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Supplementary information

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Targeted therapies plus radiotherapy for diffuse intrinsic pontine glioma: the randomized phase 2 BIOMEDE trial

In the format provided by the authors and unedited

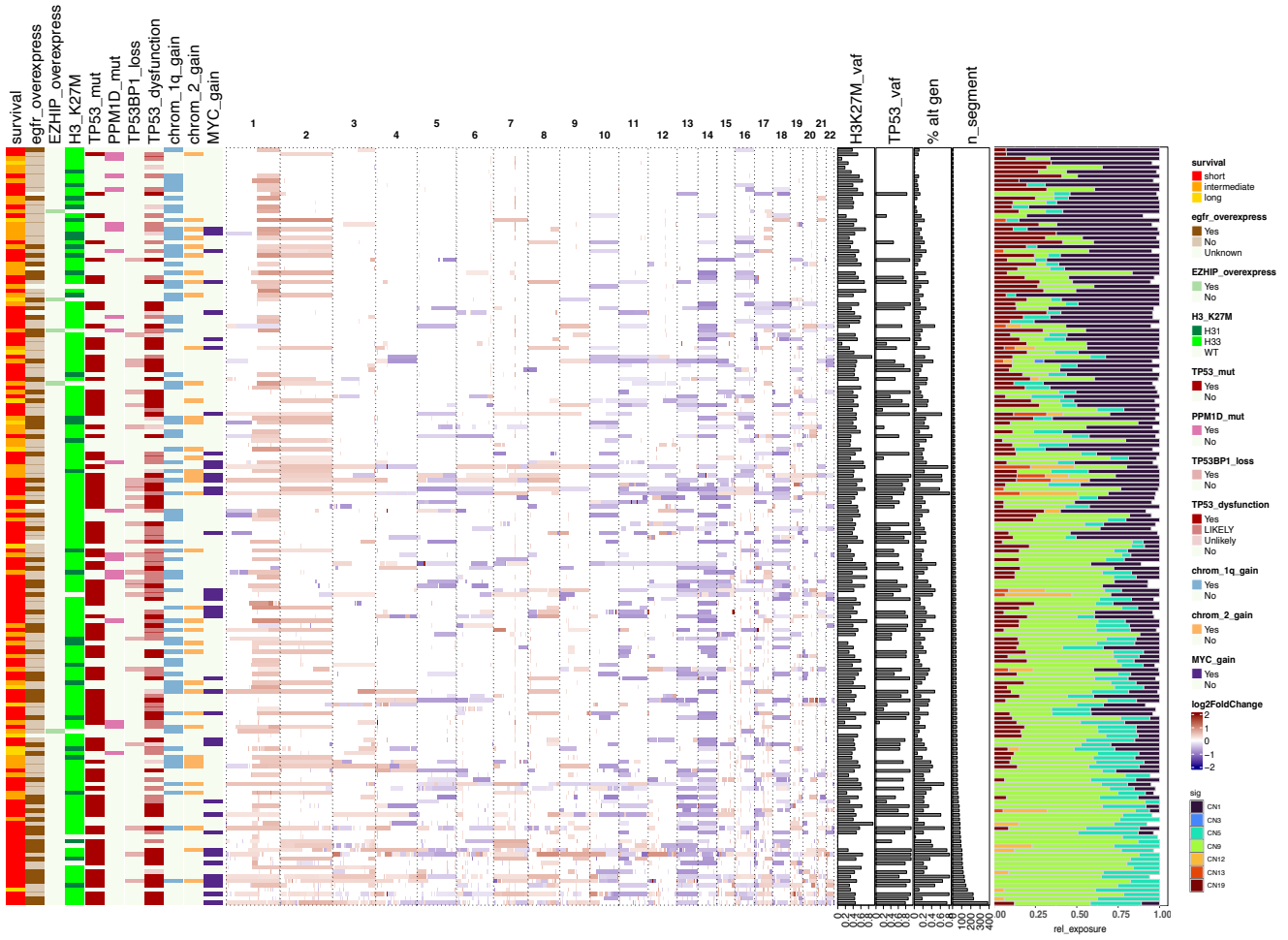

Figure S1
Heatmap showing copy number genomic alterations in DIPG patients with DIPG profiled by WES, as presented in ExtendedFigure8B but with samples ranked according to the number of segments detected by CNA analysis.

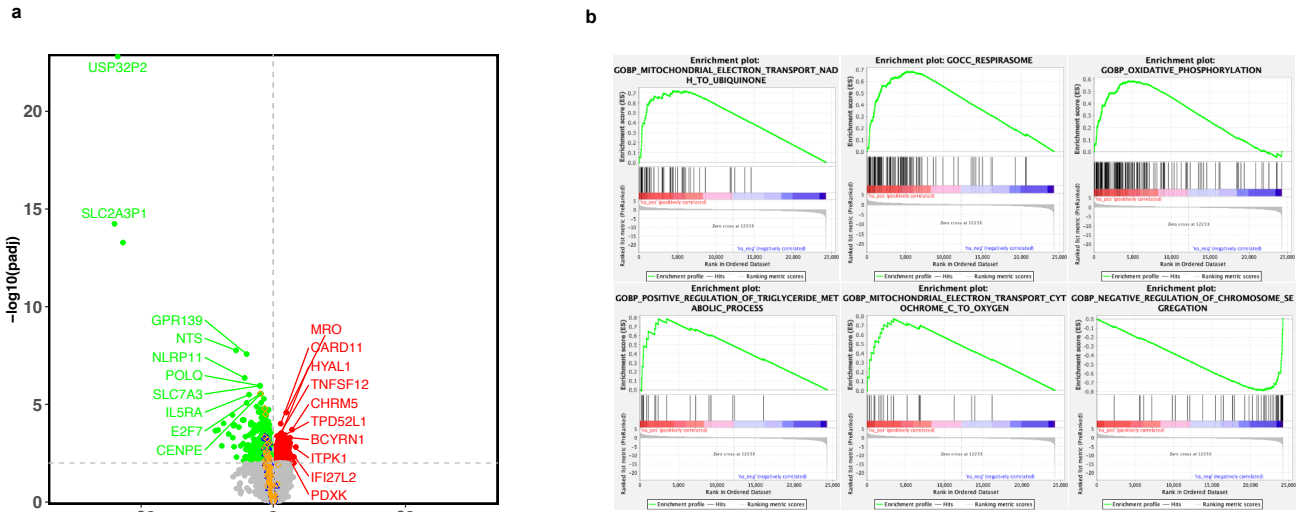

Figure S2

A. Volcano plot of differential analysis in patients with a long *versus* short overall survival. DEGs associated with adjusted pvalue <0.001 are color-coded in red or green.

B. GSEA plots showing Gene Ontology biological processes significantly enriched in either upregulated genes or downregulated genes (*e.g.,* negative regulation of chromosome segregation) in patients with a long- *versus* short-term overall survival.

Table S1: Screen failures related to diagnosis

BIOMEDE ID	Differential Diagnosis (Histology, IHC)	Molecular finding (frozen)
37	Non Diffuse Glioma, Ganglioglioma	H3.3 K27M/BRAF V600E
78	Biopsy not tumoral, vasculitis	
81	HG-NET MycN	MycN/ID2 x 79, TP53 MUT
91	Angiocentric Glioma	QKI translocation
112	Biopsy not tumoral, MRI not typical	Two NF1 mutations
116	Biopsy not tumoral	ACVR1 R206H, NRAS G12S
140	Pilomyxoïd Astrocytoma	NA
144	HG-NET MycN	MycN/ID2 amplification
147	Pilocytic Astrocytoma	NA
175	Biopsy not tumoral	NA
179	Angiocentric Glioma	NA
187	Normal Cerebellar Parenchyma	NA
205	ETMR	C19MC + (FISH)
209	High-grade glioma	NA
211	High-grade glioma	FISH MycN - , EGFR -, p53 -
212	Diffuse low-grade glioma	FISH MYB no fusion
223	Diffuse low-grade	FISH MYB no fusion
228	Diffuse low grade	Olig2 postive
233	Pilocytic astrocytoma	FISH BBRAF no fusion
262	DIPG rejected (error)	H3K27M mutation
269	DIPG rejected	NA
279	ETMR suspected (Lin28 positive)	FISH C19MC not amplified
288	Diffuse low-grade glioma (MYB/MYBL1 suspected)	FISH MYB no fusion

Central pathology review was performed before randomization in the all three treatment arms, with a median turnaround time of five days. Twenty-three patients were considered screen after this review, and their alternative diagnoses are indicated. Diagnosis was established according

to the WHO classification by the lead pathologist (PV) based on morphology (diffuse), histology (glial neoplasm), and loss of trimethylation at position K27 of the histone H3. H3K27M IHC was also performed, but positivity was not required for study entry. EZHIP staining was performed *a posteriori* to confirm the diagnosis when the assay becomes available.

Three more patients were not randomized for other reasons (one received dasatinib without randomization due to the absence of biomarkers for erlotinib or everolimus, 2 elligible for arm R2 had a contraindication to dasatinib and received everolimus). ETMR: Embryonal Tumor with Multilayered Rosettes; HGG: high-grade glioma; HG-NET: High-Grade NeuroEpithelial Tumor; NOS: not otherwise specified; FISH: Fluorescent *In Situ* Hybridization.

Table S2: Timing of the procedures

Time interval (days) between	Median	Q1-Q3
- **Initial symptoms and radiological diagnosis**	20	8 to 48
- **Initial symptoms and biopsy**	30	17 to 56
- **Radiological diagnosis and biopsy**	6	4 to 11
- **Informed consent and biopsy**	1	-4 to 3
- **Study entry and biopsy**	-1	-9 to 1
- **Biopsy and start of treatment**	20	14 to 24
- **Radiological diagnosis and start of treatment**	26.5	21 to 32
- **Duration of hospital stay for the biopsy**	2	1 to 5

Q1-Q3: Interquartile range

Supplementary Table S3. Summary of safety analyses

System Organ Class / Prefered Term	Erlotinib (N= 35)				Everolimus (N= 91)				Dasatinib (N= 101)				Comparison between the 3 arms	
	Gr >=1		Gr >=3		Gr >=1		Gr >=3		Gr >=1		Gr >=3		Gr >=1	Gr >=3
	N	%	N	%	N	%	N	%	N	%	N	%	p-value	p-value
Any type	35	*100*	29	*83*	91	*100*	69	*76*	100	*99*	80	*79*	1.00	0.67
Blood and lymphatic system disorders	**30**	***86***	**9**	***26***	**83**	***91***	**28**	***31***	**87**	***86***	**34**	***34***	**0.5**	**0.68**
Anemia	8	*23*	2	*6*	32	*35*	0	*0*	58	*57*	9	*9*		
Hyperleukocytosis	2	*6*	1	*3*	1	*1*	0	*0*	1	*1*	0	*0*		
Leukopenia	3	*9*	0	*0*	11	*12*	0	*0*	8	*8*	1	*1*		
Lymphocytosis	0	*0*	0	*0*	0	*0*	0	*0*	1	*1*	0	*0*		
Lymphopenia	28	*80*	6	*17*	80	*88*	28	*31*	76	*75*	23	*23*		
Neutropenia	7	*20*	2	*6*	22	*24*	3	*3*	25	*25*	7	*7*		
Neutrophilia	1	*3*	0	*0*	1	*1*	0	*0*	1	*1*	0	*0*		
Thrombocytopenia	2	*6*	0	*0*	16	*18*	0	*0*	37	*37*	4	*4*		
Thrombocytosis	0	*0*	0	*0*	1	*1*	0	*0*	4	*4*	0	*0*		
Cardiac disorders	**3**	***9***	**0**	***0***	**4**	***4***	**0**	***0***	**2**	***2***	**0**	***0***	**0.18**	**NA**
Cardiac rythm disorder	3	*9*	0	*0*	4	*4*	0	*0*	1	*1*	0	*0*		
Cardiac disorder - Other	0	*0*	0	*0*	0	*0*	0	*0*	1	*1*	0	*0*		
Ear and labyrinth disorders	**3**	***9***	**0**	***0***	**10**	***11***	**0**	***0***	**15**	***15***	**0**	***0***	**0.55**	**NA**
Ear pain	0	*0*	0	*0*	3	*3*	0	*0*	6	*6*	0	*0*		
Hearing impaired	1	*3*	0	*0*	4	*4*	0	*0*	3	*3*	0	*0*		
Tinnitus/Hyperacusis	2	*6*	0	*0*	4	*4*	0	*0*	6	*6*	0	*0*		
Ear and labyrinth disorders - Other	0	*0*	0	*0*	0	*0*	0	*0*	1	*1*	0	*0*		
Endocrine disorders	**2**	***6***	**0**	***0***	**9**	***10***	**0**	***0***	**10**	***10***	**0**	***0***	**0.73**	**NA**
Cushing's syndrome	2	*6*	0	*0*	8	*9*	0	*0*	7	*7*	0	*0*		
Diabetes insipidus	0	*0*	0	*0*	1	*1*	0	*0*	2	*2*	0	*0*		
Hypothyroidism	0	*0*	0	*0*	0	*0*	0	*0*	1	*1*	0	*0*		
Eye disorders	**28**	***80***	**2**	***6***	**32**	***35***	**1**	***1***	**39**	***39***	**1**	***1***	**<0.0001**	**0.22**
Blepharitis	1	*3*	0	*0*	0	*0*	0	*0*	0	*0*	0	*0*		
Blurred vision	11	*31*	0	*0*	11	*12*	0	*0*	11	*11*	0	*0*		
Conjunctivitis	16	*46*	0	*0*	15	*16*	0	*0*	20	*20*	0	*0*		
Diplopia	1	*3*	0	*0*	0	*0*	0	*0*	1	*1*	0	*0*		
Dry eye	8	*23*	0	*0*	10	*11*	0	*0*	9	*9*	0	*0*		
Eye pain	2	*6*	0	*0*	1	*1*	0	*0*	2	*2*	0	*0*		
Growth of eyelashes	3	*9*	0	*0*	0	*0*	0	*0*	0	*0*	0	*0*		
Keratitis/Corneal ulcer	6	*17*	1	*3*	6	*7*	1	*1*	8	*8*	1	*1*		
Papilledema	1	*3*	1	*3*	0	*0*	0	*0*	0	*0*	0	*0*		
Photophobia	1	*3*	0	*0*	1	*1*	0	*0*	1	*1*	0	*0*		
Visual acuity reduced	1	*3*	1	*3*	0	*0*	0	*0*	0	*0*	0	*0*		
Eye disorders - Other	0	*0*	0	*0*	0	*0*	0	*0*	1	*1*	0	*0*		
Gastrointestinal disorders	**35**	**##**	**8**	***23***	**86**	***95***	**17**	***19***	**98**	***97***	**35**	***35***	**0.33**	**0.038**
Abdominal distension	0	*0*	0	*0*	0	*0*	0	*0*	3	*3*	0	*0*		
Abdominal pain	20	*57*	0	*0*	31	*34*	0	*0*	64	*63*	6	*6*		
Anal mucositis	2	*6*	0	*0*	1	*1*	0	*0*	4	*4*	0	*0*		
Colitis	0	*0*	0	*0*	0	*0*	0	*0*	2	*2*	1	*1*		
Constipation	14	*40*	0	*0*	26	*29*	0	*0*	53	*52*	3	*3*		
Diarrhea	16	*46*	0	*0*	17	*19*	1	*1*	67	*66*	12	*12*		
Dry mouth	0	*0*	0	*0*	1	*1*	0	*0*	1	*1*	0	*0*		
Duodenitis	1	*3*	1	*3*	0	*0*	0	*0*	0	*0*	0	*0*		
Dyspepsia	1	*3*	0	*0*	2	*2*	0	*0*	1	*1*	0	*0*		
Dysphagia	3	*9*	2	*6*	4	*4*	1	*1*	9	*9*	2	*2*		
Gastric perforation	0	*0*	0	*0*	0	*0*	0	*0*	1	*1*	1	*1*		
Gastritis/Esophagitis	1	*3*	0	*0*	1	*1*	0	*0*	2	*2*	1	*1*		
Gastroenteritis	0	*0*	0	*0*	1	*1*	1	*1*	4	*4*	1	*1*		
Gastroesophageal reflux disease	1	*3*	1	*3*	2	*2*	0	*0*	2	*2*	0	*0*		

System Organ Class / Prefered Term	Erlotinib (N= 35)				Everolimus (N= 91)				Dasatinib (N= 101)				Comparison between the 3 arms	
	Gr >=1		Gr >=3		Gr >=1		Gr >=3		Gr >=1		Gr >=3		Gr >=1	Gr >=3
	N	%	N	%	N	%	N	%	N	%	N	%	p-value	p-value
Lipase increased	0	0	0	0	0	0	0	0	2	2	1	1		
Nausea	22	63	2	6	44	48	2	2	64	63	6	6		
Serum amylase increased	0	0	0	0	0	0	0	0	2	2	0	0		
Stomatitis/Mucositis oral	9	26	1	3	47	52	5	5	21	21	0	0		
Tooth disorder/Toothache	0	0	0	0	4	4	0	0	3	3	0	0		
Vomiting	30	86	4	11	70	77	10	11	91	90	18	18		
General disorders and administration site conditions	**34**	**97**	**7**	**20**	**85**	**93**	**11**	**12**	**97**	**96**	**23**	**23**	**0.70**	**0.15**
Anorexia	19	54	2	6	22	24	4	4	45	45	8	8		
Chills	0	0	0	0	0	0	0	0	1	1	0	0		
Edema	5	14	0	0	9	10	0	0	18	18	1	1		
Fatigue	28	80	5	14	73	80	6	7	90	89	14	14		
Fever	8	23	0	0	27	30	0	0	48	48	3	3		
Flu like symptoms	0	0	0	0	2	2	0	0	0	0	0	0		
Hypothermia	0	0	0	0	2	2	0	0	1	1	0	0		
Increased appetite	0	0	0	0	2	2	0	0	1	1	0	0		
Malaise	1	3	0	0	0	0	0	0	0	0	0	0		
Pain	2	6	1	3	3	3	0	0	3	3	0	0		
Weight gain	0	0	0	0	6	7	3	3	7	7	2	2		
Weight loss	16	46	1	3	21	23	2	2	36	36	2	2		
Hepatobiliary disorders	**14**	**40**	**3**	**9**	**31**	**34**	**4**	**4**	**30**	**30**	**1**	**1**	**0.52**	**0.069**
Hepatobiliary disorders														
Alkaline phosphatase increased	0	0	0	0	2	2	0	0	2	2	0	0		
Blood bilirubin increased	8	23	3	9	1	1	0	0	1	1	0	0		
GGT increased	4	11	0	0	18	20	2	2	12	12	0	0		
Jaundice	1	3	0	0	0	0	0	0	0	0	0	0		
Transaminases increased	9	26	0	0	26	29	3	3	27	27	1	1		
Hepatobiliary disorders - Other	0	0	0	0	0	0	0	0	1	1	0	0		
Immune system disorders	**0**	**0**	**0**	**0**	**1**	**1**	**1**	**1**	**1**	**1**	**0**	**0**	**1.0**	**0.56**
Allergic reaction	0	0	0	0	0	0	0	0	1	1	0	0		
Anaphylactic shock due to adverse food reaction	0	0	0	0	1	1	1	1	0	0	0	0		
Infections and infestations	**29**	**83**	**6**	**17**	**54**	**59**	**18**	**20**	**68**	**67**	**22**	**22**	**0.042**	**0.83**
Aspiration pneumonia	2	6	2	6	3	3	1	1	0	0	0	0		
CMV infection	0	0	0	0	0	0	0	0	1	1	0	0		
COVID-19	0	0	0	0	1	1	0	0	1	1	0	0		
Catheter related infection	0	0	0	0	2	2	1	1	0	0	0	0		
Enterocolitis infection	1	3	0	0	4	4	2	2	5	5	0	0		
Febrile neutropenia	1	3	0	0	0	0	0	0	1	1	1	1		
Folliculitis/Acne/Papulopustular rash/Skin infection	1	3	0	0	0	0	0	0	2	2	0	0		
Gastrointestinal candidiasis	0	0	0	0	2	2	0	0	5	5	0	0		
Herpes or Varicelle-Zona virus infection	1	3	0	0	7	8	2	2	4	4	1	1		
Lung infection	3	9	0	0	5	5	3	3	4	4	2	2		
Meningitis	0	0	0	0	1	1	1	1	0	0	0	0		
Otitis externa	2	6	1	3	2	2	0	0	2	2	0	0		
Peritoneal infection	0	0	0	0	0	0	0	0	1	1	1	1		
Sepsis	1	3	1	3	1	1	1	1	3	3	3	3		
Tooth infection	0	0	0	0	0	0	0	0	1	1	0	0		
Upper respiratory tract infection	16	46	0	0	29	32	5	5	43	43	9	9		
Urinary tract infection	0	0	0	0	1	1	0	0	1	1	0	0		
Vaginal infection	0	0	0	0	1	1	0	0	1	1	0	0		
Infection, Other	13	37	2	6	23	25	6	7	35	35	12	12		

System Organ Class / Prefered Term	Erlotinib (N= 35)				Everolimus (N= 91)				Dasatinib (N= 101)				Comparison between the 3 arms	
	Gr >=1		Gr >=3		Gr >=1		Gr >=3		Gr >=1		Gr >=3		Gr >=1	Gr >=3
	N	%	N	%	N	%	N	%	N	%	N	%	p-value	p-value
Injury, poisoning and procedural complications	**0**	***0***	**0**	***0***	**2**	***2***	**1**	***1***	**3**	***3***	**1**	***1***	**0.85**	**1.0**
Burn	0	*0*	0	*0*	1	*1*	0	*0*	0	*0*	0	*0*		
Fall	0	*0*	0	*0*	0	*0*	0	*0*	1	*1*	0	*0*		
Fracture	0	*0*	0	*0*	1	*1*	0	*0*	0	*0*	0	*0*		
Medical device related injuries and complications	0	*0*	0	*0*	1	*1*	1	*1*	1	*1*	0	*0*		
Wound dehiscence	0	*0*	0	*0*	0	*0*	0	*0*	1	*1*	1	*1*		
Investigations / Metabolism and nutrition disorders	**26**	***74***	**3**	***9***	**85**	***93***	**14**	***15***	**72**	***71***	**11**	***11***	**0.0003**	**0.49**
Acidosis	0	*0*	0	*0*	0	*0*	0	*0*	1	*1*	0	*0*		
Alkalosis	0	*0*	0	*0*	1	*1*	1	*1*	0	*0*	0	*0*		
Dehydration	1	*3*	1	*3*	0	*0*	0	*0*	3	*3*	0	*0*		
Hypercalcemia	1	*3*	0	*0*	4	*4*	0	*0*	1	*1*	0	*0*		
Hyperchloremia	0	*0*	0	*0*	0	*0*	0	*0*	2	*2*	0	*0*		
Hypercholesterolemia	6	*17*	0	*0*	66	*73*	9	*10*	31	*31*	0	*0*		
Hyperglycemia	12	*34*	0	*0*	53	*58*	1	*1*	48	*48*	2	*2*		
Hyperkalemia	1	*3*	0	*0*	1	*1*	0	*0*	2	*2*	0	*0*		
Hypermagnesemia	4	*11*	0	*0*	7	*8*	0	*0*	7	*7*	0	*0*		
Hypernatremia	1	*3*	0	*0*	0	*0*	0	*0*	2	*2*	0	*0*		
Hypertriglyceridemia	9	*26*	0	*0*	66	*73*	8	*9*	25	*25*	1	*1*		
Hyperuricemia	1	*3*	0	*0*	4	*4*	0	*0*	1	*1*	0	*0*		
Hypoalbuminemia	2	*6*	0	*0*	3	*3*	0	*0*	10	*10*	6	*6*		
Hypocalcemia	1	*3*	0	*0*	1	*1*	0	*0*	14	*14*	0	*0*		
Hypoglycemia	2	*6*	1	*3*	3	*3*	0	*0*	1	*1*	0	*0*		
Hypokalemia	7	*20*	2	*6*	7	*8*	1	*1*	16	*16*	3	*3*		
Hypomagnesemia	1	*3*	0	*0*	3	*3*	0	*0*	5	*5*	0	*0*		
Hyponatremia	1	*3*	0	*0*	1	*1*	0	*0*	11	*11*	4	*4*		
Hypophosphatemia	4	*11*	0	*0*	13	*14*	1	*1*	16	*16*	0	*0*		
Hypoproteinemia	0	*0*	0	*0*	0	*0*	0	*0*	1	*1*	0	*0*		
Urine phosphorus decreased	0	*0*	0	*0*	0	*0*	0	*0*	1	*1*	0	*0*		
Metabolism and nutrition disorders - Other	0	*0*	0	*0*	1	*1*	0	*0*	0	*0*	0	*0*		
Musculoskeletal and connective tissue disorders	**11**	***31***	**0**	***0***	**22**	***24***	**4**	***4***	**29**	***29***	**6**	***6***	**0.65**	**0.46**
Amyotrophy	0	*0*	0	*0*	1	*1*	0	*0*	0	*0*	0	*0*		
Arthralgia	1	*3*	0	*0*	5	*5*	1	*1*	10	*10*	1	*1*		
Back pain	1	*3*	0	*0*	1	*1*	0	*0*	5	*5*	1	*1*		
Bone pain	2	*6*	0	*0*	1	*1*	0	*0*	3	*3*	1	*1*		
Chest wall pain	2	*6*	0	*0*	0	*0*	0	*0*	1	*1*	0	*0*		
Muscle spasms	0	*0*	0	*0*	1	*1*	1	*1*	1	*1*	0	*0*		
Muscle weakness	0	*0*	0	*0*	3	*3*	1	*1*	2	*2*	2	*2*		
Myalgia	5	*14*	0	*0*	3	*3*	1	*1*	10	*10*	2	*2*		
Neck pain	1	*3*	0	*0*	3	*3*	1	*1*	2	*2*	1	*1*		
Pain in extremity	1	*3*	0	*0*	6	*7*	0	*0*	5	*5*	0	*0*		
Steroid myopathy	0	*0*	0	*0*	0	*0*	0	*0*	1	*1*	0	*0*		
Synovitis	0	*0*	0	*0*	1	*1*	0	*0*	0	*0*	0	*0*		
Torticolis	1	*3*	0	*0*	0	*0*	0	*0*	2	*2*	0	*0*		
Nervous system disorders	**31**	***89***	**13**	***37***	**82**	***90***	**27**	***30***	**87**	***86***	**35**	***35***	**0.69**	**0.65**
Amnesia/Memory impairment	0	*0*	0	*0*	0	*0*	0	*0*	1	*1*	0	*0*		
Anisocoria	0	*0*	0	*0*	0	*0*	0	*0*	1	*1*	0	*0*		
Ataxia	0	*0*	0	*0*	0	*0*	0	*0*	1	*1*	0	*0*		
Autonomic nervous system imbalance	0	*0*	0	*0*	0	*0*	0	*0*	2	*2*	2	*2*		
Cauda equina syndrome	1	*3*	1	*3*	0	*0*	0	*0*	0	*0*	0	*0*		

System Organ Class / Prefered Term	Erlotinib (N= 35)				Everolimus (N= 91)				Dasatinib (N= 101)				Comparison between the 3 arms	
	Gr >=1		Gr >=3		Gr >=1		Gr >=3		Gr >=1		Gr >=3		Gr >=1	Gr >=3
	N	%	N	%	N	%	N	%	N	%	N	%	p-value	p-value
Central nervous system necrosis	0	0	0	0	0	0	0	0	1	1	1	1		
Coma/Depressed level of consciousness	1	3	0	0	11	12	4	4	8	8	5	5		
Dizziness/Vertigo	7	20	0	0	10	11	0	0	23	23	2	2		
Drooling	2	6	0	0	0	0	0	0	2	2	0	0		
Dysarthria	0	0	0	0	2	2	0	0	3	3	2	2		
Dysesthesia	0	0	0	0	3	3	0	0	0	0	0	0		
Dysgeusia	2	6	0	0	4	4	0	0	6	6	0	0		
Edema cerebral	0	0	0	0	0	0	0	0	1	1	0	0		
Facial pain	0	0	0	0	1	1	0	0	0	0	0	0		
Facial paralysis	0	0	0	0	1	1	1	1	1	1	0	0		
Gait disturbance	0	0	0	0	0	0	0	0	2	2	1	1		
Glossopharyngeal nerve disorder	0	0	0	0	4	4	2	2	1	1	0	0		
Headache	26	74	6	17	72	79	7	8	74	73	9	9		
Hemiparesis	0	0	0	0	0	0	0	0	1	1	0	0		
Hiccups	1	3	0	0	0	0	0	0	0	0	0	0		
Hydrocephalus/Intracranial pressure increased	10	29	8	23	15	16	11	12	24	24	20	20		
Myoclonus	0	0	0	0	0	0	0	0	1	1	0	0		
Neurological status deterioration	1	3	1	3	4	4	3	3	5	5	3	3		
Neuropathic pain	1	3	0	0	4	4	1	1	3	3	1	1		
Nystagmus	0	0	0	0	2	2	0	0	0	0	0	0		
Paresthesia	0	0	0	0	0	0	0	0	1	1	0	0		
Peripheral sensory neuropathy	0	0	0	0	0	0	0	0	1	1	0	0		
Respiration abnormal (neurological sign)	0	0	0	0	2	2	1	1	1	1	1	1		
Seizure	0	0	0	0	4	4	2	2	6	6	2	2		
Tremor	1	3	0	0	0	0	0	0	1	1	0	0		
Psychiatric disorders	**4**	***11***	**1**	***3***	**15**	***16***	**0**	***0***	**22**	***22***	**2**	***2***	**0.34**	**0.38**
Agitation	0	0	0	0	2	2	0	0	3	3	0	0		
Anxiety	1	3	0	0	1	1	0	0	5	5	1	1		
Behavior disorder	0	0	0	0	1	1	0	0	2	2	0	0		
Confusion	0	0	0	0	0	0	0	0	1	1	0	0		
Depression/Mood altered	2	6	1	3	1	1	0	0	2	2	0	0		
Hallucinations	0	0	0	0	0	0	0	0	1	1	0	0		
Insomnia/Sleep disorder	1	3	0	0	12	13	0	0	12	12	0	0		
Irritability	0	0	0	0	1	1	0	0	0	0	0	0		
Personality change	0	0	0	0	0	0	0	0	1	1	1	1		
Renal and urinary disorders	**12**	***34***	**0**	***0***	**16**	***18***	**0**	***0***	**26**	***26***	**8**	***8***	**0.12**	**0.0054**
Renal and urinary disorders														
Acute kidney injury	0	0	0	0	1	1	0	0	0	0	0	0		
Blood urea increased	1	3	0	0	2	2	0	0	0	0	0	0		
Creatinine increased	7	20	0	0	6	7	0	0	4	4	0	0		
Cystitis noninfective	0	0	0	0	0	0	0	0	1	1	0	0		
Dysuria/Urinary retention	2	6	0	0	3	3	0	0	9	9	0	0		
Hematuria	0	0	0	0	0	0	0	0	2	2	0	0		
Nocturia	0	0	0	0	2	2	0	0	0	0	0	0		
Oliguria	0	0	0	0	0	0	0	0	1	1	0	0		
Pollakiuria	1	3	0	0	1	1	0	0	1	1	0	0		
Proteinuria	0	0	0	0	2	2	0	0	11	11	6	6		
Renal calculi	0	0	0	0	0	0	0	0	2	2	2	2		
Renal tubular disorder	0	0	0	0	0	0	0	0	1	1	0	0		
Urinary incontinence	2	6	0	0	0	0	0	0	0	0	0	0		
Urinary tract pain	0	0	0	0	2	2	0	0	1	1	0	0		

