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Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Giordano FA, Ganslandt O, Münter MW, et al. Dose escalation with intraoperative radiotherapy in newly diagnosed glioblastoma (INTRAGO-II): an open-label, multicentre, randomised, controlled, phase 3 trial. *Lancet Oncol* 2026; **27:** 864–78.

Appendix

Table of Contents

Table S1: Full listing of adverse events. Listing of all adverse events reported per treatment arm according to the Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0. The same adverse events could be repeatedly reported per patient.

[#]Of the 11 events in the IORT arm, four occurred peri-operatively (within 3 days of surgery, before any IORT-specific effect on steroid requirements would be expected), two occurred in patients with pre-existing diabetes mellitus, and one was retrospectively identified as a baseline condition that should not have been recorded as a treatment-emergent adverse event. Only three to four events were temporally associated with documented increases in corticosteroid dosing. Notably, one of the four SOC arm events was also explicitly attributed to steroid-induced diabetes with documented corticosteroid escalation.

		IORT arm n = 161		**SOC arm n = 137**	
		no. of AE	*%*	*no. of AE*	*%*
Blood and lymphatic system disorders					
Anemia	Grade 1	9	5·6	5	3·6
Anemia	Grade 2	5	3·1	3	2·2
Anemia	Grade 3	0	0·0	2	1·5
Anemia	Grade 4	0	0·0	1	0·7
Blood and lymphatic system disorders - Other, specify	Grade 1	2	1·2	4	2·9
Blood and lymphatic system disorders - Other, specify	Grade 2	3	1·9	2	1·5
Blood and lymphatic system disorders - Other, specify	Grade 3	1	0·6	1	0·7
Febrile neutropenia	Grade 3	1	0·6	0	0·0
Leukocytosis	Grade 1	2	1·2	2	1·5
Leukocytosis	Grade 2	1	0·6	2	1·5
Thrombotic thrombocytopenic purpura	Grade 2	0	0·0	1	0·7
Cardiac disorders					
Atrial fibrillation	Grade 3	2	1·2	0	0·0
Cardiac arrest	Grade 5	1	0·6	0	0·0
Cardiac disorders - Other, specify	Grade 1	1	0·6	1	0·7
Cardiac disorders - Other, specify	Grade 2	0	0·0	1	0·7
Myocardial infarction	Grade 5	2	1·2	0	0·0
Paroxysmal atrial tachycardia	Grade 1	0	0·0	1	0·7
Sinus bradycardia	Grade 1	2	1·2	1	0·7
Sinus bradycardia	Grade 2	1	0·6	1	0·7
Sinus tachycardia	Grade 1	3	1·9	0	0·0
Sinus tachycardia	Grade 2	0	0·0	1	0·7
Sinus tachycardia	Grade 4	1	0·6	0	0·0
Supraventricular tachycardia	Grade 2	1	0·6	0	0·0
Ear and labyrinth disorders					
Ear and labyrinth disorders - Other, specify	Grade 1	2	1·2	1	0·7
Ear and labyrinth disorders - Other, specify	Grade 2	0	0·0	1	0·7
Ear pain	Grade 1	1	0·6	0	0·0
External ear pain	Grade 1	1	0·6	0	0·0
Hearing impaired	Grade 1	2	1·2	2	1·5
Hearing impaired	Grade 2	1	0·6	1	0·7
Tinnitus	Grade 1	1	0·6	2	1·5

Vertigo	Grade 1	9	5·6	7	5·1
Vertigo	Grade 2	3	1·9	2	1·5
Endocrine disorders					
Cushingoid	Grade 1	1	0·6	0	0·0
Cushingoid	Grade 2	0	0·0	1	0·7
Hyperthyroidism	Grade 1	0	0·0	1	0·7
Eye disorders					
Blurred vision	Grade 1	4	2·5	3	2·2
Blurred vision	Grade 2	4	2·5	3	2·2
Cataract	Grade 1	1	0·6	0	0·0
Cataract	Grade 3	0	0·0	1	0·7
Conjunctivitis	Grade 1	0	0·0	2	1·5
Conjunctivitis	Grade 2	1	0·6	1	0·7
Dry eye	Grade 1	0	0·0	1	0·7
Dry eye	Grade 2	0	0·0	1	0·7
Extraocular muscle paresis	Grade 1	1	0·6	0	0·0
Eye disorders - Other, specify	Grade 1	7	4·3	7	5·1
Eye disorders - Other, specify	Grade 2	4	2·5	1	0·7
Eyelid function disorder	Grade 2	1	0·6	0	0·0
Flashing lights	Grade 1	0	0·0	1	0·7
Optic nerve disorder	Grade 1	1	0·6	2	1·5
Optic nerve disorder	Grade 2	0	0·0	2	1·5
Papilledema	Grade 1	0	0·0	1	0·7
Watering eyes	Grade 2	0	0·0	1	0·7
Gastrointestinal disorders					
Abdominal pain	Grade 1	3	1·9	0	0·0
Abdominal pain	Grade 2	1	0·6	1	0·7
Abdominal pain	Grade 3	0	0·0	1	0·7
Anal pain	Grade 2	1	0·6	0	0·0
Constipation	Grade 1	10	6·2	8	5·8
Constipation	Grade 2	6	3·7	2	1·5
Diarrhea	Grade 1	2	1·2	4	2·9
Diarrhea	Grade 2	2	1·2	1	0·7
Dry mouth	Grade 1	1	0·6	1	0·7
Dyspepsia	Grade 1	2	1·2	1	0·7
Dysphagia	Grade 1	1	0·6	1	0·7
Dysphagia	Grade 2	0	0·0	1	0·7
Esophagitis	Grade 1	1	0·6	0	0·0
Esophagitis	Grade 2	1	0·6	0	0·0
Fecal incontinence	Grade 1	3	1·9	0	0·0
Fecal incontinence	Grade 2	1	0·6	0	0·0
Gastritis	Grade 1	2	1·2	0	0·0
Gastritis	Grade 2	2	1·2	3	2·2
Gastroesophageal reflux disease	Grade 1	0	0·0	1	0·7

Gastrointestinal disorders - Other, specify	Grade 1	1	0·6	0	0·0
Gastrointestinal disorders - Other, specify	Grade 2	4	2·5	0	0·0
Gastrointestinal disorders - Other, specify	Grade 3	1	0·6	0	0·0
Gastrointestinal pain	Grade 1	1	0·6	1	0·7
Hemorrhoids	Grade 1	0	0·0	1	0·7
Lower gastrointestinal haemorrhage	Grade 3	0	0·0	1	0·7
Mucositis oral	Grade 1	1	0·6	0	0·0
Mucositis oral	Grade 2	2	1·2	0	0·0
Nausea	Grade 1	26	16·1	15	10·9
Nausea	Grade 2	14	8·7	11	8·0
Nausea	Grade 3	1	0·6	2	1·5
Obstruction gastric	Grade 1	0	0·0	1	0·7
Oral pain	Grade 1	0	0·0	1	0·7
Stomach pain	Grade 1	0	0·0	1	0·7
Toothache	Grade 1	0	0·0	1	0·7
Vomiting	Grade 1	7	4·3	1	0·7
Vomiting	Grade 2	5	3·1	2	1·5
Vomiting	Grade 3	2	1·2	0	0·0
Vomiting	Grade 4	1	0·6	0	0·0
General disorders and administration site conditions					
Chills	Grade 1	2	1·2	0	0·0
Chills	Grade 2	1	0·6	0	0·0
Death NOS	Grade 5	30	18·6	19	13·9
Edema face	Grade 1	0	0·0	1	0·7
Edema face	Grade 2	1	0·6	0	0·0
Edema limbs	Grade 1	4	2·5	0	0·0
Edema limbs	Grade 2	4	2·5	1	0·7
Edema trunk	Grade 3	3	1·9	0	0·0
Fatigue	Grade 1	35	21·7	23	16·8
Fatigue	Grade 2	34	21·1	23	16·8
Fatigue	Grade 3	4	2·5	4	2·9
Fever	Grade 1	3	1·9	2	1·5
Fever	Grade 2	2	1·2	0	0·0
Fever	Grade 3	1	0·6	0	0·0
Fever	Grade 5	1	0·6	0	0·0
Flu like symptoms	Grade 1	0	0·0	1	0·7
Gait disturbance	Grade 1	2	1·2	0	0·0
Gait disturbance	Grade 2	1	0·6	2	1·5
Gait disturbance	Grade 3	0	0·0	3	2·2
General disorders and administration site conditions - Other, specify	Grade 1	0	0·0	2	1·5
General disorders and administration site conditions - Other, specify	Grade 2	2	1·2	0	0·0
General disorders and administration site conditions - Other, specify	Grade 3	2	1·2	2	1·5

General disorders and administration site conditions - Other, specify	Unknown/ Uncoded	1	0·6	0	0·0
Irritability	Grade 1	2	1·2	0	0·0
Localized edema	Grade 1	3	1·9	3	2·2
Malaise	Grade 2	1	0·6	2	1·5
Malaise	Grade 3	2	1·2	0	0·0
Multi-organ failure	Grade 5	0	0·0	1	0·7
Non-cardiac chest pain	Grade 1	1	0·6	1	0·7
Non-cardiac chest pain	Grade 2	0	0·0	1	0·7
Pain	Grade 1	1	0·6	3	2·2
Pain	Grade 2	1	0·6	4	2·9
Pain	Grade 3	1	0·6	0	0·0
Hepatobiliary disorders					
Cholecystitis	Grade 4	0	0·0	1	0·7
Gallbladder pain	Grade 1	1	0·6	0	0·0
Hepatobiliary disorders - Other, specify	Grade 1	1	0·6	1	0·7
Hepatobiliary disorders - Other, specify	Grade 2	0	0·0	1	0·7
Immune system disorders					
Immune system disorders - Other, specify	Grade 1	1	0·6	0	0·0
Infections and infestations					
Bladder infection	Grade 2	1	0·6	0	0·0
Bronchial infection	Grade 1	0	0·0	1	0·7
Bronchial infection	Grade 2	1	0·6	0	0·0
Catheter related infection	Grade 2	1	0·6	0	0·0
Conjunctivitis infective	Grade 1	0	0·0	1	0·7
Encephalitis infection	Grade 3	1	0·6	1	0·7
Encephalitis infection	Grade 5	0	0·0	1	0·7
Endocarditis infective	Grade 3	1	0·6	0	0·0
Enterocolitis infectious	Grade 3	0	0·0	1	0·7
Infections and infestations - Other, specify	Grade 1	3	1·9	3	2·2
Infections and infestations - Other, specify	Grade 2	3	1·9	2	1·5
Lung infection	Grade 1	2	1·2	0	0·0
Lung infection	Grade 2	2	1·2	1	0·7
Lung infection	Grade 3	5	3·1	1	0·7
Lung infection	Grade 4	1	0·6	0	0·0
Lung infection	Grade 5	1	0·6	2	1·5
Meningitis	Grade 3	1	0·6	0	0·0
Meningitis	Grade 4	1	0·6	1	0·7
Mucosal infection	Grade 1	2	1·2	0	0·0
Mucosal infection	Grade 2	2	1·2	0	0·0
Otitis externa	Grade 2	1	0·6	0	0·0
Peripheral nerve infection	Grade 1	1	0·6	0	0·0
Peripheral nerve infection	Grade 2	0	0·0	1	0·7
Rhinitis infective	Grade 2	1	0·6	0	0·0
Sepsis	Grade 3	1	0·6	0	0·0

Sepsis	Grade 4	1	0·6	0	0·0
Sepsis	Grade 5	2	1·2	0	0·0
Sinusitis	Grade 2	0	0·0	1	0·7
Skin infection	Grade 2	3	1·9	2	1·5
Skin infection	Grade 3	0	0·0	1	0·7
Urethral infection	Grade 2	1	0·6	0	0·0
Urinary tract infection	Grade 1	1	0·6	1	0·7
Urinary tract infection	Grade 2	4	2·5	2	1·5
Urinary tract infection	Grade 3	5	3·1	2	1·5
Urinary tract infection	Grade 4	1	0·6	0	0·0
Wound infection	Grade 2	1	0·6	1	0·7
Wound infection	Grade 3	5	3·1	3	2·2
Injury, poisoning and procedural complications					
Ankle fracture	Grade 3	1	0·6	0	0·0
Arterial injury	Grade 3	1	0·6	0	0·0
Bruising	Grade 1	4	2·5	0	0·0
Bruising	Grade 2	1	0·6	2	1·5
Dermatitis radiation	Grade 1	12	7·5	16	11·7
Dermatitis radiation	Grade 2	3	1·9	2	1·5
Fall	Grade 1	4	2·5	2	1·5
Fall	Grade 2	2	1·2	2	1·5
Fall	Grade 3	2	1·2	2	1·5
Fracture	Grade 1	1	0·6	0	0·0
Fracture	Grade 2	2	1·2	3	2·2
Fracture	Grade 3	0	0·0	1	0·7
Fracture	Grade 5	1	0·6	0	0·0
Hip fracture	Grade 2	1	0·6	0	0·0
Injury, poisoning and procedural complications - Other, specify	Grade 1	2	1·2	0	0·0
Injury, poisoning and procedural complications - Other, specify	Grade 2	1	0·6	2	1·5
Injury, poisoning and procedural complications - Other, specify	Grade 3	0	0·0	1	0·7
Intraoperative hemorrhage	Grade 1	1	0·6	1	0·7
Intraoperative hemorrhage	Grade 2	0	0·0	2	1·5
Intraoperative hemorrhage	Grade 4	1	0·6	0	0·0
Intraoperative neurological injury	Grade 1	4	2·5	8	5·8
Intraoperative neurological injury	Grade 2	2	1·2	1	0·7
Intraoperative neurological injury	Grade 3	1	0·6	3	2·2
Intraoperative skin injury	Grade 1	2	1·2	1	0·7
Intraoperative urinary injury	Grade 1	1	0·6	0	0·0
Intraoperative venous injury	Grade 3	0	0·0	1	0·7
Postoperative hemorrhage	Grade 1	4	2·5	0	0·0
Postoperative hemorrhage	Grade 3	3	1·9	1	0·7
Postoperative hemorrhage	Grade 4	0	0·0	3	2·2
Postoperative hemorrhage	Grade 5	1	0·6	0	0·0

Seroma	Grade 1	0	0·0	1	0·7
Spinal fracture	Grade 2	1	0·6	0	0·0
Vascular access complication	Grade 3	1	0·6	0	0·0
Wound complication	Grade 1	3	1·9	1	0·7
Wound complication	Grade 2	2	1·2	0	0·0
Wound complication	Grade 3	3	1·9	1	0·7
Wound dehiscence	Grade 1	1	0·6	0	0·0
Wound dehiscence	Grade 2	1	0·6	0	0·0
Wound dehiscence	Grade 3	2	1·2	0	0·0
Investigations					0·0
Activated partial thromboplastin time prolonged	Grade 2	1	0·6	0	0·0
Alanine aminotransferase increased	Grade 1	5	3·1	3	2·2
Alanine aminotransferase increased	Grade 2	5	3·1	0	0·0
Alanine aminotransferase increased	Grade 3	3	1·9	0	0·0
Aspartate aminotransferase increased	Grade 1	2	1·2	1	0·7
Aspartate aminotransferase increased	Grade 2	1	0·6	0	0·0
Blood bilirubin increased	Grade 1	0	0·0	1	0·7
Creatinine increased	Grade 1	6	3·7	2	1·5
Creatinine increased	Grade 3	1	0·6	0	0·0
GGT increased	Grade 1	1	0·6	1	0·7
GGT increased	Grade 2	1	0·6	1	0·7
GGT increased	Grade 3	1	0·6	2	1·5
Investigations - Other, specify	Grade 1	9	5·6	6	4·4
Investigations - Other, specify	Grade 2	0	0·0	3	2·2
Investigations - Other, specify	Grade 3	2	1·2	0	0·0
Investigations - Other, specify	Grade 5	1	0·6	0	0·0
Lymphocyte count decreased	Grade 1	2	1·2	2	1·5
Lymphocyte count decreased	Grade 2	1	0·6	1	0·7
Lymphocyte count decreased	Grade 3	0	0·0	1	0·7
Lymphocyte count decreased	Grade 4	0	0·0	2	1·5
Neutrophil count decreased	Grade 2	0	0·0	1	0·7
Neutrophil count decreased	Grade 3	0	0·0	1	0·7
Neutrophil count decreased	Grade 4	1	0·6	2	1·5
Platelet count decreased	Grade 1	8	5·0	11	8·0
Platelet count decreased	Grade 2	8	5·0	12	8·8
Platelet count decreased	Grade 3	5	3·1	7	5·1
Platelet count decreased	Grade 4	2	1·2	4	2·9
Weight gain	Grade 1	1	0·6	0	0·0
Weight loss	Grade 1	5	3·1	2	1·5
Weight loss	Grade 2	3	1·9	0	0·0
Weight loss	Grade 3	1	0·6	1	0·7
White blood cell decreased	Grade 1	1	0·6	4	2·9
White blood cell decreased	Grade 2	2	1·2	5	3·6
White blood cell decreased	Grade 3	4	2·5	3	2·2

White blood cell decreased	Grade 4	1	0·6	2	1·5
Metabolism and nutrition disorders					
Anorexia	Grade 1	8	5·0	5	3·6
Anorexia	Grade 2	5	3·1	3	2·2
Dehydration	Grade 2	2	1·2	0	0·0
Dehydration	Grade 3	3	1·9	1	0·7
Hypercalcemia	Grade 1	2	1·2	0	0·0
Hyperglycemia [#]	Grade 1	3	1·9	2	1·5
Hyperglycemia [#]	Grade 2	4	2·5	0	0·0
Hyperglycemia [#]	Grade 3	2	1·2	2	1·5
Hyperglycemia [#]	Grade 4	2	1·2	0	0·0
Hyperkalemia	Grade 1	0	0·0	1	0·7
Hypernatremia	Grade 1	1	0·6	0	0·0
Hyperuricemia	Grade 1	1	0·6	2	1·5
Hyperuricemia	Grade 2	1	0·6	0	0·0
Hypoalbuminemia	Grade 1	1	0·6	1	0·7
Hypocalcemia	Grade 1	3	1·9	1	0·7
Hypocalcemia	Grade 2	0	0·0	1	0·7
Hypokalemia	Grade 1	4	2·5	1	0·7
Hypokalemia	Grade 2	0	0·0	4	2·9
Hypokalemia	Grade 3	1	0·6	2	1·5
Hyponatremia	Grade 1	7	4·3	2	1·5
Hyponatremia	Grade 2	0	0·0	1	0·7
Hyponatremia	Grade 3	0	0·0	1	0·7
Metabolism and nutrition disorders - Other, specify	Grade 1	1	0·6	1	0·7
Obesity	Grade 2	0	0·0	1	0·7
Musculoskeletal and connective tissue disorders					
Arthralgia	Grade 1	3	1·9	3	2·2
Arthralgia	Grade 2	3	1·9	0	0·0
Arthritis	Grade 2	1	0·6	0	0·0
Arthritis	Grade 3	0	0·0	1	0·7
Back pain	Grade 1	1	0·6	1	0·7
Back pain	Grade 2	2	1·2	1	0·7
Flank pain	Grade 1	0	0·0	1	0·7
Flank pain	Grade 2	1	0·6	0	0·0
Generalized muscle weakness	Grade 1	0	0·0	3	2·2
Generalized muscle weakness	Grade 2	3	1·9	4	2·9
Generalized muscle weakness	Grade 3	2	1·2	3	2·2
Joint range of motion decreased	Grade 1	1	0·6	1	0·7
Muscle weakness left-sided	Grade 1	5	3·1	2	1·5
Muscle weakness left-sided	Grade 2	7	4·3	1	0·7
Muscle weakness left-sided	Grade 3	4	2·5	7	5·1
Muscle weakness left-sided	Grade 4	0	0·0	3	2·2
Muscle weakness lower limb	Grade 1	3	1·9	1	0·7

Muscle weakness lower limb	Grade 2	6	3·7	2	1·5
Muscle weakness lower limb	Grade 3	3	1·9	1	0·7
Muscle weakness lower limb	Grade 4	1	0·6	0	0·0
Muscle weakness right-sided	Grade 1	2	1·2	2	1·5
Muscle weakness right-sided	Grade 2	0	0·0	2	1·5
Muscle weakness right-sided	Grade 3	1	0·6	4	2·9
Muscle weakness right-sided	Grade 4	0	0·0	1	0·7
Muscle weakness trunk	Grade 1	0	0·0	1	0·7
Muscle weakness upper limb	Grade 1	2	1·2	0	0·0
Muscle weakness upper limb	Grade 2	1	0·6	0	0·0
Muscle weakness upper limb	Grade 3	1	0·6	0	0·0
Musculoskeletal and connective tissue disorder - Other, specify	Grade 1	1	0·6	2	1·5
Musculoskeletal and connective tissue disorder - Other, specify	Grade 2	3	1·9	2	1·5
Myalgia	Grade 1	1	0·6	1	0·7
Myalgia	Grade 2	1	0·6	0	0·0
Neck pain	Grade 1	1	0·6	3	2·2
Neck pain	Grade 2	3	1·9	0	0·0
Pain in extremity	Grade 1	2	1·2	0	0·0
Pain in extremity	Grade 2	2	1·2	0	0·0
Neoplasms benign, malignant and unspecified (incl cysts and polyps)					
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	Grade 1	1	0·6	0	0·0
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	Grade 2	0	0·0	2	1·5
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	Grade 3	2	1·2	5	3·6
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	Grade 5	1	0·6	1	0·7
Nervous system disorders					
Gait disturbance	Grade 1	1	0·6	3	2·2
Gait disturbance	Grade 3	1	0·6	1	0·7
Amnesia	Grade 1	0	0·0	3	2·2
Amnesia	Grade 2	2	1·2	1	0·7
Amnesia	Grade 3	0	0·0	2	1·5
Aphonia	Grade 2	3	1·9	0	0·0
Aphonia	Grade 3	1	0·6	0	0·0
Ataxia	Grade 1	5	3·1	2	1·5
Ataxia	Grade 2	1	0·6	2	1·5
Ataxia	Grade 3	4	2·5	2	1·5
Central nervous system necrosis	Grade 1	5	3·1	3	2·2
Central nervous system necrosis	Grade 2	4	2·5	3	2·2
Central nervous system necrosis	Grade 3	10	6·2	3	2·2
Central nervous system necrosis	Grade 4	1	0·6	0	0·0
Cerebrospinal fluid leakage	Grade 1	0	0·0	2	1·5
Cerebrospinal fluid leakage	Grade 2	0	0·0	1	0·7

Cerebrospinal fluid leakage	Grade 3	0	0·0	1	0·7
Cognitive disturbance	Grade 1	5	3·1	4	2·9
Cognitive disturbance	Grade 2	3	1·9	5	3·6
Cognitive disturbance	Grade 3	4	2·5	3	2·2
Cognitive disturbance	Grade 4	1	0·6	1	0·7
Concentration impairment	Grade 1	10	6·2	6	4·4
Concentration impairment	Grade 2	2	1·2	2	1·5
Depressed level ofconsciousness	Grade 2	1	0·6	0	0·0
Depressed level ofconsciousness	Grade 3	3	1·9	0	0·0
Dizziness	Grade 1	11	6·8	9	6·6
Dizziness	Grade 2	6	3·7	2	1·5
Dizziness	Grade 3	0	0·0	1	0·7
Dizziness	Grade 4	1	0·6	0	0·0
Dysarthria	Grade 1	7	4·3	3	2·2
Dysarthria	Grade 2	2	1·2	4	2·9
Dysarthria	Grade 3	2	1·2	1	0·7
Dysesthesia	Grade 1	3	1·9	4	2·9
Dysesthesia	Grade 3	1	0·6	0	0·0
Dysgeusia	Grade 1	7	4·3	3	2·2
Dysphasia	Grade 1	7	4·3	5	3·6
Dysphasia	Grade 2	8	5·0	8	5·8
Dysphasia	Grade 3	7	4·3	6	4·4
Edema cerebral	Grade 1	1	0·6	0	0·0
Edema cerebral	Grade 2	7	4·3	6	4·4
Edema cerebral	Grade 3	6	3·7	2	1·5
Edema cerebral	Grade 4	1	0·6	2	1·5
Encephalopathy	Grade 3	1	0·6	0	0·0
Facial muscle weakness	Grade 1	3	1·9	0	0·0
Facial muscle weakness	Grade 3	0	0·0	1	0·7
Facial nerve disorder	Grade 1	4	2·5	3	2·2
Facial nerve disorder	Grade 2	0	0·0	1	0·7
Facial nerve disorder	Grade 3	2	1·2	0	0·0
Headache	Grade 1	49	30·4	37	27·0
Headache	Grade 2	18	11·2	10	7·3
Headache	Grade 3	3	1·9	6	4·4
Hydrocephalus	Grade 1	1	0·6	0	0·0
Hydrocephalus	Grade 2	0	0·0	1	0·7
Hydrocephalus	Grade 3	2	1·2	0	0·0
Hydrocephalus	Grade 4	2	1·2	1	0·7
Hypoglossal nerve disorder	Grade 2	0	0·0	1	0·7
Intracranial hemorrhage	Grade 2	2	1·2	2	1·5
Intracranial hemorrhage	Grade 3	2	1·2	0	0·0
Intracranial hemorrhage	Grade 4	3	1·9	1	0·7
Ischemia cerebrovascular	Grade 1	4	2·5	1	0·7

Ischemia cerebrovascular	Grade 2	1	0·6	2	1·5
Lethargy	Grade 1	2	1·2	2	1·5
Lethargy	Grade 2	2	1·2	0	0·0
Lethargy	Grade 3	1	0·6	0	0·0
Memory impairment	Grade 1	11	6·8	8	5·8
Memory impairment	Grade 2	6	3·7	5	3·6
Memory impairment	Grade 3	1	0·6	1	0·7
Memory impairment	Grade 4	1	0·6	1	0·7
Movements involuntary	Grade 1	1	0·6	1	0·7
Movements involuntary	Grade 2	1	0·6	0	0·0
Nervous system disorders - Other, specify	Grade 1	17	10·6	7	5·1
Nervous system disorders - Other, specify	Grade 2	2	1·2	7	5·1
Nervous system disorders - Other, specify	Grade 3	6	3·7	4	2·9
Nervous system disorders - Other, specify	Grade 4	2	1·2	0	0·0
Nervous system disorders - Other, specify	Grade 5	2	1·2	0	0·0
Oculomotor nerve disorder	Grade 2	1	0·6	1	0·7
Oculomotor nerve disorder	Grade 3	1	0·6	0	0·0
Paresthesia	Grade 1	7	4·3	6	4·4
Paresthesia	Grade 2	5	3·1	3	2·2
Paresthesia	Grade 3	1	0·6	0	0·0
Peripheral motor neuropathy	Grade 1	1	0·6	0	0·0
Peripheral sensory neuropathy	Grade 1	0	0·0	1	0·7
Pyramidal tract syndrome	Grade 1	4	2·5	2	1·5
Pyramidal tract syndrome	Grade 2	2	1·2	1	0·7
Pyramidal tract syndrome	Grade 3	5	3·1	2	1·5
Pyramidal tract syndrome	Grade 4	1	0·6	0	0·0
Seizure	Grade 1	9	5·6	9	6·6
Seizure	Grade 2	23	14·3	13	9·5
Seizure	Grade 3	20	12·4	8	5·8
Seizure	Grade 4	1	0·6	1	0·7
Somnolence	Grade 1	1	0·6	1	0·7
Somnolence	Grade 2	2	1·2	0	0·0
Somnolence	Grade 3	1	0·6	3	2·2
Somnolence	Grade 4	1	0·6	0	0·0
Spasticity	Grade 1	0	0·0	1	0·7
Stroke	Grade 1	1	0·6	0	0·0
Stroke	Grade 4	1	0·6	0	0·0
Syncope	Grade 2	1	0·6	1	0·7
Syncope	Grade 3	4	2·5	2	1·5
Tremor	Grade 1	4	2·5	3	2·2
Tremor	Grade 2	0	0·0	1	0·7
Tremor	Grade 3	0	0·0	1	0·7
Vasovagal reaction	Grade 3	1	0·6	0	0·0
Pregnancy, puerperium and perinatal conditions					

Unintended pregnancy	Grade 2	1	0·6	0	0·0
Psychiatric disorders					
Agitation	Grade 2	1	0·6	0	0·0
Anxiety	Grade 1	5	3·1	1	0·7
Anxiety	Grade 2	2	1·2	1	0·7
Confusion	Grade 1	4	2·5	4	2·9
Confusion	Grade 2	3	1·9	3	2·2
Confusion	Grade 3	3	1·9	1	0·7
Delirium	Grade 3	0	0·0	1	0·7
Depression	Grade 1	3	1·9	0	0·0
Depression	Grade 2	5	3·1	1	0·7
Depression	Grade 4	1	0·6	0	0·0
Euphoria	Grade 2	1	0·6	0	0·0
Hallucinations	Grade 1	0	0·0	2	1·5
Hallucinations	Grade 2	0	0·0	2	1·5
Insomnia	Grade 1	4	2·5	2	1·5
Insomnia	Grade 2	2	1·2	2	1·5
Mania	Grade 2	0	0·0	1	0·7
Personality change	Grade 1	1	0·6	0	0·0
Personality change	Grade 2	0	0·0	1	0·7
Psychosis	Grade 2	0	0·0	1	0·7
Psychosis	Grade 3	0	0·0	1	0·7
Restlessness	Grade 2	1	0·6	0	0·0
Suicide attempt	Grade 4	1	0·6	0	0·0
Renal and urinary disorders					
Acute kidney injury	Grade 1	1	0·6	0	0·0
Acute kidney injury	Grade 3	1	0·6	0	0·0
Hematuria	Grade 1	1	0·6	0	0·0
Hematuria	Grade 2	2	1·2	1	0·7
Renal and urinary disorders - Other, specify	Grade 1	0	0·0	1	0·7
Renal and urinary disorders - Other, specify	Grade 5	0	0·0	1	0·7
Urinary frequency	Grade 1	2	1·2	1	0·7
Urinary frequency	Grade 2	1	0·6	0	0·0
Urinary incontinence	Grade 1	2	1·2	2	1·5
Urinary incontinence	Grade 2	4	2·5	0	0·0
Urinary incontinence	Grade 3	0	0·0	1	0·7
Urinary retention	Grade 2	1	0·6	1	0·7
Urinary tract pain	Grade 1	2	1·2	0	0·0
Urinary tract pain	Grade 3	1	0·6	0	0·0
Reproductive system and breast disorders					
Vaginal hemorrhage	Grade 1	1	0·6	0	0·0
Respiratory, thoracic and mediastinal disorders					
Aspiration	Grade 2	2	1·2	0	0·0
Aspiration	Grade 3	1	0·6	0	0·0

Cough	Grade 1	2	1·2	0	0·0
Dyspnea	Grade 1	4	2·5	0	0·0
Hiccups	Grade 3	1	0·6	0	0·0
Hoarseness	Grade 1	1	0·6	0	0·0
Hoarseness	Grade 2	0	0·0	1	0·7
Pneumonitis	Grade 2	2	1·2	0	0·0
Pneumonitis	Grade 4	1	0·6	1	0·7
Respiratory, thoracic and mediastinal disorders - Other, specify	Grade 1	0	0·0	1	0·7
Respiratory, thoracic and mediastinal disorders - Other, specify	Grade 2	0	0·0	1	0·7
Sore throat	Grade 2	1	0·6	0	0·0
Skin and subcutaneous tissue disorders					
Alopecia	Grade 1	22	13·7	15	10·9
Alopecia	Grade 2	14	8·7	22	16·1
Alopecia	Grade 3	4	2·5	0	0·0
Dry skin	Grade 1	4	2·5	4	2·9
Dry skin	Grade 3	0	0·0	1	0·7
Erythema multiforme	Grade 1	3	1·9	0	0·0
Erythema multiforme	Grade 2	0	0·0	3	2·2
Erythroderma	Grade 1	3	1·9	4	2·9
Erythroderma	Grade 2	2	1·2	2	1·5
Erythroderma	Grade 3	0	0·0	1	0·7
Hyperhidrosis	Grade 2	0	0·0	1	0·7
Periorbital edema	Grade 1	2	1·2	1	0·7
Periorbital edema	Grade 2	1	0·6	0	0·0
Pruritus	Grade 1	1	0·6	2	1·5
Pruritus	Grade 2	0	0·0	1	0·7
Purpura	Grade 1	1	0·6	0	0·0
Rash acneiform	Grade 1	2	1·2	0	0·0
Rash maculo-papular	Grade 1	1	0·6	1	0·7
Rash maculo-papular	Grade 2	2	1·2	0	0·0
Skin and subcutaneous tissue disorders - Other, specify	Grade 1	5	3·1	3	2·2
Skin and subcutaneous tissue disorders - Other, specify	Grade 2	1	0·6	5	3·6
Skin atrophy	Grade 2	1	0·6	0	0·0
Skin hyperpigmentation	Grade 1	4	2·5	3	2·2
Skin ulceration	Grade 2	2	1·2	0	0·0
Skin ulceration	Grade 3	1	0·6	0	0·0
Social circumstances					
Social circumstances - Other, specify	Grade 1	1	0·6	0	0·0
Social circumstances - Other, specify	Grade 2	1	0·6	0	0·0
Surgical and medical procedures					
Surgical and medical procedures - Other, specify	Grade 2	1	0·6	2	1·5
Surgical and medical procedures - Other, specify	Grade 3	1	0·6	2	1·5
Vascular disorders					

Hematoma	Grade 1	1	0·6	1	0·7
Hematoma	Grade 2	0	0·0	1	0·7
Hematoma	Grade 3	1	0·6	0	0·0
Hematoma	Grade 4	1	0·6	0	0·0
Hematoma	Grade 5	1	0·6	0	0·0
Hypertension	Grade 1	1	0·6	3	2·2
Hypertension	Grade 2	2	1·2	1	0·7
Hypotension	Grade 1	2	1·2	1	0·7
Thromboembolic event	Grade 1	3	1·9	1	0·7
Thromboembolic event	Grade 2	6	3·7	2	1·5
Thromboembolic event	Grade 3	4	2·5	3	2·2
Thromboembolic event	Grade 4	3	1·9	2	1·5
Vascular disorders - Other, specify	Grade 1	1	0·6	0	0·0
Vascular disorders - Other, specify	Grade 3	1	0·6	0	0·0

Abbreviations: AE: adverse event, SOC: standard of Care, IORT: intraoperative radiotherapy

Table S2: Reasons for PFS censoring. IORT=intraoperative radiotherapy; PFS=progression-free survival; SOC=standard of care.

Reason for censoring	**IORT arm**	**SOC arm**
Withdrawal by participant	10	20
Lost to follow-up	6	3
Protocol violation	0	4
Unrelated adverse event	1	0
Synchronous second tumor	0	1
Discontinuation with unknown reason	1	0
IORT not feasible after randomisation	1	0
On-Study	13	12
Total	**32**	**40**

Table S3: Subsequent anti-cancer treatment triggering PFS endpoint. IORT=intraoperative radiotherapy; LITT=Laser interstitial thermal therapy; PFS=progression-free survival; SOC=standard of care.

Subsequent treatment	IORT arm	SOC arm
Lomustine/CCNU	4	3
Temozolomide	4	2
Bevacizumab	1	4
Lomustine + Bevacizumab	0	2
LITT	2	0
Reirradiation	0	2
Regorafenib	1	0
Fotemustine	0	1
Total	**12**	**14**

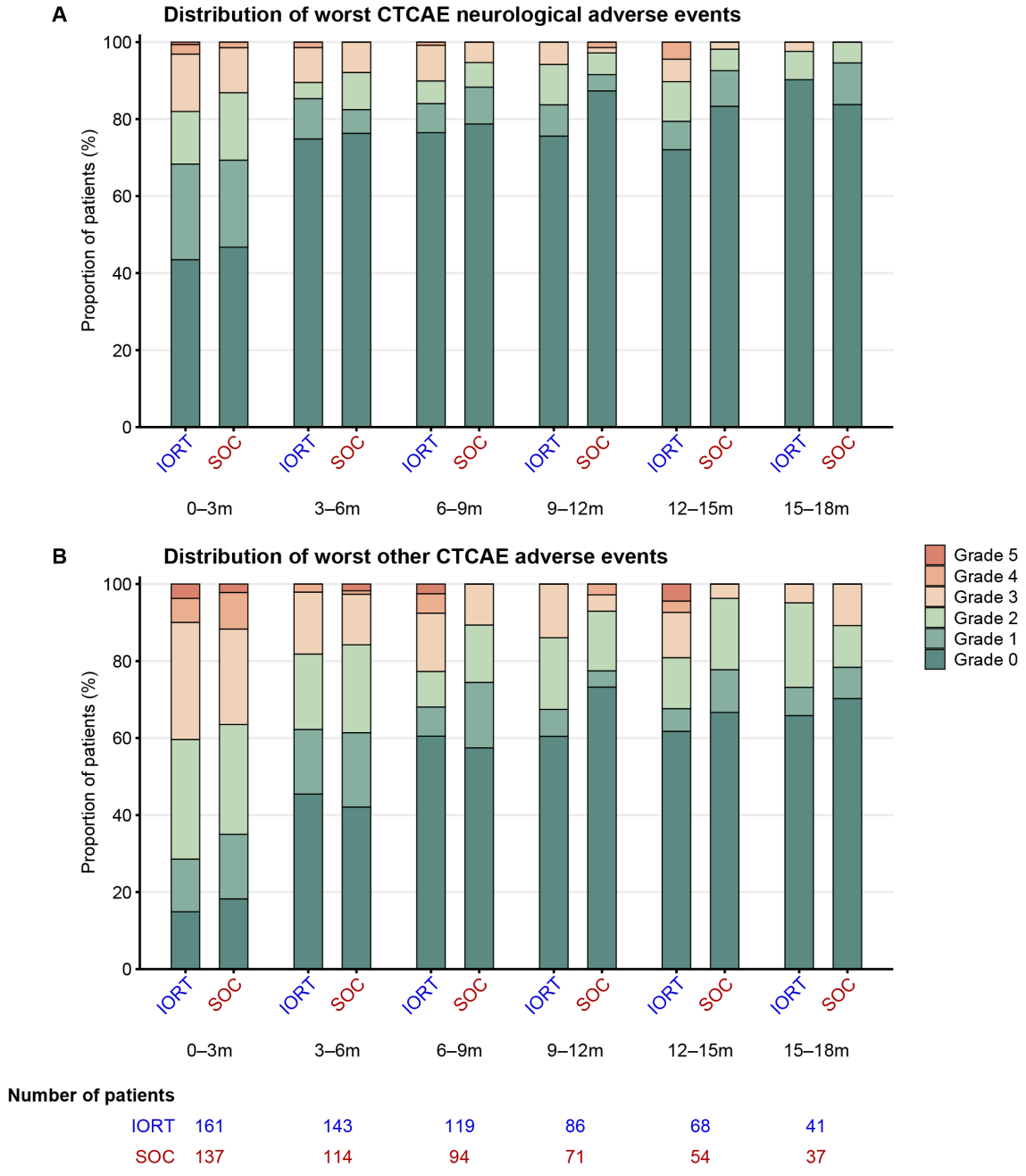


Figure S1: Distribution of worst CTCAE adverse events. Grouped stacked bar charts showing the distribution of worst-grade CTCAE adverse events for (A) neurological adverse events (corresponding to the CTCAE v4.0 'Nervous system disorders' System Organ Class) and (B) other adverse events. For each adverse event category, the maximum CTCAE grade per patient was considered. Grade 5 events were included only when assignable to a specific CTCAE term. CTCAE=Common Terminology Criteria for Adverse Events (version 4.0); IORT=intraoperative radiotherapy; m=months; SOC=standard of care.

File S1: Substantial changes to the protocol

Initial protocol:

- Protocol version 2.1, date 15 June 2016

Amendment 1:

- Protocol version 2.2, date 25 July 2016
- Substantial changes: none, only administrative changes

Amendment 2:

- Protocol version 2.3, date 16 November 2017
- Substantial changes:

Page 12 and page 31: Inclusion Criteria

- V2.2: Age ≥18 and ≤ 70 years
- V2.3: Age ≥18 and ≤ 75 years

Page 12 and page 32: Exclusion Criteria

- V2.2: #4, Use of alternating electrical fields (e.g., Novo-TTF)
- V2.3: exclusion criteria #4 removed

Amendment 3:

- Protocol version 2.4, date 28 September 2018
- Substantial changes:

Page 12 and page 31: Inclusion Criteria, Age

- V2.3: Age ≥18 and ≤ 75 years
- V2.4: Age ≥18 and ≤ 80 years

Page 12 and page 31: Inclusion Criteria, Organ Functions

- V2.3:
  Bone marrow function:
  - Platelets ≥ 145.000/µL
  - WBC ≥ 4.000/µL
  - Hemoglobin ≥ 12.0 g/dL

  Liver function:
  - ASAT and ALAT ≤ 1.5 times ULN (upper limit of normal)
  - Total Serum Bilirubin < 1.0 times ULN
- V2.4
  Bone marrow function:
  - Platelets ≥ 75.000/µL
  - WBC ≥ 3.000/µL
  - Hemoglobin ≥ 10.0 g/dL

  Liver function:
  - ASAT and ALAT ≤ 3.0 times ULN (upper limit of normal)
  - Total Serum Bilirubin < 1.5 times ULN

Page 12 and page 31: Inclusion Criteria, histology

- V2.3: Histologically confirmed (frozen sections) of GBM
- V2.4: Histology supports diagnosis of GBM

Page 14: Schedule

- V2.3: Histologic confirmation of GBM
- V2.4: Histology assessment of GBM

Page 34: Intra-operative screening

- V2.3: Frozen sections must confirm the diagnosis of Glioblastoma multiforme. Participating centers must provide the infrastructure to allow frozen sections. All histopathological procedures should be conducted according to local standards.
- V2.4: Frozen sections and/or smear preparations should support the preliminary diagnosis of glioblastoma

Page 71: Appendix III, modified RANO Assessment

- V2.3: radiological PD should be associated with an increase with more than 25% in rBV compared with the preceding scan to qualify for having reached the PFS endpoint.
- V2.4: radiological PD should be associated with an increase with more than 25% in rBV compared with the nadir to qualify for having reached the PFS endpoint.

Amendment 4:

- Protocol version 2.5, date 3 September 2020
- Substantial changes:

Cover page and page 2: change sponsor

- V2.4: University Hospital Heidelberg
- V2.5: University Hospital Bonn

Page 37: Primary Objective

V2.4 The primary end point is the median progression free survival rate (PFS).

V2.5 The primary end point is the median progression free survival rate (PFS). PFS is defined from the day of randomization to the day of local tumor progression or recurrence. The patient has met the PFS endpoint if any of the following criteria are met:

- Radiological progression per modified RANO criteria
- The initiation of any new or salvage treatment
- Progressive disease based on clear clinical deterioration not attributable to other causes apart from the tumor
- Death

Page 38: Trial Duration and Schedule

V2.4	Total trial duration:	72 months
	Duration of the clinical phase:	48 months
	FPI (First Patient In):	Q3 2016
	LPI (Last Patient In):	Q3 2020
	LPO (Last Patient Out):	Q3 2022
	Analyses completed:	Q1 2023
V2.5	Total trial duration:	84 months
	Duration of the clinical phase:	60 months
	FPI (First Patient In):	Q4 2016
	LPI (Last Patient In):	Q4 2021
	LPO (Last Patient Out):	Q4 2023

Analyses completed: Q1 2024

Page 40: Criteria for Withrawel

V2.4 Reasons leading to the withdrawal of a patient can include the following (one primary reason must be determined):

- Patient died
- Adverse Events (need to be specified)
- Withdrawal by Subject
- Pregnancy
- Protocol Violation
- Lost to Follow-Up
- Study Terminated by Sponsor
- Other (needs to be specified)

In all patients who finish the study prematurely, a withdrawal examination at least with respect to the primary endpoint should be carried out. Drop-outs will not be replaced.

V2.5 Reasons leading to the withdrawal of a patient can include the following (one primary reason must be determined):

- Withdrawal by Subject
- Lost to Follow-Up
- Study Terminated by Sponsor
- Other (needs to be specified)

Note: deviations from the post-operative Standard of Care protocol (eg, Stupp) are not a reason for study discontinuation. In addition, EBRT given at another facility is as well not considered a reason for study discontinuation if all follow-up data can still be obtained.

In all patients who finish the study prematurely, a withdrawal examination at least with respect to the primary endpoint should be carried out. Discontinuations will not be replaced.

