability of the tumor was demanded only in Study 3). The different outcomes in these studies, partly based on different definitions of the inclusion criterion, “resectability” clearly shows that visualization is not the only factor determining EOR. Surgical decisions, based on location and size of the tumor are other important determining factors. It may be questionable to use PFS or OS as a surrogate marker for clinical usefulness of 5-ALA, as PFS and OS are driven by EOR and not by the use of 5-ALA.

1.7 Safety of 5-ALA

The safety summary for the NDA includes integrated safety data from clinical studies 3, 8, 28, 30, and 32. Data from Study 20 are not included in the integrated safety analyses because this study was conducted in healthy subjects, who did not undergo major brain surgery. Altogether, in clinical studies, 527 glioma patients received 20 mg/kg bw 5-ALA and are included in the full safety population. The median age of patients was 61 years (range 19-80); approximately two-thirds of patients were male (62.8%) and one-third of patients were female (37.2%). Median body weight at dosing was 78.0 kg (range 37-130 kg). The demographics of the patient populations were comparable across studies. A summary of Treatment-Emergent Adverse Events (TEAEs) in patients who received 5-ALA (by patient) is provided in Table 3. TEAEs are defined as those that start or worsen at, or during the time of, or after 5-ALA administration through the last study visit and include all procedural-related adverse events (AEs) independent

of causality. Not unexpectedly for this patient population, the rate of TEAEs was greater than 55% across the studies; none of the TEAEs led to study discontinuation and very few were deemed to be drug related.

Table 3. Summary of TEAEs in Patients who Received 5-ALA (by Patient)

Number of Patients with Any:	Study N (%)					
	Study 8 (N = 7)	**Study 28 (N = 36)**	**Study 30 (N = 40)**	**Study 3 5-ALA (N = 201)**	**Study 32 (N = 243)**	**Pooled Studies[a] (N = 527)**
Treatment-emergent Adverse Event (TEAE)	7 (100%)	27 (75.0%)	22 (55.0%)	135 (67.2%)	126 (51.9%)	317 (60.2%)
Total Events	37	90	48	348	279	802
Drug-related TEAE	2 (28.6%)	6 (16.7%)	0 (0%)	7 (3.5%)	3 (1.2%)	18 (3.4%)
Total Events	4	6	0	8	5	23
Severity						
Mild TEAE	0 (0%)	1 (2.8%)	2 (5.0%)	33 (16.4%)	30 (12.3%)	66 (12.5%)
Moderate TEAE	5 (71.4%)	10 (27.8%)	15 (37.5%)	26 (12.9%)	50 (20.6%)	106 (20.1%)
Severe TEAE	2 (28.6%)	14 (38.9%)	5 (12.5%)	45 (22.4%)	34 (14.0%)	100 (19.0%)
Life-Threatening TEAE	0 (0%)	0 (0%)	0 (0%)	13 (6.5%)	12 (4.9%)	25 (4.7%)
Fatal TEAE	0 (0%)	2 (5.6%)	0 (0%)	18 (9.0%)	0 (0%)	20 (3.8%)
Serious Adverse Event	1 (14.3%)	8 (22.2%)	4 (10.0%)	68 (33.8%)	49 (20.2%)	130 (24.7%)
Adverse Event Leading to Discontinuation	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Adverse Event Leading to Death	0 (0%)	2 (5.6%)	0 (0%)	18 (9.0%)	5 (2.1%)	25 (4.7%)

TEAE = treatment-emergent adverse event, N = number of patients

*Patients reporting a particular adverse event (preferred term) more than once are counted only once by preferred term and system organ class.

[a] Pooled column includes patients treated with 20 mg/kg bw 5-ALA from Studies 8, 28, 30, 3, and 32

AEs leading to death (recorded by the investigator as fatal) occurred in 25 patients; none of the deaths that occurred in study patients were considered related to 5-ALA. Because of 5-ALA's short half-life (approximately 3 hours), deaths and AEs that occur in the early postoperative period have the greatest probability of being related to the treatment drug. Very few deaths (5) occurred during the early postoperative period, and for the most part these were related to the underlying disease with its incumbent high rate of mortality during the first year after diagnosis.

The most commonly reported TEAEs immediately following surgery were nervous system disorders, which included aphasia (8% of patients), hemiparesis (7.8%), hemianopia (3.2%), and headache (2.7%). Data from the clinical studies revealed the overall rate of nervous system disorders for the period immediately following resection surgery for the pooled studies was 29.4%; the frequency decreased to 9.8% during the period 1 week to 6 weeks following surgery, and to 12.5% for more than 6 weeks following surgery. These TEAEs are not unexpected for this patient population after brain major surgery. A total of 11 patients (2.1%) experienced TEAEs within the first week after surgical resection that were determined by the investigator to be at least possibly related to 5-ALA.

Data from clinical studies indicate that rare risks associated with brain surgery using 5-ALA include brain edema, hemianopia, hypoaesthesia, pyrexia, chills, photosensitivity reaction, solar dermatitis, hypotension, abnormal liver function test, diarrhea, and venous thrombosis. These risks are described in the proposed product labeling.

Safety data reveal no effect of 5-ALA on clinical laboratory values, electrocardiogram (ECG) findings, QT interval corrected (QTc) prolongation potential, vital signs, or physical findings. However, across studies, patients experienced increases in alanine aminotransferase (ALT) and gamma-glutamyl transferase (GGT) within the first week after surgery, which continued at 6 weeks in 3-8% of patients. These increases were not considered clinically relevant.

NXDC conducted a thorough literature search and identified peer reviewed publications describing studies in which 5-ALA was utilized to visualize glioma during resection surgery; the search covered the period 1 January 1999 through 9 June 2016. Relevant articles were obtained and those that met the stated inclusion criteria (clinical studies containing data on the safety of 20 mg/kg bw 5-ALA for glioma visualization, investigator calculation of PPV, or sufficient data from which to calculate PPV were reviewed and summarized. The AE data from these studies were found to be consistent with the AE data from the studies in the clinical trial safety database.

The safety of 5-ALA is further supported by postmarketing safety data available from several countries. From March 8, 2012 to March 7, 2015, more than 58,000 patients have received 5-ALA prior to surgical resection for malignant glioma, as described in the EU Periodic Safety Update Report (PSUR). The PSUR did not present any unexpected AEs that would, due to their frequency and/or nature of cases in which they occur, suggest new risks with 5-ALA that are not previously known and acknowledged in the clinical trials or product labeling.

Taken together, a large safety database for 5-ALA is available that draws from controlled clinical trials, literature, and postmarketing data. 5-ALA is well tolerated and most TEAEs are expected in a population undergoing major brain surgery. Because of observations from clinical studies regarding the potential for photosensitization; reduction in blood pressure, particularly in patients receiving blood pressure medication; and transient elevations of certain liver enzymes in patients who had resection surgery with 5-ALA, the proposed product labeling cautions to minimize the potential for photosensitivity by avoiding direct light exposure, as well as the administration of 5-ALA in combination with hepatotoxic drugs and in patients with blood pressure concerns.

1.8 Benefit Risk

5-ALA in combination with blue light has shown to improve the visualization of malignant tissue during glioma surgery. The consistently high values for biopsy-based PPV (typically greater than 90% from clinical studies and supporting literature) show that what is fluorescent during surgery contains tumor cells in histopathological analysis. 5-ALA-induced fluorescence provides the neurosurgeon with accurate, high-resolution information on the location and extent of the tumor from which he or she can make decisions regarding surgical resection.

Surgical resection carries risks and complications. The complications and risks of resection surgery can be divided into neurologic, regional, and systemic, including direct cortical and vascular injury, surgical wound complications, and postsurgical medical complications (Jackson et al. 2016). As expected, patients in the clinical studies experienced AEs consistent with those associated with resection surgery.

To minimize the likelihood of risks associated with surgery with 5-ALA, a risk management training program (the 5-ALA Medicines Management Program) has been developed, which is predicated on restrictions or conditions with regard to the safe use of 5-ALA imposed by the EMA. The risk management strategy is intended to provide a product distribution, training, and certification program to limit the use of 5-ALA to neurosurgeons who have an understanding of the benefits and risks of FGS surgery in the management of patients with gliomas and to

neurosurgeons who have demonstrated the ability to visualize 5-ALA-induced fluorescence using the specified operating microscopes and filters.

1.9 Conclusions

5-ALA-induced fluorescence provides the neurosurgeon with detailed information on the location and extent of the tumor from which he or she can make informed decisions about the resection based on surgeon skill, experience, and anatomic and functional knowledge of the brain. 5-ALA-induced fluorescence facilitates this decision.

In summary:

- More than 79,000 new cases of primary brain tumors are diagnosed each year in the U.S. and approximately 25% are gliomas.
- High grade (malignant) gliomas are universally lethal with a 1-year survival from time of diagnosis at less than 50%; LGGs frequently transform into malignant gliomas.
- Gliomas with MR imaging suggestive of being LGGs (no significant MRI contrast enhancement), often (up to 50%) have malignant, or anaplastic foci present within the tumor that are difficult to detect by standard microscopy.
- MRI is initially utilized to image a brain tumor; biopsy or tumor resection is required to establish the diagnosis and stage the tumor properly.
- Maximal safe, surgical resection is the standard of care for malignant gliomas and may be undertaken for LGG as well.
- The standard of care involves postoperative radiation and/or chemotherapy to aggressively attempt to delay disease recurrence. Survival following resection surgery is impacted upon by numerous factors including patient age, performance status, tumor aggressiveness, genetics, surgical success, and patient response to therapy.
- Surgical resection is challenging because gliomas have no distinct borders but show infiltrative growth into normal brain.
- Preoperative and intraoperative neuronavigation techniques can assist the neurosurgeon in identifying the location and extent of the tumor but they have limitations (brain shift and interruption of surgical flow).
- 5-ALA is an imaging agent to facilitate the real time detection and visualization of malignant tissue during glioma surgery (for both LGG and high grade gliomas).
- In the body, 5-ALA metabolizes to PpIX, which selectively accumulates in tumor tissue but not normal brain tissue. Upon excitation with the appropriate light source, PpIX, with a Stokes-Einstein shift in fluorescence signal, allows the neurosurgeon to identify tumor tissue in the presence of normal brain tissue.

- PPV (the probability that fluorescent areas contain histologically confirmed tumor tissue) is an appropriate measure of efficacy (specifically diagnostic accuracy) for an intraoperative medical imaging agent. PPV data from the clinical studies, supported by published literature, support PPV values that are typically greater than 90%.
- Efficacy is also supported by the clinical usefulness of 5-ALA as demonstrated by clinical data from the studies and from the published literature that demonstrate the ability of 5-ALA to aid surgeons in identifying tumor tissue under fluorescent light that might be overlooked under white light.
- 5-ALA is well tolerated by patients. Safety data from a large number of patients from the clinical studies, supported by published literature, and postmarketing surveillance data indicate that the rare risks associated with resection surgery with 5-ALA include pyrexia, chills, photosensitivity reaction, solar dermatitis, hypotension, abnormal liver function test, diarrhea, edema, hemianopia, hypoaesthesia, and venous thrombosis. These risks will be described in the proposed product labeling.
- To ensure that any potential risks related to the ingestion of 5-ALA remain low, a product distribution, training, and certification program has been developed by NXDC, which is intended to minimize the risks that may be associated with surgical resection with 5-ALA to visualize malignant brain tissue. The focus of the program is to limit access to 5-ALA only to neurosurgeons who have been trained in the use of the drug to support glioma resection surgery.

The approval of 5-ALA will allow highly trained neurosurgeons to intraoperatively locate tumors that are difficult to visualize under standard conditions, remove doubt, provide confirmation of the tumor's border, overcome technical limitations associated with brain shift, and enhance the surgeon's ability to distinguish malignant tissue from health brain tissue.

2 INTRODUCTION

NXDC is seeking marketing approval for 5-ALA for oral solution to use as an imaging agent to facilitate the real time detection and visualization of malignant tissue during glioma surgery.

photonamic – in cooperation with its licensee medac GmbH ("medac") – developed 5-ALA (Gliolan®) for the intraoperative visualization of malignant glioma (WHO grade III and IV). In late 2007, the EMA granted marketing authorization for Gliolan® for this neurosurgical indication. The marketing authorization in the EU is held by medac GmbH, Wedel, Germany. Gliolan® is approved for marketing in approximately 40 countries outside of the U.S., including the EU, Japan, Israel, Australia, Hong Kong, South Korea, Taiwan, Ukraine, and Kuwait. In addition, numerous studies demonstrating the suitability of 5-ALA or Gliolan® as an imaging

agent have been published in scientific literature and postmarketing surveillance has been conducted, which includes approximately 58,000 patients.

NXDC and photonamic have collaborated to prepare 5-ALA for commercial deployment in the U.S. NXDC submitted a NDA for 5-ALA on December 6, 2016. In the course of preparing the NDA, NXDC participated in several meetings and document exchanges with the U.S. Food and Drug Administration (FDA) Division of Medical Imaging Products. On the basis of those discussions, NXDC implemented agreed upon clinical safety and efficacy evaluations from clinical trials and literature assessments of 5-ALA.

3 MEDICAL NEED

3.1 Malignant Glioma

Malignant glioma is an invasive type of tumor that occurs in the brain and spinal cord. Gliomas originate in the glial cells that surround and support neurons in the brain and vary in aggressiveness, or malignancy. Gliomas may be slow growing and sometimes curable, as in the case of pilocytic astrocytomas, or, fast-growing, invasive, heterogeneous, difficult to treat, and likely to recur. Gliomas are classified according to the type of glial cell involved in the tumor: anaplastic astrocytoma (AA), anaplastic oligodendroglioma (AO), and GBM. GBM is the most common, accounting for approximately 60% to 70% of malignant gliomas. Anaplastic astrocytomas account for 10% to 15% and anaplastic oligodendrogliomas and anaplastic oligoastrocytomas account for 10% (Huang et al. 2017). Gliomas are further categorized according to their grade (WHO classification of tumors of the central nervous system [CNS]; Louis et al. 2016), which is determined by pathologic evaluation. Tumors are graded from I (least advanced disease and best prognosis) to IV (most advanced disease and worst prognosis). Low-grade gliomas (LGG, WHO grade II) are well differentiated (not anaplastic) and tend to exhibit benign tendencies, however, they can recur and increase in grade over time. HGG (WHO grade III-IV) are undifferentiated or anaplastic, are malignant, and carry a worse prognosis.

In general, malignant brain tumors have a poor prognosis and can significantly affect a patient's quality of life and cognitive function (Sizoo et al. 2010, Omuro & DeAngelis 2013).

3.2 Standard of Care

The clinical presentation of patients with newly diagnosed malignant glioma can vary greatly depending on the size and location of the tumor (Young et al. 2015, Ahmed et al. 2014). Nonspecific complaints include headache, dizziness, nausea, lethargy, seizures, hemiparesis, vision loss, stroke-like symptoms, memory problems, or personality changes (Young et al. 2015).

If a brain tumor is suspected, a brain scan (computerized tomography [CT] and MRI) is performed. A biopsy may also be performed before or during resection surgery.

There is currently no cure for malignant glioma and patient survival is limited. Primary malignant brain tumors are very difficult to treat due to the heterogeneity and level of infiltration. The standard of care for glioma patients is universal and has not appreciably changed over time. Current treatment modalities include surgery, radiation, and chemotherapy, but despite their use, the median survival of patients diagnosed with GBM is only 12 to 15 months (Wen & Kesari 2008). Resistance to radiation and chemotherapeutic agents is a concern in patients treated for primary brain cancers, which makes it difficult to identify a successful treatment (Wen & Kesari 2008). In addition, despite maximal surgical resection followed by adjuvant chemoradiation, the median time to tumor recurrence is 8 months (Chaichana et al. 2014).

Surgical removal of brain tumor masses is the most commonly used approach in the management of brain tumors (Huang et al. 2017), and is often the first line of treatment (Halani & Adamson 2016). Guidelines for the management of malignant glioma from both the U.S. and Europe recommend surgery, as long as there is no great risk for additional neurological dysfunction (Stupp et al. 2014, Weller et al. 2014, Olson et al. 2009, NCCN 2016, NCI 2016, Wen & Kesari 2008, Ryken et al. 2008). Unfortunately, there are known risks associated with resection surgery itself and it is often difficult to obtain discernable tumor margins to achieve complete resection using standard operating room white light. The precise delineation of normal tissue from tumor tissue during resection is of paramount performance and remains difficult for neurosurgeons using standard microsurgical techniques (Huang et al. 2017).

3.3 Medical Imaging

During resection surgery, imaging tools are used to identify areas of tumor tissue for removal as well as to ensure areas considered eloquent are not removed (i.e., minimally invasive resection). Prior to craniotomy, the brain tumor and margin are usually assessed using CT and MRI, with and without contrast agents, which are considered anatomical information tools. These, along with histological and functional information, aid the surgeon in making a decision of whether or not the tissue should be resected (Tamura et al. 2015).

These current imaging tools used during resection surgery have limitations. CT can provide anatomical location of the tumor; however, with GBM, small tumors may be missed. At present in the U.S., iMRI is rarely available and is restricted to a few centers of excellence capable of supporting such tools. In addition, iMRI is not a real time tool and requires longer periods of time in which surgery is interrupted. Conventional neuronavigation relies on a prior MRI scan

that is obtained days before surgery and registered with the patient at surgery. Brain shift upon initiating the surgical procedure is a well-known occurrence in all brain tumor operations which renders neuronavigation systems inaccurate. In addition, malignant glioma cells are usually found beyond the margins of the area signaled on the MRI.

In addition to the tools described above, contrast enhancing agents have been used to define the boundaries of tumor infiltration. For example, gadolinium (Gd)-enhancing regions on an MRI have been used. However, Gd contrast-enhancement relies on the disruption of the blood-brain barrier and, therefore, infiltrative glioma regions at the tumor margin are typically not shown with the MRI (Hadjipanaysis et al. 2015).

Overall, despite the use of a variety of imaging tools for assessment of resection, there is still an unmet medical need for a safe and effective tool that provides neurosurgeons with directly visible, accurate, unambiguous information about the location and extent of the tumor in real time, with a high resolution to enable microsurgery.

4 5-AMINOLEVULINIC ACID HYDROCHLORIDE (5-ALA)

4.1 Description of Drug

5-ALA (pharmaceutical ingredient) is a porphyrin precursor intended for oral solution. 5-ALA is supplied as 1.5 g of lyophilized 5-ALA HCl (salt form) corresponding to 1.17 g of 5-ALA. 5-ALA is reconstituted by dissolving 1 vial (1.5 g) in 50 mL drinking water (30 mg 5-ALA HCl per mL) and is administered to patients as 20 mg/kg bw (0.666 mL solution per kg body weight), corresponding to 15.6 mg 5-ALA per kg bw. It is administered to patients orally 3 hours before induction of anesthesia.

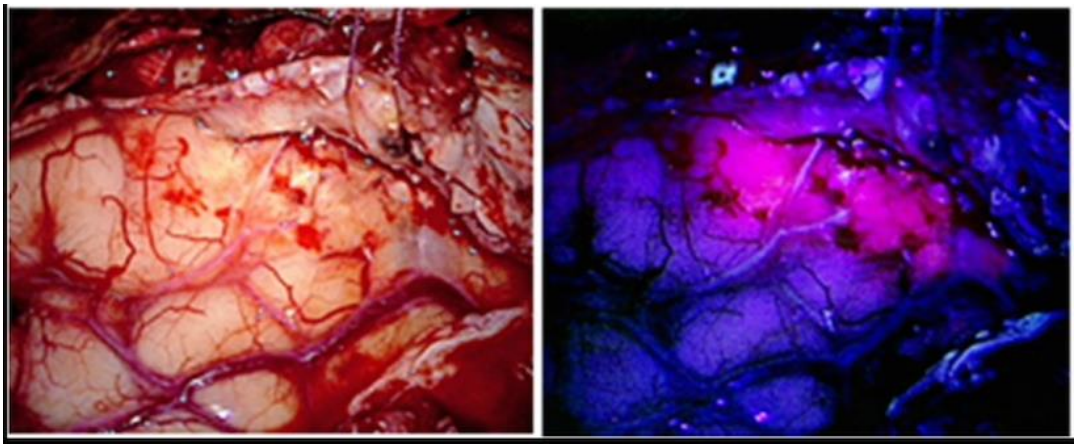
4.2 Mechanism of Action

5-ALA is a naturally occurring metabolite that is formed in the mitochondria from succinyl-CoA and glycine (Ajioka et al. 2006). The concept of administration of 5-ALA as prodrug, which is metabolized intracellularly to form the fluorescent molecule protoporphyrin IX (PpIX), has been developed in various indications since the late 1980s. 5-ALA synthesis is regulated by an intracellular pool of free heme via a negative feedback mechanism. The exogenous administration of 5-ALA leads to a highly selective accumulation of PpIX in tumor cells and epithelial tissues. During glioma surgery, PpIX, which has selectively accumulated in malignant brain tissue, can be stimulated by fractions of normal white light using excitation with blue light (wavelength range from 375 nm to 410 nm, and for observation of red emission from the tissue

in the upper wavelength from 620 to 710 nm). PpIX has a unique excitation/emission spectrum that results in emission of a red-violet light that is visible to the neurosurgeon (Figure 1).