System Organ Class / Prefered Term	Erlotinib (N= 35)				Everolimus (N= 91)				Dasatinib (N= 101)				Comparison between the 3 arms	
	Gr >=1		Gr >=3		Gr >=1		Gr >=3		Gr >=1		Gr >=3		Gr >=1	Gr >=3
	N	%	N	%	N	%	N	%	N	%	N	%	p-value	p-value
Reproductive system and breast disorders	**0**	***0***	**0**	***0***	**3**	***3***	**0**	***0***	**4**	***4***	**1**	***1***	**0.77**	**1.0**
Hydrocele	0	*0*	0	*0*	1	*1*	0	*0*	0	*0*	0	*0*		
Irregular menstruation	0	*0*	0	*0*	1	*1*	0	*0*	2	*2*	1	*1*		
Painful erection	0	*0*	0	*0*	1	*1*	0	*0*	0	*0*	0	*0*		
Vaginal itching	0	*0*	0	*0*	0	*0*	0	*0*	2	*2*	0	*0*		
Respiratory, thoracic and mediastinal disorders	**16**	***46***	**3**	***9***	**37**	***41***	**11**	***12***	**52**	***51***	**8**	***8***	**0.32**	**0.60**
Allergic rhinitis	1	*3*	0	*0*	1	*1*	0	*0*	0	*0*	0	*0*		
Chylothorax	0	*0*	0	*0*	0	*0*	0	*0*	2	*2*	1	*1*		
Cough	12	*34*	0	*0*	22	*24*	0	*0*	43	*43*	1	*1*		
Dyspnea	4	*11*	2	*6*	15	*16*	7	*8*	15	*15*	1	*1*		
Hypoxia	0	*0*	0	*0*	1	*1*	0	*0*	0	*0*	0	*0*		
Laryngeal inflammation	1	*3*	0	*0*	0	*0*	0	*0*	0	*0*	0	*0*		
Pleural effusion	0	*0*	0	*0*	0	*0*	0	*0*	4	*4*	2	*2*		
Respiration abnormal	0	*0*	0	*0*	3	*3*	1	*1*	2	*2*	1	*1*		
Respiratory distress	1	*3*	1	*3*	4	*4*	3	*3*	3	*3*	3	*3*		
Respiratory, thoracic, mediastinal disorders-Other	0	*0*	0	*0*	0	*0*	0	*0*	1	*1*	1	*1*		
Skin and subcutaneous tissue disorders	**34**	***97***	**10**	***29***	**66**	***73***	**0**	***0***	**85**	***84***	**4**	***4***	**0.004**	**<0.0001**
Alopecia	15	*43*	0	*0*	43	*47*	0	*0*	59	*58*	0	*0*		
Bruising	0	*0*	0	*0*	0	*0*	0	*0*	2	*2*	0	*0*		
Dermatitis radiation	3	*9*	0	*0*	3	*3*	0	*0*	14	*14*	0	*0*		
Dermo-hypodermitis	0	*0*	0	*0*	0	*0*	0	*0*	1	*1*	1	*1*		
Dry skin	27	*77*	3	*9*	23	*25*	0	*0*	35	*35*	0	*0*		
Folliculitis/Acne/Papulopustular rash/Skin infection	26	*74*	6	*17*	20	*22*	0	*0*	42	*42*	0	*0*		
Hair depigmented	0	*0*	0	*0*	0	*0*	0	*0*	21	*21*	0	*0*		
Hirsutism/Hypertrichosis	3	*9*	0	*0*	4	*4*	0	*0*	4	*4*	0	*0*		
Onychorrhexis	0	*0*	0	*0*	0	*0*	0	*0*	1	*1*	0	*0*		
Palmar-plantar erythrodysesthesia syndrome	0	*0*	0	*0*	1	*1*	0	*0*	0	*0*	0	*0*		
Panniculitis	0	*0*	0	*0*	0	*0*	0	*0*	1	*1*	1	*1*		
Paronychia	11	*31*	1	*3*	3	*3*	0	*0*	9	*9*	0	*0*		
Pruritus	13	*37*	0	*0*	8	*9*	0	*0*	15	*15*	0	*0*		
Skin atrophy	1	*3*	0	*0*	0	*0*	0	*0*	0	*0*	0	*0*		
Skin hyperpigmentation	0	*0*	0	*0*	1	*1*	0	*0*	0	*0*	0	*0*		
Skin hypopigmentation	0	*0*	0	*0*	1	*1*	0	*0*	8	*8*	0	*0*		
Skin lesion	1	*3*	0	*0*	0	*0*	0	*0*	0	*0*	0	*0*		
Skin rash other than folliculitis	18	*51*	4	*11*	26	*29*	0	*0*	46	*46*	3	*3*		
Skin striae	0	*0*	0	*0*	0	*0*	0	*0*	1	*1*	0	*0*		
Skin ulceration	0	*0*	0	*0*	2	*2*	0	*0*	3	*3*	0	*0*		
Sweating abnormal	1	*3*	0	*0*	0	*0*	0	*0*	2	*2*	0	*0*		
Urticaria	1	*3*	0	*0*	1	*1*	0	*0*	0	*0*	0	*0*		
Skin and subcutaneous tissue disorders - Other	0	*0*	0	*0*	0	*0*	0	*0*	2	*2*	0	*0*		
Vascular disorders	**14**	***40***	**1**	***3***	**32**	***35***	**5**	***5***	**33**	***33***	**4**	***4***	**0.73**	**0.91**
Cold extremities	0	*0*	0	*0*	0	*0*	0	*0*	2	*2*	0	*0*		
Frostbite	0	*0*	0	*0*	0	*0*	0	*0*	1	*1*	0	*0*		
Hematoma	1	*3*	0	*0*	0	*0*	0	*0*	0	*0*	0	*0*		
Hemorrhage	0	*0*	0	*0*	0	*0*	0	*0*	0	*0*	0	*0*		
Hemorrhage, digestive	2	*6*	1	*3*	0	*0*	0	*0*	10	*10*	2	*2*		
Hemorrhage, epistaxis	5	*14*	0	*0*	16	*18*	0	*0*	5	*5*	0	*0*		
Hemorrhage, intracranial	0	*0*	0	*0*	2	*2*	1	*1*	1	*1*	0	*0*		

System Organ Class / Prefered Term	Erlotinib (N= 35)				Everolimus (N= 91)				Dasatinib (N= 101)				Comparison between the 3 arms	
	Gr >=1		Gr >=3		Gr >=1		Gr >=3		Gr >=1		Gr >=3		Gr >=1	Gr >=3
	N	%	N	%	N	%	N	%	N	%	N	%	p-value	p-value
Hemorrhage, other	2	*6*	0	*0*	1	*1*	0	*0*	7	*7*	2	*2*		
Hot flashes/Flushing	0	*0*	0	*0*	3	*3*	0	*0*	2	*2*	0	*0*		
Hypertension	6	*17*	0	*0*	17	*19*	3	*3*	10	*10*	0	*0*		
Telangiectasia	1	*3*	0	*0*	0	*0*	0	*0*	1	*1*	0	*0*		
Vasculitis	0	*0*	0	*0*	1	*1*	1	*1*	0	*0*	0	*0*		

Distribution of the maximum grade of adverse events (0-2 *vs*. >=3), by System Organ Class and PT, according to the treatment arm, regardless of causality, over the whole treatment duration. p-value: p-value of the test comparing the distribution of toxicity (grade >=1 or grade >=3, respectively) between the three treatment groups using 2-sided tests, Chi-square tests with 2 degrees of freedom, or Fisher exact tests, as appropriate.

Table S4: Clinical response according to subgroup

Best clinical response / Treatment group	Improved	Stable	Worse	Total
Erlotinib	26 (74%)	8 (23%)	1 (3%)	35
Everolimus	72 (79%)	12 (13%)	7 (8%)	91
Dasatinib	71 (71%)	24 (24%)	5 (5%)	100
Total	169 (75%)	44 (19%)	13 (6%)	226

The best clinical response at any time was assessed by the treating physician and categorized as improved, stable, worse. Data are reported for the 226 patients who initiated treatment (4 did not) and for whom a clinical evaluation was available (3 missing data). The distribution of best clinical response did not differ significantly between treatment groups (Fischer Exact test, p=0.315).

Table S5. Summary of prognostic factors analyses of overall survival

Patient and tumor characteristics	**Univariate analyses**			**Multivariable model [(1)]**	
	Median OS (95%CI)	HR (95%CI)	p-value	HR (95%CI)	p-value
Age at study entry			0.0093		0.022
< 5 years	13.9 (10.8-15.7)	0.79 (0.53-1.16)		1.72 (1.04-2.86)	
5-10 years	8.8 (7.7-10.3)	1.34 (0.99-1.80)		1.64 (1.13-2.38)	
≥ 10 years	10.7 (9.0-12.9)	1 (ref)		1 (ref)	
Histone mutation (MD=41)			0.064		0.49 [(2)]
Wild Type	12.1 (6.6-14.9)	1 (ref)		1 (ref)	
H3.1 K27M	14.8 (11.3-18.6)	0.81 (0.46-1.42)		0.66 (0.33-1.33)	
H3.3 K27M	9.4 (8.3-10.5)	1.28 (0.81-2.03)		0.79 (0.43-1.48)	
***TP53* mutation (MD=52)**			<.0001		<.0001[(3)]
No	14.3 (12.3-15.7)	1 (ref)		1 (ref)	
Yes	8.1 (7.0-9.4)	2.44 (1.78-3.34)		2.84 (1.92-4.20)	

OS: Overall Survival

HR: Hazard Ratio of death

95%CI: 95% confidence interval

MD: number of patients with missing data

p-value: 2-sded p-value of the Wald test of the Cox models

1 (ref): group considered as reference for the estimation of hazard ratios

1) Multivariable model including age at study entry (<5 years *versus* 5-10 *versus* ≥10 years), Histone mutation (Wild type *versus* H3.1 K27M *versus* H3.3 K27M), *TP53* mutation (yes *versus* no), EGFR status (overexpression *versus* EGFR-negative *versus* EGFR unknown) PTEN status (PTEN loss *versus* no PTEN loss *versus* PTEN unknown) and treatment group (erlotinib, everolimus, dasatinib). This multivariable model includes 180 patients as 53 had missing data for histone mutation only (n=1), *TP53* mutation (n=12) or both (n=40).

2) Results regarding Histone mutation are relatively stable when the multivariable model includes all the variables except the age: $HR_{(H3.1\ K27M\ /WT)}$=0.77 (0.39-1.53); $HR_{(H3.3\ K27M\ /WT)}$=0.86 (0.46-1.62); p-value=0.62
3) Results regarding *TP53* mutation are relatively stable when the multivariable model includes all the variables except the age: $HR_{(TP53mutation)}$=2.42 (1.68-3.46); p-value<0.0001

Table S6: Association between Histone mutation and *TP53* mutation

Histone mutation / ***TP53* mutation**	**Wild Type**	**H3.1 K27M**	**H3.3 K27M**	Missing data	**Total**
No	10 (71.4%)	22 (75.9%)	44 (32.1%)	0	76
Yes	4 (28.6%)	7 (24.1%)	93 (67.9%)	1	105
Missing data	8	0	4	40	52
Total	22	29	141	41	233

Chi-square test, 2 degrees of freedom, two-sided p-value <0.0001

Table S7: Association between age and Histone mutation, *TP53* mutation or the combination of both

Age category / Biological characteristics	< 5 years (N=42)	5-10 years (N=117)	≥ 10 years (N=74)	Total (N=233)	P-value
Histone mutation					.001
Wild type	4 (11.4%)	10 (10.3%)	8 (13.3%)	22 (11.5%)	
H3.1 K27M	14 (40.0%)	12 (12.4%)	3 (5.0%)	29 (15.1%)	
H3.3 K27M	17 (48.6%)	75 (77.3%)	49 (81.7%)	141 (73.4%)	
Missing data	7	20	14	41	
***TP53* mutation**					<.0001
No	25 (75.8%)	34 (37.4%)	17 (29.8%)	76 (42.0%)	
Yes	8 (24.2%)	57 (62.6%)	40 (70.2%)	105 (58.0%)	
Missing data	9	26	17	52	
Histone combined with *TP53* mutation					<.0001
Wild type or H3.1 K27M and no *TP53* mutation	14 (42.4%)	13 (14.3%)	5 (8.9%)	32 (17.8%)	
H3.3 K27M and no *TP53* mutation	11 (33.3%)	21 (23.1%)	12 (21.4%)	44 (24.4%)	
TP53 mutation	8 (24.2%)	57 (62.6%)	39 (69.6%)	104 (57.8%)	
Missing data	9	26	18	53	

Two-sided p-value of the Chi-square test, with 4 degrees of freedom for Histone mutation and Histone combined with *TP53* mutation, and 2 degrees of freedom for *TP53* mutation.

Table S8: Description of H3K27 wild type patients.

Biomede number	H3K27M (WES)	H3K27M (IHC)	H3K27me3 loss (IHC)	*ACVR1* mutation	EZHIP positive (RNAseq)	EZHIP positive (IHC)	Additional information
BIOMEDE_002	no	no	yes	yes	yes	yes	
BIOMEDE_007	no	no	yes	yes	yes	yes	
BIOMEDE_077	no	no	yes	yes	yes	yes	
BIOMEDE_079	no	no	NA	yes	NA	NA	Necrotic sample
BIOMEDE_088	no	no	yes	yes	yes	yes	
BIOMEDE_128	no	no	no	no	yes	NA	
BIOMEDE_193	no	yes	yes	no	NA	yes	DMG-EGFR
BIOMEDE_194	no	no	yes	no	no	yes	Cluster in DMG-EZHIP+ subgroup in methylome analysis
BIOMEDE_242	no	yes	yes	no	no	NA	Non contributive biopsy as no detection of SNV and CNA
BIOMEDE_283	no	no	yes	yes	yes	yes	EZHIP expression <1tpm and *TP53* mutation

Five patients harbored *ACVR1*-mutated and were EZHIP positive, as previously reported[8]. One tumor with H3K27me3 loss lacked RNA-seq data but clustered with "DMG-EZHIP+ subgroup" in methylome analysis (DMG for Diffuse Midline Glioma). One frozen tumor biopsy was non-contributive, showing no detectable SNVs or copy number alteration (CNA) although IHC was positive for H3K27M. One patient had a DMG-EGFR with positive EZHIP expression by IHC, as recently described[72]. One biopsy was necrotic, allowing only DNA analysis, which revealed a canonical *ACVR1* mutation. Finally, one tumor exhibited *EZHIP* expression level below the threshold of 1 tpm (transcript per million) and harbored a *TP53* mutation.

Table S9: PI3K/AKT/MTOR pathway genelist

Gene symbol	Impact on PIK3/AKT/ mTOR pathway	Source	Alteration in cancer
ACACA	activating	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	circ-ACACA regulates PIK3 pathway in NSCLC
ACTR2	inhibition	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	downregulation of PTEN in pancreatic cancer cells
ACTR3	inhibition	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	overexpression in cervical cancer
ADCY2	activating	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	overexpression in bladder cancer
AKT1	activating	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	mutation in cancer (driver)
AKT1S1	inhibition	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	AKT1 substrate
AKT2	activating	Gleeson et al (doi.org/10.18632/oncotarget.18750)	overexpression in cancer
AKT3	activating	Gleeson et al (doi.org/10.18632/oncotarget.18750)	mutated in cortical dysplasia
AP2M1	activating	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	expression associated with chemoresistance
ARF1	inhibition	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	overexpression in ovarian cancer
ARHGDIA	activating	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	AKT phosphorylation in glioblastoma
ARID1A	inhibition	Mullen et al (doi: 10.1016/j.ctrv.2021.102287)	loss of function mutations
ARPC3	target	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	overexpression in breast cancer
ATF1	target	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	translocation
BRAF	activating	Christen et al (DOI: 10.1186/s12964-024-01808-2)	activating mutation
CAB39	activating	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	cisplatin resistance
CAB39L	activating	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	bladder carcinogenesis
CALR	activating	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	mutations
CAMK4	activating	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	overexpressed in liver cancer
CBP	Target	Iyer et al (doi: 10.1038/sj.onc.1207118)	mutations (Rubinstein-Taybi)

CDK1	target	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	overexpression/amplification
CDK2	target	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	overexpression/amplification
CDK4	target	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	overexpression/amplification
CDKN1A	target	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	downregulation
CDKN1B	inhibition	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	mutations
CFL1	activating AKT	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	overexpression
CLTC	activating	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	fusion
CSMD3	Unknown	Gleeson et al (doi.org/10.18632/oncotarget.18750)	mutations
CSNK2B	activating	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	colorectal cancer
CXCR4	activating	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	angiogenesis
DAPP1	target	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	methylation in poor prognosis CRC
DDIT3	activating	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	fusion
DEPDC5	inhibition	Du et al (doi: 10.1007/s11427-022-2313-1)	loss of function / repression
DEPTOR	inhibition	Duan et al (doi: 10.1038/s41420-024-02263-1)	loss of function / repression
DNMT1	Target	Zhang et al (doi: 10.1016/j.yexcr.2019.111502)	overexpression
DUSP3	activating	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	loss of function alterations
E2F1	Target and activating	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	overexpression
ECSIT	unknown	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	loss of function repression
EEF2	target inactivated by mTOR	Gleeson et al (doi.org/10.18632/oncotarget.18750)	aberrant expression in many cancers
EGFR	activating	Glaviano et al (doi: 10.1186/s12943-023-01827-6.);HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	mutation/amplification
EIF4E	target inactivated by mTOR	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	increased activity in many cancers
EIF4G1	activating mTOR	Lu et al (doi: 10.1111/jcmm.16340. Epub 2021 Feb 1)	overexpression in NSCLC
EP300/P300 HAT	Target of AKT	Kim et al (doi: 10.1038/s44321-025-00243-1)	fusion
EZH2	AKT inhibits EZH2	Liu et al (doi: 10.1007/s12032-023-02025-6)	EZH2 inhibits PTEN

	reducing H3K27me3		
FASLG	target of AKT	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	downregulation
FGF17	activating	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	prognostic factor in AML
FGF22	activating	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	invasion in pancreatic cancer
FGF6	activating	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	amplification/mutation
FGFR	activating	Glaviano et al (doi: 10.1186/s12943-023-01827-6.)	mutation/amplification
FOXO3	AKT target	Tsuji et al (doi: 10.1038/s41388-021-01757-x)	loss of function
GNA14	activating	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	mutation in cavernous hemangioma
GNGT1	activating	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	overexpression
GPC4	activating mTOR	Gleeson et al (doi.org/10.18632/oncotarget.18750)	overgrowth syndrome
GRB2	activating mTOR pathway	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	cancer progression
GRK2	activating	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	overexpression
GSK3A	regulate AKT	Hermida et al (doi: 10.1016/j.jbior.2017.06.003.)	overexpression
GSK3B	Regulate AKT	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	overexpression
HGF	activating	Glaviano et al (doi: 10.1186/s12943-023-01827-6.)	mutation/amplification
HRAS	activating	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	activating mutation
HSP90B1	inhibition	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	expression in HNSC
IGF2-AS	activating	Jin et al (doi: 10.1007/s10815-022-02638-2)	tumor suppressor gene
IL2RG	actrivating	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	immunodeficiency
IL4	activating	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	follicular lymphoma
INSR	activating	Glaviano et al (doi: 10.1186/s12943-023-01827-6.)	mutation/amplification
IRAK4	activating	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	activation in CRC
ITPR2	regulate mTOR pathway	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	hallmarks of cancer
KDM5A	AKT inhibits KDM5A	Spangle et al (doi: 10.1016/j.celrep.2016.05.046)	mutations

	increasing H3K4me3 (target)		
KIF23	Unknown	Gleeson et al (doi.org/10.18632/oncotarget.18750)	mutated in cortical dysplasia
KRAS	activating	Gleeson et al (doi.org/10.18632/oncotarget.18750)	mutations
LAMTOR1	activating	genecards.org	overexpression
LAMTOR2	activating	genecards.org	overexpression
LAMTOR3	activating	genecards.org	overexpression
LAMTOR4	activating	genecards.org	overexpression
LAMTOR5	activating	genecards.org	overexpression
LCK	activating	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	pediatric T cell ALL
LTK	activating	Glaviano et al (doi: 10.1186/s12943-023-01827-6.)	mutation/amplification
MAP2K3	activating	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	mutations
MAP2K6	activating	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	mutations
MAP3K7	activating	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	mutations
MAPK1	activating	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	mutations
MAPK10	activating	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	mutations
MAPK8	activating	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	mutations
MAPK9	activating	Gleeson et al (doi.org/10.18632/oncotarget.18750)	mutations
MAPK9	activating	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	mutations
MAPKAP1	activating	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	mutations
miR-20b	activating	Ilhan et al (doi: 10.1186/s12964-022-01019-7)	deregulated / tumor supressor
miR-21	inhibition	Wang et al (PMID: 36494326)	overexpression
miR-421	activating	Xu et al (doi: 10.2217/epi-2021-0229)	overexpression
MKNK1	activating	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	EIF4E regulation
MKNK2	activating	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	EIF4E regulation
MLST8	mTORC complex subunit	Fu et al (DOI: 10.1128/mbio.00899-23)	translation in cancer cells
MTOR	regulator	Gleeson et al (doi.org/10.18632/oncotarget.18750)	overexpression/activating mutation
MYD88	activating	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	lymphoma
NCK1	activating	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	EIF2 activation

NF1	activating	Gleeson et al (doi.org/10.18632/oncotarget.18750)	mutation
NFKBIB	effector	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	target of miR-20a
NGF	activating	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	overexpression
NOD1	activating mTOR	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	Ras Lung Cancer
NPRL2	inhibitor of mTOR	Gleeson et al (doi.org/10.18632/oncotarget.18750)	overexpression
NPRL3	inhibitor of mTOR	Gleeson et al (doi.org/10.18632/oncotarget.18750)	overexpression
NRAS	activating	Brandhorst et al (doi: 10.1016/j.drup.2024.101182)	activating mutation
PAK4	target	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	overexpression
PDGFR	activating	Glaviano et al (doi: 10.1186/s12943-023-01827-6.)	mutation/amplification
PDK1	activating	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	dysregulated in multiple cancers
PFN1	activating	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	tumor supressor gene
PIK3C2G	PI3K complex	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	
PIK3C3	PI3K complex	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	
PIK3CA	activating	Gleeson et al (doi.org/10.18632/oncotarget.18750)	activating mutation/amplification
PIK3CB	PI3K complex	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	
PIK3CD	PI3K complex	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	
PIK3CG	PI3K complex	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	
PIK3R1	regulating	Glaviano et al (doi: 10.1186/s12943-023-01827-6.)	inactivating mutations
PIK3R2	regulating	Glaviano et al (doi: 10.1186/s12943-023-01827-6.)	inactivating mutations
PIK3R3	regulating	Gleeson et al (doi.org/10.18632/oncotarget.18750); HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	inactivating mutations
PIKFYVE	inhibition	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	pancreatic cancer
PIN1	inhibition	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	hepatocellular carcinoma
PIP	activating	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	target of PTEN
PITX2	activating AKT	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	gastric cancer
PLA2G12A	target	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	

PLCB1	activating	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	cholangiocarcinoma
PLCG1	activating	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	mutations
PPP1CA	activating	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	mutations
PPP2R1B	repressor of AKT	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	mutations
PPP2R2B	repressor of AKT	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	mutations
PRAS40	inhibition	Yang et al (doi: 10.1038/nature25023)	tumor progression
PRKAA2	activating	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	NSCLC
PRKAG1	activating	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	
PRKAR2A	activating	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	
PRKCB	activating	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	
PTEN	negative regulator	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	mutation/loss/promoter hypermethylation
PTPN11	activating	Gleeson et al (doi.org/10.18632/oncotarget.18750);HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	
RAC1	regulator of mTORC	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	
RAF1	activating	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	
RAGA	activating	Zhao et al (doi: 10.1186/s12974-019-1610-5)	
RALB	activating	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	
RAPTOR	MTOR complex regulator	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	
RHEB	MTOR complex regulator	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	
RHOA	RAS homolog activating	Gleeson et al (doi.org/10.18632/oncotarget.18750)	
RICTOR	MTOR complex regulator	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	
RIPK1	target	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	
RIT1	activating	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	Noonan
RPS6KA1	target	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	

RPS6KA3	target	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	
RRAGC	Unknown	Ying et al (doi: 10.1158/1078-0432.CCR-16-0609)	mutations in follicular lymphoma
SCGB3A1	repressor of AKT	Mazumdar et al (doi: 10.1073/pnas.1001296107)	Kras Lung cancer
SFN	regulator of mTORC	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	
SLA	target	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	
SLC2A1/GLUT1	effector	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	MPNST
SMAD2	effector	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	gastric cancer progression
SQSTM1	target	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	
STAT2	activating	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	Castleman
TBC1D7	inhibition	Schrotter et al (doi: 10.1016/j.celrep.2022.110824)	TSC complex
TBK1	activating	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	thyroid cancer
THEM4	activating	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	glioma
TIAM1	activating	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	pancreatic cancer
TNFRSF1A	activating AKT	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	breast cancer
TRAF2	mTORC2 regulation	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	hepatocellular carcinoma
TRIB3	activating	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	
TSC1	inhibits mTOR pathway	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	inactivating mutations
TSC2	inhibits mTOR pathway	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	inactivating mutations
UBE2D3	mTORC regulation	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	
UBE2N	mTORC regulation	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	
VAV3	activating	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	ALL, breast cancer
VEGFR	activating	Glaviano et al (doi: 10.1186/s12943-023-01827-6.)	mutation/amplification
YWHAB	inhibition	HALLMARK_PI3K_AKT_MTOR_SIGNALING, msigdb v7.0	colon cancer

Table S10 : List of investigators of the study

Investigator name	Site name	Nb Patients enrolled
ABBOU	VILLEJUIF	2
ADAMSKI	BIRMINGHAM-CH	1
ANDRE	MARSEILLE	6
BAILEY	NEWCASTLE	1
BAUTISTA	MADRID-HUNJ	1
BERGER	SAINT ETIENNE	2
BERLANGA	VILLEJUIF	3
BERTOZZI	TOULOUSE	16
BLANC	POITIERS	1
BLANCO	OXFORD	1
BLOMGREN	STOCKHOLM	13
BLOUIN	TOURS	1
BODET	CAEN	1
BORRDEAUT	PARIS	1
BRENNAN	MANCHESTER	1
BROUGHAM	EDINBURGH	2
CAMPBELL	MELBOURNE-RCH	1
CARCELOR	SUTTON	1
CHAPPE	RENNES	9
CHASTAGNER	NANCY	8
COHEN	PARIS	1
DANDAPANI	NOTTINGHAM	1
DE CARLI	ANGERS	6
DE CARLY	ANGERS	1
DEFACHELLES	LILLE	1
DEVOLDERE	AMIENS	4
DUCASSOU	VILLEJUIF	1
DUFOUR	VILLEJUIF	3
DUHIL DE BENAZE	NICE	2
DUPRAZ	POITIERS	1
DUPUY	NICE	1
ELLIOT	LEEDS	2
ENGLISH	BIRMINGHAM-CH	2
ENTZ-WERLE	STRASBOURG	4
EVANS	LEEDS	1
FAURE CONTER	LYON	5
FORESTO	BRISBANE-LCCH	1
FOUYSSAC	NANCY	5
FRAPPAZ	LYON	5
GALLEGO MELCON	BARCELONA-VDH	1
GAMBART	TOULOUSE	5
GOURMEL	AMIENS	3
GRAY	SOUTHAMPTON	3
GRILL	VILLEJUIF	39
GRUNDY	NOTTINGHAM	3

GUERRINI	VILLEJUIF	1
HANSFORD	MELBOURNE-RCH	3
HARGRAVE	LONDON-GOSH	4
HARO	BREST	1
HASSALL	BRISBANE-LCCH	1
HOWELL	LIVERPOOL	2
ICHER	BORDEAUX	7
ISFAN	CLERMONT FERRAND	2
JIMENEZ	PARIS	1
JOHNSTON	BIRMINGHAM-CH	1
KANOLD	CLERMONT FERRAND	4
KIRBY	ADELAIDE-WCH	1
KONALD LASTAWIECKA	CLERMONT FERRAND	1
LAITHIER	BESANCON	1
LEBLOND	LILLE	13
LEBLOND	LYON	6
LERVAT	LILLE	3
LOWIS	BRISTOL	3
MARIE-CARDINE	ROUEN	1
MCCOWAGE	SYDNEY-CHW	1
MICHALSKI	LONDON-GOSH	1
MICHON	PARIS	1
MILLOT	POITIERS	1
MINCKES	CAEN	3
MURPHY	GLASGOW	2
NYSOM	COPENHAGEN	17
OBERG	STOCKHOLM	2
ORBACH	PARIS	1
PACQUEMENT	PARIS	1
PAGNIER	GRENOBLE	3
PALENZUELA	MONTPELLIER	1
PICTON	LEEDS	4
PILLARY	LONDON-GOSH	1
PLANTAZ	GRENOBLE	1
PROUST	ANGERS	9
PUISEUX	RENNES	1
REDONDO	MADRID-HUNJ	2
ROME	MARSEILLE	1
ROUJEAU	MONTPELLIER	2
RUBIO	MADRID-HUNJ	1
SABEL	STOCKHOLM	1
SASTRY	GLASGOW	2
SCHNEIDER	ROUEN	1
SHANKAR	LONDON-UCLH	4
SIRVENT	MONTPELLIER	2
STANHOPE	MELBOURNE-RCH	1
SUDOUR	LILLE	4
SZYCHOT	SUTTON	1
TANDONNET AREGUI	BORDEAUX	8

THOUVENIN-DOULET	SAINT ETIENNE	2
TSUI	AUCKLAND-SCH	2
VAN DER LUGT	UTRECHT-PMC	1
VERSCHUUR	MARSEILLE	2
WILNE	NOTTINGHAM	2
WILSON	OXFORD	1
WOOD	MELBOURNE-MCH	1
YONGLIN HOU	SYDNEY-SCH	1
YVERT-GILLIBERT	TOURS	4
ZEBIAN	LONDON-KCH	1

Section/topic	No	CONSORT 2025 checklist item description	Reported on page no.
Title and abstract			
Title and structured abstract	1a	Identification as a randomised trial	1
	1b	Structured summary of the trial design, methods, results, and conclusions	3,4
Open science			
Trial registration	2	Name of trial registry, identifying number (with URL) and date of registration	End Abstract page 4
Protocol and statistical analysis plan	3	Where the trial protocol and statistical analysis plan can be accessed	7-9 (results) 4-56 (meths) 61,62
Data sharing	4	Where and how the individual de-identified participant data (including data dictionary), statistical code and any other materials can be accessed	EGA 55, 59
Funding and conflicts of interest	5a	Sources of funding and other support (eg, supply of drugs), and role of funders in the design, conduct, analysis and reporting of the trial	Acknowledgements 23,24
	5b	Financial and other conflicts of interest of the manuscript authors	COI reported 24-25
Introduction			
Background and rationale	6	Scientific background and rationale	5
Objectives	7	Specific objectives related to benefits and harms	6
Methods			
Patient and public involvement	8	Details of patient or public involvement in the design, conduct and reporting of the trial	Role of Patricia Blanc, co-author
Trial design	9	Description of trial design including type of trial (eg, parallel group, crossover), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	50-51
Changes to trial protocol	10	Important changes to the trial after it commenced including any outcomes or analyses that were not prespecified, with reason	Amendment 5 of the trial provided supplementary information
Trial setting	11	Settings (*e.g,* community, hospital) and locations (eg, countries, sites) where the trial was conducted	Supplementary Table S10
Eligibility criteria	12a	Eligibility criteria for participants	46-49
	12b	If applicable, eligibility criteria for sites and for individuals delivering the interventions (eg, surgeons, physiotherapists)	10
Intervention and comparator	13	Intervention and comparator with sufficient details to allow replication. If relevant, where additional materials describing the intervention and comparator (eg, intervention manual) can be accessed	Protocol provided

Outcomes	14	Prespecified primary and secondary outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome	55,56
Harms	15	How harms were defined and assessed (eg, systematically, non-systematically)	ExtData6 (legend p 40)
			Reported on page no.
Sample size	16a	How sample size was determined, including all assumptions supporting the sample size calculation	50-51
	16b	Explanation of any interim analyses and stopping guidelines	50-51
Randomisation:			
Sequence generation	17a	Who generated the random allocation sequence and the method used	Meths 50
	17b	Type of randomisation and details of any restriction (eg, stratification, blocking and block size)	Meths 50
Allocation concealment mechanism	18	Mechanism used to implement the random allocation sequence (eg, central computer/telephone; sequentially numbered, opaque, sealed containers), describing any steps to conceal the sequence until interventions were assigned	Meths 50
Implementation	19	Whether the personnel who enrolled and those who assigned participants to the interventions had access to the random allocation sequence	Meths 50
Blinding	20a	Who was blinded after assignment to interventions (eg, participants, care providers, outcome assessors, data analysts)	nobody
	20b	If blinded, how blinding was achieved and description of the similarity of interventions	Not applicable
Statistical methods	21a	Statistical methods used to compare groups for primary and secondary outcomes, including harms	Extended Data1
	21b	Definition of who is included in each analysis (eg, all randomised participants), and in which group	Table 1
	21c	How missing data were handled in the analysis	No imputation
	21d	Methods for any additional analyses (eg, subgroup and sensitivity analyses), distinguishing prespecified from post hoc	58-59
Results			
Participant flow, including flow diagram	22a	For each group, the numbers of participants who were randomly assigned, received intended intervention, and were analysed for the primary outcome	Table 1
	22b	For each group, losses and exclusions after randomisation, together with reasons	Figure 1
Recruitment	23a	Dates defining the periods of recruitment and follow-up for outcomes of benefits and harms	6
	23b	If relevant, why the trial ended or was stopped	7
Intervention and comparator delivery	24a	Intervention and comparator as they were actually administered (eg, where appropriate, who delivered the intervention/comparator, how participants adhered, whether they were delivered as intended (fidelity))	Figure 1
	24b	Concomitant care received during the trial for each group	7-8
Baseline data	25	A table showing baseline demographic and clinical characteristics for each group	Table 1
Numbers analysed, outcomes and estimation	26	For each primary and secondary outcome, by group: ● the number of participants included in the analysis ● the number of participants with available data at the outcome time point ● result for each group, and the estimated effect size and its precision (such as 95% confidence interval) ● for binary outcomes, presentation of both absolute and relative effect size	Table 1
Harms	27	All harms or unintended events in each group	10,11

Ancillary analyses	28	Any other analyses performed, including subgroup and sensitivity analyses, distinguishing pre-specified from post hoc	8,9
Discussion			
Interpretation	29	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	20-23
Limitations	30	Trial limitations, addressing sources of potential bias, imprecision, generalisability, and, if relevant, multiplicity of analyses	23

Citation: Hopewell S, Chan AW, Collins GS, Hróbjartsson A, Moher D, Schulz KF, et al. CONSORT 2025 Statement: updated guideline for reporting randomised trials. BMJ. 2025; 388:e081123. https://dx.doi.org/10.1136/bmj-2024-081123
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*We strongly recommend reading this statement in conjunction with the CONSORT 2025 Explanation and Elaboration and/or the CONSORT 2025 Expanded Checklist for important clarifications on all the items. We also recommend reading relevant CONSORT extensions. See www.consort-spirit.org.