Page 43: Imaging studies

V2.4 -
V2.5 Additional phrasing:

In case no quantitative rBV data is available, the on-site response assessments may include the qualitative interpretation of the rBV map to distinguishing tumor recurrence from pseudoprogression. Although sites are encouraged to perform a quantitative evaluation of the perfusion scan (eg, changes in corrected normalized rBV values), a qualitative assessment is accepted taking into account a central post-processing step will be performed for all patients after suspected progression.

Page 45: Follow-Op MRI

V2.4 In case a new/increasing T1-Gd enhancing lesion or an increase in T2/FLAIR is noted and perfusion shows an increase in rCBV in FU scans, all centers must adhere to a pre-defined algorithm (see **Fehler! Verweisquelle konnte nicht gefunden werden.** and Appendix II and III).
V2.5 In case a new/increasing T1-Gd enhancing lesion or an increase in T2/FLAIR is noted and the enhancing lesion shows a rCBV ≥ 1, all centers must adhere to a pre-defined algorithm (see **Fehler! Verweisquelle konnte nicht gefunden werden.** and Appendix II).

Page 49: Concomittant Chemotherapy

V2.4 -
V2.5 Added:

The concomitant application of the CeTeG chemotherapy regimen (CCNU plus elevated dose-temozolomide) in patients with MGMT promoter hypermethylation is allowed if considered standard of care for these patients at the individual institution.

Page 50: Adjuvant Chemotherapy

V2.4 -

V2.5 Added:

The application of the CeTeG chemotherapy regimen (CCNU plus elevated dose-temozolomide) in patients with MGMT promoter hypermethylation is allowed if considered standard of care for these patients at the individual institution.

Page 50: Diagnosis of Radionecrosis

V2.4 -

V2.5 Added:

A central pathology review will as well be performed for patients with diagnosed radionecrosis after they had surgery for suspected tumor progression. Tissue obtained during the surgery should be stored until the end of the study:

Ideally,

- one HE section and a paraffine block containing representative tissue material OR
- one HE section with representative tumor material and 10 unstained sections
- one HE section of all the blocks to confirm the absence recurrent tumor.
    -

Sites will be informed by the sponsor when the tissue can be send for central pathology review. Sites will be provided with shipment forms and labels. Patients that consented for the shipment of tissue for central pathology will be tracked by the sites.

Page 52: Primary Target variable

V2.4 -

V2.5 Added:

The primary target variable of the trial is the median progression-free survival (PFS). The patient has met the PFS endpoint if any of the following criteria are met:

- Radiological progression per modified RANO criteria
- The initiation of any new or salvage treatment
- Progressive disease based on clear clinical deterioration not attributable to other causes apart from the tumor
- Death

Page 52: Primary Target variable

V2.4 The initiation of salvage chemotherapy or re-irradiation for GBM in the absence of diagnosed tumor progression by RANO or recurrence will be considered as protocol violation and will not be counted as an event for PFS (the case will be censored at the date the treatment was initiated).

V2.5

The initiation of any new or salvage treatment for GBM will be considered as an event for PFS. Salvage treatment include:

1) Re-resection of recurrence

2) Re-irradiation

3) Invasive treatments (eg, Laser interstitial thermal therapy (LITT), seeds, etc)

4) Chemotherapy

- Alkylators (e.g. CCNU 2nd line, TMZ re-challenge)
- TIs (Topotecan, Irinotecan)
- Targeted therapies (EGFR-I, ..)

Page 52: Primary Target variable

V2.4 -
V2.5

Added:

Patients receiving bevacizumab for radionecrosis will be censored for tumor response (eg, pseudoresponse) and the assigned radiological timepoint response should be “Not Evalable” starting from start treatment with a wash-out period of 6 weeks. Any observed decreases in enhancement during this period will not be considered as nadir for the response assessments of successive scans.The above is omitted in case increased enhancement is observed during treatment with bevacizumab meeting the progressive disease definitions.

Page 52: Appendix II

V2.4 -

V2.5 Added:

Modified RANO criteria updated to incorporate the response criterion „rCBV ≥ 1“ consistently. In addition, clarifications added that the PD criteria for new enhancing lesions are the same regardless if the new enhancing lesion is located within or outside the irradiation field.

Amendment 5:

- Protocol version 3.0, date 28 March 2022
- Substantial changes:

Page 3: Investigators and Administrative Structure

V2.5 -

V3.0 ENDPOINT ADJUDICTION COMMITTEE

The Endpoint Adjudication Committee (EAC) periodically reviews the efficacy endpoints for compliance with the definitions for PFS as described in the protocol and the SAP. Members of the EAC include:

Whitney Pope, Neuroradiologist, David Geffen School of Medicine, UCLA

Kevin Petrecca, Neurosurgeon, Montreal Neurological Institute and Hospital

Christina Tsien, Clinical Director, Johns Hopkins National Proton Center, Sibley

Frank Giordano, Radiation oncologist and Principal Investigator, University Hospital

Page 11, 30: Secondary Objective, page 49 Efficacy Parameters

V2.5 PFS within a 1 and 2 cm margin around the cavity determined by serial contrast-enhanced MRI scans using modified RANO criteria.

V3.0 Outcome (PFS, OS) with respect to:

- Site of failure/patterns of recurrence:
  - PD within < 1 cm margin around the cavity
  - PD within ≥ 1 cm – 2.0 cm margin around the cavity
  - PD within ≥ 2.0 cm margin around the cavity

Page 11, 30: Secondary Objective, page 49 Efficacy Parameters

V2.5 Outcome (PFS, OS) with respect to thickness of anticipated T1-Gd-enhancing (remaining) tumor margin and extent of resection (given as sum of all maximum diameters of residual lesions in cm)

V3.0 Outcome (PFS, OS) with respect to:

- Volume of residual disease
  - 0.0 - 0.2 cm
  - ≥ 0.2 cm

Page 11, 30: Secondary Objective, page 49 Efficacy Parameters

V2.5 -

V3.0 Outcome (PFS, OS) with respect to:

- Pattern of progressive disease:
  - PD based on non-measurable and/or measurable enhancing residual lesions
  - PD based on new enhancing lesions
  - PD based on new non-enhancing lesions

Page 11, 30: Secondary Objective, page 49 Efficacy Parameters

V2.5 -

V3.0 Outcome (PFS, OS) with respect to:

- Appplicator size
  - ≤ 3.5 cm
  - > 4 cm

Page 11, 30: Secondary Objectives, page 49 Efficacy Parameters

V2.5 -

V3.0

- Radiological progression per modified RANO criteria determined by the off-site central radiology review

Page 31: Trial Duration and Schedule

V2.5	Total trial duration:	84 months
	Duration of the clinical phase:	60 months
	FPI (First Patient In):	Q4 2016
	LPI (Last Patient In):	Q4 2021
	LPO (Last Patient Out):	Q4 2023
	Analyses completed:	Q1 2024
V3.0	Total trial duration:	96 months
	Duration of the clinical phase:	72 months
	FPI (First Patient In):	Q4 2016
	LPI (Last Patient In):	Q4 2022
	LPO (Last Patient Out):	Q4 2024
	Analyses completed:	Q1 2025

Page 32: Selection of Subjects and Centers

V2.5 As calculated in section 9.2. (Sample Size Calculation) 314 subjects should be enrolled in the clinical trial, i.e. 157 subjects per treatment group. Recruitment and treatment of subjects is expected to be performed in 20 centers.

V3.0 As calculated in section 9.2. (Sample Size Calculation) 314 subjects should be enrolled in the clinical trial, i.e. 157 subjects per treatment group. Recruitment and treatment of subjects is expected to be performed in 20 centers. After 200 randomized patients the original assumptions for sample size calculation will be proven using an interims analysis of non-binding futility stop.

Page 45: Centralized review

V2.5 6.5.1.1 Diagnosis of Radionecrosis

A central pathology review will as well be performed for patients with diagnosed radionecrosis after they had surgery for suspected tumor progression.

V3.0 6.5.1.2 Diagnosis of Radionecrosis and tumor recurrence

A central pathology review will as well be performed for patients with diagnosed radionecrosis/tumor recurrence after they had surgery for suspected tumor progression.

Page 55, 56 Statistical Procedures

V2.5 -

V3.0 Interim analysis

The trial is planned with an adaptive interims analysis after 200 patients have been randomization. The purpose of the interim analysis is to check the assumptions made during the study planning phase (especially the survival and censoring rates) and potentially modify the design correspondingly.

Although the trial is an open one the interim analysis will be performed by an independent board and details of the analysis will not be presented to persons directly involved in the conduct of the trial.

The analysis will apply the inverse normal combination of the p-values of the two stages with an O'Brien-Fleming type alpha-spending function and information rate of 50%. The corresponding stage-wise significance levels are 0.0088 and 0.0467. The primary efficacy endpoint (PFS) will guide the decision process but OS, adverse events and the attrition rates in the two treatment groups will also be presented.
The possible decisions during the interim look includes a stop for efficacy or futility, a sample size re-estimation or change of other design parameters like the randomization ratio.

Amendment 6:

- Protocol version 4.0, date 18 January 2023
- Substantial changes:

Cover page and page 2: change sponsor

- V3.0: University Hospital Bonn
- V4.0: University Medical Center Mannheim, Medical Faculty Mannheim, University Heidelberg

Page 35: Secondary Objectives

V3.0 Volume of residual disease

- 0.0 – 0.2 cm
- ≥ 0.2 cm

V4.0 Volume of residual disease

- Non-measurable disease (< 0.5 * 0.5 cm)
- Measurable disease (≥ 0.5 * 0.5 cm)

Page 36/37: Trial Duration and Schedule

V3.0	Total trial duration:	96 months
	Duration of the clinical phase:	72 months
	FPI (First Patient In):	Q4 2016
	LPI (Last Patient In):	Q4 2022
	LPO (Last Patient Out):	Q4 2024
	Analyses completed:	Q1 2025
V4.0	Total trial duration:	120 months
	Duration of the clinical phase:	96 months

FPI (First Patient In):	Q4 2016
LPI (Last Patient In):	Q4 2024
LPO (Last Patient Out):	Q4 2026
Analyses completed:	Q1 2027

Page 51/52: Outcome (PFS, OS) with respect to strata:

V3.0 Volume of residual disease

- 0.0 – 0.2 cm
- ≥ 0.2 cm

V4.0 Volume of residual disease

- Non-measurable disease (< 0.5 * 0.5 cm)
- Measurable disease (≥ 0.5 * 0.5 cm)

File S2: Site activation and Ethics Committees approvals

City	Site name	Date SQV	Date SIV	Open for enrollment	EC approval	EC approval reference
Mannheim	University of Heidelberg	na	na	1/Nov/16	28/Jul/16	2015-582S-MA
Montreal	Montreal Neurological Institute	waived	30/Jan/18	28/Feb/18	28/Sep/16	F11-4079
Augsburg	Clinical Center Augsburg	10/Nov/16	05/Dec/17	16/Jan/18	13/Jul/16	BO 16037
Stuttgart	Clinical Center Stuttgart	11/Nov/16	26/Jun/18	30/Aug/18	16/Nov/16	B-F-2026-090
Morgantown	West Virginia University	24/Jan/17	06/Jul/17	21/Jul/17	29/Dec/16	1604101139A001
Phoenix	Barrow Neurological Institute	17/Nov/16	05/Aug/22	26/Oct/22	04/Mar/19	PHX-17-0106-70-12
Maywood	Loyola University Medical Center	10/Nov/17	02/Aug/18	30/Aug/18	20/Dec/17	209099122017
Barcelona	Catalan Institute of Oncology	26/Mar/18	10/Oct/18	04/Jun/18	07/Sep/17	No reference
Munich	Technical University Munich	07/Nov/18	22/Feb/18	11/Apr/18	12/Apr/17	143/17 S
New York	Northwell Health: North Shore	30/Jan/18	26/Feb/18	08/Mar/18	14/Sep/17	17-0633
São Paulo	Hospital Alemão Oswaldo Cruz	17/Nov/20	09/Dec/20	28/Dec/20	23/Jun/20	22972119.4.0000.0070
Cordoba	Hospital Reina Sofía	12/Feb/20	10/Mar/20	25/Mar/20	06/Mar/20	AC010/17
Leipzig	Clinic Center St. Georg	24/Oct/19	07/Feb/20	06/Mar/20	21/Aug/19	EK-BR-60/19-1
Bonn	University Clinic Bonn	12/Feb/20	30/Sep/20	09/Oct/20	29/Jul/20	232/20
Berlin	Charite	07/May/20	12/Nov/20	16/Apr/21	follows EC approval coordinating site	not applicable
Seoul	Gangnam Severance Hospital	15/Oct/21	18/Dec/20	15/Apr/21	02/Apr/21	3-2021-0048
Beijing	Beijing Tiantan Hospital	24/Nov/20	01/Jun/21	07/Jun/21	18/May/21	no reference
Bochum	Ruhr University Bochum	waived	26/Jan/23	23/Feb/23	07/Apr/22	22-7536-MPG

File S3: Enrolment per site

City	Institute	Investigator	Enrolled	Randomized
Stuttgart	Clinical Center Stuttgart	Dr. O. Ganslandt	55	47
Munich	Technical University Munich	Dr. S. Combs	77	44
Bonn	University Hospital Bonn	Dr. E. Gkikka	48	40
Augsburg	Clinical Center Augsburg	Dr. H. Kahl	48	39
Montreal	Montreal Neurological Institute	Dr. K. Petrecca	24	23
New York	Northwell Health: North Shore	Dr. M. Schulder	22	21
Barcelona	Catalan Institute of Oncology	Dr. A. Lucas	26	21
Mannheim	University Medical Center Mannheim	Dr. F. Giordano	31	20
Cordoba	Hospital Reina Sofia	Dr. S. Cabezas	14	13
Morgantown	West Virginia University	Dr. C. Cifarelli	16	11
Beijing	Beijing Tiantan Hospital	Dr. Wang Jia	16	10
Leipzig	Clinic Center St. Georg	Dr. O. Sorge	9	9
Seoul	Gangnam Severance Hospital	Dr. Jun Won Kim	8	4
Berlin	Charité - University Hospital Berlin	Dr. J. Onken	4	4
Chicago	Loyola University Medical Center	Dr. Chundury	6	3
Phoenix	Barrow Neurological Institute	Dr. K. Smith	2	2
Bochum	Ruhr University Bochum	Dr. K. Schmieder	3	2
São Paulo	Hospital Alemão Oswaldo Cruz	Dr. D. Guedes	1	1
New York	Lenox Hill Hospital	Dr. J. A. Boockvar	1	0
Schwerin	Helios Hospital Schwerin	Dr. O. Heese	0	0
		Total:	**411**	**314**

File S4: Study Group, Investigators and study sites

City	Institute	Investigator
Mannheim	University Hospital Mannheim	Frank Giordano
		Stefanie Brehmer
		Nima Etminan
		Yasser Aboumadian
		Katharina Fleckenstein
		Judit Boda-Heggenmann
		Arne Ruder
		Michael Ehmann
		Elena Sperk
		Frank Schneider
		Michael Platten
		Johannes Bohme
Montreal	McGill University	Kevin Petrecca
		Christina Tsien
		Luis Souhami
		Piotr Pater
		Valerie Panet-Raymond
		Marie-Christine Guiot
Augsburg	Clinical Centre Augsburg	Henning Kahl
		Heiko Muller
		Ehab Shiban
		Georg Stüben
		Christoph Maurer
		Ute Grossert
		Michal Hock
		Sebastian Ritter
		Philipp Emanuel Krauss
		Christoph Maurer
Stuttgart	Clinical Centre Stuttgart	Oliver Ganslandt
		Marc Münter
		Stefan Baumbach
		Minou Nadij-Ohl
		Guido Nikkah
		Elina Henkes
New York	Lenox Hill Hospital	John Boockvar
		Anuj Goenka
Morgantown	West Virginia University	Chris Ciffarelli
		Geraldine Jacobsen
Chicago	Loyola University Medical Center	Anupama Chundury
		Tamer Abdelrhman
		Anand Germanwala
		Willium Small
		Bahman Emami
		Vikram Prabhu
		Edward Melian
		Courney Hentz
Barcelona	Catalan Institute of Oncology	Anna Lucas
		Gerard Plans
		Miquel Macia Garau
		Ferran Guedea

Munich	Technical University Munich	Carles Aquilera Carles Majos Stephanie Combs Bernard Meyer Christian Diehl Jens Gempt Steffi Pigorsch Christoph Straube Hendrik Dapper Melanie Barz Benedikt Wiestler Artur Wagner
New York	Northwell Health; North Shore	Michael Schulder Anuj Goenka
São Paulo	Hospital Alemão Oswaldo Cruz	Douglas Guedes de Castro Rodrigo Hanriot
Cordoba	Hospital Reina Sofia	Sonia Garcia Cabezas Juan Solivera Vela Amalia Palacios Eito
Leipzig	Clinical Center St. Georg	Oliver Sorge Andre Liebmann
Berlin	Charité Berlin	Julia Onken Dirk Bohmer Peter Vajkoczy
Bonn	University Hospital Bonn	Eleni Gkika Ulrich Herrlinger Julian Layer Harmut Vatter Matthias Schneider Motaz Hamed Christopher Schmeel Gustavo Sarria Cas Dejonckheere Katharina Layer Youness Nour David Scafa Stephan Garbe Mümtaz Köksal David Koch Roberth Nemeth
Seoul	Gangnam Severance Hospital	Jun Won Kim Ik Jae Lee Hun Ho Park Jason Joon Bock Lee
Beijing	Beijing Tiantan Hospital	Wang Jia Zhan Xue
Bochum	Ruhr University Bochum	Kirsten Schmieder Eveline Popanda Jan Boström Kathleen Jetschke Mortimer Gierthmühlen Dorothea Miller

Phoenix	Barrow Neurological Institute	Kris Smith
		Randall Porter
		William Kennedy
		Igor Barani
Schwerin	Helios Hospital Schwerin	Oliver Heese
		Christian Cöster
		Marciana Duma
		Max Bulsara
Lünen	St. Marien Hospital Lünen	Martin Glas
Dresden	University Hospital Dresden	Dietmar Krex
Phoenix	St. Joseph's Hospital and Medical Center	Kris Smith

Methods S1: Quality assurance and monitoring

On-site qualification visits were performed by the sponsor prior the site study participation to ensure the site capabilities to comply with all study-specific procedures. Sites were opened for enrolment after the completion of the remote site initiation visit where the site study team was trained on the protocol, the study logistics, the imaging acquisition and submission guidelines and the eCRF.

Prior to enrolment of the first patient, each participating centre completed a mandatory certification process comprising hands-on training of the surgical and radiation oncology team with the INTRABEAM system, dosimetric verification of the applicator output, and a supervised dry-run procedure. On-site support by the coordinating team was provided for the first patient treated at each centre. Before each treatment, the INTRABEAM system performed automated calibration and dosimetric verification, and all treatment parameters, including applicator size, treatment time, and prescribed surface dose were electronically documented. After sites enrolled their first patient, an on-site monitoring visit was scheduled. Further on-site monitoring visits were scheduled based on the number of patients enrolled and if any quality issues were observed during the regular remote monitoring visits. Seventy-five on-site monitoring visits were performed. In addition, remote medical monitoring was performed for all reported adverse events for medical soundness prior presenting the data to the DSMB. All submitted MRI scans were assessed for quality upon receipt for compliance with the imaging acquisition guidelines and were reconciled with the clinical eCRF to ensure completeness of the data sets.

Study management was performed by the sponsoring universities (Heidelberg and Bonn). The required study IT infrastructure was maintained by Clinflows UG, Hüllhorst, Germany operating ISO 9001. The utilized study software tools were Castor (eCRF); decidemedical (imaging portal); Mint Lesion (read software for the RANO response assessments); Olea Sphere (post-processing DSC-MRI scans). Monitoring, Site Management, Data Management and Imaging Workflow Management was performed by Medwave Clinical Research B.V., Harlingen, The Netherlands.

Independent oversight was performed by the installed data safety monitoring board (DSMB) that was meeting on a bi-annually basis. A planned interim analysis was performed in May 2023 and the DSMB determined the timing of the primary analysis in October 2025.

CLINICAL TRIAL PROTOCOL

Version/Date: V 2.1, 05/17/2016

A MULTICENTER RANDOMIZED PHASE III TRIAL ON INTRAOPERATIVE RADIOTHERAPY IN NEWLY DIAGNOSED GLIOBLASTOMA MULTIFORME (INTRAGO II)

Clinical Trial Code:	INTRAGO II
Indication:	Glioblastoma Multiforme (WHO Grade IV)
Planned intervention:	Intraoperative Radiotherapy
Status and Date:	Version 2.1, dated 05/17/2016
Amendments and Date:	Amendment 1, filed for amendment on 05/17/2016
ClinicalTrials.gov ID:	NCT02685605
Clinical Phase:	Phase III

Sponsor:

Department of Radiation Oncology
University Medical Center Mannheim
University of Heidelberg
Theodor-Kutzer-Ufer 1-3
68167 Mannheim
Phone: +49 621 383 4960
Fax: +49 621 383 2476

PI (CORRESPONDING):

Frank A. Giordano, MD
Department of Radiation Oncology
University Medical Center Mannheim
University of Heidelberg
Theodor-Kutzer-Ufer 1-3
68167 Mannheim
Phone: +49 621 383 4960
Fax: +49 621 383 2476
E-Mail: frank.giordano@umm.de

INVESTIGATORS AND ADMINISTRATIVE STRUCTURE

PRINCIPLE INVESTIGATOR	**Frank A. Giordano, MD** Department of Radiation Oncology University Medical Center Mannheim University of Heidelberg Theodor-Kutzer-Ufer 1-3 68167 Mannheim Phone: +49 621 383 4960 Fax: +49 621 383 2476 E-Mail: frank.giordano@umm.de
TRIAL COORDINATING CENTER:	University Medical Center Mannheim University of Heidelberg Theodor-Kutzer-Ufer 1-3 68167 Mannheim
BIOMETRICIAN	**Grit Welzel, MSc** Department of Radiation Oncology University Medical Center Mannheim University of Heidelberg Theodor-Kutzer-Ufer 1-3 68167 Mannheim Phone: +49 621 383 4494 Fax: +49 621 383 2476 E-Mail: grit.welzel@umm.de
DATA MANAGEMENT AND MONITORING COORDINATION	**Anke Keller, MSc** Department of Radiation Oncology University Medical Center Mannheim University of Heidelberg Theodor-Kutzer-Ufer 1-3 68167 Mannheim Phone: +49 621 383 6020 Fax: +49 621 383 2476 E-Mail: anke.keller@umm.de
CLINICAL RESEARCH ORGANIZATION	**ClinFlows UG & CoKG** Grossenberkener Str.40 32609 Hüllhorst Germany Phone: +49 5744 5098 80 Fax: +49 57 44 5098 82 E-Mail: info@clinflows.com
CRO PROJECT LEADER	**John Uiters, PhD** ClinFlows UG & CoKG Grossenberkener Str.40 32609 Hüllhorst Germany Phone: +49 5744 5098 80 Fax: +49 57 44 5098 82 E-Mail: info@clinflows.com

STEERING COMMITTEE (SC)

The steering committee comprises the lead investigator and supporting co-investigators, clinical experts not directly involved in the clinical trial and the responsible biometrician. The steering committee is responsible for the scientific integrity of the study protocol, the quality of the study conduct as well as for the quality of the final study report.

Members of the SC:

Frank A. Giordano, MD (Chair) Department of Radiation Oncology University Medical Center Mannheim University of Heidelberg Theodor-Kutzer-Ufer 1-3 68167 Mannheim, Germany Phone: +49 621 383 6020 Fax: +49 621 383 2476 E-Mail: frank.giordano@umm.de	**Kevin Petrecca, MD, PhD, FRCS(C), FAANS (Co-Chair)** Department of Neurology and Neurosurgery, Montreal Neurological Institute and Hospital, McGill University 3801 University Avenue, Montreal, Quebec, Canada H3A 2B4. Phone: +1 514 398 2591 Fax: +1 514 398 2811 E-mail: kevin.petrecca@mcgill.ca
Bahman Emami, MD, FACR, FASTRO (DSMB Chair) Departments of Radiation Oncology & Otolaryngology Loyola University Medical Center 2160 S. 1st Avenue Maguire Center 2944 Maywood, Illinois 60153, USA E-Mail: BEMAMI@lumc.edu	**Daniel Haenggi, MD** Department of Neurosurgery University Medical Center Mannheim University of Heidelberg Theodor-Kutzer-Ufer 1-3 68167 Mannheim, Germany Phone: +49 621 383 2360 Fax: +49 621 383 2004 E-Mail: daniel.haenggi@umm.de
Frederik Wenz, MD Department of Radiation Oncology University Medical Center Mannheim University of Heidelberg Theodor-Kutzer-Ufer 1-3 68167 Mannheim, Germany Phone: +49 621 383 4960 Fax: +49 621 383 2476 E-Mail: frederik.wenz@umm.de	**Grit Welzel, MSc** Department of Radiation Oncology University Medical Center Mannheim University of Heidelberg Theodor-Kutzer-Ufer 1-3 68167 Mannheim, Germany Phone: +49 621 383 4494 Fax: +49 621 383 2476 E-Mail: grit.welzel@umm.de
Bassam Abdulkarim, MD, PhD Division of Radiation Oncology McGill University Health Centre 974 Ste-Marguerite Montreal, Quebec H4C 2X8 Canada Phone: + 1 514 934 1934 Fax: + 1 514 934 8392 E-Mail: bassam.abdulkarim@mcgill.ca	**Pedro Lara, MD, PhD** Radiation Oncology Department University Hospital Gran Canaria Dr. Negrín, Barranco de La Ballena s/n 35010 Las Palmas de Gran Canaria Spain Phone: + 34 92 845 0284 Fax: + 34 92 845 9127 E-Mail: plara@dcc.ulpgc.es
N.N.[1]	**N.N.**[1]
N.N.[1]	**N.N.**[1]

[1] Four positions in the SC are available for PIs from study centers that qualify, e.g. by strong(est) patient recruitment. All new memberships must be approved by a bare majority of the current SC.

DATA SAFETY MONITORING BOARD (DSMB)

The Data Safety Monitoring Board (DSMB) is an independent panel of experts that periodically reviews clinical study data, incidental event reporting, and clinical study design.

The DSMB will meet once annually. Interim meetings and/or conference calls may be held at the request of DSMB members or the study leadership.

Members of the DSMB:

Bahman Emami, MD (Chair) Department of Radiation Oncology Loyola University Medical Center Maywood, IL, USA	**Luis Souhami, MD** Division of Radiation Oncology McGill University Health Centre Montreal, QC, Canada
Max Bulsara, PhD Institute of Health and Rehabilitation Research Notre Dame University Freemantle, Australia	**Martin Glas, MD** Division of Clinical Neuro-Oncology Department of Neurology University of Bonn Medical Center Bonn, Germany

WRITING AND PRESENTATION COMMITEE

The Writing and Presentation Committee has access to all study-related data arising from the design of the protocol until the final analysis. It will decide when and how information on end points of the trial will be disseminated. The committee will also decide on passing information to press, patient groups or patient advocacy organizations. The chairperson holds all mastercopies.

Details on the determination of the order of authorship are specified in the “Publication” section.

Members of the WPC:

Frank A. Giordano, MD (Chair) Department of Radiation Oncology University Medical Center Mannheim	**Kevin Petrecca, MD, PhD** Department of Neurology and Neurosurgery, Montreal Neurological Institute and Hospital, McGill University
Grit Welzel, MSc Department of Radiation Oncology University Medical Center Mannheim	**Frederik Wenz, MD** Department of Radiation Oncology University Medical Center Mannheim
N.N.[1]	**N.N.**[1]
N.N.[1]	

[1] Three positions in the WPC are available for PIs from study centers that qualify. All new memberships must be approved by a bare majority of the current WPC.

CENTRAL REVIEW

Central technical facilities to be involved in the conduct of the trial, e.g. laboratory or other technical facilities in which the measurement or assessment of the main evaluation criteria are centralized:

CENTRAL PATHOLOGY
(NEUROPATHOLOGY REVIEW)

Marie-Christine Guiot, MD
Montreal Neurological Institute and Hospital
Room 639
3801 University
Montreal, H3A 2B4
Quebec , Canada

CENTRAL RADIOLOGY
(NEURORADIOLOGY REVIEW)

Whitney B. Pope, MD, PhD
Department of Radiological Sciences
David Geffen School of Medicine,
University of California
757 Westwood Plaza 1621E
Los Angeles, CA 90095
USA
Phone: 310-267-9783

PARTICIPATING SITES (COUNTRIES IN ALPHABETICAL ORDER)

Canada
Montreal Neurological Institute and Hospital, McGill University, Montreal

Germany
Augsburg Medical Center, Augsburg
University Medical Center Mannheim, University of Heidelberg, Mannheim

Peru
National Institute of Neoplastic Diseases, Lima

Spain
University Hospital Dr. Negrin, Las Palmas de Gran Canaria
Catalan Institute of Oncology, University of Barcelona, Barcelona

United States of America
Barrow Neurological Institute, Phoenix, AZ
Cleveland Clinic, Cleveland, OH
Loyola University Medical Center, Maywood, IL
West Virginia University Hospital, Morgantown, WV

TABLE OF CONTENTS

PROTOCOL SYNOPSIS

TITLE:	A multicenter randomized phase III trial on **INT**raoperative **RA**diotherapy in newly diagnosed **G**li**O**blastoma multiforme (INTRAGO II)
SHORT TITLE:	IORT in GBM
SPONSOR:	Department of Radiation Oncology University Medical Center Mannheim University of Heidelberg
CLINICAL TRIAL CODE:	INTRAGO II
REGISTRATION	Clinicaltrials.gov ID: NCT02685605
OTHER CODES:	ARO-2016-1
PROTOCOL VERSION:	Version 2.1, dated 05/12/2016
INDICATION:	Newly diagnosed glioblastoma multiforme (WHO grade IV) [MedDRA: 10018337, ICD-10: C71]
OBJECTIVES:	Primary • Median PFS according to modified RANO criteria
	Secondary • Median OS • PFS within a 1 and 2 cm margin around the cavity determined by serial contrast-enhanced MRI scans using modified RANO criteria and serial perfusion imaging. • Outcome (PFS, OS) with respect to strata age, KPS, thickness of anticipated T1-Gd-enhancing (remaining) tumor margin and extent of resection (given as sum of all maximum diameters of residual lesions in cm) • Outcome (PFS, OS) with respect to molecular subgroups (e.g., MGMT, IDH1) • QoL / ADL • Radiation-related (acute / early delayed / late) neurotoxicity
STUDY DESIGN:	Randomized, controlled, open, 2-Arm, multicentric, phase III
INTERVENTION	Arm A (experimental): Standard surgery **plus intraoperative radiotherapy** (20-30 Gy) followed by radiochemotherapy (EBRT: 60 Gy, 75 mg/m^2/d temozolomide) and adjuvant chemotherapy with 150-200 mg/m^2/d temozolomide per cycle (5/28 days). Arm B (control): Standard surgery followed by radiochemotherapy (EBRT: 60 Gy, 75 mg/m^2/d temozolomide) and adjuvant chemotherapy with 150-200 mg/m^2/d temozolomide per cycle (5/28 days).
RANDOMIZATION	Patients will be 1:1 randomized into both arms. Stratified block randomization will be performed for each center. Strata are: • Age (<65 vs. ≥ 65 years) • Initial KPS (80-100% vs. 60-70%) • Thickness of anticipated T1-Gd-enhancing (remaining) tumor margin as per the discretion of the surgeon (margin ≥0.5 cm or multiple spots of residual tumor within the cavity vs. <0.5 cm)

STUDY POPULATION:	Inclusion Criteria 1. Age ≥18 and ≤ 70 years 2. Karnofsky Performance Score (KPS) ≥ 60% 3. Supratentorial T1-Gd enhancing lesion(s) amenable to total resection 4. Legal capacity and ability of subject to understand character and individual consequences of the clinical trial 5. Patient's written IC obtained at least 24h prior to surgery 6. For women with childbearing potential: adequate contraception 7. Patients must have adequate organ functions Bone marrow function: - Platelets ≥ 145.000/µL - WBC ≥ 4.000/µL - Hemoglobin ≥ 12.0 g/dL Liver Function: - ASAT and ALAT ≤ 1.5 times ULN - ALP ≤ 2.5 times ULN - Total Serum Bilirubin < 1 times ULN Renal Function: - Serum Creatinine ≤ 1.5 times ULN Inclusion Criteria Related to Surgery: 8. IORT must be technically feasible 9. Histologically confirmed (frozen section) GBM (WHO grade IV) Exclusion Criteria 1. Multicentric disease (e.g. in both hemispheres) or non-resectable satellite lesions 2. Previous cranial radiation therapy 3. Cytostatic therapy / chemotherapy for cancer within the past 5 years 4. Use of alternating electrical fields (e.g., Novo-TTF) 5. History of cancers or other comorbidities that limit life expectancy to less than five years 6. Previous therapy with anti-angiogenic substances (such as bevacizumab) 7. Technical impossibility to use MRI or known allergies against MRI and/or CT contrast agents 8. Participation in other clinical trials testing cancer-derived investigational agents/procedures. 9. Pregnant or breast feeding patients 10. Fertile patients refusing to use safe contraceptive methods during the study Exclusion Criteria Related to Surgery: 11. Active egress of fluids from a ventricular defect 12. In-field risk organs and/or IORT dose >8 Gy to any risk organ
SAMPLE SIZE:	157 patients per arm, 314 patients in total
TRIAL DURATION:	Total trial duration: 48 months Accrual: 24 months FU: 24 months FSI (First Subject In): Q3 2016

	LSI (Last Subject In): Q3 2018 LSO (Last Subject Out): Q3 2020 Analyses completed: Q1 2021
STATISTICAL ANALYSIS:	This study will detect a 50% improved median progression-free survival (from 9 to 13.5 months) with 80.2% (actual) power with a 1-sided p of 0.05. Considering a drop-out/loss to FU rate of 6.5%, the study must recruit 157 patients per arm, or 314 patients in total.
BIOMETRICAL METHODS FOR DESIGN AND ANALYSIS	A 1-tailed log-rank test with an overall sample size of 314 patients (157 in the IORT group and 157 in the control group) achieves 80.2% power at a 0.050 significance level to detect a hazard ratio of 0.667 when the median PFS in the observation group is 9 months. The study duration will be 4 years, whereas patient accrual (entry) occurs in the first 2 years. The proportion dropping out of the observation group is 6.5%. The proportion dropping out of the IORT group is 6.5%.

SCHEDULE

Table 1. Study Assessments and Visits

	Pre-Treatment	Treatment Phase						Follow-Up		Survival
Assessments	≤ 10 d prior to surgery/IORT	Surgery ± IORT	<3d post op	≤ 10 d prior to RCT	Radio-Chemotherapy		21-28d after EBRT	42-49d after EBRT	q90±10d thereafter	life-long after PD (quarterly)
					Week 1	Week 6				
Visit #	screening	1	2	3	4	5	6	7	≥8	N
Pre-op IC / EC	X									
Written informed consent	X									
Pregnancy test[1]	X									
KPS / ECOG	X				X	X	X	X	X	
PE	X				X	X	X	X	X	
CBC, basic chemistry	X			X[3]						
RFT, LFT	X									
Brain MRI +/- contrast	X[4]		X	(X)[5]				X[6]	X	
EORTC QLQ-C30 / BN20	X				X			X	X	
Barthel ADL index	X				X			X	X	
Basic neurological exam	X				X			X	X	
Intra-op IC / EC		X								
Histologic confirmation of GBM[7]		X								
Technical feasibility of IORT		X								
Randomization		X								
IORT with 20-30 Gy[8]		X[9]								

[1] Serum or urine pregnancy test in women of child bearing potential
[3] No study-specific requirement (part of the clinical routine).
[4] Planning MRI (for neuronavigation) should be performed <96 h prior to surgery
[5] Not mandatory
[6] Includes confirmatory MRI scan(s) in case of suspected PD
[7] Frozen section
[8] Prescribed to the applicator surface
[9] Experimental arm only

	Pre-Treatment	Treatment Phase						Follow-Up		Survival
Assessments	**≤ 10 d prior to surgery/IORT**	**Surgery ± IORT**	**<3d post op**	**≤ 10 d prior to RCT**	**Radio-Chemotherapy**		**21-28d after EBRT**	**42-49d after EBRT**	**q90±10d thereafter**	**life-long after PD (quarterly)**
					Week 1	**Week 6**				
EoR (sum of all max diameters of residual lesions in cm)			X							
Adverse events		X	X	X	X	X	X	X	X	(X)[1]
MGMT and IDH1 Status[2]				X						
Treatment planning CT with contrast				X						
EBRT (60 Gy) 5x/week					X					
daily (7d per week) TMZ 75 mg/m/d[2]					X					
cyclic TMZ 150-200 mg/m/d/cycle[2]							X			
Second line interventions and treatment(s)[3]										X
Vital Status										X

[1] After end of study, only ongoing SAEs and SAEs occurring up to 3 months later are followed. Thereafter, no SAEs except radionecrosis will need to be reported.
[2] No study-specific requirement. Centers are encouraged to assess these markers.
[3] Includes histology/pathology results after any second biopsy/surgery

ABBREVIATIONS

ACNU	Amino-Methyl-Chlorethyl-Nitroso-Urea (Nimustine)
ADL	Activities of Daily Living
ADR	Adverse Drug Reaction
AE	Adverse Event
ALA (5-ALA)	5-Aminolevulinic Acid
ANOCEF	Association des Neuro-OnCologues d'Expression Française
BCNU	Bis-Chlorethyl-Nitroso-Urea (Carmustine)
BTSG	Brain Tumor Study Group
BTCG	Brain Tumor Cooperative Group
CBC	Complete Blood Count
CCNU	Chlorethyl-Cyclohexyl-Nitroso-Urea (Lomustine)
CED	Convection-Enhanced Delivery
CSF	Cerebrospinal Fluids
CTC-AE	Common Terminology Criteria for Adverse Events
CTV	Clinical Target Volume
CV	Curriculum Vitae
CVLT	California Verbal Learning Test
DNA	Deoxyribonucleic Acid
DLT	Dose-Limiting Toxicity
EBRT	External Beam Radiotherapy
ECG	Electrocardiography
ECOG	Eastern Cooperative Oncology Group
eCRF	electronic Case Report Form
EoR	Extent of Resection
EORTC	European Organization for Research and Treatment of Cancer
EQD2	Equivalent Dose Delivered in 2 Gy per Fraction
EC	Ethics Committee
FET	Fluorethyl-L-Tyrosine
FLAIR	Fluid-Attenuated Inversion Recovery
FPI	First Patient In
FU	Follow-up
GBM	Glioblastoma Multiforme
GCP	Good Clinical Practice
GTR	Gross Tumor Resection
GTV	Gross Tumor Volume
ICH	International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICH GCP	ICH Harmonized Tripartite Guideline on GCP
IDH1	Isocitrate Dehydrogenase 1
IMRT	Intensity-Modulated Radiotherapy
IOERT	Intraoperative Electron Radiotherapy
IORT	Intraoperative (Photon) Radiotherapy

ISF	Investigator Site File
ITR	Incomplete Tumor Resection
ITT	Intention To Treat
KPS	Karnofsky Performance Status
LFT	Liver Function Tests
LINAC	Linear Accelerator
LPI	Last Patient In
LPO	Last Patient Out
MRI	Magnetic Resonance Imaging
MGMT	O6-Methylguanine-DNA Methyltransferase
MP-RAGE	Magnetization-Prepared Rapid Gradient-Echo
MTD	Maximum Tolerated Dose
NANO	Neurologic Assessment in Neuro-Oncology
NCF	Neurocognitive Functions
NCIC	National Cancer Institute of Canada
OAR	Organ at Risk
OR	Operating Room
OS	Overall Survival
PD	Progressive Disease
PDT	Photodynamic Therapy
PE	Physical Exam
PET	Positron-Emission Tomography
PFS	Progression-Free Survival
PTV	Planning Target Volume
QLQ-C30/BN20	Quality of Life Questionnaire Brain Module
RANO	Response Assessment in Neuro-Oncology
RBE	Relative Biological Effectiveness
RCT	Radiochemotherapy
RFT	Renal Function Tests
RTOG	Radiation Therapy Oncology Group
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SC	Steering Committee
SDV	Source Data Verification
SOP	Standard Operating Procedure
SRS	Stereotactic Radiosurgery
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMF	Trial Master File
TMZ	Temozolomide
TVC	Target Volume Coverage
ULN	Upper Limit of Normal
WBC	White Blood Cell
WHO	World Health Organization

1. INTRODUCTION

1.1. Scientific Background

1.1.1. Incidence and Standard Treatment of Glioblastoma Multiforme

Glioblastoma multiforme (GBM) resembles the most common primary brain tumor in adults. It is a rare disease with an incidence of 3 cases in 100.000 residents in Europe and the US (Porter et al, 2010). However, the peak incidence lies around 65 years and, in the light of an ageing population and improved therapy strategies for cardiovascular diseases, the number of patients diagnosed with GBM is expected to considerably rise over the next decades (Ostrom *et al*, 2013).

Although therapy improved with the introduction of the alkylating agent temozolomide, it to date still displays a highly lethal disease with a median progression-free survival (PFS) of 5-6 months and a median overall survival (OS) of 12-15 months (Stupp et al, 2009; Stupp et al, 2005). The currently accepted standard of care for GBM was defined by the pivotal EORTC/NCIC study in 2005 (Stupp et al, 2005). It consists of surgery, radiochemotherapy (involving a total radiation dose of 60 Gy) and the parallel application of temozolomide, followed by 6 cycles of adjuvant chemotherapy with temozolomide in a 5-day-on, 23-days-off scheme. GBM was treated with nitroseureas for decades and the remarkable improvement of overall- and long-term survival rates seen with temozolomide carried the excitement to eventually further improve treatment by intensifying its application. Although there was encouraging data from single-arm phase II trials on recurrent GBM (Perry et al, 2008; Tosoni et al, 2008; Wick et al, 2007), this hypothesis was rejected after a negative randomized phase III trial conducted by the Radiation Therapy Oncology Group (Gilbert et al, 2013).

1.1.2. Factors Influencing Survival

Age, clinical performance status, methylation of the O^6-methylguanine-DNA methyltransferase (MGMT) promoter and the extent of resection (EoR) have been consistently shown to be key prognostic factors in GBM (Gorlia et al, 2008; Lacroix et al, 2001; Lamborn et al, 2004).

Age

Roughly 50% of patients with GBM are 65 years and older (Ostrom *et al*, 2013). However, the EORTC-NCIC trial excluded patients older than 70 years (Stupp *et al*, 2005) and thus the treatment of GBM in elderly patients is to date controversially discussed. As evidence for a specific treatment is rare, decisions are mainly based on the nature and number of co-morbidities, medication or socio-economic situations (Iwamoto et al, 2008). It is therefore not surprising that either treatment modality is frequently omitted despite compelling data on the benefit (Barker et al, 2012; Brandes et al, 2009; Gerstein et al, 2010; Minniti et al, 2012; Noorbakhsh et al, 2014; Reyngold et al, 2012; Vuorinen et al, 2003). Even more, best supportive care (BSC)was a valid and widespread practice in elderly until the French ANOCEF trial showed that radiotherapy improves survival compared with supportive care alone (Keime-Guibert et al, 2007). In the following years, two trials (the NOA-08 and the NORDIC trial) suggested that survival after chemotherapy alone was non-inferior to that after radiotherapy alone (Malmstrom et al, 2012; Wick et al, 2012). It has to be mentioned that in the NOA-08 trial, there was a trend towards superior event-free survival with RT alone (Figure 1) and, as cross-over to other arm was allowed in case of progression, OS might have not been the adequate primary end point.

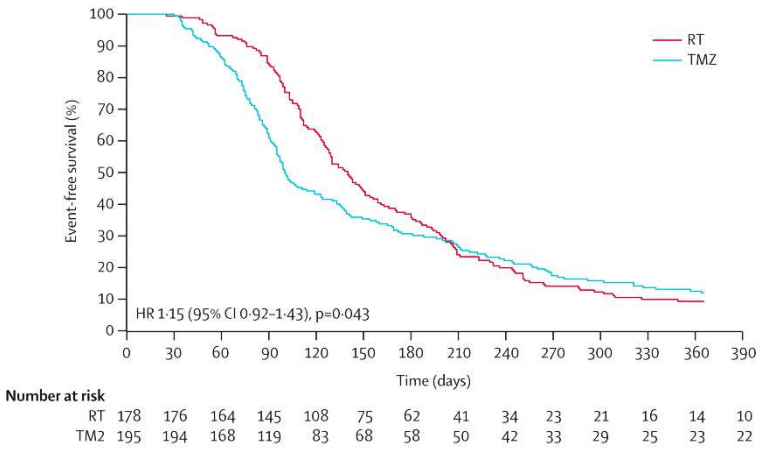


Figure 1. Event-free survival (non-proportional curves) of patients in the NOA-08 trial. Adapted from Wick *et al.* 2012.