The preferential accumulation of PpIX allows for identification of glioma tissue for removal, as determined by the neurosurgeon. This includes both HGG and areas of anaplastic foci from which malignant progression may occur in LGGs. This imaging agent has been used over the last decade by numerous surgeons throughout the world in more than 50,000 recorded brain cancer cases in the EU and in 9 other countries. Among the benefits of 5-ALA are that it provides real time delineation of malignant tissue, is well tolerated by patients, is appropriate for use in patients with primary or recurrent brain tumors, can be used with existing standard operating neurosurgical microscopes, and results in greater surgeon confidence to identify tumor tissue.

Figure 1. Intraoperative Fluorescence Image



White light image of resection cavity (left) and corresponding blue light image (right) of resection cavity with area of strong (red), weak (pink) and no fluorescence (Zeiss Pentero, white light 25%, focus 255 mm, zoom 2.7 x, blue light: 100%)

4.3 Use in Clinical Practice

4.3.1 Devices Used for Visual Assessment of 5-ALA-induced Fluorescence

FGS of glioma using 5-ALA is accomplished through the use of the following technology:

- A standard surgical microscope; these are classified as Class 1 (general controls) medical devices exempt from premarket notification procedures (21 CFR 878.4700);
- A standard white, xenon light source for use with a surgical microscope; and
- An accessory kit for the standard microscope consisting of the filters that allow the passage of blue wavelength projected to the surgical field to visualize the fluorescence of

PpIX in the excitation wavelength range from 375 nm to 410 nm, and for observation of red emission from the tissue in the upper wavelength from 620 to 710 nm. The accessory kit has excitation and emission filters with slightly overlapping transmissions integrated into its optical configuration. The filters are designed to transmit red porphyrin fluorescence, as well as a fraction of backscattered blue excitation light necessary for distinguishing nonfluorescing tissue, thus, allowing for the differentiation of normal brain tissue (appearing blue in the surgical field) from malignant glioma (appearing red and pink in the surgical field).

4.3.2 Use in Surgery

5-ALA is used as an imaging tool to help neurosurgeons in visualizing malignant tumors in real time under blue light that may otherwise be overlooked during conventional resection. Identification, localization, and demarcation between tumor and normal brain tissue remains a difficult task for neurosurgeons, as there is no real time imaging tool to allow them to accurately define tumor margins for safe resection. Therefore, 5-ALA would be used as an informational tool to accurately define tumor location and differentiation from normal brain to aid in safe resection.

5-ALA is convenient to use. The oral solution (20 mg/mL) is administered to the patient 3 hours prior to anesthesia for resection surgery. The surgeon would then utilize standard operating procedures; however, after craniotomy and tumor tissue assessment under standard white light microscopy, the neurosurgeon has the option to select the insertion of adjunctive filters in front of the white light source on the operating microscope to allow the passage of only blue light (375-410 nm) illuminating the surgical field to search for fluorescent areas. These are the areas the neurosurgeon will consider for resection based on his or her training and other tools, such as neuronavigation and iMRI, to avoid vital regions of the brain (e.g., motor tracts and other key centers) identified in surgical mapping prior to approaching the tumor. Therefore, 5-ALA provides the surgeon with an accurate visualization tool to ensure that the tissue they deem resectable is indeed malignant glioma.

4.3.3 Patient Population

As previously discussed, malignant gliomas are categorized according to type and WHO grade. Recommendations for patient treatment often depend on tumor classification. The clinical studies supporting efficacy of 5-ALA are largely comprised of patients with suspected WHO grade III and IV glioma; however, 5-ALA has demonstrated utility in detection of lower grade tumors in the supportive peer-reviewed literature. 5-ALA is efficacious for use in patients with

primary or recurrent disease. Importantly, treatment with 5-ALA does not interfere with the safety or efficacy of oncology treatments. In addition, clinical studies with 5-ALA show no impact of patient demographics on safety and efficacy.

4.3.4 5-ALA and Resection Surgery for Malignant Glioma Patients

Resection surgery is the standard of care treatment for malignant glioma. The extent and completeness of glioma resection is related to several variables including the ability to differentiate tumor tissue from healthy brain tissue, the size of the tumor, the location of the tumor (avoiding damage to eloquent structures of the brain), and surgical skill. The use of 5-ALA during FGS for glioma contributes to providing the neurosurgeon with real time information relating to the location and margins of the tumor tissue. By providing this information, surgeons are better able to determine what and how much tissue to remove, thereby minimizing the potential of removing healthy brain tissue.

Similarly, several variables contribute to the OS or PFS of glioma patients. These include tumor grade, tumor size and location, age at diagnosis (younger patients often receive more aggressive treatment), functional status, amount of residual tumor, histologic genomic factors governing response to chemotherapies as well as mutations that alter tumor metabolism that may not be known to the treating physician, and response to radiation and chemotherapy, etc. (Walid 2008) As such, it is difficult to tease out the strength of the relationship between extent of surgical resection and overall survival in glioma patients.

Despite the use of the endpoints of PFS, OS, and EOR in previous regulatory submissions ex-U.S. and in the literature, the Sponsor does not assume that these factors are truth principles (see Section 6). As such, the current label and supporting data do not rely on the classic oncology endpoints that are presented; these endpoints are presented in this briefing package to provide the totality of the data which demonstrates a positive impact on PFS and EOR. 5-ALA is being presented solely as a real time imaging agent for the visualization of glioma with no claims toward benefit of resection or survival. The predictive accuracy of 5-ALA in identifying what is malignant, will be an invaluable tool in the neurosurgeons' tools in approaching brain tumor removal.

5 CLINICAL BACKGROUND

5.1 Overview of the Clinical Development Program

An overview of the clinical data comprised in the 5-ALA NDA are provided in Table 4. The 5-ALA clinical development program consists of a total of 6 clinical studies conducted in

Germany and meeting International Council for Harmonisation Good Clinical Practice requirements: 1 Phase 1 study, 1 Phase 1/2 study, 2 Phase 2 studies, and 2 Phase 3 studies. The key features of the 6 clinical studies are provided in Table 5.

Table 4. Overview of Clinical Safety and Efficacy Data Sources

	Study 28	Study 30	Study 3	Study 8	Study 32	Study 20	Scientific Literature[c]	EU Postmarketing
Patients Contributing Efficacy Data	N=33	N=36	N=176–FL	--	--	--	N=377	--
Patients Contributing Safety Data	N=36[a]	N=40	N=201-FL	N=7	N=243	N = 21[d]	N≅2,000	N≅58,000

FL = fluorescent-light group (i.e., received 5-ALA); N = number of patients; WL= white-light group (i.e., did not receive 5-ALA HCl)

[a] For Study 28, N=39 patients enrolled but n=3 were discontinued prior to receiving 5-ALA HCl

[c] Evaluation of scientific literature did not include analysis of raw data

[d] Study 20 was not included in the safety pooled analyses

Table 5. Clinical Studies Supporting 5-ALA Development

Study Type/Study Identifier	Objective of the Study	Test Product; Dosage Regimen; Route of Administration	Number of Enrolled Subjects[a]	Healthy Subjects or Diagnosis of Patients
Study 20 Phase 1 BA	To assess absolute bioavailability of oral (p.o.) single doses (20 mg/kg body weight [bw]) of 5-aminolevulinic acid (5-ALA) compared to intravenous (i.v.) administration (2 mg/kg bw) in healthy male subjects and to evaluate duration of photosensitization of the skin	5-ALA HCl solution 1 x 20 mg/kg bw p.o.; 1 x 2 mg/kg bw i.v.	N = 21 n = 12 p.o. and i.v. (subgroup 1) n = 9 p.o. (subgroup 2)	Healthy male subjects
Study 8 Phase 1/2 Dose Finding	To detect a dose-efficacy relationship between dose levels (0.2, 2, and 20 mg/kg bw of 5-ALA), to detect extent and quality of fluorescence of the tumor core, and to determine pharmacokinetics (PK) and safety of 5-ALA	5-ALA HCl solution 1 x 0.2, 2, or 20 mg/kg bw p.o.	N = 21 n = 7 0.2 mg/kg bw n = 7 2 mg/kg bw n = 7 20 mg/kg bw	Malignant glioma
Study 28 Phase 2 Safety and Efficacy	To determine the positive predictive value of tissue fluorescence and safety of 5-ALA	5-ALA HCl solution 1 x 20 mg/kg bw p.o.	N = 39	Malignant glioma
Study 3 Phase 3 Safety and Efficacy	To determine the efficacy and safety of fluorescence-guided resection of malignant gliomas with 5-ALA (FL group) compared to conventional resection (WL group) and to assess the clinical usefulness of this method	5-ALA HCl solution 1 x 20 mg/kg bw p.o. (FL group)	N = 415[a] n = 207 FL group n = 208 WL group	Malignant glioma
Study 30 Phase 2 Safety and Efficacy	To determine the positive predictive value of tissue fluorescence and safety of 5-ALA	5-ALA HCl solution 1 x 20 mg/kg bw p.o.	N = 40	Progressive/Recurrent malignant glioma

Study Type/Study Identifier	Objective of the Study	Test Product; Dosage Regimen; Route of Administration	Number of Enrolled Subjects[a]	Healthy Subjects or Diagnosis of Patients
Study 32 Phase 3 Safety	To determine the incidence of adverse events after fluorescence-guided resection of malignant gliomas using 5-ALA	5-ALA HCl solution 1 x 20 mg/kg bw p.o.	N = 245	Malignant glioma (newly diagnosed or progressive/recurrent)

5-ALA = 5-aminolevulinic acid; 5-ALA HCl = 5-aminolevulinic acid hydrochloride; BA = bioavailability; bw = body weight; FL group = fluorescence-guided resection group; i.v. = intravenous; PK = pharmacokinetics; p.o. = per os; by mouth or orally; WL group = conventional resection group.
[a] For Study 3, N = number of patients randomized; 3 of these did not receive 5-ALA HCl

Peer-reviewed published scientific literature also contributed to the demonstration of efficacy and safety. NXDC conducted a formal literature search of the PubMed, Medline and Embase medical literature databases to identify studies published between January 1, 1995 and June 9, 2016 that provide information on the use of 5-ALA to identify malignant tumor during glioma surgery. Clinical studies involving 5-ALA administration prior to glioma surgery were identified. The abstracts were reviewed for relevance to safety and efficacy and complete articles were retrieved and reviewed for further consideration. Studies were considered for inclusion regardless of whether the findings were positive or negative for the efficacy or safety of 5-ALA.

There were 12 peer-reviewed publications included to support the efficacy of 5-ALA (PPV and clinical usefulness). Studies from which sufficient data were provided to report or to calculate PPV at the biopsy level, and for which the methodology clearly noted that the surgeon's visual assessment of fluorescence took place during the resection, were included in this NDA as supportive efficacy data (N=11). Studies were considered supportive of clinical usefulness (i.e., data demonstrating the ability of 5-ALA to aid surgeons in the identification of malignant tumor under fluorescent light that may otherwise be overlooked during conventional white light microsurgical resection) if resection was first completed under white light before switching to blue light to view fluorescence (N=4).

Peer-reviewed publications included for safety were comprised of approximately 29 publications consisting of ~2,000 patients. Publications included clinical studies and case reports.

The safety assessment of 5-ALA also included EU postmarketing surveillance data from 2007-2015 as presented in the EU PSUR.

5.2 Clinical Pharmacology

5.2.1 Dose Justification

Within the dose-range finding study (Study 8), only the highest 5-ALA dose used in this trial (20 mg/kg bw) led to a sufficient tumor visualization both by subjective description as well as spectrophotometric/histological investigations and lead to a pronounced simplification of tumor surgery in the judgement of the principal investigator. This highest dose was the most efficient with respect to fluorescent quality and extent of the tumor core (Jonckheere-Terpstra test for fluorescence quality: $p < 0.0001$; test for fluorescence extent: $p < 0.0001$).

Overall, macroscopic fluorescence in the tumor core was highest in the 20-mg/kg group and in all cases the surgeon estimated that the entire viable tumor core to fluoresce. In the 2-mg/kg group, fluorescence was generally perceived as weak and the entire tumor core was not found to fluoresce. No fluorescence was perceived in the 0.2-mg/kg group, indicating a monotone, nonfalling dose-efficacy relationship. In addition, a significant positive correlation (Spearman Correlation Coefficient 0.5, p = 0.011) between tumor cellularity and spectrometrically determined fluorescence intensity was observed only in the 20 mg/kg group.

The timing of the dose was determined based on PK data from Phase 1 Study 20 and Phase 1/2 Study 8, which included 28 subjects (healthy volunteers and patients) who were treated orally with 20 mg/kg 5-ALA, the recommended dose for use in glioma surgery. Study 8 and Study 20 demonstrated that the absorption of exogenous 5-ALA occurs rapidly with maximum plasma concentrations reached approximately 1 hour after oral administration of 20 mg/kg bw 5-ALA. In healthy volunteers (Study 20), an absolute bioavailability of the orally administered dose of 20 mg/kg bw of 5-ALA is shown in Table 6.

Table 6. Absolute Bioavailability of 5-ALA (Geometric Means [Dispersion Factor]) (Study 20)

Parameter	Treatment Geometric Mean (Dispersion Factor)		Absolute BA (%)*
	b_1 (20 mg/kg p.o.)	b_2 (2 mg/kg i.v.)	[Ratio b_1 / b_2]
$AUC_{0-\infty}$ [mg x h/L]	33.13 (1.26)	3.31 (1.30)	100.02
$A_{e,ur}$ [mg]	420.62 (108.16)	45.17 (17.01)	104.79

$A_{e,ur}$ = amount excreted in urine; AUC = area under the plasma concentration curve; BA = bioavailability; i.v. = intravenous; p.o. = oral
*After dose adjustment

In Study 8, 5-ALA was rapidly absorbed after p.o. administration and eliminated quickly (Table 7). The PK parameters of PpIX are also presented in Table 8.

Table 7. Pharmacokinetic Parameters of 5-ALA (Study 8)

Parameter		Dose Level of 5-ALA		
		0.2 mg/kg	2 mg/kg	20 mg/kg
Number of patients:		7	7	7
$t_{1/2}$ [h]	Geo Mean (DF) Median (range)	0.85 (1.71) 0.75 (0.51–2.38)	1.12 (2.00) 0.84 (0.45–2.44)	3.05 (2.09) 1.94 (1.60–10.04)
T_{max} [h]	Geo Mean (DF) Median (range)	0.50 (1.75) 0.50 (0.23–1.00)	0.61 (1.77) 0.50 (0.25–1.47)	0.94 (1.51) 1.00 (0.52–2.00)
C_{max} [mg/L]	Geo Mean (DF) Median (Range)	256.9 (1.20) 274.7 (196.2–310.8)	2,103.6 (1.57) 1,862.6 (987.3–3,757.9)	8,272.2 (1.11) 8,239.1 (7,416.8–9,700.16)
AUC_{inf} [mg x h/L]	Geo Mean (DF) Median (Range)	539.9 (1.98) 424.2 (246.8–1,779.7)	3,326.0 (1.60) 2,901.3 (1,602.2–5,880.1)	26,914.9 (1.19) 27,143.5 (20,413.0–34,625.8)

5-ALA = 5-aminolevulinic acid; AUC = area under the plasma concentration time curve; C_{max} = mean peak plasma concentration; DF = dispersion factor; Geo Mean = geometric mean; $t_{1/2}$ = plasma concentration half-life time; T_{max} = time to reach C_{max}

Table 8. Pharmacokinetic Parameters of PpIX (Study 8)

Parameter		Dose Level of 5-ALA		
		0.2 mg/kg	2 mg/kg	20 mg/kg
Number of patients:		7	7	7
$t_{1/2}$ [h]	Geo Mean (DF) Median (range)	Not calculated	2.90 (1.36) 3.19 (1.62 – 3.83)	2.61 (1.63) 3.38 (1.52 – 4.08)
T_{max} [h]	Geo Mean (DF) Median (range)	Not calculated	4.81 (1.37) 4.92 (2.90 – 6.92)	5.73 (1.58) 5.48 (2.97 – 11.92)
C_{max} [mg/L]	Geo Mean (DF) Median (range)	Not calculated	32.28 (2.28) 27.44 (9.87 – 83.20)	Not calculated 101.71 (0.00 - 258.83)
AUC_{inf} [mg x h/L]	Geo Mean (DF) Median (range)	Not calculated	255.80 (2.46) 318.94 (54.97 – 572.60)	779.90 (2.73) 862.04 (247.96 – 2,655.06)

5-ALA = 5-aminolevulinic acid; AUC = area under the plasma concentration time curve; C_{max} = mean peak plasma concentration; DF = dispersion factor; Geo Mean = geometric mean; $t_{1/2}$ = plasma concentration half-life time; T_{max} = time to reach C_{max}

The highest concentrations of 5-ALA and PpIX were reached after 0.76 and 4.04 hours. For clinical use in brain tumor surgery, 5-ALA HCl is administered orally 3±1 hours before induction of anesthesia. Since PpIX is responsible for the tissue fluorescence, this dosing schedule for 5-ALA HCl is justified. It ensures that at the time of start of tumor resection the plasma concentration of PpIX is at its maximum and remains rather high for at least 6-8 hours.

5.2.2 Drug Interactions

5-ALA was not found to be a potent inhibitor of the major human cytochrome p450 isoforms (1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 3A) in human liver microsomes. All median inhibitor concentration values were > 300 μM.

The pharmacokinetic profile of subjects who received 5-ALA concurrently with another drug has not been formally evaluated. Due to the risk of possible phototoxic reactions, the label includes a statement that phototoxic substances (e.g., certain antibiotics [tetracylines, sulfonamides, fluoroquionolones], neuroepileptics [hypericin extracts], should not be used for up to 24 hours perioperatively after administration of 5-ALA. In addition, the label includes a statement that, within 24 hours of administration, potentially hepatotoxic drugs should be avoided.

6 CLINICAL EFFICACY DATA

6.1 Key Primary Endpoints

Based on the totality of the data, NXDC has elected to present the efficacy of 5-ALA as demonstrated by the following endpoints:

- predictive accuracy as measured by biopsy-based PPV and
- clinical usefulness as identified by the ability to aid surgeons in the identification of malignant tumor under fluorescent light that may otherwise be overlooked during conventional microsurgical resection.

In addition to clinical data concerning PPV and clinical usefulness, the clinical studies described in the NDA include information relating to the efficacy of 5-ALA as demonstrated by its effect on EOR, PFS, and OS. These data are described in Section 6.5. However, EOR for any patient depends on several factors including tumor location (eloquent areas), tumor size, patient condition, surgical skill, and the ability to visualize tumor margins. Further patient survival depends on several prognostic factors. As such, these endpoints are not an appropriate measure of the performance of 5-ALA. The use of PPV and clinical usefulness as relevant efficacy endpoints for 5-ALA have been extensively discussed with the FDA review division.

6.2 Sources of Efficacy Data

6.2.1 Pivotal Clinical Studies

In accordance with previous discussion with FDA, NXDC utilized 3 studies of the 6 clinical studies to demonstrate efficacy of 5-ALA based on biopsy-based PPV and clinical usefulness. Thus, for demonstration of efficacy for this NDA, Study 28, Study 30, and Study 3 are the pivotal studies. These studies include efficacy data on 245 patients who received 20 mg/kg bw 5-ALA, underwent resection surgery, had histologically confirmed grade III or IV glioma, and had available efficacy data (i.e., full analysis set [FAS]). The original efficacy endpoints (per each study protocol) are summarized in Table 9, with the primary efficacy endpoints for this NDA highlighted (biopsy-based PPV and clinical usefulness). At the request of FDA, the data

are presented in the NDA at a biopsy-based level and at the patient-based level. All data sets have been electronically submitted in an agreed upon analysis plan for FDA independent review of the data.