BIOMEDE

Biological Medicine for Diffuse Intrinsic Pontine Glioma (DIPG) Eradication

Protocol N° : CSET2014/2126
EudraCT N° : 2014-001929-32

Version 5.0 – November 28th 2018

Confidentiality Statement

Information and data included in this protocol contain trade secrets and privileged or confidential information which is the property of the sponsor. No person is authorized to make it public without written permission of the sponsor. These restrictions on disclosure will apply equally to all future information supplied to you which is indicated as privileged or confidential. This material may be disclosed to and used by your staff and associates as may be necessary to conduct the clinical study.

SPONSOR	**Gustave Roussy** **114 rue Edouard Vaillant** **94 805 Villejuif** **France**	**Signature of the Head of the Clinical Research Department:** **Pr Gilles Vassal**
COORDINATING INVESTIGATOR	**Dr Jacques Grill** **Gustave Roussy Villejuif** **Paediatric Oncology Department** **Gustave Roussy** **Tel: +33 1 42 11 62 09** **Fax: +33 1 42 11 52 75** **jacques.grill@gustaveroussy.fr**	

SIGNATURE PAGE

BIOMEDE
"Biological Medicine for Diffuse Intrinsic Pontine Glioma (DIPG) Eradication"

Protocol N° : CSET2014/2126
EudraCT N° : 2014-001929-32

Version 5.0 – November 28th 2018

Investigator centre: .....

Department: .....

Name and address: .....

.....

.....

I ..... certify that I have read the protocol named « BIOMEDE »

And I certify to conduct this study in accordance with the Good Clinical Practice, the local applicable regulation and the study Protocol.

Date: ___ / ___ / 20__

Signature:

BIOMEDE GROUP

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GLOSSARY OF ABBREVIATIONS AND DEFINITIONS OF TERMS

AE adverse event
ALP alkaline phosphatase
ALT (SGPT) alanine aminotransferase
ANC absolute neutrophil count
AST (SGOT) aspartate aminotransferase
AUC area under the plasma concentration-time curve
BBB blood brain barrier
BP blood pressure
BSA body surface area
CHF congestive heart failure
CNS central nervous system
CSF cerebral spinal fluid
CT computed tomography
CTCAE Common Terminology Criteria for Adverse Events
CTV clinical target volume
CVAD central venous access device
DIPG Diffuse Intrinsic Pontine Glioma
DLT dose-limiting toxicity
DMG diffuse midline glioma (including DIPG and non-brainstem midline glioma H3K27M mutant)
DNA deoxyribonucleic acid
DSCE dynamic susceptibility contrast imaging
DTI diffusion tensor imaging
ECG electrocardiogram
eCRF electronic case report form
EFS event-free survival
EGFR epidermal growth factor receptor
EMA European Medicines Agency
ESF Eligibility Screening Form
EU European Union
FDA U.S. Food and Drug Administration
FFPE formalin-fixed paraffin-embedded
GCP Good Clinical Practice
G-CSF granulocyte colony-stimulating factor
GTV gross tumour volume
H0 null hypothesis
H1 alternative hypothesis
5-HT3 5-hydroxytryptamine-3
HUI Health Utility Index
ICF informed consent form
ICH International Conference on Harmonisation
IDH1 isocitrate dehydrogenase 1 gene
IDMC Independent Data Monitoring Committee
IHC immunohistochemistry
IND investigational new drug
IMP investigational medicinal product
IRB/IEC Institutional Review Board/Independent Ethics Committee
IB Investigators' Brochure
ITCC Innovative Therapies for Children with Cancer

ITH intratumoural haemorrhage
IV intravenous
IVRS Interactive Voice Response System
KPS Karnofsky Performance Scale
LMWH low-molecular weight heparin
LOCF last observation carried forward
MGMT O6-methylguanine-DNA methyltransferase
MRI magnetic resonance imaging
MRS magnetic resonance spectroscopy
NB-DMG Non-brainstem diffuse midline glioma H3K27M mutant
NCI National Cancer Institute
NCI-C National Cancer Institute of Canada
NYHA New York Heart Association
OS overall survival
PBTC Pediatric Brain Tumor Consortium
pCV prednisone, lomustine (CCNU), and vincristine
PD progressive disease
psPD pseudoprogression
PDGF platelet-derived growth factor
PFS progression-free survival
PICC peripherally inserted central line
PlGF placental growth factor
PMA population modelling analysis
PS performance status
PR partial response
PTEN phosphatase and tension homolog
PTV planning target volume
q2w every 2 weeks
RAC Response Assessment Criteria in HGG
RANO Response Assessment in Neuro-Oncology
RECIST Response Evaluation Criteria in Solid Tumours
SAE serious adverse event
SADR Serious adverse event drug reaction
SD stable disease
SUSAR Suspected unexpected serious adverse reaction
T1/2 half-life
TIA transient ischemic attack
ULN upper limit of normal
WHO world health organisation

SYNOPSIS

SPONSOR CODE	CSET2014/2126	**VERSION AND DATE**	5.0 of the 28th November 2018
EUDRACT NUMBER	2014-001929-32		
ACRONYM	BIOMEDE		
TITLE	Biological Medicine for Diffuse Intrinsic Pontine Glioma (DIPG) Eradication : BIOMEDE		
SPONSOR	Gustave Roussy 114, Rue Eduard Vaillant 94 805 Villejuif Cedex France		
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STUDY POPULATION	Children, adolescents and young adults with newly diagnosed diffuse intrinsic pontine glioma		
CLINICAL PHASE	Randomised Phase II		
STUDY CENTRES	70 sites : 25 sites in France, 36 in Europe, 7 in Australia, 1 in New Zealand.		
RATIONALE FOR THE CLINICAL TRIAL	Diffuse Intrinsic Pontine Gliomas (DIPG) appear almost exclusively in children and adolescents, representing 15 to 20% of posterior fossa tumours. Even if it is one of the most common malignant brain tumours, there are only 30 to 40 new cases per year in France. Their clinical presentation is stereotyped with a short clinical history and a unique MRI appearance that was usually considered as sufficient to establish the diagnosis. The prognosis of DIPG is always unfavourable; median overall survival is 9 to 10 months in general and most patients will die within two years after diagnosis (Kaplan 1996, Hargrave 2006). Malignant gliomas infiltrating the brainstem represent the greatest challenge of paediatric oncology; despite numerous collaborative studies performed, patients' survival has not significantly increased in thirty years (Hargrave 2009). There is no validated prognostic factor. There is currently no validated treatment except radiotherapy. Several targeted agents have been tested in DIPG (Pollack 2007 Haas-Kogan 2008, Geoerger, 2011), without knowing whether the target was present in the tumour. A critical review of the paradigms of these trials tells us that there are long term survivors in these studies that is to say patients who may have benefited from the tested therapy, but they are few. So far, the new therapies that have been tried were evaluated one after the other in search of a treatment that would be effective for all patients, measuring the treatment effect on median survival. They were all rejected as ineffective. However we can challenge the endpoint to evaluate efficacy in these trials as the existence of long term survivors (> 18 months, for example) and their		

	number should not been ignored, especially if targeted therapies are considered. We propose a paradigm shift in the choice of treatment; the issue raised would be to give to each patient the treatment associated with the highest likelihood of efficacy based on the specific biological tumour profile. The development of targeted therapies for malignant gliomas infiltrating the brainstem has been hampered by the absence of biological data. It is therefore crucial to better understand the biology of these tumours. Despite the safety of the biopsy in brainstem tumours, most teams of paediatric neurosurgery limit the use of stereotactic biopsy only for clinically or radiologically unusual forms. Until recently, there has been no systematic genetic study at diagnosis to date and the few available data were confounded by the inclusion of autopsies or clinically and radiologically unusual cases (Louis, 1993; Gilbertson 2003; Okada, 2008; Zarghooni 2010; Broniscer, 2010; Wu, 2012 and Schwartzentruber, 2012). French teams gathered in the French Society of Paediatric Oncology and the European consortium "Innovative Therapies in Children with Cancer (ITCC)" decided a few years ago to perform biopsies of these tumours for diagnostic confirmation and to ensure the presence of certain therapeutic targets prior to a possible inclusion in a trial evaluating a targeted therapy (Geoerger, 2009; Geoerger, 2010). Part of this experiment was reported by the team of the Necker Hospital in Paris, confirming the low rate of complications of stereotactic biopsy procedure (Roujeau, 2007). The biopsy specimen analysis allowed practicing immunohistochemical, genomic (CGHarray), gene expression (transcriptome) and direct sequencing of candidate genes studies. These data (Puget, 2012) serve as biological data background for the targeted therapy protocol presented here. We have demonstrated that malignant gliomas infiltrating the brainstem are biologically different from those of supratentorial regions with the exception of some tumours of the midline (thalamus). We have identified two distinct groups based on the biological profile: one mesenchymal type overexpressing pro-angiogenic and neural stem cells markers and the other having an oligodendroglial look and overexpressing PDGFRA sometimes after gene amplification. Three potentially actionable targets are known to be frequently expressed in these tumours: 1) Gain/amplification or more frequently overexpression of EGFR protein (+++): this abnormality is found in 50% of tumours analyzed in a European ITCC study (Geoerger, 2011) and in almost all tumours grade III and IV analyzed in the Memphis study (Gilbertson,2003). 2) Activation of the mTOR pathway: in our experience, loss of PTEN expression detected at immunohistochemistry was found in around 90% of patients with malignant brain stem tumour (Geoerger, 2010). This loss of PTEN expression is accompanied by activation of the mTOR pathway (Zarghooni, 2010). Deletion of *PTEN* occur in less than 5% of the cases while loss are more frequent, up to 60% in the small series of the NCI (Zarghooni, 2010; Warren, 2012; Puget, 2012). Moreover, mutations in the PI3K/AKT/mTOR pathway are seen in 45% of cases (Taylor et al, Nature Genetics 2014). 3) Gain or amplification of PDGFRA: This abnormality is found in 20% of the tumours analyzed by our group at diagnosis (Puget 2012) and

	even more frequently (36%) at autopsy by the Toronto team (Zarghooni 2010). More recent data from our group indicated however that the amplification of PDGFRA may not be as frequent at diagnosis (Taylor et al, Nature Genetics in revision). Cases analysed after radiotherapy in the north-american series may have overestimated the rate of *PDFRA* amplification which is preferentially associated with secondary glioblastomas (Paugh 2010). The prognostic value of biomarkers has been studied retrospectively in the patients treated in previous trials (Geoerger 2010, Chassot 2011). EGFR expression is not linked to progression-free survival (PFS) or overall survival (OS), neither PTEN loss of expression. PDGFRA amplification or PDGFRA-driven transcriptomic signature is however linked to an adverse prognosis with 70% of children dying before the median survival of the entire population of DIPG studied; median survival is 7.5 months. Other potential targets have been reported and may open new possibilities of drug development in this disease, as detailed below: 4) Activating mutations of the ACVR1/ALK2 has been recently identified with whole genome sequencing (Taylor et al, Nature Genetics 2014). These mutations activate the Bone Morphogenic Protein (BMP) pathway and can be targeted by specific inhibitors currently in preclinical development for a congenital bone disease named Fibrodysplasia Ossificans Progressiva (FOP) (Yu et al, Nature Medicine 2008; Sanvitale et al, PLoS ONE 2013). These agents may become available later during the conduct of the study. Interestingly ACVR1 is also a target of dasatinib. 5) MET mutations and amplifications/gains: mutations exist in 5% of cases (Taylor et al, Nature Genetics 2014) and amplifications/gains in 15% of cases frequently associated with PDGFRA amplification (Puget 2012, Paugh et al, J Clin Oncol 2011). Several MET inhibitors are under preclinical development but none is yet authorized to treat pediatric brain tumors even within a trial. These agents may become available later during the conduct of the study. 6) The most attractive target due to their almost constant alterations in DIPG would be the mutations of the histones H3 variants and their consequences, ie a loss of the trimethylation of the H3 histones at the Lysine 27 (Truffaux et al, submitted; Bender et al, Cancer Cell 2013, Lewis Science 2013; Chan 2013). There is however no therapeutic agents targeting these alterations at the moment. These agents may become available later during the conduct of the study. The presence of theses histones H3 alterations will however be evaluated as diagnostic biomarker. Indeed, almost all bona fide DIPG have either a mutation in one of the H3 variant genes or the consequence of this mutation (Castel et al, in preparation). Moreover, the type of histone H3 mutated may have a prognostic impact since retrospective data indicated that mutations in the H3.3 gene, ie *H3F3A*, have a worst prognosis (Khuong-Quang, Acta Neuropathol 2012 & Castel et al, in preparation). Three classes of drugs have been evaluated in children with DIPG, EGFR TKI inhibitors (Porkka 2010; Geoerger 2011), PDGFRA TKI inhibitors (Pollack 2007, Geoerger 2009, Broniscer 2013) and mTOR inhibitors (Geoerger 2012). The suggested dose of erlotinib in

	combination with radiotherapy is 125 mg/m2 once daily (Geoerger et al 2011). The recommended dose in children for dasatinib and everolimus given as single agents are 85 mg/m2 twice daily and 5 mg/m2/day, respectively. Responses in brain tumors associated with Tuberous Sclerosis Complex have been observed at this dose level of everolimus. It was deemed unnecessary to have a formal dose-finding phase I for everolimus and dasatinib given in combination with radiotherapy as both treatment components (targeted agent and radiotherapy) are known to have different toxicity profiles. Moreover, dasatinib has already been tested in combination with radiation therapy plus vandenatib in children with DIPG and the dose of 65 mg/m2 x 2 per day of dasatinib was considered safe (Broniscer 2013). The combination with vandetanib precluded to reach the MTD of dasatinib when used as monotherapy. No unexpected toxicity of radiotherapy was encountered with the addition of dasatinib. Regarding mTOR inhibitors, temsirolimus and everolimus have been used in combination with radiotherapy in adult glioblastomas (Sarkaria 2010 & 2011; Chinnaiyan 2013), and could be administered without increased toxicity other than the expected increased risk of infection. Anyway, toxicity will be carefully monitored and the dose recommended at the beginning of the trial may be re-evaluated in the course of the trial based on accumulated toxicity data. **In this study, the majority of patients will receive a treatment assumed to specifically target a biological abnormality identified on the biopsy. More importantly, patients will not receive a drug for which the identified target is absent.** Based on previous knowledge, this design is expected to increase the likelihood of finding a positive efficacy signal if these targeted agents are truly efficient to inhibit the corresponding oncogenic signaling pathways. As *in vitro* data suggest that dasatinib could be one of the most effective drugs in DIPG, regardless of PDGFRA status, a comparative evaluation of its efficacy is planned versus erlotinib in patients with EGFR overexpression, and versus everolimus in patients with PTEN-loss. These treatments will be allocated by randomisation stratified by marker status, which provides unbiased evidence about the relative efficacy of these drugs. Although PDGFRA-negative patients will be eligible for the dasatinib arm, the number of patients that can reasonably be included in the randomised trial will limit the evaluation of the predictive value of PGFRA on dasatinib efficacy. We acknowledge the fact that this design will not allow us to evaluate the predictive value of the EGFR overexpression or PTEN-loss with respect to the effect of erlotinib and everolimus respectively, as patients without the biomarker will not be eligible for the comparative evaluation of these drugs. Indeed, based on prior data, it was deemed unethical to give erlotinib to patients without EGFR overexpression (Geoerger 2011, Andreiuolo submitted) and consequently everolimus to patients without PTEN-loss. In this first step of the protocol, the patients will thus be allocated to one of the three treatment groups as follows: - If the tumor overexpresses EGFR without PTEN loss of expression, patients may receive erlotinib or dasatinib allocated by randomisation (R1 randomisation).

	- If the tumor shows loss of PTEN expression without EGFR overexpression, patients may receive everolimus or dasatinib allocated by randomisation (R2 randomisation). - If the tumor shows both EGFR overexpression and loss of PTEN expression, patients may receive erlotinib, everolimus or dasatinib by randomisation (R3 randomisation). - If the tumor shows neither EGFR overexpression nor loss of PTEN expression (a very rare situation in our experience), patients will receive dasatinib (no randomisation). - If the biopsy assessment is not contributive, the treatment will be allocated by randomisation between erlotinib, everolimus and dasatinib (R3 randomisation). In order to offer to the patients the best therapeutic option corresponding to a given biomarker within a research program and to avoid gaps between protocols, this protocol is an overarching protocol using an adaptive design allowing for the evaluation of new drugs evaluation according to the presence of other targets when the corresponding drugs and companion tests become available for testing. Similarly to the flexible approach developed by Sydes et al. for randomised controlled trials allowing for stopping arms for lack-of-benefit and adding research arms mid-trial in a multi-arm multi-stage design (Sydes et al, Trials 2012, 13:168), we propose to drop inefficient treatment arms based on interim analyses, and add new treatment arms. This flexible biomarker-driven design is a unique opportunity to introduce quickly promising agents in this devastating and rare disease. The current clinical trial that is focused on erlotinib, everolimus and dasatinib evaluation represents the first step of this overarching protocol. <u>Background for diffuse midline glioma K27M mutant</u> In 2016, the WHO classification of glioma created a new entity named “diffuse midline glioma, K27M mutant” grouping all midline gliomas with this specific histone mutation and including diffuse intrinsic pontine gliomas (Louis et al, Acta Neuropathol 2016). When treated with classical anti-glioma therapy, ie radiotherapy + temozolomide after surgery, their outcome is poor justifying new therapeutic considerations (Grill et al, J Clin Oncol 2018). Further work showed that these thalamic gliomas have a dismal prognosis similar to the DIPG (Ryall et al, Acta Neuropathol Commun 2016; Karremann et al, Neuro-Oncol 2018; Mackay et al, Cancer Cell 2018; Castel et al, Acta Neuropathologica Communications in press). Moreover, transcriptome and methylome of these diffuse midline gliomas appeared to be similar, irrespective of the location of the tumor (Castel et al, Acta Neuropathologica Communications in press). Taking into account these data, it was considered acceptable to offer these patients a treatment according to the same therapeutic paradigm than the one offered to DIPG in the BIOMEDE trial. However, there is uncertainty about their response to therapy compared to DIPG, owing for example that some of these tumours may undergo partial surgical debulking that may interfere in the evaluation of response to treatment, as well as the impact of micro-environment that may differ according to tumour site. It was thus decided not to incorporate them in the primary

	analysis of the trial that will be limited to children with brainstem gliomas. Patients with other midline tumours will be analysed apart and specific therapeutic modifications will be implemented in the protocol for the patients with spinal tumors who will not receive the study drug during the spinal radiotherapy.
STUDY OBJECTIVES	**Primary objective** The main objective of this study is to evaluate the efficacy in terms of overall survival (OS) of erlotinib, everolimus and dasatinib in combination with radiation therapy in patients with a DIPG, both - compared to the other experimental treatments by comparisons between randomised subsets of patients selected according to the biological abnormalities found in their tumour (pairwise direct comparisons), and - compared to historical control. **Secondary objectives** The secondary objectives are: - To compare the 2-year OS between randomised subsets of patients with DIPG (pairwise comparisons) and compared to historical controls; - To evaluate the safety profile of each of the three drugs when administered in combination with radiotherapy, and over the whole duration of treatment, as well as the feasibility of a continuous administration of these drugs; - To evaluate the efficacy in terms of progression-free survival (PFS) of erlotinib, everolimus and dasatinib in combination with radiation therapy, comparatively between randomised subsets of patients with DIPG, and also compared to historical controls; - To evaluate the whole strategy by estimating the overall and progression-free survival of the entire cohort of patients with DIPG (by pooling the different treatment arms) and comparing it to historical controls. **Exploratory objectives** - To study the association between known biomarkers of the targeted activation pathway (a preliminary list is given below) and response to treatment (OS, PFS) as well as to describe prospectively new tumour abnormalities that will emerge during the course of the study and evaluate their correlation with response to treatment; - To describe the effect of therapy with morphological (MRI) and functional imaging (multimodal MRI). The rate of pseudo-progression, in particular, will be assessed since it could be as frequent as 25% (Chassot, 2011). - To describe the outcome of the cohort of non-brainstem diffuse midline gliomas, H3K27M mutant, in terms of PFS and OS, overall and according to treatment arm, and in comparison with DIPG.
STUDY DESIGN	The current study is a multicenter, randomised open-label phase II trial comparing efficacy of three investigational products (i.e erlotinib, everolimus, and dasatinib). As all patients are not eligible for the three-

drug comparison, the study includes three subtrials

- R1: erlotinib versus dasatinib
- R2: everolimus versus dasatinib
- R3: erlotinib versus everolimus versus dasatinib

Eligibility criteria for the different subtrials will be based on EGFR overexpression and PTEN loss of expression detected by immunohistochemistry.

It is an adaptive design because interim efficacy analyses will be performed in the course of the trial to have the possibility of dropping one treatment arm for lack of benefit, in particular if a new experimental agent is available for testing in this disease.

Treatment allocation according to biomarker profile

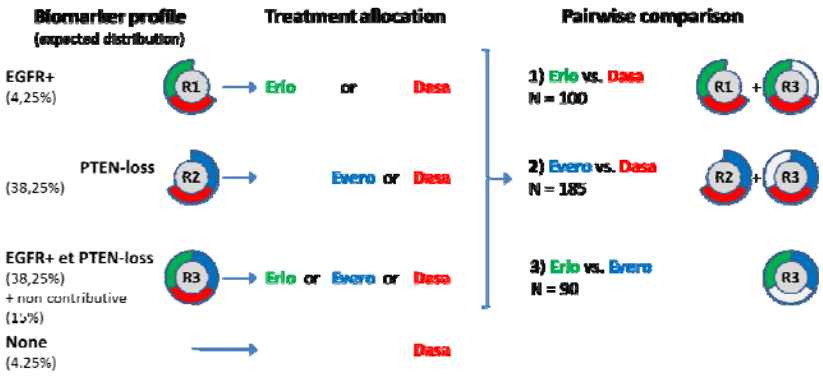
Biomarker Profile	%	Subtrials and Allocated treatment
EGFR+ only	4.25%	R1: erlotinib or dasatinib
PTEN-loss only	38.25%	R2: everolimus or dasatinib
EGFR+ and PTEN-loss	38.25%	R3: erlotinib, everolimus or dasatinib
None	4.25%	Cohort dasatinib (not randomised)
Inconclusive	15%	R3: erlotinib, everolimus or dasatinib

This expected distribution of biomarker profiles leads to the following distribution of patients per pairwise randomised comparison (cf. figure below):

- 40% included in the direct comparison “erlotinib versus dasatinib” (Er-D comparison: 100% of R1 + 2/3 of R3 randomisations), including 30% of patients with a known EGFR positive tumour and 10% of patients with a non-informative tumour.

- 74% included in the direct comparison “everolimus versus dasatinib” (Ev-D comparison: R2 + R3 randomisations) including 64% of patients with a known PTEN-loss in the tumour and 10% of with a non-informative tumour.

- 36% included in the direct comparison “erlotinib versus everolimus” (Er-Ev comparison: R3 randomisation) including 26% of patients with a known EGFR positive + PTEN-loss tumour and 10% of with a non-informative tumour.



NB: Patients with EGFR status unknown and no PTEN-loss will be eligible for the R1 randomisation. Patients with EGFR-negative and PTEN status unknown will be eligible for the R2 randomisation.