MGMT

Approximately half of all GBMs show de-methylation of the MGMT promoter region with subsequent over-expression of the MGMT protein. Alkylators such as temozolomide induce DNA lesions and subsequent cellular apoptosis by alkylating (or methylating, respectively) the O^6-position of guanine (Roos et al, 2007). MGMT removes the methyl group by irreversibly transferring it into its active center (a functionality which is often referred to as "suicide" activity) and thereby saves the affected cell from persistent DNA damage and subsequent cell cycle arrest or apoptosis (Pegg et al, 1995). The peculiar biological property of tumors to counteract alkylator therapy by MGMT expression has immediate clinical consequences: methylation of the MGMT promoter (referred to as 'epigenetic silencing') is associated with improved survival (Hegi et al, 2005). Furthermore, approximately 14% of these patients may even survive for more than 5 years while in contrast, the portion of patients with promoter de-methylation surviving this period is about 8% (Stupp et al, 2009).

EoR

Although GBM grows highly invasive and the tumor cells are able to migrate along pre-existing structures such as blood vessels or white matter tracts (Holland, 2000; Scherer, 1940), most (if not all) tumors paradoxically recur in close proximity (2-3 cm) to the resection margin even after intensive radio- and chemotherapy (Choucair et al, 1986; Gaspar et al, 1992; Petrecca et al, 2013; Wallner et al, 1989). Thus, although novel surgical techniques (such as fluorescence-guided resection) may have improved the rates of *macroscopic* complete resections (Stummer et al, 2006), no single or combined approach is to date sufficient to deplete *microscopically* dispersed tumor cells around the tumor cavity (Orringer et al, 2012).

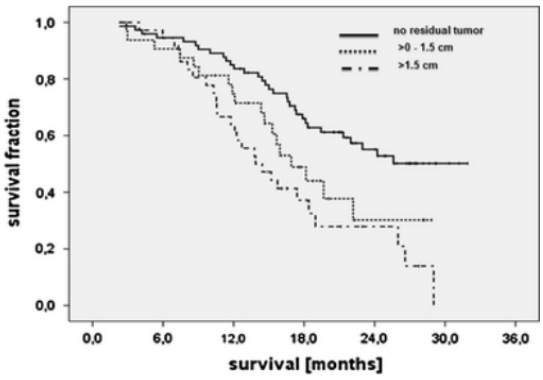


Figure 2. Overall survival of patients with various residual tumor volumes. Adapted from (Stummer et al, 2012)

More than a decade ago, a retrospective analysis on more than 400 patients with GBM suggested that at least 98% of the tumor have to be removed to achieve a statistically significant effect on survival (Lacroix et al, 2001). This figure was later challenged and a much lower threshold of 78% was proposed (Sanai et al, 2011).

Prospective data on the EoR is available from a subgroup analysis of patients from the 5-ALA study (Stummer et al, 2012). These analyses showed that patients with only minimal residual tumor volume (i.e., as little as a single T1 contrast-enhancing voxel in a postoperative MRI

scan) show dramatically OS impairment (median OS with no residual tumor was 24 mo; with diameters >0 - 1.5 cm: 17 mo and 14 mo with more than 1.5 cm; p<0.0001; Figure 2).

However, any increase in the EoR has to be balanced against the risks to irreversibly cause new neurological deficits. A common rule of thumb is that for each new neurological deficit that is introduced by resection, the expected survival time decreases by approximately 3 months (McGirt et al, 2009). Thus, the current understanding of surgery in the setting of GBM follows a "maximum safe resection" attempt with providing as much cytoreduction as possible but at the same time accepting to leave (e.g. eloquently located) portions of the tumor untouched for the sake of neurological functions (Marko et al, 2014).

1.1.3. Local therapy intensification approaches – EBRT dose escalation

To tackle the high rate of macroscopic and microscopic incomplete resections, which subsequently lead to local failure in almost every case, several strategies involving local therapy intensification have been evaluated. The probably most practical approach was to apply higher EBRT doses to the involved field, as there is an evident dose-response correlation in GBM (Table 2).

Table 2. Studies evaluating EBRT dose escalation

Study	N of Pat	RT Dose	Median OS	Significance
BTSG[1)]	194	No RT	4.5 m	**Low doses p=0.003**
	61	</= 45 Gy	3.9 m	**50 vs. 60 Gy p=0.004**
	56	50 Gy	7.0 m	
	33	55 Gy	9.0 m	
	270	**60 Gy**	**10.5 m**	
MRC[2)]	144	45 Gy	9 m	**p=0.007**
	299	**60 Gy**	**12 m**	
Michigan[3)]	34	90 Gy	11.7 m	
Tokyo[4)]	60	60 Gy	12.4 m	**80-90 vs. 60 Gy p=0.014**
	48	80 Gy	16.2 m	
	13	**90 Gy**	**19.6 m**	
ROTG 9803[5)]	55	66 Gy	11.6 m	
	46	72 Gy	11.8 m	
	62	78 Gy	11.8 m	
	40	**84 Gy**	**19.3 m**	

1. Walker MD *et al.*, Int J Radiat Oncol Biol Phys. 1979
2. Bleehen NM *et al.*, Br J Cancer. 1991
3. Chan JL *et al.*, J Clin Oncol 2002
4. Tanaka M *et al.*, Lancet Oncol. 2005
5. Tsien C *et al.*, Int J Radiat Oncol Biol Phys. 2009

One of earliest landmark studies that suggested dose dependency was conducted by the Brain Tumor Study Group (BTSG) in 1979 (Walker et al, 1979). Within this study, more than 600 patients with high-grade gliomas were allocated to different doses of EBRT (up to 60 Gy). It turned out that patients receiving less than 45 Gy had the worst median survival (4.5 months) and escalation of the dose lead to a significant survival increase (7 months in the cohort receiving 50 Gy *vs*. 10.5 months in the cohort receiving 60 Gy, p=0.004). Similar effects were seen in the MRC trial in 1991, where 144 patients received 45 Gy and 299 patients 60 Gy. Here, dose escalation also lead to a statistically significant increase in median survival (9 vs. 12 months; p=0.007) (Bleehen & Stenning, 1991).

The question then was whether or not doses above the commonly applied 60 Gy will still increase survival. This was tested by two non-randomized trials: one prospective study conducted by the RTOG (trial 9803) and one retrospective analysis by a Japanese group from

Tokyo. The RTOG 9803 study investigated if the application of higher EBRT doses above the commonly applied 60 Gy are tolerated and efficient if applied with (3D-) conformal radiotherapy. In this trial, patients treated with 66 Gy EBRT showed the worst (11.6 months) and those that were receiving 84 Gy showed the best survival rates (19.3 months) (Tsien et al, 2009).

The Japanese group analyzed outcomes of 121 patients with glioblastoma, whereas 61 patients received high-dose (80-90 Gy) and 60 conventional (60 Gy) EBRT. They saw that patients receiving 80-90 Gy had significantly longer overall survival rates compared with those who received conventional EBRT (16.2 vs. 12.4 months; p=0.014), whereas survival did not differ between the cohorts receiving 80 Gy or those treated with 90 Gy (Tanaka et al, 2005).

Although the RTOG trial did not report elevated rates of brain toxicities 'high-dose' EBRT to large treatment volumes (mostly including the edema around the tumor) does not only bear a high risk of inducing acute side effects such as nausea, vomiting and fatigue, but also causes long-term neurocognitive deficits (Aoyama H, 2007; Giordano et al, 2012; Goetz P, 2012; Sun A, 2011). Thus, although dose intensification may be beneficial, the corresponding treatment volumes may need to be considerably narrowed down.

1.1.4. Local therapy intensification approaches – SRS

With the advent of sub-millimeter precision in the application of high single doses (e.g. by using LINAC-based, Gamma Knife®, X-Knife® or CyberKnife® radiosurgery), the question arose whether or not stereotactic radiosurgery (SRS) may add benefit to the outcome in GBM. Although several retrospective mono-institutional studies have revealed promising outcome of a SRS boost following EBRT (Kondziolka et al, 1997; Loeffler et al, 1992; Nwokedi et al, 2002; Shrieve et al, 1999) a randomized phase III study conducted by the RTOG (RTOG 9305) showed no benefit (although in contrast to the retrospective studies mentioned, the RTOG protocol required the application of the boost within 5 weeks after surgery and 1 week prior to EBRT) (Souhami et al, 2004).

RTOG 9305 included patients with postoperatively visible contrast-enhancing lesions (the maximum diameter of the lesion had to be below 4 cm). 89 patients received an upfront SRS boost (15-24 Gy) and radiochemotherapy (60 Gy + carmustine/BCNU) and 97 patients of a control arm received radiochemotherapy only (60 Gy + carmustine/BCNU). It turned out that local relapse was still the predominant failure pattern despite SRS (>90 % of patients in both arms) and that survival in both arms did not significantly differ (13.5 vs. 13.6 months; p=0.57).

1.1.5. Local therapy intensification approaches – Chemotherapy

The application of local chemotherapy was tested either with implantable biodegradable polymers (wafers) or by flushing the cavity using convection-enhanced delivery (CED). Both options bypass the blood-brain barrier and directly expose remaining tumor cells and health brain tissue to high concentrations of cytotoxic chemotherapeutic agents or recombinant proteins.

Wafers are small polymer discs that slowly (within 3-6 weeks) decay and thereby release an alkylating agent (carmustine/BCNU) into the cavity (Sampath & Brem, 1998) thus allowing high local levels of a cytostatic drug without the need for the substance to cross the blood-brain barrier and with (almost) no systemic effects (Fleming & Saltzman, 2002). Wafers carried a "wave" of excitement when improved survival rates were reported from a randomized study that included patients that underwent re-operation for recurrent glioma (Brem et al, 1995). However, as the impact on GBM (the study included AA and GBM) was marginally improving survival in the primary setting and elevated complication rates (including infections, wound healing abnormalities, CSF leak) were reported, their actual role is to date controversially discussed (Bregy et al, 2013; Gutenberg et al, 2013).

For CED, transcranial catheters are stereotactically placed in the tumor cavity and a cytotoxic substance is delivered by extracranial pumps with constant flow (Debinski & Tatter, 2009). Although the concept of CED appears sound and rational and a randomized study found it to be equally effective as wafers (Kunwar et al, 2010), it bears a high risk (~20%) of unwanted intracerebral distribution of the delivered substances (Varenika et al, 2008). Thus, the experience with CED is preliminary and it has to date no role in the treatment of brain tumors.

1.1.6. Local therapy intensification approaches – Brachytherapy

Another approach to curb local tumor recurrence involved brachytherapy by temporary or permanent implantation of radioactive sources into the tumor or the resection cavity. Specifically the radioisotope ^{125}I, which is emitting low-energy (28 keV) photons carried the excitement of enabling high-dose irradiation of the resection cavity while sparing deeper tissue. On the basis of a variety of single-center studies that were showing encouraging results (Gutin et al, 1984; Halligan et al, 1996; Malkin, 1994; Mundinger et al, 1991; Scharfen et al, 1992), two randomized trials have been initiated to test whether or not this approach is beneficiary to patients with high-grade gliomas:

The first study that shall be mentioned here was conducted by a collaborative group from Toronto (PMH and TGH) from 1986 to 1996 (Laperriere et al, 1998). In this trial, 71 patients received postoperative EBRT followed by stereotactical implantation of iodine seeds and adjuvant chemotherapy. In the control arm, 69 patients were treated with postoperative EBRT and chemotherapy. This study could not detect a statistically significant difference in median survival rates in both treatment arms (13.8 and 13.2 months; p=0.49). It is noteworthy that nearly a quarter of patients treated with brachytherapy experienced significant complications and the dexamethasone doses in the experimental arm were twice as high as in the control arm.

The second study was conducted by the Brain Tumor Cooperative Group (BTCG). The study randomized patients with mostly unresected (84% biopsy only) high-grade gliomas (85% GBM) to either the control arm with surgery or biopsy, followed by EBRT and BCNU chemotherapy (n=137) or to the experimental arm with surgery or biopsy, followed by stereotactic implantation of ^{125}I seeds, EBRT and BCNU chemotherapy (n=133) (Selker et al, 2002). The median overall survival appeared to be better in the treatment arm with additional brachytherapy boost (17 mo) than in the control arm (14.7 mo), but the difference did not reach statistical significance (p=0.101).

Although both studies were performed in a multicentric and randomized fashion, the outcomes must be interpreted with caution due to major conceptual and technical shortcomings:

First, the Toronto protocol foresaw randomization *before* EBRT and the application of brachytherapy *after* EBRT. This timing not only lead to high complication rates, but also to a high rate of protocol violations as only 63 of 71 patients (89%) assigned to the experimental arm actually underwent brachytherapy after EBRT. Eight patients experienced either progression during EBRT or died of comorbidities but had to be included in the intent-to-treat analysis. When looking at the outcomes of actually implanted patients only (“as treated”), there was a clear trend for improved survival for the implant arm (p=0.07).

Second, both the Toronto and the BTCG protocols initially required EBRT to be applied with a total dose of 50 Gy, which, in the light of a biopsy-only ratio of 84% is very likely hardly sufficient to add any significant benefit at all (see above) and thus a relatively weak basis for a prospective clinical trial. However, while the Toronto group did not amend the protocol, the BTCG increased the dose to 60 Gy after enrolment of 64 patients.

Third, all tumor evaluations and stereotactical implantation procedures were performed on the basis of (at that time contemporary) postoperative contrast-enhanced CT scans, which is far from the accuracy, sensitivity and specificity modern high-resolution modalities bring along. This

together with the (still) relatively challenging implantation procedure of ^{125}I seeds (the Toronto group did not even use 3D-planning) bears a very high risk of ending up with considerable dose inhomogeneities and inadequate target volume coverage.

1.1.7. Local therapy intensification approaches – IORT

Similarly to brachytherapy, local radiation dose escalation can be achieved by intraoperative radiotherapy (IORT). In the early 1980s, in a time far before the era of neuronavigation, temozolomide, intensity-modulated radiotherapy (IMRT) and MRI- or PET-based diagnostics, successful application of IORT with electron beams (IOERT) on patients with supratentorial gliomas was reported (Abe et al, 1971; Goldson et al, 1982; Goldson et al, 1984; Matsuda, 1983; Matsutani, 1989; Matsutani et al, 1984). Since then, several small-sized, non-randomized institutional studies were published, many of which revealed encouraging results (Fujiwara et al, 1995; Gouda et al, 1997; Matsutani et al, 1994; Nemoto et al, 2002; Ortiz de Urbina et al, 1995; Sakai et al, 1991; Sakai et al, 1989; Schueller et al, 2005; Shibamoto et al, 1994; Wagner et al, 1995).

The most promising data was seen in the early Japanese trials. Sakai *et al.* published results of a study on 11 patients with GBM that received 15-50 Gy IOERT prescribed to 1-2 cm beneath the tumor cavity (referred to as “depth”), followed by 50 Gy of EBRT (Sakai et al, 1989). Although EBRT was underdosed (see above), they reported 1- and 2-year survival rates of 80 and 50% without increased rates of adverse effects - even not at the highest doses (six patients received IOERT multiple times). A second paper was published two years later and included 26 patients with GBM, whereas all patients also received adjuvant radiochemotherapy and were followed for at least 12 months (Sakai et al, 1991). Here, IOERT provided a significant survival benefit compared to a control group (22.4 vs. 15.9 months, $p<0.01$). Of note, 14 of these patients received IOERT multiple times (after recurrence) and, although follow-ups were based on low-sensitivity CT imaging, the group did not report serious complications (such as brain necrosis).

Similar mesmerizing results were reported by Matsutani and colleagues, who treated 30 GBM patients with IOERT (median dose 18.3 Gy, 20 MeV) and EBRT (mean dose 58.5 Gy). They reported a median overall survival of roughly 30 months and a 2-year survival rate of 61% (Matsutani et al, 1994). Interestingly, the median survival of patients with extensive postoperative (radio) necrosis was reported to be even significantly longer compared to patients with no necrosis.

Fujiwara et al. conducted a study that included 20 patients (10 primary GBM, 1 recurrent GBM, 7 anaplastic and 2 low-grade astrocytomas), which were treated with IOERT at doses of 20 or 25 Gy (prescribed to a depth of 2-3 cm, energies: 6-10 MeV), followed by (therapeutically insufficient) 50 Gy EBRT and chemotherapy (ACNU/cisplatin) (Fujiwara et al, 1995). The median survival turned out to be significantly better with IOERT (14 vs. 10 months of a historical in-house control).

The group of Felipe Calvo and his colleagues from Pamplona published the first European data on IOERT for malignant brain tumors. They applied IOERT (10-20 Gy, 6-20 MeV) in a broad variety of tumors, including several cases of primary and recurrent high grade gliomas. Especially the outcome in the recurrent setting was remarkable: nearly half (47%) of the patients treated lived 18 months or longer, the median time to progression and the median overall survival were 11 and 13 months, which was considered to be highly superior to any other salvage therapy available at this time (Calvo et al, 1989; Ortiz de Urbina et al, 1995; Sierrasesumaga et al, 1989).

The encouraging data from Japan and Spain could not be reproduced in the following years, neither by another Japanese (Nemoto et al, 2002) nor a German IOERT study conducted by the group of Norman Willich (Schueller et al, 2005). Nemoto and colleagues included 21 patients

with GBM and applied IOERT with doses of 12-15 Gy followed by EBRT with a median dose of 60 Gy. They found no significant difference in the survival rate between IOERT -treated patients and a historical control (Nemoto et al, 2002). The German study reported on outcomes of 45 patients with GBM that were treated with IOERT at doses of 15-25 Gy followed by 60 Gy of EBRT. Although both, the median and the 1-year survival rate were slightly in favor for IOERT, there was no statistical difference detectable that would prove the efficiency of the procedure (Schueller et al, 2005).

However, both study teams faced either logistical (the Japanese group transferred patients from the OR to the LINAC rooms) or technical challenges which they suspected to contribute to non-superiority of combined IOERT/EBRT over EBRT only. Consequently, the German group established methods for postoperative dose reconstruction and recognized that a significant amount of included patients exhibited areas of inadequate target volume coverage (TVC). The predominant reasons were wrongly selected electron energies (mostly too low), inappropriate cone sizes (mostly undersized) and angle errors (Figure 3). As expected, they found that patients with adequate TVC showed a significantly improved median survival and 2-year survival rate as compared to patients with inadequate TVC (15.2 vs. 9.3 months and 9.3% vs. 0%, P=0.02) (Schueller et al, 2005; Schueller et al, 2008).

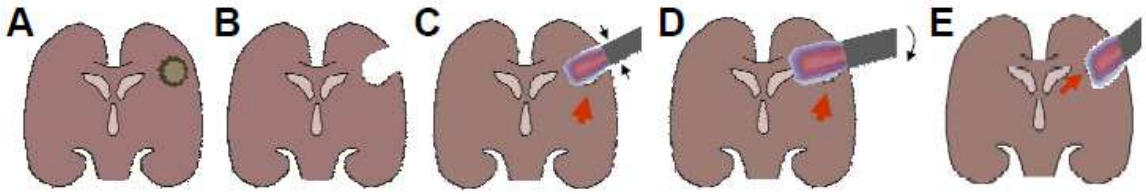


Figure 3 Graphical illustration of possible technical limitations of electron-based intraoperative radiotherapy (IOERT). (A) This graph schematically depicts a mass in the parietal lobe of the left hemisphere and (B) shows the remaining tumor cavity after (subtotal) tumor removal (surgery). (C,D) Depict examples for incomplete target volume coverage due to irradiation with an inadequate electron cone size (C), due to angle errors (D), or inadequately low energies (E). The red arrows depict areas that are at risk for underdosage after IOERT.

1.1.8. IORT with the INTRABEAM-System

The INTRABEAM® system (PRS 500 / XRS4; Carl Zeiss Meditec AG, Germany) was initially developed for interstitial radiosurgery of brain tumors before stereotactic (external) radiosurgery (or stereotactic ablative radiotherapy) entered the stage. In the setting of interstitial radiotherapy for brain metastasis (applied doses were 10-20 Gy in 2 mm depth), INTRABEAM achieved local control in up to 80% of cases with delayed necrosis appearing in less than 5% (Curry et al, 2005; Di Lorenzo et al, 2004; Gallina et al, 2002). These interstitial irradiations were performed with a needle-shaped applicator that was mounted on a stereotactic frame and inserted into the center of a mass without prior resection. In the breast cancer setting, IORT is delivered by the use of spherical plastic applicators, which are fitted into the tumor cavities in the conserved breast. Consequently, in contrast to the previously used forward-beaming electron cones, spherical irradiation sources are specifically attractive in brain tumor IORT, where post-resection cavities are normally of complex shape (Figure 4).

Logically, even at a time before the actual launch of the TARGIT trial, IORT using spherical applicators has been assessed in adult primary brain tumors: Takakura and Kubo intraoperatively irradiated 55 high-grade gliomas with the applicators and saw 2-year survival rates of 89% and 42% for patients with anaplastic astrocytoma or GBM, which was at that time superior to the control rates from the Japanese tumor registry (77% and 21%) (Takakura & Kubo, 2000).

The putatively most widely recognized paper reporting on outcomes after IORT for brain tumors with the INTRABEAM applicators was published by John Kalapurakal and his colleagues from Chicago's Northwestern Memorial Hospital. The group treated 14 children (4-21 years of age) with recurrent primary brain tumors (mostly ependymomas, n=13) with 10 Gy in 2 and 5 mm depth or 12 Gy in 2 mm depth) using spherical applicators (Kalapurakal et al, 2006). With a median follow-up of 6 months (range, 5-40 months) the group had three cases of radiation necrosis and all were occurring in previously unirradiated patients that were treated at the higher dose level (10 Gy in 5 mm depth). Although none of these patients died and all remained asymptomatic after treatment (2 were treated conservatively, 1 required surgery), the group decided to stop the dose escalation and limit the dose to 10 Gy in 2 mm depth. As no other late toxicity appeared and local control was achieved in 8 out of 14 patients (57%) with the best response observed in previously unirradiated tumors (local control was achieved in 5 of 6 patients; 83%), IORTs with INTRABEAM applicators can be judged as feasible and safe procedures even in children, where brain tissue is most sensitive to irradiation (Moore, 2005; Mulhern & Palmer, 2003).

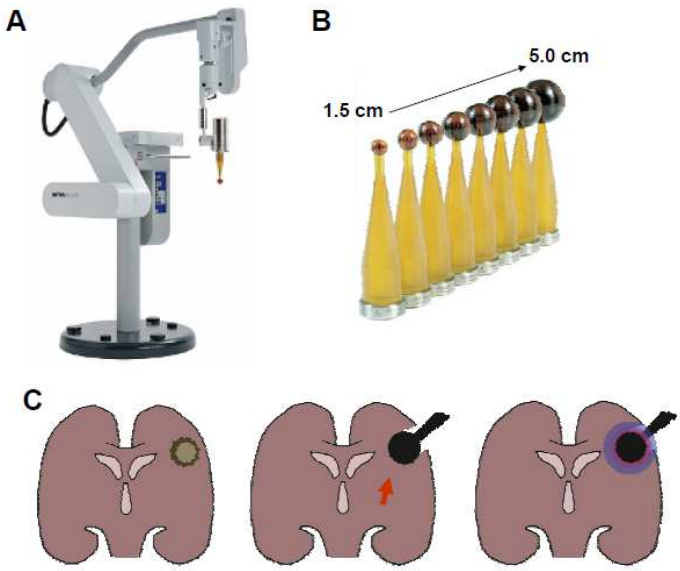


Figure 4. IORT with spherical irradiation sources. (A) The INTRABEAM System (Carl Zeiss Meditec AG, Oberkochen, Germany) consists of a floor stand with a mounted X-ray source. (B) Five applicators, ranging from 1.5 to 5 cm in diameter can be placed on the source. (C) shows how the system may be used to deliver IORT after surgery in the setting of GBM.

The most recent data that reported on the use of spherical applicators in patients with GBM was contributed by a group of the University of Dundee, which assessed whether IORT could improve the effects of photodynamic therapy (PDT) (Lyons et al, 2012). PDT makes use of photosensitizers that selectively enrich in GBM cells, allowing irradiating the tumors (or the remaining tumor tissue) with lasers, thereby exciting the sensitizer in a way that singlet oxygen is produced causing subsequent cellular damage (Eljamel et al, 2008; Stylli & Kaye, 2006). The group treated 73 patients with standard therapy (ST), which was unfortunately not further elucidated, ST and PDT, ST and IORT (mean dose 11.1 Gy prescribed to 0.5 cm depth) or ST, IORT and PDT. Although they saw no statistically significant benefit of IORT alone, the combined application of IORT and PDT almost doubled the median survival compared to PDT alone (79 vs. 39.7 months).

1.2. Trial Rationale/ Justification

1.2.1. Significance of local therapy

Almost ten years after the pivotal EORTC/NCIC study on the systemic use of temozolomide was published, only very little progress was made in improving the treatment of primary GBM. A variety of studies testing novel systemic therapies interfering with intra- and extracellular signaling cascades, adhesion molecules or intratumoral angiogenesis that were all believed to play key roles in GBM cells have failed (Chinot et al, 2014; Gilbert et al, 2014; Stupp et al, 2014). In turn, strategies directed at the removal of residual tumor cells in the tumor cavity indicate a more prominent significance of local control in GBM (Stummer et al, 2006). It is likely

that in GBM, as in many tumor diseases, the application of a more intensive initial local therapy is an indispensable prerequisite for preventing dissemination.

1.2.2. Waiting time to adjuvant therapies influences survival

Although the 'maximum safe resection' approach may be in sum beneficiary it is doubtless that it leaves an occultly populated battlefield. Thus, any delay in starting adjuvant therapies results in inevitable re-growth of the tumor, making subsequent therapies more or less incapable of controlling or removing the residual tumor burden. Consequently, a variety of studies have suggested that the waiting time for EBRT correlates with overall survival in GBM (Burnet et al, 2006; Do et al, 2000; Irwin et al, 2007). This is altogether not surprising since the mean doubling time of an untreated (residual) mass is little more than two weeks (Hoshino et al, 1992; Wang et al, 2009).

1.2.3. Evidence for effects on non-irradiated tumor cells

One might argue that GBM is highly invasively growing and, in many cases, already occultly disseminated at the time of diagnosis (Sahm et al, 2012), which logically would render many additional local therapies insignificant. This might hold true for all strategies with no direct or indirect systemic effects. IORT, in contrast, delivers extraordinarily high radiation doses in a narrow space of time, which not only causes instantaneous sterilization of the treated volume, but also elicits a variety of newly discovered effects on non-irradiated cells that are located *adjacent* (bystander effect and cohort effect) and *distant* (abscopal effects) to the initial tumor site (Herskind & Wenz, 2014; Park et al, 2014). Specifically the discovery of the prominent role of the immune system is tempting to believe in achieving distant tumor control with local therapy. Clinically, there is long standing evidence for systemic abscopal effects after high-dose radiotherapy as the multiple applications of radiosurgery in metastasized malignancies has not only been reported to achieve outstanding local, but also systemic control rates (Cheung et al, 2014; Tree et al, 2013).

1.2.4. High single doses deplete endothelial cells

The sprouting of endothelial cells into tumors, commonly referred to as angiogenesis, is a long standing target of anticancer therapy (Folkman, 1971). Solid tumor xenografts in mice with radiation-resistant endothelial cells are less sensitive to radiation than tumors grown in wild-type mice with radiation-sensitive endothelium, proving that endothelial cells can protect stem cells and tumor cells from radiation damage (Garcia-Barros et al, 2003; Paris et al, 2001). While conventionally fractionated radiotherapy (with single doses ranging from 2-3 Gy) largely preserves the intratumoral vasculature, radiosurgery with single doses larger than 10 Gy induces endothelial apoptosis and leads to thrombus formation in surviving vessels (Park et al, 2012; Schneider et al, 1997), which efficiently cuts off tumors from the blood supply. Of note, mathematical modeling suggests that endothelial apoptosis make up 20-30% of the efficiency of radiosurgery (Kocher et al, 2000). Thus, as GBM are well vascularized and characterized by excess endothelial proliferation, the application of high-doses in the treatment of GBM may be peculiarly promising.

1.2.5. Timing: an upfront boost may prevent a pro-proliferative microenvironment in the cavity

Several boost trials including the RTOG 9305 or the brachytherapy trials have failed to improve local control rates (see above). We believe that the major underlying cause for this lies in an inadequate timing of the application of the boost.

There is compelling evidence that any wounded site (such as a tumor bed) creates a specific stimulatory environment to promote healing, which inadvertently provides remaining cancer cells with strong pro-proliferative, pro-migratory and anti-apoptotic stimuli (Fisher et al, 1989; Tsuchiya et al, 2003). This has been peculiarly shown in breast cancer, where a variety of growth factors are released into the cavity if no boost was applied (Belletti et al, 2008). In turn, this unwanted response of the injured microenvironment was efficiently abrogated by upfront boosting with IORT (Belletti et al, 2008).

Table 3. Local failure rates with respect to timing of IORT in the TARGIT trial.

	Local recurrence rate	p value
IORT in initial surgery		
EBRT (Control)	1.1 %	0.31
IORT	2.1 %	
IORT in second surgery		
EBRT (Control)	1.7 %	**0.069**
IORT	**5.4 %**	

Most importantly, this had direct consequences on the clinical outcome on patients within the TARGIT trial: the cohort that received IORT in a second (sequential) operation, i.e. after the first surgery confirmed the diagnosis ('post-pathology' cohort) showed higher local recurrence rates (5.4%) compared with patients that received IORT during first surgery (2.1%; 'pre-pathology' cohort) (Table 3) (Vaidya et al, 2014).

Whether or not traumatic brain injury has a similar strong influence on GBM cell proliferation post-surgery as observed in breast cancer has not been elucidated yet. However, as the injured brain is very well known to respond with an impressive cytokine cocktail that efficiently promotes astrocytic activation and proliferation (Goodman et al, 2008; Hutchinson et al, 2007; Schultzberg et al, 2007), we believe that a similar (and, regarding survival, likely highly beneficial) quenching of the pro-proliferative tumor microenvironment is achievable with IORT.

1.2.6. Improved target volume coverage using geometry-adapted irradiation

As outlined above, target volume coverage was a key determinant of efficiency in brachytherapy and IOERT (see above). Thus, in order to achieve adequate coverage, geometry-adapted irradiation is mandatory. The INTRABEAM® system used for IORT in the INTRAGO II trial makes use of a spherically irradiating source which may be equipped with applicators of various sizes (see above).

1.2.7. Safety and evidence for high efficiency of the approach

The suggested benefits of IORT, namely instant sterilization of the tumor cavity at the time of resection, the creation of an anti-proliferative environment in the treatment-free time between surgery and adjuvant therapy and the eventual induction of systemic anti-tumor response are countered by the risk of side effects. IORT using INTRABEAM has been proven feasible in pediatric (Kalapurakal et al, 2006) and adult primary brain tumors (Lyons et al, 2012; Takakura & Kubo, 2000). However, as there were no prospective data on safety and tolerability of IORT, we have recently initiated INTRAGO I/II, a phase I/II study built on the experience from past trials in several of which proof-of-principle has been demonstrated. INTRAGO I/II is a prospective, open-label, single-arm dose-escalation study (NCT02104882) (Giordano et al, 2014). Dose escalation is conducted in a "classical" 3+3 manner with three patients entering each dose level. The primary goal is to determine the maximum tolerated dose (MTD), which was assessed on the basis of pre-defined dose-limiting toxicities (DLT).

Two types of DLT were defined in the trial: early and early-delayed DLTs. Early DLTs (≤ 3 weeks after IORT) were wound infections or wound healing difficulties requiring surgical

intervention and IORT-related cerebral bleeding or ischemia. Early-delayed DLTs (≤ 3 months after IORT) were defined as symptomatic brain necrosis requiring surgical intervention or the need for early termination of EBRT before the envisaged dose of 60 Gy due to radiotoxicity. DLTs were assessed on the basis of clinical presentation (physical examination, KPS, current medication), imaging studies (MRI) and on the basis of a neurological assessments using the Late Effects in Normal Tissues Subjective, Objective, Management and Analytic (LENT-SOMA) scales defined by the EORTC/RTOG (Pavy et al, 1995a; Pavy et al, 1995b).

At the time of finalization of this protocol, the trial has not established the MTD. A total of 7 patients have received IORT so far, 4 have been treated with 20 Gy and 3 patients with 30 Gy to the applicator surface. Due to absence of DLT at this escalation level, the dose has been further escalated to 40 Gy.

To get a first image of general efficiency of the approach, an interim analysis of the patients included in the INTRAGO trial (n=7) and all patients that were offered to receive IORT in compassionate use off trial (n=3) prior to initiation of INTRAGO I/II has been performed. The median age of the cohort was 65 years, 8 patients (80%) received incomplete resections or partial biopsies, 3 (30%) showed satellite lesions at the time of diagnosis and 1 patient entirely refused to undergo any other adjuvant therapy.

Despite these unfavorable characteristics, almost all resection cavities that contained residual T1-enhancing lesions remain stable (Figure 5 exemplary shows a patient with a significant amount of residual tumor and freedom of disease for more than 12 months).

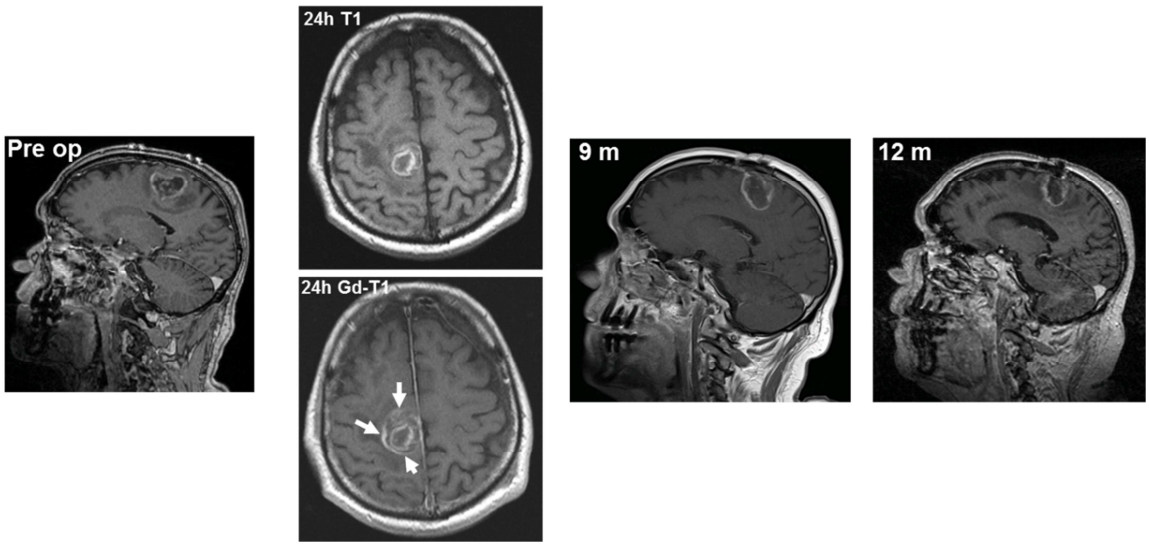


Figure 5. T1-weighted imaging studies of a patient with residual tumor after IORT. This patient is a 63 year old male presenting with a right frontal lobe GBM (methylated MGMT promoter). Multiple areas with residual tumor (white arrows) were seen in a postoperative T1-MRI scan without (upper) and with (lower) contrast. Despite considerable amounts of residual tumor after surgery and IORT, there is no recurrence within 12 months.

Head-to-head (intra-patient) comparisons of IORT plus standard-of care versus standard-of-care alone are possible where the cumulated amount of residual lesions targeted by IORT and RCT (e.g. in and around the cavity) is roughly equal (or higher) than the volume of lesions that are targeted by RCT alone (e.g. satellites). INTRAGO I/II included patients with satellites (only multifocality was excluded) and Figure 6 exemplary shows imaging studies of a pre-central GBM and a dorsal satellite. Despite a significant amount of residual tumor in close proximity to the cavity (treated by IORT with 20 Gy), progression was limited to the (unresected) dorsal satellite that was fully covered by EBRT.

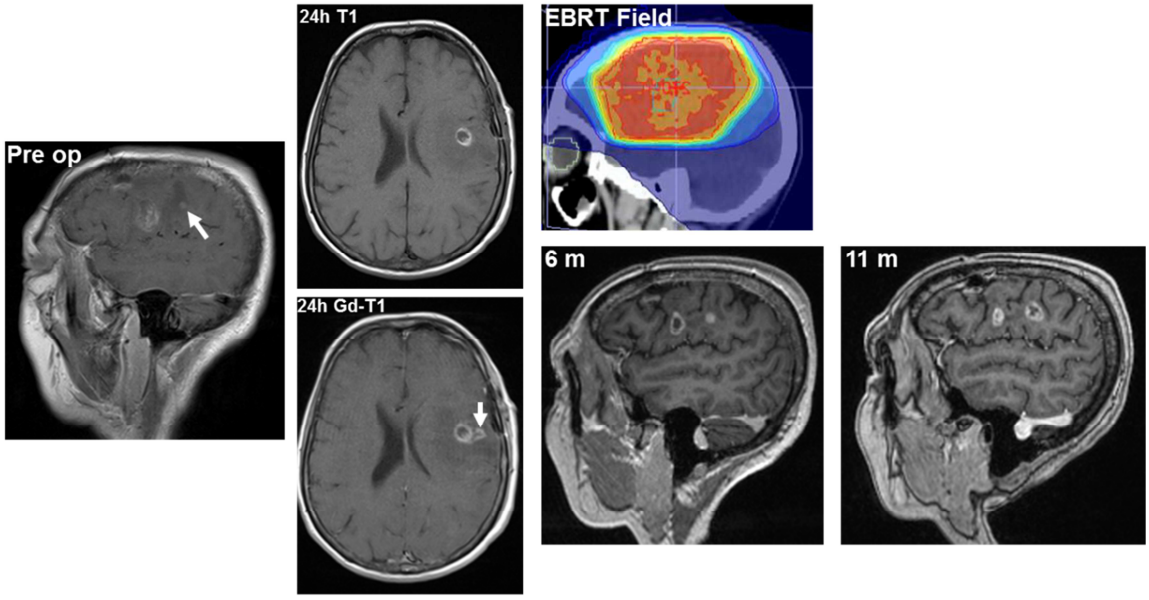


Figure 6. T1-weighted imaging studies of a patient with a satellite lesion. Shown are images of a 65 year old female patient with a left hemispheric/pre-central GBM (methylated MGMT promoter). A considerable amount of residual tumor (white arrow) after resection an IORT (20 Gy surface dose) was seen in a postoperative T1-MRI scan without (upper) and with (lower) contrast. Although the cumulated volume of the residual tumor exceeds the volume of the satellite (which was included in the EBRT field), only the dorsal satellite grew under adjuvant chemotherapy (cycling temozolomide).

Distant failure is an extremely rare event in GBM. Figure 7 shows a series of images of a patient with an incompletely resected (dural enhancement) left-temporal GBM with unmethylated MGMT promoter. This patient experienced progressive disease with distant failure 16 months after initial therapy – resulting in a PFS that is roughly four times longer than expected for this highly unfavorable subgroup. This shift in PFS points to a highly effective local therapy rather than for the induction of a migratory phenotype.

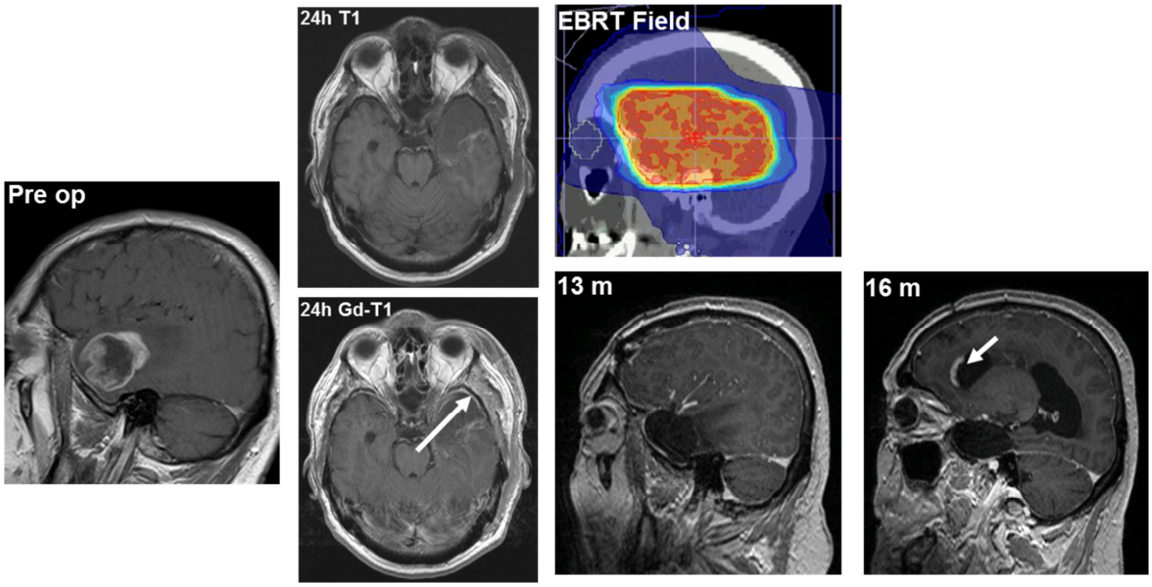


Figure 7. T1-weighted imaging studies of a patient with distant recurrence. Shown are images of a 60 year old male patient with a left temporal GBM (unmethylated MGMT promoter). Recurrence occurred in-field with regard to EBRT and out-of-field with regard to IORT (20 Gy surface dose).

1.2.8. Rationale for Patient Selection

INTRAGO enrolls patients suitable for gross or subtotal tumor resection, a population that is eventually able to achieve long-term survival. However, macroscopic or microscopic residual tumors may re-grow within few months, even if a favorable molecular subtype is present. Intensified radiotherapy treatment to areas of residual disease may thus be beneficial for these patients. The most suitable type of additional radiotherapy is IORT, as it spares healthy tissue.

2. TRIAL OBJECTIVES AND PURPOSE

2.1 Primary Objective

The primary end point is the median **progression free survival rate** (PFS).

The rationale for choosing this endpoint is that in glioblastoma, PFS and OS are strongly correlated (making PFS a surrogate for OS). PFS offers earlier assessment than OS and higher statistical power at the time of analysis (Han et al, 2014).

2.2 Secondary Objectives

The secondary objectives encompass important aspects of loco-regional and global efficiency (OS, patterns of relapse), prognostic factors (age, KPS, MGMT, etc.), as well as quality of life and safety parameters.

Secondary objectives are:

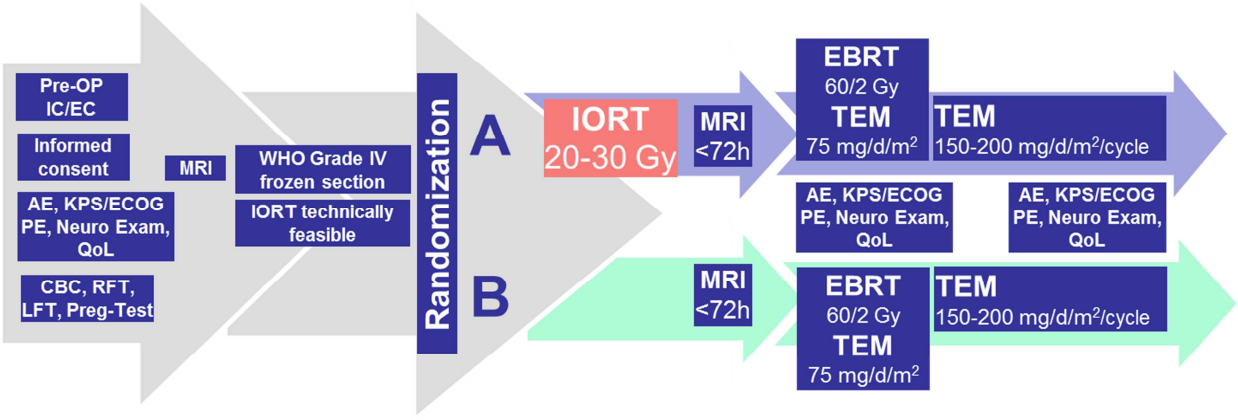
- Median overall survival from the time of randomization
- PFS within a 1 and 2 cm margin around the cavity (determined by serial contrast-enhanced MRI scans using modified RANO criteria and serial perfusion imaging)
- Outcome (PFS, OS) respect to age, KPS, tumor volume and extent of resection (as per central review of postoperative T1-MRIs, given as sum of all maximum diameters of residual lesions)
- Outcome (PFS, OS) with respect to molecular subgroups (e.g. MGMT)
- QoL/ADL
- Radiation-related (acute / early delayed / late) neurotoxicity

3. TRIAL DESIGN AND DESCRIPTION

3.1. Trial Design

INTRAGO II resembles a multicentric, prospective, randomized, 2-arm, open-label clinical phase III trial which tests if the median PFS of patients with newly diagnosed GBM can be improved by the addition of IORT to standard radiochemotherapy.