Table 9. Original Pivotal Study Endpoints

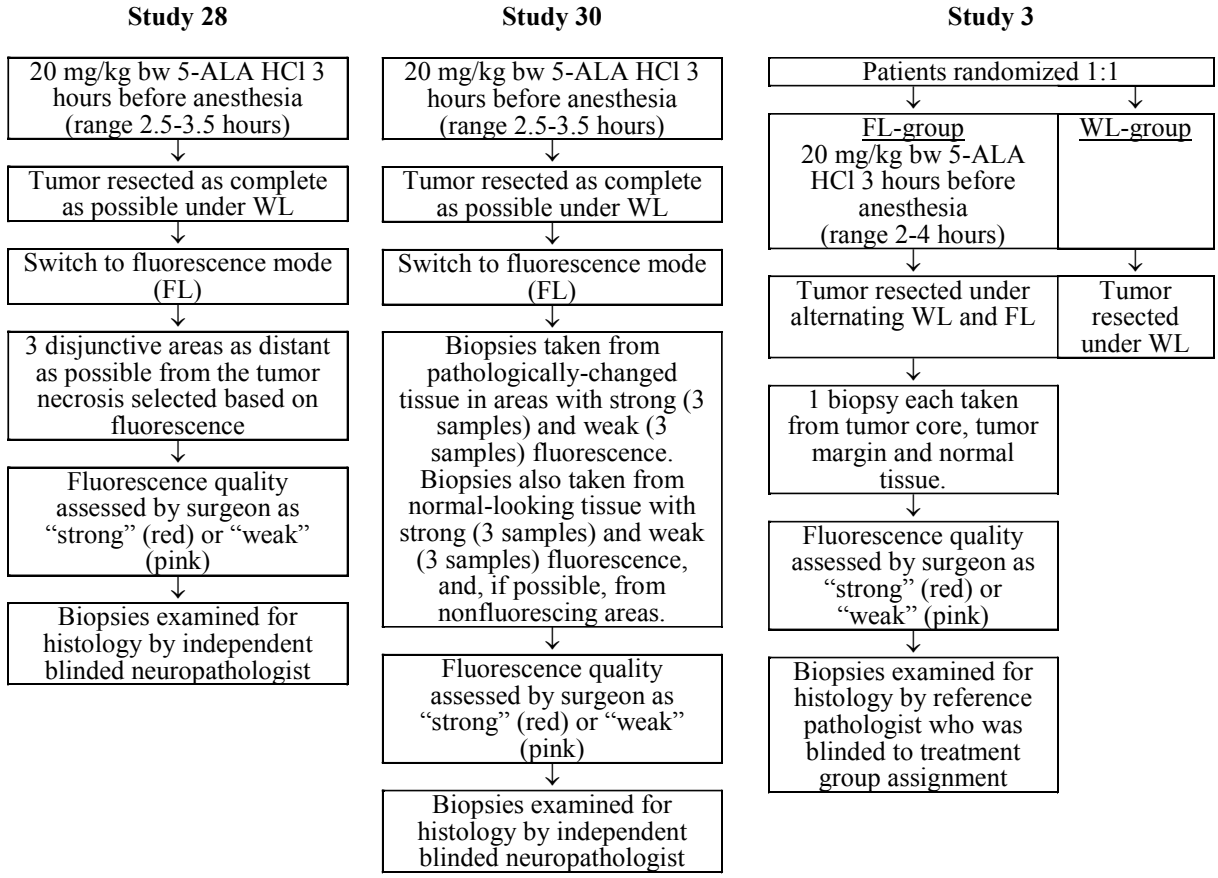
	Study 28 Single-arm; all 5-ALA	Study 30 Single-arm; all 5-ALA	Study 3 Randomized WL vs. FL [5-ALA]
Primary	• PPV: Pt-based	• PPV: Pt-based	• PFS @ 6 mo • % of patients without residual tumor
Secondary	• **PPV: Biopsy-based** • Evaluation of fluorescence quality by proximity to tumor • Fluorescence intensity measured spectrometrically • tumor cell quantity • histological differentiation • Evaluation of the simplification of resection using FGR • Assessment of location of intraoperative residual fluorescence by neuronavigation compared to post-op MRI (within 72 hrs)	• **PPV: Biopsy-based** • % of patients without residual tumor • OS	• Volume of residual tumor • PFS @ 9, 12, 15, 18 mo • OS up to 18 mo • Neurological condition 7 days, 6 and 12 weeks, 6, 9, 12, and 18 mo • Comparison of fluorescence diagnostics (intraoperative residual tumor) with early postoperative MRI (radiological residual tumor)
Evaluated for NDA; not defined in Original Study	• NPV: Biopsy based • NPV: Pt-based • **Clinical usefulness (qualitative)**	• NPV: Biopsy based • NPV: Pt-based • **Clinical usefulness (qualitative)**	• **PPV: Biopsy-based** • NPV: Biopsy based • PPV: Pt-based • NPV: Pt-based

Highlighted endpoints of PPV: Biopsy-based and Clinical usefulness were utilized to demonstrate efficacy of 5-ALA in NDA
5-ALA = 5-aminolevulinic acid; FGR=fluorescence guided resection; FL = fluorescent light; MRI=magnetic resonance imaging; NPV=negative predictive value; OS=overall survival; PFS=progression-free survival; PPV=positive predictive value; Pt=patient; WL = white light

6.2.2 Overview of Design of Pivotal Clinical Studies

The pivotal Studies 28, 30 and 3 are briefly described in the sections that follow with key design features compared and detailed in Table 10. The timing of the assessment of fluorescence and acquisition of biopsies is depicted in Figure 2.

Figure 2. Visualization of Fluorescence and Selection of Biopsies in Pivotal Studies



5-ALA HCl = 5-aminoevulinic acid hydrochloride; FL=fluorescence light; WL=white light

6.2.2.1 Study 28 Design Overview

Study 28 was a Phase 2, prospective, single-arm, uncontrolled multicenter study in primary glioma patients initiated on October, 29, 2002 and completed on June 10, 2004 conducted in Germany. The study included males or females aged 18 to 75 years with cranial MRI justifying diagnosis of malignant glioma (WHO grade III and IV) who received 20 mg/kg bw 5-ALA 3 hours before the induction of anesthesia. The primary objective was to determine the patient-based PPV of tissue fluorescence, defined as the percentage of patients showing positive

tumor cell identification in all biopsies taken from areas of weak and strong fluorescence. Biopsy-based PPV was a prespecified secondary endpoint.

Following administration of the study drug, basic treatment consisted of surgical removal of the tumor as completely as possible. After exposing the tumor using conventional white light illumination, the surgeon switched to the fluorescence mode of the operating microscope to visualize the 5-ALA-induced tissue fluorescence and to assess the fluorescence quality and intensity. To evaluate efficacy, 3 test regions remote from one another and as distant as possible from tumor necrosis were selected for each fluorescence quality. The fluorescence quality in the selected region was measured spectrometrically to determine fluorescence.

6.2.2.2 Study 30 Design Overview

Study 30 was a Phase 2, prospective, single-arm, uncontrolled multicenter study in recurrent glioma patients initiated on June 12, 2003 and completed on September 7, 2005 conducted in Germany. The study included males or females aged 18 to 75 years with cranial MRI justifying diagnosis of malignant glioma (WHO grade III and IV) for whom repeat surgery was indicated. Patients received 20 mg/kg bw 5-ALA 3 hours prior to induction of anesthesia. The primary objectives were to determine the patient-based PPV of tissue fluorescence based on total number of biopsies and to obtain an idea of the completeness of tumor resection (percentage of patients without residual contrast enhancing tumor on early postoperative MRI). Biopsy-based PPV was a prespecified secondary endpoint.

To evaluate efficacy, the fluorescence quality was evaluated in 2 different tissue areas distinguishable under normal white light: in abnormal, pathologically changed tissue and normal-looking tumor margins. For each, 3 separate areas for biopsy as distant as possible from any tumor necrosis were to be selected under white light. After switching to fluorescence mode, spots showing strong or weak fluorescence were selected and biopsies were collected. When all of pathologically altered tissue was removed, normal appearing tissue came into sight. Using standard white light illumination only, this would mark the end of resection. Per the study protocol, the surgeon was to switch to blue light (FL mode), reassessing the resection margins and to sample additional, fluorescing biopsies. At the end of the operation the surgeon was to assess whether all fluorescence-positive areas or areas suspicious for tumor under conventional white light had been resected and, if not, to describe the anatomical area of residual tumor.

6.2.2.3 Study 3 Design Overview

Study 3 was a Phase 3, randomized, group-sequential, rater-blinded, parallel-group, controlled multicenter study initiated on October 8, 1999 and completed on July 19, 2004 conducted in Germany. The study included males or females aged 18 to 72 years with cranial MRI justifying a diagnosis of uniocular malignant glioma (WHO grade III and IV). Patients were randomized to 1 of 2 treatment arms: a fluorescent light group or a standard white light operating group. Patients in the fluorescent light group received 20 mg/kg bw 5-ALA 3 hours before induction of anesthesia. The primary objectives were to determine the percentage of patients with a histologically confirmed malignant glioma without definite residual contrast-enhancing tumor seen in early postoperative control MRI and PFS at the 6-month visit after primary surgical resection. Biopsies were to be collected during the surgical procedure from the tumor core, tumor margin, and areas with normal tissue. For these biopsies, fluorescence was evaluated as weak or strong and histopathological analysis of tumor cell content was performed. Although PPV was not a prespecified endpoint, estimates of PPV and NPV were computed post hoc for the NDA.

Table 10. Study Design Comparison of the Pivotal Clinical Studies

Design Feature	Study 28	Study 30	Study 3

Number of Patients Enrolled	A total of 39 patients were assigned to undergo fluorescence-guided resection to yield 33 patients qualifying for the Full Analysis Set (FAS) within the final analysis.
	
	• Planned: 33 patients in the FAS
	• Efficacy: 33 patients in the FAS.
	• Safety: 36 patients in the Safety Analysis Set (3 patients did not receive 5-ALA)
		A total of 40 patients were assigned to undergo fluorescence-guided resection to yield 36 patients qualifying for the FAS within the final analysis.
		
		• Planned: 36 patients in the FAS
		• Efficacy: 36 patients in the FAS.
		• Safety: 40 patients in the Safety Analysis Set
			A total of 415 patients were randomly assigned to undergo either fluorescence-guided resection (FL group N=207 assigned) or standard white light surgery (WL group N=208 assigned) in a 1:1 ratio.
			
			• Final analysis:
			  • Efficacy: 349 patients (176 FL group, 173 WL group) in the FAS
			  • Safety: 374 patients (201 FL group, 173 WL group)

Key Entry Criteria:	• Radiological suspicion of a (primary) malignant glioma • Indication for surgical tumor resection • KPS ≥ 60% • Age 18-75 years	• Radiological suspicion of progressive/recurrent malignant glioma • Indication for surgical tumor resection • KPS ≥ 60% • Age 18-75 years	• Radiological suspicion of a (primary) malignant glioma • Indication for surgical tumor resection • KPS ≥ 70% • Age 18-72 years
5-ALA Dose	Single dose of 20 mg/kg bw 5-ALA HCl orally 3 hours (range 2.5-3.5 hours) before anesthesia	Single dose of 20 mg/kg bw 5-ALA HCl orally 3 hours (range 2.5-3.5 hours) before anesthesia	Single dose of 20 mg/kg bw 5-ALA HCl orally 3 hours (range 2-4 hours) before anesthesia
Biopsy Sampling Methods	Biopsies were to be taken from 3 different regions with strong fluorescence and 3 different regions with weak fluorescence as well as 2 regions of nonfluorescing tissue at the tissue border and 2 nonfluorescing regions in the cortex. .	Biopsies were to be taken from abnormal, pathologically-changed tissue in areas with strong (3 samples) and weak (3 samples) fluorescence. Biopsies were also taken from normal-looking tissue with strong (3 samples) and weak (3 samples) fluorescence, and, if possible, from nonfluorescing areas. Very few biopsies were obtained from nonfluorescing areas.	Per protocol, biopsies were to be taken from the tumor core, tumor margin, and areas with normal tissue and fluorescence was documented. For most patients, 1 biopsy each was taken from the tumor core, tumor margin and from normal tissue.

Design Feature	Study 28	Study 30	Study 3
Microscope/ Filter Information	An operating microscope (Zeiss OPMI Neuro FL) with a strong light source was used for fluorescence-guided resection. The xenon short arc lamp, fitted with emission filters, emitted blue light with a wavelength range from 380 - 440 nm. A yellow long-pass filer was inserted into the lens system, reducing the blue portion of the fluorescence excitation light and intensifying the contrast between red fluorescing and nonfluorescent areas.	An operating microscope (Zeiss OPMI Neuro FL) with a strong light source was used for fluorescence-guided resection. The xenon short arc lamp, fitted with emission filters, emitted blue light with a wavelength range from 380 - 440 nm. A yellow long-pass filer was inserted into the lens system, reducing the blue portion of the fluorescence excitation light and intensifying the contrast between red fluorescing and nonfluorescent areas.	An operating microscope (Zeiss OPMI Neuro FL) with a strong light source was used for fluorescence-guided resection.
Prespecified definition of positive tumor cell identification	Protocol defined a biopsy as demonstrating "positive tumor cell identification" if neuropathological review stated a tumor cell content of more than 0%. Centralized pathology review by 2 independent neuropathologists who were blinded to the fluorescence assessments of biopsies.	Protocol defined a biopsy as demonstrating "positive tumor cell identification" if neuropathological review stated a tumor cell content of more than 0%. Centralized pathology review by 2 independent neuropathologists who were blinded to the fluorescence assessments of biopsies.	Defined post hoc as the number of tumor positive biopsies for which tumor cell content was greater than 0% for any tumor localization (core, margin, normal tissue), among all biopsies with strong or weak florescence observed taken from patients treated with 5-ALA. Histological confirmation was provided by the reference pathologist, who was blinded at all times with respect to the assignment of the patient to treatment group.
PPV of tissue fluorescence at the biopsy level	Defined *a priori* (prespecified secondary endpoint) as the number of tumor positive biopsies among all biopsies taken from areas of weak and strong fluorescence	Defined *a priori* (prespecified secondary endpoint) as the number of tumor positive biopsies among all biopsies taken from areas of weak and strong fluorescence	Defined post hoc as the number of tumor positive biopsies, for which tumor cell content was greater than 0% for any tumor localization (core, margin, normal tissue, other tissue), among all fluorescing biopsies taken
Duration of Follow-Up	28 days	6 months	18 months

5-ALA = 5-aminolevulinic acid; FAS = full analysis set; FL =- fluorescent light; KPS = Karnofsky Performance Scale; MRI = magnetic resonance imaging; PPV = positive predictive value; WHO = World Health Organization; WL = white light

6.2.2.4 Patient Disposition

Few patients discontinued from the 3 studies, with respect to the clinical efficacy endpoints for the NDA. The 3 pivotal clinical efficacy studies provide efficacy data for 245 patients (FAS) who received 5-ALA.

6.2.2.5 Demographic, Baseline Clinical and Preoperative Characteristics in Pivotal Clinical Trials

The demographics and baseline characteristics of patients included in the 3 pivotal clinical studies, in general, were representative of patients with high-grade glioma. The median age was 60-61 years and the majority had a high KPS rating of 90 or 100. In comparison with other reported studies (Stupp et al. 2005, Chinot et al. 2014), the median age in newly diagnosed malignant gliomas was slightly lower than in the general malignant glioma population but higher than in recently published large randomized multicenter studies on GBM.

6.2.2.6 Summary of 5-ALA Exposure

The dosing regimen was generally the same for the FAS, given as 20 mg/kg bw orally, 2-4 hours before anesthesia. The average time from administration to start of surgery was consistent across studies (mean ± standard deviation [SD] 2.93 hours ± 0.29 in Study 28, 2.70 hours ± 0.89 in Study 30 and 2.39 hours ± 0.75 in Study 3). The weight-adjusted amount of 5-ALA received (19-21 mg/kg with the mean and median both being 20 mg/kg) deviated very little from the planned total dose in each study (mean deviation of 0.06 or less).

6.3 Definition of Endpoints Demonstrating Efficacy of 5-ALA

Efficacy of 5-ALA is demonstrated by the following endpoints:

- predictive accuracy as measured by biopsy-based PPV and
- clinical usefulness as identified by the ability to aid surgeons in the identification of malignant tumor under fluorescent light that may otherwise be overlooked during conventional microsurgical resection.

6.3.1 Biopsy-based PPV

The predictive accuracy of 5-ALA as measured by biopsy-based PPV was analyzed in the pivotal clinical studies. In Studies 28, 30, and 3, the surgeons conducting glioma resection identified and recorded the fluorescence quality of biopsies as “strong” if they appeared red, “weak” if they appeared pink, or “none” if no fluorescence was observed. In subsequent pathological

assessment of tumor biopsies by a blinded pathologist, a biopsy was defined as demonstrating "positive tumor cell identification" if neuropathological review stated a tumor cell content of more than 0%.

Biopsy-based PPV data from clinical studies 28 and 30 were prespecified secondary endpoints. Post hoc biopsy-based PPV was computed for Study 3. Estimates of NPV from each study were also included in the NDA. Figure 3 illustrates how biopsy-based PPV and NPV were computed from the data in the pivotal studies. Biopsy-based PPV was defined as the number of fluorescence-positive biopsies with positive identification of tumor among all fluorescence-positive biopsies. Biopsy-based NPV was defined (post hoc) as the number of nonfluorescing biopsies devoid of tumor cells among all nonfluorescent biopsies.

Figure 3. Biopsy-based Predictive Accuracy of 5-ALA

		Disease	**No Disease**	
		(> 0 tumor cells by pathologist)	**(0 tumor cells by pathologist)**	**Total**
Test Results (Fluorescence Assessment)	Positive (strong or weak fluorescence)	True Positive (TP) (biopsies with > 0 tumor cells and fluorescence = strong or weak)	False Positive (FP) (biopsies with 0 tumor cells and fluorescence = strong or weak)	Total Test positive = TP+FP
	Negative (no fluorescence)*	False Negative (FN) (biopsies with > 0 tumor cells and fluorescence = none)	True Negative (TN) (biopsies with 0 tumor cells and fluorescence = none)	Total Test negative = FN + TN
		Total Disease = TP+FN	Total Non-Disease = FP+TN	Total

FN = false negative; FP = false positive; TN = true negative; TP = true positive
*missing fluorescence results were excluded from assessments, only fluorescence indicated as "None" by investigator assessment on clinical study case report forms were counted as having no fluorescence.
PPV = TP/(TP+FP); NPV= TN/(FN+TN); Sensitivity=TP/(TP+FN); Specificity=TN(FP+TN)

In addition, the studies included patient-based estimates of PPV. For the endpoint of patient-based PPV, all biopsies exhibiting fluorescence from a given patient had to have been histologically confirmed as glioma for the patient to be counted as a "true positive" case. A single fluorescing biopsy where tumor-cell identification was negative renders a patient as a "false positive." Thus, estimates of patient-based PPV that are comprised of an assessment of multiple biopsies are, in part, biased by the number of biopsies obtained. As a result, biopsy-based PPV is a more accurate demonstration of predictive accuracy.

6.3.2 Clinical Usefulness

In alignment with pre-NDA meetings with FDA, NXDC provided data in the NDA for 5-ALA that demonstrates the use of 5-ALA for enhancing delineation of tumor resection margins. FDA noted that this assessment of clinical usefulness could be accomplished with patients undergoing maximal tumor resection using the optimal surgical procedures under white light and then undergoing additional FGS with 5-ALA.

The clinical usefulness of 5-ALA is *qualitatively* demonstrated by its ability to aid surgeons in the real time identification of malignant tumor under fluorescent light that may otherwise be overlooked during conventional microsurgical resection. Studies 28 and 30 provide evidence that 5-ALA fluorescence enables surgeons to identify residual areas of tumor seen in real time under blue light which were not identified under white light. Studies from peer-reviewed literature were considered supportive of clinical usefulness if resection was performed under standard white light and after the surgeon deemed the resection complete, the light source was switched to blue-violet light to assess residual malignant tissue. Studies in which the surgeon intermittently switched from white to blue light were not included because, although such procedures highlight how 5-ALA is utilized by surgeons during a resection, such studies were not optimal for demonstrating the additional utility of 5-ALA to detect malignant tissue that otherwise may have be overlooked during resection.

6.4 Efficacy Results

The primary efficacy results of biopsy-based PPV and clinical usefulness of FGS with 5-ALA are discussed in the sections that follow. In the NDA, the results of the other efficacy endpoints that were originally evaluated in each of the pivotal studies are provided (such as patient-based PPV). Also provided in the NDA are the exploratory analyses of biopsy-based and patient-based NPV that were conducted for the NDA.