	The expected distribution of treatment groups is as follows: - 20% will receive erlotinib (50% of R1 + 33% of R3 randomisation) including 15% of patients with a known EGFR positive tumour ("targeted" erlotinib cohort) and 5% of patients randomly assigned to this treatment with a non-informative tumour. - 37% will receive everolimus (50% of R2 + 33% of R3 randomisation) including 32% of patients with a known PTEN-loss in the tumour ("targeted" everolimus cohort) and 5% of patients randomly assigned to this treatment with a non-informative tumour. - 43% will receive dasatinib (50% of R1, 50% of R2, 33% of R3 randomisations, and 100% of patients not randomised), including 8% PDGFRA+ patients. We cannot a priori define a "targeted" dasatinib cohort since we do not know which biomarker(s) defines best the tumours targeted by dasatinib. Considering the cohort of non-brainstem diffuse midline gliomas, the distribution of the biomarkers used for randomisation is not known precisely albeit our preliminary data indicate that both biomarkers have been shown in these tumours. These patients will not contribute to the primary analysis.
ELIGIBILITY CRITERIA	**Eligibility criteria for the BIOMEDE study (pre-screening for the randomised subtrials)** - Diagnosis of DIPG (clinical and radiological, or histological in case the biopsy was performed before study entry). - Non-brainstem diffuse midline gliomas, H3K27M mutant (NB-DMG), will be eligible for the trial after biopsy or surgery. As biopsy and surgery is considered as standard practice for these locations, informed consent for the biopsy will not be necessary. Patient will sign the consent after the diagnosis to allow central review and biomarkers assessment thereafter. - DIPG or NB-DMG at diagnosis: no prior chemotherapy for the present cancer; no prior cerebral radiation therapy - NB: Metastatic disease allowed. Patient with metastatic disease are eligible for the study (including the randomised trial if diagnosis of DIPG/NB-DMG confirmed). In this situation, radiotherapy will have to start within three weeks after the biopsy while targerted treatment will start **at the end** of the irradiation - Age > 6 months and < 30 years. For children below the age of 3 years, inclusion in the study and medical decisions should be discussed with the coordinating investigator. - Eligible for a biopsy, or biopsy performed for diagnostic purpose and material available for the biomarker assessment - Eligible for cerebral radiotherapy - Patient covered by an health insurance if national requirement - Written informed consent given by patient and/or parents/legal representative for biomarkers assessment and registration in the study. **Non eligibility criteria for the study** - Spontaneous massive intratumour bleeding. Patients with post-operative bleeding will be allowed to enter the study provided the hemmorhage is controled. Same rule applies for the other post-operative complications (infection, CSF leakage, absence of wound closure, subdural collection…). - Any other concomitant anti-cancer treatment not foreseen by this protocol

	- Any other cancer during the last 5 years - Uncontrolled intercurrent illness or active infection - Any other co-morbid condition that in the investigator's opinion would impair study participation - Unable for medical follow-up (geographic, social or mental reasons) - Patient not fulfilling one of the previous eligibility criteria. - Patient previously treated with irradiation on the brainstem for another neoplasm - Patient with congenital galactose intolerance, Lapp lactase deficiency or glucose-galactose malabsorption. - Patient not covered by a social security agreement accepted in the treating country if national requirement. - Pregnant or breast feeding women - NB: A patient with known hypersensitivity for one the drug or its excipients could still participate to the study and receive one of the other drug(s) **Common eligibility criteria for the BIOMEDE randomised subtrials** - Eligibility criteria for the study (see above) - Confirmed histological diagnosis of diffuse intrinsic pontine glioma (grade II, III, IV WHO), or NB-DMG confirmed by central pathology review (including the assessment of the loss of H3K27me3 by immunohistochemistry or the presence of a mutation in the histone H3 variant genes). Patients without classical clinical and radiological diagnostic criteria who fulfil the histological and biological criteria of DIPG are eligible for the trial. Pilocytic astrocytoma and ganglioliomas are not eligible. Patients with a suspected DIPG but no histological confirmation could be randomised **if and only if** the imaging is typical of a DIPG as well as the short clinical history. This would need to be reviewed and agreed centrally. Confirmation of the diagnosis of non-brainstem diffuse midline gliomas, H3K27M mutant, by central review, is needed before the randomisation inclusion of cases of NB-DMG. - Life expectancy > 12 weeks after the start of study treatment - Karnofsky performance status scale or Lansky Play Scale > 50%. The PS should not take the neurologic deficit per se into account. NB: Children and young adults with a worse performance status due to glioma-related motor paresis can be included. - Absolute neutrophil count > 1.5 x 10^9/l, Platelets > 100 x 10^9/l - Total bilirubin < 1,5 x ULN, AST and ALT< 2,5 x ULN - Serum creatinine < 1,5 X ULN for age. If serum creatinine > 1,5 ULN, creatinine clearance must be > 70 ml/min/1,73 m² (EDTA radioisotope GFR or 24 hours urines collection) - Normal coagulation tests within the local reference ranges - No current organ toxicity > grade 2 according to the NCI-CTCAE version 4.0 especially cardiovascular, pulmonary or renal disease (including but not limited to: congenital long QT syndrome, nephrotic syndrome, glomerulopathy, uncontrolled high blood pressure despite adequate treatment, interstitial lung disease, pulmonary arterial hypertension).In case of known or possible cardiac disease, a cardiological advice will be required prior to the inclusion in the randomized trial as a preexisting cardiopathy represents a contra-indication to dasatinib. - Effective and appropriate contraception for patients (male and female) of reproductive potential during their entire participation in the study and during 6 months after the end of treatment. Effective contraception are defined in CTFG Guidelines "Recommendations

	related to contraception and pregnancy testing in clinical trials" (Appendix 7) - Negative pregnancy test (serum beta-HCG or urinary test) evaluated in the last week in sexually actives females of reproductive potential - Written informed consent given by patient and/or parents/legal representative for treatment and randomization **Eligibility criteria for the subtrials** Eligibility criteria for the different subtrials will be mainly based on biomarkers assessment as detailed in the table above. In addition, contra-indication to specific drugs will be considered.
INVESTIGATIONAL MEDICINAL PRODUCTS	Erlotinib: 25 mg and 100 mg tablets. The prescribed dose is 125 mg/m²/day orally, once daily. Dose will be capped at 150mg/day for the first cycle and 200 mg/day from the second cycle onwards for adolescents and young adults. Everolimus: 2.5 mg tablets. The prescribed dose is 5 mg/m²/day, orally, once daily. Dose will be capped at 10mg once daily for adolescents and young adults. Dasatinib: 20 mg and 50 mg tablets. The prescribed dose is 85 mg/m²/dose, orally, twice daily, i.e. 170 mg/m2/day. Dose will be capped at 100mg BID for the first cycle, further escalation is allowed depending on tolerance but to a maximum dose of 150mg BID for adolescents and young adults. If, due to the formulation of the prescribed drug, the daily dose does not correspond to the planned dose, the dose will be distributed over the week, to achieve a correct weekly dose. The weekly dose cannot deviate for more than 10% of the planned dose. Duration of treatment Treatment will be continued until unacceptable toxicity, tumour progression, and/or withdrawal of patient, parents or legal representative consent. In case of doubt with a pseudo-progression, the patient will continue his treatment until subsequent MRI performed within 2 months. The maximum treatment duration allowed is 18 months (excepted for patients benefiting from the study drugs at the end of this 18 months period). Radiotherapy All patients will be treated with 30 conventional single daily fraction of 1.8 Gy to a total of 54 Gy over a planned period of 6 weeks. The target volume will include all the areas of abnormality on T2/FLAIR sequences with a 2-cm margin. Radiation will be typically delivered by megavoltage photon beams. Radiotherapy will have to start ideally within three weeks (MAXIMUM four weeks) after the biopsy / initial surgery. Radiotherapy should be planned as soon as the biopsy date is known (between 10 and 21/28 days after the planned date of biopsy). Targeted treatment will start on the first day of irradiation.

	<u>In case of metastatic disease</u>, patients can be included in the study. In this situation, radiotherapy will have to start within three weeks after the biopsy while targerted treatment will start **<u>at the end</u>** of the irradiation.
BIOMARKER DEFINITION	Biomarkers will be assessed on formalin-fixed paraffin-embedded samples. Consecutive slides will be analysed. The percentage of tumour cells will be defined centrally by counting the percentage of cells with loss of histone H3 trimethylation on Lysine 27 (H3K27me3). EGFR IHC will be measured according to the Hirsch index. A positive score for EGFR will correspond to an index above or equal to 100. PTEN IHC will be assessed using the vessels as internal controls. The vessels should be postive. Tumours cells should be negative. PDGFRA amplification will be judged by two colour FISH with CEP7 control using the Colorado System.
PRIMARY ENDPOINTS	The overall survival is the primary efficacy endpoint for the different randomised Phase II sub-trials. For the pairwise comparisons (internal comparisons of randomised groups), the overall survival is the time between date of randomisation and death whatever the cause. For the comparison to historical controls, the overall survival is computed from the date of biopsy (or initial surgery). The main analysis will be based on the whole survival curve.
SECONDARY ENDPOINTS	**Effcicacy endpoints** **2-year overall survival** The 2-year OS will be estimated from the Overall Survival curves (Kaplan Meier estimate). **Progression-free survival** Progression-free survival from the date of biopsy/initial surgery (or date of randomisation for the comparison of randomised groups) to the date of unequivocal clinical or radiological progression, or death whatever the cause. Progression will be defined according to the RANO criteria. **Toxicity** The safety assessment will be conducted using the NCI-CTC V4 on the whole duration of treatment in all treatment arms, using a list of selected toxicity items for the most frequent expected adverse events and text fields for unexpected events and other less frequent toxicities. For each experimental treatment given in combination with radiotherapy, adverse events will be carefully monitored, based on the estimated proportion of patients experiencing Grade 3 / 4 CTCAE adverse event reported as a Serious Adverse Event, both in the first 8 weeks of treatment and on the whole treatment duration, excluding symptoms that are related to tumour progression or pseudo-progression (neurological deterioration, increased intracranial pressure): Each of these adverse events must be communicated directly to the trial manager team and the sponsor coordinator.

	Feasibility - duration of treatment and description of the reasons for treatment discontinuation, - number of treatment temporary stops and dose-reductions and the description of the reasons for these treatment modifications, and - estimate of mean dose per week over the whole treatment duration. **Translational study** Response to therapy (PFS and OS) will be correlated to the biomarkers identified before the start and during the course of the study (including whole exome sequencing and RNAsequencing). **Correlation of Multimodal Imaging with Response to Therapy** The value of multimodal imaging to predict response to therapy will be evaluated. - T1-weighted post-gadolinium imaging will be correlated with outcome since the presence of enhancement could be correlated with survival in one recent study (Hipp 2011). - MRS: monovoxel or multivoxel proton spectroscopy will be used to assess metabolic profile especially citrate as well as Cho:Cr and Cho:NAA ratios, the latter being correlated to prognosis (Hipp 2011, Steffen-Smith 2011 & 2012). - DCE-MRI: dynamic contrast-enhanced MRI will be used to assess perfusion since increased perfusion could predict survival (Hipp 2011); the sequences will be analysed centrally with the OLEA software. - ASL: arterial spin-labelling perfusion MRI will be assessed as a possible surrogate for DCE-MRI. Guidelines from the "ASL Network" (http://www.asl-network.org) will be implemented. - DWI: diffusion-weighted imaging will be used to assess the apparent diffusion coefficient as a surrogate of tumour cellularity (Gauvain 2001).
STATISTICAL CONSIDERATIONS	**General considerations** As DIPG is a very rare disease and there is an important need of new drug evaluation, we consider that little unbiased evidence is better than none. This led us to propose randomised trials with relaxed decision rules. We acknowledge the fact this trial is an exploratory trial and not a confirmatory trial. The main analysis will be based on direct pairwise comparisons considered separately (erlotinib versus dasatinib from R1 and R3 randomisations; everolimus versus dasatinib from R2 and R3 randomisations; and erlotinib versus everolimus from R3 randomisation). Two-sided tests will be used with alpha=0.20 for these comparisons between experimental arms. The three pairwise comparisons will be considered without correction for multiple testing, as recommended by Freidlin et al. for multi-arm trials (Freidlin, 2008). **Main endpoint estimate** The overall survival has been chosen as the primary endpoint. The data accumulated in previous studies allow concluding a certain stability of the results: median survival not exceeding 1 year so far, and less than 5% of survivors at 2 years, from initial diagnosis. The OS and PFS curves will be estimated using Kaplan-Meier method. The two-year OS difference between randomised groups and its confidence interval will also be computed using Kaplan Meier

	estimates and Greenwood variance if some data are censored before two years. Else, if all patients have two years of follow-up (final analysis with no patient lost-to-follow-up), it will be computed as the difference of two proportions. **Analysis of treatment effect on the efficacy outcome** Treatment effects on the overall survival curves between randomised groups of patients with DIPG (Hazard Ratio, HR, for death for the OS curves; HR for progression for the PFS) will be tested with two-sided logrank tests and estimated in Cox models, stratified by EGFR and PTEN biomarkers status. As an example, for the erlotinib versus dasatinib comparison, three strata will be considered: (i) patients with EGFR over-expression but no PTEN-loss (R1 randomisation), (ii) patients with EGFR over-expression and PTEN-loss (R3 randomisation), (iii) patients included in the R3 randomisation because of inconclusive results. The proportional hazards assumption underlying the HR estimate in Cox models will be evaluated, using graphic methods and models including interaction with time. Appropriate methods for treatment effect estimates will be used if the proportional hazard assumption appears violated or questionable (use of restricted mean survival as published by Royston and Parmar). Each treatment group of patients with DIPG will also be considered as a single-arm phase 2 trial. For each treatment group separately, we will consider that a probability of survival equal to or lower than 5% at 2 years after the date of biopsy (or initial surgery) is insufficient (H0: p0 = 5%).The observed survival probability at 2 years will be tested against this null hypothesis. For each treatment group, the whole OS curve from initial biopsy (or initial surgery) will be also compared to theoretical curves based on historical data, as there is no concurrent control group in the randomised trials. For this purpose, we will use the one-sample logrank test (Finkelstein, 2003). The main issue is to have a similarly selected historical population. The main comparison will use series including only biopsy-confirmed DIPG (Necker's cohort, personal data and subgroup of the European Society for Pediatric Oncology DIPG registry, Hoffman JCO 2018). One-sided tests will be used with alpha=0.05 for the comparisons to the historical control. For DIPG patients, the whole strategy will be evaluated by pooling the different treatment groups and analysing them together (estimation of OS and PFS curves of the whole population and comparison to historical controls). **Analysis of safety and feasibility outcome** The analysis of safety and feasibility will be primarily descriptive (toxicity profile, incidence of the different types of toxicity...). Adverse events will be reported considering (i) the first 8 weeks of treatment when treatment is given in combination with radiotherapy, (ii) the whole treatment duration. Adverse events will be reported per 28-day cycle and per patient. Adverse events will be carefully monitored, based on the estimated proportion of patients experiencing an adverse event as defined in the endpoint subsection (7.2.2), as well as the feasibility. A safety report will be performed at least every 6 months with an analysis per treatment group and overall. The main analysis of safety data will include all patients who have started the study treatment, regardless of tumour site (DIPG and NB-DMG). However safety data of DIPG and

	NB-DMG may be presented separately for publication purposes. **Other analyses** The prognostic and/or the predictive value of biomarkers on the response to treatment (OS, PFS) will be investigated using Cox models, with interaction terms. Forest plots will illustrate the heterogeneity of treatment effect by biomarker status. The association between pseudo-progression detected on the morphological MRI and the outcome will be evaluated using time-dependent Cox models. The association between change in functional MRI and the outcome will be investigated in Cox models. Efficacy outcomes (PFS, OS) of the cohort of non-brainstem NB-DMG, H3K27M mutant will be analysed separately as exploratory analyses. This analysis will be mainly descriptive as the numbers will not allow a formal comparison of the three treatments. PFS and OS will be estimated on the whole group of NB-DMG and by treatment groups. Relative treatment effect of the experimental drugs will also be estimated (hazard ratios) in NB-DMG. Difference between DIPG and NB-DMG will be described in terms of PFS and OS curves. Heterogeneity of treatment effect according to tumour site will be assessed using interaction tests and illustrated by forest plots, although we acknowledge that the limited sample precludes any conclusion. A pooled analysis of efficacy outcomes (PFS, OS) DIPG and NB-DMG will be performed if there is no evidence of difference between both strata. **Analysed population** The main analysis of efficacy outcomes of each comparison between treatment groups will be based on all patients with a DIPG allocated by randomisation to the compared treatments: - R1 + part of R3 subtrial for erlotinib versus dasatinib comparison - R2 + part of R3 subtrial for everolimus versus dasatinib comparison - Part of the R3 subtrial for erlotinib versus everolimus comparison All patients with DIPG will be considered for the main efficacy analyses (Intention-to-treat). The same principle will be followed for the comparison of each treatment group of patients with DIPG to the historical control. For both types of analyses of the efficacy results in DIPG (direct pairwise comparisons and comparison to the historical control), a sensitivity analysis will be performed after exclusion of patients recruited with non-informative biomarkers results and patients with a metastatic disease at study entry. The efficacy outcomes of the cohort of non-brainstem DMG, H3K27M mutant will be analysed separately. Safety will be evaluated on the treated population (at least one dose of allocated treatment) considering the whole study population, including the DIPG and the NB-DMG, H3K27M mutant. However safety data of DIPG and NB-DMG may be presented separately for publication purposes.

SAMPLE SIZE AND POWER CALCULATION	Considering the initial assumption regarding the distribution of biomarkers within the DIPG cohort, we could expect that 36% of the trial population will contribute to the comparison of everolimus versus erlotinib, 40% to the comparison of erlotinib versus dasatinib, and 74% to the comparison of everolimus versus dasatinib. The expected number of patients participating in the pairwise comparisons is derived from the expected distribution in the different subtrials (cf subsection 8.1.3) As the distribution across the different pairwise comparisons is not equal, the initial sample size of the study for DIPG was computed in order to ensure a minimum sample size of 90 patients, for the pariwise comparison with the smallest sample size, i.e. everolimus versus erlotinib. In the pairwise comparison of everolimus to erlotinib, a total of 90 patients is required to have an 80%-power in the comparison of the whole OS curves if the Hazard Ratio of death is 0.62, equivalent to a 10.6% increase in the two-year OS (5% versus 15.6%) assuming proportional hazards (logrank test, 79 events, East software with an exponential survival distribution and an expected accrual of 22.5 patients per year. This sample size is also associated with an 80%-power to conclude a significant benefit if one experimental arm leads to a 17% increase in the two-year OS compared to the other one (5% versus 22%) with a two-sided alpha=0.20 (Casagrande & Pike). Considering the expected distribution across the different trials, this number of patients with DIPG should be achieved by recruiting 250 patients in the randomised trial to get 250 randomised trial patients. This target number of 250 randomised trial patients with DIPG is achievable over a 5 year accrual period with approximately 63 patients per year. With 250 randomised trial patients, we also expect approximately 100 patients for the erlotinib-dasatinib comparison and 185 patients for the everolimus-dasatinib comparison. Considering the current screen failure rate (approximately 88% of the patients registered in the study are finally allocated to one treatment arm by randomisation), a total of 285 patients with DIPG may be required to achieve the total of 250 randomised patients with DIPG. For the erlotinib-dasatinib comparison, a total of 100 patients is associated with a 80% power to conclude a significant benefit if the Hazard Ratio of death is 0.64, equivalent to a 9.7% increase in the two-year OS (5% versus 14.7%) assuming proportional hazards (logrank test, 91 events, East software with an exponential survival distribution and an expected accrual of 25 patients per year). This sample size is also associated with an 80%-power to conclude a significant benefit if one arm leads to a 16% increase in the two-year OS (5% versus 21%) with a two-sided alpha=0.20. For the everolimus-dasatinib comparison, a total of 185 patients is associated with a 80% power to conclude a significant benefit if the Hazard Ratio of death is 0.72, equivalent to a 6.6% increase in the two-year OS (5% versus 11.6%) assuming proportional hazards (logrank test, 167 events, East software with an exponential survival distribution and an expected accrual of 45 patients per year. This sample size is also associated with an 80%-power to conclude a significant benefit if one arm leads to a 10.5% increase in the two-year OS (5% versus 15.5%~~)~~ with a two-sided alpha=0.20. We acknowledge the fact that the targeted treatment effect is relatively

	optimistic for the first two randomised comparisons, meaning that these trials are underpowered for a smaller treatment effect that would be more reasonable and still clinically meaningful. Accrual in the randomised trial will stop when the target of 250 patients with a DIPG is achieved, even if less than 90 patients have been accrued in the smallest comparison. Hence, due to the observed biomarker distribution in the first patients, the proportion of patients enrolled in the erlotinib arm is smallest than anticipated. However recruiting patients in the current trial beyond the target of 250 seems not acceptable. The main analysis of the trial will be performed 6 months after recruitment of the last patient, or earlier if the target number of 167 events is already achieved in the everolimus-dasatinib comparison. Updated analyses will be performed 2 years after the end of accrual, and later on (for long-term follow-up). Considering the comparison of each treatment group to the historical control (secondary analysis testing against the null hypothesis, 2-year OS p0=5%, with a one-sided alpha=0.05), if we assume that 20% of patients receive erlotinib (approximately 50 patients), 37% everolimus (~92 patients) and 43% dasatinib (~108 patients), the trial will have an 80%-power to detect a 9.5% increase in the two-year OS for erlotinib (5% versus 14.5%) a 6.6% increase for everolimus (5% versus 11.6%) and a 6% increase for dasatinib (5% versus 11%). The addition of NB-DMG, H3K27M mutant will change neither the sample size calculation nor the power or the type-1 error rate for the main analysis of DIPG since NB-DMG will be analysed apart in a prospective observational cohort with a descriptive purpose. Considering the estimated remaining accrual duration in the randomised trial for DIPG patients to get 250 patients, we may recruit up to 30 patients in the non-brainstem DMG H3K27M mutant cohort. Thus, the study may recruit up to 315 patients (285 DIPG plus 30 non brainstem DMG) to get 250 randomised DIPG patients.
STUDY DURATION	Planned recruitment period: 5 years Treatment period per patient: 18 months (the median expected treatment duration is 9 to 12 months) (excepted for patients benefiting from the study drugs at the end of this 18 months period) Follow-up period: 2 years after inclusion of the last patient (6 months after end of treatment if 18 months of treatment) Planned study duration: 7 years A long-term follow-up is also planned.

SCHEDULE OF ASSESSEMENTS

Visit	**Screening**	**Cycle 1**				**Cycle 2**				**Cycles 3-18[9]**	**End of Treatment**	**Follow-Up**
Day of visit	**-4 to -1 week**	**D1**	**D8**	**D15**	**D22**	**D1***	**D8**	**D15**	**D22**	**D1***	**28 days (+/- 5 days) after the end of treatment**	**Every 3-4 months**
Written Informed Consent	X											
Demographics, Inclusion/exclusion criteria, Medical History[1]	X											
Performance Status[2]	X	X	X	X	X	X	X	X	X	X	X	X
Pregnancy Test (if indicated)[3]	X	X										
Physical Examination (including vital signs)	X	X	X	X	X	X	X	X	X	X	X	X
ECG[5]	X											
Neurologic Examination	X	X				X				X	X	X
Height / Weight / ± Body surface[4]	X	X				X				X	X	X
Steroid medication (type and dose)	X	X	X	X	X	X	X	X	X	X	X	X
Chest X Ray	X											
Hematology[6]/Biochemistry [6]	X	X	X	X	X	X	X	X	X	X	X	
Coagulation test	X											
Adverse Events	X	X	X	X	X	X	X	X	X	X	X	
MRI [7]	X						X			Every 2 months	(X)	X
Functional MRI[7]	X							X		Every 2 months if possible	(X) if possible	X if possible
Blood genetic analysis [8]	X											
Archival tumour sample (FFPE + fresh-frozen sample)	X											

*D1 of a cycle = D29 of previous cycle

1. Relevant past medical history, and current medical conditions not related to the DIPG. Information related to diagnosis of the DIPG, any disease related symptoms present at baseline.
2. Performance status will be assessed using Karnofsky performance status scale for patients >12 years of age or Lansky play score for patients ≤ 12 years for age (see Appendix 1)
3. Serum β-HCG or urinary test within 7 days before study enrolment (sexually active menarchal patients only); further controls if indicated
4. Body Surface Area will be calculated, for treatment dosing, with the Mosteller formula (m2) = √ [Height(cm) x Weight(kg) / 3600] or according to national guidance.
5. A cardiac echography at baseline is not requested except in case of known or possible cardiac disease, which may lead to a contra-indication to dasatinib.
6. Complete Blood Count, White Blood Count, Absolute Neutrophil count, Hemoglobin, Platelet count, Serum Creatinin, Urea, AST (SGOT), ALT (SGPT), Total and conjugate bilirubin, Gamma-GT, Alkaline Phosphatase, Potassium, Magnesium. Blood Glucose and Blood Lipids will be performed only for patients receiving Everolimus. Within 7 days before study enrolment, then once a week during the first 2 cycles, then before each cycle (±3 days), and if possible at the first post-discontinuation visit. The frequency of the blood assessment can be adapted on the patient's clinical situation.
7. For radiological imaging: Investigators must consistently use the same imaging modality.
   - ⇨ At Baseline: Preferably 1 to 3 weeks, but no more than 4 weeks before enrolment. MRI with at least two plans (axial, sagittal or coronal) of gadolinium enhanced T1 sequences are mandatory.
   - ⇨ During study treatment period: Every two cycles, prior to the new cycle (apart from the week 7 early evaluation if functional MRI is performed).
   - ⇨ During post-discontinuation period: every 3 to 4 months until death or for at least 6 additional months (minimum follow-up duration of 2 years from start of treatment) if patient’s disease has not progressed.

   Multimodal MRI including Dynamic Contrast Enhanced – MRI should be obtained at baseline and at week 7, then if possible, every 2 months during treatment and every 3 months after the end of treatment in order to be able to monitor perfusion inside the tumor (see Appendix 3).
8. 5 ml Blood PAXgene® DNA tube (stored at room temperature) for genotyping assessment (see Section 6.5.2).
9. The maximum treatment duration allowed is 18 months (excepted for patients benefiting from the study drugs at the end of this 18 months period).

1 INTRODUCTION AND RATIONALE OF THE STUDY

1.1 Introduction

As a group, paediatric central nervous system (CNS) tumours are the most common solid tumours of childhood and the second most common cancer after haematological malignancies. These tumours account for approximately 20% of all primary paediatric tumours and are the leading cause of cancer deaths of children, adolescents and adults under 40 years of age.

Diffuse Intrinsic Pontine Gliomas (DIPG) are one of the most frequent brain neoplasms in this age group. Most of the patients die within two years after diagnosis because these tumours are not operable, almost always chemoresistant and only transiently controlled with irradiation. They represent the biggest therapeutic challenge in paediatric oncology (Warren K. et al, 2012).

Clinical (short disease course with an association between long tracts involvement signs and cranial nerve palsies) and radiological (infiltrating and intrinsic pontine tumour with involvement of more than 50% of its volume) signs are usually sufficient to establish their diagnosis. General policy has therefore been to replace biopsies by MRI for the last 20 years (Albright et al., Neurosurgery 1993).

Numerous clinical trials of new agents and novel therapeutic approaches have been performed over the course of several decades in efforts to improve the outcome of children with DIPG, yet without success. The paradigm of their treatment has been based on that of supratentorial high-grade gliomas in adults as the biology of these lesions were presumed to be similar.

The biology of these tumours remained for years largely unknown because biopsies were considered dangerous and useless as diagnostic procedure. However, recent pivotal studies mostly from autopsy samples shading lights in their oncogenesis demonstrate that DIPGs appear to be an own entity (Zarghooni et al., J Clin Oncol, 2010; Paugh et al., J Clin Oncol., 2011; Monje et al., 2011; Barrow et al., Neuro-Oncol, 2011; Puget et al., 2012; Grill et al., 2012; Wu et al., 2012; Schwartzentruber et al., 2012; Warren et al., 2012; Khuong-Quang et al., 2012; Sturm et al., 2012; Saratsis et al., 2013). Most of the DIPG have a K27M mutation in the regulatory tail of an histone H3 variant which is unique to this disease (Castel et al, 2015).

In addition, these studies have established that a significant heterogeneity exists among patients with DIPG. Most of these data, but those from the two French studies (Puget et al., 2012; Grill et al., 2012) were obtained from autopsy cases or a few cases at diagnosis with unusual clinical or radiological presentation. Simply identifying the fact that DIPG have their specific biology releases a number of constraints and opens new opportunities for biologic investigations of these lesions, setting the stage for more specific treatments of DIPG taking into account their biological hallmarks.

In addition, other diffuse midline gliomas (spinal cord, medulla oblongata, pedoncular and thalamic) may harbor a H3K27M mutation. The 2016 update of the WHO classification of brain tumors has created therefore a new entity named “difuse midline glioma, H3K27M mutant” grouping these later tumors with classical DIPG (Louis et al, 2016). This grouping could subsequently be justified by additional studies showing that these tumors share the same dismal prognosis (Grill et al, 2018; Mackay et al, 2018; Kannermann et al, 2018; Castel et al, Acta Neuropathologica Communications, in press). Moreover, gene expression and methylation analyses with microarrays showed that the H3K27M mutant DMG share the same profiles which is clearly distinct from other malignant gliomas (Mackay et al, Cancer Cell 2017; Castel et al, Acta Neuropathologica Communications, in press).

1.2 Current therapeutic strategies for newly diagnosed DIPG

1.2.1 Diagnosis

DIPG is a disease occurring generally in the middle childhood between 5 and 10 years. Most patients will present with brainstem dysfunction (motor deficit, gait disturbances and/or cranial nerve palsies) or CSF obstruction. Typically, interval between first symptoms and diagnosis is short (i.e. < 1 months). The diagnosis is based on characteristic CT and MRI findings. On CT, the brainstem is hypo dense and enlarged, with sometimes local ring contrast enhancement. On MRI, the pons is occupied by a large expansive and infiltrative lesion.

The tumour is intrinsic and centred in the pons, rarely and then only partly exophytic. It should involve more than 50% of the pons and centered in this structure. The tumour is homogeneously hypo intense on T1 and hyper intense on T2 and FLAIR. Diffusion Tensor Imaging (DTI) fibre tracking can differentiate demyelinising diseases from diffuse brain stem glioma (Giussani et al., 2010). MR spectroscopy may show and increased Choline:N-Acetyl Aspartate and Choline:Creatinine ratio compared to normal brainstem (Laprie et al., 2005; Thakur et al., 2006; Hipp et al., 2011). ADC values are frequently low (Löbel et al., 2011). Pinpoints haemorrhages can be detected with susceptibility-weighted imaging or alternatively T2*-weighted gradient-echo imaging (Löbel et al., 2010).

Although making a diagnosis based on radiographic evidence alone represents a well-established management paradigm for children with suspected DIPG, a recent survey in the USA demonstrated considerable inconsistency on the part of paediatric neurosurgeons in the application of this diagnostic strategy to individual patients. As such, the practice of diagnosing DIPG based on imaging characteristics and clinical history alone does not reach the appropriate threshold to be considered a standard of care (Hankinson et al., 2011).

Improving diagnostic methods are therefore mandatory in these tumours since inaccurate imaging diagnosis may lead to misinterpretation of the trial results.

1.2.2 Surgery and biopsy

Surgery of intrinsic brainstem lesion is dangerous and resection often impossible in case of infiltrative neoplasms. This treatment modality is therefore not routinely considered in the management of these tumours.

Anecdotal reports have shown that not all intrinsic brainstem lesions are DIPG (Zagzag et al., 2000; Sufit et al., 2012). Biopsy is therefore advocated to comfort the diagnosis in case of unusual clinical presentation or radiological aspect. Numerous series have been published showing the low mortality and morbidity of stereotactic biopsies (Samadani et al., 2003; Roujeau et al., 2007). This procedure is now considered safe enough in well-trained hands to be incorporated in prospective trials (Puget S, et al., 2012).

Two approaches for biopsying these lesions are possible either transcerebellar or transfrontal. Feasibility and safety of these systematic procedures in the context of prospective trials has been shown in France and Europe in several studies (Geoerger et al., 2009; Geoerger et al., 2011; and the ongoing CILENT study). A minimal set of biomarkers could be acertained with immunohistochemistry and FISH. In many cases, sufficient material was available to be snap-frozen or cultured *in vitro* (Puget et al., 2012). A median of 3 microgrammes of DNA and 2 microgrammes of RNA could be extracted from these samples. One to three samples were needed to extract sufficient nucleic acids depending on the tumor infiltration. The rate of non informative biopsies is inferior to 10% in experienced hands. In the experience of the Necker group, 70% of the tumor samples were fully representative of the tumor, ie containing the supposely most agressive part of the tumor based on the appearance of the lesion on MRI (Pascale Varlet, personal communication).

1.2.3 Radiotherapy

The standard of care for children with newly diagnosed DIPG is focal irradiation therapy, using a 2 cm margin to cover microscopic disease, to a total dose of 54-60 Gy administered over 6 weeks, usually in daily fractions of 180 to 200 cGy. About two third of patients will have some neurological improvement in neurological symptoms in response to radiation therapy. Radiation therapy appears to control tumour growth for a short period of time, prolonging survival by a mean of 3 months (Haas-Kogan et al., 2011). In early studies, patients receiving doses under 50 Gy with conventional fractionation have a worse outcome than those receiving higher doses (Warren et al., 2012). Various hypofractionated regimen over 3 to 4 weeks have been proven to be safe without jeopardizing survival (Negretti et al., 2011; Janssens et al., 2013). Total radiation doses higher than 60 Gy have been evaluated utilizing hyperfractionated schedules. Even doses up to 78 Gy have failed to improve survival (Packer et al., 1994; Mandell et al.,1999). No radiosensitizing agent tested (etanidazole, topotecan, carboplatin, carbogen) has been able to increase the efficacy of radiation therapy (for review see Warren et al., 2012).

Usually, radiation therapy is limited to the infiltrating lesion albeit neuraxis dissemination has been frequently reported in this disease (Donahue et al.,1998; Gururangan et al., 2006; Wagner et al., 2006). Prospective neuraxis MRI surveillance revealed a risk of leptomeningeal dissemination as high as 56% in one study (Sethi et al., 2011). These authors therefore advocated routine craniospinal imaging at diagnosis and follow-up. The impact of concomitant use of anti-angiogenic therapies on the rate of metastasis, suspected in adult supratentorial glioblastomas has yet to be determined. Almost all failures however are also local and brainstem dysfunction is usually the cause of death. These failures occur usually within 3 to 8 months after completion of radiation therapy and the absence of effective treatment at relapse explains that death occurs within a few months after relapse in most of the cases.