All patients included in the study will initially undergo standard surgical resection. In the experimental arm, a single radiation dose of 20-30 Gy will be delivered by IORT. Patients randomized into the control arm will not receive IORT. In both arms standard radiochemotherapy and adjuvant chemotherapy (according to the EORTC/NCIC protocol) will be applied after resection.



To prevent biases, all patients will be randomized into one of both arms (A – experimental arm with IORT, B – control arm) after surgical removal of the tumor and after the diagnosis of GBM is established by frozen sections.

3.2. Trial Duration and Schedule

The overall duration of the trial is expected to be approximately 4 years. Recruitment of subjects will start in Q3/2016. The actual overall duration or recruitment may vary. The study end is defined as “last patient out” (LPO).

Total trial duration:	48 months
Duration of the clinical phase:	24 months
FPI (First Patient In):	Q3 2016
LPI (Last Patient In):	Q3 2018
LPO (Last Patient Out):	Q3 2020
Analyses completed:	Q1 2021

4. SELECTION OF SUBJECTS AND CENTERS

4.1. Number of Subjects

As calculated in section 9.2. (Sample Size Calculation) **314** subjects should be enrolled in the clinical trial, i.e. **157** subjects per treatment group. Recruitment and treatment of subjects is expected to be performed in 8-12 centers.

4.2. Centers

The study will be conducted on a multinational and multicenter basis. It is intended that the study will take place as multi-center trial (at the time of writing this document: 7 confirmed and 5 interested centers) in 4 countries (Canada, Germany, Spain, USA).

4.3. Inclusion Criteria

Subjects meeting all of the following criteria will be considered for admission to the trial:

1. Age ≥18 and ≤ 70 years
2. Karnofsky Performance Score (KPS) ≥ 60%

3. Supratentorial T1-Gd enhancing lesion(s) amenable to total resection
4. Legal capacity and ability of subject to understand character and individual consequences of clinical trial
5. Patient's written informed consent (must be obtained at least 24h prior to surgery)
6. For women with childbearing potential: adequate contraception (for a list of highly effective contraceptive methods see Appendix I)
7. All patients must have adequate organ functions:

   Bone marrow function:
   - Platelets ≥ 145.000/µL
   - WBC ≥ 4.000/µL
   - Hemoglobin ≥ 12.0 g/dL

   Liver Function:
   - ASAT and ALAT ≤ 1.5 times ULN
   - ALP ≤ 2.5 times ULN
   - Total Serum Bilirubin < 1 times ULN

   Renal Function:
   - Serum Creatinine ≤ 1.5 times ULN

Inclusion criteria considered during surgery:

8. IORT must be technically feasible
9. Histologically confirmed (frozen section) glioblastoma multiforme (WHO grade IV)

4.4. Exclusion Criteria

Subjects presenting with any of the following criteria will not be included in the trial:

1. Multicentric disease (e.g. in both hemispheres) or non-resectable satellite lesions
2. Previous cranial radiation therapy
3. Cytostatic therapy / chemotherapy for cancer within the past 5 years
4. Use of alternating electrical fields (e.g., Novo-TTF)
5. History of cancers or other comorbidities that limit life expectancy to less than five years
6. Previous therapy with anti-angiogenic substances (such as bevacizumab)
7. Technical impossibility to use MRI or known allergies against MRI and/or CT contrast agents
8. Participation in other clinical trials testing cancer-derived investigational agents/procedures.
9. Pregnant or breast feeding patients
10. Fertile patients refusing to use safe contraceptive methods during the study

Exclusion criteria considered during surgery:

11. Active egress of fluids from a ventricular defect
12. In-field risk organs and/or IORT dose >8 Gy to any risk organ

4.5. Criteria for Withdrawal

4.5.1. Withdrawal of Patients from Treatment

Any patient can withdraw from the study at any time without personal disadvantages and without having to give a reason. Patients who discontinue participation in the clinical study on their own or patients who are withdrawn by the investigator, for reasons other than disease

progression (e.g. protocol violations, etc.), will be defined as premature withdrawals. Premature withdrawals will not be replaced. The time of treatment discontinuation and, if known, the reason for withdrawal must be documented on the eCRF. Reasons leading to the withdrawal of a patient can include the following (**one primary reason must be determined**):

- Patient died
- Adverse Events (need to be specified)
- Withdrawal by Subject
- Pregnancy
- Protocol Violation
- Lost to Follow-Up
- Study Terminated by Sponsor
- Other (needs to be specified)

In all patients who finish the study prematurely, a withdrawal examination at least with respect to the primary endpoint should be carried out. Drop-outs will not be replaced.

4.5.2. Premature Closure of the Clinical Trial

If serious safety concerns arise, the principal investigator must terminate or interrupt the study. If new information on the risk-to-benefit ratio of the intervention used in the study (IORT) is obtained in the meantime, the lead investigator reserves the right to interrupt or terminate the project.

Premature termination is also possible if the principal investigator notices and agrees upon that patient recruitment is insufficient and that this cannot be expedited by appropriate measures.

Premature termination of a single center is also possible if the principal investigator notices that the conduction of the trial is not according to the protocol, and/or the quality of the data is insufficient.

The ethics committee (EC) must be informed about the premature closure of the trial or one of the treatment arms. Furthermore, the ethics committee(s) themselves may decide to stop or suspend the trial.

All involved investigators have to be informed immediately about a cessation / suspension of the trial. The decision is binding to all trial centers and investigators.

5. SCREENING AND RANDOMIZATION

5.1. Screening

5.1.1. Pre-operative screening

Within 10 days before surgery, a screening visit must be scheduled, in which the eligibility checklist (available on the website of the eCRF-System) is completed. Pre-operative inclusion and exclusion criteria are assessed and the following tasks are performed:

- Signed informed consent and signed data protection declaration after hand-out of patient information (country-specific documents)
- Medical history including prior cancer diseases, as well as previous treatments thereof
- Assessment of KPS (preferably immediately prior to surgery)
- Physical examination and a neurologic (baseline) assessment
- Assessment of the Barthel ADL index (eCRF)
- Completion of the EORTC QLQ-C30 / BN20 questionnaires (eCRF)
- If not performed yet (e.g., in case only CT scanning was performed), a MRI scan of the brain is required using sequences specified in 6.3.1. The MRI scan must allow to **rule**

out unresectable satellites or multicentric disease (by Gd-T1 and/or T2 and/or T2-FLAIR):

- *Multifocal* disease is defined as two or more lesions within the same hemisphere but separated by white matter tracts. Multifocal lesions may be resected in the same surgery as the primary lesion, thus patients may be eligible for surgery and IORT.
- In turn, multicentric disease is defined as two or more lesions in opposite hemispheres. These patients are not eligible for surgery and IORT.

- The following laboratory studies will have to be performed:
  - Pregnancy test for women of childbearing potential
  - CBC and basic chemistry (LFT, RFT) to assess adequate organ functions (see 4.3, inclusion criteria)

5.1.2. Intraoperative screening

Patients who gave informed consent to participate in the trial will undergo surgery. During surgery, the following inclusion/exclusion criteria apply:

1. **Frozen sections must confirm the diagnosis of Glioblastoma multiforme.** Participating centers must provide the infrastructure to allow frozen sections. All histopathological procedures should be conducted according to local standards. However, at least three out of four criteria defined by the World Health Organization (WHO) grading system must be fulfilled:
   - high cellularity / high proliferation rate
   - cellular and/or nuclear pleomorphism
   - microvascular proliferation
   - necrosis
2. **IORT must be technically feasible:**
   Locally established intraoperative imaging (e.g. ultrasound, neuronavigation, ioMRI, etc.) should be used to identify risk structures after resection (to account for brain shifts). In case risk structures cannot be adequately identified, or doses to risk structures are exceeding dose constraints (see 6.4.4), IORT is considered as technically not feasible.

In case patients meet the intraoperative inclusion criteria (in addition to the pre-operative IC), the eligibility checklist will be completed (using the eCRF system) and the screening is completed.

5.1.3. Screening failures

For screening failures, i.e. patients who gave informed consent but do not meet randomization criteria (e.g., in case frozen sections reveal lower grade astrocytic tumors), the major reason for screening failure must be documented in the eCRF. Screening failures retain their identification codes (e.g. randomization code, if already given).

Screen failures will be replaced.

5.2. Randomization

5.6.1 Randomization Timing

Randomization must take place after surgical resection of the tumor and after the surgeon gives a statement on the extent of resection. It will be performed intraoperatively by using the web interface (eCRF).

The patient will then be assigned to one of the two treatment arms (A – experimental arm with IORT, B – control arm) and will be given a 6-digit **randomization code** (consisting of the 5-digit screening number and an additional letter indicating the arm, e.g. 01-001A for a patient randomized into the IORT arm).

5.6.2 Randomization Method

Stratified block randomization will be performed for each center to achieve equal group sizes per center. The details of randomization will be kept safe and confidential. The algorithm will be implemented into the eCRF.

Strata used in the algorithm are:

1. Age (<65 vs. ≥ 65)
2. KPS (90-100% vs. 70-80%)
3. Thickness of anticipated T1-Gd-enhancing (remaining) tumor margin as per the discretion of the surgeon (margin ≥0.5 cm or multiple sites of residual tumor at the cavity borders vs. <0.5 cm)

6. STUDY ASSESSMENTS AND INTERVENTIONS

6.1. Assessment of QoL and ADL

To assess Quality of life, all patients are requested to complete the Barthel ADL Index and the EORTC QLQ-C30/BN20 questionnaires at the screening visit (documents are downloadable from the eCRF web page), during the first week of radiotherapy, 6 weeks after radiotherapy and quarterly thereafter (at follow-ups visits).

6.2. Basic Neurological Exam

Acute (within 3 weeks from IORT), early-delayed (within 3 months from IORT) and late (>3 months after IORT) toxicity will be assessed throughout the whole study period on the basis of clinical presentation (physical examination, KPS), imaging studies (MRI) and on the basis of a basic neurological assessment (per NANO-Scale).

6.3. Imaging Studies

6.3.1 General Requirements

All patients must be suitable candidates to undergo repetitive contrast-enhanced MRI scans as the initial evaluation, as well as all follow-up evaluations are based on cranial MRI imaging studies.

Each center is required to be able to perform T2, T2-FLAIR, dynamic susceptibility contrast (DSC), DWI and 3D T1 volumetric imaging. Slice thickness should be 3-5 mm for 2D sequences. Standardized brain tumor protocols that can be downloaded directly to the scanners are available at http://cvib.ucla.edu/standardized-brain-tumor-mri-protocols/.

Perfusion imaging is mandatory for each time point (see Appendix II) and shout be performed according to ASFNR recommendations for clinical performance of MR DSC perfusion imaging (http://www.ajnr.org/content/early/2015/04/23/ajnr.A4341.full.pdf). In case PD is suspected, **longitudinal analysis** of DSC sequences for distinguishing tumor recurrence from pseudoprogression must be performed.

In brief, relative cerebral blood volume (rCBV) maps need to be computed and the absolute change in normalized rCBV from the time at initial progressive enhancement to the first subsequent follow-up must be calculated. Changes in rCBV (∆rCBV) values ≥ 0 indicate tumor progression, whereas ∆rCBV values < 0 indicate pseudoprogression. Of note, pseudoprogression may be even seen in lesion that show an increase in T1-enhancing volume but a decrease in perfusion over time.

6.3.2 Pre-Operative (neuronavigation) MRI

To allow frameless stereotactic surgery (also referred to as image-guided surgery or neuronavigation surgery), pre-operative MRI scans are mandatory. Before any pre-operative MRI scanning is performed, **all patients are required to first consent to participation in the trial**. The pre-operative scan should be performed within 96 h before surgery.

Beside generally required sequences (including T2, T2-FLAIR, etc. - see 6.3.1) specifically the obtainment of T1-weighted magnetization-prepared rapid gradient-echo (MP-RAGE) or MP-RAGE equivalent (such as SPGR for GE scanners) acquired as a “volumetric” acquisition that will allow reformatting in any plane with a slice thickness of max. 1.2 mm and axial T1-weighted sequences before and after contrast medium (Gd) is required.

To maximize detection of the total extent of the tumor (e.g. satellites and multifocal disease), scans with higher doses of contrast agent (such as Multihance, Dotarem, Gadavist) and a minimum time between injection and imaging of 5 minutes are recommended.

6.3.3 Early Postoperative MRI

Early postoperative MRI must be performed **<72 h after surgery** and must be analyzed in a standardized assessment as follows: T1-hyperintense lesions without contrast must be used to evaluate residual blood/heme. Specific sequences to more accurately assess acute blood (i.e. SWI) are encouraged. Next, (T1-)contrast-enhancing masses or nodules are indicative for residual tumor tissue. To determine the extent of resection (secondary end point, see 7.1.2.3), the maximal tumor diameter is obtained and then a second diameter is obtained at a right angle to the first. Both, the maximal diameter and the product of the two measurements must be noted (this also applies if the lesion is smaller than 1 cm, which would be classified as “non-measurable”).

Perfusion imaging is mandatory.

6.3.4 MRI for Treatment Planning

For planning of external beam radiotherapy (EBRT), MRI scans are **advised but not mandatory**. Sequences should be performed with and without contrast should be obtained not later than 7 days before the planned start of EBRT. The T1 -MRI and T2-FLAIR sequences will be co-registered on the planning-CT. For required sequences see 6.3.1.

6.3.5 CT for Treatment Planning

Postoperative MRI or (ideally) treatment planning MRI scans should be co-registered with a contrast-enhanced CT scan of the entire head region using the smallest possible axial slice thickness (≤3 mm) to define clinical and planning target volumes. The treatment-planning CT scan must be acquired with the patient in the same position and with the same immobilization device (e.g., thermoplast mask) as for treatment. The planning should be completed within 10 days prior to initiation of radiochemotherapy.

6.3.6 Follow-up MRI scans

The first follow-up MRI scan will take place 6 weeks after radiochemotherapy. Imaging must be performed in accordance with criteria specified in 6.3.1. Thereafter, all patients will receive quarterly MRI scans unless progression is suspected. MRIs have to be assessed using **modified RANO criteria and perfusion imaging as specified in 7.1.1.** Relevant imaging studies documenting disease relapse (i.e. the most actual and 1-2 MRI scans from previous FU visits including perfusion scans) will undergo centralized review to confirm progressive disease (see imaging site manual for details).

In case a new/increasing T1-Gd enhancing lesion or an increase in T2/FLAIR is noted and perfusion shows an increase in rCBV in FU scans, all centers must adhere to a pre-defined algorithm (**see 7.1.1 and Appendix II and III**). As a part of this algorithm, confirmatory scans are requested in 4-6 weeks. Once a patient is diagnosed with PD in the absence of radiographic criteria (e.g., if any other tumor-specific therapy was initiated or clinical deterioration occurred), confirmatory scans are advised.

6.3.7 Other Imaging Studies during the Trial

Any center is free to decide whether or not an included patient requires additional imaging (e.g. PET imaging), if appropriate. **The images obtained in this context are not mandatory for centralized review** but may help the central reader in the diagnosis of progressive disease versus pseudoprogression.

6.4. Treatments

6.4.1 Device used for IORT

It is mandatory that the INTRABEAM® System PRS500 with the x-ray source XRS 4 (Carl Zeiss Meditec AG, Oberkochen, Germany) must be used for IORT in this trial.

Each center has to specify:

- the serial number of the machine (once) and

- the serial number of the applicator used for used for each treatment in the eCRF (for each IORT case).

6.4.2 Pre-operative IORT Planning

Prior to surgery, radiation oncologists have to analyze all obtained pre-operative images (preferably contrast-enhanced T1-weighted MP-RAGE sequences) and identify all potentially involved risk structures. It should be taken into account that structures may displace intraoperatively after resection as a consequence of the reduced intracerebral pressure.

For two risk structures (Optical nerve/chiasm and brain stem), adapted dose constraints of 10 Gy apply during IORT.

Therefore, all risk structures that are located ≤1.5 cm to a T1-enhancing lesion in pre-operative MRIs have to be documented in the eCRF and searched for intraoperatively using neuronavigation, intraoperative ultra-sound or in-room imaging CT/MRI.

Depending on the applicator size, a depth of 1.5 cm correlates to a single dose of 3-7 Gy if 30 Gy are prescribed to the surface (Figure 8).

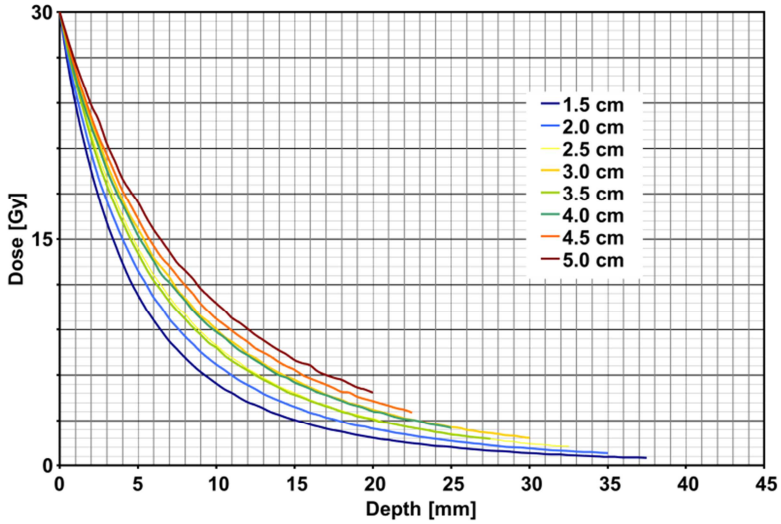


Figure 8. Dose depth curves (measured) for spherical applicators (diameters of 1.5-5.0 cm) of the Intrabeam system (exemplary surface dose of 30 Gy).

6.4.3 Surgery

The resection procedure should be performed as frameless stereotactic surgery with techniques that meet individual center standards and preferences. These techniques may include suction, bi-/monopolar cautery or ultrasound aspiration. Intraoperative application of 5-aminolevulinic acid (5-ALA) is optional. A maximum safe resection approach is recommended, but not mandatory.

Due to the possibility of CSF accumulation or retention around the applicator and subsequent lowering of doses to the target volume, any accumulation of fluids during and after surgery should be avoided.

Ventricular opening is *per se* no exclusion criterion. However, **no active egress of fluids originating from the ventricle is tolerable.** Placement of hemostatic agents (such as Surgicel®) is allowed to achieve a blood- and transudation-free cavity.

Once the diagnosis is established by frozen sections and a transudation-free cavity is achieved, the surgeon must estimate the thickness of the anticipated T1-Gd-enhancing (remaining) tumor margin (margin ≥0.5 cm or multiple sports of remaining tumor vs. <0.5 cm). All data is then entered into the eCRF and the patient is randomized into one of both arms.

No further tumor resection is permitted after randomization. Hemostasis or stopping of transudation is allowed.

Following IORT, surgery is continued as per local standards. Touching of the resection cavity should be avoided whenever possible.

Each patient should receive intraoperative i.v. steroids (10-20 mg dexamethasone as single dose).

6.4.4 IORT (experimental arm only)

6.4.4.1 Technical issues

Following preparation of the cavity (see section 6.4.3) and randomization into the experimental arm, the most suitable INTRABEAM applicator size has to be chosen to provide the highest degree of target volume coverage (“tightest fit rule”). **In case risk organs have been identified in ≤1.5 cm proximity to the initial T1-enhancing lesions in the preoperative planning (see 6.4.1), these structures have to be identified either visually or using techniques like**

neuronavigation, intraoperative ultrasound or in-room CT/MRI. Adequate documentation is mandatory. Screenshots documenting their depth are recommended.

The final distances to these structures have to be determined and documented. The expected IORT radiation doses (D_{IORT}) to risk structures are defined intraoperatively on the basis of the dose-depth profiles of the corresponding applicator provided in the INTRABEAM® workstation software.

If any risk structure is likely to receive >8 Gy, IORT has to be omitted due to "technical impossibility" and the case is recorded as screening failure. In case the applicator has direct contact to a risk structure, distancing (e.g. with wet cotton patties) or shielding (e.g. using tungsten-covered silicone strips) may be an option.

When risk structure allocation and protection is ensured, the applicator is inserted into the cavity in a way that surface contact to the whole cavity wall is established. If this is not achievable, the most likely area of failure has to be at least adequately covered and must have tight contact to the applicator surface. Identification of these areas is part of the training prior to initiation of new treatment centers.

Following placement of the applicator and closing of all interlocks, IORT is applied.

6.4.4.2 IORT Dose and Machine Settings

IORT with a surface dose of 30 Gy is recommended. However, should the proximity to any risk structure not allow to apply 30 Gy, a dose reduction by up to 10 Gy (resulting in a surface dose of 20 Gy) is allowed. **IORT with less than 20 Gy is not permitted.**

IORT will be applied using the following machine settings:

Beam voltage [kV]: 50.0	Applicator Type: Spherical
Current [µA]: 40	Applicator Size [cm]: 1.5 to 5.0
Prescription Dose [Gy]: 20.0 - 30.0	Prescription Depth [mm]: 0.00

6.4.4.3 IORT-Related Radiation Protection Issues

All centers have to adhere to federal, state and/or local regulations on radiation protection. IORT with the INTRABEAM® System does not require structural alterations of the operating room.

6.4.5 Radiotherapy

6.4.5.1 Schedule

Radiochemotherapy (RCT) should be initiated 3-5 weeks after surgery (with or without IORT). The craniotomy wound must be adequately healed and all sutures, stitches or drains must be removed. If any condition (e.g., wound healing difficulties, infections) does not permit the initiation of RCT within this timeframe, a further postponement of one week is allowed (minor violation). If RCT can still not be applied after this extension period (6 weeks after surgery), the patient is withdrawn from the trial.

6.4.5.2 Technical Requirements for EBRT

EBRT requires LINACs with photon energies ≥ 6 MV capable of delivering static or dynamic intensity modulation. **Intensity-Modulated Radiotherapy (IMRT) is preferred**. Acceptable IMRT modalities include helical tomotherapy or LINAC-based IMRT involving static gantry angles or modulated arc therapy. Electron or particle irradiation is not allowed. Image-guided radiotherapy (IGRT), e.g. with cone-beam verification, is favored. Treatment verification and documentation should be carried out based on institutional policy.

6.4.5.3 Target Volumes

For treatment planning, a T1-Gd-MRI MRI sequence has to be co-registered on the planning-CT acquired in treatment position. The accuracy of co-registration has to be confirmed by the radiation oncologist. Target volume delineation should follow RTOG guidelines.

The *gross tumor volume* 1 (GTV1) resembles the edema (if visible) in T2-FLAIR sequences and must also cover the complete resection cavity and any contrast-enhancing lesion in the T1-post contrast sequences. The *clinical target volume 1* (CTV1) is then defined as the GTV1 plus a 2 cm margin. The CTV1 may be reduced up to 0.5 cm around natural margins for tumors (e.g., skull, falx, etc.). The *planning target volume 1* (PTV1) then resembles the CTV1 plus an additional margin of 2-5 mm.

All (T1-)contrast-enhancing lesions/abnormalities (which must include the cavity margins) will be defined as boost volume (GTV2). The boost clinical target volume (CTV2) will be the GTV2 plus a margin of 2 cm. The CTV2 may be reduced up to 0.5 cm around natural margins for tumors (e.g., skull, falx, etc.). The *boost planning target volume* (PTV2) resembles the CTV2 plus an additional margin of 2-5 mm.

6.4.5.4 Dose Specification and Compliance Criteria

All patients will receive EBRT with a total dose of 46 Gy to the PTV1 and a conedown (boost) of 14 Gy to the PTV2. EBRT is given in daily doses of 2 Gy, 5 days per week (6 weeks total duration time).

EBRT may be interrupted for a maximum period of **seven consecutive days**. The reason for interruption and the duration must be documented in the eCRF. Any longer treatment pause is considered as unacceptable protocol violation.

It is recommended that 95% of the PTV receives 100% of the prescribed dose.

The following compliance criteria apply:

Compliance Criteria for PTV2		
Per Protocol:	95% covered by 60 Gy	99% covered by 54 Gy
Acceptable:	90% covered by 60 Gy	97% covered by 54 Gy
Unacceptable:	<90% covered by 60 Gy	<97% covered by 54 Gy

6.4.5.5 Critical Structure Constraints

Lenses, retinae, optical nerves and/or chiasm and brain stem have to be defined as *organ at risk* (OAR). The corresponding *planning risk volume* (PRV) is the OAR plus a 3 mm safety margin.

In case an OAR was pre-irradiated during IORT, the D_{IORT} has to be converted into an equivalent dose delivered in 2 Gy per fraction (EQD2) using an α/β ratio of 2.5 (Tsien et al. Clin Cancer Res 2012) and considering an RBE of 1.3 as follows:

$$\mathbf{EQD2 = 1.3 * D_{IORT} * (D_{IORT} + \alpha/\beta) / (2 + \alpha/\beta) = 1.3 * D_{IORT} * (D_{IORT} + 2) / 4}$$

Exemplary D_{IORT} and EQD2 values:

D_{IORT}	EQD2
3	4.8
4	7.5
5	10.8
6	14.7

7	19.2
8	24.3

The following point dose (dose to a volume > 0.03 cm^3) constraints apply for the OAR:

Risk Organ	Maximum Point Dose (EQD2)
Lenses	7 Gy
Retinae	50 Gy
Optical nerves / chiasm	55 Gy
Brain stem	66 Gy

In case the PTV includes a PRV which would in consequence not receive a dose within the given constraints, a specific risk-organ PTV (PTV-R) has to be delineated to allow separate dose reconstruction.

6.4.6 Chemotherapy

6.4.6.1 Concomitant Chemotherapy

All patients will receive oral temozolomide from day 1 to the last day of radiotherapy (7 days per week) at a daily dose of 75 mg/m^2. The body surface area (in m^2) is calculated **once** on the basis of the patient's pre-EBRT (post-surgery) weight and height. The resulting dose can be rounded to the nearest 10 mg.

The concomitantly applied temozolomide chemotherapy may be interrupted or terminated whenever clinically indicated for any given period of time (e.g., in case of hematotoxicity). The reason for interruption and the duration must be documented in the eCRF.

As part of the regular clinical routine, monitoring of chemotherapy-related side effects by performing complete blood counts (CBC), basic chemistry, RFT and LFT within 10 days prior to RCT and weekly during RCT is strongly advised. Only clinically significant abnormalities need to be recorded as AE in the CRF.

6.4.6.2 Adjuvant Chemotherapy

All patients should receive **a minimum of six cycles** of adjuvant chemotherapy (more than six cycles are allowed) unless there is evidence of tumor progression or treatment-related toxicity. Weekly blood workups should be obtained during adjuvant chemotherapy in line with the locally established clinical routine (see 6.4.6.1). Only clinical significant abnormalities needs to be recorded as AE in the CRF.

Adjuvant chemotherapy should be initiated 4 weeks after the last day of **irradiation** (per protocol: 4 weeks; acceptable ≤6 weeks; major violation >6 weeks). Ideally, the first day of chemotherapy is a Monday. Chemotherapy is performed with six 28-day cycles consisting of 5 consecutive days of temozolomide at doses of 150-200 mg/m^2 and a 23-day therapy break.

The body surface area in m^2 is calculated on the basis of the height obtained at the pre-surgery visit and the most actual weight (determined before each cycle).

The first cycle will be performed with 150 mg/m^2/d/cycle (absolute dose limit: 300 mg/d/cycle) of temozolomide. If there are no treatment-related toxicities, the dose may be escalated to 200 mg/m^2/d/cycle (absolute dose limit: 400 mg/d/cycle) in subsequent cycles.

6.4.6.3 Other Medication during concomitant and adjuvant Chemotherapy

Any non-cytotoxic or cytostatic side medication that is medically indicated is allowed. The intake of corticosteroids has to be documented as part of the response assessment. Non-enzyme-inducing anticonvulsive drugs are preferred over enzyme-inducing drugs. 5-HT3 antagonists are

preferred antiemetics. The intake of herbs (e.g. incense, St. John's wort, etc.), homeopathic remedies or vitamins should be avoided.

6.5. Centralized review

6.5.1 Pathology review

After closure of the trial, a central pathology review will be performed at the Department of Pathology at McGill University to confirm the diagnosis of GBM. Therefore, each participating center must send tissue to a central reviewer.

Ideally,

one HE section and a block containing representative tumor material

or

one HE section with representative tumor material and 10 unstained sections

should be sent to:

Mary-Cristine Guiot, MD
Montreal Neurological Institute and Hospital
Room 639
3801 University
Montreal, H3A 2B4

Contact for questions/advice: Phone: + 1514-398-5319
Fax: +1 514-398-5825

6.5.2 Centralized Neuroradiology Review

Per protocol analysis of the MRI data according to modified RANO will be performed by an independent reviewer (Dr. Whitney B. Pope, Department of Radiological Sciences, David Geffen School of Medicine, UCLA, 757 Westwood Plaza 1621E, Los Angeles, CA 90095, USA).

Participating sites will have to submit pseudonymized MRI images (as further outlined in the imaging site manual) of each randomized patient (further instructions on the submission of images for central review are described in the imaging site manual).

In accordance to RANO criteria (Wen et al, 2010), clinical deterioration related to the tumor alone may define progressive disease. In such cases, the MRI scans with closest temporal relation to the date of progression must be uploaded. Once a patient is diagnosed with PD in the absence of radiographic criteria (e.g., if any other tumor-specific therapy was initiated or clinical deterioration occurred), confirmatory scans are advised but not mandatory.

Documentation of the radiation field must also be provided to the central review board as a prerequisite for correctly assessing pseudoprogression (e.g. a PDF of the treatment plan).

Upload of these data sets will be performed using specific online software provided by the Coordinating Center (University Medical Center Mannheim).

6.6 Risks of Trial Treatment(s)/ Intervention(s)

All surgical procedures carry general risks, including

- adverse/allergic reaction to anesthesia
- cardiovascular depression
- nausea and emesis
- blood loss
- cosmetic problems (e.g., exuberant scars)
- infections and septic shock

Specifically brain (tumor) surgery is associated with the following risks:

- ischemic or hemorrhagic stroke
- edema, brain swelling, increased intracerebral pressure and incanceration
- loss of consciousness, coma
- impaired speech, vision, motor function, coordination, balance or memory
- focal or generalized seizures, grand-mal

Cranial irradiation with IORT or EBRT carries the risk of acute, early-delayed and late (side) effects including:

- symptoms of mass effects (e.g., headache, nausea, worsening neurological symptoms).
- radionecrosis with symptoms similar to tumor progression
- optical nerve impairment: loss of vision
- brain stem injury: reduced ability to respond to stimuli, changes in muscle tone, difficulties in respiration and other vital functions

Chemotherapy with the alkylating drug temozolomide is associated with specific risks:

- hematotoxicity: anemia, bleeding disorders, infections, fever, chills, sore throat, flu symptoms, dry cough, weight loss, night sweats, UTI, dysuria
- loss of appetite, unusual or unpleasant taste in the mouth, nausea and vomiting, unusual weakness, diarrhea or constipation
- seizures, numbness or tingling sensations, dizziness, blurred vision
- liver damage, jaundice
- hair loss, skin rash
- insomnia

6.7 Emergency Treatment

During and following a subject's participation in the trial, the investigator should ensure that adequate medical care is provided to a subject for any AE including clinically significant laboratory values. The investigator should inform a subject when medical care is needed for intercurrent illness(es) of which the investigator becomes aware.

6.8 Planned Treatment After Study End

After progressive disease is noted (or confirmed), second-line treatment modalities are at the local investigator's discretion. All second-line treatments should be reported to the study central lifelong, until withdrawal or until the patient is lost to follow up. **A second application of IORT is not recommended.**

The investigator will continue to observe patients withdrawn from the study because of intolerable AEs until the findings have been clarified.

Any SAEs occurring within 3 months after the end of study need to be reported. The occurrence of radionecrosis will have to be reported lifelong or until the patient is lost to FU.

7 METHODS

7.1. Efficacy Parameters

7.1.1. Primary target variable

The primary target variable of the trial is the median progression-free survival (PFS). PFS is defined from the day of randomization to the day of local tumor progression or recurrence. Recurrence definition is based on a pathological MRI evaluated using RANO criteria (Wen et al, 2010) with specific modifications to differentiate radiation-related effects from tumor progression (Table 4). These modifications to RANO are necessary as dose-escalated radiotherapy may cause alterations in MRI scans similar to tumor progression.

In case PD is suspected according to modified RANO criteria (including longitudinal perfusion imaging as outlined in Appendix II) and the confirmatory MRI scan does not confirm pseudoprogression (see flowchart in Appendix III), **the corresponding imaging studies have to be submitted for independent central review**. The central reviewer will make the final decision. In case the central review confirmed PD, the date of PD becomes the date of the MRI of the initially suspected progression (prior to the 2 confirmatory scans). If PD is not confirmed by the central reviewer, the patient will have no event for the endpoint PFS.

In addition to central image review, further efforts should be made to distinguish radiation-related effects from *real* tumor progression. Stereotactic biopsy or re-surgery provide the most reliable diagnosis in this regard.

In case PD was confirmed by the central reviewer but surgery/histology reveals that there are no areas of vital tumor (areas with positivity for Ki-67 or presence of endothelial proliferation), the diagnosis of PD is omitted.

Table 4. Modified RANO criteria.

Criterion	CR	PR	SD	PD
T1-Gd enhancing disease[1]	None	≥ 50% ↓	< 50% ↓ but < 25% ↑	≥ 25% ↑ + ΔrCBV≥0[2]
T2/FLAIR[3]	Stable or ↓	Stable or ↓	Stable or ↓	↑
New lesion[4]	None	None	None	Present
Corticosteroids	None	Stable or ↓	Stable or ↓	NA[5]
Clinical status	Stable or ↑	Stable or ↑	Stable or ↑	↓[6]
Requirement for response	All	All	All	Any

[1] Refers to the %-change in the sum of the products of perpendicular diameters of enhancing lesions compared with the smallest tumor measurement obtained either at baseline or the time point with the best response.

[2] Any significant increase in T1-enhancing disease **must be additionally analyzed by longitudinal analysis of DSC MR perfusion scans** (see Appendix II). In case a decrease in perfusion is noted over time (ΔrCBV<0) tumor progression is not confirmed.

[3] The increase in T2/FLAIR should be significant (25%) and should not be attributable to other non-tumor causes (e.g., radiation therapy, demyelination, ischemic injury, etc.). Advanced MRI imaging is advised.

[4] new measurable lesions must be visible on two or more axial slices (slice thickness ≤ 5 mm).

[5] Increase in corticosteroids alone will not be taken into account in determining progression in the absence of persistent clinical deterioration.

[6] Clinical deterioration that is not attributable to other causes apart from the tumor (e.g., radionecrosis, medication adverse effects, complications of therapy, cerebrovascular events, etc.).

The initiation of salvage chemotherapy or re-irradiation for GBM in the absence of diagnosed tumor progression by RANO or recurrence will be considered as protocol violation and will not be counted as an event for PFS (the case will be censored at the date the treatment was initiated). In case re-surgery is performed for suspected progressive disease, the presence of areas of viable tumor cells with positivity for Ki-67 or the presence of endothelial proliferation is required to be considered as an event for PFS.

Radionecrosis Statements:

- Surgical resection or biopsy for histological confirmation of radionecrosis is recommended. **Surgery for radionecrosis will not be considered as endpoint for PFS unless (local) pathology reveals that the specimens contain tumor tissue** (e.g. presence of areas of viable tumor cells with positivity for Ki-67 in some of these cells or presence of endothelial proliferation).
- **Patients receiving Bevacizumab for radionecrosis (diagnosed by any modality) are not considered to have an event for the endpoint PFS.**

7.1.2. Secondary target variables

7.1.2.1. Median overall survival

Median survival is defined as the time after which 50% of patients have died by any cause. This figure can be estimated using the Kaplan-Meier method.

7.1.2.2. PFS within a 1 and 2 cm margin around the cavity

IORT with 20-30 Gy at the applicator surface will result in doses of 6-8 Gy in 1 cm depth (see 6.3.3 and Figure 8), which corresponds to equivalent doses delivered in 2 Gy per fraction (EQD2) of 16-26 Gy (α/β ratio of 2.5). To assess whether these doses are effectively prolonging local control, margins of 1 and 2 cm will be analyzed for tumor progression by serial contrast-enhanced MRI scans using modified RANO criteria and serial perfusion imaging (see 7.1.1).

7.1.2.3. Outcome (PFS, OS) with respect to strata

PFS and OS will be calculated (or estimated) in the following subgroups:

- Age (<65 vs. ≥ 65 years)
- Initial KPS (80-100% vs. 60-70%)
- Thickness of anticipated T1-Gd-enhancing (remaining) tumor margin as per the discretion of the surgeon (margin ≥0.5 cm or multiple spots of residual tumor within the cavity vs. <0.5 cm)
- Extent of resection (as per central reviewer):
    - All study centers must analyze early postoperative MRI scans (see 6.3.3) to determine the extent of resection (EoR). The EoR is given as sum of the maximum diameters of all lesions in cm.
    - PFS and OS will be calculated for the following groups:
        - Max Diameter group 0: 0 cm (no residual tumor)
        - Max Diameter group 1: >0 to ≤1.5 cm (cumulative if multiple residual lesions)
        - Max Diameter group 2: >1.5 cm (cumulative if multiple residual lesions)

7.1.2.4. Outcome (PFS, OS) with respect to molecular subgroups

Assessment of molecular markers is not mandatory, but strongly recommended. PFS and OS will be calculated (or estimated) in the following subgroups:

- MGMT: promoter methylation present vs. absent
- IDH1 mutation (R132H): present vs. absent

7.1.2.5. Quality of life (QoL) and Activities of daily living (ADL)

QoL will be determined using the EORTC quality of life of cancer patients brain module questionnaire (EORTC-QLQ-C30/BN20) and activities of daily living (ADL) will be assessed using the Barthel Index (Mahoney & Barthel, 1965). The questionnaires should be completed ≤1 week prior surgery, within the first week of EBRT, at the 6-week visit after the end of radiochemotherapy and quarterly thereafter.

Even if the patient progresses or recurs before the last scheduled QoL assessment, QoL forms should still be completed.

7.2. Safety Parameters

7.2.1. General (not IORT-Specific) Safety Parameters

As for patients not included in the trial, each center must ensure trial patient safety based on institutional policy. This is in particular including (but not limited to) regular physical exams and blood workups (e.g. to monitor side effects of temozolomide chemotherapy).

7.2.2. Radiotherapy-related Toxicity

7.2.2.1. Definitions and Assessment

As a general principle in radiotherapy, toxicity is defined as acute or sub-acute if manifesting up to 90 days after the start of radiotherapy or as late toxicity if manifesting after this period. Dose intensification, e.g. by IORT to the brain, may cause acute, subacute or late neurotoxicity.

Neurotoxicity will be assessed throughout the whole study period on the basis of clinical presentation (physical examination, KPS), imaging studies (MRI) and on the basis of a basic neurological assessment.

7.2.2.2. Acute and Subacute Neurotoxicity

Radiotherapy to the brain as applied in this trial (both arms) may be associated with the following acute or subacute radiotherapy-associated neurotoxicities:

- Nausea
- Vomiting
- Alopecia
- Skin toxicity
- Vertigo
- Problems with attention or concentration
- Worsening of pre-existing neurological symptoms
- Somnolence syndrome (periods of drowsiness, lethargy, loss of appetite, irritability)

7.2.2.3. Late Neurotoxicity

the following radiotherapy-associated late toxicities may appear:

- Radionecrosis
- Leukoencephalopathy
- Cognitive (neuropsychological) impairment, such as attention deficit, memory deficit

7.2.3. Handling of Radionecrosis

Common radiotherapy-related toxicities (such as headache, nausea, vomiting) can be treated according to locally established standards (e.g. antiemetics, steroids, etc.). For radionecrosis, which can occur several months to many years following treatment, specific recommendations apply:

1. Radionecrosis should be first-line treated with steroids and/or surgery.
2. Surgery for radionecrosis will not be considered as endpoint for PFS unless (local) pathology reveals that the specimens contain tumor tissue (e.g. presence of areas of viable tumor cells with positivity for Ki-67 in some of these cells or presence of endothelial proliferation).
3. Bevacizumab (e.g. 7.5 mg/kg BW given q3w) is an option in case patients do not respond to first-line treatments. **Patients receiving bevacizumab for radionecrosis will not be considered as having an event for the endpoint PFS (see 'Methods' section).**

8. ADVERSE EVENTS

8.1. Definitions

8.1.1. Adverse Events

An adverse event (AE) is defined as any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medical treatment or procedure regardless of whether it is considered related to the medical treatment or procedure (attribution of unrelated, unlikely, possible, probable, or definite) (NCI, 2013).

AE will be interrogated for at each contact between the responsible investigator and the study subject until the endo of the study (diagnosis of PD). Ongoing AEs will be followed until recovery.. Wherever possible, adverse events will be reported on the basis of CTC-AE v4.0.

A pre-existing disease or symptom (including pathological laboratory values) will not be considered an adverse event unless there will be an untoward change in its intensity, frequency or quality. This change will be documented by an investigator.

Surgical procedures themselves are not AEs; they are therapeutic measures for conditions that require surgery. The condition for which the surgery is required may be an AE. Planned surgical measures permitted by the clinical trial protocol and the condition(s) leading to these measures are not AEs, if the condition leading to the measure was present prior to inclusion into the trial.

AEs are classified as "non-serious" or "serious".

8.1.2. Serious Adverse Event

A serious adverse event (SAE) is any *untoward medical occurrence* that at any dose:

- results in death,
- is life-threatening (the term life-threatening refers to an event in which the subject was at risk of death at the time of event and not to an event which hypothetically might have caused death if it was more severe),
- requires hospitalization or prolongation of existing hospitalization[1],

[1]Hospitalization for performing protocol-required procedures is not classified as an SAE. Hospitalizations due to tumor progression (will be recorded elsewhere [primary endpoint of this study]), for disease-related procedures (e.g., surgery, radiotherapy, adjustment of anti-tumor and/or concomitant medication, imaging, laboratory tests) or for any

- results in persistent or significant disability/ incapacity,
- is a congenital anomaly/birth defect or
- is otherwise medically relevant.

Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations - such as important medical events that may not be immediately life threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed above. These should also usually be considered serious (examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse).

8.2. Grading of AEs

The classification of AE-intensity in this trial will be carried out on the basis of a 3-grade scale as follows:

Mild:	signs and symptoms which can be easily tolerated. Symptoms can be ignored or disappear when the subject is distracted.
Moderate:	symptoms cause discomfort but are tolerable, they cannot be ignored and affect normal activity.
Severe:	symptoms strongly affect normal activity.

8.3. Relationship to radiotherapy

The investigator at each site will evaluate each AE that occurred regarding the coherency with radiotherapy (RT):

Definitely related:	There is a reasonable possibility that the event may have been caused by RT. A certain event has a **strong temporal relationship** and an alternative cause is unlikely.
Probable:	An AE that has a reasonable possibility that the event is likely to have been caused by RT. The AE has a **timely relationship** and **follows a known pattern of response**, but a potential alternative cause may be present.
Possible:	An AE that has a reasonable possibility that the event may have been caused by RT. The AE has a **timely relationship** to RT; **however, the pattern of response is untypical**, and an alternative cause seems more likely, or there is significant uncertainty about the cause of the event.
Unlikely:	Only a remote connection exists between RT and the reported adverse event. Other conditions including concurrent illness, progression or expression of the disease state or reaction of the concomitant medication appear to explain the reported adverse event.
Not related:	An AE that does not follow a reasonable temporal sequence related to RT and is likely to have been produced by the subject's clinical state, other modes of therapy or other known etiology.
Not assessable:	There is insufficient or incomplete evidence to make a clinical

procedures that were planned before entry into the study are not considered SAEs. Hospitalizations for social and/or custodial reasons in the absence of an adverse event are not classified as SAEs either.

judgment of the causal relationship.