6.4.1 Biopsy-based Estimates of PPV

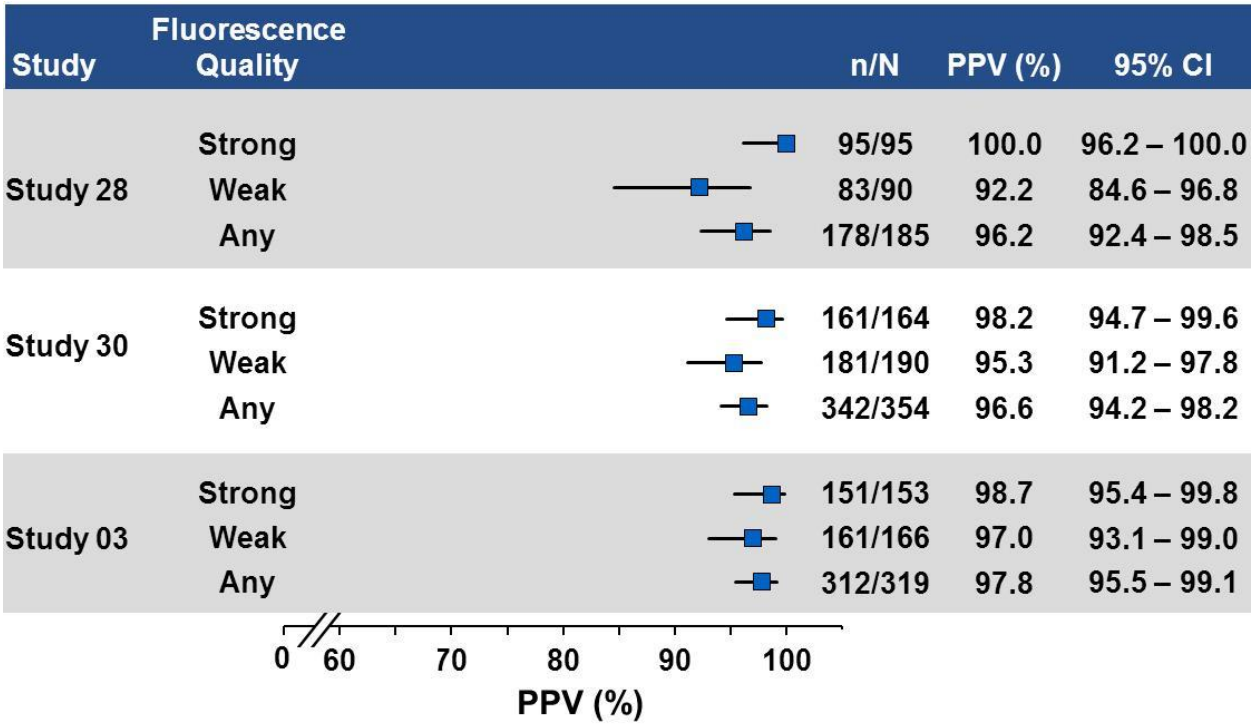
The biopsy-based PPV from all 3 pivotal trials shows high predictive accuracy of 5-ALA for any fluorescence (i.e., red or pink): 96.2% in Study 28, 96.6% in Study 30, and 97.8% in the FL group of Study 3 (Table 11 and Figure 4). The PPV for strong (i.e., red) fluorescence was slightly higher in each study compared to the PPV for weak (i.e., pink) fluorescence with the lowest PPV reported for weak fluorescence in Study 28 (92.2%).

Table 11. Biopsy-based Estimates of Positive Predictive Value Stratified by Fluorescence Quality

Fluorescence Quality of Biopsies	Study 28	Study 30	Study 3
Any Fluorescence:			
n/N (PPV)	178/185 (96.2%)	342/354 (96.6%)	312/319 (97.8%)
95% CI of PPV	92.4-98.5	94.2-98.2	95.5-99.1

n = number of fluorescent biopsies having tumor cells > 0%; N = number of fluorescent biopsies; PPV = positive predictive value (n/N*100); CI = confidence interval.
Exact 95% CIs are presented using the Clopper-Pearson method;
Only patients who received 5-ALA HCl are presented for Study 3.

Figure 4. Biopsy-based Estimates of PPV Stratified by Fluorescence Quality



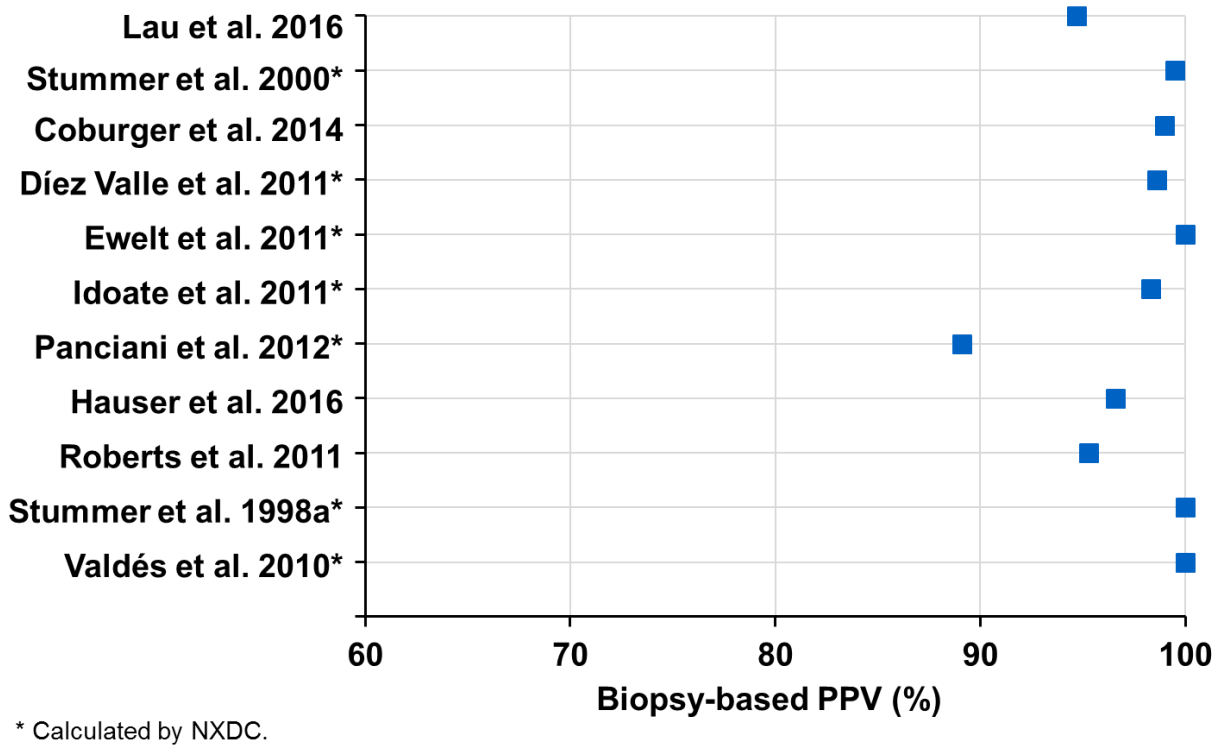
n = number of fluorescent biopsies having tumor cells > 0%; N = number of fluorescent biopsies; PPV = positive predictive value (n/N*100); CI = confidence interval.

The PPV of 5-ALA-induced fluorescence is supported by 11 peer-reviewed published studies (Figure 5; Coburger et al. 2014, Díez Valle et al. 2011, Ewelt et al. 2011, Hauser et al. 2016, Idoate et al. 2011, Lau et al. 2016, Panciani et al. 2012, Roberts et al. 2011, Stummer et al. 1998, Stummer et al. 2000, Valdés et al. 2010). From the systematic review conducted by NXDC, these publications either reported PPV at the biopsy level following FGS with 5-ALA or contained the necessary data from which biopsy-based PPV could be calculated for one or more biopsy subgroups, depending on the details of the study. Detailed information about these publications and PPV calculations are provided in the NDA.

The estimates of PPV from 11 public sources range from 69.0% to 100% for identification of malignant glioma by 5-ALA fluorescence under a variety of study conditions and study subgroups. The lowest PPV reported by Coburger et al. (2014) of 69.0% was computed for a subgroup of biopsies from solid tumor only using a strict criteria whereby 5-ALA strong positive biopsy samples among biopsy samples from the infiltration zone were considered false positive samples, even though these contained some (less than 20%) tumor cells and tumor proliferation detected by histology. If such samples were considered true positive along with histologically confirmed solid tumor samples, the PPV was 99.0%.

Figure 5 depicts the consistently high PPV (typically > 90%) rates at the biopsy level for most inclusive sets of biopsies (e.g., biopsies from solid tumor plus infiltration zone) reported in each publication.

Figure 5. Biopsy-based Estimates of Positive Predictive Value from Scientific Literature



* PPV not reported by authors but data presented in publication allowed for calculation by NXDC

All studies utilized the proposed dosing regimen of 5-ALA, i.e., a single dose of 20 mg/kg bw administered 2 - 4 hours prior to surgery. Standard neuronavigation techniques were typically employed. Although some studies also aimed to examine the utility of additional imaging techniques such as intraoperative MRI, contrast-enhanced MRI, or fluoroethyl-L-tyrosine (FET),

the PPVs reported reflect 5-ALA identification of tumor rather than the combination of tumor identification techniques. The visualization of fluorescence by the surgeon yields a high PPV confirming that the fluorescent tissue removed is confirmed to be malignant glioma.

Limitations of the literature studies include the following. Details about the sites selected for biopsy varied, however, these investigations typically included histological assessment of both fluorescent and nonfluorescent areas and sampled from both solid tumor and the tumor margin. Given that biopsy samples were generally obtained (per the individual study protocols) from sites that the surgeon identified as solid tumor or infiltration or tumor margin areas, the high PPVs are computed among a population of biopsies with a high pretest probability of disease, i.e., the likelihood of histologically confirmed tumor tissue in the population of biopsy samples selected. This high prevalence of glioma tissue in the population in question (i.e., the biopsy samples) results in a lower false positive rate.

In summary, NXDC identified, from a thorough search of the scientific literature, publications containing data from which PPV could be calculated. Without exception, the data from these studies support efficacy findings in the pivotal clinical studies of a high PPV (> 92%) of 5-ALA-induced fluorescence to visually identify malignant glioma.

6.4.2 Evidence of Clinical Usefulness

The clinical usefulness of 5-ALA is *qualitatively* demonstrated by its ability to aid surgeons in the real time identification of malignant tumor under fluorescent light that may otherwise be overlooked during conventional microsurgical resection. Studies 28 and 30 demonstrated the visual identification of malignant glioma tissue with 5-ALA, following what was deemed to be a complete resection by the surgeon utilizing conventional white-light neuronavigation techniques.

Several of the prespecified secondary efficacy analyses in Study 28 provide evidence of the clinical usefulness of 5-ALA. Per the protocol, after exposing the tumor using conventional white light illumination the surgeon switched to the fluorescence mode of the operating microscope to visualize tissue fluorescence and to determine the fluorescence quality and intensity. The fluorescence observed after switching to the fluorescence mode was classified subjectively by the surgeon in terms of its fluorescence quality (strong/weak). At the end of the operation the surgeon was to assess whether all fluorescence-positive areas or areas suspicious for tumor under conventional white light had been resected and, if not, to estimate the area and the quality (strong/weak) of residual tumor. Moreover, regions of residual tumor were to be documented by using neuronavigation and obtaining appropriate screenshots. This was to be

performed for a later comparison of residual tumor, as determined intraoperatively, with the location of contrast-enhancing tumor on early postoperative MRI.

Complete resection of tumor/fluorescent tissue, as assessed intraoperatively by the surgeon, was achieved in only 30.3% of patients. In 69.7%% of patients residual tumor/fluorescent tissue was left behind mainly due to potential harm to functional areas if these parts of the tumor would have been resected. Some of the intraoperative sites with suspected residual tumor were visible only under fluorescent conditions (but not using standard white light). Other positions were suspicious both under fluorescent conditions and using standard white light. Altogether, intraoperatively 42 sites (100%) with suspected residual tumor were recorded. Thirty-two sites (76%) were detected only with the aid of fluorescence, whereas 10 sites (24%) could be detected under standard conditions (and fluorescent conditions), indicating the clinical usefulness of the fluorescence method.

These 32 regions were further analyzed with respect to fluorescence area and quality. Large areas (> 3 cm^2) were seen in 2 of these positions, which were both seen on early postoperative MRI. Medium (1-3 cm^2) and small (< 1 cm^2) residual fluorescence could be identified in MRI only in 28.6% and 26.1% of cases, respectively. Two of 5 (40%) and 8 of 27 (29.6%) areas with strong or weak residual fluorescence could be detected by contrast enhancement on early postoperative MRI. From the 10 residual areas seen intraoperatively under white and fluorescence light, 4 (40%) showed contrast enhancement in early postoperative MRI. Ten residual lesions in 10 patients could only be detected with early postoperative MRI. Of these lesions, 2 were rather large (14.6 and 33.1 cm^3). In these patients, residually enhancing tumor was located at a distance to the resection cavity and was not expected to be detectable during surgery from within the cavity.

In Study 30, a multicenter study of 36 recurrent glioma patients, surgeons performed standard microsurgical resection under white light of recurrent malignant gliomas. After tumor resection, the resection cavity was examined with standard white light illumination. Abnormal, pathologically appearing tissue and adjacent, normal-appearing tissue were identified. The microscope was then switched to the fluorescent mode for fluorescence visualization and fluorescent tissue was sampled for histopathologic analysis. Thirty-four (34) of 36 patients (94.4%) showed fluorescence in at least 1 selected area, which looked normal under white light. PPV results showed that in normal appearing areas under white light, fluorescent tissue is malignant in 93% of the cases. In 35 of 36 patients, all acquired biopsies, irrespective of fluorescence quality (strong or weak), were positive for tumor cells. In abnormal tissue, fluorescence had a PPV of 97% for malignant glioma tissue in 35 out of 36 patients. More

importantly, areas appearing normal under white light, but displaying strong fluorescence, had a high PPV of 92% for malignant glioma tissue in 22 out of 24 patients.

In addition to the clinical studies, 4 peer-reviewed scientific publications were identified that support the clinical usefulness of 5-ALA for visualization of glioma (Table 12). Studies were considered to contain evidence of clinical usefulness if resection was performed under standard white light and after the surgeon deemed the resection complete, the light source was switched to blue-violet light to assess residual malignant tissue. Studies in which the surgeon intermittently switched from white to blue light were not included because although such procedures highlight how 5-ALA is utilized by surgeons during a resection, they cannot qualify the ability of 5-ALA to detect malignant tissue that otherwise may have been overlooked during resection.

Table 12. Clinical Usefulness of 5-ALA Evidenced by Scientific Literature

Publication	Objective of the Study/ Study Design	Number of Subjects (Biopsies) and Diagnosis of Patients	Test Product; Dosage Regimen; Route of Administration	Clinical Usefulness
Coburger et al. 2014	The objective of this prospective study was to evaluate whether 5-aminolevulinic acid (5-ALA) fluorescence provides an additional benefit in detection of high-grade gliomas (HGGs) and metastasis (MET) compared with intraoperative MRI (iMRI).	42 (136 biopsy samples) 34 World Health Organization (WHO) grade IV glioblastoma multiforme (GBM) (114 biopsies) 8 MET (13 biopsies)	5-ALA 20 mg/kg/ body weight (bw) oral (p.o.) 4 hours before surgery	Tumor detection was significantly higher with 5-ALA than with iMRI, especially at the tumor margin where both sensitivity (tumor positive) and specificity (tumor negative) were superior.
Hefti et al. 2008	The objective of this study was to report on the feasibility of 5-ALA induced intracellular protoporphyrin IX (PpIX) fluorescence to assist in the resection of high grade gliomas in daily clinical practice, and to report on the benefits for patient and surgeon, and the technical limitations and ways of overcoming those limitations.	71 (unknown number of samples) 57 glioma: 47 GBM 5 anaplastic astrocytoma (AA) 4 fibrillary astrocytomas 1 anaplastic ependymoma 14 tumors of other histological origin	5-ALA 20 mg/kg bw p.o. 5-6 hours before surgery	Residual positive fluorescence was visible and required further resection in 38/42 cases with intended gross tumor removal.
Panciani et al. 2012	The objective of this study was to analyze advantages and limitations of fluorescence- and image-guided resection.	23 (92) HGGs	5-ALA 20 mg/kg/ bw p.o. 2-4 hours before anesthesia	18 of 23 patients (78.3%) were found to have tissue fluorescence outside the area of neuronavigation that was consistent with tumor.
Stummer et al. 1998	The objective of this study was to investigate the value of fluorescent porphyrins that accumulate in malignant tissue after administration of a precursor (5-aminolevulinic acid) for labeling of malignant gliomas	9 (89 biopsy samples) 8 newly diagnosed GBM 1 AA	5-ALA 10 mg/kg/ bw p.o. 5 hours before surgery	In 7/9 cases of primary malignant glioma, the border appeared inconspicuous under white light but showed areas of PpIX fluorescence when illuminated by violet blue light. These areas which were resected revealed tumor in histological examinations.

5-ALA= 5-aminolevulinic acid; AA = anaplastic astrocytoma; bw = body weight; GBM = glioblastoma multiforme; HGGs = high-grade gliomas; iMRI = intraoperative MRI; MET = metastasis; PpIX = protoporphyrin IX; p.o. = oral; WHO = World Health Organization

In summary, clinical usefulness of 5-ALA was demonstrated by the pivotal studies and from peer-reviewed publications as evidenced by the identification of malignant glioma tissue under blue light after 5-ALA administration that was not detected during resection under white light.

6.4.3 Comparison of Results of Subpopulations

None of the efficacy analyses for PPV stratified by age revealed any obvious pattern suggesting an interaction with age. Information on race and ethnicity were not collected; however, high estimates of biopsy-based PPV as described in the 3 studies of German patients were consistent with PPV estimates reported in studies from published sources with a variety of patient populations. None of the efficacy analyses stratified by gender revealed any obvious pattern suggesting an interaction with gender.

6.4.4 Identification of Anaplastic Foci in Gliomas

5-ALA is useful in identifying the presence of anaplastic foci in gliomas. LGG, even when they appear homogenous on imaging, are genetically heterogenic and often contain areas of anaplasia denoting focal malignant degeneration to a WHO grade III or even grade IV tumor. In up to 50% of cases such tumors lack contrast-enhancement (Jaber et al. 2016, Kunz et al. 2011, Stockhammer et al. 2009, Muragaki et al. 2008, Jackson et al. 2001) and appear as grade II gliomas radiologically. Surgically, these tumors are not resected en bloc. Rather, they are decompressed carefully from within, with much of the tissue being removed by suction and not being available for histopathological assessment. Even when using the surgical microscope areas of anaplasia are rarely evident to the eye and are easily overlooked. Importantly, if such regions of anaplasia are not specifically interrogated pathologically, tumors will be under graded and necessary adjuvant treatments for HGG will be withheld, resulting in under treatment of patients. 5-ALA-derived tumor fluorescence allows better identification of HGG tissue intraoperatively for driving resection.

An important use of 5-ALA is its propensity for identifying areas of anaplasia in HGG which mimic LGG, showing no or only insignificant enhancement on imaging. This has been demonstrated by several studies (Floeth et al. 2011, Jaber et al. 2016, Marbacher et al. 2014, Widhalm et al. 2010, Widhalm et al. 2013) which are detailed below.

Floeth et al. (2011) compared the presurgical 18F-fluoroethyl-L-tyrosine (^{18}F-FET) uptake and diethylenetriaminepentaacetic acid enhancement on MRI (Gd) with intraoperative 5-ALA fluorescence in cerebral gliomas in a group of 30 patients newly diagnosed with WHO grade II-IV gliomas and previously resected LGG. Patients received 20 mg/kg bw p.o. Gliolan® (5-ALA)

2-4 hours before surgery. Resection yielded a total of 38 biopsies. Biopsies were taken from areas positive for enhancement (FET-PET) and fluorescence was determined ex vivo. 5-ALA fluorescence was observed in 57% (12/21) of the high-grade glioma biopsies and in 6% (1/17) of the LGG biopsies.

In a recent compilation of 10 nonenhancing grade III gliomas, 7 (70%) revealed PpIX fluorescence in areas of anaplasia. In the single GBM analyzed in this series no enhancement was found on MRI, whereas the malignant region was highlighted by intraoperative fluorescence (Jaber et al. 2016).

Marbacher et al. (2014) assessed the frequency of positive 5-ALA fluorescence in a cohort of patients with primary brain tumors and metastases in a single-center, retrospective analysis of 531 patients. A total of 458 cases qualified for final analysis. The highest percentage of 5-ALA-positive fluorescence in open resection was found in GBMs (96%, n = 99/103). Among other tumors, 5-ALA-positive fluorescence was detected in 88% (n = 21/32) of anaplastic gliomas (WHO grade III), 40% (n = 8/19) of LGG (WHO grade II), no WHO grade I gliomas (n = 0/3), and 77% (n = 85/110) of meningiomas.