There has been one anecdotal report of the use of a short course of re-irradiation (18 to 20 Gy in 2 Gy fractions) with effective symptoms control in some cases for up to 5 months (Fontanilla et al., 2012).

1.2.4 Chemotherapy

In an attempt to improve the efficacy of treatment, most trials have associated chemotherapy before, during and/or after radiotherapy. These trials including also high-dose chemotherapy regimen with stem cell rescue (Bouffet et al., 2000), have failed to improve significantly the outcome of these patients. Contemporary studies limiting enrolment to patients with DIPG generally report median PFS of 5 to 8 months and 2 year-OS rates of less than 10%. Median OS is between 9 and 10 months usually (Warren et al., 2012). Temozolomide was the chemotherapy most extensively studied with several trials, including one with systematic biopsies at diagnosis (Chassot et al., 2012), showing no advantage over radiotherapy only. The combination of O6-benzylguanine with temozolomide in order to counteract one of the resistance mechanisms to this drug failed also to improve significantly the results of the treatment (Warren et al., 2011).

Only one trial of pre-irradiation chemotherapy has shown interesting results in terms of survival but the cost of infection and hospitalization deserve a cautious evaluation of the risk-benefit ratio (Frappaz et al., 2008); in addition, eleven patients had a pre-diagnostic duration of symptoms longer than usual questioning the true nature of the diseases treated since none of the patients were biopsied.

1.2.5 Supportive care

To control symptoms before and during treatment, patients are receiving corticosteroids that may interfere with drug metabolism and cause numerous known side effects, including immunosuppression. The prescription of anti-epileptic drugs is not routinely given to these patients, especially if no seizures were encountered. To control hydrocephalus occurring in at least 20% of the children, ventriculo-peritoneal shunting may be effective and could allow a more limited use of corticosteroids (Roujeau et al., 2011). The insertion of a central line would be dependent on the need for intravenous access to deliver treatment or for supportive care in case the

oral or enteral route could not be used; this procedure is therefore not performed systematically in all patients at diagnosis.

1.3 Current therapeutic strategies for newly diagnosed NB-DMG, K27M mutant

Non-brainstem supratentorial diffuse midline gliomas used to be treated like the other supratentorial gliomas with a combination of surgery, radiation and chemotherapy with temozolomide. Now that this entity is recognized, the results could be distinguished from the other, usually hemispheric gliomas. They appear to be as poor as DIPG with a median PFS below one year and no survivor beyond two years (Mackay et al, 2018).

These results justify a change in the treatment paradigm. As they seem to form a uniform entity with DIPG, there is a consensus to start to apply to these tumors the same strategy than for DIPG.

This needs however some adaptations. In spinal tumors, the risk of radionecrosis using radiosensitization is not acceptable and the patients would therefore start the medication only after completion of the radiotherapy, as already requested for the metastatic patients. In the case of large supratentorial thalamo-peduncular tumors that could not underwent debulking surgery, it may be preferable not to use concommitent radiosensitizing drugs. This can be discussed with the coordinating investigator and the study management team.

1.4 Future directions for the management of newly diagnosed DIPG and NB-DMG, H3K27M mutant: new designs, new agents

1.4.1 Statistical considerations

The majority of recent trials are early (i.e. Phase I) clinical trials or single-arm Phase II studies that rely on historical controls for comparison. Although most, if not all, studies demonstrate the recognizable ski-slope Kaplan-Meyer survival curve, a true historical cohort has not been defined. Those studies that include younger children (ie below the age of two) or older children (ie above the age of ten) (Bailey et al., 2013), children with a long history of symptoms prior to diagnosis, and children with Neurofibromatosis Type 1 (NF-1) may favourably bias results, as will those excluding patients with herniation, disseminated disease and intratumoral haemorrhage. The eligibility criteria for previous and ongoing studies differ, and the definition of typical and atypical DIPG is not standardized. In order to avoid selection bias, evaluation of new drugs should preferably be performed through randomized trials. Designing a randomized trial including a control arm with radiation therapy only could provide unbiased estimates of new drugs efficacy; however this is deemed not acceptable because of the severity of the disease and the hope of patients, families or treating physicians willing to participate in a trial evaluating new agents. We thus propose randomized trials comparing new drugs to reliably estimate their relative benefit.

Performing DIPG-specific randomized clinical trials that have adequate power and standard level of evidence to detect modest differences in outcome between treatment groups is generally precluded by the relatively small number of available patients. As we consider that little unbiased evidence is better than none, we propose randomised Phase II trials with relaxed decision rules. We acknowledge the fact this

trial is an exploratory trial, and not a confirmatory trial. The main objective of the trial is to evaluate the relative efficacy of erlotinib, everolimus and dasatinib in combination with radiation therapy, between randomised subsets of patients selected according to the biological abnormalities found in their tumour (pairwise direct comparisons).

Pragmatic considerations have driven the sample size and power calculations. In order to increase the number of patients participating in these comparative trials, we are considering the extension of the trial in several other European countries.

We will also evaluate the interest of the whole strategy by estimating the outcome of the entire cohort (by pooling the different treatment arms) and comparing it to that of historical controls. We acknowledge the fact that a positive signal in a treatment cohort may disappear at the level of the whole trial population (dilution effect) but this issue is handled by the treatment effect estimate of each cohort. On the other hand, a weak signal in each subtrial may translate in a stronger signal considering all patients together.

In order to offer to the patients the best therapeutic option corresponding to a given biomarker within a research program and to avoid gaps between protocols, this protocol is an overarching protocol using an adaptive design allowing for the evaluation of new drugs evaluation according to the presence of other targets when the corresponding drugs and companion tests become available for testing. Similarly to the flexible approach developed by Sydes et al. for randomized controlled trials allowing for stopping arms for lack-of-benefit and adding research arms mid-trial in a multi-arm multi-stage design (Sydes 2012), we propose to drop inefficient treatment arms based on interim analyses, and add new treatment arms. By avoiding gaps between clinical studies, this flexible “rolling” biomarker-driven design is a unique opportunity to introduce quickly promising agents in this devastating and rare disease. Introduction of new subtrials may lead to modify eligibility criteria for the different subtrials (non-inclusion of patients exhibiting a special biomarker profile who would become eligible for a new subtrial). However for each of the pairwise comparisons planned in the current protocol, the distribution of biomarkers profiles should remain comparable as treatment will be allocated by random. Any of these changes will be subject of a discussion within the steering committee who will propose the introduction of a new trial and with an independent DMC who will see the accumulated trial data. Any of these changes would be subject of a study amendment.

Another issue when trying to compare results between different studies is that definition of endpoints may vary across studies leading to an information bias. As such, the definition of response or disease progression varies between, and within, paediatric consortia. There is frequently mismatch between clinical and radiographic findings for an individual patient; while some adhere to radiographic definition of response and progression, others adjust treatment based on clinical findings alone (Hayward et al., 2008). Tumour response assessed on radiology is not a suitable criterion for this type of study because of (i) the infiltrative character of such tumours that makes measurements highly uncertain (ii) the joint action of radiation (iii) the

mechanism of drug action allowing waiting for a stabilization rather than a tumour shrinkage in 2 months.

Progression-free survival is also a questionable endpoint in this context given the difficulties of interpretation of clinical deterioration not correlated with radiographic progression or reverse if the image is evocative of radiological progression in a child getting better clinically.

The primary efficacy endpoint for the different randomised Phase II sub-trials will thus be the overall survival at 2 years.

1.4.2 Role of targeted agents in DIPG and NB-DMG, H3K27M mutant

Due to the high recurrence rate of DIPG, these patients are often enrolled in phase I and phase II trials that have rarely brought meaningful data for efficacy. Of note a recent study with temsirolimus has shown efficient tumour control in some recurrent DIPG (Geoerger et al., 2011).

Biologic response modifiers have been explored quite extensively also at diagnosis: pegylated recombinant β-inteferon, interferon alpha 2b, tipifarnib, gefitinib, erlotinib, vandetanib, imatinib, thalidomide, tamoxifen, nimotuzumab (Warren et al., 2012). Although some trials showed encouraging increments in terms of survival, these were small, limited to a few months and were not significantly above historical controls. In addition, no information was provided if a subset of patients could benefit more from the treatment with a given targeted agent.

Most of these biological modifiers were chosen without specific justification in DIPG due to the lack of knowledge of DIPG oncogenesis.

1.4.2.1 **Combination of targeted agents with radiotherapy in DIPG and NB-DMG, K27M mutant**

Only a handful of studies have explored the combination of TKIs with radiation therapy in DIPG. It is difficult to conclude from these phase I studies about the efficacy of these approaches despite the report of a few long-term survivors (Broniscer et al., 2010; Pollack et al., 2011; Geoerger et al., 2010, Broniscer et al., 2013). The most recent study from St Jude has explored a combination of two TKI, vandetanib and dasatinib (Broniscer et al., 2013). Twenty-five patients were treated. Phosphorylated 70S6K decreased during therapy in PBMCs. Treatment was well tolerated. Diarrhea was the most significant toxicity. Three patients experienced substantial myelosuppression. No changes in cartilaginous growth plates were detected by either knee x-ray or magnetic resonance imaging (MRI). Osteonecrosis was seen by knee MRI in two patients 4 and 8 months after the start of therapy; both had received dexamethasone. Treatment with vandetanib has been shown to induce bone changes, mainly osteonecrosis but also growth plate abnormalities in 20% of patients explored (Kaste et al., 2013). It is difficult to exclude a significant role of concomitant steroids in some of these side effects. In terms of efficacy, median duration of treatment was 184 days. The one and two-years overall survivals were 52% +/- 10% and 9% +/-6%, respectively. Five patients completed more than 1 year of treatment and two patients completed more than two years of treatment. The

authors concluded that further studies are needed to explore further dasatinib and other PDRGFR inhibitors alone or in combination.

In the European study of the ITCC consortium, a trend in favour of a better PFS and OS for patients with EGFR positive tumours was observed when treated with erlotinib compared to a historical control cohort treated without EGFR TKI, whereas no benefit was observed with erlotinib in EGFR negative tumours (see below). In the control cohort, EGFR-positive DIPG was not associated with a better outcome, compared to EGFR-negative tumours.

Although some rare radiation recall lesions have been recently documented (Levy et al., 2013), the combination of TKI and radiotherapy has been considered generally to be safe but deserving further explorations (Niyazi et al., 2012). Digestive toxicity is of particular concern in patients whose radiation fields encompass the digestive tracts.

The combination of targeted agents with radiotherapy was proven to be safe for most of the agents tested except imatinib. Phase I studies combining EGFR TKI (erlotinib or gefitinib) in DIPG did not show an increased toxicity compared to historical controls and previous trial (Pollack et al. ,2011, Geoerger et al., 2010). A few long-term survivors (over two years) were observed in both studies and median overall survival was slightly better than in historical control (ie 12 mo vs 9 mo). In the study with imatinib, an unexpected frequent rate of intratumoral bleeding lead the investigators to differ the administration of imatinib after radiation therapy (Pollack et al., 2007). However, intratumoral bleeding is not a rare event in the course of high-grade gliomas, also in the brainstem. Petechial hemorrhages can be observed in up to 20% of cases (Broniscer et al., 2006; Löbel et al., 2010). This rate of bleeding in the imatinib trial could therefore not be attributed to the interaction between this drug and radiotherapy.

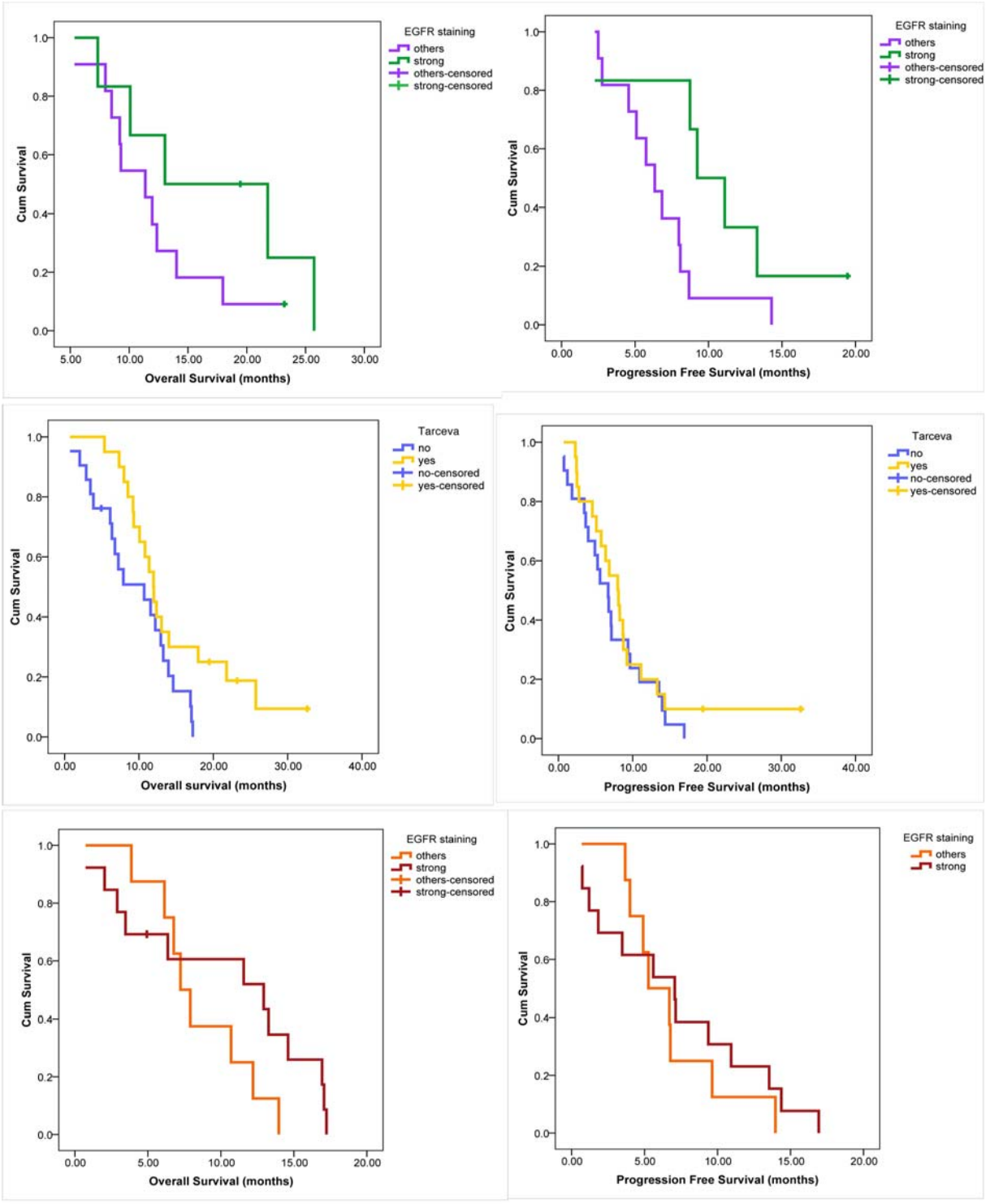


Figure 1: Effect of EGFR immunopositivity on the survival of patients with DIPG treated (panels A & B) or not treated (panels E & F) with erlotinib (TARCEVA). Panels C and D describe the OS and PFS of children treated or not treated with erlotinib.

1.4.2.2 Pediatric Experience with the Targeted Agents Chosen for the Study

Three classes of drugs have been evaluated in children with DIPG, EGFR TKI inhibitors (Porkka 2010; Geoerger 2011), PDGFRA TKI inhibitors (Pollack 2007, Geoerger 2009, Broniscer 2013) and mTOR inhibitors (Geoerger 2012).

The suggested dose of erlotinib in combination with radiotherapy is 125 mg/m2 once daily (Geoerger 2011). Of note, these doses of erlotinib to be administered in combination with radiation therapy are identical to the recommended dose in monotherapy. The main side effects, observed on the long run, are fatigue, skin rashes and diarrhea. Long-term toxicity of the use of erlotinib in the adjuvant phase will need to be documented in the trial.

The recommended dose in children for dasatinib and everolimus given as single agents are 85 mg/m2 twice daily and 5 mg/m2/day, respectively (Demitri et al, Clin cancer res 2009, Aplenc et al., 2011; Zwaan et al., 2013; Fouladi 2007). Responses in brain tumors associated with Tuberous Sclerosis Complex have been observed at this dose level of everolimus (Krueger 2010). Both drugs could be administered for long periods with good tolerance; main side effects were diarrhea and hypokalaemia for dasatinib and diarrhea and mucositis for everolimus. Significant changes in Fractional Anisotropy and radial diffusivity were observed on MRI after treatment with everolimus in patients with TSC, suggesting that the genetic defect of TSC in the brain may be modified pharmacologically, even in normal-appearing white matter (Tillema et al., 2012). These changes will have to be tracked with multimodal MRI. It was deemed unnecessary to have a formal dose-finding phase I for everolimus and dasatinib given in combination with radiotherapy as both treatment components (targeted agent and radiotherapy) are known to have different toxicity profiles. Moreover, dasatinib has already been tested in combination with radiation therapy plus vandenatib in children with DIPG and the dose of 65 mg/m2 x 2 per day of dasatinib was considered safe (Broniscer 2013). The combination with vandetanib precluded to reach the MTD of dasatinib when used as monotherapy. No unexpected toxicity of radiotherapy was encountered with the addition of dasatinib.

Regarding mTOR inhibitors, temsirolimus and everolimus have been used in combination with radiotherapy in adult glioblastomas (Sarkaria 2010 & 2011; Chinnaiyan 2013), and could be administered without increased toxicity other than the expected increased risk of infection. Anyway, toxicity will be carefully monitored and the dose recommended at the beginning of the trial may be re-evaluated in the course of the trial based on accumulated toxicity data.

1.5 Special considerations for children below the age of three

The diagnosis of DIPG is rarely made in children below the age of three. ATRT and PNET may be more frequent at this age, justifying the need for histological confirmation of the diagnosis. Stereotactic biopsy may be difficult due to the difficulty of using a stereotactic frame at this age. Although it is difficult to set up a lower age limit for inclusion in DIPG trials, infants below the age of six months will not be eligible for this study since they may have a more favorable course, with even spontaneous regression (Cappellano et al., 2011: Shah et al., 2008). Children between 6 months and 3 years will be discussed on a case by case basis for

inclusion in the study. Inclusion in the randomized trial is allowed, provided irradiation is considered feasible and the diagnosis of DIPG is formally proven by molecular pathology (loss of H3K27me3 expression on IHC and/or presence of a K27M mutation in one of the two histone H3 variants).

1.6 Special considerations for children with Type 1 Neurofibromatosis

Brainstem lesions in children with NF1 can be diffuse and infiltrating but their course is usually indolent and the symptoms are limited (Bilaniuk et al.,1997; Ulrich et al., 2011). Despite a characteristic imaging, children with NF1 will only be included in case of short tumor history and/or grade III/IV histology and/or the presence of an immunohistochemical proof of H3K27me3 loss in the tumor cells.

1.7 Biological targets in DIPG

Recent studies performed on biopsy samples of DIPG at diagnosis as well as other data obtained from mixed cohorts described in the literature have indicated that the most frequent abnormalities found at diagnosis concerned p53 (Louis et al.,1993; Puget et al., 2012; Grill et al., 2012), EGFR (Gilbertson et al., 2003; Geoerger et al, 2010; Li et al., 2012), PDGFRA (Geoerger et al., 2009; Zarghooni et al., 2010; Paugh et al., 2011; Puget et al., 2012) and the mTOR pathway via loss of expression of PTEN (Geoerger et al., 2010) or PI3KCA and PI3KR1 mutations (Grill et al., Pediatr Blood Cancer, 2012, Taylor et al, Nature Genet 2014).

1.7.1 EGFR

Different alterations of EGFR have been reported in DIPG. The first abnormalities shown in DIPG was an EGFR overexpression associated in some cases with EGFR amplification as shown by FISH (Gilbertson et al., CCR 2003) later confirmed by another study (Geoerger et al., 2010); in this later study EGFR copy number changes were rarely observed. Other reports (Paugh et al., 2010; Puget et al., 2012) did also not find frequent EGFR gain/amplification while another autopsy study showed frequent gains but rare amplification (Warren et al., 2012). EGFRvIII mutations have been recently described in a significant number of patients (11 out of 16) (Li et al., 2012). This data still needs to be confirmed because mutant EGFRvIII was not detected neither by immunohistochemistry (Geoerger et al., 2010) nor by sequencing (Wu et al., 2012, Taylor et al, in revision Nature Genet). Our preliminary data indicate that EGFR mutations can be found in the external domain as previously shown in adult glioblastoma (Lee et al., 2006).

The consequences of these alterations will usually lead to the activation of the receptor and its downstream pathway. In our preliminary screens of DIPG biopsy samples by immunohistochemistry and FISH, the observed frequency of strong EGFR staining was 45% compatible with previous reports from our group (Geoerger et al., 2010).

1.7.2 mTOR

Activation of the mTOR pathway, measured by immunohistochemistry for phospho-mTOR, was first described by the Toronto group (Zarghooni et al., 2010) and found positive in 11 of 11 samples tested. Subsequently, we showed that PTEN expression was frequently absent on immunohistochemistry in more than 70% of the samples (Geoerger et al., 2010) albeit the gene was rarely deleted in DIPG (Puget et al., 2012; Paugh et al., 2011; Warren et al., 2012). PTEN is the major downregulator of the mTOR pathway and its loss may also contribute to the activation of the pathway. In addition, mutations in the PI3K pathway can also activate this pathway as it has been shown in 15% of the DIPG (Grill et al., 2012). In a recent study analysing whole genome sequencing of DIPG (Taylor et al, Nature Genet 2014), we have found that 47% of DIPG have alterations in the PI3K/AKT/PTEN/mTOR pathway.

Our preliminary screen with immunohistochemistry yielded an 90% frequency of PTEN loss.

1.7.3 PDGFRA

Gain or amplification of PDGFRA is found in 20% of the tumours analyzed by our group at diagnosis (Puget 2012) and even more frequently (36%) at (Zarghooni 2010, Warren 2012, Paugh 2011). More recent data from our group indicated however that the amplification of PDGFRA may not be as frequent at diagnosis (Taylor et al, Nature Genetics in revision). The preliminary screen peformed in the conditions of this trail showed that the frequency of any PDGFRA gain/amplification was 12%. Frequent overexpression by immunohistochemistry has also been described in DIPG (Zarghooni 2010, Puget 2012, Geoerger et al., 2009). PDGFRA is overexpressed compared to normal brainstem at the mRNA level and the level of expression is dependent on the copy number of the gene (Puget et al., 2012). PDGFRA can be activated also by intragenic deletions (Osawa et al., 2010) or possibly by mutations in the external domain as for EGFR (Puget et al., 2012, Paugh et al., 2013).

Cases analysed after radiotherapy in the north-american series may have overestimated the rate of PDFRA amplification which is preferentially associated with secondary glioblastomas (Paugh 2010). In addition, FISH may miss some of the amplifications/gains when they are present only in a subset of cells (Paugh 2011, Puget 2012). Several preclinical studies indicate that inhibitors of tyrosine kinase PDGFRA as imatinib or dasatinib are radiosensitizer (Geng Li 2006 Holdhoff 2005, Russell 2003). In the ITCC phase II study of imatinib evaluating only relapsing patients with PDGFRA overexpression detected by immunohistochemistry, 1 out of 3 patients affected with a malignant brainstem glioma showed a volumetric response (-31% according to MacDonald) persisting for 10 months. We explored the role of dasatinib rather than imatinib because of its better bioavailability in brain tissue (Porkka 2008), its greater affinity for the PDGFRA receptor with a dasatinib IC5O of 28 nM, versus 380 nM for imatinib (Bantscheff 2007) and a broader spectrum of action on ephrins receptors and Src family tyrosine kinase (especially Fyn) which can also be overexpressed and activated in malignant gliomas (Du 2009; Angers-Loustau 2004). Src phosphoproteins may therefore be considered as interesting targets in glioblastoma (Du et al, Nature Biotechnol 2009; De Groot & Milano, J Neuro-oncol 2009; Ahluwalia M et al, Cancer Letters 2010). In our preclinical drug screen,

dasatinib appears to be one of the most effective drug in vitro (Grasso submitted). Its efficacy in vitro on DIPG cell lines is not related to the presence of a PDGFRA alteration questioning therefore which biomarker should be used for patient selection (Truffaux submitted).

1.7.4 Other potential targets to be explored

The number of potential therapeutic targets in DIPG is rapidly growing as data from genome wide studies become available. To name a few: sonic hedgehog pathway (Monje et al., 2010), MET (Paugh et al., 2011; Puget et al., 2012) or histone H3 mutations (Wu et al., 2012; Schwartzentruber et al., 2012; Sturm et al., 2012). It will be a component of the study to explore prospectively them on the biological material left over after the initial biomarkers screen.

In addition, due to the adverse prognostic impact of the mutation of Histone H3.3 in brainstem glioma (Khuong-Quang et al., 2012), we will have to sequence these mutation in the patients samples as a possible confounding factor in the analysis of the effect of the various targeted therapies used in the trial. Indeed PDGFRA amplification is associated with K27M mutation of the H3.3 specifically with a synergistic effect on tumor growth (Lewis et al., 2013). In our series of gliomas and DIPG, 7 out 9 tumors with PDGFRA amplification have a K27M mutation on H3.3 while only 24 out of 77 tumors without PDGFRA amplification have a K27M mutation on H3.3, p=0.005. It is therefore likely that the group of patients with PDGFRA amplification would have a worse prognosis.

As a whole in our case series, median survival of histone H3.3 mutated DIPG is 11 months versus 15 months for the patients with H3.1 mutated DIPG ($p<0.01$, log-rank test).

Activating mutations of the ACVR1/ALK2 has been recently identified with whole genome sequencing by our group (Taylor et al, Nature Genetics 2014). These mutations activate the Bone Morphogenic Protein (BMP) pathway and can be targeted by specific inhibitors currently in preclinical development for a congenital bone disease named Fibrodysplasia Ossificans Progressiva (FOP) (Yu et al., 2008; Sanvitale et al., 2013). In vitro, these agents have been shown to decrease the level of activity of the BMP pathway in DIPG cell lines (Taylor et al., in revision in Nature Genetics). These agents may become available later during the conduct of the study. Of note, ACVR1 is also a target of dasatinib that can effectively downregulate the effect of ACVR1 downstream on pSMAD, albeit at higher concentrations (Truffaux in press).

MET mutations and amplifications/gains: mutations exist in 5% of cases (Taylor et al, in revision in Nature Genetics) and amplifications/gains in 15% of cases frequently associated with PDGFRA amplification (Puget et al., 2012, Paugh et al., 2011). Several MET inhibitors are under preclinical development but none is yet authorized

to treat pediatric brain tumors even within a trial. These agents may become available later during the conduct of the study.

The most attractive target due to their almost constant alterations in DIPG would be the mutations of the histones H3 variants and their consequences, ie a loss of the trimethylation of the H3 histones at the Lysine 27 (Truffaux et al, submitted; Bender et al, Cancer Cell 2013, Lewis Science 2013; Chan 2013). There is however no therapeutic agents targeting these alterations at the moment. These agents may become available later during the conduct of the study. The presence of theses histones H3 alterations will however be evaluated as diagnostic biomarker. Indeed, almost all bona fide DIPG have either a mutation in one of the H3 variant genes or the consequence of this mutation (Castel et al, in preparation). Moreover, the type of histone H3 mutated may have a prognostic impact since retrospective data indicated that mutations in the H3.3 gene, ie *H3F3A*, have a worst prognosis (Khuong-Quang, Acta Neuropathol 2012 & Castel et al, in preparation).

1.8 Specific Aspects of the Study Design

1.8.1 Biopsy

Papers on stereotactic biopsies in the brainstem published in the last 20 years represent a substantial amount of knowledge, yet unfortunately often report mixed series of adults and children with a wide range of diagnoses. These mixed series cite morbidity rates between 0% and 10% and mortality rates between 0% to 3% (Puget and al., 2012). However, when biopsy data on paediatric patients with DIPG are extracted, the diagnostic yield ranges from 96% to 100%, with no mortality and morbidity less than 5% for the largest series. In a series of 30 transcerebellar stereotactic biopsies for infiltrating pontine neoplasms, 2 patients (7%) experienced a transient and mild worsening of their neurological condition and asymptomatic punctuated haemorrhage was observed on the post-biopsy imaging in 2 other patients (Roujeau et al., 2007). Material suitable for pathological diagnosis was obtained in all but one patient.

Two routes have been described for brainstem biopsies: the transcerebellar approach and the transfrontal approach. The transfrontal route is longer and allows sampling of masses located in all the segments of the brainstem. The positioning of the entry site has to be chosen carefully to avoid the ventricles, the vascular structures, and the tentorium. The use of software is recommended for planning this trajectory. The transcerebellar approach is shorter, through the middle cerebellar peduncle, and has less eloquent structures in its trajectory. It can be used only for pontine and upper medullary masses, and because of this, the transcerebellar route for DIPG is preferred.

In a large series of stereotactic brain biopsy in 270 adults, it has been shown that increasing numbers of specimens obtained per trajectory and brainstem lesions were not significant risk factors for morbidity (McGirt et al., 2005). We have recently shown that transcerebellar stereotactic biopsies could allow multiple biopsies samples (up to

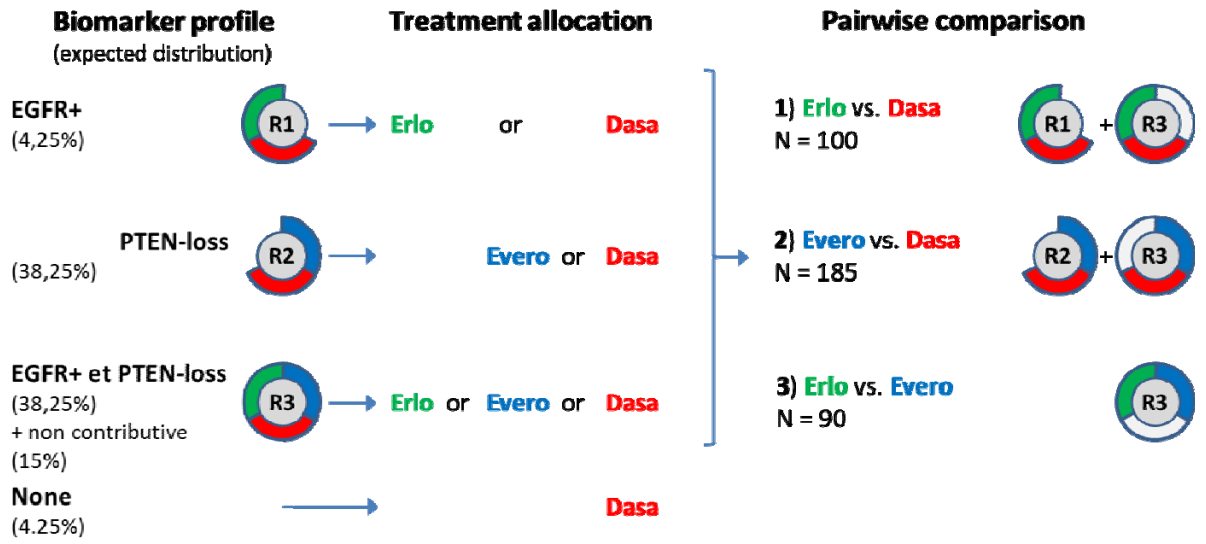
eight) to provide enough tissue for further genomic analyses and stem-cell culture (Puget et al., 2012). In this series, one or two biopsies were used for histologic diagnosis and immunohistochemistry. The remaining biopsies were snap-frozen with cytologic control smears within minutes of surgical removal, and nucleic acids were extracted later.