8.4. Countermeasures

The term “countermeasures” refers to the specific actions taken to treat or alleviate adverse events or to avoid their sequels. Following categories will be used to categorize the countermeasures to adverse events:

None:	No action taken.
Drug treatment:	Newly-prescribed medication or change in dose of a medication.
Others:	Other countermeasures, e.g. an operative procedure.

8.5. Period of Observation and Outcomes of adverse events

During the trial, all subjects who have reportable AEs, whether considered associated with IORT or not, **must be monitored to determine the outcome**.

The clinical course of the (S)AE will be followed up until resolution or normalization of changed laboratory parameters or until it has changed to a stable condition. After PD (End of Study) or withdrawal, ongoing (S)AEs should be followed until resolution or normalization. In addition, any new SAEs occurring within 3 months after End of Study should be reported. **Except for radionecrosis**, no other AEs need to be reported during the survival follow-up period.

The clinical course of the AE will be followed up until resolution or normalization of changed laboratory parameters or until it has changed to a stable condition. This also holds for ongoing AEs/SAEs of withdrawn subjects.

The outcome of an AE at the time of the last observation will be classified as:

Recovered / resolved:	All signs and symptoms of an AE disappeared without any sequels at the time of the last interrogation.
Recovering / resolving:	The intensity of signs and symptoms has been diminishing and / or their clinical pattern has been changing up to the time of the last interrogation in a way typical for its resolution.
Not recovered/not resolved:	Signs and symptoms of an AE are mostly unchanged or worsened at the time of the last interrogation.
Recovered / resolved with sequel:	Actual signs and symptoms of an AE disappeared but there are sequels related to the AE.
Fatal:	Resulting in death. If there are more than one adverse event only the adverse event leading to death (possibly related) will be characterized as ‘fatal‘.
Unknown	The outcome is unknown or implausible and the information cannot be supplemented or verified.

8.6. Reporting of Serious Adverse Events by Investigator

All SAEs must be reported to the Sponsor by the investigator within 24 hours after the SAE becomes known using the "Serious Adverse Event" form in the eCRF or by sending a report by E-mail to studien.strahlen@medma.uni-heidelberg.de or via fax to +49 621 383 2022. When a patient has completed or withdrawn from the study, all SAEs occurring within 4 weeks after completion/withdrawal should be reported.

Additionally, in accordance with local (national) regulations, SAEs have to be reported to the corresponding oversight authorities.

8.7. Handling of Serious Adverse Events by the Sponsor

All unexpected and related or possibly related adverse events will be reported via the SAE manager of the Sponsor to the DSMB, which will then decide on further actions.

9. STATISTICAL PROCEDURES

9.1. Statistical Analysis Plan

The section “Statistical Procedures” shall give a brief overview on the statistical considerations on this trial. All planned analyses in this trial are specified in detail in a separate statistical analysis plan (SAP). The SAP will be carefully reviewed by statisticians and programmers.

9.2. Sample Size Calculation

This study will detect a 50% improved median progression-free survival (from 9 to 13.5 months) with 80.2 % (actual) power with a 1-sided p of 0.05. Considering a drop-out/loss to FU rate of 6.5% per month, the study must recruit 157 patients per arm, or 314 patients in total.

9.3. Analysis Variables

Primary variables: median progression free survival rate (PFS).

Secondary variables:

- Median OS from the time of randomization
- PFS within a 1 and 2 cm margin around the cavity
- Outcome (PFS, OS) with respect to strata age, KPS, tumor volume and extent of resection (based on analyses of postoperative T1-MRIs with and without contrast, given as the sum of maximum diameters of all lesions in cm)
- Outcome (PFS, OS) with respect to molecular subgroups (e.g. MGMT, IDH1)
- QoL/ADL
- Radiation-related (acute / early delayed / late) neurotoxicity

9.4. Definition of Trial Population to be analyzed

The primary analysis will be performed for the **full analysis set** which comprises all patients randomized into the trial. In this set, every patient is analyzed according to the group randomized into. Exceptions to this rule are made for patients for whom non-eligibility became apparent after randomization and/or treatment (e.g. when central neuropathology judges tumors to be anaplastic astrocytomas or central neuroradiology review detects satellite lesions in pre-operative scans).

Due to electronic data capture (eCRF) in the trial, protocol violations will be readily detectable and a **per-protocol set** will be defined. This set comprises all patients who were treated according to the randomized treatment as outlined in the protocol.

The **safety set** will comprise all patients who have received IORT, regardless of further therapy.

9.5. Statistical Methods

9.5.1. Study Design

The study design calls for an accrual period of 2 years and a follow-up period of 2 years. Patients are entered and then followed until either (a) the terminal event occurs, or (b) they drop out of the study, or (c) the study ends and the patient is censored while still being actively followed.

9.5.2. Effect size

Computation of power is based on a median progression-free survival rate of 13.5 months for the IORT-treated group versus 9 months for the control group. This effect (50% improvement) was selected as the smallest effect that would be important to detect, in the sense that any smaller effect would not be of clinical or substantive significance.

9.5.3. Sample size and Patients per Center

Patients will be entered into the observation group at the rate of 157 per year for 2 years yielding a total of 314 patients. Patients will be entered into the IORT arm at the rate of 73-84 per year for 2 years. All centers should recruit equal numbers of patients.

9.5.4. Attrition

The computation assumes an attrition rate of 6.5% per year. This attrition (or drop-out) rate resembles the calculated average considering each center's individual experience of the last 3-5 trials. It is separate from the censoring of patients that takes place when the study ends.

9.5.5. Alpha and Tails

The criterion for significance (alpha) has been set at 0.050. The test is 1-tailed, which means that only an effect in the expected direction will be interpreted.

9.5.6. Power

For this study design, sample size, attrition rate, alpha and tails, and the population effect size described above, the study will have power of 80.2% to yield a statistically significant result.

9.5.7. Statistical methods

All variables will be described by descriptive statistics (frequencies or mean and standard deviation, median, minimum and maximum). Kaplan-Meier curves and log-rank tests will be used to estimate OS and PFS. Analyses of variance (ANOVAs) with repeated measures or Friedman's one-way ANOVAs will be used to analyze the QoL/ADL assessments. If not stated differently, the tests for statistical significance will be reported on a one-sided 5.0% significance level for OS and PFS, and on a two-sided 5.0% significance level for the QoL/ADL assessments.

10. DATA MANAGEMENT

10.1. Data Management Plan

Data management will adhere to a separate document, a data management plan, which shall ensure compliance with good clinical data management practices throughout the entire clinical trial.

10.2. Data Collection

All findings including clinical and laboratory data will be documented in the subject's medical record and in the eCRF. Source documents must mention that the patient has been included in an investigational study. There must be no data that are inconsistent between eCRF and source documents.

All protocol-required information collected during the trial must be entered by the investigator, or a designated (competent/trained) representative, in the eCRF. Patient data will be documented pseudonymously. The investigator, or a designated representative, should complete the eCRF pages as soon as possible after the information is collected, preferably on the same day when a trial subject is seen for an examination, treatment, or any other trial procedure. The investigator must certify that the data entered into the eCRF are complete and accurate.

10.3. Data Handling

Data entries will undergo an automatical online check for plausibility and consistency. In case of implausibilities, 'warnings' will be reported. A responsible investigator will be obliged either to correct the implausible data or to confirm its authenticity and to give appropriate explanation. If not corrected, the data will be flagged, rendering a check of all questionable entries conveniently possible. A responsible monitor will check all flagged data and will generate questions, that will be send back to the responsible investigator. The investigator will have to resolve all 'discrepancies'.

All missing data or inconsistencies will be reported back to the center(s) and clarified by the responsible investigator. If no further corrections are to be made in the database it will be declared closed and used for statistical analysis.

10.4. Archiving of Essential Documents

The investigators have to arrange the retention of the subject identification codes and all other documentation pertaining to the trial in accordance with country-specific regulations (Germany: at least 30 years after the completion or termination of the trial in accordance with the Radiation Protection Act). Patient files and other source data shall be kept for the maximum period of time permitted by the hospital.

Any change of data ownership shall be documented. All data shall be made available if requested by relevant authorities.

The investigator(s) will archive all trial data (source data and investigator site file (ISF) including subject identification list and relevant correspondence) according to the section 4.9 of the ICH Consolidated Guideline on GCP (E6) and to local law or regulations.

11. ETHICAL AND LEGAL ASPECTS

11.1. Good Clinical Practice and ISO 14155

This trial will be carried out in compliance with the protocol, the principles laid down in the Declaration of Helsinki, version as of October 1996 (as long as local laws do not require to follow other versions), in accordance with the ICH Harmonised Tripartite Guideline for Good Clinical Practice (GCP), the harmonized standards for Medical Devices (ISO 14155) and all other applicable regulatory requirements (local, regional and global).

Standard medical care (prophylactic, diagnostic and therapeutic procedures) remains in the responsibility of the treating physician of the patient.

11.2. Approval of Trial Protocol and Amendments by the Ethics Committee

Before enrollment of the first patient, the trial protocol, informed consent document, and any other appropriate documents must be submitted to the independent Ethics Committee (EC).

A written favorable vote of the local EC is a mandatory prerequisite for initiation of this clinical trial.

The statement of EC should contain the title of the trial, the trial code, the trial site, and a list of reviewed documents. It must mention the date on which the decision was made and must be officially signed by a committee member. This documentation must also include a list of members of the EC present on the applicable EC meeting and a GCP compliance statement.

Before the first subject is enrolled in the trial, all ethical and legal requirements must be met. All planned substantial changes will be submitted to EC in writing as protocol amendments. They have to be signed by the sponsor and biometrician and approved by the EC.

The investigator will keep a record of all communication with the EC.

11.3. Approval by the Federal Office for Radiation Protection (Germany only)

After approval of the independent EC, appropriate documents will be submitted to the Federal Office for Radiation Protection (Bundesamt für Strahlenschutz, BfS). A written favorable vote of the BfS is a prerequisite for initiation of this clinical trial in Germany. Substantial changes will be submitted to the BfS in writing as protocol amendments. The investigator will also keep a record of all communication with the BfS.

11.4. Subject Information and Informed Consent

Before being admitted to the clinical trial, the subject must consent to participate after the nature, scope, and possible consequences of the clinical trial have been explained in a form understandable to him or her. The subject must give consent in writing. The signed informed consent form will be filed by the investigator.

A copy of the signed informed consent document must be given to the subject. The documents must be in a language understandable to the subject and must specify who informed the subject.

The subjects will be informed as soon as possible if new information may influence his/her decision to participate in the trial. The communication of this information should be documented.

Before being enrolled in the clinical trial, every patient will be informed that participation in this trial is voluntary and that he/she can withdraw from the study at any time without giving a reason and without having to fear any loss in his/her medical care. The patient will be informed about the study intervention(s) and the possible side effects. At the same time, the purpose, significance and scope of the study will be explained to him. The explanation also includes informing the patient about the insurance protection and the obligations of the insured.

The patient's consent must be obtained in writing before the start of the study.

The patient will have sufficient time and opportunity to clarify any unresolved questions. Furthermore, the patient will be handed over a "Patient Informed Consent Form" containing all the important information in written form (in local language) and a copy of the signed informed consent form.

By signing the informed consent form, the patient declares that he/she is participating voluntarily and intends to follow the study protocol instructions and the instructions of the investigator, and to answer the questions asked during the course of the trial. The investigator will keep a copy of the signed and dated written patient informed consent form in the designated place in the investigator's file.

11.5. Reimbursement of Trial Patients

Trial patients will not be compensated for participation.

11.6. Patient insurance

Patient insurance will be issued by the sponsor.

The insurance will ensure that any damage to health suffered by patients, which is directly related to this clinical trial, is financially covered. The investigator, or an individual who is designated by the investigator, will inform the patient of the existence of the insurance, including the obligations arising from it. Additionally, the patient information informs the patient about the insurance protection and the obligations of the insured (see 11.4). All trial patients must have access to insurance documents and provided with a copy of the general conditions of insurance on request.

12. QUALITY CONTROL AND QUALITY ASSURANCE

12.1. Confidentiality

During the clinical trial, subjects will be identified solely by means of their individual identification code (randomization code). Trial findings stored on a computer will be stored in accordance with local data protection law and will be handled in strictest confidence. For protection of these data, organizational procedures are implemented to prevent distribution of data to unauthorized persons. The appropriate regulations of local data legislation will be fulfilled in its entirety.

The subject consents in writing to release the investigator from his/her professional discretion in so far as to allow inspection of original data for monitoring purposes by authorized persons (monitors, auditors). Authorized persons (clinical monitors, auditors) may inspect the subject-related data collected during the trial ensuring the appropriate effective data protection law.

The investigator will maintain a subject identification list (subject numbers with the corresponding subject names) to enable records to be identified. Subjects who did not consent to circulate their pseudonymized data will not be included into the trial.

This protocol, the eCRFs, other result forms, laboratory data must be handled with strict confidentiality and not be disclosed to third parties except with the express prior consent of Lead Principle Investigators. Staff of the investigators involved in this study is also bound by this agreement.

12.2. Training, Initiation, Monitoring and Audits

12.2.1. Training and Initiation

The sponsor will ensure adequate site training with a focus on technical implications of IORT and response assessment.

12.2.2. Monitoring and Audits

Monitoring and audits will be performed according to a pre-specified **monitoring and audit plan**.

12.3. Data Safety Monitoring Board

A Data Safety Monitoring Board will be regularly meeting to monitor safety and other data related to this study. The Board members may receive confidential patient information, but they will not receive any name or other information that would allow them to identify the patient by name.

13. ADMINISTRATIVE AGREEMENTS

13.1. Financing of the Trial

13.1.1. Principle Funding Statement

INTRAGO II is an academic-driven study.

13.1.2. Web Interface and Data Management

The web interface for screening, randomization and follow-up using electronic case report forms will be provided by the Sponsor free of charge. Also, data management will be performed by the Coordinating Center free of charge.

13.1.3. Centralized Review

Centralized Review of imaging studies will be funded by the Sponsor. Central pathology review after closure of the trial will be funded by the Montreal Neurological Institute Montreal, Quebec, Canada.

13.1.4. Initiation, Monitoring and Audits

Monitoring will be financed and performed by the Sponsor.

13.1.5. Insurance, IRB fees

Patient insurance is provided by the sponsor. IRB fees are covered by the sponsor.

13.2. Reports

After completion of the analysis by the responsible biostatistician the final integrated medical and statistical report will be prepared and signed jointly with the biostatistician.

The final study report will be written and signed in co-operation between the lead and the coordinating investigator and the trial coordinator.

13.3. Registration of the Trial

The trial will be registered with clinicaltrials.gov before enrollment of the first patient.

13.4. Publication

13.4.1. General issues

All information concerning the trial is **strictly confidential** before publication.

Publication and/or presentation of the study results are planned. The right of publication rests with the lead investigators (FW, FG). All data collected within the trial will be treated in confidence by the trial coordinating center and all others involved in the study, until publication. Unscheduled interim data and final results may only be published (orally or in writing) with the

agreement of the lead investigators and the Writing Committee. They have to be provided with a draft of the abstract and/or manuscript at least 30 days prior to planned submission and/or presentation.

13.4.2. Publication of Primary End Points

Reporting of the primary end points of the trial will be done by the Writing and Presentation Committee. The order of authorship in publications, abstracts, conference papers and oral presentations will be as follows:

(1) The first, corresponding and/or presenting author will be the Principle Investigator, Dr. Frank A. Giordano. Second author will be Dr. Frederik Wenz. Presentations that cannot be given by the PI may be delegated to another PI of the trial.

(2) All PIs of participating sites will be listed after the second author in an order reflecting (a) patient recruitment and (b) extent of intellectual and/or scientific contributions (i.e. contributions to the design of the protocol, conduct of the trial, contributions to research aspects, etc.).

(3) Both, senior and second senior authorship will be provided to McGill University. Depending on contributions, authorships may be indicated as “shared”.

13.4.3. Publication of Secondary End Points

Reporting of **secondary end points** will be also done by the Writing Committee. However, in contrast to reporting of primary end points, the order of authorship in publications, conference papers and oral presentations on secondary end points is determined by the extent of contribution of each PI to the corresponding analysis.

13.4.4. Publication of Other Data and Data Linked to End Points

Publication of “other data”, that is data that do not involve the pre-specified primary and secondary end points (e.g. from additional imaging studies or cell- and molecular biological workups) can be published at any time from any center/PI that obtained the data without a need to obtain permission by the Writing and Presentation Committee.

However, should there be any link between “other data” and study end points (for example a new molecular marker was found that correlates with PFS), the center/PI that obtained the “other data” must obtain written permission to publish this data by the Writing and Presentation Committee. To this end, the center/PI must provide all information on the gathering and analysis of data (also raw data, if requested) to the Writing and Presentation Committee at least 1 month prior to the planned submission date.

14. SIGNATURES

The present trial protocol was subject to critical review and has been approved in the present version by the persons undersigned. The information contained is consistent with:

- the current risk-benefit assessment of the study intervention(s)
- the moral, ethical, and scientific principles governing clinical research as set out in the latest relevant version of Declaration of Helsinki and the applicable legal and regulatory requirements

The investigator will be supplied with details of any significant or new finding including AEs relating to treatment with the study intervention(s).

Date: 07/21/15 Signature: _____
Name (block letters): Frank Anton Giordano, MD
Function: Principle Investigator

Date: 07/21/15 Signature: _____
Name (block letters): Frederik Wenz, MD
Function: Co-Principle Investigator

Date: 07/21/15 Signature: _____
Name (block letters): Kevin Petrecca, MD, PhD
Function: Co-Principle Investigator

Date: 07/21/15 Signature: _____
Name (block letters): Grit Welzel, MSc
Function: Biometrician

15. DECLARATION OF PRINCIPLE INVESTIGATOR(S) OF PARTICIPATING CENTERS

I have read the above trial protocol and confirm that it contains all information to conduct the clinical trial.

I pledge to conduct the clinical trial according to the protocol.

I will enroll the first subject only after all ethical requirements are fulfilled. I pledge to obtain written consent for trial participation from all subjects.

I pledge to retain all trial-related documents and source data as described. I will provide a Curriculum Vitae (CV) before trial start. I agree that the CV may be submitted to the responsible EC.

Date _____	**Signature**	_____
	Name (block letters)	_____
	Function	Principle Investigator
	Trial Center (full address and contact)	_____
		_____
Date _____	**Signature**	_____
	Name (block letters)	_____
	Function	_____
	Trial Center (full address and contact)	_____
		_____
Date _____	**Signature**	_____
	Name (block letters)	_____
	Function	_____
	Trial Center (full address and contact)	_____
		_____

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17. APPENDICES

Appendix I.

Highly-effective contraceptive methods

Highly-effective contraceptive methods (this may also apply to the trial subject's partner).

The following contraceptive methods with a Pearl Index lower than 1% are regarded as highly-effective:

- Oral hormonal contraception ('pill') (as far as its efficacy is not expected to be impaired during the trial, e.g. with drugs that cause vomiting and diarrhea, adequate safety cannot be assumed)
- Dermal hormonal contraception
- Vaginal hormonal contraception (NuvaRing®)
- Contraceptive plaster
- Long-acting injectable contraceptives
- Implants that release progesterone (Implanon®)
- Tubal ligation (female sterilization)
- Intrauterine devices that release hormones (hormone spiral)
- Double barrier methods

This means that the following are not regarded as safe: condom plus spermicide, simple barrier methods (vaginal pessaries, condom, female condoms), copper spirals, the rhythm method, basal temperature method, and the withdrawal method (coitus interruptus).

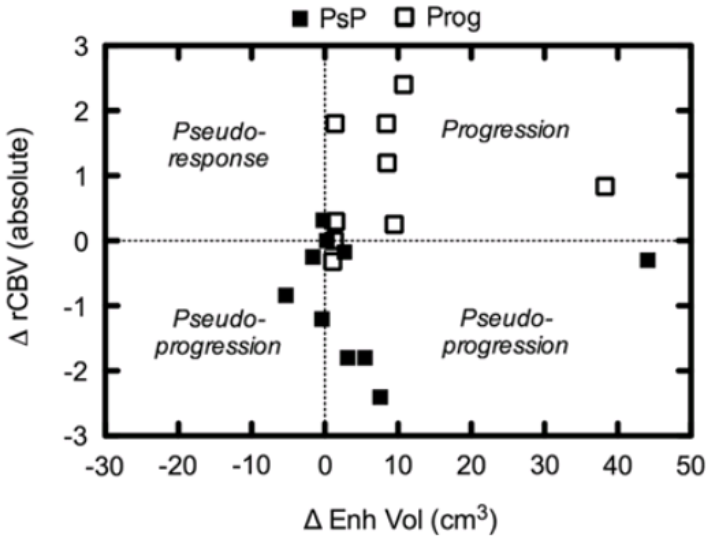
The regulations for contraception are derived from Guideline ICH E8 Chapter 3.2.2.1 Selection of subjects together with ICH M3 Note 4

Appendix II

In case PD is suspected, longitudinal dynamic susceptibility contrast MRI (DSC-MRI) for distinguishing tumor recurrence from pseudoprogression must be performed.

Compliance with American Society of Neuroradiology recommendations for clinical performance of MR DSC perfusion imaging is advised (available as open access paper at http://www.ajnr.org/content/early/2015/04/23/ajnr.A4341.full.pdf)

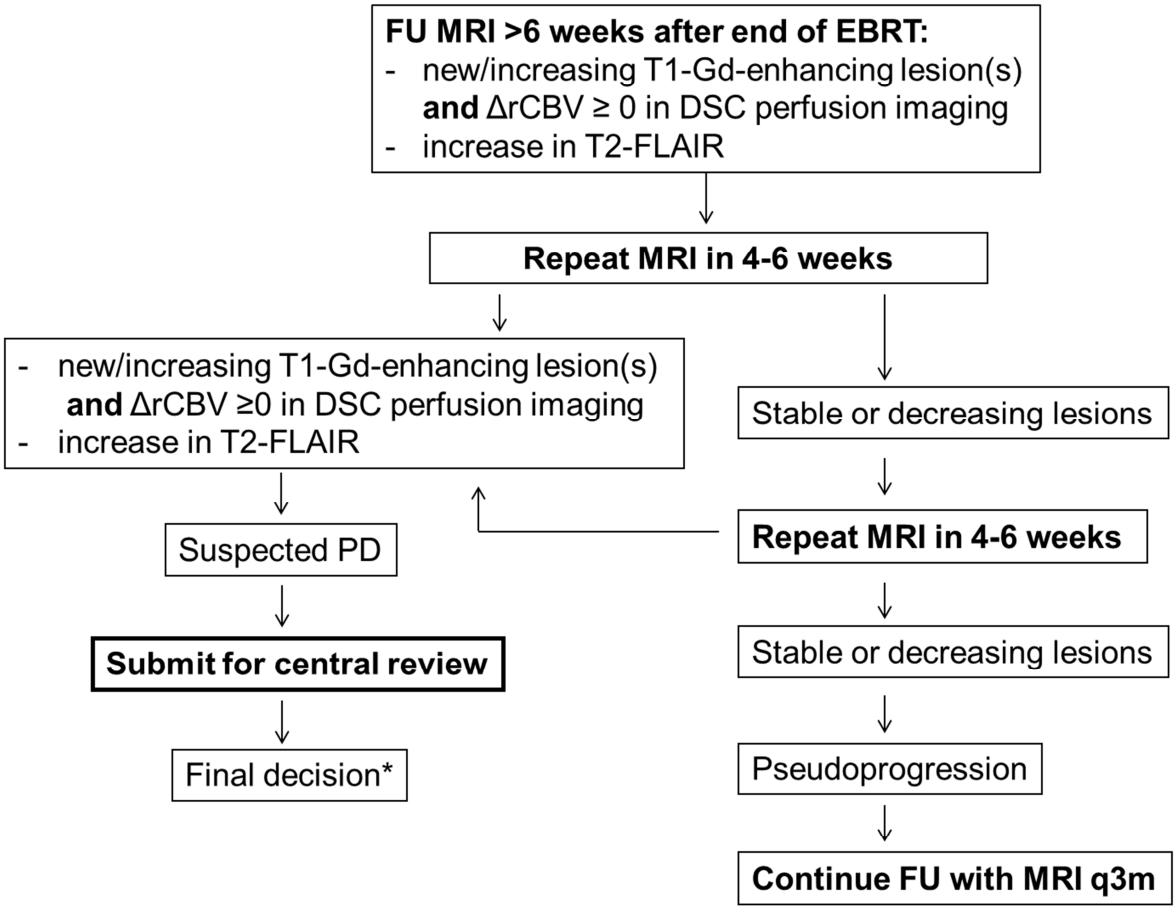
In brief, relative cerebral blood volume (rCBV) maps need to be computed and the absolute change in normalized rCBV from the time at initial progressive enhancement to the first subsequent follow-up must be calculated. Changes in rCBV (∆rCBV) values ≥ 0 indicate tumor progression, whereas ∆rCBV values < 0 indicate pseudoprogression (**Figure A1**). Of note, pseudoprogression may be even seen in lesion that show an increase in volume but a decrease in perfusion over time.



A 1. Longitudinal change in normalized rCBV in Patients with PsP (n = 10) and PD (n = 9). Shown is the change in rCBV (∆rCBV) from time at initial progressive enhancement to first subsequent follow-up versus change in enhancing volume over the same time interval for lesions evolving like PsP (closed squares) and PD (open squares). Initial change in rCBV appears to be helpful for predicting PsP versus PD in the setting of an initial increase in enhancing volume. PD indicates progressive disease; PPX, paclitaxel poliglumex; PsP, pseudoprogression; rCBV, relative cerebral blood volume.

Appendix III

Algorithm for evaluation of follow-up MRI scans.



***may be overruled by results of biopsy or re-surgery**

CENTRAL IMAGE REVIEW CHARTER

Study Title	A MULTICENTER RANDOMIZED PHASE III TRIAL ON INTRAOPERATIVE RADIOTHERAPY IN NEWLY DIAGNOSED GLIOBLASTOMA MULTIFORME
Study acronym:	INTRAGO II
Protocol Version:	V2.1, 15 June 2016
EudraCT No.	NTC02685605
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Sponsor:	Department of Radiation Oncology University Medical Center Mannheim University of Heidelberg Theodor-Kutzer-Ufer 1-3 68167 Mannheim Phone: +49 621 383 4960 Fax: +49 621 383 2476

Author:	John Uiters M.Sc, Imaging Project Manager, Clinflows
Reviewers:	Prof. Whitney Pope, Central Reader, University of California Dr. Alex Förster, Neuro-radiologist, University Hospital Mannheim
Approval:	Prof. Fredrik Wenz, Head Radiation Oncology, University Hospital Mannheim Dr. Frank Giordano, Principal Investigator, University Hospital Mannheim

APPROVAL FORM

CENTRAL IMAGE REVIEW CHARTER

Study Title	A MULTICENTER RANDOMIZED PHASE III TRIAL ON INTRAOPERATIVE RADIOTHERAPY IN NEWLY DIAGNOSED GLIOBLASTOMA MULTIFORME (INTRAGO II)
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SIGNATURES:

Author, John Uiters

Date (dd/mmm/yyyy):_____ Signature:_____

Reviewer, Whitney Pope

Date (dd/mmm/yyyy): 21/Jun/2017 Signature: Wg Pope

Reviewer, Alex Förster

Date (dd/mmm/yyyy): 17/Jul/2017 Signature:

Approver, Frank Giordano

Date (dd/mmm/yyyy): 19/07/2017 Signature:

Approver, Fredrik Wenz

Date (dd/mmm/yyyy): 20/Jul/2017 Signature:

Table of Contents

1. DEFINITIONS OF TERMS

Target lesion	A target lesion is defined as a measurable lesion. If there are multiple measurable lesions at baseline (eg, the post-operative scan), a minimum of the two largest lesions and a maximum of 5 target lesions should be measured. The sum of the products of the perpendicular diameters (SPD) of these lesions should be determined at each timepoint.
Non-target lesion	A non-target lesion is a non-measurable lesion or a measurable lesion that was not categorized by the reader as a target lesion.
Measurable lesions	Measurable disease is defined as bidimensionally contrast-enhancing lesions with clearly defined margins by CT or MRI scan, with two perpendicular diameters (PD) of at least 10 mm, visible on two or more axial slices that are preferably, at most, 5 mm apart with 0 mm skip. In the event the MRI is performed with thicker slices, the size of a measurable lesion at baseline should be two times the slice thickness. Measurement of tumor around a cyst or surgical cavity Lesions around a cyst or surgical cavity are considered non-measurable unless there is a nodular component measuring at least 10 mm in diameter. The cystic or surgical cavity should not be measured in determining response.
Non-measurable lesion	Nonmeasurable disease is defined as either unidimensionally measurable lesions, masses with margins not clearly defined, or lesions with maximal perpendicular diameters less than 10 mm.
Updated RANO	Tumor response determined by radiological response criteria based on the updated RANO criteria (Wen et. al JCO 2010). In summary, target and non-target lesions are evaluated serially, and comparative analysis of changes in the area of contrast enhancement, as well as the non-enhancing component is performed.
Intrago-RANO	Intrago-RANO are based on the updated RANO criteria for the radiological tunor assessments. To discriminate between pseudo-progression and true progression, the following progression parameters were incorporated in the tumor response assessments: - Initial PD requires a confirmatory scan after 4-6 weeks and - the successive scan (confirmatory scan) shows a 25% increase in SPD compared with the nadir. A new enhancing lesion can only constitute to PD if the new lesion is/becomes measurable. - at least 1 lesion has an increase of $\geq$ 25% in rBV compared with the preceding scan (if enhancing disease constitute to PD) - the rBV values of the final scan is $\geq$1 - A new enhancing lesion can only constitute to PD if the new lesion is measurable.

CR	<u>Complete response per updated RANO:</u> Radiological Complete Response requires all of the following: complete disappearance of all enhancing measurable and non-measurable disease sustained for at least 4 weeks; no new lesions and stable or improved non-enhancing (T2/FLAIR) lesions. In the absence of a confirming scan 4 weeks later, this response will be considered only stable disease. <u>Complete response per Intrago-RANO:</u> Same as above.
PR	<u>Partial response per updated RANO:</u> Partial response requires all of the following: 50% decrease, compared with baseline, in the sum of products of perpendicular diameters (SPD) of all measurable enhancing lesions sustained for at least 4 weeks; no progression of non-measurable disease; no new lesions; stable or improved non-enhancing (T2/FLAIR) lesions on same in the absence of a confirming scan 4 weeks later, this response will be considered only stable disease. <u>Partial response per Intrago- RANO:</u> Same as above.
SD	<u>Stable disease per updated RANO:</u> Stable disease occurs if the patient does not qualify for complete response, partial response, or progression. <u>Stable disease per Intrago-RANO:</u> Same as above. However, in case radiological PD was not supported by the rBV progression criteria, the response is SD.
PD	Updated RANO Intrago-RANO: confirmed PD per updated RANO AND contrast enhancing component constitute to PD meets the rBV criteria AND new contrast enhancing lesion should be measurable.
NE	Not Evaluable
New lesion	<u>Updated RANO:</u> appearance of a new enhancing lesion or the appearance of a new non-enhancing lesion Intrago-RANO: appearance of a new **measurable** contrast-enhancing lesion or the appearance of a new non-enhancing lesion
Pseudo-progression	Initial PD was not confirmed by the on-site assessments or by the central reader.

True progression	Confirmation of PD by the central reader per Intrago-RANO
PFS	Progression Free Survival PFS endpoint has been met if the central review confirmed radiological PD. Time of initial PD will be used to calculate the duration of PFS. For non-radiological PD (start salvage treatment, clinical progression), duration of PFS will be calculated based on the event date.
SAP	Statistical Analysis Plan
Radiology eCRF	Electronic Case Report Form The radiology eCRF is an auditable electronic record of information. For this study, the eCRF is directly associated within the imaging review system and is used by radiology reviewers to report the tumor evaluations for each study subject.

2. INTRODUCTION

For the Intrago study, a prospective central imaging review will be performed. The results of the central image review will be conclusive for the patient treatment. The imaging charter describes the imaging workflow and contains a more technical and detailed elaboration of the implementation of the RANO criteria and the incorporation of the rBV values to discriminate between pseudo-progression and true progression.

The imaging charter will be finalized and signed prior start of the image evaluations by the reviewer.

3. CONTRIBUTING PARTIES

Sponsor representative

Frank A. Giordano, MD
Department of Radiation Oncology
University Medical Center Mannheim
University of Heidelberg
Theodor-Kutzer-Ufer 1-3
68167 Mannheim
Phone: +49 621 383 4960
E-Mail: frank.giordano@umm.de

Imaging CRO representative

John Uiters, M.Sc.
Imaging Project Manager, Clinflows
Veerkade 15
3016 DE, Rotterdam
Mobile: +31 (0) 6 3192 9657
E-mail: juiters@clinflows.com

Radiology reviewer

A single radiology read is performed for this study. The following radiology reviewer has been selected by the sponsor to perform the central reads:

Whitney B. Pope, MD, PhD
Department of Radiological Sciences
David Geffen School of Medicine,
University of California
757 Westwood Plaza 1621E
Los Angeles, CA 90095

The central reader will be contracted by the sponsor.

4. SCHEDULE AND LOCATION OF THE CENTRAL REVIEW

Patients will be submitted for central radiology review when the on-site tumor assessments show suspected PD. The central read results will be provided to the site within 5 working days counting from the day that suspected PD was recorded by the site in the EDC and taking into account all required images were uploaded and all pending critical imaging queries are resolved. Hence, the site is instructed to upload the images 2 days after acquisition.

The radiology reviewer will be provided with a local workstation to allow image review at the reviewer's facility: 12719 Woodgreen St., Los Angeles, CA 90066.

It is anticipated that the radiology review will start in Q2_2017. The central imaging review for this study is completed if all randomized patients have reached the PFS endpoint which is anticipated by December 2020.

Clinflows will have remote access to the readers' workstation to coordinate the central review process.

4.1 Central Reader Back-Up Plan

The central reader committed to complete a read case within 3 working days after assignment. In case of any significant delays to complete a read case (eg, due to sickness, logistical considerations, etc), an ad-hoc procedure will be implemented. The ad-hoc procedure will imply that a neuro-radiologist from the University of Heidelberg, who is the study contact person for all imaging related questions, will perform the review:

Alex Förster, MD

Neuro-radiologist, Dept. of Neuroradiology

Mannheim Medical Faculty, University of Heidelberg

Phone: +49 621 383-2443 (Secretary)

Mail: Alex.Foerster@umm.de

Dr. Alex Förster will be granted access to the local workstation located at the central reader facility. A separate reader account will be created in Mint Lesions and the corresponding read cases that are delayed will be assigned to Dr. Alex Förster. Dr. Alex Förster will perform the central review in accordance with the read methodology described in the imaging charter. The read results will be communicated to the site which will be conclusive to decide if the patient has reached PD based on the radiological image evaluations. The central reader, Dr. Whitney Pope, will repeat the evaluations which will imply that the central read results in the DB will originate from a single reader. Any discrepancies between both readers will be discussed case-by-case.

5. ACQUISITION AND POST-PROCESSING GUIDELINES

5.1 Imaging Timepoints

Per protocol, tumor imaging will be performed at the following time points:

Tumor Imaging Timepoint	Timepoint label	Timepoint window
Visit 2	Post-operative MRI	2-3 days after surgery
Visit 7	Follow-up MRI	6 weeks after EBRT
Visit => 8	Follow-up MRI	Quarterly thereafter until endpoint PFS

If at a scheduled tumor imaging initial PD is observed, the site is required to acquire a confirmatory scan. The confirmatory scans will be acquired in accordance with the following algorithm:

Tumor Imaging Timepoint	Timepoint window
Confirmatory scan A	4-6 weeks after initial PD
Confirmatory scan B	4-6 weeks after confirmatory scan A (may coincide with the scheduled tumor imaging timepoint)

- If the confirmatory scan A confirms PD, the patient will be submitted for central review.
- If the confirmatory scan A shows non-PD, another confirmatory scan is required. This confirmatory scan B will be acquired 4-6 weeks after confirmatory scan A
- If the confirmatory scan B shows PD, the patient will be submitted for central review.
- If the confirmatory scan B show non-PD, the initial PD is considered a pseudo-progression case. The site can continue pt treatment and there is no need to submit the patient for central review. If at any of the successive tumor imaging timepoints PD is observed, the confirmatory scan algorithm is repeated.

- **Note 1**: in case PD is observed based on clinical deterioration, no confirmatory scans are required. The site assessments will be conclusive that the patient has reached the PFS endpoint. However, the case will be submitted for central review to ensure that central image review will be available for analysis for all patients. This read will not be time-critical.
- **Note 2:** in case PD is based on a new lesion outside the irradiation field, the site is not required to perform a confirmatory scan. If the central review confirms the presence of the new lesion, the patient has reached the PFS endpoint.
- **Note 3:** the optional treatment planning MRI is not considered as a scheduled tumor imaging timepoint. However, when unequivocal progression is observed based on this scan, the patient is considered having reached the endpoint PFS based on the on-site assessments. However, the case will be submitted for central review to ensure that central image review will be available for analysis for all patients but the central read will not be time critical.
- **Note 4:** The central reader will not be aware which of the above-described scenarios is applicable.

5.2 Tumor image Acquisition Guidelines

Each center will acquire the images per standardized brain tumor protocols and preferably import these directly to the scanners from the following reference:

http://cvib.ucla.edu/standardized-brain-tumor-mri-protocols/.

At each tumor imaging assessment, at least the following series will be acquired:

- T2
- T2-FLAIR
- 3D T1 volumetric imaging
- DSC

Perfusion imaging will be performed by the sites in accordance to ASFNR recommendations for clinical performance of MR-DSC perfusion imaging:

(http://www.ajnr.org/content/early/2015/04/23/ajnr.A4341.full.pdf).

5.3 On-site Post-Processing of the DSC Sequence

The site will perform the post-processing step in accordance with the local routine practice. The rBV values are required for the on-site tumor evaluations but these do not need to be provided for central review as the central reviewer will repeat the post-processing step.

5.4 Central Post-Processing of the DSC Sequence

Olea Sphere is used as post-processing software by the central reviewer. The following steps will be performed for each timepoint that requires a post-processing step:

1) The central reader will import the timepoint from the Olea DB. The Olea DB will have a separate folder for each patient that contain all submitted images. The file name of an image stored in the Olea DB has the following syntax: "Intrago_patient-id_timepoint_date submission". To match a timepoint reviewed in Mint lesions with the Olea DB, the central reader should display the image Dicom tags in Mint Lesions (right click in "serie"). The dicom tag "patient name" contains the patient-id and timepoint label (eg, 001-01_w7). The reader can now select and import the correspond image in Olea from the Olea DB. See for detailed instructions/screenshots appendix I.
2) When importing the image, the reader will select which series should be included in the import in case the software not automatically has selected the required sequences. Please refer to appendix I for detailed information/screenshots.
3) The default settings for the post-processing will be used without any customizations.
4) In the analysis mode, a selection of the parametric maps is made. In the analysis mode, the anatomical T1 post-contrast, the corrected rBV and the rBV are displayed. Please refer to appendix I for detailed information/screenshots.
5) The ROIs are placed on the anatomical image (T1 post-contrast) for the relevant contrast-enhancing lesions identified in Mint Lesions. The central reader will exclude any necrotic tissue from the analysis.

6) A reference ROI is placed on the contralateral site. The reference ROI will have a minimal size of 10x10 mm.
7) Olea Sphere will automatically calculate the normalized corrected rBV values. The corrected normalized rBV values are entered in Mint Lesions at the comment section of the corresponding lesion (rBV = 1.9, ROI#) including the ROI number. Please refer to appendix I for detailed information/screenshots.
8) Prior closing the post-processing, the central reader will run the PDF report that contains all parametric maps, the ROIs and the ROI statistics and will file the report at the corresponding patient in the Olea DB (D:/Olea DB/Patient id/results). The post-processed image can now be closed and automatically saved. Please refer to appendix I for detailed information/screenshots.

5.4.1 Request for Re-Scans

Clinflows will not request for any repeat scans in case of significant quality issues. Images of suboptimal quality will be included in the central read and it remains to the discretion of the reviewer to decide if the image can be used for tumor response.

If imaging sequences are missing, the site will be informed to resubmit these. However, if these sequences were by error not performed, the site will not be required to repeat the scan.

6. SITE QUALIFICATION, SITE TRAINING AND IMAGE COLLECTION

6.1 Site qualification

Sites were identified by the sponsor. The site qualification process is described in the following functional plan:

- Site qualification and central monitoring guidelines, effective date: 10 October 2016

Qualification visit reports were prepared by the sponsor representative and are archived in the TMF.

6.2 Site training

Site training is performed by the Clinflows representative at the site initiation visit. The site initiation visit procedures are described in the following functional plan:

- Site qualification and central monitoring guidelines, effective date: 10 October 2016

The imaging logistics and the tumor response evaluations are part of the site training. For each site initiation visit, a report will be prepared and filed in the TMF.

6.3 Image Collection

Sites will use the imaging portal (decidemedical) to transfer images to Clinflows. Clinflows operates for decidemedical a certified quality management system based on ISO 9001:2008 (TÜV Süd).

The person at the site that is responsible for the imaging logistics will receive an email notification with a code that is required to create a log-in account for the imaging portal. For this study, only images in DICOM format should be uploaded. Images can be uploaded by the "advanced uploader" or by the "standard uploader". The advanced uploader requires JAVA (1.7 or higher). If the site cannot run JAVA, the images scan be uploaded by compressing (zip/rar) the content and then select the standard uploader. An overview of the browsers that support JAVA is listed below:

Browser	Version	JAVA supported	Standard Uploader	Advanced Uploader
Chrome	all	No	Yes	No
Firefox	v51	Yes	Yes	Yes
Firefox	≥ v52	No	Yes	No
Safari	≥ v10	Yes	Yes	Yes
Explorer	≥ IE9	Yes	Yes	Yes
Edge	all	No	Yes	No

For each site, a training will be scheduled where a demo of the imaging portal is provided. Slides of the site training are available on the imaging portal for further reference (link: decidemedical.com; folder: Documents)

6.3.1 Pseudo-anonymization

Prior uploading any DICOM images, the site should ensure that the images have been de-identified in compliance with the local patient privacy rules. Prior the upload, the site should ensure that the DICOM tag "PatientID (0010,0020)", contains the patient number as assigned in the EDC (Castor) for the Intrago study. The following DICOM tags do not need to be anonymized by the sites:

DICOM tag	Entry
0010-0020, PatientID	Patient study number (EDC)
0010,0040, PatientSex	Actual
0010,1010, PatientAge	Actual
0010,1030, PatientWeight	Actual
0010,1020, PatientSize	Actual

The DICOM tags that will be automatically anonymized for each image by the upload tool (decidemedical) are described in appendix II. The file name of the uploaded image will be assigned by the system using the following syntax: *"Intrago_patient id_timepoint_date upload".* The file name will be used by the central reader for selecting the images that needs to be analyzed in the post-processing software (Olea Sphere).

6.4 Image quality checks

Images will be uploaded in an ongoing manner by the site and upon receipt of the image, an administrative image QC will be performed:

- Readability of images
- All required series available
- Anatomical coverage and number of slices
- Correct patient identifiers
- Correct scan date
- No annotations or markings
- Correct pseudo-anonymization

The QC step is performed within decidemedical using the integrated preview tool and by generating an overview of all dicom tags. Query handling/tracking and requests for due images are done via decidemedical. If an image has passed the QC step, which is recorded in decidemedical, the image will be transferred to the image DB on the local workstation of the central reader.

7. PATIENT INCLUSION IN CENTRAL REVIEW

Approximately 314 randomized patients will be enrolled in this study. All randomized patients who completed the study will be included in the central review. A patient has completed the study when reaching the PFS endpoint. Patients who discontinued the study will not be submitted for central review.

The central read results will be conclusive for the patient treatment and the central reads are therefore time critical with exception of the following scenarios:

1) Patient reached the PFS endpoint based on clinical progression (tumor related)
2) Patient reached the PFS endpoint based on the unequivocal progression of the optional treatment planning MRI
3) Patient reached the PFS endpoint based on initiation of salvage treatment

In addition, patients will be included in the radiology review regardless:

1) The reporting of any violations concerning imaging related inclusion/exclusion criteria
2) Permanent discrepancies with the imaging acquisition guidelines
    a. Missing anatomies
    b. Missing sequences
    c. Inconsistent/incorrect acquisition parameters

Hence, it will remain to the discretion of the independent reviewer to decide if the patient/timepoint can be evaluated with the available images despite any observed deficiencies during the QC process that could not be resolved. If a timepoint is considered not evaluable (NE), the reviewer will document the rationale for NE in the radiology eCRF.