Widhalm et al. (2010) aimed to clarify whether 5-ALA might serve as a marker for visualization of anaplastic foci in diffusely infiltrating gliomas with nonsignificant contrast enhancement for precise intraoperative tissue sampling in 17 patients with diffusely infiltrating gliomas with nonsignificant contrast enhancement. Focal 5-ALA fluorescence was observed in 8/9 patients with WHO grade III diffusely infiltrating gliomas. All grade II diffusely infiltrating gliomas were 5-ALA negative.

In another study, Widhalm et al. (2013) analyzed whether PpIX fluorescence can detect anaplastic foci according to WHO criteria in 59 patients with diffusely infiltrating gliomas (WHO grade II and III). A total of 215 biopsies were collected. In 27 of 59 patients (mostly grade III) PpIX fluorescence was visible, which allowed for different biopsy sampling. Specifically, in 23 of 26 cases (85%) of grade III gliomas with nonsignificant ["*none* or *patchy/faint* (=unspecific)"] contrast-enhancement demonstrated intraoperative fluorescence.

An exception to this is the study of Ewelt et al. (2011) who performed FET-PET imaging in 30 consecutive patients with intracerebral lesions suggestive of diffuse gliomas on MRI with or without areas of contrast-enhancement. Of these, 13 were WHO grade II (LGG), 15 were WHO grade III and 2 were grade IV (GBM). Prior to surgery patients were given 5-ALA at a dose of 20 mg/kg bw. A total of 40 biopsy samples were collected from the patients, and based on the

data provided, NXDC calculated a biopsy-based PPV of 93.3% if WHO grade II samples were considered false positives and 100.0% if considered true positives. A small number of samples that were negative by Gd-MRI and FET positive were positive for 5-ALA-induced fluorescence (2/17 = 11.8%). Although in this study biopsies did not show any 5-ALA-induced fluorescence when Gd-MRI and FET were both negative, it is important to note that the use of FET-PET imaging for glioma surgery is not widespread.

6.5 Progression-free Survival and Overall Survival Data from Clinical Studies

The goal of glioma resection is to safely remove as much of the tumor tissue as is possible without compromising brain function. This is intended to increase the success of adjuvant treatments with chemotherapy and radiotherapy, and therefore, increase PFS or OS (Stummer et al. 2008, Brown et al. 2016). Several investigations have concluded that complete gross total tumor resection has a greater benefit on patient survival than subtotal resection (Table 13), and a recent meta-analysis found that in over 41,117 newly diagnosed GBM patients, gross total resection substantially improved OS and PFS compared to subtotal resection (Brown et al. 2016).

Table 13. Summary of Published Literature Supporting the Beneficial Effect of the Removal of All Enhancing Tumor on PFS or Progression-free Intervals in Patients with High-grade Glioma

Reference	Patient Population	Extent of Resection	Effect on Patient Survival
Albert et al. 1994	GBM 60 AA 31	gross total resection: 18 % subtotal resection: 70 % unknown: 12%	Patients with a residual tumor postoperatively had a 6.6-times higher risk of death in comparison to patients without a residual tumor
Ammirrati et al. 1987	GBM 21 AA 10	gross total resection: 61% subtotal resection: 39%	Longer median survival (90 vs 43 weeks; P < 0.001); improved post-operative functional ability (P = 0.006) and Karnofsky index (P = 0.002)
Bricolo et al. 1990	GBM 86 AA 21	gross total resection: 74% subtotal resection: 26%	Increased 1-year survival (60 vs 24%); Improved Karnofsky index
Forsting et al. 1993	GBM 68	gross total resection: 23% subtotal resection: 77%	Reduced progression rate (36% vs 75%)
Lacroix et al. 2001	GBM 416	≥ 98% resection: 47% < 98% resection: 53%	Increased survival (13 vs 8.8 months, P < 0.0001)
Obwegeser et al. 1995	GBM 151	gross total resection: 62% subtotal resection: 38%	Increased survival (P = 0.003)
Stummer et al. 2006	GBM 52	gross total resection: 63% subtotal resection: 37%	Increased survival (P = 0.045)
McGirt et al. 2009	GBM 700 AA/AO 249	gross total resection: 35% near total resection: 41% subtotal resection: 24%	Longer median survival (13 vs 11 vs 8 months; significant difference for gross total vs subtotal resection (P = 0.05)
van den Bent et al. 2005	GBM 573	complete resection: 39% partial resection: 44%	Increased median survival (18.3 vs 13.5 months)*
Stummer et al. 2012	GBM 143	Complete resection: 52% Small residual tumor (< 1.5 cm): 32% Large residual tumor (> 1.5cm): 25%	Median overall survival not reached in patients with complete resections (95% CI: 21.4-25.9), as opposed to 16.9 (13.3-21.5) or 13.9 (10.3-17.5, p < 0.0001) months for small or large residual tumors*
Brown et al. 2016	GBM 1229	Complete resection: 71% Subtotal resection: (> 78% - < 100%): 29%	Increased median survival time (15.2 vs 9.8 months, P < 0.001)
* in patients receiving postoperative RT plus temozolomide			

The EOR for any patient depends on several factors including tumor location (eloquent areas), tumor size, patient condition, surgical skill, and the ability to visualize tumor margins. As a result, an effect of EOR on patient survival is not consistently observed.

Even for experienced surgeons, due to the infiltrative nature of gliomas, it is very difficult to identify the margins of the tumor, because often there is no sharp demarcation between tumor and normal tissue. This can result in the surgeon completing partial resection or unintentionally removing healthy tissue. The use of neuronavigation techniques such as MRI and iMRI, as well as the investigation of optical markers for the detection of tumors (tetracycline, methylene blue, or synthetic porphyrins), have improved surgeons' ability to identify tumor tissue. However, their use is limited in allowing the surgeon to locate and accurately identify malignant tumor during surgery, thus allowing the surgeon to make the best possible decision as to what to remove and how much to remove.

6.5.1 Extent of Resection and Survival Endpoints from the Clinical Studies

In the pivotal efficacy trials in the NDA (Study 30 and Study 3) completeness of resection was evaluated as the percentage of patients without residual tumor on early postoperative MRI (Table 14).

Table 14. Percentage of Patients without Residual Tumor on Early Postoperative MRI (Study 30 and Study 3)

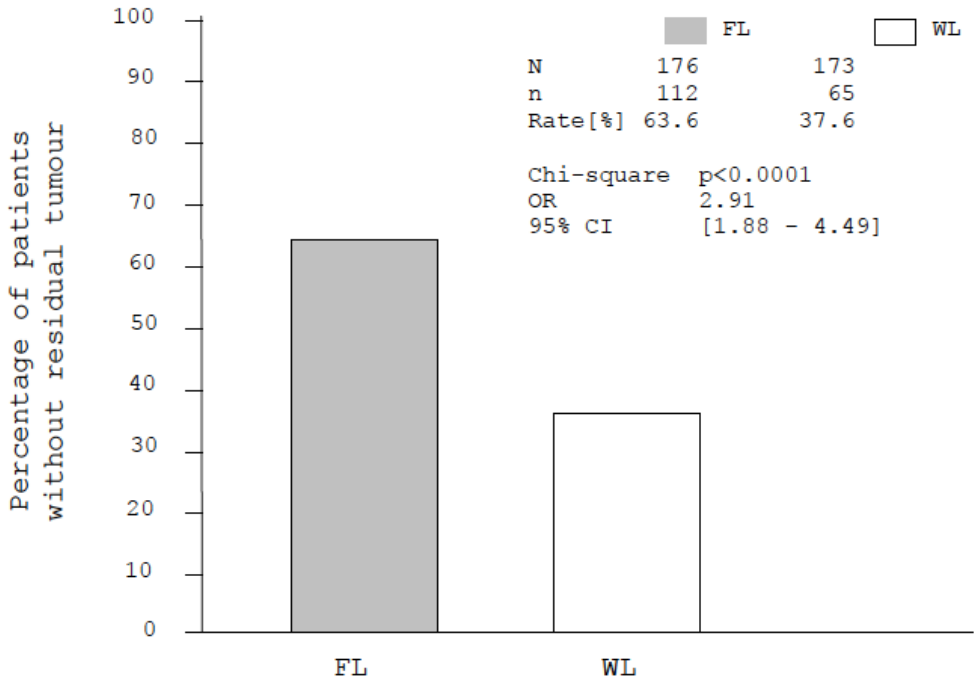
Study and Treatment Arm	Patient Population	% Patients without Residual Tumor @ Early Postop MRI (n/N)
Study 30 (5-ALA)	Recurrent glioma; Previous surgical treatment including open craniotomy	19.4% (7/36)
Study 3 (5-ALA FL Group)	Primary glioma; First operation of the tumor, no other tumor-specific pretreatment	63.6% (112/176)*
Study 3 (WL Group)		37.6% (65/173)

* $p < 0.0001$ FL vs. WL (Chi-square test); 5 patient(s) (2 in FL and 3 in WL arm) with not evaluable/missing early postoperative MRI data were/was included as patient(s) with residual tumor

The primary efficacy analysis in Study 3 included the percentage of patients without residual tumor on early postoperative MRI. For the FAS, 63.6% of all patients in the FL group and 37.6% of all patients in the WL group did not show residual tumor on early postoperative MRI (Chi-square test, $p < 0.0001$).

Significantly more patients in the experimental arm were tumor-free on early postoperative MRI than in the control group (FAS: 63.6% vs 37.6%; $P < 0.0001$) (Figure 6).

Figure 6. Complete resection rates



A very similar result was obtained for the Per-Protocol-Set. In the FL group, 63.8% of patients were operated on without residual tumor on the early MRI versus 39.2% of patients in the control-arm. This result was statistically significant ($p < 0.0001$) with a crude odds ratio of 2.73 (95% CI: 1.75 - 4.28). Thus, results were homogenous in both patient sets analyzed.

Classification of subgroups were chosen according to the criteria for randomization, i.e. age (55 vs > 55 years), KPS (80% vs > 80%), and endangerment of eloquent areas (no vs yes). There were more patients without residual tumor on early postoperative MRI if they were young (55 years: FL group: 74.5% vs WL group: 48.1%; $p = 0.0049$), had a better preoperative KPS (> 80%: FL group: 66.9% vs WL group: 40.2%; $p < 0.0001$), or had tumors without endangerment of eloquent brain areas (FL group: 69.1% vs WL group: 47.2%; $p = 0.0060$).

Odds ratios of almost all analyzed subgroups indicated a higher percentage of patients without residual tumor in the FL group compared to the WL group. Pronounced heterogeneities between subgroups could not be observed.

In Study 30, the single-arm study of recurrent glioma patients, central neuroradiological review of early postoperative MRIs demonstrated complete removal of areas with contrast enhancement in 7 patients (19.4%). In the majority of patients (25/36; 69.4%), complete resection of all areas

with contrast enhancement was not possible, leaving a total of 41 enhancing positions unresected in these 25 patients.

In Study 28 (single-arm study of primary glioma patients), locations of intraoperative residual fluorescence by neuronavigation were compared to post-op MRI (within 72 hours) (Table 15). Complete resection of tumor/fluorescing tissue as assessed intraoperatively by the surgeon was achieved in about a third of patients (10/33, 30.3%). In 69.7% of patients (23/33), residual tumor/fluorescence was left behind to avoid potential harm to functional areas. In 65.2 % of these patients (15/23), residual contrast enhancement in early postoperative MRI could be detected. In contrast, among patients considered by the surgeon to be macroscopically tumor-free after tumor resection (n = 10; 30.3%), the reference radiologist detected 2 patients with postoperative MRI contrast-enhancing areas (6 patients tumor-free; 2 patients not evaluable). In both patients, the area of enhancement on MRI corresponded to a second tumor at a distance from the operated tumor with intervening, nonenhancing brain.

Table 15. Intraoperative Assessment of Residual Tumor Compared to Contrast-Enhancing Residual Tumor on Early Postoperative MRI

Intraoperative assessment		Reference radiology		
Complete resection		Any enhancing residual tumor?		Specify
No	23 (69.7)	No	8 (34.8)	
		Yes	15 (65.2)	
		Total	23 (100.0)	
Yes	10 (30.3)	No	6 (60.0)	
		Yes	2 (20.0)	
		n.e.	2 (20.0)	- motion artefacts on MRI mage - preparation of postoperative MRI was omitted due to the patient's poor general condition. In a computer tomography bihemispheric infarction was found.
		Total	10 (100.0)	

MRI = magnetic resonance imaging; n.e. = not evaluable

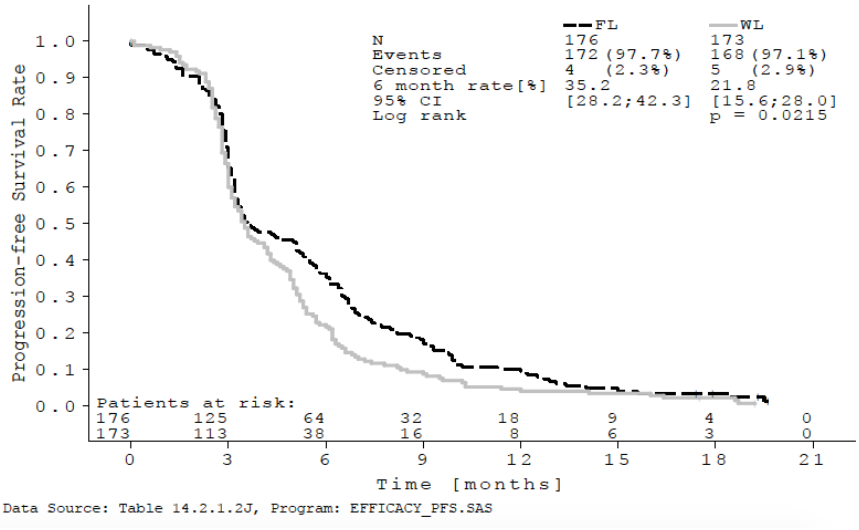
In Study 3, with the help of 5-ALA supported fluorescence-guided resection, significantly more patients in the experimental arm became tumor-free than in the control group (Full-Analysis-Set: 63.6% vs 37.6%; $P < 0.0001$). This was also true for all analyzed subgroups of patients (age, KPS, endangerment of eloquent areas, study surgeon, and study center). Logistic regression models showed that fluorescence-guided resection had the most important influence on the percentage of patients without residual tumor ($P < 0.0001$). Study 3 is the first study that prospectively showed that fluorescence-guidance increases the completeness of resection of malignant gliomas.

It must however be emphasized that fluorescing tissue cannot always be removed completely if eloquent areas are involved. In Study 28, in 69.7% of patients, residual tumor/ fluorescence was left behind to avoid potential harm to functional areas. In 65.2 % of these patients, residual contrast enhancement in early postoperative MRI could be detected. This was much higher than in the 5-ALA-arm of Study 3 because of different inclusion criteria (resectability of the tumor was demanded only in Study 3). The different outcomes in these studies, partly based on different definitions of the inclusion criterion, "resectability" clearly shows that visualization is not the only factor determining EOR. Surgical decisions, based on location and size of the tumor are other important determining factors.

Impact of 5-ALA on Progression-free Survival

In Study 3, the second primary efficacy parameter was PFS rates at the 6-month visit. Main outcomes are reported for completeness, but not to support the indication for 5-ALA. In the FAS, 20.5% of patients in the FL group and 11.0% of patients in the WL group were alive at the 6-month visit without progression. This difference was statistically significant using the Chi-square test ($p = 0.0152$) with a crude odds ratio of 2.08 (95% CI: 1.12 - 3.88) (Figure 7).

Figure 7. PFS Rates at 6 Months (Study 3)



In the FAS, 20.5% of patients in the FL group and 11.0% of patients in the WL group were alive at the 6-month visit without progression. This difference was statistically significant using the Chi-square test (p = 0.0152) with a crude odds ratio of 2.08 (95% CI: 1.12 - 3.88). In the Per-Protocol-Set, the PFS rate after at the 6-month visit was 22.5% in the FL group and 10.8% in the WL group. This result was statistically significant (p = 0.0047) with a crude odds ratio of 2.39 (95% CI: 1.27 - 4.50).

The PFS rate at 6-month visit was further analyzed by stratifying for the prognostic factors age (55 vs > 55 years), KPS (80% vs > 80%), endangerment of eloquent areas (no vs yes), and combinations thereof.

More patients were alive and progression-free at the six-month visit if they were young (55 years: FL group: 25.5% vs WL group: 13.5%; p = 0.1185), had a better preoperative KPS (> 80%: FL group: 21.6% vs WL group: 10.6%; p = 0.0143), or tumors without endangerment of eloquent brain areas (FL group: 27.2% vs WL group: 9.7%; p = 0.0060).

Odds ratios of almost all analyzed subgroups indicated superiority of the FL group compared to the WL group. The beneficial treatment effect was equally present within the subgroups. Odd ratios and their confidence intervals did not change when adjusted for the prognostic factors age, KPS, endangerment of eloquent areas, and combinations thereof, but were all of about the same size as the crude odds ratio. P-values adjusted for the prognostic factors using the Cochran-Mantel-Haenszel test were statistically significant with respect to the treatment effect. Similar results were obtained for the Per-Protocol-Set.

PFS rates at 9, 12, 15, and 18 months always favored the experimental arm with odds ratios clearly above 1, however, the differences did not reach the level of statistical significance.

Impact of 5-ALA on Overall Survival

Overall survival was a secondary endpoint in Studies 3 and 30. It is included for completeness, and for review of historical data.

In Study 30 of recurrent glioma patients who underwent FGS with 5-ALA median OS was 7.9 months (95% CI: 4.5 – 13.2 months). In Study 3 (randomized study of primary glioma patients), OS was planned as a secondary efficacy parameter. Because patients were taken off study after radiological progression has been diagnosed, post-study treatment was totally uncontrolled and. therefore, analysis of OS in this study was of explorative nature only. Median OS was 14.3 months (95% CI: 12.1 - 16.2 months) for patients in the FL group compared to 13.7 months (95% CI: 12.3 - 14.9 months) in the WL group. Differences did not reach statistical significance (p = 0.9170, log-rank test) and the crude hazards ratio was 0.99 (95% CI: 0.78 - 1.24). Survival rate one year after study surgery was 58% in both study groups.

The OS data provided needs to be interpreted with care. Study 3 was not powered to detect any differences in OS between groups. In addition, at the time of executing the Study 3, adjuvant radiotherapy was considered as a standard treatment option for patients with malignant gliomas. Approximately 90% of patients in both study arms received adjuvant postoperative radiotherapy. Furthermore, some kind of chemotherapy was administered to approximately 10% of patients postoperatively and (off-study) to further 55% of patients after tumor progression/recurrence. In the control arm, more patients underwent one (30.1 vs 39.3%) or two (5.1 vs 12.1%) re-operations. Furthermore, more patients in the control arm received temozolomide (FL vs WL: 45.8 vs 53.7%). The time to such re-intervention after radiological progression was prolonged in patients of the FL group compared to the control-group (9.0 months vs 7.1 months; log-rank-test P = 0.0863).

Nevertheless, OS was analyzed as a secondary endpoint in Study 3 and was shown to be comparable in both treatment arms (FL vs WL: 14.3 vs 13.7 months; P = 0.9170, log-rank test). Survival rate one year after study surgery was 58% in both study groups. Similarly to other studies (Albert et.al 1994, Ammirrati et al. 1987, Bricolo et al. 1990, Forsting et al. 1993, Obwegeser et al. 1995) those patients with complete resection in both treatment groups had a significantly better OS than those with an incomplete resection (16.7 vs 12.0 months; P < 0.0001 (Stummer et al. 2008).

It may be questionable to use PFS or OS as a surrogate marker for clinical usefulness of 5-ALA, as PFS and OS are driven by EOR and not by the use of 5-ALA. Surgeons were not able to achieve complete resections in all patients in the FL group of Study 3. In many patients in the WL group, complete resections were achieved without using 5-ALA, thus diluting the effects that can be derived from this novel tool.