1.8.2 Treatment Allocation Procedure

In most cases, treatment will be allocated by randomisation. In the current protocol (first step of an overarching protocol), the allocation rule will be based on EGFR overexpression and PTEN loss of expression detected by immunohistochemistry: For each biomarker profile, we have identified which drugs were deemed worthy of evaluation. As erlotinib will not be evaluated in known EGFR-negative tumours and everolimus will not be evaluated in known cases without PTEN-loss, randomisation may concern two of the three drugs (R1-randomisation: erlotinib versus dasatainib; R2-randomisation: everolimus versus dasatinib), or the three drugs (R3 randomisation). If the biomarker study is not contributive (ie no information for randomisation is obtained) but the diagnosis of DIPG is ascertained either by a typical short clinical history and typical radiological appearance or by the histology of diffuse glioma with H3K27M trimethylation loss and eventually the detection of the H3K27M mutation by immunohistochemistry, the patient will be included in the trial and included in the R3 randomisation, ie for the three drugs.

In this study, the majority of patients will receive a treatment assumed to specifically target a biological abnormality identified on the biopsy. More importantly, patients will not receive a drug for which the identified target is absent. Based on previous knowledge, this design is expected to increase the likelihood of finding a positive efficacy signal if these targeted agents are truly efficient to inhibit the corresponding oncogenic signalling pathways.

The expected distribution of biomarker profiles and consequently the expected distribution of patients per pairwise randomised comparison is detailed in 8.1.3.



NB: Patients with EGFR status unknown and no PTEN-loss will be eligible for the R1 randomisation. Patients with EGFR-negative and PTEN status unknown will be eligible for the R2 randomisation.

1.8.3 Evaluation of Tumour Progression/Recurrence and Assessment of Pseudoprogression

Progression is an event difficult to assess in patients treated for a brain tumour (Dhermain et al., 2010). The phenomenon of pseudoprogression has been widely recognised also in children although its diagnostic criteria may not be straitforward to define (Chawla et al., 2012). In DIPG specifically, this phenomenon has been observed as well at high frequency (Chassot et al., 2011). In this series, 25% of the children were diagnosed as having a pseudoprogression. During the first six months after radiotherapy, where this phenomenon is the most frequently seen, the diagnostic of progression should be based on a combination of clinical and radiological criteria as suggested in the RANO criteria (see below). The use of multimodal imaging is recommended and the images will be reviewed centrally.

1.8.3.1 Clinical Evaluation

The worsening of existing neurological signs or the appearance of new neurological signs has to be considered in patients with a stable dose of steroids. Indeed, tapering the steroids may induce a deterioration of the neurological status. Other potential causes of this neurological deterioration (seizures, haemorrhage, CSF blockade, medications) have to be excluded before considering that the symptoms could be due to tumour progression. Not all pseudoprogressions are asymptomatic, especially in the brainstem (Chassot et al., 2011). However, there is usually dissociation between the importance of the images on MRI and the relative limitation of the clinical deterioration.

1.8.3.2 Radiological Evaluation

The most widely used response criteria for malignant glioma (Macdonald criteria) address only the contrast-enhancing component of the tumour. These criteria use a significant increase (considered by convention to be 25% or more) in the surface area of the contrast-enhancing lesion as a reliable surrogate marker for tumour progression. With the use of anti-angiogenic therapy and the increased incidence of pseudoprogression (psPD) after concomitant radio chemotherapy, it has become increasingly apparent that contrast enhancement is nonspecific (i.e., it can be influenced and induced by a variety of non-tumoral processes) and not always pathognomonic of tumour response or progression. In addition, it has also become progressively evident that there is a need to account for progressive non-enhancing disease.

The Response Assessment in Neuro-Oncology (RANO) Working Group was established to address the recognised and accepted limitations of the current Macdonald criteria and to recommend new criteria that will permit the accurate assessment of the efficacy of novel therapies in High-Grade Glioma (HGG). These

updated response-assessment criteria (Wen et al., 2010) will be implemented in this trial to assess patients for the development of tumour progression and recurrence.

In case specific radiological criteria for paediatric DIPG become available during the course of the study through the RANO endeavour, they will be implemented at the stage of retrospective central review

However, RANO criteria may not be sufficient to judge for a possible pseudoprogression. There is increasing evidence that multimodal imaging could be of help in the follow-up of patients with DIPG.

1.8.3.3 Multimodal Imaging

MRI is thought to be a central component of clinical trials evaluation in DIPG (Poussaint et al., 2011). However, there is a wide inter-observer variability in the measurements of DIPG (Hayward et al., 2008). Measurements on the FLAIR sequences appear to be the most reproducible ones and the transversal diameter of the brainstem as single measure may be the most reliable one.

Spectroscopy of DIPG can be useful at diagnosis and at relapse. Citrate peak have been considered as pathognomonic of infiltrating gliomas in the brain (Seymour et al., 2009). At baseline, creatine and total choline (tCho) were significantly lower in DIBSGs than in astrocytomas elsewhere in the CNS. Serial MRS in individual subjects revealed increasing levels of tCho and lipids and reduced ratios of N-acetylaspartate, creatine, and myoinositol relative to tCho. Metabolic progression defined by increased tCho concentration in serial MRS preceded clinical deterioration by 2.4 to 2.7 months (Panigrahy et al., 2008; Laprie et al., 2005). Other subsequent reports have emphasized the usefulness of this modality for follow-up (Hipp et al., 2011; Steffen-Smith et al., 2011, 2012).

To differentiate pseudoprogression from true progression, the use of perfusion imaging has been shown to be useful showing usually that true progressions are associated with increased perfusion (high relative Cerebral Blood Volume) on the contrary to radionecrosis (Meyzer et al., 2010; Dhermain et al., 2010). However, very early post-irradiation imaging, i.e. before or right after the end of irradiation, can be misleading showing a paradoxical increase in perfusion that may be associated with the response to irradiation (Essok-Burns et al., 2011; Sedlacik et al., 2013).

The assessment of diffusion-weighted images for patients with increases of measurable enhancing regions 2 months after radiotherapy completion is useful for differentiating true progression from pseudoprogression (Lee et al., 2012).

Finally arterial spin labelling (ASL) sequences can also be used to differentiate pseudoprogression from progression (Choi et al., 2013).

With reference to the perfusion imaging, it has been shown that an early increase of perfusion after radiotherapy could be associated with a better outcome in the recent Saint-Jude trial with vandetanib in DIPG (Sedlacik et al., 2013). An MRI with perfusion sequences will be required right after the end of radiotherapy.

It is therefore recommended to combine the different modalities of MR imaging to ascertain the diagnosis.

2 STUDY OBJECTIVES

2.1 Primary objective

The main objective of this study is to evaluate the efficacy in terms of overall survival (OS) of erlotinib, everolimus and dasatinib in combination with radiation therapy in patients with DIPG, both

- compared to the other experimental treatments by comparisons between randomised subsets of patients selected according to the biological abnormalities found in their tumour (pairwise direct comparisons), and
- compared to historical control.

2.2 Secondary objectives

The secondary objectives are:

- To compare the 2-year OS between randomised subsets of patients with DIPG (pairwise comparisons) and compared to historical controls;
- To evaluate the safety profile of each of the three drugs when administered in combination with radiotherapy, and over the whole duration of treatment, as well as the feasibility of a continuous administration of these drugs;
- To evaluate the efficacy in terms of progression-free survival (PFS) of erlotinib, everolimus and dasatinib in combination with radiation therapy, comparatively between randomised subsets of patients with DIPG, and also compared to historical controls;
- To evaluate the whole strategy by estimating the overall and progression-free survival of the entire cohort of patients with DIPG (by pooling the different treatment arms) and comparing it to historical controls.

2.3 Exploratory objectives

The exploratory objectives are:

- To study the association between known biomarkers of the targeted activation pathway (a preliminary list is given below) and response to treatment (OS, PFS) as well as to describe prospectively new tumour abnormalities that will emerge during the course of the study and evaluate their correlation with response to treatment;
- To describe the effect of therapy with morphological (MRI) and functional imaging (multimodal MRI). The rate of pseudo-progression, in particular, will be assessed since it could be as frequent as 25% (Chassot, 2011).
- To describe the outcome of the cohort of non-brainstem diffuse midline gliomas, H3K27M mutant, in terms of PFS and OS, overall and according to treatment arm, and in comparison with DIPG.

3 METHODOLOGY

3.1 Description of the design

The current study is a multicenter, randomised open-label phase II trial comparing efficacy of three investigational products (i.e erlotinib, everolimus, and dasatinib). As all patients are not eligible for the three-drug comparison, the study includes three subtrials

- R1: erlotinib versus dasatinib
- R2: everolimus versus dasatinib
- R3: erlotinib versus everolimus versus dasatinib

Eligibility criteria for the different subtrials will be based on EGFR overexpression and PTEN loss of expression detected by immunohistochemistry.

It is an adaptive design because interim efficacy analyses will be performed in the course of the trial to have the possibility of dropping one treatment arm for lack of benefit, in particular if a new experimental agent is available for testing in this disease.

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3.2 Study centres

70 sites will be open for the study (≈ 25 in France and 36 in Europe, 7 in australia and 1 in New Zealand).

Treatment will be administered in all the participating institutions but the biopsy procedure will be restricted to some centres.

3.3 Expected number of patients

The sample size of patients with DIPG to be recruited in the randomised trial was initially computed at 250 patients to achieve a total of 90 patients in the smallest pairwise comparison. Accrual in the randomised trial will stop when the target of 250 patients with a DIPG is achieved, even if less than 90 patients have been accrued in the smallest comparison.

Considering the current screen failure rate, a total of 285 patients with DIPG may be required to achieve the total of 250 randomised patients with DIPG.

Considering the estimated remaining accrual duration in the randomised trial for DIPG patients to get 250 patients, we may recruit up to 30 patients in the non brainstem DMG H3K27M mutant cohort.

Thus, the study may recruit up to 315 patients (285 DIPG plus 30 non brainstem DMG) to get 250 randomised DIPG patients.

3.4 Study duration

Planned recruitment period: 5 years

Treatment period per patient: 18 months (the median expected treatment duration is 9 to 12 months).

Follow-up period: 2 years after inclusion of the last patient (6 months after end of treatment if 18 months of treatment) (excepted for the patients benefiting from the study drugs at the end of this 18 months period).

Planned study duration: 7 years

A long-term follow-up is also planned.

4 SELECTION OF PATIENTS

4.1 Eligibility criteria for the BIOMEDE study (pre-screening for the randomised subtrials)

- Diagnosis of DIPG (clinical and radiological, or histological in case the biopsy was performed before study entry).
- Non-brainstem diffuse midline gliomas, H3K27M mutant (NB-DMG), will be eligible for the trial after biopsy or surgery. As biopsy and surgery is considered as standard practice for these locations, informed consent for the biopsy will not be necessary. Patient will sign the consent after the diagnosis to allow central review and biomarkers assessment thereafter.
- DIPG or NB-DMG at diagnosis: no prior chemotherapy for the present cancer; no prior cerebral radiation therapy
- NB : Metastatic disease allowed. Patient with metastatic disease are eligible for the study (including the randomised trial if diagnosis of DIPG/ NB-DMG confirmed). In this situation, radiotherapy will have to start within three weeks after the biopsy while targerted treatment will start **at the end** of the irradiation
- Age > 6 months and < 30 years. For children below the age of 3 years, inclusion in the study and medical decisions should be discussed with the coordinating investigator.
- Eligible for a biopsy, or biopsy performed for diagnostic purpose and material available for the biomarker assessment
- Eligible for cerebral radiotherapy
- Patient covered by an health insurance if national requirement
- Written informed consent given by patient and/or parents/ legal representative for biomarkers assessment and registration in the study.

4.2 Non eligibility criteria for the study

- Spontaneous massive intratumour bleeding. Patients with post-operative bleeding will be allowed to enter the study provided the haemorrhage is controlled. Same rule applies for the other post-operative complications (infection, CSF leakage, absence of wound closure, subdural collection…).
- Any other concomitant anti-cancer treatment not foreseen by this protocol
- Any other cancer during the last 5 years
- Uncontrolled intercurrent illness or active infection
- Any other co-morbid condition that in the investigator's opinion would impair study participation
- Unable for medical follow-up (geographic, social or mental reasons)
- Patient not fulfilling one of the previous eligibility criteria.
- Patient previously treated with irradiation on the brainstem for another neoplasm

- Patient with congenital galactose intolerance, Lapp lactase deficiency or glucose-galactose malabsorption.
- Patient not covered by a social security agreement accepted in the treating country if national requirement
- Pregnant or breast feeding women
- NB: A patient with known hypersensitivity for one the drug or its excipients could still participate in the study and receive one of the other drug(s)

4.3 Common eligibility criteria for the BIOMEDE randomised subtrials

- Eligibility criteria for the study (see above)
- Confirmed histological diagnosis of diffuse intrinsic pontine glioma (grade II, III, IV WHO), or NB-DMG confirmed by central pathology review (including the assessment of the loss of H3K27me3 by immunohistochemistry or the presence of a mutation in the histone H3 variant genes). Patients without classical clinical and radiological diagnostic criteria who fulfil the histological and biological criteria of DIPG are eligible for the trial. Pilocytic astrocytoma and ganglioliomas are not eligible. Patients with a suspected DIPG but no histological confirmation could be randomised **if and only if** the radiology is typical of a DIPG as well as the short clinical history. This would need to be reviewed and agreed centrally. Confirmation of the diagnosis of non-brainstem diffuse midline gliomas, H3K27M mutant, by central review, is needed before the randomisation of cases of NB-DMG.
- Life expectancy > 12 weeks after the start of study treatment
- Karnofsky performance status scale or Lansky Play Scale > 50%. The PS should not take the neurologic deficit per se into account. NB: Children and young adults with a worse performance status due to glioma-related motor paresis can be included.
- Absolute neutrophil count > 1.5 x 109/l, Platelets > 100 x 109/l
- Total bilirubin < 1,5 x ULN, AST and ALT< 2,5 x ULN
- Serum creatinine < 1,5 X ULN for age. If serum creatinine > 1,5 ULN, creatinine clearance must be > 70 ml/min/1,73 m² (EDTA radioisotope GFR or 24 hours urines collection)
- Normal coagulation tests within the local reference ranges
- No current organ toxicity > grade 2 according to the NCI-CTCAE version 4.0 especially cardiovascular, pulmonary or renal disease (including but not limited to: congenital long QT syndrome, nephrotic syndrome, glomerulopathy, uncontrolled high blood pressure despite adequate treatment, interstitial lung disease, pulmonary arterial hypertension). In case of known or possible cardiac disease, a cardiological advice will be required prior to the inclusion in the randomized trial as a preexisting cardiopathy represents a contra-indication to dasatinib.
- Effective and appropriate contraception for patients (male and female) of reproductive potential during their entire participation in the study and during 6

months after the end of treatment. Effective contraception are defined in CTFG Guidelines “Recommendations related to contraception and pregnancy testing in clinical trials” (Appendix 7)

- Negative pregnancy test (serum beta-HCG or urinary test) evaluated in the last week in sexually active females of reproductive potential
- Written informed consent given by patient and/or parents/legal representative for treatment and randomization

4.4 Eligibility criteria for the subtrials

Eligibility criteria for the different subtrials will be mainly based on biomarkers assessment as detailed in the table below. In addition, contra-indication to specific drugs will be considered.

- Definition of the eligibility for the different subtrials according to biomarkers assessment

Biomarker status	EGFR overexpression	EGFR negative	EGFR unknown*
PTEN loss	R3	R2	R3
PTEN positive (no PTEN loss)	R1	No Randomisation ⇨ dasatinib	R1
PTEN unknown	R3	R2	R3

*EGFR status should be rarely unknown.

R1 subtrial: erlotinib versus dasatinib

R2 subtrial: everolimus versus dasatinib

R3 subtrial: erlotinib versus everolimus versus dasatinib

- Check of contraindications to each drug evaluated in the selected subtrial or its excipient
    - If biomarker profile eligible for R1 but erlotinib administration is contraindicated or not recommended ⇨ No randomisation ⇨ dasatinib
    - If biomarker profile eligible for R1 but dasatinib administration is contraindicated or not recommended ⇨ No randomisation ⇨ erlotinib
    - If biomarker profile eligible for R2 but everolimus administration is contraindicated or not recommended⇨ No randomisation ⇨ dasatinib
    - If biomarker profile eligible for R2 but dasatinib administration is contraindicated or not recommended ⇨ No randomisation ⇨ everolimus

- If biomarker profile eligible for R3 but erlotinib administration is contraindicated or not recommended
  ⇨ randomisation R2 ⇨ everolimus or dasatinib
- If biomarker profile eligible for R3 but everolimus administration is contraindicated or not recommended
  ⇨ randomisation R1 ⇨ erlotinib or dasatinib
- If biomarker profile eligible for R3 but dasatinib administration is contraindicated or not recommended
  ⇨ No randomisation ⇨ everolimus

- These eligibility criteria lead to the following algorithm

Biomarker status		Potentially eligible for	Contra-indication to	Eligible for
EGFR Positive (or unknown)	PTEN-positive	R1	-	R1
			erlotinib	No Rand ⇨ dasatinib
			dasatinib	No Rand ⇨ erlotinib
EGFR Negative	PTEN-loss (or unknown)	R2	-	R2
			everolimus	No Rand ⇨ dasatinib
			dasatinib	No Rand ⇨ everolimus
EGFR Negative	PTEN-positive (no PTEN-loss)	None	-	Dasatinib
All other cases		R3	-	R3
			erlotinib	R2
			everolimus	R1
			dasatinib	No Rand ⇨ everolimus

Patients with EGFR status unknown and no PTEN-loss will be eligible for the R1 randomisation. Patients with EGFR-negative and PTEN status unknown will be eligible for the R2 randomisation.

If the biomarker study is not contributive (ie no information for randomisation is obtained) but the diagnosis of DIPG is ascertained either by a typical short clinical history and typical radiological appearance or by the histology of diffuse glioma with H3K27M trimethylation loss and eventually the detection of the H3K27M mutation by immunohistochemistry, the patient will be included in the trial and randomised in the R3 randomisation, ie for the three drugs.

5 TREATMENTS

Study treatments should start ideally within three weeks after biopsy and no later than four weeks after biopsy / surgery. The study medication will be started at Day 1 of radiotherapy.

Radiotherapy will have to start within three weeks (maximum four weeks) after the biopsy/surgery. Radiotherapy should be planned as soon as the biopsy date is known (between 10 and 21/28 days after the planned date of biopsy).

In case of metastatic disease, spinal tumors and large supratentorial thalamo-peduncular tumors that could not undergo debulking surgery, patients can be included in the study. In this situation, radiotherapy will have to start within three to four weeks after the biopsy while targerted treatment will start **at the end** of the irradiation.

Study treatment (ie Erlotinib, Everolimus and Dasatinib) will be provided by the sponsor through the laboratories.

Laboratory tests and monitoring

Renal function, hepatic function, electrolytes balance (including potassium and magnesium level), blood glucose, blood lipids (including cholesterol and triglycerides level) and of complete blood count will be monitored as described in paragraph 6.2.3 Laboratory Investigations.

If these laboratory results are abnormal (i.e hypokaliemia, hypomagnesemia..), treatment may be discontinued and appropriate treatment initiated as necessary until return to grade ≤ 1.

5.1 Identification of the investigational medicinal products

5.1.1 Erlotinib hydrochloride

The chemical name is N-(3-ethynylphenyl)-6,7bis(2-methoxy)-4-quinazolinamine, monohydrochloride. The molecular weight is 429.90.

Erlotinib is off-white to pale yellow powder formulated in tablets of 25 mg and 100 mg (6 and 9 mm diameter, respectively). Tablets are supplied in white, high density polyethylene bottles, amber glass bottles or Triplex blister packs and should be stored at temperature between 15°C and 30°C.

The suggested dose in this study is 125 mg/m²/day.

If due to the formulation of the drug, the daily dose does not correspond to the planned dose, the dose will be distributed over the week, to achieve a correct weekly dose. The weekly dose can not deviate for more than 10% of the planned dose.

Orally, once daily, the tablets should be swallowed as a whole with water once daily, on an empty stomach at least one hour before or two hours after the ingestion of food.

For patients who are unable to swallow tablets, the tablet may be dispersed in one teaspoon of apple sauce or compote or nonfat plain yogurt and should be taken immediately. Not more than one teaspoon of apple sauce and no food other than apple sauce or nonfat plain yogurt must be used.

A nasogastric tube may be also used.

5.1.2 Everolimus

Everolimus is an m-TOR inhibitor.

Everolimus is available as tablets (2.5 mg).

The prescribed dose is 5 mg/m²/day.

If due to the formulation of the drug, the daily dose does not correspond to the planned dose, the dose will be distributed over the week, to achieve a correct weekly dose. The weekly dose can not deviate for more then 10% of the planned dose.

Orally, once daily, the tablets should be swallowed with water, with or without a meal but consistently, at the same time every day. Alternatively, they can be dissolved in water.

If needed, the tablet may be dispersed in one glass of water and should be taken immediately. The glass should be rinsed with the same volume of water and the rinse should be completely swallowed to ensure that the entire dose is administered.

5.1.3 Dasatinib

Dasatinib is a potent, broad spectrum ATP-competitive inhibitor of 5 critical oncogenic tyrosine kinases/kinase families: BCR-ABL, SRC, c-KIT, PDGFR, and ephrin (EPH) receptor kinases.

Dasatinib is available as tablets (20 mg and 50 mg).

The prescribed dose is 85 mg/m² x 2 / day. The daily dose can be unequally distributed between morning and evening intake. The daily dose can not deviate for more then 10% of the planned dose.

Orally, twice daily, the tablets should be swallowed as a whole with water, with or without a meal, at the same time every day.

For patients who are unable to swallow tablets, the tablet may be dispersed in one teaspoon of apple sauce or compote or nonfat plain yogurt and should be taken immediately. Not more than one teaspoon of apple sauce and no food other than apple sauce or nonfat plain yogurt must be used.

A nasogastric tube may be also used.

5.2 Dosage and schedule of treatment

The drugs will be administered daily at the following doses:

- Erlotinib: 125 mg/m² x 1 / day
- Everolimus: 5 mg/m² x 1 / day
- Dasatinib: 85 mg/m² x 2 / day

A cycle is defined as 28 days.

If the patient vomits within 20 minutes after taking the tablets or if the tablets can actually be seen and counted, the dose is replaced. If a patient misses a dose normally taken in the morning, s/he may take the dose any time during the same day. However the missed dose should not be taken on a subsequent day. Patient will be instructed to notify study site personnel of missed doses.

If, due to the formulation of the prescribed drug, the daily dose does not correspond to the planned dose, the dose will be distributed over the week, to achieve a correct weekly dose. The weekly dose cannot deviate for more than 10% of the planned dose.

5.3 Capping doses for adolescents and young adults (depending on the body surface area)

Patients should be dosed according to their body surface area. However, for adolescents and young adults, doses should be capped as described below to avoid overdosing.

If, due to the formulation of the prescribed drug, the daily dose does not correspond to the planned dose, the dose will be distributed over the week, to achieve a correct weekly dose. The weekly dose cannot deviate for more than 10% of the planned dose.

- **Dasatinib: BIOMEDE prescribed dose is 85 mg/m²/day orally, twice daily.**
  - EMA approved dose of dasatinib for adult patients is 100-140 mg/day (chronic myelogenous leukemia)
  - The initial phase II dose of dasatinib in an adult trial of patients with recurrent glioblastoma was of 100 mg twice daily (Lassman et al, Neuro-Oncology 2015). Due to infrequent treatment-related toxicity the trial was redesigned to allow intrapatient escalation by 50 mg daily every cycle as tolerated. Escalation was tolerable in 10/17 patients.
  - **Proposal for BIOMEDE.** Maximum starting dose 100 mg twice daily during the first cycle. Then, depending on the body surface area and in case of no grade 2/3 toxicity, escalate to a maximum of 100 mg in the morning and 150 mg at night in the second cycle and to a maximum of 150 mg twice daily from the third cycle onwards.

- **Erlotinib. BIOMEDE prescribed dose is 125 mg/m²/day orally, once daily.**
  - EMA approved dose of erlotinib for adult patients is 100 mg (pancreatic cancer) or 150 mg (NSCLC), once daily.

- In most adult trials, phase II dose of erlotinib was of 150 mg once daily. In a randomized phase II trial of erlotinib vs temozolomide in patients with recurrent glioblastoma, erlotinib was started at 150 mg daily, with dose escalation to 200 mg daily if no or minimal toxicity was experienced (van den Bent et al, JCO 2009).
- **Proposal for BIOMEDE**. Maximum starting dose 150 mg once daily during the first cycle. Then, depending on the body surface area, and in case of no grade 2/3 toxicity, escalate to a maximum of 200 mg once daily from the second cycle onwards.

- **Everolimus: BIOMEDE prescribed dose is 5 mg/m²/day orally, once daily**.
- EMA approved dose of everolimus for adult patients is 10 mg (hormone receptor-positive breast cancer, neuroendocrine tumors of pancreatic/gastrointestinal/lung origin, renal cell carcinoma)
- **Proposal for BIOMEDE**. Maximum dose 10 mg once daily.

5.4 Adaptation of doses and management of side effects

5.4.1 Dose adjustment

The dose will be re-adjusted every month if necessary depending on the variation of the weight or stature in order to maintain a weekly dose at the planned dose +/-10%.

5.4.2 Dose modification

In case of grade 3/4 toxicity, the drug will be discontinued. The drug may be restarted at a lower dose only if the toxicity has resolved to a grade <2. The first dose reduction will be a one third decrease (considering the weekly dose). The second dose reduction will be half of the initial dose and no further dose reduction will be allowed. If the treatment cannot be restarted within three weeks, the drug should be permanently discontinued.

In case of persistent grade 2 causing a significant impairment of the quality of life of the patient, the dose can be reduced by one third.

In case of mucositis occurring with everolimus, plasma levels of everolimus should be measured, because mucositis may be indicative of overdosing. In case its level is above 10 ng/mL, a temporary interruption or a decrease in the dose of everolimus should be considered provided it is associated with clinical signs of toxicity. Topical treatments are recommended, but alcohol- or hydrogen peroxide-containing mouthwashes should be avoided as they may exacerbate the condition. Antifungal agents should not be used unless fungal infection has been diagnosed.

Cases of pneumocystis jirovecii pneumonia (PJP) have been reported with everolimus and of non-infectious pneumonitis (including interstitial lung disease), some with fatal outcome, have been reported in patients who received everolimus or erlinotib.

Patients should be advised to report promptly any new or worsening respiratory symptoms. In patients who develop acute onset of new and/or progressive unexplained pulmonary symptoms such as dyspnoea, cough and fever erlitonib or everolimus therapy should be interrupted pending diagnostic evaluation. A diagnosis of non-infectious pneumonitis should be considered in patients presenting with non-specific respiratory signs and symptoms such as hypoxia, pleural effusion, cough or dyspnea, and in whom infectious, neoplastic, and other non-medicinal causes have been excluded by means of appropriate investigations.

For patients who require use of corticosteroids for treatment of non-infectious pneumonitis, prophylaxis for pneumocystis jirovecii pneumonia (PJP) may be considered.

For cases of grade 3 non-infectious pneumonitis, interrupt everolimus/erlotinib until resolution to less than or equal to grade 2. Everolimus/erlitonib may be re-initiated at a lower dose one third decrease. If the treatment cannot be restarted within three weeks, the drug should be permanently discontinued.

If toxicity recurs at grade 3, consider discontinuation of Everolimus/erlotinib. For cases of grade 4 noninfectious pneumonitis, Everolimus/erlotinib therapy should be discontinued.

Gastrointestinal perforation

Patients receiving Erlotinib are at increased risk of developing gastrointestinal perforation, which was observed uncommonly (including some cases with a fatal outcome). Patients receiving concomitant anti-angiogenic agents, corticosteroids, NSAIDs, and/or taxane based chemotherapy, or who have prior history of peptic ulceration or diverticular disease are at increased risk. **Erlotinib should be permanently discontinued in patients who develop gastrointestinal perforation.**

Bullous and exfoliative skin disorders

Bullous, blistering and exfoliative skin conditions have been reported with erlotinib, including very rare cases suggestive of Stevens-Johnson syndrome/Toxic epidermal necrolysis, which in some cases were fatal. Erlotinib treatment should be interrupted or discontinued if the patient develops severe bullous, blistering or exfoliating conditions. Patients with bullous and exfoliative skin disorders should be tested for skin infection and treated according to local management guidelines. As indicated earlier in this chapter in case of grade 3/4 toxicity, the drug will be discontinued. The drug may be restarted at a lower dose only if the toxicity has resolved to a grade <2.

Ocular disorders

Erlotinib should be used with caution in patients with a history of keratitis, ulcerative keratitis or severe dry eye.

Patients receiving erlotinib and presenting with signs and symptoms suggestive of keratitis such as acute or worsening: eye inflammation, lacrimation, light sensitivity, blurred vision, eye pain and/or red eye should be referred promptly to an

ophthalmology specialist. If a diagnosis of ulcerative keratitis is confirmed, treatment with erlotinib should be interrupted or discontinued. If keratitis is diagnosed, the benefits and risks of continuing treatment should be carefully considered**.**

Fluid retention

Dasatinib is associated with fluid retention including grade 3 or 4 pleural and pericardial effusion, ascites, generalised oedema and pulmonary oedema.

Patients who develop symptoms suggestive of pleural effusion such as dyspnoea or dry cough should be evaluated by chest X-ray. Grade 3 or 4 pleural effusion may require thoracocentesis and oxygen therapy. Fluid retention events were typically managed by supportive care measures that include diuretics and short courses of steroids. In case of grade 3/4 toxicity, the drug will be discontinued. The drug may be restarted at a lower dose only if the toxicity has resolved to a grade <2.

Cardiac adverse reactions/ QT Prolongation

Dasatinib has the potential to prolong cardiac ventricular repolarisation (QT Interval). Dasatinib should be administered with caution to patients who have or may develop prolongation of QTc. These include patients taking anti-arrhythmic medicinal products or other medicinal products which lead to QT prolongation, and cumulative high dose anthracycline therapy. **Hypokalaemia or hypomagnesaemia should be corrected prior to dasatinib administration.**

In case the toxicities that have led to the dose reduction have resolved and did not happen again, an attempt to give a higher dose is allowed.

No increase above the starting dose will be allowed except for adjusting to weight and stature changes.

All dose adjustments and modifications will be recorded in the CRF.