The central reader will not be aware which of the scenarios described above are applicable for the assigned read case.

8. IMAGE PREPARATION FOR CENTRAL REVIEW

8.1 Reconciliation with the Clinical Database

Incoming images are tracked by Clinflows via the imaging portal. The imaging tracking database is reconciled with the corresponding fields in the clinical database. Consistency between the two databases include the following fields:

- Patient identifiers
- Missing imaging timepoints
- Scan date
- Age

Any observed discrepancies between the imaging tracking database and the clinical database will be queried to the site. Critical queries should be resolved prior the patient can be submitted for central review.

8.2 Prior Radiotherapy

Information on prior radiotherapy is redundant for the tumor evaluations. Target lesions can be located in an irradiated field. In accordance with updated RANO, these lesions are evaluable for response.

8.3 Image Review Methodology

Two software tools are used for the tumor assessments; Mint Lesions and Olea Sphere. Mint Lesions will be used for the identification of the contrast-enhancing lesions and the non-contrast enhancing lesions. Olea Sphere is used for the post-processing of the DSC sequence to generate the rBV values. rBV values will be generated for contrast enhancing lesions that contributed to PD as timepoint response per updated RANO.

The central reader will start the tumor response assessments in Mint lesions. The reviewer is not blinded for patient and timepoint. The imaging timepoints are reviewed and displayed sequentially based on scan date.

The read will start with the review of the "baseline" scan (post-operative MRI) by identifying the presence of any target and/or non-target lesions. Once the reviewer has completed the review of the baseline scan and sign-off for completion, the successive timepoint is displayed. This workflow is continued until all timepoints have been reviewed. Hence, the preceding scans are available as reference including the identified lesions but no changes can me made retrospectively. If the reader needs to make a change on a previous evaluated timepoint, upon request Clinflows can re-open the case via the so-call "data change process" that is described in detail in the Imaging Operational Manual.

Mint Lesions automatically calculate the timepoint response per updated RANO. If the timepoint response is PD, the central reader will perform a post-processing (prior sign-off the timepoint) step to generate the normalized corrected rBV values of the corresponding contrast-enhancing lesions that constitute to PD. The rBV values are entered in Mint Lesions at the comment section for the lesion of concern. If the response criteria including the rBV values do not support PD as timepoint response, the central reader will manually overrule the timepoint response in Mint Lesions, and subsequently will sign-off the timepoint. A response table is available in Mint with all the individual assessments that supports the reader to decide if the default timepoint response (per updated RANO) should overruled. Please refer to appendix II for detailed information/screenshots.

The central reader should continue the review of a read case until all available timepoints have been evaluated. This as well implies if PD was confirmed and there are any successive scans available

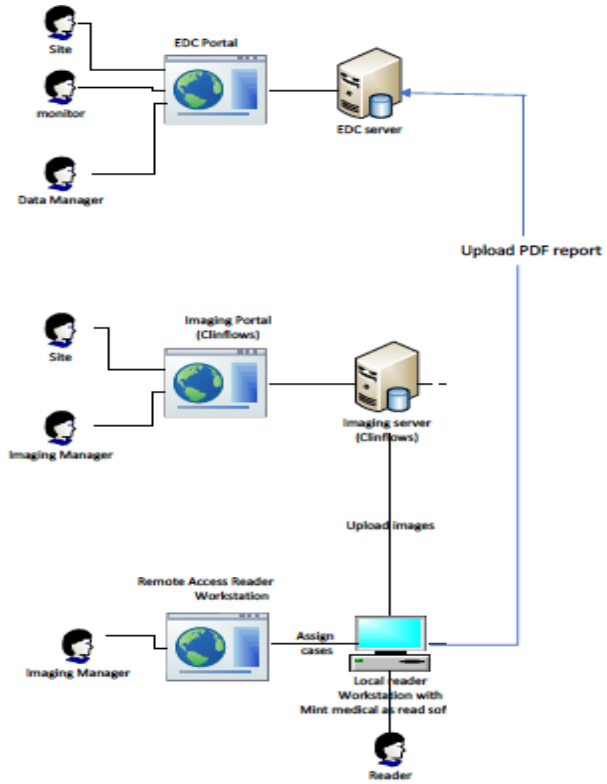
Although the sites should schedule a confirmatory scan after initial PD, the central reader will not know which timepoint was performed as a confirmatory scan. For the tumor response assessments, this is not of concern as the tumor load will be compared with baseline/nadir and the increase of the rBV value is calculated in reference to the preceding scan.

9. SOFTWARE USED FOR CENTRAL REVIEW WORKFLOWS

The systems that are used in relation to the central review workflows are listed below:

Activity	System	Provider
Upload images	decidemedical	Clinflows
Image QC	decidemedical	Clinflows
Image query handling	decidemedical	Clinflows
Storage/archiving images	Server	ovh
Central Review (updated RANO)	Mint Lesions	Mint Medical
Post-processing (rBV)	Olea Sphere	Olea Medical
Remote access reader workstation	GoToMyPC	Citrix
Import central read results in EDC for site acknowledgement	Castor	Ciwit

9.1.1 IT Infrastructure for the imaging handling and central review



Clinflows has established the IT infrastructure for the Intrago study including the EDC, the image upload tool and the central read system. In brief, all acquired images are submitted to Clinflows in an ongoing manner and are transferred after QC to the local workstation from the central reader. The site will record in the EDC when a patient has suspected PD which will trigger the central read. A read case will be remotely assigned to the central reader and upon completion of the read, the read results will be uploaded in the EDC for the site acknowledgement.

9.1.2 Back-ups system of the local workstation

Mint Lesion

By default, every night a job is executed that creates a full dump of the database and stores this to C:\mint\backup. A nightly back-up to the PostgreSQL database server is performed.

The back-up files, are regularly checked:

- the modified date
- the file size for plausibility
- revision history

Olea Sphere

Each timepoint that is post-process, will be saved in Olea Sphere so the source data is available for reference. In addition, a PDF report is generated for each post-processing step including the parametric maps, the ROIs and the ROI statistics (corrected rBV, normalized rBV). The PDF reports at filed at the local workstation and a back-up of this directory is done nightly to the Clinflows server. This PDF report is considered as the source for this study.

10. CENTRAL REVIEW ENDPOINTS AND EVALUATIONS

10.1 Endpoints

The following imaging related efficacy endpoints are defined for this study:

- Progression Free Survival (PFS)
- PFS within a 1 – 2 cm margin around the cavity
- Response with respect to strata:
    - Age,
    - KPS,
    - Thickness of anticipated T1-Gd-enhancing (remaining) tumor margin

The imaging endpoint analysis is described in the protocol and will be further elaborated in the Statistical Analysis Plan (SAP).

10.2 Evaluations by the Central Reader

The central review will include the following evaluations:

- Radiological response per updated RANO
    - Response measurable contrast enhancing lesions
    - Response non-measurable contrast enhancing lesions
    - Response non-enhancing lesions
    - Presence new contrast enhancing lesions
    - Presence new non-enhancing lesions
- Post-processing step to generate the rBV values of the corresponding measurable contrast-enhancing lesions
- Timepoint response assessments per updated RANO including the rBV criteria

11. INDEPENDENT RADIOLOGY ASSESSMENTS

11.1 Check of Patient Status at Baseline

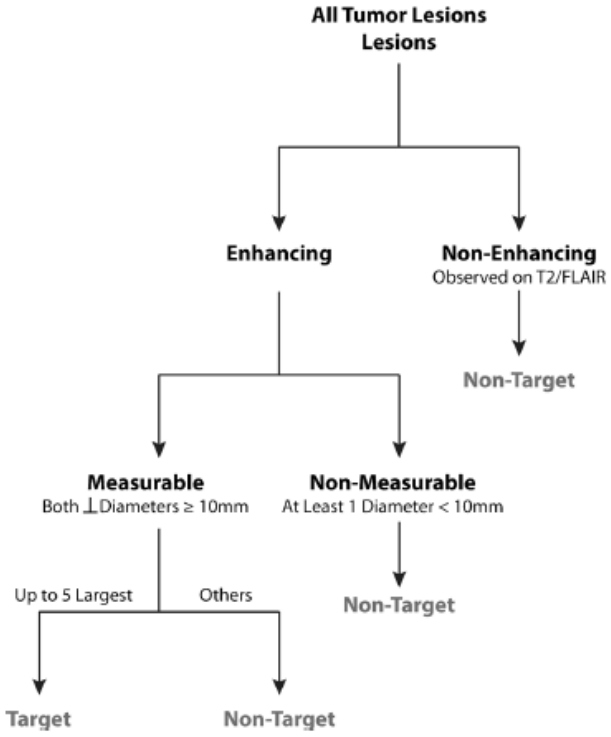
The central reviewer will not evaluate any imaging related inclusion or exclusion criteria. The following imaging related inclusion/exclusion criteria are verified by the site:

- IC: Supratentorial T1-Gd enhancing lesion(s) amenable to total resection
- EC: Multifocal disease or non-resectable satellite lesions

The thickness of anticipated T1-Gd-enhancing (remaining) tumor margin is visually assessed by the neurosurgeon and recorded in the EDC as stratification parameter for the randomization.

11.2 Definition of Radiology Response Criteria

Tumor response will be determined by radiological response criteria based on the updated RANO criteria (Wen et. al JCO 2010). The RANO criteria distinguish between measurable and non-measurable contrast-enhancing lesions. Measurable lesions are defined as bi-dimensional measurements (long axis / short axis) with clearly defined margins by CT or MRI scans and can be entitled either as target or non-target lesions. Non-measurable lesions are defined as unidimensionally measurable lesions, masses with margins not clearly defined or lesions with maximal perpendicular diameters less than 10mm and can be entitled as Non-target lesions. Furthermore, Non-target lesions are divided into contrast-enhancing and non-contrast-enhancing categories. The contrast-enhancing lesions are evaluated on T1-weighted scans. For the non-contrast-enhancing (pathological) non-target lesions, T2/Flair weighted scans are used for evaluation. Target and non-target lesion assessments by the central reader are depicted below:



To discriminate between pseudo-progression and true-progression, study specific adaptations to the updated RANO were defined that include the incorporation of the normalized corrected rBV value as additional progression parameter and the confirmation of PD. The customizations to RANO are referred in this document as "Intrago-RANO". These customized response criteria are described in more detail in the remaining part of this chapter.

11.2.1 Measurable Contrast-Enhancing Lesions

Radiological Progression (measurable contrast-enhancing lesions):

In case measurable contrast enhancing lesions are present at the post-operative MRI scan, progression is defined by 25% increase in sum of the products of perpendicular diameters (SPD) of the contrast-enhancing lesions in any follow-up scan compared with the nadir.

Furthermore, progression based on an increase of SPD should be associated with an increase of at least 1 lesion of ≥ 25% in rBV to qualify for PD. Furthermore, the rBV value of the final scan should be ≥1 before any delta rBV can be considered as a valid progression parameter. If, under these given constraints, there is no increase of ≥ 25% in rBV, the response for non-measurable lesions is SD.

If these tumor response criteria for the contrast-enhancing lesions result in initial PD, the successive scan should meet the above PD criteria again before the patient can be considered having reached the PFS endpoint:

- the successive scan (confirmatory scan) shows a 25% increase in SPD compared with the nadir
- at least 1 lesion has an increase of ≥ 25% in rBV compared with the preceding scan
- the rBV values of the final scan is ≥1

If there is an increase of SPD ≥ 25% and the rBV values of the enhancing lesion do not qualify for progression (per Intrago-RANO), but at the next scan show progression, the date of progression is the date of the prior scan. In the example below, date of progression is the date of follow-up 2.

If after initial PD, no successive scan is present, the patient will have an unconfirmed PD. This will imply that progression is not confirmed by the central reader and that the site can remain the patient in the study as the PFS endpoint has not yet been reached. Example:

	Baseline	Follow-up 1	Follow-up 2	Follow-up 3	Follow-up 4
Target lesion	present	SPD < 25%	SPD ≥ 25%	SPD ≥ 25%	SPD ≥ 25%
rBV	na	(0.6)	0.9	1.5	2.0
Delta rBV	na	na	≥ 25%	≥ 25%	≥ 25%
RANO	na	SD	PD	PD	PD
Intrago-RANO	na	SD	SD	PD (initial)	PD (confirmed)

Radiological Stable Disease (measurable contrast-enhancing lesions):

Stable disease occurs if the patient does not qualify for complete response, partial response, or progression. If radiological PD is not associated with an increase in rBV, the response of the measurable contrast enhancing lesion should be SD.

If there is an increase of ≥ 25% in rBV (with rBV values of at least 1) between two successive time points for at least 1 single lesion, while SPD shows SD, the increase in rBV alone would not count as progressive disease and the response remains SD.

Radiological Partial Response (measurable contrast-enhancing lesions):

In case measurable contrast enhancing lesions are present in the post-operative MRI scan, Partial Response requires at least a 50% decrease in successive scans, compared with the post-operative MRI, in the sum of products of perpendicular diameters (SPD) of all measurable enhancing lesions. If there is an increase of ≥ 25% in rBV (with rBV values of at least 1) between two successive time points for at least 1 single lesion, while SPD shows PR, the increase in rBV alone would not count as progressive disease and the response remains PR.

Radiographic Complete Response (measurable contrast enhancing lesions):

In case measurable contrast enhancing lesions are present at the post-operative MRI scan, Complete Response requires complete disappearance of all enhancing measurable lesions.

11.2.2 **Non-measurable Contrast-Enhancing Lesions**

Radiological Progression (non-measurable contrast-enhancing lesions):

In case non-measurable contrast enhancing lesions are present at the post-operative MRI scan, initial progression (per Intrago-RANO) occurs when the following conditions are met:

- At least one non-measurable contrast-enhancing lesions became measurable and increased significantly in size.
- at least 1 lesion has an increase of ≥ 25% in rBV compared with the preceding scan
- the rBV values of the final scan is ≥1

Progression for the non-target lesion(s) is confirmed if the successive scan shows PD based on the same criteria. The time point response will be SD if the rBV criteria for progression are not met. Example:

	Baseline	Follow-up 1	Follow-up 2	Follow-up 3	Follow-up 4
Non-target lesion	present	present	unequivocal progression	unequivocal progression	unequivocal progression
rBV	na	(0.5)	0.6	1.5	2.0
Delta rBV	na	na	< 25%	≥ 25%	≥ 25%
RANO	na	SD	PD	PD	PD
Intrago-RANO	na	SD	SD	PD (initial)	PD (confirmed)

If there is "unequivocal progression" and the rBV values of the enhancing lesion(s) do not qualify for progression, but at the next scan show progression, the date of progression is the date of the prior scan.

Radiographic Stable Disease (non-measurable contrast enhancing lesions):

Stable disease occurs if the patient does not qualify for complete response or progression. If radiological PD is not associated with an increase in rBV, the response of the non-measurable contrast enhancing lesion should be SD.

If there is an increase of $\geq$ 25% in rBV between two successive time points for at least 1 single lesion, and the non-measurable contrast enhancing lesions are SD (per RANO), the increase in rBV alone would not count as progressive disease and the response remains SD.

Radiographic Complete Response (non-measurable contrast enhancing lesions):

In case non-measurable contrast enhancing lesions are present at the post-operative MRI scan, Complete Response requires complete disappearance of all enhancing non-measurable lesions.

11.2.3 Non-Enhancing Lesions

Radiological Progressive Disease (non-enhancing lesions):

In case non-enhancing lesions are present at the post-operative MRI scan, Unequivocally Worsening is defined as a significant visual increase of one of the non-enhancing lesions. Note, the rBV values are not of concern for defining progression of the non-enhancing lesions. The status Unequivocally Worsening will constitute to PD and the patient has reached the PFS endpoint. Progression based in unequivocally worsening of a non-enhancing lesion requires no confirmation at a successive scan.

Radiological Stable Disease (non-enhancing lesions):

In the situation non-enhancing lesions are present at the post-operative MRI scan, Stable Disease Is defined as visual stable or improved non-enhancing (T2/FLAIR) lesions.

Radiological Complete Response (non-enhancing lesions):

In case non-enhancing lesions are present at the post-operative MRI scan, Complete Response is defined as resolution of all T2/FLAIR signal abnormality attributable to bulk tumor. This does not require resolution of T2/FLAIR signal abnormality attributable to gliosis, radiation effect, infection, ischemia, etc.

11.2.4 **New Lesions**

New contrast-enhancing lesions

A single new contrast-enhancing lesion occurring after the post-operative scan will constitute to progression if the new lesion has become measurable and has an increase of rBV $\geq$ 25% compared with the preceding scan. Furthermore, the rBV value of a new lesion should be $\geq$1 before the delta rCBV can be taken into account as a valid progression parameter. If the new lesion does not meet the criteria for PD, the lesion status is SD.

The patient has reached the PFS endpoint if at the successive scan progression is confirmed (same response criteria apply as above.

If a new contrast enhancing lesion does not qualify for progression based on the above-mentioned criteria, but at the next scan meet the criteria for true progression, the date of progression is the date of the prior scan.

New contrast-enhancing lesions located outside the irradiation field

The occurrence of a new measurable contrast-enhancing lesion located outside the irradiation field by default qualifies for PD. No confirmation is required for PD at a successive scan nor the response criteria defined for the rBV values needs to be applied.

New non-enhancing lesions

The appearance of any new non-enhancing lesions will constitute to progression. The patient has reached the PFS endpoint and the presence of a non-enhancing lesion requires no confirmation at a successive scan.

12. RESPONSE ALGORITHM MINT LESIONS (RANO)

The read software will provide the sum of the product of the PD for all lesions. In addition, for each target and non-target lesion and new-measurable lesion, the lesion status at the corresponding timepoint will be assigned by the reviewer.

- Presence
- Disappeared
- Not evaluable
- New

If a lesion has not been measured and/or was not assigned as "disappeared" or "Not evaluable", the status "not assessed" is assigned by default by the read software. This lesion status will result in a query during the QC process as this status most likely represents a reader error.

12.1 Response Algorithm for the Target Lesions

Condition criterion measureable contrast enhancing lesions (Targets)	Response
No target lesions defined at baseline	"Undefined"
>= 25% increase of SPD compared to nadir	Progressive disease
Reappearance of at least one target lesion (previous target response was CR)	Progressive disease
At least one target lesion state is "Not evaluable"	Not evaluable
SPD equals 0.0 mm^2 (all target lesions have state "disappeared"	Complete response
>= 50% decrease of SPD compared to baseline	Partial Response
All other cases	Stable disease

12.2 Response Algorithm for the Non-Targets (contrast-enhancing lesions)

Condition criterion non-measureable contrast enhancing lesions	Response
No non-targets enhancing lesions identified at baseline	"Undefined"
At least one non-target lesion shows "unequivocal progression"	Progressive disease
Reappearance of at least one non-target enhancing lesion if previously evaluable non-target enhancing response was CR	Progressive disease
At least one non-target enhancing lesion state is "Not evaluable"	Not evaluable
All non-target enhancing lesions have state "Disappeared"	Complete response
All other cases	Stable disease

12.3 Response Algorithm for the Non-Targets (non-enhancing lesions)

Condition criterion non-enhancing lesions	Response
No non-enhancing lesions identified at baseline	"Undefined"
At least one non-enhancing lesion shows "unequivocal progression"	Progressive disease
Reappearance of a non-enhancing lesion that had state "disappeared" previously	Progressive disease
At least one non-enhancing lesion state is "Not evaluable"	Not evaluable
All non-target enhancing lesions have state "Disappeared"	Complete response
All other cases	Stable disease

12.4 Response Algorithm Overall Timepoint Response

Timepoint response will be assigned by the read system in accordance with the following response table below:

Target enhancing Response	Non-target enhancing response	Non-target non-enhancing response	New lesion present	Timepoint response Updated RANO
PD	any	any	any	Progressive disease
any	PD	any	any	Progressive disease
any	any	PD	any	Progressive disease
any	any	any	Yes	Progressive disease
CR	CR or undefined	SD or undefined	No	Complete response
CR	SD	SD or undefined	No	Partial response
PR	Non-PD	SD or undefined	No	Partial response
undefined	Non-PD	SD or undefined	No	Stable disease
All other cases				Stable disease
At least one "Not evaluable"				Not evaluable

12.5 Timepoint Response Table per Intrago-RANO for Contrast-Enhancing Lesions

Timepoint response RANO	At least 1 target lesion with ≥ 25% increase in rBV with an rBV values of ≥1	At least 1 measurable non-target lesion (CE) with ≥ 25% increase in rBV with an rBV values of ≥1	At least 1 new measurable lesion (CE) with ≥ 25% increase in rBV with an rBV values of ≥1	Timepoint response Intrago-RANO
PD	yes	any	any	PD
PD	any	yes	any	PD
PD	any	any	yes	PD
PD	no	no	no	SD
PD[1]	any	any	any	PD
SD	any	any	any	SD
PR	any	any	any	PR
CR	any	any	any	CR
Not evaluable	any	any	any	Not evaluable
PD	Not evaluable	Not evaluable	Not evaluable	Not evaluable

The reviewer will be able to overrule the assigned RANO timepoint response by the read software. If the reviewer changes the timepoint's response, the reader will need to record the rationale in the radiology eCRF. Once a timepoint is signed-off for approval, no changes can be made anymore by the reader.

[1] PD based on a new lesion outside the irradiation field

12.6 Reader Rules in Addition to the Response Criteria

1) If the timepoint response is PD per updated RANO and no pMRI sequence is available, the timepoint response is Not Evaluable (NE). However, if no successive scans were acquired, the patient will be considered as "True progression".
2) Until new contrast enhancing lesions becomes measurable, the new lesions should be recorded under "finding" at the eCRF. Only when the new lesions become measurable, the lesion should be captured in Mint Lesions under the eCRF category "new lesions". The response algorithm in Mint lesions will ignore any recording captured under "finding".
3) If the non-target lesions do not individually qualify for unequivocal progression, but as entity the non-targets should be considered as unequivocal progression, the lesion status for the individual lesions should be "unequivocal progression".
4) The read software does not allow to retrospective changes once the timepoint(s) have been signed off by the reader. However, if the reader concludes during the review a change needs to be made, Clinflows will be informed by providing the rationale for re-opening the read case to allow this change. This process will be documented in the audit trail.
5) For target lesions, the lesion state "Too small to measure" should be assigned if the lesions becomes smaller than 5x5mm
6) In case of multiple non-target lesions present, the reader can group the lesions and evaluate the grouped lesions accordingly.
7) If progression is confirmed (two timepoints with PD per Intrago-RANO), the reader nevertheless should continue the reads until all available timepoints have been reviewed.
8) If no disease is present at baseline and no disease is present at the successive follow-up timepoints, the default timepoint response is "undefined". The reader should manually change the timepoint response for these cases in SD.
9) Any scenarios that may occur during the central read that are not clarified in the imaging charter will be discussed case-by-case between the reader, the Clinflows representative and the sponsor representatives. The outcome of the discussions will be documented and when required, the imaging charter will be revised.

13. REVIEWER TRAINING

13.1 Training Session

The central reader and the reader back-up will be trained on the read software and the read processes. This training will be performed shortly before the actual reads are expected. In addition, the reader will be provided with the imaging charter, the protocol, the manual for Olea Sphere and the manual for Mint Lesions.

13.2 Test Cases

As part of the reader training, 2 test cases will be completed by the reviewer. If the test cases demonstrate the reader is familiar with the read software and the read processes, the central read can start.

14. CONTROL OF INTRA-REVIEWER VARIABILITY

No intra-reader variability assessments will be performed for the Intrago study.

15. CENTRAL REVIEW DATABASE, QUALITY CONTROL AND TRANSMISSION TO SPONSOR

The Data Management handling procedures for the imaging data are described in the Data Management Plan. All read results will be imported as a PDF file in the EDC for the site to acknowledge the read results.

Mint Lesions: the originating "raw" reviewed images including the measurements can be made available as "secondary Dicom" as part of the study file. The data entered in the radiology eCRF will be exported as XML file. The XML file can be imported in SAS for further data analysis required for the publication.

Olea Sphere: the post-processed images are saved upon completion in the system and are available for future reference. A PDF report will be generated for each post-process image containing the parametric maps, the ROI placements and the ROI statistics. These PDF report will be part of the study file.

A quality control step will be performed to verify if the rBV value has been correctly entered by the central reader in Mint Lesions at the corresponding lesion.

16. ANALYSIS

Analysis of the independent review results is described in the statistical section of the protocol. Additional analysis and the development of any TFLs will be described in the SAP.

17. DOCUMENT REVISION HISTORY

Initial version.

18. REFERENCES

1) Study Protocol
2) Manual Mint Lesions
3) Manual Olea Sphere

APPENDIX I: READER MANUAL

Reader Manual

Introduction

The reader manual includes screenshots of the study specific workflow showing each step in the central read process. In addition, the user manual from Mint Lesions and from Olea Sphere are available for detailed information on the features of the read software. The manuals can be opened within the software. In case of specific questions, the reader can contact directly the application specialists:

Mint Medical
Kelie H. Luby, VP Clinical Trials Software
Email: k.luby@mint-medical.com
Phone: (+1) 844 200 6468 ext 1 (timezone:EST)
Mobile: (+1) 860 918 7016

Olea Medical
Jeremy Gros-Frauca, application specialist
Email: jeremy.gros-frauca@olea-medical.com
Phone: +33 4 42 71 24 20 (timezone: CET)
Mobile: (+33) 6 5237047

For all communication with the application specialist, the project manager from Clinflows should be copied. In case of any issues occurring during the read, contact directly John Uiters via email or phone:

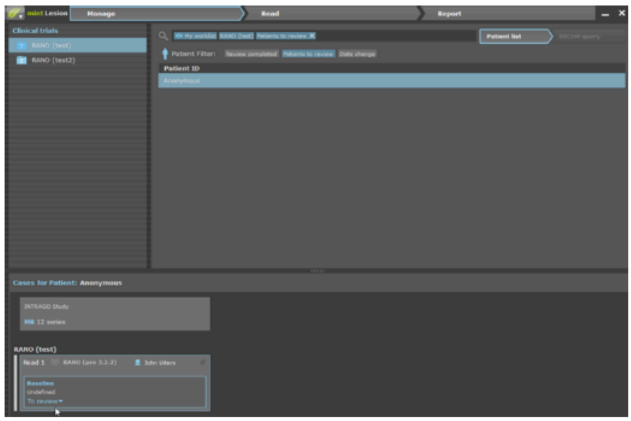
Clinflows
John Uiters, Project Manager
Mobile: +31 6 31929657 (Timezone: CET)
Email: john.uiters@clinflows.com

Reference materials for the central reader:

1) Study protocol
2) Imaging charter
3) User manual Mint Lesions
4) User manual Olea Sphere
5) Tutorial for manual series identification in Olea Sphere

Step 1: Start central read

An email notification will be send to the central reviewer specifying the patient that is assigned for central review in Mint Lesions. The reader will select the patient from his worklist in Mint Lesions. The images will be displayed based on the scan date starting with the post-operative scan (Baseline scan).



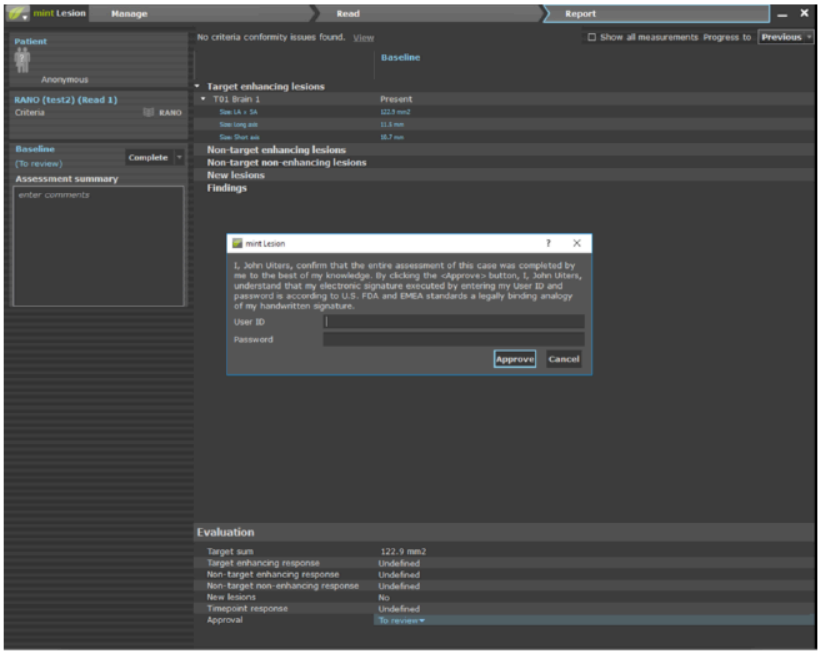
Step 2: start review "baseline" scan

A read case will start with the review of the "baseline" scan. The baseline scan will correspond with the earliest scan acquisition. The reader will identify at baseline the lesions and categorize these as target or non-target lesions in accordance with the RANO definitions. For the target lesions, bi-dimensional measurements should be provided. Non-target enhancing lesions should preferable be identified by providing a measurement although the diameters may be less than 10 mm. Alternative, the non-target enhancing lesions can be identified using the arrow tool. The non-enhancing lesions can be identified using an arrow. The reader can use keyboard shortcuts to the measurement tool of concern. See below (eg, all keyboard shortcuts are listed in the manual):

Measurements	Use the left mouse button to select and use a measurement tool
L	Bookmark current slice A bookmarked slice can be later upgraded to a measured observation
A S	Draw diameter / bidimensional diameter
D F	Mark a region-of-interest (ROI) / smooth ROI Double click to complete the ROI measurement
G	Perform a volumetric measurement Place a seed point inside the lesion with a left mouse click, while keeping the mouse button pressed, move the mouse over the outer lesion bounderies, and release the button
H	Perform an interpolated volumetric measurement Like a ROI / smooth ROI measurement, several slices in original image orientation can be marked, which are then interpolated to a volume
Z U	Perform a volumetric PET measurement Place a seed point inside the lesion with a left mouse click, while keeping the mouse button pressed, move the mouse over the outer lesion bounderies, and release the button
J	Set a marker The first left mouse click places the arrow head, the second determines arrow length and direction

In the read module, the reader will be able to view, edit, and assess image series in accordance with the selected response evaluation criteria (updated RANO), and to compare them historically.

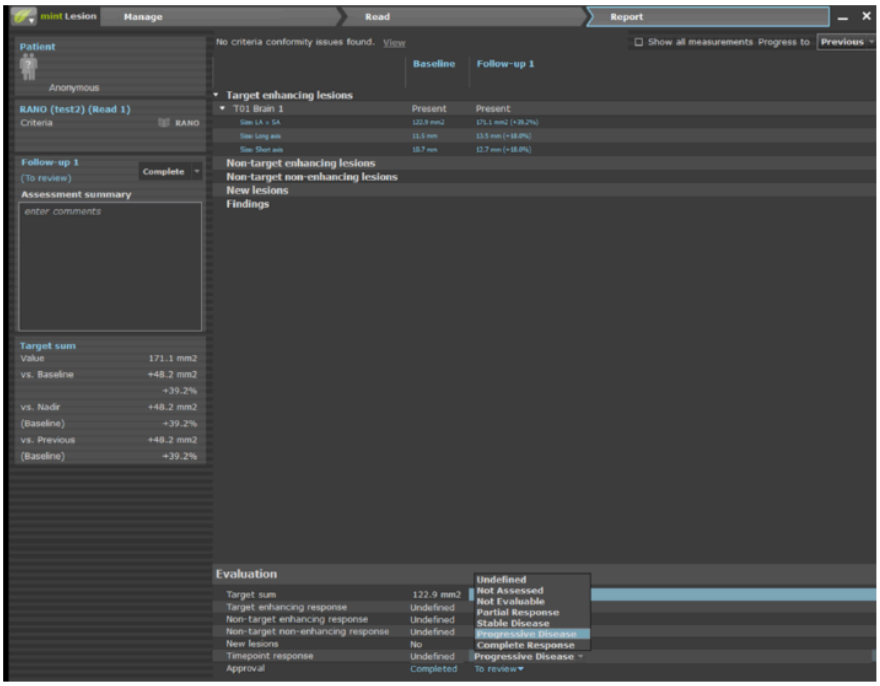
When the review of the baseline scan is completed, the assessments can be verified in the report module. If all assessments are accurate, the reader can sign-off the timepoint. If changes should be made, the reader can switch back to the read module. If no disease was present at baseline, the reader should add a comment at the assessment summary and subsequently signs-off in the report module.



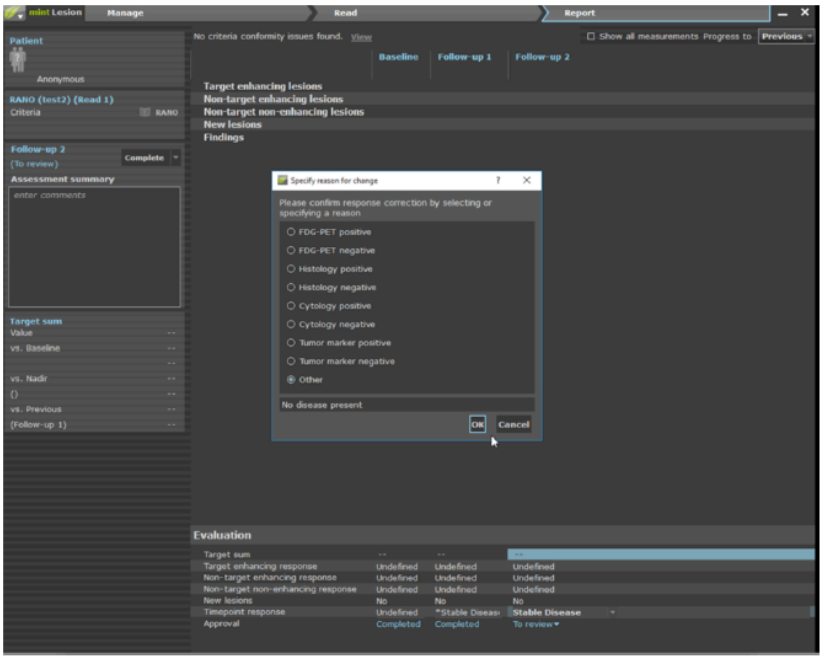
Step 3: Review "follow-up" scan

When a scan is reviewed, and signed-off, the software will display the successive scan. The software will allow to refer to the previous measurements of the completed scans. The reader should evaluate at each timepoint the contrast-enhancing and the non-enhancing component. The software will calculate the timepoint response based on the evaluations entered in the radiology eCRF. New contrast-enhancing lesions should be categorized by the reader as "new" if the lesion has become measurable. If, this criterion does not apply, the lesion should be recorded at "finding". Recordings at "finding" will not be used by the software to calculate the timepoint response. For the non-measurable contrast-enhancing lesions, at least 1 non-target lesion should become measurable and should have increased significantly to constitute to PD (eg, reader assignment for lesion state: unequivocal progression). Although the sites should schedule a confirmatory scan after initial PD, the central reader will not know which timepoint was performed as a confirmatory scan. For the tumor response assessments, this is not of concern as the tumor load will be compared with baseline/nadir and the increase of the rBV value is calculated in reference to the preceding scan

When the review of a timepoint is completed, and if the reader is agreeing with the calculated timepoint response in Mint Lesions as shown in the report module, the reader can sign-off the follow-up timepoint. If the reader disagrees with the calculated timepoint response, the reader can overrule the timepoint response. However, a rationale for changing the calculated timepoint response should be provided. If a timepoint response needs to be manually overruled, the reader should go to the report module and click on the timepoint response.



The timepoint response should as well manually be assigned if there was no disease present at baseline and no disease was present at the follow-up timepoint. The timepoint response for these cases should then be changed from "undefined" to "Stable disease". See below.

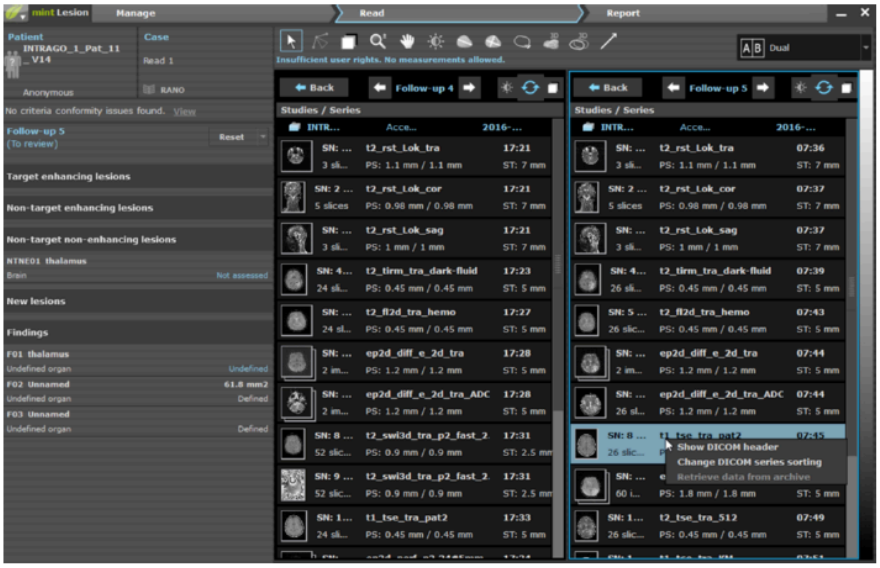


Step 4: Incorporation of the rBV values in the tumor response assessments

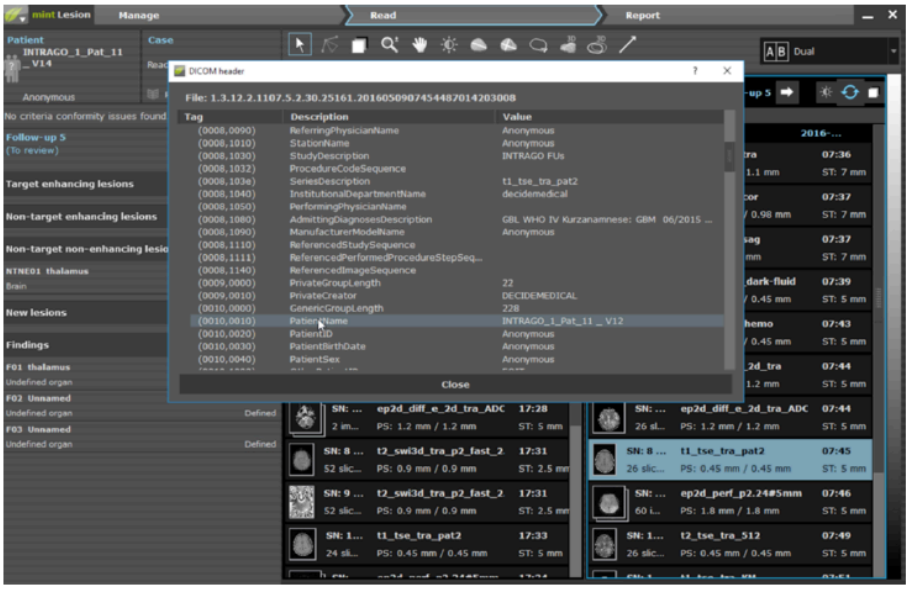
If per updated RANO, the calculated timepoint response in Mint Lesions is PD, the central reader will perform a post-processing step for the contrast-enhancing lesions that constitute to PD. If PD was based on new non-enhancing lesions, no additional post-processing step is required.

The reader should import in Olea Sphere the corresponding timepoint from the image DB (D/Olea DB/pt id). The scans in the image DB are labelled with the timepoint response per protocol (V7, V8, V9, etc) which differs from the timepoint labels in Mint lesions (baseline, follow-up 1, follow-up 2, etc). To select the corresponding scan, follow the steps as displayed below.

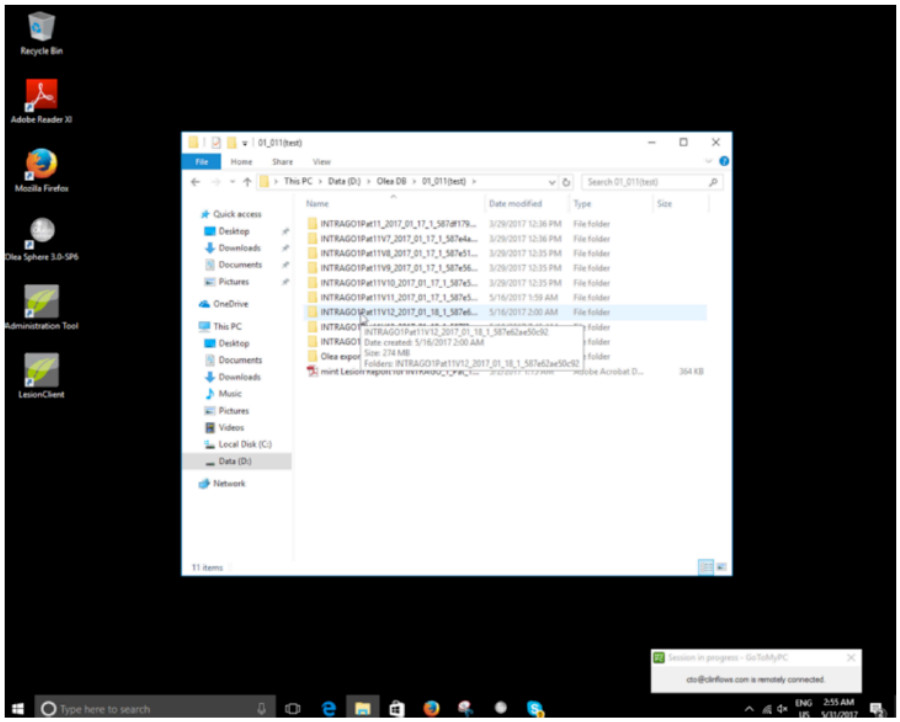
In the read module, click on "studies/series". Then right mouse click and select "show DICOM header":



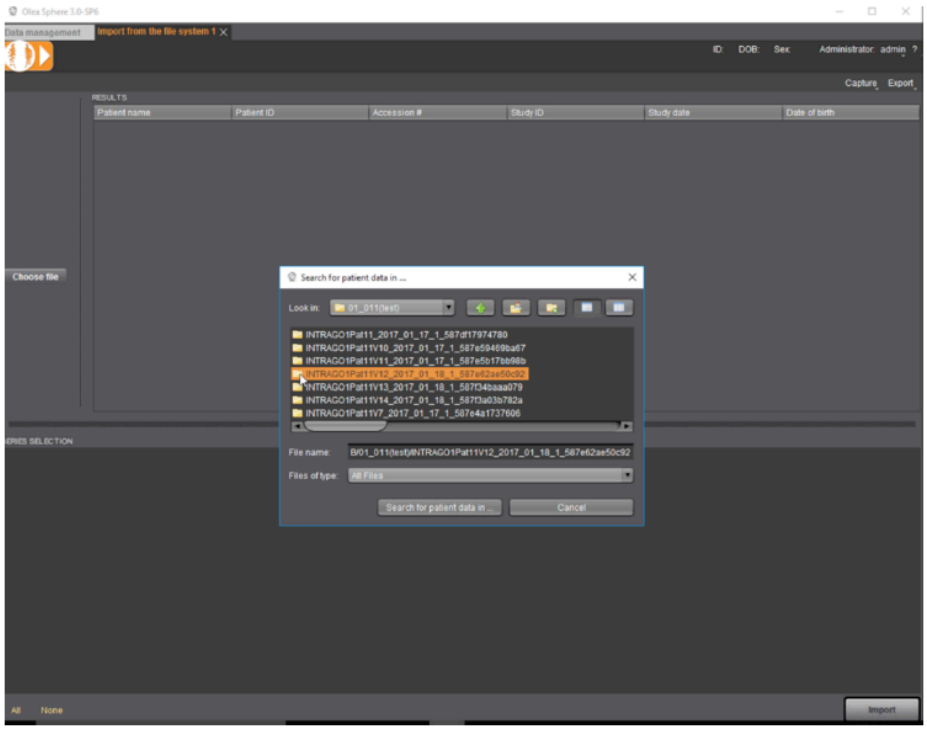
At the DICOM tag "patient name", the per protocol timepoint is shown:



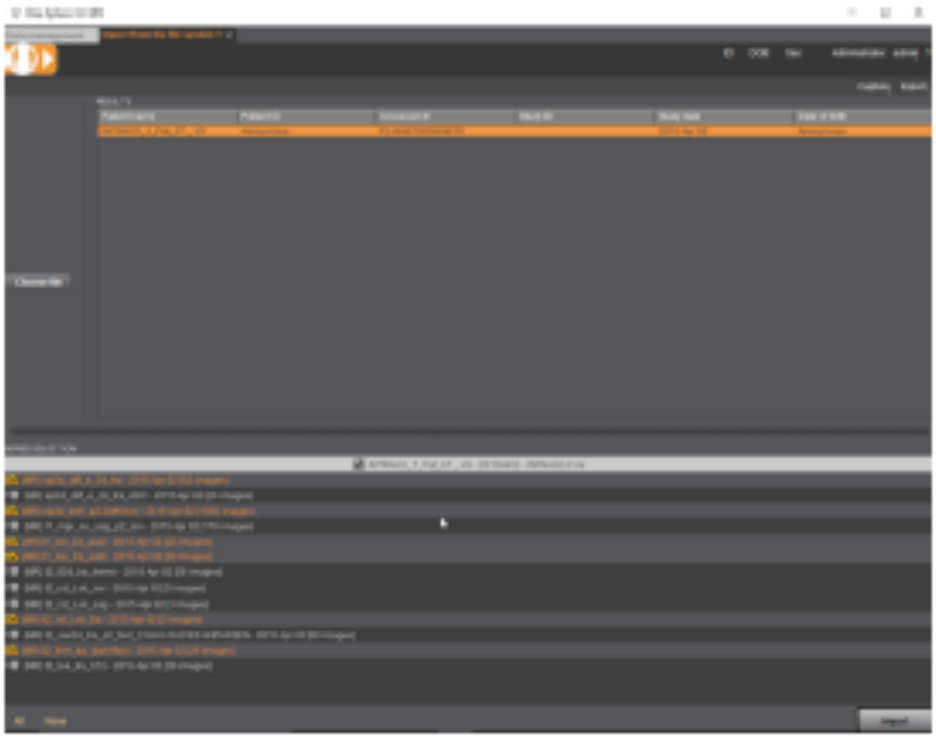
From the image DB, the reader can now select the corresponding scan to be imported in Olea Sphere (in our example: Pt11_V12).