6.5.2 Other Clinical Efficacy Endpoints from Clinical Studies

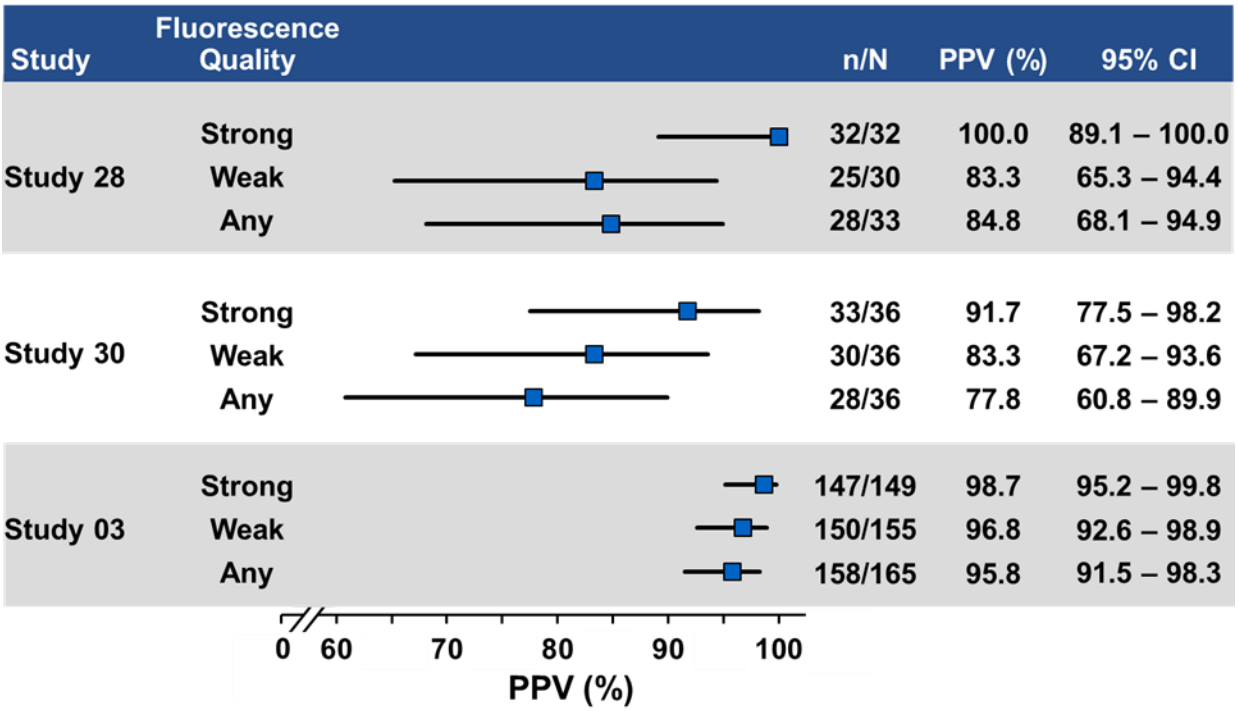
In the following, a number of endpoints are described, that were included as part of Study 3, Study 28, and Study 30. Some of these endpoints (patient-based PPV) were among the predefined objectives of these studies. Patient-based PPV and NPV endpoints are included here for completeness, but are not considered as pivotal to support the claim of clinical efficacy for 5-ALA, due to the methodological concerns of sampling normal brain tissue.

Patient-based PPV

The patient-based PPV of tissue fluorescence is defined as the percentage of patients with positive tumor cell identification in all biopsies taken from areas with weak and strong fluorescence. A biopsy was considered positive for tumor cell identification if the reference tumor cell content greater than 0%. For a patient to be considered a "true positive" for a particular fluorescence quality (weak or strong) all biopsies of that particular fluorescence quality must have been positive for that patient. Within the patient-based analyses, a single negative biopsy defines a patient as false positive, irrespective of the number of true-positive specimens, which explains the lower patient-based PPV estimates compared to the biopsy-based PPV estimates.

The patient-based estimates of PPV for any fluorescence (strong or weak) were consistently high across the 3 pivotal studies: 84.8% in Study 28, 77.8% in Study 30, and 95.8% in the FL group of Study 3 Figure 8).

Figure 8. Patient-based Estimates of Positive Predictive Value Stratified by Fluorescence Quality (Population: Full Analysis Set)



In each study, the number of patients where all fluorescing biopsies showed tumor (true-positive specimens) was higher when the quality of fluorescence was strong rather than weak.

Biopsy-Based NPV and Patient-Based NPV

Biopsy-based NPV was defined post-hoc as the number of nonfluorescing biopsies with 0% tumor cells identified during histology, among all nonfluorescing biopsies. Although the setting of glioma resection does not allow the collection of normal brain tissue in a systematic manner to fully characterize the NPV, the estimates were similar in the 3 studies (Table 16 for biopsy-based PPV and Table 17 for patient-based PPV)

Table 16. Biopsy-based Estimates of Negative Predictive Value (Biopsy-Based Full Analysis Set)

Fluorescence Quality of Biopsies	Study 28	Study 30	Study 3
n/N (NPV)	27/112 (24.1%)	3/16 (18.8%)	30/160 (18.8%)
95% CI of NPV	16.5-33.1	4.0-45.6	13.0-25.7

n = number non-fluorescent biopsies having 0 tumor cells; N = number of non-fluorescent biopsies; NPV = negative predictive value (n/N*100); CI = confidence interval.
Exact 95% CIs are presented using the Clopper-Pearson method.
Only patients who received 5-ALA HCl are presented for Study 3

Table 17. Patient-based Estimates of Negative Predictive Value (Patient-Based Full Analysis Set)

	Study 28	Study 30	Study 3
n/N (NPV)	2/33 (6.1%)	2/12 (16.7%)	29/143 (20.3%)
95% CI of NPV	0.7-20.2	2.1-48.4	14.0-27.8

n = number of patients with all non-fluorescent biopsies having 0 tumor cells; N = number of patients with any non-fluorescent biopsies; NPV = negative predictive value (n/N*100); CI = confidence interval.
Exact 95% CIs are presented using the Clopper-Pearson method.
Only patients who received 5-ALA HCl are presented for Study 3

Many of the peer-reviewed scientific literature providing biopsy-based PPV also reported NPV, or NPV could be computed. As summarized in Table 18 these estimates ranged from 20% - 91%.

Table 18. Summary of Biopsy-based NPV of 5-ALA from Public Sources

Citation	Biopsy-based NPV
Coburger et al. 2014	43% (solid tumor) 22% (tumor and infiltration zone)
Díez Valle et al. 2011	66%
Ewelt et al. 2011	NR
Hauser et al. 2016	12.5%
Idoate et al. 2011	67%
Lau et al. 2016	37.7%
Panciani et al. 2012*	91.3%
Roberts et al. 2011	26%
Stummer et al. 1998a*	75.0
Stummer et al. 2000*	50.0%
Valdés et al. 2010	20%

NPV = negative predictive value; NR=not reported
Source: Abstracts of Clinical Studies from the Public Literature Demonstrating the PPV of 5 ALA
* Calculated by NXDC

Patient-based NPV was defined as the number of patients in whom all nonfluorescing biopsies had 0% tumor cells identified during histology, among patients with any nonfluorescing biopsies. Although the setting of glioma resection does not allow the collection of normal brain tissue in a systematic manner to fully characterize the NPV, the estimates were similar in the 3 studies ranging from 6.1% (95% CI: 0.7% - 20.2%) in Study 28 to 20.3% (95% CI: 14.0% - 27.8%) in Study 3.

NXDC believes the NPV of 5-ALA-induced fluorescence is not a meaningful parameter for assessing the efficacy of 5-ALA HCl because the setting of glioma resection precludes the collection of normal brain tissue in a manner that can produce an unbiased estimate. Different

biopsy algorithms in different studies at various distances from the main tumor mass with varying tumor cell prevalence explain the high variability observed in the literature regarding NPV. In addition, NPV is of far less utility to the surgeon than PPV. Study 28 and other studies (Stummer et al. 2000, Coburger et al. 2015) have demonstrated fluorescence to exceed the area of enhancement on the MRI. Since the aim of surgery is resection of enhancing tumor, it is not necessary to visualize more tumor than what is visible during fluorescence.

The advantages of 5-ALA for making GBM tumors more visible are its endogenous origin (from heme metabolism) and good tolerability after local, oral, or intravenous use. Exogenously administered 5-ALA induces selective accumulation of the direct heme precursor, PpIX, in neoplastic cells, such as those of malignant gliomas. Upon exposure to violet-blue light, PpIX becomes activated which results in red-light fluorescence. The scope of the administration of 5-ALA is neither to "diagnose malignant gliomas" nor to "test the tumor stage or WHO grading". The aim of the use of 5-ALA is to visualize malignant lesions as an adjunct to neurosurgery. Recent studies have defined the complexity of using EOR as an endpoint, since it is dependent on multiple factors. In alignment with the Agency, NXDC finds considerable controversy in using EOR as a measureable endpoint, despite statistical significance in the studies included in the clinical database

Likewise, the influence of EOR on survival endpoints remains controversial. Brown et al. (2016) recently assessed whether EOR is associated with improved 1- and 2-year OS and 6-month and 1-year PFS in patients with GBM. This meta-analysis across 37 studies with 41,117 unique patients revealed decreased mortality in patients that underwent gross total resection as compared to subtotal resection. At 1 year (RR, 0.62; 95%CI, 0.56-0.69; $P < 0.001$; number needed to treat [NNT], 9) and 2 years (RR, 0.84; 95%CI, 0.79-0.89; $P < 0.001$; NNT, 17). The likelihood of disease progression was decreased with GTR compared with STR at 6 months (RR, 0.72; 95%CI, 0.48-1.09; $P = 0.12$; NNT, 14) and 1 year (RR, 0.66; 95%CI, 0.43-0.99; $P < 0.001$; NNT, 26). This analysis represents the largest systematic review and the only quantitative systematic review to date performed on this subject. Compared with STR, GTR substantially improves overall and PFS, but the quality of the supporting evidence is "moderate to low".

5-ALA-assisted more complete resection of malignant gliomas may result in statistically significant prolongation of PFS and reports of increase in OS. Although the data in the clinical database in our NDA package confirms improvements in the completeness of resection and increases in PFS (6 months) NXDC, in conjunction with the Agency and other reports, decided to rely on diagnostic accuracy and clinical usefulness as the primary endpoints for the use of 5-

ALA as an imaging agent to delineate structural elements, because of the multifactorial aspects of EOR and survival endpoints.

Based on these facts and the evidence presented in the NDA and the briefing package, NXDC intends to apply for the stated indication of the visualization of malignant tissue during surgery for malignant glioma. It is self evident that such a tool that could in real time identify malignant tissue would provide important and highly useful information for the neurosurgeon in decision making during surgery and therefore would be a much-needed tool.

6.6 Efficacy Conclusions

PPV (the probability that fluorescent areas contain histologically confirmed tumor tissue fluoresces) is an appropriate measure of efficacy (specifically predictive accuracy) for an intraoperative medical imaging agent. PPV data from the clinical studies, supported by published literature, support PPV values that are typically greater than 90%. Efficacy is also supported by the clinical usefulness of 5-ALA as demonstrated by clinical data from the studies and from the published literature that demonstrate the ability of 5-ALA to aid surgeons in identifying tumor tissue under fluorescent light that might be overlooked under white light.

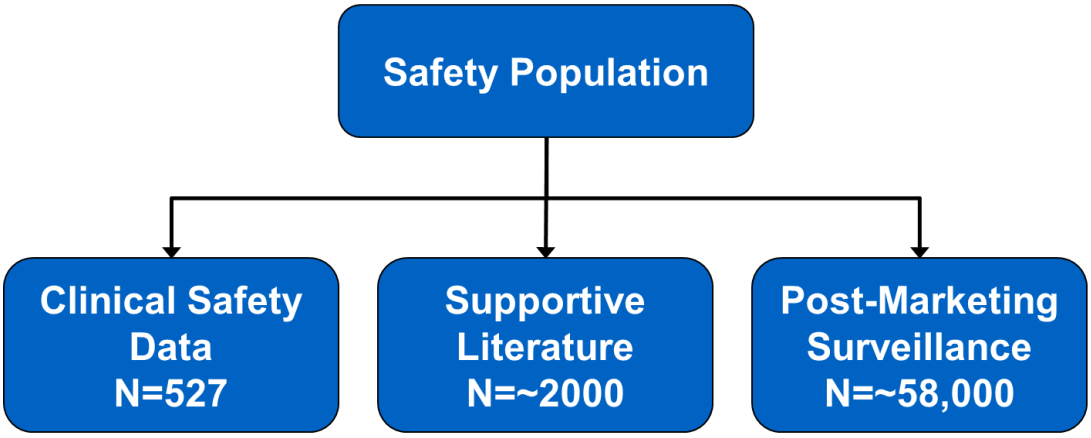
Exogenously administered 5-ALA induces selective accumulation of the heme precursor, PpIX, in neoplastic cells such as those of malignant gliomas. Upon exposure to violet-blue light, PpIX becomes activated which results in red-light fluorescence. The scope of the administration of 5-ALA is neither to “diagnose malignant gliomas” nor to “test the tumor stage or WHO grading”. The aim of the use of 5-ALA is to visualize malignant lesions as an adjunct to neurosurgery. PPV (the probability that fluorescent areas contain histologically confirmed tumor tissue fluoresces) is an appropriate measure of efficacy (specifically predictive accuracy) for an intraoperative medical imaging agent. The pivotal clinical studies, supported by published literature, demonstrate that the PPV of 5-ALA-induced fluorescence is typically greater than 90%. Efficacy is also supported by the clinical usefulness of 5-ALA. The pivotal clinical studies supported by published literature demonstrate the ability of 5-ALA to aid surgeons in identifying tumor tissue under fluorescent light that might be overlooked under white light. A product that can identify malignant tissue with a high degree of accuracy and distinguish such from normal brain tissue in real time would provide a much-needed tool to impact the surgical management of patients with glioma.

7 CLINICAL SAFETY DATA

7.1 Pivotal Studies Supporting Safety

The clinical safety data consists of integrated safety data from 527 patients (safety analysis set) who received at least 1 dose of 20 mg/kg bw 5-ALA HCl in Studies 8, 28, 30, 3, and 32 (Figure 9 and Table 19). Study 20 was described in the NDA, but was not included in the pooled safety analysis database because the data were derived from healthy normal volunteers. In addition, safety data are supported by 29 peer-reviewed published literature articles identified by a systematic literature search (~2,000 patients) and postmarketing surveillance data summarized in an EU PSUR (~58,000 patients) (Figure 9).

Figure 9. Clinical Safety Population



Postmarketing surveillance included recorded data from 2007 to 2015.

Table 19. Number of Subjects in Completed Studies in the Safety Analysis Set

Study Number	Phase	Patients who Received at Least 1 Dose of 20 mg/kg bw 5-ALA (N)
Full Safety Population (pooled studies)		527
Study 8	1/2	7
Study 28	2	36
Study 30	2	40
Study 3	3	201
Study 32	3	243

5-ALA = 5-aminolevulinic acid; N = number of patients

7.2 Inherent Risks of Resection Surgery

Surgical resection carries its own risks and complications. The complications and risks of resection surgery can be divided into neurologic, regional, and systemic, including direct cortical and vascular injury, surgical wound complications, and postsurgical medical complications (Jackson et al. 2016).

In the clinical studies it was expected that AEs would occur that are consistent with those associated with resection surgery. Therefore, for the purposes of the NDA, emphasis was placed on events that occurred within 6 weeks of resection surgery. Clinically, it is unlikely that TEAEs with an onset in the mid-term or long-term period were drug related. Pharmacokinetic studies of both 5-ALA and PpIX show that the increased plasma levels rapidly decrease and return to baseline with half-lives of 1-3 hours for 5-ALA and 3-4 hours for PpIX. Neurological TEAEs are more likely to be due to the course of the disease and/or the surgical procedure. It should be noted that the rate of postoperative deterioration in neurological function in single-armed trials was not different from what was found without the use of 5-ALA in the Glioma Outcome Project (Chang et al. 2010).

7.3 Safety Data

7.3.1 Adverse Events

AEs were classified using standard terminology from the verbatim description according to MedDRA Coding Dictionary (Version 18.1). TEAEs were defined as those that start or worsen at, or during the time of, or after 5-ALA administration through the last study visit. TEAEs were reported as mild, moderate, severe, life-threatening, or fatal. Serious adverse events (SAEs) without grade were imputed as follows:

a) If reason for classifying as a SAE on a case report form was indicated as fatal or outcome was recorded as resulting in death then grade was set to 5 (fatal).

b) If SAE was not graded as fatal as described in condition a) and reason for classifying as a SAE on the case report form included ‘life-threatening’ then grade was set to 4 (life-threatening).

c) If SAE was not graded as fatal or life-threatening as described in conditions a) or b) and reason for classifying as a SAE included ‘requires hospitalization’ or ‘leads to disability or deformity’ then grade was set to 3 (severe).

All other TEAEs with missing grade were reported as missing. TEAEs were reported in the integrated safety database as being related or not related to study drug. Events with a relationship of certain, probable, or possible were considered related to study drug. TEAEs with unknown relationship to study drug were counted as related to study drug.

All summary tables include only patients with TEAEs. All TEAE summaries present data separately based on time of occurrence. Patients with short-term TEAEs, mid-term TEAEs, and long-term TEAEs are presented separately:

- Short-term safety data is defined as data collected within 1 week of the resection operation;
- Mid-term safety data is defined as data collected more than 1 week but within 6 weeks of the resection operation;
- Long-term safety data is defined as data collected more than 6 weeks after the resection operation.

The denominator for incidence of events in each follow-up period is the total number of patients that entered the phase.

The overall summary presentations will display the number of patients. Incidence presentations include summaries of the number of patients and percentage of patients by system organ class (SOC) and preferred term (PT). Incidence presentations include summaries of the number of patients and percentage of patients by SOC, PT, and maximum severity and relatedness with any TEAE. The worst severity (i.e., mild, moderate, severe, life-threatening) and causality (i.e., related to study drug) were counted if a patient experienced a TEAE more than once. Severity was not reported for SAEs for Study 28, Study 30, and Study 8, and was imputed per rules outlined in the Statistical Analysis Plan. Severity for all other TEAEs is presented as reported by the investigator. Summaries of TEAEs occurring in ≥ 1% of patients receiving 5-ALA are reported.

7.3.1.1 Treatment-Emergent Adverse Events

All analyses of AEs are presented as the incidence of TEAEs by patient. A total of 802 TEAEs were experienced by 317 patients in the pooled safety population (N = 527). The majority of TEAEs were not related to 5-ALA and no patients discontinued the study due to nonfatal AEs.

Table 20 summarizes patients with TEAEs occurring in all phases (short, mid and long-term) of the studies. Most patients experienced TEAEs in the first week after surgery (short-term phase)

compared to the mid and long-term phases (41.6% versus 24.6% within 6 weeks after surgery and 18.3% of patients more than 6 weeks after surgery). TEAEs more than 6 weeks after surgery were captured primarily in Study 3 (42.5%), which was expected because the subject follow-up period was 18 months. TEAEs occurring within the first week following surgical resection are presented in Table 21. Mid-term and long-term TEAEs are presented in Table 22 and Table 23.

Table 20. Summary of TEAEs across All Phases of Study (by Patient)

Number of Patients with Any:	Study N (%)					
	Study 8 (N = 7)	**Study 28 (N = 36)**	**Study 30 (N = 40)**	**Study 3 (N = 201)**	**Study 32 (N = 243)**	**Pooled Studies[a] (N = 527)**
Treatment-emergent Adverse Event (TEAE)	7 (100%)	27 (75.0%)	22 (55.0%)	135 (67.2%)	126 (51.9%)	317 (60.2%)
Total Events	37	90	48	348	279	802
Drug-related TEAE	2 (28.6%)	6 (16.7%)	0 (0%)	7 (3.5%)	3 (1.2%)	18 (3.4%)
Total Events	4	6	0	8	5	23
Severity						
Mild TEAE	0 (0%)	1 (2.8%)	2 (5.0%)	33 (16.4%)	30 (12.3%)	66 (12.5%)
Moderate TEAE	5 (71.4%)	10 (27.8%)	15 (37.5%)	26 (12.9%)	50 (20.6%)	106 (20.1%)
Severe TEAE	2 (28.6%)	14 (38.9%)	5 (12.5%)	45 (22.4%)	34 (14.0%)	100 (19.0%)
Life-Threatening TEAE	0 (0%)	0 (0%)	0 (0%)	13 (6.5%)	12 (4.9%)	25 (4.7%)
Fatal TEAE	0 (0%)	2 (5.6%)	0 (0%)	18 (9.0%)	0 (0%)	20 (3.8%)
Serious Adverse Event	1 (14.3%)	8 (22.2%)	4 (10.0%)	68 (33.8%)	49 (20.2%)	130 (24.7%)
Adverse Event Leading to Discontinuation	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Adverse Event Leading to Death	0 (0%)	2 (5.6%)	0 (0%)	18 (9.0%)	5 (2.1%)	25 (4.7%)

N = number of patients; TEAE = treatment-emergent adverse event

*Patients reporting a particular adverse event (preferred term) more than once are counted only once by preferred term and system organ class.