5.5 Treatment duration – treatment discontinuation

Treatment will be continued until unacceptable toxicity, tumour progression, and/or withdrawal of patient, parents or legal representative consent.

In case of doubt with a pseudo-progression, the patient will continue his treatment until subsequent MRI performed within 2 months. Pseudo-progression will be explored by functional imaging if possible. A central radiological review will be performed when a pseudo-progression is suspected.

The maximum treatment duration allowed is 18 months.

The investigator is ultimately responsible for the safety and the well-being of the patients. If at any time during the study it is believed that the constraints of this protocol are detrimental to the patient's health, the patient may be withdrawn from the study.

In this event:

- Sponsor must be notified;

- The reason(s) for withdrawal should be documented in the case report form;
- Follow-up should be reported.

At the end of the 18 months of treatment, patients benefiting from the study drugs may be offered by the investigator to continue study treatment. The Sponsor and the Coordinating investigator will decide, on a case by case basis, to provide continued access to the study drug to a subject enrolled and still benefiting from the study drug and his/her wellbeing requires continued administration of the study drug.

Subjects will be permitted to continue treatment as long as they meet the following criteria:

- Investigator-assessed clinical benefit

and

- Subject is well tolerating the study drug.

5.6 Concomitant treatments

5.6.1 Prohibited Therapies

Except for the protocol-defined chemotherapy, radiotherapy, and study medication, no other anti-tumour treatment is allowed before the diagnosis of tumour progression/recurrence has been established excepted Bevacizumab for the management of pseudo-progression, as defined in Appendix 8.

During participation in this study, patients will not receive any other clinical investigational drugs.

5.6.2 Permitted Therapies and Procedures, including side effect treatment and prevention

Anti-Convulsants

If anticonvulsants are currently administered, the dosing regimen must be stable within 1 week prior to the first dose of study treatment; antiepileptic drugs must be non-enzyme inducing drugs.

Corticosteroids

Corticosteroid therapy is permitted for the treatment of raised intracranial pressure and for other clinical conditions as indicated. The dose and schedule of corticosteroid therapy must be recorded in the CRF.

If corticosteroids are administered, the dosing regimen must be stable ≥ 5 days prior to the first dose of study treatment.

Antiemetics

Patients will not receive prophylactic treatment for emesis except during the radiation therapy phase in case of necessity. In case of nausea-vomiting, 5-HT3 antagonists (e.g ondansetron or granisetron or according to local practice) 15-30 min before the radiation session is advocated. Dexamethasone should be avoided if possible due its known effects on cytochrome P450 functions.

Anti-diarrheal medications

Previous trials have shown that the frequency and severity of diarrhoea rarely hindered administration of erlotinib or dasatinib and could be managed with loperamide. Should diarrhoea develop, patients > 2-15 yrs should be treated with loperamide 0.03 mg/kg every 4 hours, patients > 15 yrs with 4 mg at first onset, followed by 2 mg every 2 – 4 hours or according to local practice for acute drug induced diarrhoea. Continue until the patient has a 12 hour diarrhoea free interval.

Appropriate measures should be taken to treat vomiting/diarrhea associated with dehydration and/or hypokalaemia by IV rehydration and potassium replacement. Renal function and serum electrolytes should be monitored in case of severe episodes of vomiting/diarrhea, in patient at risk of dehydration.

Treatment and prevention of mucositis

No prevention of mucositis with mouth wash will be applied albeit this complication has been encountered with both erlotinib and everolimus. With everolimus however, mucositis may be indicative of overdosing (cf. 5.4.2, page 57).

Prevention of skin toxicity

Erlotinib is known to induce skin toxicity. Its management should comply with the guidelines described by Lacouture (Lacouture 2007) or local / national specialist recommandations.

http://www.skintherapyletter.com/2007/12.6/1.html

Interaction with other medicinal products

Enzyme inhibitors and inducers

Studies are currently ongoing to investigate the potential interaction between TKI and everolimus, and a number of commonly used concomitant drugs that are inhibitors or inducers of CYP 450-3A4. Data, however, are not yet available. Potent inducers of CYP3A4 may reduce the efficacy of these drugs whereas potent inhibitors of CYP3A4 may lead to increased toxicity. Concomitant treatment with potent inducers/inhibitors of CYP3A4 should be avoided if possible. However if the use of these drugs is judged necessary, caution and careful monitoring in using such compounds with erlotinib and dasatinib is recommended and all concomitant medications taken by the patient are to be recorded in the case report forms (Appendix 5).

Please note that while caution and careful monitoring is recommended if the use of these compounds is necessary, usage does NOT exclude the patient from participating in the study.

The following agents are considered to be the most **potent inhibitors** that may increase toxicity;

- Systemic antifungals – (e.g. ketoconazole, itraconazole, miconazole etc.)
- Erythromycin, clarithromycin, roxithromycin, and troleandomycin
- SSRI (e.g. Nefazodone),
- Grapefruit juice
- Some Ca++ channel blockers (e.g. verapamil, diltiazem)

The following agents are considered to be the most common **inducers**, these are of concern as they would decrease levels of erlotinib and hence decrease efficacy but they probably do not represent a safety concern (although antiepileptic levels may need to be monitored for effect on those drugs).

- Antiepileptics (e.g. carbamazepine, phenobarbital, phenytoin etc.),
- Rifampicin,
- Troglitazone, pioglitazone
- Dexamethasone

Other known interactions

Antacids

Non-clinical data demonstrate that the solubility of dasatinib is pH-dependent. In healthy subjects, however, when antacids were administered 2 hours prior to a single dose of Dasatinib, no relevant changes in dasatinib concentration or exposure were observed. Thus, antacids may be administered up to 2 hours prior to or 2 hours following Dasatinib.

Histamine-2 antagonists and proton pump inhibitors

Long-term suppression of gastric acid secretion by H2 antagonists or proton pump inhibitors (e.g. famotidine and omeprazole) is likely to reduce dasatinib exposure. The use of antacids should be considered in place of H2 antagonists or proton pump inhibitors in patients receiving Dasatinib therapy.

Vaccination

The use of live vaccines should be avoided during treatment with Everolimus. For paediatric patients who do not require immediate treatment, completion of the recommended childhood series of live virus vaccinations is advised prior to the start of therapy according to local treatment guidelines.

Erlotinib and coumarin-derived anticoagulants

Interaction with coumarin-derived anticoagulants including warfarin leading to increased International Normalized Ratio (INR) and bleeding events, which in some cases were fatal, have been reported in patients receiving erlotinib. Patients taking coumarin-derived anticoagulants should be monitored regularly for any changes in prothrombin time or INR.

5.7 Radiotherapy

All patients will be treated with 30 conventional single daily fraction of 1.8 Gy to a total of 54 Gy over a planned period of 6 weeks. The clinical target volume will include all the areas of abnormality on T2/FLAIR sequences with a 1.5-cm margin. Radiation will be typically delivered by megavoltage photon beams. Guidelines are detailed below.

No other fractionation will be allowed.

5.7.1 Patient Positioning and Immobilisation

Patients should be in a supine position to allow for improved immobilisation and adequate airway access for general anaesthesia (if required) during planning and treatment. An individualised and appropriate immobilisation device such as a head shell, according to departmental policies, must be used.

5.7.2 Radiotherapy Equipment

Megavoltage photons with a nominal energy ≥ 4 MV must be used. Treatment with 60Co is not permitted. IMRT techniques are allowed. Protontherapy is not advised.

5.7.3 Target Volume Definition

Gross tumour volume (GTV), clinical target volume (CTV), and planning target volume (PTV) will be determined according to ICRU 50/62 criteria. Computed tomography (CT) planned delineation of all target volumes is mandatory and based on a planning CT and/or CT-MRI fusion. In case of absence of CT-MRI fusion, CT should be performed with IV contrast. The target volumes and organs at risk will be outlined on each slice of the planning scan.

5.7.4 Radiological Parameters for Target Volume Definition

<u>Definition for GTV, CTV and PTV</u>

GTV will be defined by the T2/FLAIR hyper-intensity at the time of RT planning. Thus, the GTV will contain any residual enhancing tumour. The GTV will have to take into account any anatomical shift or changes after surgery. Planning CT and CT/MR merge is recommended.

The CTV will include an additional margin of 1.5-cm in directions of potential tumour spread, (with a limit of 1 cm in cerebellum, cerebral peduncles and thalamus, and medulla oblongata if macroscopically not involved and anatomically confined). The PTV is a geometric expansion of CTV. The PTV margin will be 0.3-0.5 cm in all directions according to department policies.

Organs at risk

All possible organs at risk must be identified and contoured, in particular:

- Pituitary glands
- Cochlea, left and right
- Lens, left and right
- Optic chiasm
- Optic nerve, left and right
- Spinal cord

5.7.5 Treatment Technique

Patients will be treated using conformal radiation therapy treatment planning and delivery techniques. Generally a conformal 3−4 field arrangement should be used with a multileaf collimator (MLC), mini−multi-leaf collimator (mMLC), or individual customized divergent lead shielding blocks. In case of tumours involving both hemispheres -for example, bithalamic tumours- a parallel opposed conformal pair is likely to be the best technical solution. Exceptionally, for patients requiring urgent treatment for progressive symptoms, treatment may need to start with a straightforward technique and must not be delayed by the use of complex planning or production of shielding blocks. This technique should be modified as soon as possible thereafter, usually within the first week of treatment. IMRT techniques will be allowed under the assumption that appropriate departmental QA procedures are available and approved by the study coordinator. All patients must be treated on isocentric machines with a minimum source-to-axis distance (SAD) of 80 cm. For a treatment to be considered as conformal the following criteria must be met:

- Three dimensional imaging data (CT ± MRI) are acquired with the patient in the treatment position with a slice thickness no more than 5 mm (ideally, 2.5 mm or less).
- Three dimensional treatment planning software must be used to determine beam arrangements and dosimetry.
- Image data are used to delineate and reconstruct a GTV, CTV, PTV, and organs at risk (OR) in 3 dimensions.
- Radiation beams can be freely orientated in three dimensions usually not entering or exiting through the eyes and structures traversed by the beams can be visualised with the beam's-eye view facility.
- The distribution of dose relative to the target volume or any structure is computable on a point-by-point basis in 3-dimensional space.

5.7.6 Dose Prescription

Dose prescription is to the isocenter for 3D conformal radiotherapy and to the median PTV dose for IMRT dose prescriptions. All patients will be treated with 30 conventional single daily fraction of 1.8 Gy to a total of 54 Gy.

5.7.7 Metastatic Disease and specific tumour sites

Patients should be irradiated at 36 Gy on the cranio-spinal axis followed by a boost of 18 Gy on the primary tumor (*i.e.* a total of 54 Gy on the brainstem). In case of bulky metastases an additional boost of 9 Gy (spinal metastasis) – 18 Gy (brain supratentorial metastasis) may be considered whenever possible outside the brainstem. The drug will start after the completion of radiotherapy, ie after the first follow-up and MRI and the verification of the haematological criteria

Patients with spinal cord tumours will be irradiated at the maximum tolerated dose without any concomitant drug. The drug will start after the completion of radiotherapy, ie after the first follow-up and MRI and the verification of the haematological criteria.

Patients with large supratentorial tumours unable to undergo surgical debulking, will be treated with radiation only at the maximum tolerated dose. The drug will start after the completion of radiotherapy, ie after the first follow-up and MRI and the verification of the haematological criteria.

5.7.8 Dose Uniformity

The primary tumour should be treated through use of a suitable technique that allows for the least amount of normal brain tissue and organs to be at risk from exposure to high-dose irradiation. Dose variations across the target volume should be within + 7% and − 5% of the prescription point according to ICRU 50/62 recommendations. It is highly recommendable (usually technically achievable) to keep the dose variation within ± 5%.

In case of the use of IMRT techniques (either as step and shoot or arching techniques), the monitor units generated by the IMRT planning system must be independently checked before the start of treatment. Measurements in a QA phantom can suffice for a check as long as the plan’s fluency distributions can be recomputed for a phantom geometry.

5.7.9 Field Shaping

The use of customised divergent beam blocks or multi-leaf collimators with use of beam’s eye view facilities is mandatory.

5.7.10 Dose to organs at risk

Spinal cord : At a cumulative dose of 45 Gy, a volume reduction under the foramen magnum is recommended in order to limit the dose to the spinal cord under 50 Gy. In case of use of IMRT, sequential boost technique should be preferred to an integrated boost in aim to limit the dose to the spinal cord.

Optic chiasm and optic nerve : Dmax < 54 Gy

Cochlea : Dmean < 45 Gy and if possible < 35 Gy at least one cochlea

Lens : Dmean < 10 Gy

6 PLAN OF THE STUDY

6.1 Recruitment procedures

Patients will be identified for potential recruitment for the study, in the Department of Neurosurgery of the participating institutions based on an MRI meeting the clinical and radiologic criteria for the diagnosis (see above). In case of atypical clinical history or unusual MRI characteristics, the biopsy is advised to ascertain the diagnosis and the patient may be recruited in the trial based on the results of the biopsy. It is the responsibility of the local investigators to record in a log book all the potential patients considered for recruitment in the trial.

6.1.1 Biopsy

Informed consent for the biopsy will be obtained by the neurosurgeon or the paediatric oncologist but all patients must be registered by the onco paediatric centers.

Biopsy, within the framework of the study, will be offered to every patient able to undergo the procedure and fulfilling the clinical and radiological criteria for DIPG:

- short clinical history (i.e. less than three months between first symptoms and diagnosis);
- intrinsic brainstem lesion centred to the pons and occupying more than 50% of the volume of the pons.

Patients with a DIPG will be included in the study whatever the grade of the tumour (grade II, III or IV WHO). Pilocytic astrocytoma or gangliogliomas are not eligible for the study.

Atypical tumours (i.e. unusual clinical or radiological characteristics) may be biopsied in order to ascertain the diagnosis. They will be included in the study only in case the histology is compatible with a DIPG (including the assessment of the loss of H3K27me3 by immunohistochemistry or the presence of a mutation in the histone H3 variant genes).

The pathology will be centrally reviewed before inclusion in the randomised subtrial together with the biomarker assessment procedure allowing for the definition of the randomised subtrial.

Patients with a non informative biopsy should be randomised (without histological confirmation) **if and only if** the radiology is typical of a DIPG as well as the short clinical history.

Frontal approach should be avoided and stereotactic trans-cerebellar route is the recommended approach.

Complications of the biopsy (ie neurological worsening or new symptoms occurring during the first 48 hours after the biopsy) will be carefully monitored and reported in the CRF and documented with the surgical report and the report of the hospital stay in the neurosurgical ward. For those cases, imaging will be sent to the Sponsor for central review.

6.1.2 Neuropathology review

Once a potential patient has been identified and biopsied, his or her eligibility will be assessed according to the inclusion and exclusion criteria including pathology review.

Confirmation of the local histological DIPG diagnosis by the designated reference neuropathologist as well as biomarker assessment is required **before** randomisation.

Tumour tissue collected from the biopsy will be ideally divided as such:

- one part fixed in formalin and embedded in paraffin for histology review and IHC
- any additional material frozen

Although a local pathology report will constitute adequate documentation of histology for consideration to participate in the study, eligibility for the randomised subtrial will require confirmation of the institution's histopathologic diagnosis of DIPG by the independent designated reference neuropathologist as well as biomarker assessment.

Therefore, the submission of the formalin-fixed paraffin-embedded (FFPE) tumour tissue blocks to the designated reference pathologist to confirm the local DIPG diagnosis is mandatory before inclusion in the randomised subtrial. If only a limited biopsy was performed (or if it is not possible to send FFPE tumour tissue blocks), at least 6 unstained, uncovered slides must be sent. In any cases, stained slides must be provided for pathology review whenever possible.

Material for histopathologic review and biomarkers assessment is to be sent to:

Pr Pascale Varlet

Neuropathology Department, Hôpital Sainte-Anne,

1 rue Cabanis, 75014 Paris.

Frozen material will be sent on dry ice to the central laboratory doing the assessment of additional molecular markers of the disease, i.e.

Dr Marie-Anne Debily

UMR CNRS 8203 "Vectorologie et Nouvelles Thérapeutiques Anticancéreuses",

114, rue Édouard-Vaillant, 94805 Villejuif.

6.1.3 Assessment of biomarkers

The presence of the biomarkers will be assessed centrally in the Neuropathology Department of the Hospital Sainte-Anne, 1 rue Cabanis, Paris 75014 under the supervision of Pr Pascale Varlet within 10 working days from the receipt of the sample.

Results will be sent to the responsible oncopaediatrician. An **anonymized copy** will be sent to the data center.

Biomarkers will be assessed on formalin-fixed paraffin-embedded samples. Consecutive slides will be analysed.

The percentage of tumour cells will be defined centrally by counting the percentage of cells with loss of histone H3 trimethylation on Lysine 27 (H3K27me3).

EGFR will be assessed by immunochemistry and scored according to the Hirsch index, combining the percentage of positive cells and the intensity of the staining (on a semi-quantitative scale of 0 to +++). The cellular location of the EGFR staining will not be considered. The score covers therefore a range from 0 to 300. A positive score for IGR will correspond to an index above or equal to 100.

PTEN will be assessed by immunochemistry using the vessels as internal positive controls. Loss of staining of the tumour cells while staining is conserved in the vessels (PTEN-loss) will be considered as an indication of mTOR pathway.

PDGFRA amplification will be judged by two colour FISH with CEP7 control using the Colorado System. PDGFRA FISH-positive specimen will be considered as follows:

(A) Specimens that have $\geq$ 40% of cells displaying >4 copies of the PDGFRA signal.

(B) Specimens that display PDGFRA gene amplification, defined according to one of the following criteria:

(a) a PDGFRA to CEP7 ratio >2 over all scored nuclei and calculated using the sum of PDGFRA divided by the sum of CEP7 when mean CEP7 per cell is 2 copies;

(b) the presence of gene cluster (>4 spots) in 10% of tumour cells;

(c) at least 15 copies of the PDGFRA signals in 10% of tumour cells. PDGFRA FISH-negative specimens that do not display gene amplification according to the criteria defined above and with <40% of cells displaying 4 copies of the PDGFRA signal.

6.1.4 Patient registration and randomisation of treatment

A Registration Form documenting the patient's fulfilment of the eligibility criteria for the study is to be completed by the investigator and faxed to the data manager in the biostatistics unit of Gustave Roussy at number:

Data manager of the study: Moussa Angokaï
Fax: +33 (0) 1 42 11 67 46, from Monday to Friday: 9 am – 5 pm

The registration will be performed before biopsy if the biopsy is performed for the purpose of the trial. The registration can be performed after the biopsy if the biopsy has already been performed for the purpose of diagnosis. The registration in the study must be performed before material is sent for central review and biomarkers assessment.

For all eligible patients, a confirmation of inclusion will be automatically sent to the treating centre including the patient study number. The patient study number should be used on all future correspondence related to this patient.

A second form "Randomisation" will have to be sent for the inclusion of patient in one of the subtrials, using the same procedures. The confirmation of randomisation will

be automatically sent to the treating centre, including the treatment allocated by randomisation if eligible for one randomised trial.

Both “Registration in the study” and “Randomisation” forms can be sent together if the patient has already been biopsied and results of biomarkers are already available.

6.2 Baseline assessments

6.2.1 Medical history

The medical history should include:

- Demographic data (screening only)
- Medical conditions (diseases) and symptoms, NOT related to the DIPG (in particular, those that have been diagnosed during the 3 months before screening); whether resolved or unresolved.
- Any new diseases/symptoms not related to the DIPG that develop after the start of treatment must be reported as an AE.
- Symptoms related to the DIPG with special emphasis on the first symptoms related to the disease.
- Menarche status and the menstrual history (screening only)
- Surgical history and extent of surgery (biopsy/total or near total resection/others)
- Corticosteroid medication (type and dose)

6.2.2 Clinical Evaluation

The clinical examination should include

- Performance status (PS) will be assessed using Karnofsky performance status scale for patients >12 years of age or Lansky play score for patients ≤ 12 years for age. It is preferable that, throughout the study, the same person assesses the patient’s PS. The PS should not take the neurologic deficit per se into account.
- Vital signs, including blood pressure, pulse rate, temperature
- General examination including:
    - Body weight and height (standing length), as well as body surface area (BSA) computed using Mosteller formula (or according to national guidance),
      $BSA\ (m^2) = \sqrt{[Height(cm) \times Weight(kg) / 3600]}$
- Systematic examination including
    - Documented signs related to the DIPG and
    - Signs not related to the DIPG

- Chest X-Ray (screening visit)

- ECG **at screening only** (cardiac echography is not requested except in case of previous cardiac disease, which may lead to a contra-indication to dasatinib.)

6.2.3 Laboratory Investigations (once a week during the first 2 cycles, and then before each cycle (+/-3 days))

- Haematology
  - Complete Blood Count (CBC)
  - White blood cell count
  - Absolute neutrophil count (ANC)
  - Haemoglobin
  - Platelet count
- Biochemistry
  - Serum creatinine/urea
  - AST (SGOT) / ALT (SGPT); total and conjugate bilirubin
  - Gamma-GT
  - Alkaline Phosphatase
  - Potassium
  - Magnesium
  - Blood Glucose (**for patients receiving EVEROLIMUS only)**
  - Blood lipids (cholesterol & triglyceride) (**for patients receiving EVEROLIMUS only)**
- Pregnancy test (**for sexually active menarchal patients only at screening and before C1D1**)
  - Serum β-HCG result or urinary test
- Coagulation test (**at screening only**)
- Hepatitis B virus testing before initiating treatment (**at screening only**)

6.3 Clinical follow-up

Clinical investigations, including a monitoring of corticosteroid type and dose will be performed:

- weekly during the first two cycles (radiotherapy treatment), for metastatic patients, weekly during the first three cycles,
- at the start of each 28-day cycle for the subsequent cycles,
- at the first post-discontinuation visit, 28 days (+/- 5 days) after the end of treatment,

- then every 3-4 months.

Haematology and biochemistry investigations will be performed as detailed in the previous section, following the same schedule as clinical investigations up to the end of treatment (once a week during the first 2 cycles or 3 cycles for metastatic disease, then before each cycle).

Special warning for patients treated with Dasatinib (BCR-ABL Tyrosine Kinase Inhibitors) and Everolimus

Cases of reactivation of Hepatitis B virus (HBV) have occurred in patients who are chronic carriers of HBV after they received BCR-ABL tyrosine kinase inhibitors (TKIs). Reactivation of Hepatitis B virus have also been described in patients taking everolimus. Some cases of HBV reactivation resulted in acute hepatic failure or fulminant hepatitis leading to liver transplantation or a fatal outcome. Recommendations:

- Patients must be tested for HBV infection before initiating treatment with Dasatinib (BCR-ABL TKI) or Everolimus
- Consult experts in liver disease before treatment in patients with positive HBV serology (including those with active disease) is initiated and for patients who test positive for HBV infection during treatment
- Closely monitor patients who are carriers of HBV requiring treatment with Dasatinib / Everolimus for signs and symptoms of active HBV infection throughout the treatment and for several months following the end of treatment.

6.4 Tumour imaging

6.4.1 Minimal Brain MRI

A whole brain minimal MRI scan must be performed at diagnosis and should comprise at least two orthogonal planes (axial plane and sagittal plane) of T1 weighted images, axial T2 weighted images, coronal T2-FLAIR weighted images, and axial and sagittal T1 weighted images after the intravenous administration of paramagnetic contrast agent (gadolinium chelates) at a standard dose of 0.1mMol/kg. For 3.0T scanners, to minimize SAR exposure, the sagittal T1 weighted images after a 3D T1 weighted gradient echo volume can replace paramagnetic contrast injection.

Post-biopsy imaging must be performed routinely within 48 hours to ensure the biopsy was taken within the tumor. An MRI with at least an axial T2 weighted series is recommended.

An early post-RT MRI is to be performed the week after the end of radiotherapy (~week 7).

Both the diagnostic MRI and the early post-RT MRI should include susceptibility perfusion images (see below) besides morphological images (inclusion of DSC perfusion imaging to the standard minimal MRI protocol).

Thereafter the MRI follow up should be performed every 2 cycles during treatment, and then every 3 to 4 months, unless otherwise indicated to document tumour progression, when clinically suspected.

6.4.2 Functional Imaging

Functional imaging includes dynamic susceptibility contrast (DSC) perfusion, diffusion weighted imaging (DWI), diffusion tensor weighted imaging (DTI), arterial spin labeling (ASL), and spectroscopy.

Both the diagnostic MRI and the early post-RT MRI (performed in the week following the end of radiotherapy) require the inclusion of DSC perfusion imaging to the standard minimal MRI protocol.

However, multimodal functional imaging for each follow-up MRI is encouraged in order to document pseudo-progression or progression during follow-up.

Please refer to Appendix 3 for scanning parameter guidelines.

6.5 Translational research

6.5.1 Overview of Translational Research Science

Blood, tumour tissue and derivatives thereof (e.g., DNA, RNA proteins/ peptides) will be collected from all patients participating in the trial and stored in an academic laboratory.

The Translational Research Science repository specimen will be used to better understand the pathogenesis, course, and outcome of DIPG.

Plasma biomarker assays may include determination of markers of angiogenesis, inflammation, and macrophage recruitment, as well as polymorphisms in constitutional DNA. Analysis of tumour tissue will include whole genome and transcriptome sequencing, DNA methylation and miRNA profiling, proteomics, in situ hybridisation and IHC.

6.5.2 Specimen Types

Whole blood sample for genetic analysis

A sampling for exome sequencing and genotyping assessment will be performed at study entry (5 ml of blood in PAXgene® DNA tube, stored at room temperature).

Data generated from the genetic analyses may undergo initial exploratory analyses in order to generate hypotheses identifying genes associated with treatment response, adverse events, or disease risk. Genetic hypotheses may be confirmed in future clinical studies within this therapeutic area.

DNA will be prepared from whole blood samples in order to determine the somatic nature of the genetic alterations observed in the tumour.

Tumour Tissue

The use of tumour samples for translational academic research builds upon recent work that has begun to unravel the key biological differences between tumours in children and adults. The analyses that will be performed may provide extremely valuable additional information regarding the underlying biology of the disease, and provide further insight to treatment response.

DNA will be prepared from tumour tissue in order to determine the genetic alterations observed.

Formalin-fixed paraffin-embedded material will be utilized, after histology review, for IHC and FISH. After analysis, any remaining sample and/or derivatives thereof will be included for long-term storage and potential additional exploratory assessments. In the event that the entire tumour block cannot be obtained, a partial block should be obtained. Tumour blocks collected will be sent back to the study site after analysis. These blocks will be used for molecular profiling, either WES or targeted next-generation sequencing in case there is no frozen material available.

Fresh tumour biopsy and/or resection will also be collected from patients enrolled in the study according to best local practice, above and beyond the material required for routine diagnosis.

6.6 End of study

The end of the study is defined as the Last Patient Last Visit. LPLV is defined as the date of the last protocol-specified visit/assessment for the last subject in the study (i.e. 6 months after the end of treatment of the last patient included if 18 months treatment OR 1 month after the end of treatment if more than 18 months of treatment whichever occur the latest).

A Clinical Study Report will be issued 6 months after the LPLV occurred.

After the end of the study, a long term Follow-Up study is planned in order to gather data regarding Overall Survival. Information on patient outcomes, information on post-study anticancer therapies will be recorded in the CRF during the follow-up period as reasonably possible.

The investigator (or designee) will contact the patient in order to record data regarding progression. All efforts must be undertaken by the study sites to determine if there is progression but no additional protocol visit can be required.

Results of this long-term follow-up will be reported separately and will not be part of the Clinical Study Report.

7 EVALUATION CRITERIA

7.1 Primary endpoint

The overall survival is the primary efficacy endpoint for the different randomised Phase II sub-trials.

For the pairwise comparisons (internal comparisons of randomised groups), the overall survival is the time between date of randomisation and death whatever the cause. For the comparison to historical controls, the overall survival is computed from the date of biopsy (or initial surgery). The main analysis will be based on the whole survival curve.

Patients still on treatment at the end of the 18 months treatment period will be considered as success for the evaluation of the endpoints.

All patients with DIPG will be considered for the main efficacy analyses (Intention-to-treat).

7.2 Secondary endpoints

7.2.1 Efficacy

2-year overall survival

The 2-year OS will be estimated from the Overall Survival curves (Kaplan Meier estimate).

Progression-free survival

Progression-free survival will be determined from the date of biopsy / initial surgery (or date of randomisation for the comparison of randomised groups) to the date of unequivocal clinical or radiological progression, or death whatever the cause. Progression will be defined according to the RANO criteria (Appendix 4).

Clinical progression will be defined by the occurrence of new symptoms or the worsening of existing symptoms, with stable or increasing steroids. This clinical deterioration must be attributable to the tumour and not to any other causes (e.g., seizures, medication adverse effects, complications of therapy, cerebrovascular events, infection, and steroids decrease...).

Radiological progression will be defined, in patients on stable or increasing doses of steroids, based on the following findings:

- on T1-weighted post-gadolinium: 50% increase in the sum of the products of perpendicular diameters of the contrast enhancing lesions compared with the smallest measurement obtained either at baseline (if no decrease was observed afterwards) or best response during the treatment, and
- on T2/FLAIR: significant increase in the non-enhancing lesion compared with the baseline scan or best response after the initiation of therapy; the increase in the T2/FLAIR signal must have occurred and not be the result of co-morbid

events (e.g., radiation therapy, demyelination, ischemic injury, seizures, post-operative changes, or other treatment effect).

In case there is only a progression of the contrast enhancing part or the appearance of a contrast enhancing lesion without significant change of the size T2-FLAIR signal alterations, pseudo-progression should be considered, especially if this phenomenon is observed during the first six months after radiation therapy.

Any new tumour lesion outside the radiation field will be considered as a progression.

In any case of radiological progression of the contrast enhancing lesion(s) occurring in the first 6 months post radiotherapy, **pseudo-progression (Appendix 8)** will be explored by functional imaging if possible. In case of doubt, the patient will continue his treatment until subsequent MRI performed within 2 months. If progression is then confirmed, the timing of progression will be back dated. A central radiological review will be performed when a pseudo-progression is suspected.

7.2.2 Safety and feasibility

Toxicity

The safety assessment will be conducted using the NCI-CTC V4 on the whole duration of treatment in all treatment arms, using a list of selected toxicity items for the most frequent expected adverse events and text fields for unexpected events and other less frequent toxicities.

For each experimental treatment given in combination with radiotherapy, adverse events will be carefully monitored, based on the estimated proportion of patients experiencing Grade 3-4 CTCAE adverse event reported as a Serious Adverse Event both in the first 8 weeks of treatment and on the whole treatment duration, excluding symptoms that are related to tumour progression or pseudo-progression (neurological deterioration, increased intracranial pressure).

In addition to being reported in the study data base, each of these adverse events must be communicated directly to the trial manager team and the sponsor coordinator.

Safety will be evaluated on the treated population (at least one dose of allocated treatment) considering the whole study population including the DIPG and the non-brainstem DMG, H3K27M mutant.

Feasibility

The feasibility of the treatment will be evaluated by

- the duration of treatment and description of the reasons for treatment discontinuation,
- the number of treatment temporary stops and dose-reductions and the description of the reasons for these treatment modifications, and
- the estimate of mean dose per week over the whole treatment duration.