In Olea, select "Import from file system" to start the import.

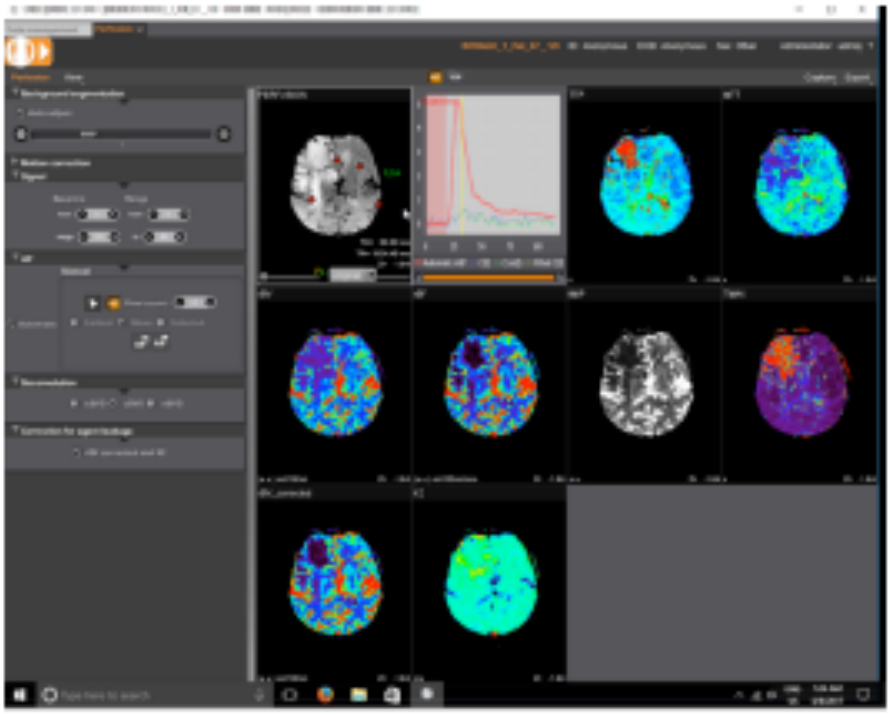


Select the file and click on "search for patient data in". Extend where needed the automatically selected series that will be imported. Click on import to start the import of the selected series.

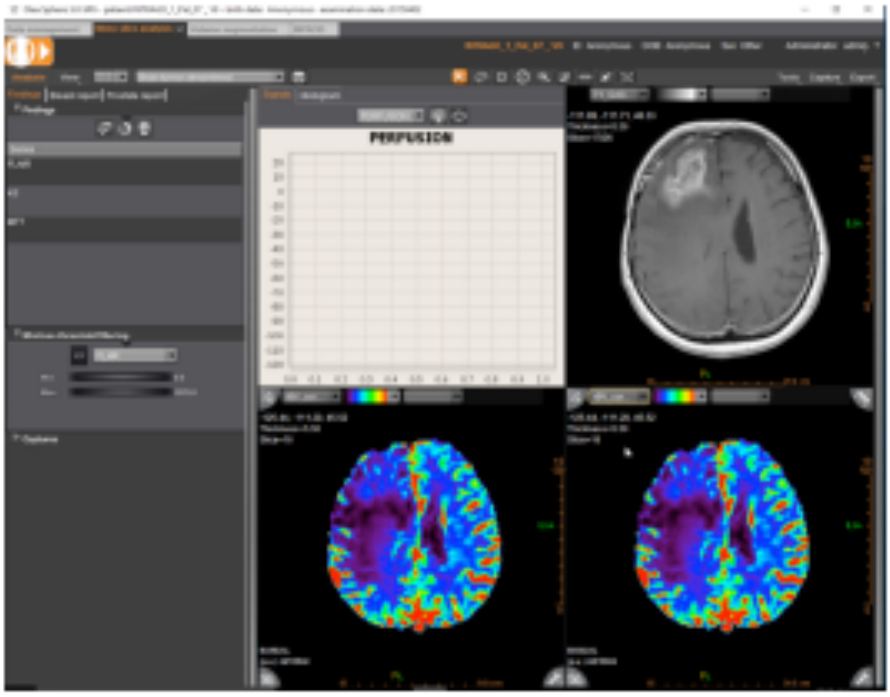


After the import, the imported timepoint is listed in the Data management overview. Double click on the record and select the perfusion plug-in.

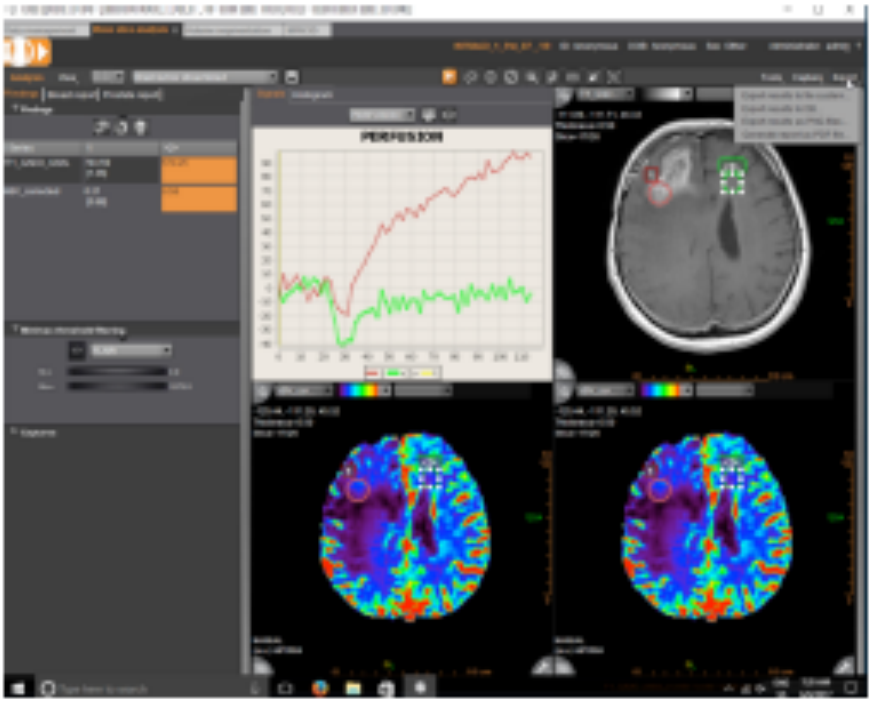
The perfusion module will generate the parametric maps with the default perfusion settings. As standard rule, the default perfusion settings assigned by the software tool do not need to be customized.



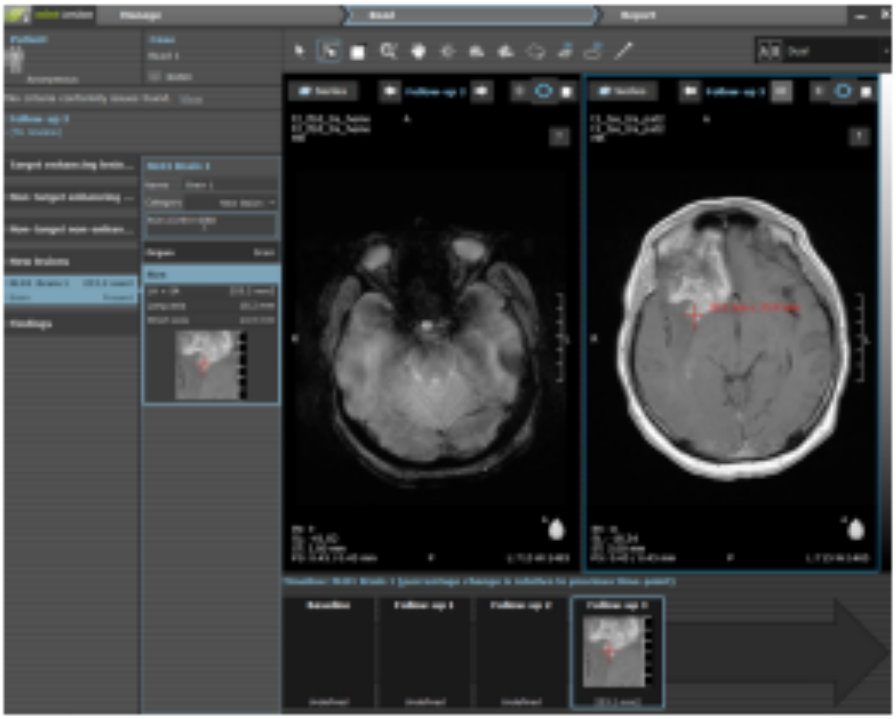
After the parametric maps have been generated, open the plug-in analysis. In the analysis mode, the ROIs can be placed. In the analysis mode, display the T1-post contrast anatomical image for the ROI placement and the corrected rBV and the rBV parametric maps as show below:



For all target lesions that did constitute to PD per updated RANO, the reader will draw a corresponding ROI. A reference ROI should be drawn at the contralateral side with a minimum diameter of 5mmx5mm.

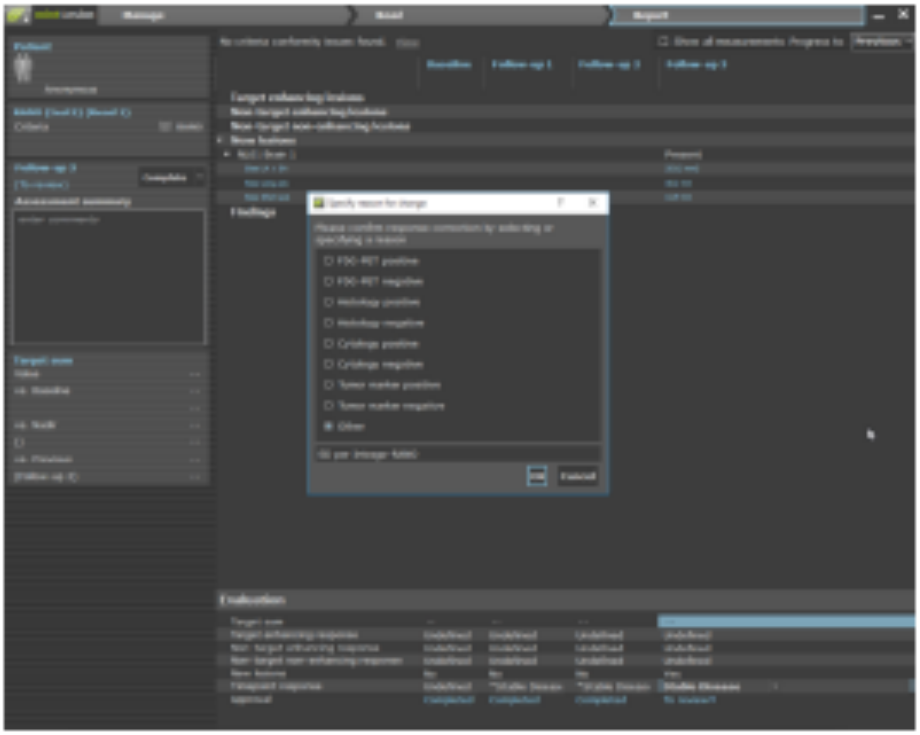


The system will automatically calculate the normalized rBV values that are listed at the left column. The normalized corrected values for rBV should be entered in Mint Lesions for the corresponding target lesion. When entering the rBV value at the corresponding lesion (in our case a new enhancing lesion), please indicate as well the originating ROI number. In our example: ROI-2: rBV=0.88.



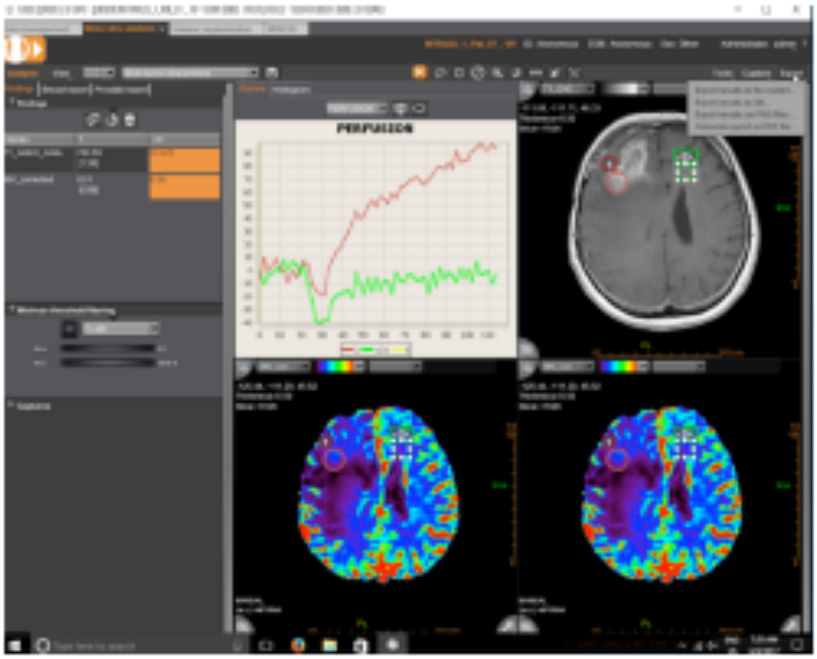
Note: If the timepoint response was SD per updated RANO and at the successive timepoint the response is PD, this would require the implementation of the rBV values for both timepoints of the corresponding lesion to calculate the delta rBV. Taking into account the preceding scan was already signed-off and no data can be added anymore, the reader should make a statement in the assessment summary if the delta rBV would qualify for PD.

A new lesion would constitute to PD per updated RANO. However, as the normalized rBV value in our example was < 1, the new lesion would not constitute to PD per Intrago-RANO. Consequently, the timepoint response will be changed to SD with rationale: "SD per Intrago RANO". See below.

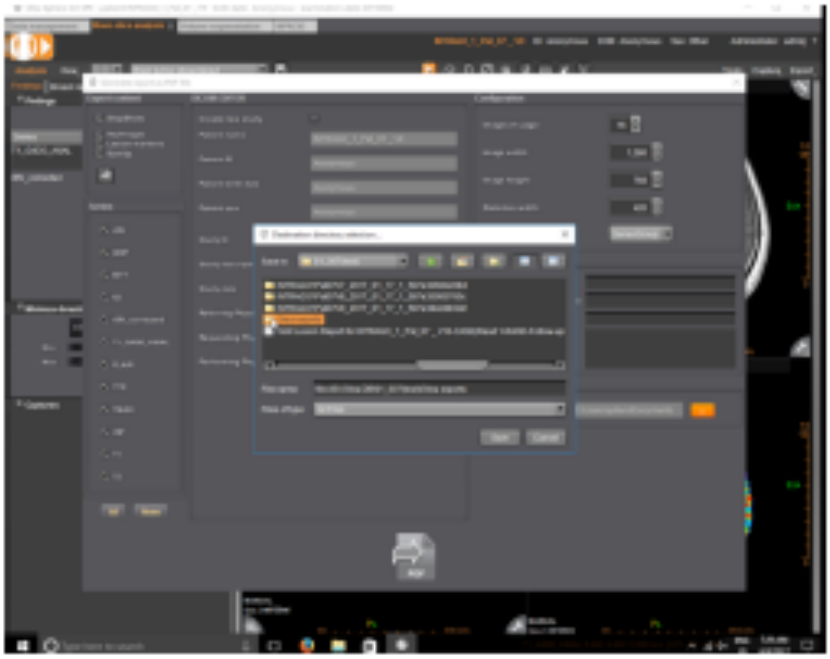


By clicking on the PDF icon at the report module, the reader can generate an overview with all measurements and entered rBV values and the performed manual timepoint response assessments:

Upon completion of the post-processing step, a PDF report should be generated with the resu archive the results, click on export and select "Generate report as PDF file".



Archive the PDF report in the Olea DB at the corresponding patient folder under "Olea exports"



As file name, use the following syntax: *patient id_timepoint_scan date_reader initials.* (Example: 001-05_V8_18AUG2017_WP)

When closing Olea, confirm the alert message to save the post-processed file.

APPENDIX II: ANONYMIZATION PROTOCOL

DICOM tag	Tag description	Value (site entry)
0010-0020,	Patient ID	Actual (Patient study number (EDC))
0010,0040,	Patient Sex	Actual
0010,1010,	Patient Age	Actual
0010,1030	Patient Weight	Actual
0010,1020	Patient Size	Actual

DICOM tag	Tag description	Value after anonymization
0008-0080	Institution Name	decidemedical
0008-0081	Institution Address	anonymized
0008-0090	Referring Physician's Name	anonymized
0008-0092	Referring Physician's Address	anonymized
0008-0094	Referring Physician's Telephone Number	anonymized
0008-1040	Institutional Department Name	decidemedical
0008-1050	Performing Physician Name	anonymized
0008-1070	Operator's Name	anonymized
0008-1030	Study description	Intrago
0010-0010	Patient Name	Case reference ("Patient id" + timepoint)
0010-0030	Patient's Birth Date	anonymized
0010-1000	Other patient IDs	anonymized
0010-1001	Other patient names	anonymized
0010-1005	Patient birth name	anonymized
0010-1040	Patient address	anonymized
0010-1050	Insurance Plan Identification	anonymized
0010-1060	Patient Mother Birth Name	anonymized
0010-2154	Patient telephone number	anonymized
0010-4000	Patient Comments	anonymized
0010,2160	Ethnic Group	anonymized
0010,2297	Responsible Person	anonymized
0010,2299	Responsible Organization	anonymized

STATISTICAL ANALYSIS PLAN

Version 1.4, 3 March 2026

A Multicenter Randomized Phase III Trial On Intraoperative Radiotherapy in Newly Diagnosed Glioblastoma Multiforme (Intrago II)

Clinical Trial Code:	INTRAGO II
Indication:	Glioblastoma Multiforme (WHO Grade IV)
Planned intervention:	Intraoperative Radiotherapy
ClinicalTrials.gov ID:	NCT02685605
Clinical Phase:	Phase III

Sponsor:
Department of Radiation Oncology
University Medical Center Mannheim
Medical Faculty Mannheim
University of Heidelberg
Theodor-Kutzer-Ufer 1-3, 68167 Mannheim
Phone: +49 (0) 621 383-1549

Principal Investigator:
Frank A. Giordano, MD
Department of Radiation Oncology
Medical Center Mannheim; Medical Faculty Mannheim
University of Heidelberg
Theodor-Kutzer-Ufer 1-3, 68167 Mannheim
Phone: +49 (0) 621 383-1549
E-Mail: frank.giordano@umm.de

BIOMETRICIAN:
Dr. Robert Németh
University Hospital of Bonn
Venusberg-Campus 1, D-53127 Bonn
Tel. No.: +49 (0) 228-287-15424
Fax No.: +49 (0) 228-287-15032
Email: robert.nemeth@ukbonn.de

Table of contents

Signatures

Prepared by:

University Hospital Bonn	Gustavo Sarria (Mar 3, 2026 16:41:55 GMT+1) Dr. Gustavo Sarria Medical Monitor	03/03/26 Date (DD MMM. YYYY)
Study Centre Bonn (SZB)	Dr. Robert Németh Biostatistician	04/03/26 Date (DD MMM YYYY)

Reviewed by:

Study Centre Bonn (SZB)	Jennifer Schmitz Biostatistician	04/03/26 Date (DD MMM YYYY)
Medwave Clinical Research	John Uiters John Uiters Project Manager	03/03/36 Date (DD MMM YYYY)

Approved by:

Medical Center Mannheim	Frank Giordano (Mar 3, 2026 21:04:48 GMT+1) Prof. Frank Giordano Principal Investigator	03/03/26 Date (DD MMM YYYY)

1 LIST OF ABBREVIATIONS

ADL	Activities of Daily Living
AE	Adverse Event
CCNU	Chlorethyl-Cyclohexyl-Nitroso-Urea (Lomustine)
CSF	Cerebrospinal Fluids
CTC-AE	Common Terminology Criteria for Adverse Events
CTV	Clinical Target Volume
EBRT	External Beam Radiotherapy
ECOG	Eastern Cooperative Oncology Group
eCRF	electronic Case Report Form
EoR	Extent of Resection
EORTC	European Organization for Research and Treatment of Cancer
FLAIR	Fluid-Attenuated Inversion Recovery
GBM	Glioblastoma Multiforme
GCP	Good Clinical Practice
ICH GCP	ICH Harmonized Tripartite Guideline on GCP
IDH1	Isocitrate Dehydrogenase 1
IORT	Intraoperative (Photon) Radiotherapy
KPS	Karnofsky Performance Status
MRI	Magnetic Resonance Imaging
MGMT	O6-Methylguanine-DNA Methyltransferase
NANO	Neurologic Assessment in Neuro-Oncology
NCIC	National Cancer Institute of Canada
OS	Overall Survival
PD	Progressive Disease
PE	Physical Exam
PFS	Progression-Free Survival
PPS	Per Protocol Set
PR	Partial Response
PsP	Pseudo-progression
QLQ-C30/BN20	Quality of Life Questionnaire Brain Module
RANO	Response Assessment in Neuro-Oncology
RCT	Radiochemotherapy
RFT	Renal Function Tests
SAE	Serious Adverse Event
SC	Steering Committee
TMZ	Temozolomide

2 List of Key Terms

Initial PD	When the time point response is progressive disease. Initial PD should be followed by a confirmatory scan
Suspected PD	The term suspected PD is used when the on-site response assessment of the confirmatory scan shows again PD. The site should request an off-site radiology review to confirm the on-site response assessment that the patient reached the PFS endpoint
Confirmed PD	The term confirmed PD is used to indicate the off-site radiology review confirmed the on-site response assessments, that the patient reached the PFS endpoint.
Pseudoprogression (PsP):	The term pseudoprogression is used if initial PD is not confirmed by the on-site or the off-site response assessments.
Treatment phase	Period from surgery to completion radiochemotherapy
Follow-Up phase	Period from completion radiochemotherapy to study completion (PFS/study discontinuation)
Survival phase	Period from study completion to death
Study Completion	A patient has completed the study when having reached the PFS endpoint or discontinued.
Clinical progression	Progression based on clinical deterioration not attributable to concurrent medication or comorbid conditions
Randomized	A subject who was randomized to receive study treatment.
Modified RANO	The Response Assessment in Neuro-Oncology (RANO) criteria that were modified for the INTRAGO ll study to discriminate between Pseudo progression and True progression are referred to in the SAP as mRANO.
Enrolled	Subjects who have signed informed consent, and have performed (part of) the screening visit.
Screening failure	Screened subject who did not fulfill the pre-operative and/or intra-operative protocol inclusion and/or exclusion criteria, or decided not to participate anymore (withdrew consent) prior to the planned surgery.
Subject number	Number assigned to each enrolled subject by the eCRF

3 Introduction

This Statistical Analysis Plan (SAP) contains a more technical and detailed elaboration of the principal features of the analysis described in the protocol for the clinical study INTRAGO II, A Multicenter Randomized Phase III Trial On Intraoperative Radiotherapy In Newly Diagnosed Glioblastoma Multiforme.

The contents of this SAP are based on protocol version 4.0, dated 18JAN2023 and includes detailed procedures for executing the statistical analysis of the primary and secondary efficacy variables and analysis of safety variables. The SAP will be finalized and signed prior to database lock.

4 Flow chart and visit schedule

	Pre-Treatment	Treatment Phase						Follow-Up		Survival
Assessments	≤ 10 d prior to surgery/IORT	Surgery ± IORT	≤ 3d post op	≤ 10 d prior to RCT	Radio-Chemotherapy Week 1[2] (28d after surgery)	Radio-Chemotherapy Week 6	28d after EBRT[1]	42d after EBRT	q90±10d thereafter	life-long after PD (quarterly)
Visit #	screening	1	2	3	4	5	6	7	≥8	N
Pre-op IC / EC	X									
Written informed consent	X									
Pregnancy test[3]	X									
KPS	X				X	X	X	X	X	
PE	X				X	X	X	X	X	
CBC, basic chemistry	X			X[4]						
RFT, LFT	X									
Brain MRI +/- contrast	X[5]		X	(X)[6]				X[7]	X	
EORTC QLQ-C30 / BN20	X				X			X	X	
Barthel ADL index	X				X			X	X	
Basic neurological exam	X				X			X	X	
Intra-op IC / EC		X								
Histologic assessment of GBM[8]		X								
Technical feasibility of IORT		X								
Randomization		X								
IORT with 20-30 Gy[9]		X[10]								
EoR (sum of all max diameters of residual lesions in cm)			X							
Adverse events		X	X	X	X	X	X	X	X	(X)[11]
MGMT and IDH1 Status[12]				X						
Treatment planning CT with contrast				X						
EBRT (60 Gy) 5x/week					X					
daily (7d per week) TMZ 75 mg/m/d[2]					X					
cyclic TMZ 150-200 mg/m/d/cycle[2]							X			

[1] Acceptance window after last day of RT: d21-d42
[2] Acceptance window after surgery: d21-d42
[3] Serum or urine pregnancy test in women of child bearing potential
[4] No study-specific requirement (part of the clinical routine).
[5] Planning MRI (for neuronavigation) should be performed <96 h prior to surgery
[6] Not mandatory
[7] Includes confirmatory MRI scan(s) in case of suspected PD
[8] Frozen section and/or smear preparation.
[9] Prescribed to the applicator surface
[10] Experimental arm only
[11] After end of study, only ongoing SAEs and SAEs occurring up to 3 months later are followed. Thereafter, no SAEs except radionecrosis will need to be reported.
[12] No study-specific requirement. Centers are encouraged to assess these markers.

	Pre-Treatment	Treatment Phase						Follow-Up		Survival
					Radio-Chemotherapy					
Assessments	**≤ 10 d prior to surgery/IORT**	**Surgery ± IORT**	**≤ 3d post op**	**≤ 10 d prior to RCT**	**Week 1[2]** (28d after surgery)	**Week 6**	**28d after EBRT[1]**	**42d after EBRT**	**q90±10d thereafter**	**life-long after PD (quarterly)**
Second line interventions and treatment(s)[3]										X
Vital Status										X

[1] Includes histology/pathology results after any second biopsy/surgery

4.1.1 Screening phase

Initially, subjects will provide signed informed consent, which must be obtained at least 24 hours before surgery. The pre-operative screening should be done within 10 days before surgery and consists of a physical and neurological examination, pregnancy test (women of childbearing potential), functional assessments (KPS, Barthel ADL index), quality of life assessments (EORTC QLC-C30 / BN20) and laboratory studies (complete blood count and basic chemistry to assess adequate organ functions).

A pre-operative risk assessment must be done. If not performed yet (e.g., in case only CT scanning was performed), an MRI scan of the brain is required. The MRI scan must allow to rule out unresectable satellites or multicentric disease (by Gd-T1 and/or T2 and/or T2-FLAIR):

During surgery, an additional screening will be performed, which is described below in Chapter 5.1.2 of the trial protocol.

4.1.2 Treatment phase

Briefly, the treatment phase consists of surgery, a post-operative MRI and standard radiochemotherapy and adjuvant chemotherapy according to the EORTC-NCIC (Stupp) protocol. The concomitant application of the CeTeG chemotherapy regimen (CCNU plus elevated dose-temozolomide) in patients with MGMT promotor hypermethylation is allowed if considered standard of care for these patients at the individual institution.

Deviations from the Stupp protocol (see also Ch. 1.2 of the trial protocol) that have the potential to impact the efficacy endpoint are considered major protocol deviations, and are described 8.2.1.

Visit 1 - Surgery ± IORT

During the surgery, patients that signed the informed consent and passed initial screening will undergo an additional screening:

- The preliminary diagnosis of glioblastoma should be confirmed by frozen section and/or smear preparations.
- IORT should be deemed technically feasible.

Intraoperative imaging should be used to identify risk structures after resection. In case risk structures cannot be adequately identified, or doses to risk structures are exceeding dose constraints, IORT is considered as technically not feasible.

In case patients meet the intraoperative inclusion criteria (in addition to the pre-operative IC), the eligibility checklist will be completed (using the eCRF system) and the screening is completed. Subjects

who don't fulfill the pre-operative and intra-operative inclusion criteria or withdraw their consent before surgery will be counted as screening failures.

After resection and meeting the intraoperative inclusion criteria, the patient is assigned to one of the two treatment arms (A – experimental arm with IORT, B – control arm).

It is planned to randomize 314 patients in total, 157 randomized patients per treatment arm.

Visit 2 – Post-operative MRI

Early postoperative MRI must be performed ≤72 h after surgery and must be analyzed in a standardized assessment as follows: T1-hyperintense lesions without contrast must be used to evaluate residual blood/heme. Specific sequences to more accurately assess acute blood (i.e. SWI) are encouraged. Next, (T1-) contrast-enhancing masses or nodules are indicative for residual tumor tissue. This MRI scans serves as the base for the initial evalution, and is the baseline (efficacy baseline) for follow-up evaluations described in chapter 3.3.

Visit 3 - Treatment planning

Within 10 days prior to start of external beam radiotherapy (EBRT) a CT scan should be performed for treatment planning purposes. A contrast-enhanced CT scan is required of the entire head region using the smallest possible axial slice thickness (≤3 mm). Ideally, treatment planning MRI scans should be co-registered with treatment planning MRI scans, but this is not mandatory.

Visit 4 and 5 - Radio-Chemotherapy

Radiochemotherapy (RCT) should be initiated 3-5 weeks after surgery (with or without IORT), and consists of a combination of external beam radiotherapy (EBRT) and oral Temozolomide

The craniotomy wound must be adequately healed and all sutures, stitches or drains must be removed. If any condition (e.g., wound healing difficulties, infections) does not permit the initiation of RCT within this timeframe, a further postponement of one week is allowed.

Radiotherapy - EBRT

All patients will receive EBRT with a total dose of 46 Gy to the PTV1 and a conedown (boost) of 14 Gy to the PTV2. EBRT is given in daily doses of 2 Gy, 5 days per week . The duration of EBRT is 6 weeks in total.

EBRT may be interrupted for a maximum period of seven consecutive days. The reason for interruption and the duration must be documented in the eCRF. Any longer treatment pause is considered as an unacceptable (major) protocol violation, and these patient will not be included in

the Per protocol set analysis. All major protocol deviations that lead to exclusion of the per protocol set analysis are described in table 1, chapter 8.2.1.

Chemotherapy – oral Temozolomide

All patients will receive oral temozolomide (TMZ) from day 1 to the last day of radiotherapy (7 days per week) at a daily dose of 75 mg/m^2. The body surface area, in m^2, is calculated once on the basis of the patient's pre-EBRT (post-surgery) weight and height. The resulting dose can be rounded to the nearest 10 mg.

The concomitantly applied temozolomide chemotherapy may be interrupted or terminated whenever clinically indicated for any given period of time (e.g., in case of hematotoxicity). The reason for interruption and the duration must be documented in the eCRF.

The concomitant application of the CeTeG chemotherapy regimen (CCNU plus elevated dose-temozolomide) in patients with MGMT promotor hypermethylation is allowed if considered standard of care for these patients at the individual institution.

Visit 6 - Start adjuvant chemotherapy

After initial radiochemotherapy all patients should receive a minimum of six cycles of adjuvant chemotherapy (more than six cycles are allowed) unless there is evidence of tumor progression or treatment-related toxicity. Weekly blood workups should be obtained during adjuvant chemotherapy in line with the locally established clinical routine.

Adjuvant chemotherapy should be initiated 4 weeks after the last day of irradiation (with an acceptance window of ± 1 week). Chemotherapy is performed with six 28-day cycles consisting of 5 consecutive days of temozolomide at doses of 150-200 mg/m^2 and a 23-day therapy break.

The body surface area in m^2 is calculated on the basis of the height obtained at the pre-surgery visit and the most actual weight (determined before each cycle). The first cycle will be performed with 150 mg/m^2/d/cycle (absolute dose limit: 300 mg/d/cycle) of temozolomide. If there are no treatment-related toxicities, the dose may be escalated to 200 mg/m^2/d/cycle (absolute dose limit: 400 mg/d/cycle) in subsequent cycles.

4.1.3 Follow-Up phase

The first follow-up MRI scan for follow-up evaluations should take place 6 weeks +/- 2 weeks after radiochemotherapy (visit 7). Thereafter, all patients will receive quarterly MRI scans unless progression is suspected. MRIs have to be assessed using modified RANO criteria and perfusion imaging. Relevant imaging studies documenting disease relapse (i.e. the most actual and 1-2 MRI scans from previous FU visits including perfusion scans) will undergo centralized review to confirm progression.

In case a new/increasing T1-Gd enhancing lesion or an increase in T2/FLAIR is noted and the enhancing lesion shows a rCBV ≥ 1, all centers must adhere to a pre-defined algorithm (see 7.1.1 and Appendix II of the protocol). As a part of this algorithm, confirmatory scans are requested in 4-6 weeks (usually resembling one additional scan within a 12-week FU-interval). Once a patient is diagnosed with PD in the absence of radiographic criteria (e.g., if any other tumor-specific therapy disease was initiated or clinical deterioration occurred), confirmatory scans are advised.

As the first of six 28-day cycles of TMZ is administered at visit 6, the remaining 5 (or more) cycles are expected to be administered through visit 8.

4.1.4 Survival Phase

After the PFS endpoint or discontinuation, the patient will be followed up every 90 days ± 10 days until death or end-of-study. During survival follow-up, radionecrosis events should be reported and initiation of any salvage treatment. Furthermore, any SAEs occurring within 3 months after study completion/discontinuation needs to be reported.

5 Study objectives and Design

5.1 Study Objectives

The primary objective of the study is to evaluate the efficacy of the addition of intra-operational radiotherapy to standard radiochemotherapy versus standard radiochemotherapy alone in newly diagnosed glioblastoma multiforme (WHO grade lV).

The primary endpoint to measure the efficacy for the primary objective is the median progression free survival rate (PFS). The primary endpoint will be determined based on the on-site assessments entered in the eCRF. The rationale for choosing this endpoint is that in glioblastoma, PFS and Overall Survival (OS) are strongly correlated, making PFS a surrogate for OS. PFS offers earlier assessment than OS and higher statistical power at the time of analysis.

The patient has met the PFS endpoint if one of the below criteria are met.

- Radiological progression per modified RANO criteria
    - Date PFS based on the on-site response assessments and suspected PD subsequently confirmed by the off-site reading
- The initiation of any new OR salvage treatment
- Progressive disease based on clear clinical deterioration not attributable to other causes apart from the tumor
- Death
    - Reported during the treatment/follow-up period regardless cause

The on-site assessments of suspected radiological progression requires the confirmation from the off-site reader to qualify as a PFS endpoint. If radiological progression is not confirmed, the patient should be kept in the study. Hence, the on-site radiological response assessments dictate the date of radiological progression which is the date of the MRI showing initial PD that is confirmed at the successive (even if the off-site response assessments showed initial PD at an earlier timepoint).

The initiation of any new or salvage treatment for GBM will be considered as an event for PFS. Salvage treatment include:

- Re-resection of recurrence
- Re-irradiation
- Invasive treatments (eg, Laser interstitial thermal therapy (LITT), seeds, etc)
- Chemotherapy
    - Alkylators (e.g. CCNU 2nd line, TMZ re-challenge)
    - TIs (Topotecan, Irinotecan)
    - Targeted therapies (EGFR-I, ..)

In case the local tumor board decides to start salvage therapy in absence of radiological progression per mRANO response criteria, the date of PFS will be censored in accordance with the rules described in 9.1.1.

The initiation of salvage treatment in the absence of radiological progression by mRANO will be considered a protocol deviation. These protocol deviations will be reviewed case-by-case by the study board members, and classified as major protocol violation if the patient should be excluded from the PPS. Definitions for major protocol violations are described in 8.2.1.

The secondary objectives encompass important aspects of loco-regional and global efficiency (OS, patterns of relapse), prognostic factors (age, KPS, MGMT, etc.), as well as quality of life and safety parameters. Secondary objectives are:

- Median OS
- Outcome (PFS, OS) with respect to:
    - Site of failure/patterns of recurrence:
        - PD within < 1 cm margin around the cavity
        - PD within ≥ 1 cm – 2.0 cm margin around the cavity
        - PD within ≥ 2.0 cm margin around the cavity
- Pattern of progressive disease:
    - PD based on non-measurable and/or measurable enhancing residual lesions
    - PD based on new enhancing lesions
    - PD based on new non-enhancing lesions
- Volume of residual disease
    - Non-measurable disease (<0.5 * 0.5cm)
    - Measurable disease (≥ 0.5 * 0.5 cm)
- Applicator size
    - ≤ 3.5 cm
    - > 4 cm
- Stratification criteria Age and KPS
- Molecular subgroups (e.g., MGMT, IDH1)
- QoL / ADL
- Radiation-related (acute / early delayed / late) neurotoxicity

5.2 Study design

INTRAGO II is a multicentric, prospective, randomized, 2-arm, open label clinical phase 3 trials which investigates if the median PFS of patients with newly diagnosed GBM can be improved by the addition of IORT to standard radiochemotherapy.

Briefly, all patients included in the study will initially undergo standard surgical resection. In the experimental arm, a single radiation dose of 20-30 Gy will be delivered by IORT. Patients randomized into the control arm will not receive IORT. In both arms standard radiochemotherapy and adjuvant chemotherapy (according to the EORTC/NCIC protocol) will be applied after resection (Figure 1). The concomitant application of the CeTeG chemotherapy regimen (CCNU plus elevated dose-temozolomide) in patients with MGMT promotor hypermethylation is allowed if considered standard of care for these patients at the individual institution.

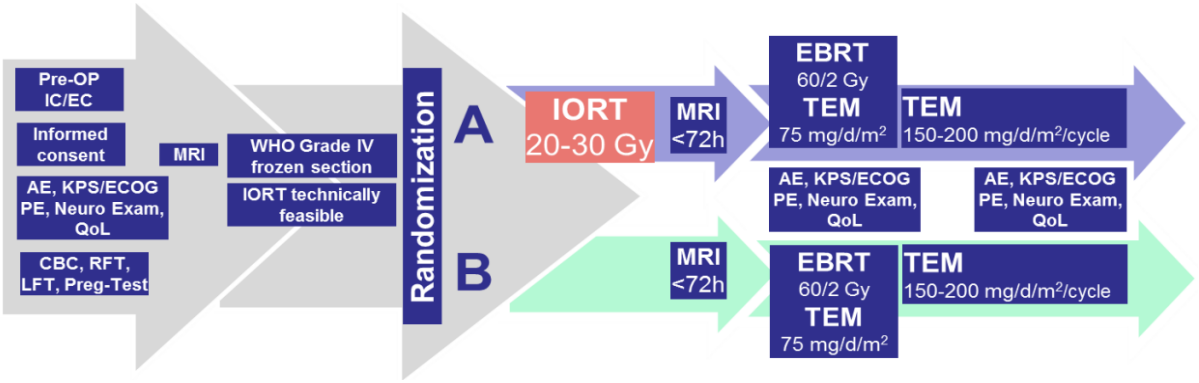


Figure 1 INTRAGO ll study design.

To prevent biases, all patients will be randomized into one of both arms (A – experimental arm with IORT, B – control arm) after surgical removal of the tumor and after the diagnosis of GBM is established by frozen sections.

It is planned to recruit subjects from approximately 20 sites in approximately 9 countries worldwide. In total 314 subject should be enrolled in the clinical trial, i.e., 157 subject per treatment group.

5.2.1 Study Committees

The following oversight committees are installed for this study:

- A steering committee will review study performance, make recommendations regarding the conduct of the study, assist in the resolution of problems, and will also be involved in the analysis and reporting of study data.
- A DSMB will be installed to act in an advisory capacity to the sponsor to monitor participant safety and data quality
- A separate independent endpoint adjudication committee will be installed to review the primary PFS endpoint. The adjudication committee will be blinded for treatment arm.
- A classification committee will be installed to review the protocol violations and to determine if the patients can be included in the PPS.

5.3 Randomization

Stratified block randomization (4, 6, 8) will be performed for each center to achieve equal group sizes per center. Assignment to the treatment group will be done by the respective site staff member using the randomization tool in the eCRF (CASTOR).

Strata used in the randomization algorithm are:

1. Age (<65 vs. ≥ 65)
2. KPS (90-100% vs. 70-80%)
3. Thickness of anticipated T1-Gd-enhancing (remaining) tumor margin as per the discretion of the surgeon (margin ≥0.5 cm or multiple sites of residual tumor at the cavity borders vs. <0.5 cm).

Randomization is performed after surgical resection of the tumor and after the surgeon gives a statement on the extent of resection. The patient will be assigned by the eCRF to one of the two treatment arms:

- Arm A – experimental arm with IORT
- Arm B – control arm

5.3.1 Blinding and Unblinding

Not Applicable.

6 Sample size

Progression-free survival has been chosen as the primary efficacy endpoint for this phase 3 study. The rationale for choosing this endpoint is that in glioblastoma, PFS and OS are strongly correlated (making PFS a surrogate for OS). PFS offers earlier assessment than OS and higher statistical power at the time of analysis (Han, Ren et al., 2014).

A 1-tailed log-rank test with an overall sample size of 314 patients (157 in the IORT group and 157 in the control group) achieves 80.2% power at a 0.050 significance level to detect a hazard ratio of 0.667 when the median PFS in the observation group is 9 months, equivalent to a 50% improved median progression-free survival (from 9 to 13.5 months). The study duration will be 7 years, whereas patient accrual occurs in the first 5 years. The proportion discontinuations of the observation group is 6.5% per month. The proportion discontinuations out of the IORT group is 6.5% per month.

6.1 Background information for sample size justification

Several, mostly small retrospective single-institutional studies have shown efficacy of the IORT approach. However, those pioneering studies were using IOERT and thus faced classical technical challenges that come along with a forward-scattering irradiation system in a setting with cylindrical or spherical tumor cavities. Postoperative dose reconstructions from the Munster University group demonstrated that many patients receiving IOERT exhibited areas of inadequate coverage (due to inaccurately selected electron energies, inappropriate cone sizes or angle errors). As expected, they found that median and 2-year survival significantly improved with better coverage (MS: 15.2 with adequate coverage vs. 9.3 months with inadequate coverage; 2-year survival: 9.3% vs. 0%, p = 0.02).

Computation of power is based on a median progression-free survival rate of 13.5 months for the IORT-treated group versus 9 months for the control group. This effect (50% improvement) was selected as the smallest effect that would be important to detect, in the sense that any smaller effect would not be of clinical or substantive significance.