**For TEAE by severity patients are counted once on the highest severity of all TEAE experienced by the patient.

[a] Pooled column includes patients treated with 20 mg/kg bw 5-ALA from Studies 8, 28, 30, 3, and 32

Table 21. Summary of TEAEs – Short Term (by Patient)

Number of Patients with Any	Study N (%)					
	Study 8 (N = 7)	**Study 28 (N = 36)**	**Study 30 (N = 40)**	**Study 3 (N = 201)**	**Study 32 (N = 243)**	**Pooled Studies[a] (N = 527)**
Treatment-emergent Adverse Event (TEAE)	7 (100%)	25 (69.4%)	22 (55.0%)	69 (34.3%)	96 (39.5%)	219 (41.6%)
Total Events	27	66	42	108	170	413
Drug-related TEAE	2 (28.6%)	4 (11.1%)	0 (0%)	3 (1.5%)	2 (0.8%)	11 (2.1%)
Total Events	4	4	0	3	4	15
Severity						
Mild TEAE	0 (0%)	1 (2.8%)	2 (5.0%)	25 (12.4%)	22 (9.1%)	50 (9.5%)
Moderate TEAE	6 (85.7%)	9 (25.0%)	15 (37.5%)	15 (7.5%)	45 (18.5%)	90 (17.1%)
Severe TEAE	1 (14.3%)	13 (36.1%)	5 (12.5%)	19 (9.5%)	25 (10.3%)	63 (12.0%)
Life-Threatening TEAE	0 (0%)	1 (2.8%)	0 (0%)	7 (3.5%)	4 (1.6%)	12 (2.3%)
Fatal TEAE	0 (0%)	1 (2.8%)	0 (0%)	3 (1.5%)	0 (0%)	4 (0.8%)
Serious Adverse Event	0 (0%)	8 (22.2%)	4 (10.0%)	26 (12.9%)	31 (12.8%)	69 (13.1%)
Adverse Event Leading to Discontinuation	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Adverse Event Leading to Death	0 (0%)	1 (2.8%)	0 (0%)	3 (1.5%)	1 (0.4%)	5 (0.9%)

N = number of patients; TEAE = treatment-emergent adverse event
Short term = within 1 week after resection surgery
*Patients reporting a particular adverse event (preferred term) more than once are counted only once by preferred term and system organ class.
**For TEAE by Severity patients are counted once on the highest severity of all TEAE experienced by the patient.
[a] Pooled column includes patients treated with 20 mg/kg bw 5-ALA from Studies 8, 28, 30, 3, and 32

Table 22. Summary of TEAEs – Mid Term (by Patient)

Number of Patients with Any	Study N (%)					
	Study 8 (N = 7)	**Study 28 (N = 35)**	**Study 30 (N = 39)**	**Study 3 (N = 198)**	**Study 32 (N = 241)**	**Pooled Studies[a] (N = 520)**
Treatment-emergent Adverse Event (TEAE)	3 (42.9%)	13 (37.1%)	2 (5.1%)	54 (27.3%)	56 (23.2%)	128 (24.6%)
Total Events	10	24	6	80	105	225
Drug-related TEAE	0 (0%)	2 (5.7%)	0 (0%)	2 (1.0%)	1 (0.4%)	5 (1.0%)
Total Events	0	2	0	2	1	5
Severity						
Mild TEAE	1 (14.3%)	4 (11.4%)	1 (2.6%)	11 (5.6%)	17 (7.1%)	34 (6.5%)
Moderate TEAE	0 (0%)	6 (17.1%)	1 (2.6%)	10 (5.1%)	20 (8.3%)	37 (7.1%)
Severe TEAE	2 (28.6%)	2 (5.7%)	0 (0%)	21 (10.6%)	11 (4.6%)	36 (6.9%)
Life-Threatening TEAE	0 (0%)	0 (0%)	0 (0%)	5 (2.5%)	8 (3.3%)	13 (2.5%)
Fatal TEAE	0 (0%)	1 (2.9%)	0 (0%)	7 (3.5%)	0 (0%)	8 (1.5%)
Serious Adverse Event	1 (14.3%)	5 (14.3%)	1 (2.6%)	25 (12.6%)	20 (8.3%)	52 (10.0%)
Adverse Event Leading to Discontinuation	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Adverse Event Leading to Death	0 (0%)	1 (2.9%)	0 (0%)	7 (3.5%)	4 (1.7%)	12 (2.3%)

N = number of patients; TEAE = treatment-emergent adverse event
Mid term = more than 1 week but within 6 weeks of resection surgery
*Patients reporting a particular adverse event (preferred term) more than once are counted only once by preferred term and system organ class.
**For TEAE by Severity patients are counted once on the highest severity of all TEAE experienced by the patient.
[a] Pooled column includes patients treated with 20 mg/kg bw 5-ALA from Studies 8, 28, 30, 3, and 32

Table 23. Summary of TEAEs – Long Term (by Patient)

Number of Patients with Any	Study N (%)					
	Study 8 (N = 0)	**Study 28 (N = 0)**	**Study 30 (N = 36)**	**Study 3 (N = 183)**	**Study 32 (N = 231)**	**Pooled Studies[a] (N = 453)**
Treatment-emergent Adverse Event (TEAE)	0 (0%)	0 (0%)	0 (0%)	79 (42.5%)	4 (1.7%)	83 (18.3%)
Total Events	0	0	0	160	4	164
Drug-related TEAE	0 (0%)	0 (0%)	0 (0%)	2 (1.1%)	0 (0%)	2 (0.4%)
Total Events	0	0	0	3	0	3
Severity						
Mild TEAE	0 (0%)	0 (0%)	0 (0%)	22 (11.8%)	3 (1.3%)	25 (5.5%)
Moderate TEAE	0 (0%)	0 (0%)	0 (0%)	20 (10.8%)	1 (0.4%)	21 (4.6%)
Severe TEAE	0 (0%)	0 (0%)	0 (0%)	25 (13.4%)	0 (0%)	25 (5.5%)
Life-Threatening TEAE	0 (0%)	0 (0%)	0 (0%)	3 (1.6%)	0 (0%)	3 (0.7%)
Fatal TEAE	0 (0%)	0 (0%)	0 (0%)	9 (4.8%)	0 (0%)	9 (2.0%)
Serious Adverse Event	0 (0%)	0 (0%)	0 (0%)	33 (17.7%)	0 (0%)	33 (7.3%)
Adverse Event Leading to Discontinuation	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Adverse Event Leading to Death	0 (0%)	0 (0%)	0 (0%)	9 (4.8%)	0 (0%)	9 (2.0%)

N = number of patients; TEAE = treatment-emergent adverse event
Long term = more than 6 weeks after resection surgery
*Patients reporting a particular adverse event (preferred term) more than once are counted only once by preferred term and system organ class.
**For TEAE by Severity patients are counted once on the highest severity of all TEAE experienced by the patient.
[a] Pooled column includes patients treated with 20 mg/kg bw 5-ALA from Studies 8, 28, 30, 3, and 32

The most common TEAEs, regardless of causality, were nervous system disorders, which occurred in 29.4% of patients within the first week after surgery (Table 24). Neurological TEAEs are likely due to the surgical procedure itself. Other TEAEs that occurred in > 1% of patients in the week following surgery were pyrexia, hypertension, nausea, and vomiting.

Table 24. Most Common Nervous System Disorders TEAEs – Short Term (by Patient)

Adverse Event	Patients, % N=527
Aphasia	8.0
Hemiparesis	7.8
Hemianopia	3.2
Headache	2.7
Seizure	1.9
Hemiplegia	1.9
Monoparesis	1.3
Hypoaesthesia	1.1

Short term = within 1 week after resection surgery

TEAEs that were determined by the investigator to be at least "possibly related" were classified as drug-related TEAEs. Drug-related TEAEs accounted for 2.1%, 1.0%, and 0.4% of all TEAEs in the short term (Table 25), mid term (Table 26), and long term (Table 27), respectively. Please note that these tables also include all TEAEs where causality was not indicated or was "unexplained".

Table 25. Related TEAEs – Short Term

System Organ Class Preferred Term	Pooled Studies N=527 n (%)
Overall	**11 (2.1)**
Nervous system disorders	
Brain edema	**1 (0.2)**
Hemianopia	**1 (0.2)**
Hypoesthesia	**1 (0.2)**
General disorders and administration site conditions	
Pyrexia	**2 (0.4)**
Chills	**1 (0.2)**
Skin and subcutaneous tissue disorders	
Photosensitivity reaction	**2 (0.4)**
Solar dermatitis	**1 (0.2)**
Vascular disorders	
Hypertension	**1 (0.2)**
Hypotension	**1 (0.2)**
Injury, poisoning and procedural complications	
Procedural complication	**1 (0.2)**
Procedural hypotension	**1 (0.2)**
Investigations	
Liver function test abnormal	**1 (0.2)**
Respiratory, thoracic and mediastinal disorders	
Respiratory failure	**1 (0.2)**

Short term = within 1 week after resection surgery
Patients reporting a particular adverse event (preferred term) more than once are counted only once by preferred term and system organ class.

Table 26. Related TEAEs – Mid Term

System Organ Class Preferred Term	Pooled Studies N=527 n (%)
Overall	**5 (1.0)**
Skin and subcutaneous tissue disorders	
Rash generalized	**1 (0.2)**
Vascular disorders	
Venous thrombosis limb	**1 (0.2)**
Gastrointestinal disorders	
Diarrhea	**1 (0.2)**
Infections and infestations	
Meningitis	**1 (0.2)**
Musculoskeletal and connective tissue disorders	
Sjogren's syndrome	**1 (0.2)**

Mid term = more than 1 week but within 6 weeks of resection surgery
Patients reporting a particular adverse event (preferred term) more than once are counted only once by preferred term and system organ class.

Table 27. Related TEAEs – Long Term

System Organ Class Preferred Term	Pooled Studies N=527 n (%)
Overall	**2 (0.4)**
Nervous system disorders	
Dizziness	**1 (0.2)**
Seizure	**1 (0.2)**
General disorders and administration site conditions	
Asthenia	**1 (0.2)**

Long term = more than 6 weeks after resection surgery
Patients reporting a particular adverse event (preferred term) more than once are counted only once by preferred term and system organ class.

7.3.1.2 Serious Adverse Events

Overall, a total of 130 patients (24.7%) experienced an SAE, which is expected in this patient population. The incidence of SAEs was highest within 1 week of surgical resection (13.1%). The most common SAEs were nervous system disorders, which were experienced by 9.3% of

patients. SAEs occurred in 10.0% and 7.3% of patients in the mid- and long-term phases respectively. Table 28, Table 29, and Table 30 summarize the SAEs occurring in the safety analysis set (N = 527) that were reported by 2 or more patients in the short, mid, and long-term time frames.

Table 28. Incidence of Serious TEAEs by SOC and PT – Short Term (by Patient)

System Organ Class	Preferred Term	Pooled Studies (N = 527) N (%)
Overall		69 (13.1%)
Nervous system disorders	Hemiparesis	23 (4.4%)
	Seizure	5 (0.9%)
	Aphasia	10 (1.9%)
	Hemiplegia	7 (1.3 %)
	Cerebral infarction	4 (0.8%)
	Partial seizures	2 (0.4%)
	Monoparesis	2 (0.4%)
Infections and infestations	Pneumonia	2 (0.4%)
Injury, poisoning and procedural complications	Post procedural complication	5 (0.9%)
	Post procedural hemorrhage	3 (0.6%)
	Extradural haematoma	2 (0.4%)
Respiratory, thoracic and mediastinal disorders	Pulmonary embolism	2 (0.4%)
General disorders and administration site conditions	Pyrexia	3 (0.6%)
Psychiatric disorders	Mental disorder due to a general medical condition	2 (0.4%)

Short term = within 1 week after resection surgery
Patients reporting a particular adverse event (preferred term) more than once are counted only once by preferred term and system organ class.

Table 29. Incidence of Serious TEAEs by SOC and PT—Mid Term (by Patient)

System Organ Class	Preferred Term	Pooled Studies (N = 520) N (%)
Overall		52 (10.0%)
Nervous system disorders	Hemiparesis	2 (0.4%)
	Seizure	5 (1.0%)
	Aphasia	2 (0.4%)
Infections and infestations	Pneumonia	3 (0.6%)
	Brain abscess	3 (0.6%)
	Meningitis	3 (0.6%)
	Sepsis	3 (0.6%)
	Subdural empyema	2 (0.4%)
Injury, poisoning and procedural complications	Post procedural complication	2 (0.4%)
Respiratory, thoracic and mediastinal disorders	Pulmonary embolism	8 (1.5%)
General disorders and administration site conditions	Impaired healing	2 (0.4%)
Vascular disorders	Deep vein thrombosis	2 (0.4%)
Skin and subcutaneous tissue disorders	Rash generalized	2 (0.4%)

Mid term = more than 1 week but within 6 weeks of resection surgery
Patients reporting a particular adverse event (preferred term) more than once are counted only once by preferred term and system organ class.

Table 30. Incidence of Serious TEAEs by SOC and PT—Long Term (by Patient)

System Organ Class	Preferred Term	Pooled Studies (N = 453) N (%)
Overall		33 (7.3%)
Nervous system disorders	Hemiparesis	3 (0.7%)
	Seizure	4 (0.9%)
	Generalized tonic-clonic seizure	6 (1.3%)
Infections and infestations	Brain abscess	2 (0.4%)
Respiratory, thoracic and mediastinal disorders	Pulmonary embolism	4 (0.9%)
General disorders and administration site conditions	Condition aggravated	2 (0.4%)

Long term = more than 6 weeks after resection surgery
Patients reporting a particular adverse event (preferred term) more than once are counted only once by preferred term and system organ class.

7.3.1.3 Deaths

As expected due to the nature and severity of glioma, where normal disease progression is often fatal, there was a total of 284 deaths among patients in the clinical studies. TEAEs leading to death as recorded by the investigator as fatal occurred in 25 patients. The fewest numbers of these patients died within the first week following surgical resection (short term). The greatest

number of patients who died as a result of a TEAE died between 1 and 6 weeks (mid term). None of the deaths were considered related to 5-ALA.

TEAEs that resulted in death are summarized in Table 31, Table 32, and Table 33 for short, mid, and long-term time frames, respectively.

Table 31. Incidence of TEAEs Leading to Death by SOC and PT—Short Term (by Patient)

System Organ Class	Preferred Term	Study N (%)					
		Study 8 (N=7)	Study 28 (N=36)	Study 30 (N=40)	Study 3 (N=201)	Study 32 (N=243)	Pooled Studies (N=527)
Overall		0 (0%)	1 (2.8%)	0 (0%)	3 (1.5%)	1 (0.4%)	5 (0.9%)
Respiratory, thoracic and mediastinal disorders	Pulmonary embolism	0 (0%)	0 (0%)	0 (0%)	1 (0.5%)	0 (0%)	1 (0.2%)
Nervous system disorders	Cerebral infarction	0 (0%)	1 (2.8%)	0 (0%)	0 (0%)	0 (0%)	1 (0.2%)
	Intracranial venous sinus thrombosis	0 (0%)	0 (0%)	0 (0%)	1 (0.5%)	0 (0%)	1 (0.2%)
Cardiac disorders	Ventricular fibrillation	0 (0%)	0 (0%)	0 (0%)	1 (0.5%)	0 (0%)	1 (0.2%)
Vascular disorders	Circulatory collapse	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (0.4%)	1 (0.2%)

Short term = within 1 week after resection surgery
Patients reporting a particular adverse event (preferred term) more than once are counted only once by preferred term and system organ class.

Table 32. Incidence of TEAEs Leading to Death by SOC and PT—Mid Term (by Patient)

System Organ Class	Preferred Term	Study N (%)					
		Study 8 (N = 7)	Study 28 (N = 35)	Study 30 (N = 39)	Study 3 (N = 198)	Study 32 (N = 241)	Pooled Studies (N = 520)
Overall		0 (0%)	1 (2.9%)	0 (0%)	7 (3.5%)	4 (1.7%)	12 (2.3%)
Respiratory, thoracic and mediastinal disorders	Pulmonary embolism	0 (0%)	0 (0%)	0 (0%)	4 (2.0%)	1 (0.4%)	5 (1.0%)
	Pneumonia aspiration	0 (0%)	1 (2.9%)	0 (0%)	0 (0%)	0 (0%)	1 (0.2%)
General disorders and administration site conditions	Condition aggravated	0 (0%)	0 (0%)	0 (0%)	1 (0.5%)	0 (0%)	1 (0.2%)
	Impaired healing	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (0.4%)	1 (0.2%)
Infections and infestations	Brain abscess	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (0.4%)	1 (0.2%)
	Pneumonia	0 (0%)	0 (0%)	0 (0%)	1 (0.5%)	0 (0%)	1 (0.2%)
	Sepsis	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (0.4%)	1 (0.2%)
Injury, poisoning and procedural complications	Brain herniation	0 (0%)	0 (0%)	0 (0%)	1 (0.5%)	0 (0%)	1 (0.2%)
	Post-procedural hemorrhage	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (0.4%)	1 (0.2%)
Vascular disorders	Deep vein thrombosis	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (0.4%)	1 (0.2%)

Mid term = more than 1 week but within 6 weeks of resection surgery
Patients reporting a particular adverse event (preferred term) more than once are counted only once by preferred term and system organ class.

Table 33. Incidence of TEAEs Leading to Death by SOC and PT—Long Term (by Patient)

System Organ Class	Preferred Term	Study N (%)					
		Study 8 (N = 0)	Study 28 (N = 0)	Study 30 (N = 36)	Study 3 (N = 186)	Study 32 (N = 231)	Pooled Studies (N = 453)
Overall		0 (0%)	0 (0%)	0 (0%)	9 (4.8%)	0 (0%)	9 (2.0%)
Respiratory, thoracic and mediastinal disorders	Pulmonary embolism	0 (0%)	0 (0%)	0 (0%)	3 (1.6%)	0 (0%)	3 (0.7%)
General disorders and administration site conditions	Condition aggravated	0 (0%)	0 (0%)	0 (0%)	1 (0.5%)	0 (0%)	1 (0.2%)
	Cardiac death	0 (0%)	0 (0%)	0 (0%)	1 (0.5%)	0 (0%)	1 (0.2%)
Infections and infestations	Broncho-pulmonary aspergillosis	0 (0%)	0 (0%)	0 (0%)	1 (0.5%)	0 (0%)	1 (0.2%)
Nervous system disorders	Cerebral hemorrhage	0 (0%)	0 (0%)	0 (0%)	1 (0.5%)	0 (0%)	1 (0.2%)
	Somnolence	0 (0%)	0 (0%)	0 (0%)	1 (0.5%)	0 (0%)	1 (0.2%)
Cardiac disorders	Cardio-pulmonary failure	0 (0%)	0 (0%)	0 (0%)	1 (0.5%)	0 (0%)	1 (0.2%)

Long term = more than 6 weeks after resection surgery
Patients reporting a particular adverse event (preferred term) more than once are counted only once by preferred term and system organ class.

7.3.1.4 Other Safety Parameters

Other safety parameter data collected included clinical laboratory evaluations (i.e., hematology, electrolytes and renal function, liver function, biochemistry), and ECG. Treatment-emergent changes were defined as an increase in ≥ 2 toxicity grade above baseline grade according to the Common Terminology Criteria for Adverse Events Version 4.0. The following was observed across the pooled safety analyses (laboratory data was not available for Study 32 which decreased the pooled analyses to N = 284):

- There was no clinically significant pattern of changes in any laboratory parameter that were associated with 5-ALA.
- A worsening of ≥2 toxicity grades of hemoglobin occurred in 7.4% of patients in the short-term assessment; few patients experienced worsening of toxicity grades in mid-term analyses.
- A worsening of ≥2 toxicity grades in electrolytes and renal function parameters was observed in the short-term assessment with 0.4%, 0.7%, and 4.9% of patients experiencing worsening in creatinine, potassium and sodium, respectively. Only a small percentage of patients had worsening of sodium in the mid-term assessments.

- No increases in liver function parameters were considered clinically relevant. Across studies patients experienced a worsening of ≥2 toxicity grades in ALT (15.8%) and GGT (11.6%) within the first week after surgery. At 6 weeks, ALT remained elevated in 2.9% of patients and GGT was elevated in 7.5% of patients. Increases were not considered clinically relevant.
- In the short-term, patients experienced a worsening of amylase (16.2%). During the mid-term assessment, a worsening of glucose was observed (2.2%).
- Analyses of ECG (Study 8) and AEs from the clinical studies show no evidence of QT prolongation or arrhythmogenic effect.