The entered and then followed until either (a) the terminal event occurs, or (b) they discontinue from the study, or (c) the study ends and the patient is censored while still being actively followed.
Computation of power is based on a median progression-free survival rate of 13.5 months for the IORT-treated group versus 9 months for the control group. This effect (50% improvement) was selected as the smallest effect that would be important to detect, in the sense that any smaller effect would not be of clinical or substantive significance.
The computation assumes a discontinuation rate of 6.5% per month. This discontinuation rate resembles the calculated average considering each center's individual experience of the last 3-5 trials. It is separate from the censoring of patients that takes place when the study ends. The type I error rate (alpha) has study design calls for an accrual period of 6 years and a follow-up period of 2 years. Patients are

been set at 0.050, one-sided. Using these assumptions, the study would have a power of 80.5% with a fixed number of subjects of 157 in both groups.
The trial design has been changed to include an adaptive interims analysis after 200 patients have been randomized. The purpose of the interim analysis was to check the assumptions made during the study planning phase (especially the survival and censoring rates) and potentially modify the design correspondingly.
The analysis was to apply the inverse normal combination of the p-values of the two stages with an O'Brien-Fleming type alpha-spending function and information rate of 50%. The corresponding stage-wise significance levels were 0.0088 and 0.0467. More details are given in the interim analysis plan.

6.2 Replacements of drop-outs

Randomized patients that discontinue the study will not be replaced with exception for those patients whose on-site final histopatology shows a different tumor type.

7 Overview of Planned Analysis

This SAP covers the analyses for efficacy and safety based on the data cut-off dates for the interim and final analyses. Statistical analyses will be performed using cleaned eCRF data as well as data of tumor assessment results as determined by the independent off-site radiology review, final histopathology as determined by the independent off-site pathology review and Quality of Life data. All data will be included up to a prospectively determined clinical cut-off date. The data cut-off for the interim and the final analysis will be prospectively determined based on monthly enrolment projections.

7.1 Sequence of Analysis

The following analyses will be performed during this trial.

- Interim analysis: after 200 enrolled patients
    - The results of the interim analysis were presented to the DSMB on the 28th of June 2024.
- Final analysis: after all patients are enrolled in 6 years with a 2 years follow-up period

There will be a partial database lock for the interim analyses. There will be ongoing interim analyses for periodic safety reviews by the DSMB.

7.2 Interim Analysis

7.2.1 Interim Safety Analysis

An DSMB will be formed and will be responsible for periodic safety evaluations of the trial as well as the evaluation of the interim efficacy analysis. The DSMB consist of a group of four independent experts:

- Radiation oncology: Bahman Emami, Chair — Loyola University Medical Center
- Neurosurgery: Dietmar Krex — University Hospital Carl Gustav Carus
- Neuro-oncology: Martin Glas — University of Duisburg-Essen
- Statistics: Max Bulsara — Notre Dame University

7.2.1 Interim Analysis

For this trial an adaptive interims analysis will be performed after randomization of 200 patients. The main purpose of the adaptive analysis is sample size reassessment based on the observed discontinuations (drop-out) of patients for any reason. The Interim analysis will be performed by a statistician and shared with the DSMB for final conclusions on the study assumptions. The purpose of the interim analysis is to check the assumptions made during the study planning phase (especially the survival and censoring rates) and potentially modify the design correspondingly. After the interim analysis it was decided not to make any changes to the study design.

7.3 Final Analysis

All planned analyses outlined in this SAP will be performed for the final analysis. A partial database lock will be performed for both the interim and the final analysis.

Subject follow-up for progression and survival will continue until 2 years after the last subject completed the study (eg, reached the PFS endpoint or discontinued). Therefore, the full database lock will take place either 2 years after the last subject completed the study or after the Sponsor decides to terminate the study.

An Endpoint Adjudication Meeting and a Classification meeting will be held prior the final analysis (for both partial and full database lock).

8 ANALYSIS SETS

The following population sets will be used for the analyses:

8.1 Full Analysis Set (FAS)

The population FAS comprises all subjects who meet the following criteria:

- Subjects who were exposed to irradiation
- Subjects with GBM as final histopathology diagnosis based on the on-site pathology review.

This population is used for summaries of demographic and baseline characteristics and all efficacy analyses. In this set, every patient is analyzed according to the group randomized into.

8.2 Per Protocol Set (PPS)

A per-protocol set will be defined. This set compromises all patients who were treated according to the randomized treatment as outlined in the protocol.

The PPS will include all subjects who meet the following criteria:

- Subjects in the FAS
- Subjects without a major protocol violation as determined during the classification meeting
- Subjects with optimal treatment adherence:
    - Arm A:
        - IORT: 20-30 Gy
        - EBRT: total dose of 50-60 Gy
        - At least 1 cycle of adjuvant chemotherapy
    - Arm B:
        - EBRT: total dose of 50-60 Gy
        - At least 1 cycle of adjuvant chemotherapy

The PPS is used for sensitivity analyses of the primary and key secondary endpoints efficacy analyses and is regarded secondary to the FAS.

8.2.1 Major Protocol Violations

Protocol violations will be identified by running scripts on the clinical and the imaging database. A protocol violation that may impact the efficacy assessments is considered a major protocol violation (MPV). Criteria for identifying a major protocol violation are described below in table 1.

Final judgments on exclusion of subjects from the PPS, based on the MPVs, will be made at the classification meeting which is held prior to database hard-lock. The following study team members will be joining the classification meeting:

- Prof. F. Giordano, Medical Center Mannheim, Principal Investigator
- Dr. Arne Ruder, Medical Center Mannheim, Medical Monitor
- Dr. Robert Németh, University Hospital Bonn, Biostatistician
- Dr. Gustavo Sarria, University Hospital Bonn, Medical Monitor
- John Uiters, Medwave, Project Manager
- Ria van Vliet, Medwave, CRA
- Jan-Albert Manenschijn, Medwave, iCRA

Table 1 : Criteria for Assessing Major Protocol Violations which could potentially impact efficacy assessments

Number	Major Protocol Violation Criteria
Eligibility Violations - inclusion criteria	
1	#1: Age ≥18 and ≤ 80 years
2	#2: Karnofsky Performance Score (KPS) ≥ 60%
3	#3: Supratentorial T1-Gd enhancing lesion(s) amenable to total resection Comments: if on the post-operative scan, the off-site radiology review determines the presence of residual enhancing disease measuring ≥ 10x10 mm, the subject will be identified with a possible major protocol violation for inclusion criterion 3
Eligibility Violations - exclusion criteria	
4	#1: Multicentric disease (e.g. in both hemispheres) or non-resectable satellite lesions

	Comments: the subject is identified with a possible major protocol violation for exclusion criterion 1 if the off-site radiology review of the post-operative MRI scan shows any of the following findings: - satellite lesion(s) or - ependymal spread or - CSF seed metastasis - multicentric disease involvement
Central Pathology	
5	The patient was treated on-site as GBM based on the local final histopathology diagnosis but (if available) the off-site histopathology diagnosis showed a different tumor type
Treatment Violations: IORT	
6	Applied IORT ≤ 20 Gy
Treatment Violations: EBRT	
7	EBRT started more than 42 days after surgery
8	EBRT interrupted for more than seven consecutive days
Treatment Violations: Adjuvant and concomitant chemotherapy (temozolomide)	
	Note: no MPV are applicable for the treatment compliance with adjuvant chemotherapy.
9	Start adjuvant chemotherapy is delayed with more than 21 days (eg, ≥ 49 days after last day of irradiation)
Imaging violations	
10	Salvage treatment was started in absence of confirmed radiological progression.
Concomitant Therapy violations	
11	Administration of concomitant therapy effecting the efficacy evaluations will be assessed during the Medical Review of the corresponding eCRF section.
Quality of study data	
12	Subject reached the PFS endpoint based on clinical deterioration in absence of radiological worsening, decline in KPS or worsening neurological examination (NANO) or symptoms.

13	Any reported protocol violations, other than the criteria defined above, that potentially effect the efficacy assessment and which are identified at the Classification Meeting prior to database lock.

8.3 Safety Analysis Set (SAF)

The safety set will comprise all patients who were randomized in the study regardless of further therapy. In analyses of the SAF, subjects will be presented by randomized/received treatment group (Arm A, Arm B).

8.4 HR-QoL Analysis Set

The HR-QoL analysis set is a subset of the FAS and includes all FAS subjects who meet all of the following criteria:

- Have one baseline HR-QoL assessment
- Have at least one post-baseline HR-QoL questionnaire completed

8.5 Subgroup Analysis Sets

Subgroup analyses will be performed on the key primary (PFS) and the key secondary efficacy endpoints (PFS, OS) within the FAS.

The following subgroups will be defined:

- Site of failure/patterns of recurrence (determined by off-site assessments):
  - PD within < 1 cm margin around the cavity
  - PD within ≥ 1 cm – 2.0 cm margin around the cavity
  - PD within ≥ 2.0 cm margin around the cavity
- Pattern of progressive disease (determined by off-site assessments):
  - PD based on non-measurable and/or measurable enhancing residual lesions
  - PD based on new enhancing lesions
  - PD based on new non-enhancing lesions
- Volume of residual disease (determined by off-site assessments):
  - - 0.2 cm
  - ≥ 0.2 cm

- Appplicator size (as surrogate for tumor volume):
  - ≤ 3.5 cm
  - 4 cm
- Age at screening
  - < 65 years
  - ≥ 65 years
- KPS at screening
  - 60 – 70 percent
  - 80 – 100 percent
- MGMT (local histopathology)
  - Unmethylated
  - Methylated
- IDH1 (local histopathology)
  - Mutation present
  - Mutation absent

The off-site assessments of the “site of failure”, “pattern of progressive disease” and “volume of residual disease” will be described in the imaging charter.

9 Efficacy evaluation

9.1 Endpoint evaluation

Primary efficacy analyses will be performed on the FAS and the PPS .

The key primary endpoint of the trial is:

- Progression-free survival (PFS)
    - as determined by the on-site assessments

The secondary key efficacy endpoints are:

- Overall Survival (OS)
- Radiological progression per Modified-RANO criteria
    - as determined by the off-site response evaluations

All variables will be described by descriptive statistics (frequencies or mean and standard deviation, median, minimum and maximum). Kaplan-Meier (KM) curves and log-rank tests will be used to estimate OS and PFS. Analyses of variance (ANOVAs) with repeated measures or Friedman's one-way ANOVAs will be used to analyze the QoL/ADL assessments. If not stated differently, the tests for statistical significance will be reported on a one-sided 5.0% significance level for OS and PFS, and on a two-sided 5.0% significance level for the QoL/ADL assessments.

Lost to follow-up will include the following subjects:

- Lost to follow-up status is collected on the eCRF page prior to the analysis cut-off;
- Subjects with the last contact date > 21 weeks prior to the analysis cut-off date (duration of 14 weeks is based on the assessment schedule of every 3 months for survival follow-up interval + 1 week window).

9.2 Primary Endpoint Analysis

9.2.1 Primary Efficacy Analysis of PFS

All data required for the calculation of time to event will be taken from the eCRF.

The following variables are used to determine PFS:

- Radiological progression per modified RANO criteria
    - On-site
- The initiation of any new or salvage treatment

- Progressive disease based on clear clinical deterioration not attributable to other causes apart from the tumor
- Death

The primary efficacy analysis will compare the PFS time between the two treatment groups, and will be performed using a one-sided stratified log rank test. PFS time is defined as the time from randomization to the date of progression. For subjects who don't experience a progression till the time of data analysis or who are lost to follow-up, PFS time will be censored at the last recorded date that the subject is known to have no progression.

Frequency (number and percentage) of subjects with an event (progression) and censoring reasons will be presented by treatment arm.
The main analysis of the primary endpoint will use the Kaplan-Meier product limit estimates of survival with the treatment group as covariate. The comparison of the groups will be done by the logrank test (by means of PROC LIFETEST of SAS) and the median PFS will be estimated together with the corresponding two-sided 90% confidence interval. The results will be shown in a KM curve incl. the number of subjects at risk and the cumulative hazard functions will be plotted. To account for the stratification factors of the randomization process these will be included in the model in a second step (as STRATA in PROC LIFETEST while testing the effect of the treatment).

An Endpoint Adjudication Committee has been installed for the study. The Endpoint adjudication Committee may censor the PFS endpoint. If the PFS endpoint is censored, the justification will be reported in the patient narratives. The local principal investigator will be asked to update the eCRF accordingly. The members of the adjudication committee are listed in Appendix II.

Radiological Progression (on-site)

Tumor response will be determined by radiological response criteria based on the updated RANO criteria (Wen et. al JCO 2010). To discriminate between pseudo-progression (radionecrosis) and true-progression, study specific adaptations to the updated RANO were defined for this trial. The following requirement is taken into account for confirmation of radiological progression:

- Increase of the enhancing lesions should be associated with an increase of the perfusion (rCBV ≥ 1) of at least 1 lesion to be considered radiological progression
- Radiological progression needs to be confirmed by the site with a confirmatory scan within 4-6 weeks after initial radiological progression to qualify for "suspected" radiological progression
- Suspected radiological progression needs to be confirmed by the independent off-site radiologist to be considered confirmed radiological progression.

The customizations to RANO are referred to as "Modified-RANO". The Modified RANO response assessments are described in detail in **Appendix I.**

The patient will have met the radiological PFS endpoint if the on-site response assessments are confirmed by the off-site radiology review. The following terms are used to describe the response evaluations:

- **Initial PD**: initial PD refers to the on-site response assessments when the timepoint response is progressive disease. Initial PD should be followed by a confirmatory scan
- **Suspected PD**: the term suspected PD is used when the on-site response assessment of the confirmatory scan shows again PD. The site should request an off-site radiology review to confirm the on-site response assessment that the patient reached the PFS endpoint
- **Confirmed radiological progression**: the term confirmed PD is used to indicate that the off-site radiology review confirmed the on-site response assessments that the patient reached the PFS endpoint.
- **Pseudoprogression (PsP)**: the term pseudoprogression is used if initial PD is not confirmed by the on-site or the off-site response assessments.

Table 1 Outcome and Event Dates for PFS based on radiological progression

#	Scenario	Date of event/censoring
1	Patients receiving Bevacizumab	• Patients receiving Bevacizumab for radionecrosis are not considered to have an event for the endpoint PFS • Patients receiving bevacizumab for radionecrosis will be censored for tumor response: • CR/PR cannot be assigned as response while treated with Bevacizumab • In absence of PD, timepoint response is SD • Patients receiving Bevacizumab for progression are considered to have an event for the endpoint PFS
2	Confirmatory scans acquired < 4 weeks after initial PD	• Progression cannot be confirmed and timepoint response becomes NE unless the confirmatory scan shows unequivocal progression compared with the preceding scans as determined by the off-site reading.
3	Confirmatory scans acquired >6 weeks after initial PD	• In case the confirmatory scan was acquired at the next quarterly imaging evaluation, this deviation will not impact the PFS assessment as date of PFS is the date of initial PD (upon confirmation)

4	Initial PD is not confirmed by the on-site response assessments	• Non-confirmed PD is considered SD and patient should remain in the study. The scan showing initial PD will therefore not act a date PFS and the patient will need to have two new successive tumor evolutions for suspected PD.
5	Suspected PD is not confirmed by the off-site response assessments	• Suspected PD is considered PsP and patient should remain in the study.
6	Perfusion data is missing	• Patient is evaluable for response (CR/PR/SD) but NE for tumor progression based on increase in enhancement (≥ 25% compared with baseline/nadir)
7	On-site suspected radiological progression confirmed by off-site response assessment but at an earlier timepoint compared to the on-site response evaluations	• Date of PFS remains the date of the MRI showing initial PD based on the on-site response assessments
8	Salvage treatment started before confirmatory scan	• If radiological progression is confirmed, date of PFS date is date of scan showing initial PD. • If radiological progression is not confirmed, patient reached the PFS endpoint in absence of radiological progression. Date of PFS is date of start salvage treatment
9	Subjects with new enhancing lesions < 12 weeks after RCT completion	• Case will be submitted to the Endpoint Adjudication Committee to determine if lesion was outside of the radiation field. • For determining the PFS endpoint while the patient is on-study, the same rules apply as for new enhancing lesions that occur ≥ 12 weeks after RCT completion.
10	On-site tumor response evaluations show PD solely based on appearance new non-enhancing lesion(s) but off-site response evaluation is non-PD.	• PFS endpoint has not been met and patient should stay in the study.
11	Patient is on-study at time of study closure	• PFS data will be censored on the date of the last adequate tumor evaluation.

initiation of new salvage treatment

The initiation of any new or salvage treatment for GBM will be considered as an event for PFS. PFS will be calculated from date randomization to event date.

Salvage treatment include:

- Re-resection of recurrence
  - Showing areas of vital tumor (areas with positivity for Ki-67 or presence of endothelial proliferation),
- Re-irradiation
- Invasive treatments (eg, Laser interstitial thermal therapy (LITT), seeds, etc)
- Chemotherapy
  - Alkylators (e.g. CCNU 2nd line, TMZ re-challenge)
  - TIs (Topotecan, Irinotecan)
  - Targeted therapies (EGFR-I, ..)

The medical monitor will review all new/salvage treatments if these qualify as new/salvage treatments.

Table 2 Outcome and Event Dates for PFS based on initiation of new/salvage treatment

#	Scenario	Date of event/censoring

1	Re-surgery	• Surgery for radionecrosis will not be considered as endpoint for PFS
		• If local pathology reveals that the specimens contain tumor tissue, the patient has reached the PFS endpoint:
		  ○ Date of PFS is date of resurgery if pre-surgery scan showed stable disease
		  ○ Date of PFS is date of pre-surgery scan showing increase in enhancement (even if no perfusion data was available)

2	In absence of radiological progression, patient started new treatment	• Date of PFS is date of start new treatment • If patient is alive > 12 months after start salvage treatment, case will be submitted to Endpoint Adjudication Committee that may request additional information from the site to censor the PFS endpoint.
3	Salvage treatment started before confirmatory scan	• If radiological progression is confirmed, date of PFS date is date of scan showing initial PD. • If radiological progression is not confirmed, patient reached the PFS endpoint in absence of

		radiological progression. Date of PFS is date of start salvage treatment

In absence of radiological progression, PFS will be calculated from date randomization to date of start salvage treatment. If initial PD was followed by re-surgery, showing areas of vital tumor, the scan date of the MRI showing initial PD will be used for calculation of PFS.

Clinical Progression

Progressive disease based on clear clinical deterioration not attributable to other causes apart from the tumor will be considered as an event for PFS.

In absence of radiological progression, PFS will be calculated from date randomization to the reported date of clincial progression by the investigator in the eCRF.

Table 3 Outcome and Event Dates for PFS based on clinical progression

#	Scenario	Date of event/censoring
1	Clinical progression is reported before the confirmatory scan.	• If radiological progression is confirmed, date of PFS is the date of the MRI scan showing initial PD • If radiological progression is not confirmed, date of PFS is the date of the reported clinical progression. • To qualify for clinical progression, the KPS should have worsened.
2	Clinical progression in absence of radiological worsening	• Clinical decline alone will not be sufficient for meeting the PFS endpoint.

Death

Patients who have died during the treatment/follow-up phase are considered having reached the PFS endpoint.

Table 4 Outcome and Event Dates for PFS based on Death

#	Scenario	Date of event/censoring
1	Patient dies after initial PD.	• Date PFS is date of death • Case will be submitted to Endpoint Adjudication Committee that may censor the PFS date to date of MRI scan showing initial PD.
2	Unknown if death was related to tumor progression	• Case submitted to Endpoint Adjudication Committee
3	Death	• If no imaging evaluations are performed for the patient anymore (eg, pt is at hospice, patient reached the PFS endpoint "death" is the death occurs within 1 month window in reference to the most recent scheduled imaging assessment • Pt is considered a "discontinuation" if no imaging assessments are anymore performed and the death occurred more than 4 months after the last scheduled imaging evaluation

9.2.2 Sensitivity Analyses of Primary Endpoint

The analysis of the primary endpoint will be repeated using the proportional hazard model of Cox (PHREG procedure in SAS). The model will include the treatment group and the stratification factors of the randomization process as covariates. The main outcome will be the estimated hazard ratio with the corresponding 90% confidence interval. The same model will be further explored including additional effects, especially the ones used in Ch. 8.5 for the subgroup definitions.

9.3 Seconday Endpoint Analysis

The secondary endpoint analysis will be performed on the FAS and the PPS.

9.3.1 Secondary Efficacy Analysis of PFS (off-site)

For all randomized patients, an independent off-site radiology review is performed in accordance with the methodology described in the imaging charter v1.0. Tumor response will be determined by radiological response criteria based on the Modified-RANO response criteria. Radiological progression (date of PFS) will not be censored with clinical data and will only be determined based on the imaging review of the independent off-site reader.

The patient will have met the radiological PFS endpoint if two successive timepoint response assessments confirm PD. The date of PFS will be calculated from the date of randomization to date initial PD. The Modified RANO response assessments are described in detail in **Appendix I** including a response table for all other possible scenarios that may occur.

Hence, it is anticipated that most patients will developed radiological progression based on the appearance of new enhancing lesions (eg, enrolled patients should be amendable for a total resections). New enhancement will result in PD if the new contrast-enhancing lesion has become measurable and the rCBV ≥ 1. If the new lesion does not meet these criteria, the lesion status is SD. The patient has reached the PFS endpoint if at the successive scan progression is confirmed (eg, new lesion remains measurable with an rCBV ≥ 1).

Table 5 Outcome and Event Dates for PFS based on radiological progression

#	Scenario	Date of event/censoring
1	Follow-up scans acquired < 4 weeks after initial PD	• Progression cannot be confirmed and timepoint response becomes NE unless the confirmatory scan shows unequivocal progression compared with the preceding scans as determined by the off-site reading.
2	Perfusion data is missing/inadequate	• Patient is evaluable for response (CR/PR/SD) but NE for tumor progression based on increase in enhancement (≥ 25% compared with baseline/nadir)
3	Subjects with new enhancing lesions < 12 weeks after RCT completion	• Case will be submitted to the Endpoint Adjudication Committee to determine if lesion was outside of the radiation field.
4	Retrospective imaging evaluations showed patient had two episodes of confirmed PD with SD for more than two imaging evaluations in between	• The initial confirmed PD could have been PsP and case will be submitted to the Endpoint Adjudication Committee to determine date of PFS
5	Patient is on-study at time of study closure	• PFS data will be censored on the date of the last adequate tumor evaluation.

The analysis methods for secondary PFS endpoint will be the same as described for the primary endpoint incl. the evaluation by the Cox model.

9.3.2 Median overall survival

All data required for the calculation of time to event will be taken from the eCRF. Randomization strata will be taken from the eCRF as well. OS time is defined as the time from randomization to the date of death, regardless of the actual cause of the subject's death. All deaths reported during the treatment and follow-up phase and during the survival follow-up up phase will be included in the OS analysis.

For subjects who are still alive at the time of data analysis or who are lost to follow-up, OS time will be censored at the last recorded date that the subject is known to be alive.

The main efficacy analysis will compare the OS time between the two treatment groups, and will be performed using a one-sided stratified log rank test.

Frequency (number and percentage) of subjects with an event (death) and censoring reasons will be presented by treatment arm. Censoring reasons are as follows:

- Alive
- Withdrawal of consent
- Lost to follow-up

Lost to follow-up will include the following subjects:

- Lost to follow-up status is collected on the eCRF page prior to the analysis cut-off;
- Subjects with the last contact date earlier than 21 weeks prior to the analysis cut-off date (duration of 14 weeks is based on the assessment schedule of every 3 months for survival follow-up interval + 1 week window).

The analysis methods for OS endpoint will be the same as described for the primary endpoint.

9.3.3 Health-Related Quality of Life

Health-related quality of life analysis will be performed on the FAS. Health-related quality of life (HRQoL) will be assessed by the European Organization for Research and Treatment of Cance questionnaire (EORTC-QLQ-C30/BN20). Activities of daily living (ADL) will be assessed using the Barthel Index (Mahoney & Barthel, 1965).

The EORTC QLQ-C30 is a questionnaire developed to assess the general aspects of health-related quality of life of cancer patients. It incorporates five functional scales (physical, role, cognitive, emotional, and social), three symptom scales (fatigue, pain, and nausea and vomiting), a global health status / QoL scale, and a number of single items assessing additional symptoms commonly reported by cancer subjects (e.g. dyspnea, loss of appetite, insomnia, constipation, and diarrhea), and financial impact of the disease.

The EORTC QLQ-BN20 is a questionnaire developed to evaluate the effects of the tumour and its treatment on symptoms, functions and health-related quality of life (HRQoL) of brain tumour patients, both in clinical trials and clinical practice.
Both EORTC scales and single items will be assessed on categorical scales. The interpretation of the scales is different:

A higher value for a functional scale represents a higher level of functioning.
A higher value for the global health status represents a higher QoL.
A higher value of a symptom scale represents a higher level of symptomatology/problems.

C30 comprises the following types of questions:

Scales and Items	**Questions**
Global health status	
Global health status/QoL	29, 30
Functional scales	
Physical functioning	1 to 5
Role functioning	6, 7
Emotional functioning	21 to 24
Cognitive functioning	20, 25
Social functioning	26, 27
Symptom scales / items	
Fatigue	10, 12, 18
Nausea and vomiting	14, 15
Pain	9, 19
Dyspnoea	8
Insomnia	11
Appetite loss	13
Constipation	16
Diarrhoea	17
Financial difficulties	28

The BN20 scale comprises the following types of questions:

Symptom scales / items	QLQ-BN20 Questions
Future uncertainty	1, 2, 3, 5
Visual disorder	6, 7, 8

Motor dysfunction	10, 15, 19
Communication deficit	11, 12, 13
Headaches	4
Seizures	9
Drowsiness	14
Itchy skin	17
Hair loss	16
Weakness of legs	18
Bladder control	20

Afterwards the scales will be linearly transformed to 0-to-100 scores in 2 steps:

1. Estimate the raw score, which is the average of the items $I_1, I_2, \dots I_n$, i.e. $RS = (I_1 + I_2 + \cdots + I_n)/n$
2. Standardise the raw scores to a range of 0-100
   - $S = \left[1 - \frac{RS-1}{range}\right] \cdot 100$ for functional scales and
   - $S = \left[\frac{RS-1}{range}\right] \cdot 100$ otherwise, where range means the range of the individual items I_i (difference between the possible maximum and the minimum response to individual items which is constant within the same category).

The Barthel Index is an ordinal scale used to measure performance in activities of daily living (ADL). Each performance item is rated on this scale with a given number of points assigned to each level or ranking. It uses ten variables describing ADL and mobility. A higher number is associated with a greater likelihood of being able to live at home with a degree of independence following discharge from hospital. The score is the sum of the items x5 resulting in a scale from 0 to 100.
Data will be analyzed for QoL and ADL parameters as change from baseline scores by means of a repeated measure ANOVA with treatment as fixed effect, baseline value as covariate and visit as repeated effect.

9.3.1 Sensitivity Analyses of Secondary Endpoint

The sensitivity analyses of the PFS and OS endpoints will be the same as for the primary endpoint. The QoL and ADL parameters will be analyzed by the above mentioned ANOVA incl. the stratification factors for the randomization process as additional fixed effects.

10 Safety Evaluations

Safety analyses will be performed on the Safety Analysis Set. The safety endpoints will be tabulated using descriptive
Statistics.

Adverse events will be reported in eCRF with the “Investigator Term” and by worst grade and “Preferred Term” according to National Cancer Institute –Common Terminology Criteria for Adverse Events (NCI-CTCAE version 4.0) per subject. The medical monitor reviews all reported AEs and ensures the Investigator Term matches with the corresponding ”Preferred Term” in accordance with the CTC criteria.

10.1 Adverse Events

The AE listings will include all reported AEs (all worsening’s compared with baseline and all new Adverse Events).

- Related Adverse Events: adverse events with relationship to study treatment:
- Relationship surgery
- Relationship chemotherapy
- Relationship radiotherapy
- Serious Adverse Events (SAE): serious adverse events with relationship disease progression
- Adverse Events Leading to Death

The overall summary of AEs table will include the following per treatment group:

- Number of AEs per SOC per Arm
- Number of SAEs per SOC per Arm
- Number of AEs per SOC per Arm per grade
- Distribution of SAEs over individual patients per Arm
- Number of study visits per Arm
- Number of AEs per SOC, per study arm, seriousness and relation to radiotherapy
- Relation (S)AE to surgery / radiotherapy / chemotherapy - all grades
- Relation (S)AE to surgery / radiotherapy / chemotherapy - grades 3-4-5

- Incidence reported adverse event per grade between the two arms regardless relationship
- Incidence reported adverse event per grade between the two arms related to radiotherapy (possible, probable or definitely)
- Incidence reported adverse event per grade between the two arms related to surgery (possible, probable or definitely)
- Incidence reported adverse event per grade between the two arms related to chemotherapy (possible, probable or definitely)
- incidence reported neurotoxicities per grade between the two arms related to radiotherapy (possible, probable or definitely)
- Overview AEs per site per Arm
- Number of AEs per SOC per visit per Arm- all AEs
- Number of AEs per SOC per visit per Arm- Grade 3-5
- NANO assessments per visit per Arm
- KPS assessments per visit per Arm
- Adverse events leading to death by SOC per Arm
- Adverse of special interest – radionecrosis

10.1.1 Adverse Events Leading to Study Discontinuation

Overall frequency tables will be prepared for the adverse events that lead to study discontinuation.

10.1.2 Serious Adverse Events

The following overall frequency tables will be prepared for serious adverse events (SAEs):

- Incidence of serious AEs by SOC and PT per study arm
- Incidence of serious AEs definitely related to irradiation by SOC and PT per study arm
- Incidence of serious AEs related to tumor progression by SOC and PT per study arm

The listings of SAEs will also be provided with the relevant information.

10.1.3 Deaths

All deaths, deaths within 30 days after surgery, 30 days after completion EBRT, deaths within 60 days after completion as well as the primary reason for death, will be tabulated based on information from the eCRF

- Number of Deaths
- Number of Deaths within 30 days after surgery
- Number of Deaths within 30 days after completion EBRT
- Number of Deaths within 60 days after completion EBRT
- Reason of Death
    - Disease progression
    - Adverse event related to study treatment
    - Adverse event not related to study treatment
    - Other
    - Unknown

10.1.4 Period of observation and Outcomes of AEs

The clinical course of the (S)AE will be followed up until resolution or normalization of changed laboratory parameters or until it has changed to a stable condition. After study completion (reach PFs endpoint or study discontinuation), ongoing (S)AEs should be followed until resolution or normalization. In addition, any new SAEs occurring within 3 months after End of Study should be reported.

Except for radionecrosis**,** no other AEs need to be reported during the survival follow-up period.

11 STATISTICAL METHODOLOGY

All confirmatory and exploratory statistical analyses and summary information are to be generated according to this analysis plan. Any deviation from this plan will be documented in the clinical study report. All computations will be performed prior to rounding. The p-values will be displayed with 2 significant digits with a maximum of 3 decimal places. Statistical significance will be determined prior to rounding the p-value.

If not stated differently, all statistical comparisons will be made using one-sided tests at the 0.05 significance level for OS and PFS, and two-sided at the 0.05 significance level for the QoL/ADL assessments.

11.1.1 Definitions

11.1.2 Treatment groups

The following treatment groups are included in the study and will be referred to as treatment groups:

- Arm A: Standard surgery plus intraoperative radiotherapy followed by radiochemotherapy and adjuvant chemotherapy (Experimental).
- Arm B: Standard surgery followed by radiochemotherapy and adjuvant chemotherapy (Control)

11.1.3 Comparisons of Treatment Groups

The comparison between the experimental and control group will be performed based on the statistical models described in the following sections (1-tailed log-rank test, Kaplan-Meier, ANOVA):

OS and PFS will be estimated with Kaplan-Meier curves and log-rank tests

QoL/ADL assessment will be analyzed with ANOVA with repeated measures or Friedman´s one-way ANOVA.

P-values will be calculated for all comparisons as described above for efficacy variables.

11.1.4 1-tailed log rank test

A 1-tailed log-rank test will be used to analyze median progression-free survival (PFS) in for the primary and secondary efficacy variables.

11.1.5 Kaplan-meier curve

The Kaplan-Meier curve will be used to estimate median overall survival (time after which 50% of patients have died of any cause) for the secondary efficacy variables.

11.1.6 ANOVA

A repeated measures ANOVA or Friedman´s one-way ANOVA will be used to analyze the secondary efficacy variable QoL/ADL on all measurements from baseline to EoT (PFS or discontinuation). The model contains terms for treatment group (2 treatment arms), baseline measurement and time (each relevant visit).

11.1.7 Analysis of Subgroups

Subgroup analyses for primary and key secondary efficacy will be performed by applying the Kaplan-Meier, log-rank and ANOVA test separately for each subgroup and variable.

11.2 Study Population

11.2.1 Disposition of Subjects

The following will be summarized overall and by treatment group, where appropriate. The summary will be presented for all FAS subjects.

- Total number of subjects enrolled overall
- Number of enrolled subjects who were not randomized and grouped by the main reason
- Number and percentage of randomized subjects in the following analysis sets:
    - FAS
    - PPS
    - Safety Analysis Set
    - HR-QoL analysis set
- Number of subjects randomized but not treated
- Number of randomized subjects still on-study
- Number of randomized subjects reached the PFS endpoint with reason
- Number of randomized subjects per reason of study discontinuation
- Number of subjects who discontinued but are still in survival follow-up

- Number of randomized subjects per site
- Number of randomized subjects by randomization strata (eCRF)

11.2.2 Demographic and Other Baseline Characteristics

The listing of demographics and baseline characteristics will include the following information:

- subject identifier
- treatment group
- age
- Sex
- height (cm)
- weight (kg)
- BMI (kg/m2)
- KPS performance status.

11.3 Study treatment

The compliance to the radio-chemotherapy will be assessed as the percentage of the received does compared to the planned dose (both for radioation and TMZ doses) will be tabulated by treatment arm (see also Ch. 4.1.2). In addition, a tabulation will be prepared using the compliance categories <80%, <90%, <95%. In case of high incompliance the main analysis may be repeated with the dose as covariate.

12 Appendix I: Modified RANO Tumor Response Criteria

Tumor response will be determined by radiological response criteria based on the updated RANO criteria (Wen et. al JCO 2010). The RANO criteria distinguish between measurable and non-measurable contrast-enhancing lesions. Measurable lesions are defined as bi-dimensional measurements (long axis / short axis) with clearly defined margins by CT or MRI scans and can be entitled either as target or non-target lesions. Non-measurable lesions are defined as unidimensionally measurable lesions, masses with margins not clearly defined or lesions with maximal perpendicular diameters less than 10mm and can be entitled as Non-target lesions. Furthermore, Non-target lesions are divided into contrast-enhancing and non-contrast-enhancing categories. The contrast-enhancing lesions are evaluated on T1-weighted scans. For the non-contrast-enhancing (pathological) non-target lesions, T2/Flair weighted scans are used for evaluation.

To discriminate between pseudo-progression and true-progression, study specific adaptations to the updated RANO were defined:

- Increase of the enhancing lesions should be associated with a rCBV ≥ 1 of at least 1 lesion to be considered radiological progression
- Progression of the enhancing lesions requires confirmation

In case no quantitative rCBV data is available, the on-site response assessments may include the qualitative (visual) interpretation of the rCBV map to distinguishing tumor recurrence from pseudoprogression. Although sites are encouraged to perform a quantitative evaluation of the perfusion scan (eg, changes in corrected normalized rCBV values), a qualitative assessment is accepted taking into account a central post-processing step will be performed for all patients after suspected progression.
The customizations to RANO are referred in this document as “Modified-RANO”. These customized response criteria are described in more detail in the remaining part of this chapter.

12.1 Tumor Response Assessments: Measurable Contrast-Enhancing Lesions

Radiological Progressive Disease (PD):
The measurable contrast enhancing lesions (target lesions) present at the post-operative MRI scan constitute to radiological progression when the following two criteria are met:

- 25% increase in sum of the products of perpendicular diameters (SPD) of the contrast-enhancing lesions compared with the nadir AND
- at least 1 lesion demonstrate a rCBV ≥ 1

If these tumor response criteria for the contrast-enhancing lesions result in initial PD, the successive scan (eg, confirmatory scan) should meet these two criteria again before the patient can be considered having a confirmed radiological progression and consequently reached the PFS endpoint.

Radiological Stable Disease (SD):
Stable disease occurs if the patient does not qualify for complete response, partial response, or progression. In case an 25% increase in SPD is not associated with a rCBV ≥ 1, the response of the measurable contrast enhancing lesion is SD.

If the rCBV ≥ 1 while the SPD shows SD, the rCBV alone would not count as progressive disease and the response remains SD.

Radiological Partial Response (PR):
Partial Response (PR) requires at least a 50% decrease in SPD compared with the post-operative MRI. Partial Response can only be assigned if the patient has measurable residual disease.

Radiographic Complete Response (CR):
Complete Response requires complete disappearance of all enhancing measurable lesions. Complete Response can only be assigned if the patient has measurable residual disease.

12.2 Tumor Response Assessments: Non-measurable Contrast-Enhancing Lesions

Radiological Progressive Disease (PD):
In case non-measurable contrast enhancing lesions are present at the post-operative MRI scan, initial progression (per Modified-RANO) occurs when the following conditions are met:

- At least one non-measurable contrast-enhancing lesions became measurable and increased significantly in size.
- at least 1 lesion has a rCBV ≥ 1

Progression for the non-target lesion(s) is confirmed if the successive scan shows PD based on the same criteria. The time point response will be SD if the rCBV criteria for progression is not met.

Radiographic Stable Disease (SD):
Stable disease occurs if the patient does not qualify for complete response or progression (eg, the enhancing lesions remain non-measurable or become measurable without a rCBV ≥ 1).

Radiographic Complete Response (CR):
In case non-measurable contrast enhancing lesions are present at the post-operative MRI scan, Complete Response requires complete disappearance of all enhancing non-measurable lesions.

12.3 Tumor Response Assessments: Non-Enhancing Lesions

Radiological Progressive Disease (PD):
In case non-enhancing lesions are present at the post-operative MRI scan, Unequivocally Worsening is defined as a significant visual increase of one of the non-enhancing lesions. Note, the rCBV values are not of concern for defining progression of the non-enhancing lesions. The status "Unequivocally Worsening: will constitute to PD and the patient has reached the PFS endpoint. Progression based in unequivocally worsening of a non-enhancing lesion requires no confirmation at a successive scan.

Radiological Stable Disease (SD):
In the situation non-enhancing lesions are present at the post-operative MRI scan, Stable Disease Is defined as visual stable or improved non-enhancing (T2/FLAIR) lesions.

Radiological Complete Response (CR):
In case non-enhancing lesions are present at the post-operative MRI scan, Complete Response is defined as resolution of all T2/FLAIR signal abnormality attributable to bulk tumor. This does not require resolution of T2/FLAIR signal abnormality attributable to gliosis, radiation effect, infection, ischemia, etc.

12.4 Tumor Response Assessments: New Lesions

New contrast-enhancing lesions:
A single new contrast-enhancing lesion (within or outside the irradiation field) occurring after the post-operative scan will constitute to progression if the new lesion has become measurable and the rCBV ≥ 1. If the new lesion does not meet these criteria, the lesion status is SD.
The patient has reached the PFS endpoint if at the successive scan progression is confirmed (eg, new lesion remains measurable with an rCBV ≥ 1).

New non-enhancing lesions:
The appearance of any new non-enhancing lesions will constitute to progression. The patient has reached the PFS endpoint and the presence of a non-enhancing lesion requires no confirmation at a successive scan.

12.5 Response Table Modified RANO: No Residual Disease Present Post-Operative

Condition criterion	Response
No new enhancing or non-enhancing lesions present	Stable Disease (SD)
New measurable enhancing lesion - **rCBV < 1**[14]	Stable Disease (SD)
New measurable enhancing lesion - **rCBV ≥ 1**[15]	Progressive Disease (PD) - Needs confirmation
New non-measurable enhancing lesion (without any other condition qualifying for PD)	Stable Disease (SD)
New non-enhancing lesion	Progressive Disease (PD) - No confirmation needed
Appearance of ependymal spread	Progressive Disease (PD) - No confirmation needed

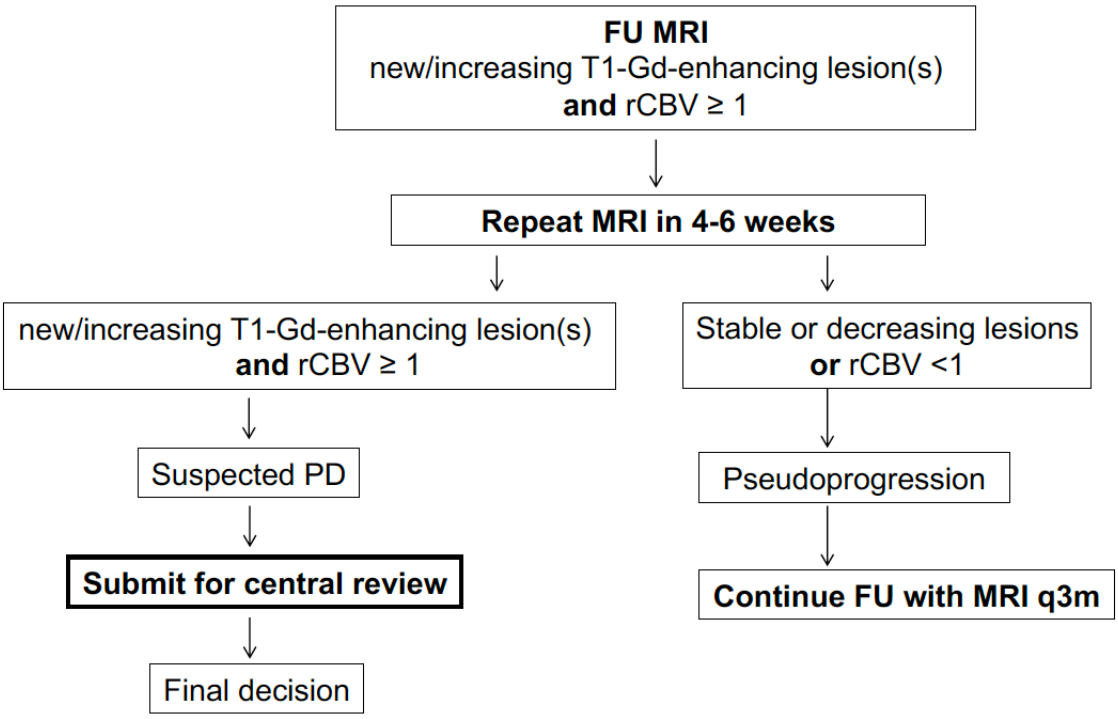
[14] Or No significant visual change in rCBV (on-site assessments)
[15] Or Significant visual increase in rCBV (on-site assessments)

12.6 Response Table Modified RANO: Residual Disease Present Post-Operative

Condition criterion	Response
Disappearance of all enhancing and non-enhancing disease	Complete Response (CR)
≥ 50% decrease in SPD compared to baseline	Partial Response (PR)
≥ 25% increase in SPD compared with nadir AND: - **rCBV < 1**[16]	Stable Disease (SD)
≥ 25% increase in SPD compared with nadir AND: - **rCBV ≥ 1**[17]	Progressive Disease (PD) - Needs confirmation
New Measurable Lesion(s) AND: - **rCBV < 1**[18]	Stable Disease (SD)
New Measurable Lesion(s) AND: - **rCBV ≥ 1**[19]	Progressive Disease (PD) - Needs confirmation
New non-measurable enhancing lesion (without any other condition qualifying for PD)	Stable Disease (SD)
New non-enhancing lesion	Progressive Disease (PD) - No confirmation needed
Ependymal spread	Progressive Disease (PD) - No confirmation needed

[16] Or no significant visual change in rCBV (on-site assessments)
[17] Or significant visual increase in rCBV (on-site assessments)
[18] Or no significant visual change in rCBV (on-site assessments)
[19] Or significant visual increase in rCBV (on-site assessments)

12.7 Flowchart for confirmatory scan and central radiology review



Note: in case the rCBV value is not available/reported, the on-site assessments may include the visual assessment of the rCBV map (eg, significant increase) to determine if the patient is progressive.

13 Appendix II Endpoint Adjudication Committee

Subjects of interest will be reviewed by the Endpoint adjudication Committee as determined the medical monitor. The Endpoint adjudication Committee will be blinded for treatment arm. The Endpoint adjudication Committee will be provided with the patient narratives and will have access to the annotated imaging database from the off-site radiology review.

The Endpoint adjudication Committee includes the following members:

- Whitney Pope, Neuroradiologist, David Geffen School of Medicine, UCLA
- Kevin Petrecca, Neurosurgeon, Montreal Neurological Institute and Hospital
- Frank Giordano, Radiation oncologist and Principal Investigator, University Hospital
- Christina Tsien, Radiation Oncologist, McGill University