7.3.1.5 Special Populations

Based on the analysis in the safety database from the clinical studies, the following were concluded:

- No notable differences in tolerability were seen based on age, gender, weight, KPS, NIH-Stroke Scale, time from dose to surgery, or total 5-ALA dose.
- Tumor Type: There was a difference in overall AE occurrence (44.7% GBM versus 29.7% non-GBM)
- Tumor Grade: There was no difference in TEAEs based on WHO tumor grade.
- Tumor Recurrence Status: Although a statistical analysis was not performed, patients with recurrent disease were deemed to be more likely to experience neurological TEAEs, consistent with the severity and course of the disease.
- Renal or Hepatic Insufficiency: No formal studies were conducted in patients with renal or hepatic impairment, and therefore, 5-ALA is not recommended for patients with renal or hepatic insufficiency.
- Pregnancy and Lactation: 5-ALA is not intended to be used in pregnant women.
- Pediatrics: 5-ALA is not currently labeled or indicated for use in the pediatric patient population.

7.3.2 Supportive Literature

Overall, the safety profile of 5-ALA from the clinical studies is consistently supported by numerous peer-reviewed published literature (29 publications in approximately 2,000 patients). In Study 3, the randomized Phase 3 trial conducted by Stummer et al. (2006, 2011), there was no significant difference in the safety profiles of the 2 groups (5-ALA compared to conventional surgery with white light). A large review of 207 patients who underwent 5-ALA-guided brain

tumor resection demonstrated a favorable safety profile of 5-ALA when patients were compared to those undergoing surgery without 5-ALA (Honorato-Cia et al. 2015).

Overall, 5-ALA is well-tolerated. Photosensitivity is an immediate reaction that was reported at low rates (< 1% of patients) in several publications (Chung & Eljamel 2013, Coburger et al. 2015, Cozzens et al. 2016, Yamada et al. 2015), and the majority of publications reported no skin sensitivity. Following administration of 5-ALA, it is recommended that exposure of eyes and skin to strong light sources (e.g., operating illumination, direct sunlight or brightly focused indoor light) be avoided for 24 hours. In addition, co-administration with potentially phototoxic substances (e.g., tetracyclines, sulfonamides, fluoroquinolones, hypericin extracts) should be avoided.

Hypotension is another immediate but uncommon side effect that was reported by Chung and Eljamel (2013), and Lau et al. (2016). In the retrospective chart review performed by Chung and Eljamel (2013), hypotension was more common in patients who had hypertension and were receiving hypertension therapy prior to surgery. Other complications related to the administration of 5-ALA reported in the literature were fever, nausea, and vomiting (Chung and Eljamel 2013, Moriuchi et al. 2013, Yamada et al. 2015).

Several studies noted increased hepatic enzyme levels within the first month after surgery, which subsequently normalized. This transient liver enzyme has not been found to be clinically significant (Stummer et al. 2000, Stummer et al. 2006, Teixidor et al. 2016). It is recommended that within 24 hours after administration, other potentially hepatotoxic medicinal products be avoided.

The use of 5-ALA-fluorescent-guided resection of gliomas was reported as being associated with an increased risk of post-surgical neurological deficits (most frequently language impairment) in both controlled and uncontrolled studies. It should be noted that the rate of postoperative deterioration in neurological function in single-armed trials is not different from what was found without the use of 5-ALA in the Glioma Outcome Project (Chang et al. 2010), where 8.1% of 408 subjects with malignant gliomas undergoing initial surgical resection were neurologically "worse" at 21 days post surgery. Others have reported that both preoperative neurological condition and improvement after corticosteroids are predictive of postoperative functional outcome (Della Puppa et al. 2013).

Teixidor et al. (2016) found a new onset of a neurological deficit or worsening of a previously detected deficit in 14 patients (18.2%). Szmuda et al. (2015) reported a new postoperative neurological deficit in 6 patients (28.6%). Stummer et al. (2000) found that pre-existing symptoms were temporarily aggravated in 3 of 52 patients and had improved or were at normal

levels by the time of discharge. This was consistent with the clinical studies from the safety database, where the patients most at risk for deterioration were those with pre-existing symptoms. Overall, similar rates of AEs and SAEs have been found between 5-ALA and control groups.

Feigl et al. (2010) performed 26 procedures in 18 patients with primary malignant tumors in eloquent areas using 5-ALA with intraoperative monitoring for cortical and subcortical stimulation. This multimodal approach resulted in 24% of resections being stopped due to the identification of a functional area or cortical tract. Two cases of hemiparesis became accentuated after surgery, and no other AEs related to 5-ALA were reported.

Based on these results, special care should be taken in patients with a tumor in the immediate vicinity of an important neurological function and pre-existing focal deficits (e.g. aphasia, vision disturbances and paresis) that do not improve on corticosteroid treatment. Unresponsiveness to corticosteroids may indicate structural involvement of functional tissue, and uncritically resecting fluorescing tissue in this situation could result in a higher risk of neurological deficits.

In all patients with a tumor in the vicinity of an important neurological function, either pre- or intraoperative measures should be used to localize and monitor function relative to the tumor in order to maintain a safe resection. This requirement is no different from surgery for malignant gliomas regardless of which technique or tool is used for resection, with deterioration being linked to resection and not to 5-ALA.

In conclusion, the literature describing the safety of 5-ALA supports the safety profile from the clinical studies, as well as the warnings and precautions detailed in the proposed label. Other than a single case of mucosal edema (Hefti et al. 2008), the literature review did not reveal any new AEs.

7.3.3 Postmarketing Surveillance

5-ALA is approved for marketing in numerous regions including Australia, the European Union, Israel, Japan, Korea, Kuwait, and Taiwan. The most recent EU PSUR covers clinical studies conducted from 2007 to 2015 and summarizes data recorded from approximately 58,000 patients. A summary of AEs that implied at least a suspicion of causality by the reporter or the current marketing authorization holder is presented in Table 34. The postmarketing safety data are consistent with the pivotal clinical study safety data; no unexpected AEs occurred.

Table 34. Summary of Postmarketing Reports by SOC and PT

System Organ Class and Preferred Term	Spontaneous, including Competent Authorities (Worldwide) and Literature		Total Spontaneous	Noninterventional Study and Reports from Other Solicited Resources
	Serious	Nonserious		Serious
Infections and Infestations				
Sepsis	0	0	0	1
Wound infection	0	0	0	1
Total AE/ADR/total patients	0/0	0/0	0/0	2/1
Blood and Lymphatic Disorders				
Thrombocytopenia	1	0	1	0
Total AE/ADR/total patients	1/1	0/0	1/1	0/0
Metabolism and Nutrition Disorders				
Metabolic acidosis	0	0	0	1
Total AE/ADR/total patients	0/0	0/0	0/0	1/1
Nervous System Disorders				
Brain edema	1	0	1	0
Convulsion	0	0	0	1
Hemiparesis	1	0	1	0
Total AE/ADR/total patients	2/2	0/0	2/2	1/1
Vascular Disorders				
Hypertension	0	0	0	1
Hypotension	0	1	1	0
Total AE/ADR/total patients	0/0	1/1	1/1	1/1
Respiratory, Thoracic, and Mediastinal Disorders				
Pulmonary embolism	1	0	1	0
Respiratory disorder	1	0	1	0
Total AE/ADR/total patients	2/2	0/0	2/2	0/0
Hepatobilliary Disorders				
Hepatitis toxic	1	0	1	0
Total AE/ADR/total patients	1/1	0/0	1/1	0/0
Skin and Subcutaneous Tissue Disorders				
Drug eruption	0	0	0	1
Erythema	0	1	1	0
Photodermatosis	1	0	1	0
Photosensitivity reaction	0	1	1	0

System Organ Class and Preferred Term	Spontaneous, including Competent Authorities (Worldwide) and Literature		Total Spontaneous	Noninterventional Study and Reports from Other Solicited Resources
	Serious	Nonserious		Serious
Total AE/ADR/total patients	1/1	2/2	3/3	1/1
General Disorders and Administration Site Conditions				
Drug ineffective	0	1	1	0
Total AE/ADR/total patients	0/0	1/1	1/1	0/0
Injury, Poisoning, and Procedural Complications				
Accidental overdose	0	1	1	0
Procedural hypotension	0	0	0	1
Vasoplegia syndrome	1	0	1	0
Total AE/ADR/total patients	1/1	1/1	2/2	1/1
Total AE/ADR	**8**	**5**	**13**	**7**
Total Patients	**8**	**4**	**12**	**4**

***** MedDRA version 17.1

AE = adverse event; ADR = adverse drug reaction; PT = preferred term; SOC = system organ class

Source: Gliolan® EU PSUR 2015, Appendix 11.2

7.4 Safety Conclusion

In summary, 5-ALA has a well-established safety profile, supported by safety data from 5 clinical studies (N = 527), 29 peer-reviewed publications (N = ~2,000), and postmarketing surveillance data (N = ~58,000). The vast majority of TEAEs were not related to 5-ALA. Many of the AEs observed were expected due to the nature of the underlying disease and complications that inherently occur during resection surgery. No deaths were considered related to 5-ALA. Caution is necessary for phototoxic effects of 5-ALA after direct light exposure, as well as administration in combination with hepatotoxic drugs and in patients with cardiovascular disease (risk of hypotension). The identified risks of 5-ALA were limited, not long-lasting, and can be effectively managed. The safety data support the use of 5-ALA for the proposed indication.

8 RISK MANAGEMENT

8.1 Risk Management Strategy: 5-ALA Medicines Management Program

The risks related to the ingestion of oral 5-ALA (20 mg/kg bw) to glioma patients 3 hours prior to anesthesia are expected to be minimal. Data from clinical studies and postmarketing surveillance efforts indicate that rare risks associated with the drug include brain edema, hemianopia, hypoaesthesia, pyrexia, chills, photosensitivity reaction, solar dermatitis, hypotension, abnormal liver function test, diarrhea, venous thrombosis and also include procedurally-related side effects such as transient neurological side effects associated with tumor removal. These risks are detailed in the proposed product labeling.

The risk management strategy is intended to provide a product distribution and training certification program for surgeons to support proper use and training on how to perform 5-ALA FGS. The risk management strategy is intended to accomplish the following:

- Assist in the understanding of the proper use of 5-ALA and facilitate an understanding of the use and limitations of FGS
- Limit the use of 5-ALA to neurosurgeons who have had experience with visualizing 5-ALA using the specified operating microscopes and filters
- Limit the use of 5-ALA to neurosurgeons who can administer 5-ALA in the correct dose and timing regimen
- Limit the use of 5-ALA to neurosurgeons who can apply 5-ALA in the correct dose and timing regimen

The 5-ALA Medicines Management Program is not a surgical teaching course but an education in the safe use of 5-ALA for the detection of malignant tissue based upon the visualization of

fluorescent tissue. It instructs the surgeon on how to identify malignant tissue and review current techniques for safe resection in the brain. The education program is not intended to be a replacement for the extensive surgical training and years of surgical experience that the board-certified neurosurgeon brings to his or her practice.

8.2 European Risk Management Model

5-ALA has been approved since 2007 in the EU and numerous countries in rest of world (a total of approximately 40 countries) as an agent for the visualization of malignant tissue in patients with malignant gliomas (WHO grade III and IV). The European approval of 5-ALA was accompanied by conditions or restrictions with regard to the safe use of 5-ALA. The EU reviewers specified that 5-ALA should be used only by neurosurgeons who have attended a training course in accordance with certain specifications. In addition, the EU Risk Management Plan included minimum requirements for a qualified trainer and minimum requirements for a qualified training center.

The European 5-ALA Risk Management Plan includes a training course in FGS to be completed by the neurosurgeons who will be using the product and who are already conversant with surgery of malignant gliomas and possess and in-depth knowledge of functional brain anatomy. The European certification course incorporates a training manual, which was developed by Professor Walter Stummer (University Hospital Münster; Münster, Germany), the global principal investigator (PI) for the regulatory studies in Europe. Professor Stummer has published multiple peer-reviewed papers on the use of 5-ALA to visualize malignant brain tumors.

8.3 U.S. Risk Management Strategy

NXDC's proposed risk management strategy for 5-ALA in the U.S. models the risk management plan for 5-ALA that is in place in Europe, updated with new information in the NDA and augmented by new processes for updating both the program and surgeon retraining. The 5-ALA Medicines Management Program includes the following:

- A training course for neurosurgeons;
- Preparation of training materials;
- A certification program to identify neurosurgeons who have successfully completed the program and re-certification program;
- Route re-evaluation of the training and certifications program; and
- Provide controlled and limited access to 5-ALA for neurosurgeons who can demonstrate certification, procedural compliance with adequate training, and access to microscope and filters that meet product specifications.

8.4 Training Course

The proposed U.S. 5-ALA Medicines Management Program includes a training course in the safe and effective use of 5-ALA for the visualization of gliomas. The training course was designed by Professor Walter Stummer and deployed in Europe and the rest of the world where Gliolan® is marketed, and updated by Professor Costantinos Hadjipanayis who are both clinical users of 5-ALA and lead trainers in Europe and the U.S.

The 5-ALA Medicines Management Program is not a surgical teaching course but an education in the safe use of 5-ALA for the detection of malignant tissue based on the visualization of fluorescent tissue. It instructs the surgeon on how to identify malignant tissue and review current techniques for safe resection in the brain. The education program is not intended to be a replacement for the extensive surgical training and years of surgical experience that the fully trained neurosurgeon brings to his/her practice.

The 5-ALA Medicines Management Program is a specific educational training course for neurosurgeons that aims to support proper use of 5-ALA in glioma patients. Only neurosurgeons who have mastered and received program certification will be able to secure 5-ALA from hospital-based pharmacies. This restricted distribution arrangement will be established as a part of the supply chain control between NXDC and the hospital-based system. Upon approval, there will be no source of 5-ALA that will be available in the U.S. outside of this hospital-based restricted distribution program following the approval of this orphan drug.

The course topics address the following:

- Education of technical principles behind fluorescence-guided resections using 5-ALA
- Identification of suitable candidates for fluorescence-guided resection
- Application of 5-ALA in the correct dosage and timing
- Light photosensitivity and patient handling after 5-ALA dosing
- Intra-operative detection of tumor fluorescence and methods to maximize visualization of tumor fluorescence during surgery
- Overview of equipment, maintenance and microscope usage and quality assessment of microscope function
- Techniques for intraoperative risk reduction
- Photobleaching and limitations
- Understanding of what fluorescence signifies in the brain regarding tumor burden

- Identification of patients at risk for neurological deficits using fluorescence-guided resection with 5-ALA with a special focus on speech difficulties (aphasia) and other critical focal deficits (motor deficits)
- Tips and pitfalls

At the completion of the course, the neurosurgeon will receive a Certificate of Training and be added to an Approved User List, which will be maintained and updated by NXDC. The approved user list will be a key agreement between the hospital-based pharmacy and NXDC on the release of the drug only to certified trained surgeons.

8.5 Certification Program/Recertification Program

Neurosurgeons are the healthcare providers intended to undergo and complete the 5-ALA Medicines Management Program. Only fully trained neurosurgeons who have completed a neurosurgical residency and are practicing neurosurgeons will be admitted to the certification program.

All surgeons will be required to recertify every 2 years or as NXDC becomes aware of new information relating to technique safety and/or efficacy. Recertification will be performed through online and/or in person training. NXDC will maintain records on all neurosurgeon training. Surgeons who are current on training will be certified and included on a current Approved User List. Any neurosurgeon whose training period expires will be removed from the Approved User List.

8.6 Re-evaluation of the Training and Certification Program

The adequacy of the training will be monitored initially during the 5-ALA Medicines Management Program with a monitored procedure by the Sponsor. NXDC will partner with professional organizations knowledgeable in neurosurgery and imaging to assure the appropriateness, adequacy, and acceptability of the training to stakeholders.

8.7 Limited Access

The 5-ALA Medicines Management Program incorporates restricted access to support safe and efficacious use. 5-ALA will be delivered only to hospitals from which at least one neurosurgeon has successfully completed the training course and is on the current Approved User List. The Approved User List is regularly updated and the dispensing pharmacist will be required to confirm that the requesting surgeon has been authorized as a 5-ALA-certified

practitioner prior to 5-ALA being dispensed. In anticipation of product approval approximately 80 surgeons in the U.S. have trained in the use of 5-ALA.

9 RISK BENEFIT SUMMARY

The initial treatment for patients with a diagnosis of malignant glioma is surgical resection. The goal of the resection is to remove as much of the tumor without affecting the eloquent areas of the brain. Surgical resection of glioma is challenging because glioma tumor margins are difficult to visualize, the tumors are often infiltrative and present in eloquent areas of the brain, and tumor localization for surgery is currently not done in real time.

5-ALA is an imaging agent to facilitate the real time detection and visualization of malignant tissue during glioma surgery. Following ingestion, 5-ALA-induced PpIX selectively accumulates in tumor cells. During glioma surgery, when exposed to blue light (wavelength 400-410 nm), the tumor tissue fluoresces and emits a red-violet light that is visible to the neurosurgeon, allowing identification of glioma tissue for removal.

The safety of 5-ALA is supported by integrated safety data from Studies 3, 8, 28, 30, and 32, which included 527 glioma patients. Safety data from the clinical studies revealed no effect of 5-ALA on AE incidence, clinical laboratory values, electrocardiogram findings, QTc prolongation potential, vital signs, or physical findings. The safety data indicate that, not unexpectedly for this patient population after major surgery, the most commonly reported TEAEs were nervous system disorders. None of the deaths that occurred in study patients were considered related to 5-ALA. Data from clinical studies and postmarketing surveillance efforts indicate that rare risks associated with brain surgery using 5-ALA include pyrexia, chills, photosensitivity reaction, solar dermatitis, hypotension, abnormal liver function test, diarrhea, edema, hemianopia, hypoaesthesia, and venous thrombosis. These risks are detailed in the proposed product labeling. NXDC has also conducted a systematic literature review to identify literature pertaining to the safety of 5-ALA for use in visualizing malignant glioma. The safety data reported in these publications are consistent with the safety data profile from the clinical studies. In addition, postmarketing safety data are available from several countries and more than 58,000 patients who received 5-ALA prior to surgical resection for malignant glioma from 2007 to 2015. No new risks were identified based on the analyses of these data.

The efficacy of 5-ALA as an imaging agent to facilitate the real time detection and visualization of malignant tissue during glioma surgery is supported by its predictive accuracy and clinical usefulness. The efficacy of 5-ALA is demonstrated by typically higher than 90% PPV of tissue fluorescence to identify glioma tissue confirmed via tumor histopathology. PPV data are

available from Studies 28, 30, and 3, which represented 245 patients who received 5-ALA, including 36 patients with recurrent glioma. PPV data are also available from peer-reviewed publications. For all studies, biopsy-based PPVs of typically greater than 90% for 5-ALA were recorded or calculated. The clinical usefulness of 5-ALA is demonstrated by its ability to aid surgeons in the real time identification of malignant tumor under fluorescent light that may otherwise be overlooked during conventional microsurgical resection. Studies 28 and 30 provide evidence that 5-ALA fluorescence enables surgeons to identify residual areas of tumor seen in real time under blue light, that are not identifiable under white light. This is also supported with data from the peer-reviewed, published literature. In addition, 5-ALA is appropriate for use by patients with primary or recurrent disease, can be used with existing standard operating microscope technology, allows the surgeon to visualize the tumor tissue in real time, and does not interfere with the flow of surgery.

The data established that 5-ALA accurately identifies malignant glioma tissue during surgical resection and is able to identify tumor tissue that could not be seen under standard white light surgical resection. Risks of FGS using 5-ALA are similar to those associated with standard glioma resection surgery, but caution is needed for phototoxic effects of 5-ALA after light exposure, as well as administration in combination with hepatotoxic drugs and in patients with cardiovascular disease. Further 5-ALA does not interfere with the safety or efficacy of anesthesia or cancer therapies. As such, the benefit risk profile for 5-ALA was established and was found favorable. To ensure that any potential risks related to the ingestion of 5-ALA remain low, a risk management strategy has been developed by NXDC, which is intended to minimize the risks that may be associated with the use of 5-ALA to visualize malignant brain tissue and associated surgical debulking procedures. The focus of the program is to limit access to 5-ALA only to neurosurgeons who have been trained in the use of the drug to support glioma resection surgery.

10 CONCLUSION

In conclusion, 5-ALA-induced fluorescence provides the neurosurgeon with detailed information on the location and extent of the tumor from which he or she can make informed decisions about the resection based on surgeon skill, experience, and anatomic and functional knowledge of the brain. 5-ALA-induced fluorescence facilitates this decision.

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