7.2.3 Translational study

Response to therapy (PFS and OS) will be correlated to the biomarkers identified before the start and during the course of the study (including whole exome sequencing and RNA sequencing).

The non-exhaustive list of biomarkers will comprise:

- oligodendroglial differentiation with Olig2 immunopositivity (Puget 2012, Andreiuolo submitted),
- vimentine immunopositivity (Puget 2012),
- EGFR mutation (vIII variant and also point mutations in the external domain) detected with immunohistochemistry or sequencing whenever suitable material is available (Li 2012),
- mutations in H3F3A or HIST1H3B (Wu 2012),
- TP53 immunopositivity (Puget 2012, Zarghooni 2010, Louis 1997).
- the pathway activation downstream EGFR and PDGFRA will be assessed by immunohistochemistry against pAKT and pMAPK (Scartozzi 2007, Andreiuolo submitted).
- the activation of the mTOR pathway will be assessed by immunohistochemistry against pS6 and pAKT.

Other biomarkers that could become new therapeutic targets will be assessed by means of direct sequencing of tumour DNA from frozen samples. The number of genes to be sequenced will depend on the amount of DNA available and on the ongoing discoveries of these target genes: PI3KCA (Grill 2012), PI3KR1 (Taylor in revision in Nature Genetics), PDGFRA (Puget 2012, Paugh 2013), H3F3A or HIST1H3B (Wu 2012) and EGFRvIII (Li 2012).

7.2.4 Functional imaging study

The value of multimodal imaging to predict response to therapy will be evaluated.

- T1-weighted post-gadolinium imaging will be correlated with outcome since the presence of enhancement could be correlated with survival in one recent study (Hipp 2011).
- MRS: monovoxel or multivoxel proton spectroscopy will be used to assess metabolic profile especially citrate as well as Cho:Cr and Cho:NAA ratios, the latter being correlated to prognosis (Hipp 2011, Steffen-Smith 2011 & 2012).
- DCE-MRI: dynamic contrast-enhanced MRI will be used to assess perfusion since increased perfusion could predict survival (Hipp 2011); the sequences will be analysed centrally with the OLEA software.
- ASL: arterial spin-labelling perfusion MRI will be assessed as a possible surrogate for DCE-MRI. Guidelines from the "ASL Network" (http://www.asl-network.org) will be implemented.
- DWI: diffusion-weighted imaging will be used to assess the apparent diffusion coefficient as a surrogate of tumour cellularity (Gauvain 2001).

8 DETERMINATION OF SAMPLE SIZE AND STATISTICAL ANALYSIS

8.1 Statistical analysis

8.1.1 General considerations

As DIPG is a very rare disease and there is an important need of new drug evaluation, we consider that little unbiased evidence is better than none. This led us to propose randomised trials with relaxed decision rules. We acknowledge the fact this trial is an exploratory trial and not a confirmatory trial.

The main analysis will be based on direct pairwise comparisons considered separately (erlotinib versus dasatinib from R1 and R3 randomisations; everolimus versus dasatinib from R2 and R3 randomisations; and erlotinib versus everolimus from R3 randomisation). Two-sided tests will be used with alpha=0.20 for these comparisons between experimental arms. The three pairwise comparisons will be considered without correction for multiple testing, as recommended by Freidlin et al. for multi-arm trials (Freidlin, 2008).

8.1.2 Randomisation Method

We use a centralised randomisation software (TENALEA). Each randomised trial will be balanced by biomarker status (and by country if several countries participate in the trial). A dynamic allocation method will be used (minimisation with a random parameter set at 80%).

8.1.3 Expected distribution of biomarker profiles and consequently expected distribution in the different subtrials, pairwise comparisons and treatment groups

In the current protocol, the allocation rule will be mainly based on EGFR overexpression and PTEN loss of expression detected by immunohistochemistry. The expected distribution of biomarker profiles and consequently the expected distribution of patients in the different subtrials and in the different pairwise randomised comparison and treatment groups is as follows:

Biomarker Profile	Expected %	Subtrials and Allocated treatment
EGFR+ (or unknown) but no PTEN-loss	4.25%	R1-trial: erlotinib or dasatinib
PTEN-loss (or unknown) but no EGFR-overexpression	38.25%	R2-trial: everolimus or dasatinib
EGFR+ and PTEN-loss	38.25%	R3-trial: erlotinib, everolimus or dasatinib
None	4.25%	Cohort dasatinib (not randomised)
Inconclusive	15%	R3-trial: erlotinib, everolimus or dasatinib

Additionally, few patients may not be eligible for any of the randomized subtrials due to contra-indication to a specific drug. They will receive erlotinib, everolimus or dasatinib as appropriate, based on their biomarker profile and their medical condition. They will be included in the study and monitored similarly to other patients, even though they will not contribute to the comparative estimate of drugs efficacy.

This expected distribution of biomarker profiles leads to the following distribution of patients per pairwise randomised comparison (cf. figure below):

- 40% included in the direct comparison “erlotinib versus dasatinib” (Er-D comparison: 100% of R1 + 2/3 of R3 randomisations), including 30% of patients with a known EGFR positive tumour and 10% of patients with a non-informative tumour.

- 74% included in the direct comparison “everolimus versus dasatinib” (Ev-D comparison: R2 + R3 randomisations) including 64% of patients with a known PTEN-loss in the tumour and 10% of with a non-informative tumour.

- 36% included in the direct comparison “erlotinib versus everolimus” (Er-Ev comparison: R3 randomisation) including 26% of patients with a known EGFR positive + PTEN-loss tumour and 10% of with a non-informative tumour.

	Subtrial				Total in the pairwise comparisons	
Pairwise comparisons	R1 (4,25%)	R2 (38,25%)	R3 (53,25%)	Not randomised (4,25%)	Percentage	Expected sample size if total N=250
Evero vs Erlo (2/3 of R3)			35,50%		36%	~90
Erlo vs Dasa (100% of R1 + 2/3 of R3)	4,25%		35,50%		40%	~100
Evero vs Dasa (100% of R2 + 2/3 of R3)		38,25%	35,50%		74%	~185

The expected distribution of treatment groups is as follows:

- 20% will receive erlotinib (50% of R1 + 33% of R3 randomisation)
including 15% of patients with a known EGFR positive tumour (“targeted” erlotinib cohort) and 5% of patients randomly assigned to this treatment with a non-informative tumour.

- 37% will receive everolimus (50% of R2 + 33% of R3 randomisation)
including 32% of patients with a known PTEN-loss in the tumour (“targeted” everolimus cohort) and 5% of patients randomly assigned to this treatment with a non-informative tumour.

- 43% will receive dasatinib (50% of R1, 50% of R2, 33% of R3 randomisations, and 100% of patients not randomised), including 8% PDGFRA+ patients. We cannot a priori define a "targeted" dasatinib cohort since we do not know which biomarker(s) defines best the tumours targeted by dasatinib.

8.1.4 Main endpoint estimate

The overall survival has been chosen as the primary endpoint. The data accumulated in previous studies allow concluding a certain stability of the results: median survival not exceeding 1 year so far, and less than 5% of survivors at 2 years from initial diagnosis.

The OS and PFS curves will be estimated using Kaplan-Meier method. The two-year OS difference between randomised groups and its confidence interval will also be computed using Kaplan Meier estimates and Greenwood variance if some data are censored before two years. Else, if all patients have two years of follow-up (final analysis with no patient lost-to-follow-up), it will be computed as the difference of two proportions.

8.1.5 Analysis of treatment effect on the efficacy outcome

Treatment effects on the overall survival curves between randomised groups of patients with DIPG (Hazard Ratio, HR, for death for the OS curves; HR for progression for the PFS) will be tested with two-sided logrank tests and estimated in Cox models, stratified by EGFR and PTEN biomarkers status. As an example, for the erlotinib versus dasatinib comparison, three strata will be considered: (i) patients with EGFR over-expression but no PTEN-loss (R1 randomisation), (ii) patients with EGFR over-expression and PTEN-loss (R3 randomisation), (iii) patients included in the R3 randomisation because of inconclusive results.

The proportional hazards assumption underlying the HR estimate in Cox models will be evaluated, using graphic methods and models including interaction with time. Appropriate methods for treatment effect estimates will be used if the proportional hazard assumption appears violated or questionable (use of restricted mean survival as published by Royston and Parmar).

Each treatment group of patients with DIPG will also be considered as a single-arm phase 2 trial. For each treatment group separately, we will consider that a probability of survival equal to or lower than 5% at 2 years after the date of biopsy (or initial surgery) is insufficient (H0: p0 = 5%).The observed survival probability at 2 years will be tested against this null hypothesis. For each treatment group, the whole OS curve from initial biopsy (or initial surgery) will be also compared to theoretical curves based on historical data, as there is no concurrent control group in the randomised trials. For this purpose, we will use the one-sample logrank test (Finkelstein, 2003). The main issue is to have a similarly selected historical population. The main comparison will use series including only biopsy-confirmed DIPG (Necker's cohort, personal data and subgroup of the European Society for Pediatric Oncology DIPG registry, Hoffman JCO 2018). One-sided tests will be used with alpha=0.05 for the comparisons to the historical control.

For both types of analyses of the efficacy results (direct pairwise comparisons and comparison to the historical control), a sensitivity analysis will be performed after exclusion of patients recruited with non informative biomarkers results and patients with a metastatic disease at study entry.

For DIPG patients, the whole strategy will be evaluated by pooling the different treatment groups and analysing them together (estimation of OS and PFS curves of the whole population and comparison to historical controls).

8.1.6 Analysis of safety and feasibility outcome

The analysis of safety and feasibility will be primarily descriptive (toxicity profile, incidence of the different types of toxicity...). Adverse events will be reported considering (i) the first 8 weeks of treatment when treatment is given in combination with radiotherapy, (ii) the whole treatment duration. Adverse events will be reported per 28-day cycle and per patient. Adverse events will be carefully monitored, based on the estimated proportion of patients experiencing an adverse event as defined in the endpoint subsection (7.2.2), as well as the feasibility. A safety report will be performed at least every 6 months with an analysis per treatment group and overall. The main analysis of safety data will include all patients who have started the study treatment, regardless of tumour site (DIPG and NB-DMG). However safety data of DIPG and NB-DMG may be presented separately for publication purposes.

8.1.7 Other analyses

The prognostic and/or the predictive value of biomarkers on the response to treatment (OS, PFS) will be investigated using Cox models, with interaction terms. Forest plots will illustrate the heterogeneity of treatment effect by biomarker status.

The association between pseudo-progression detected on the morphological MRI and the outcome will be evaluated using time-dependent Cox models. The association between change in functional MRI and the outcome will be investigated in Cox models.

The efficacy outcomes (PFS, OS) of the cohort of non-brainstem NB-DMG, H3K27M mutant will be analysed separately as exploratory analyses. This analysis will be mainly descriptive as the numbers will not allow a formal comparison of the three treatments. PFS and OS will be estimated on the whole group of NB-DMG and by treatment groups. Relative treatment effect of the experimental drugs will also be estimated (hazard ratios) in NB-DMG. Difference between DIPG and NB-DMG will be described in terms of PFS and OS curves. Heterogeneity of treatment effect according to tumour site will be assessed using interaction tests and illustrated by forest plots, although we acknowledge that the limited sample precludes any conclusion. A pooled analysis of efficacy outcomes (PFS, OS) of DIPG and NB-DMG will be performed if there is no evidence of difference between both strata.

8.2 Determination of sample size

Considering the initial assumptiom regarding the distribution of biomarkers within the DIPG cohort, we could expect that 36% of the trial population will contribute to the comparison of everolimus versus erlotinib, 40% to the comparison of erlotinib versus dasatinib, and 74% to the comparison of everolimus versus dasatinib.

The expected number of patients participating in the pairwise comparisons is derived from the expected distribution in the different subtrials (cf. subsection 8.1.3).

As the distribution across the different pairwise comparisons is not equal, the initial sample size of the study for DIPG was computed in order to ensure a minimum sample size of 90 patients, for the pariwise comparison with the smallest sample size, i.e. everolimus versus erlotinib.

In the pairwise comparison of everolimus to erlotinib, a total of 90 patients is required to have an 80%-power in the comparison of the whole OS curves if the Hazard Ratio of death is 0.62, equivalent to a 10.6% increase in the two-year OS (5% versus 15.6%) assuming proportional hazards (logrank test, 79 events, East software with an exponential survival distribution and an expected accrual of 22.5 patients per year. This sample size is also associated with an 80%-power to conclude a significant benefit if one experimental arm leads to a 17% increase in the two-year OS compared to the other one (5% versus 22%) with a two-sided alpha=0.20 (Casagrande & Pike).

Considering the expected distribution across the different trials, this number of patients with DIPG should be achieved by recruiting 250 patients in the randomised trial to get 250 randomised trial patients. This target number of 250 randomised trial patients with DIPG is achievable over a 5 year accrual period with approximately 63 patients per year. With 250 randomised trial patients, we also expect approximately 100 patients for the erlotinib-dasatinib comparison and 185 patients for the everolimus-dasatinib comparison. Considering the current screen failure rate (approximately 88% of the patients registered in the study are finally allocated to one treatment arm by randomisation), a total of 285 patients with DIPG may be required to achieve the total of 250 randomised patients with DIPG.

For the erlotinib-dasatinib comparison, a total of 100 patients is associated with a 80% power to conclude a significant benefit if the Hazard Ratio of death is 0.64, equivalent to a 9.7% increase in the two-year OS (5% versus 14.7%) assuming proportional hazards (logrank test, 91 events, East software with an exponential survival distribution and an expected accrual of 25 patients per year). This sample size is also associated with an 80%-power to conclude a significant benefit if one arm leads to a 16% increase in the two-year OS (5% versus 21%) with a two-sided alpha=0.20.

For the everolimus-dasatinib comparison, a total of 185 patients is associated with a 80% power to conclude a significant benefit if the Hazard Ratio of death is 0.72, equivalent to a 6.6% increase in the two-year OS (5% versus 11.6%) assuming proportional hazards (logrank test, 167 events, East software with an exponential survival distribution and an expected accrual of 45 patients per year). This sample size is also associated with an 80%-power to conclude a significant benefit if one arm leads to a 10.5% increase in the two-year OS (5% versus 15.5%) with a two-sided alpha=0.20.

We acknowledge the fact that the targeted treatment effect is relatively optimistic for the first two randomised comparisons, meaning that these trials are underpowered for a smaller treatment effect that would be more reasonable and still clinically meaningful. Accrual in the randomised trial will stop when the target of 250 patients with a DIPG is achieved, even if less than 90 patients have been accrued in ths smallest comparison. Hence, due to the observed biomarker distribution in the first

patients, the proportion of patients enrolled in the erlotinib arm is smallest than anticipated. However recruiting patients in the current trial beyond the target of 250 seems not acceptable. The main analysis of the trial will be performed 6 months after recruitment of the last patient, or earlier if the target number of 167 events is already achieved in the everolimus-dasatinib comparison. Updated analyses will be performed 2 years after the end of accrual, and later on (for long-term follow-up).

Considering the comparison of each treatment group to the historical control (secondary analysis testing against the null hypothesis, 2-year OS p0=5%, with a one-sided alpha=0.05), if we assume that 20% of patients receive erlotinib (approximately 50 patients), 37% everolimus (~92 patients) and 43% dasatinib (~108 patients), the trial will have an 80%-power to detect a 9.5% increase in the two-year OS for erlotinib (5% versus 14.5%), a 6.6% increase for everolimus (5% versus 11.6%) and a 6% increase for dasatinib (5% versus 11%).

The addition of NB-DMG, H3K27M mutant will change neither the sample size calculation nor the power or the type-1 error rate for the main analysis of DIPG since NB-DMG will be analysed apart in a prospective observational cohort with a descriptive cohort.

Considering the estimated remaining accrual duration in the randomised trial for DIPG patients to get 250 patients, we may recruit up to 30 in the non brainstem DMG H3K27M mutant cohort. Thus, the study may recruit up to 315 patients (285 DIPG plus 30 non brainstem DMG) to get 250 randomised DIPG patients.

8.3 Analysed population

The main analysis of efficacy outcomes of each comparison between treatment groups in DIPG will be based on all patients with a DIPG allocated by randomisation to the compared treatments:

- R1 + part of R3 subtrial for erlotinib versus dasatinib comparison
- R2 + part of R3 subtrial for everolimus versus dasatinib comparison
- Part of the R3 subtrial for erlotinib versus everolimus comparison

All patients with DIPG will be considered for the main efficacy analyses (Intention-to-treat).

The same principle will be followed for the comparison of each treatment group of patients with DIPG to the historical control.

For both types of analyses of the efficacy results (direct pairwise comparisons and comparison to the historical control), a sensitivity analysis will be performed after exclusion of patients recruited with non informative biomarkers results and patients with a metastatic disease at study entry.

The efficacy outcomes of the cohort of non-brainstem DMG, H3K27M mutant will be analysed separately.

Safety will be evaluated on the treated population (at least one dose of allocated treatment) considering the whole study population including DIPG and non-brainstem

DMG H3K27M mutant. However safety data of DIPG and NB-DMG may be presented separately for publication purposes.

8.4 Interim analyses

Data will be closely monitored with approximately one interim analysis per year as soon as we have observed 20% and 30% of the expected events in each of the pairwise comparisions have been observed.

Let us denote X, Y and Z the three treatment arms. The treatment arm X may be dropped if the the two following conditions are verified:

(i) For each treatment group, the overall survival curve will be plotted against the historical curve; stopping accrual in a treatment group will be discussed if there is evidence that outcome is worse than the historical control, such as a median OS less than 9 months.

(ii) In the pairwise comparisons, the comparative arm works significantly better in both comparisons (Y >> X and Z >> X); an alpha spending function based on O'Brien and Fleming rule will be used to define the boundary for early rejection of the null hypothesis. With this "drop the looser" approach, we do not conclude the success of the "best" arm, but we keep recruiting patients in this treatment arm, which may be compared to another new treatment if any.

The sample size is not impacted by such interim analyses as we use O'Brien and Fleming rule, which is conservative.

9 SERIOUS ADVERSE EVENTS

9.1 Definition

9.1.1 Adverse Event (AE)

An Adverse Event (AE) is any new untoward medical occurrence or worsening of a preexisting medical condition in a patient or clinical investigation subject administered an investigational (medicinal) product. An AE can therefore be any unfavourable and unintended sign (including abnormal laboratory findings for example), symptom, or disease temporally associated with the use of a medical product, whether or not a causal relationship (i.e.related/not related) with study drugs and/or study procedures (i.e biopsy, radiation therapy) is suspected.

9.1.2 Serious Adverse Event (SAE)

A **Serious Adverse Event (SAE)** is any untoward medical occurrence that at any dose:

- is fatal (results in death)
- is life-threatening
- requires or prolongs in-patient hospitalization
- results in persistent or significant disability / incapacity
- is a congenital anomaly / birth defect
- is medically significant (defined as any clinical event or laboratory result that may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the subject or may require intervention (e.g., medical, surgical) to prevent one of the other serious outcomes listed in the definition above. Examples of such events include but are not limited to, allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasia or convulsions that do not result in inpatient hospitalization, development of drug dependency or drug abuse, transmission of an infectious agent.

Although overdose and cancer are not always serious by regulatory definitions, these events should be reported on a SAE report form and sent to the sponsor in an expedited manner.

A SAE judged as potentially related to a study drug qualifies as Serious Adverse Drug Reaction (SADR).

The following are not considered to be serious adverse events (SAE):

- A visit to the emergency room or other hospital department that does not result in admission (unless considered an “important medical event” or a life-threatening event)
- Outpatient or same-day or ambulatory procedures
- Observation or short-stay units

- Hospitalization due to diagnostic procedures or standard supportive care (e.g. implant of central venous catheter)
- A pre-planned hospitalization for a condition which existed at the start of study drug and which did not worsen during the course of study drug treatment
- Social admission (e.g., subject has no place to sleep; hospice facilities)
- Administrative admission (e.g., for yearly physical examinations)
- Protocol-specified admission during a clinical trial (e.g., for a procedure required by the study protocol or for clinical research)
- Optional admission not associated with a precipitating clinical AE (e.g., for elective cosmetic surgery)

9.1.3 Expected Serious Adverse Event

An expected SAE is an event already mentioned in the most recent version of the investigator brochure or in the summary of the product characteristics, for drugs with a market authorization.

9.1.4 Unexpected Serious Adverse Event

An unexpected SAE is an event not mentioned or different by its nature, intensity and/or, evolution with respect to the investigator brochure or to the summary of the product characteristics, for drugs with a market authorization.

9.1.5 Intensity criteria

Intensity criteria must not be confused with criteria for seriousness, which serve as guidelines for definition of reporting obligations.

Intensity of events will be estimated according to the NCI-CTCAE classification, version 4.0 (toxicity score grade 1 to 5). Intensity of adverse events not listed in this classification will be evaluated according to the following terms:

- Mild (grade 1): does not affect the patient's usual daily activity
- Moderate (grade 2): perturbs the patient's usual daily activity
- Severe (grade 3): prevents the patient carrying out his usual daily activities
- Very Severe (grade 4): necessitates intensive care or is life-threatening
- Death (grade 5)

9.2 Reporting of Serious Adverse Events (SAE)

Any SAE which occurs or comes to the attention of the investigator at any time during the study since consent is given and within 30 days after the last administration of study drugs, **independent of the circumstances or suspected cause**, must be reported immediately without undue delay by fax via a SAE report form to:

Pharmacovigilance Unit Fax : +33 (0) 1 42 11 61 50

Phone : 33 (0)1 42 11 61 00

(9 a.m. - 6 p.m. from Monday to Friday, except on bank holidays)

E-mail: phv@gustaveroussy.fr

All late Serious Adverse Events (occurring after this period of 30 days) considered to be reasonably related to the study treatment(s) or the research must be reported (no time limit).

Information collected in the SAE form is crucial to assess the case. For this reason, diligence in collecting as much verifiable and reliable information is needed: both, quality and timeliness are key factors. If known, the diagnosis of the underlying illness or disorder should be recorded, rather than its individual symptoms. The following information should be captured for all SAEs: onset, duration, intensity, and seriousness, relationship to study drugs, action taken and treatment required.

The investigator must also attach the following to the serious adverse event report form, wherever possible:

- A copy of the summary of hospitalization or prolongation of hospitalization
- A copy of the post-mortem report (if applicable)
- A copy of all relevant laboratory examinations and the dates on which these examinations were carried out, including relevant negative results, as well as normal laboratory ranges.
- All other document that he judges useful and relevant.

All these documents will remain anonymous.

Further information can be requested (by fax, telephone or when visiting) by the monitor and/or the safety manager.

Follow-up information

The investigator is responsible for the appropriate medical follow-up of patients until resolution or stabilization of the adverse event or until the patient's death. This may mean that follow-up should continue once the patient has left the trial.

Follow up information about a previously reported serious adverse event must be reported by the investigator to the Pharmacovigilance Unit immediately without undue delay (on the serious adverse event report form, by ticking the box marked Follow-up

N°…). The investigator also transmits the final report at the time of resolution or stabilization of the SAE.

He retains the documents concerning the supposed adverse event so that previously transmitted information can be completed if necessary.

9.3 Reporting of pregnancy

Although not considered an adverse experience, it is the responsibility of investigators to report immediately without undue delay any pregnancy in a patient or male patient's partner which occur during the study to the sponsor (the pregnancy report form should be used).

Pregnancy is an exclusion criterion. In the event of a pregnancy occurring during the course of the study, the subject must be withdrawn from the clinical trial immediately (if the pregnancy is not interrupted). Patients who become pregnant must be followed to the completion/termination of the pregnancy. Parental and neonatal outcomes must be recorded even if they are completely normal and without AEs.

NB: If the patient decides to terminate the pregnancy and the administration of the study medication is delayed for less than 6 consecutive weeks, study treatment may be resumed.

9.4 Responsibilities of the Sponsor

The Pharmacovigilance Unit at Gustave Roussy will assess the SAE in terms of seriousness, severity (NCI-CTCAE v4.0), relationship to the study drugs and expectedness). All SAEs will be coded using MedDRA.

9.4.1 Suspected Unexpected Serious Adverse Reactions (SUSARs)

To comply with regulatory requirements, the sponsor will report to the national ethic committee and competent authority all SAEs that are related to the investigational medicinal product and unexpected (ie, not previously described in the investigator brochure or in the Summary of Product Characteristics). In the European Union, an event meeting these criteria is termed as suspected Unexpected Serious Adverse Reaction (SUSAR).

All SUSAR reports and all reports involving expected SADR that are fatal will additionally be forwarded to all study investigators and to the Independent Data Monitoring Committee. The involved study offices are responsible for information of the Ethical Review Board(s) concerned as well as of the respective investigators. The reporting procedure has to comply with the national legislation.

9.4.2 Development safety update report

The pharmacovigilance unit at Gustave Roussy will issue once a year throughout the clinical trial, or on request, the Development safety update report (DSUR) of the study, in accordance with the International Conference on Harmonisation (ICH) guidance E2F issued in August 2010, and any applicable revisions thereof.

The sponsor should submit DSUR within 60 days of the data lock point (date of the first authorisation of the concerned clinical trial by a competent authority in a member state) to the national competent authority and the national Ethic Committee according to national legislation.

10 STUDY DISCONTINUATION

The study could be interrupted or terminated by the sponsor in agreement with the coordinator and with the competent authority for the following reason:

- Frequency and/or unexpected severity of the toxicity,
- Recruitment of patients too low,
- Poor quality of the data collected,
- Request of the Data Monitoring Committee (if applicable)

11 ETHICAL AND REGULATORY ASPECTS

11.1 Rules and regulations

- The clinical trial is conducted in conformity with:
- Ethical principles stated in the Declaration of Helsinki 1964, as revised in Fortaleza, 2013
- Regulation (EU) 2016/679 of the europan parliament and of the council of 27 April 2016 on the protection of natural persons with regard to the processing of personal data and on the free movement of such data, and repealing Directive 95/46/EC (General Data Protection Regulation)
- The European Directive (2001/20/EC and 2005/28/EC) and national implementation therefore
- Appendix 13 of the E. U. Guide to Good Manufacturing Practices (revised and adopted in July 2010 by the European Commission),
- The Good Clinical Practices guidelines (International Conference on Harmonization ICH E6 and Statistical Principles for Clinical Trials (ICH E9), and the Clinical Safety Data Management guidance (ICH E2A)
- and any local Regulations, including but not limited to:
    - French Public Healthcare Law (n° 2004-806) of August 9, 2004, a partial adaptation of the European Directive (2001/20/EC) on the conduct of clinical trials,
    - French Public Healthcare Law (n° 2016-41) of January 26, 2016, about modernisation of the health system,
    - Ordinance n°2016-800 of June 16, 2016 on medical research involving human subjects,
    - French Law n° 2002-303 of March 4, 2002 relative to patients' rights and to the quality of the healthcare system,
    - French Informatics and Liberties Law (n° 78-17) of January 6, 1978 modified by Law n° 2018-493 of June 20, 20018,
    - French decree N° 2018-687 of 1 August 2018 adopted for the application of Law No. 78-17 of 6 January 1978

11.2 Committee for the Protection of Persons (CPP) – Competent Authority

This protocol was submitted to the Ethic Committee/IRB/CPP which gave its approval on the **06/08/2014**. This protocol has also been approved by the Competent Authority on the **25/08/2014.**

Gustave Roussy has taken out a legal liability insurance policy (N°124.895).

A final report on the trial will be written at the latest 6 months after the end of the trial and sent to the competent authority and to the Ethic Committee/IRB/CPP.

Gustave Roussy will maintain records of essential trial documentation in the Sponsor file for a minimum duration of 15 years after the end of the trial.

11.3 Information and Consent of Participants

For patients with DIPG, informed consent for the biopsy will be obtained by the neurosurgeon or the paediatric oncologist but all patients must be registered by the onco paediatric centers.

Biopsy, within the framework of the study, will be offered to every patient able to undergo the procedure and fulfilling the clinical and radiological criteria for DIPG.

Diffuse midline gliomas, H3K27M mutant, will be eligible for the trial after biopsy or surgery. As biopsy and surgery is considered as standard practice for these locations, informed consent for the biopsy will not be necessary. Patient will sign the consent after the diagnosis to allow central review and biomarkers assessment thereafter.

Before agreeing to participate in this trial, all patients / legal representative will be provided with sufficient information in the patient information notice/consent form.

It is the responsibility of the investigator to obtain a signed informed consent before the biopsy to enter the study, and a second signed informed consent before the random allocation of the treatment, from each patient / legal representative prior to participating in this study after adequate explanation of the aims, methods, anticipated benefits, and potential hazards of the study.

Because this study includes minor subjects who can only be enrolled with the consent of the subject's legally acceptable representative, the subject must be informed about the study in language that is non-technical and easily understood, compatible with the subject's understanding and, if capable, personally sign and date the consent form or patient information sheet.

Having read the information notice, the patient / legal representative must date and sign the **consent forms** if he/she accepts to participate**.** This consent form must also be signed by the investigator. The original consent form must be kept in the study file by the investigator and the study participant should receive a copy.

During a subject's participation in the trial, any update to the consent form and any update to the written information will be provided to the subject.

11.4 Principal Investigator Responsibilities

The principal investigator of each establishment concerned promises to conduct the clinical trial in conformity with the protocol which has been approved by the CPP and the competent authority.

The principal investigator should not modify any aspect of the protocol without prior written permission from the Sponsor nor without the approval of the proposed modifications by the Ethic Committee/IRB/CPP and the competent authority.

The Principal Investigator is responsible for:

- providing the Sponsor with his/her CV as well as that of co-investigators,
- identifying members of his/her team participating in the trial and defining their responsibilities,
- recruiting patients after receiving the Sponsor's approval,

Each investigator is responsible for:

- personally obtaining the informed consent form which has been dated and signed by the participant in the research prior to any specific trial selection procedure,
- regularly completing the case report form (CRF) for each patient included in the trial and ensuring that the Clinical Research Assistant (CRA) mandated by the Sponsor has direct access to source documents in order to validate information on the CRF,
- dating, correcting and signing the corrections on the CRF for each patient included in the trial,
- accepting regular visits from a CRA and possibly visits from auditors mandated by the Sponsor or inspectors from the regulatory authorities.

All documentation concerning the trial (protocol, consent form, case report form, investigator file, etc…), as well as the original documents (laboratory results, imaging studies, medical consultation reports, clinical examination reports, etc.) is considered confidential and should be kept in a safe place. The Principal Investigator should keep data as well as a list of patient-identifying data for at least 15 years after the end of the study.

12 DATA COLLECTION

Data will be collected via electronic case report form (eCRF) and entered into the clinical database per standard SOPs. The eCRF will be implemented using InferMed-MACRO software allowing secures online data collection. Each user will have personal identifiers (user ID / password) and data access will be strictly limited according to profiles:

- Clinical Research Assistant (CRA) profile allows data entry on the eCRF online,
- Data Manager profile allows performing a first data monitoring (completeness, consistency checks)
- Monitor profile allows source data verification,
- The investigator profile enables to enter and review the data, and sign electronically the completed CRF.

Collected data in the eCRF will be verified through use of programmed edit checks specified by the data centre. If necessary, discrepancies in the data will be brought to the attention of the investigational site personnel and to the sponsor clinical research assistant. Resolutions to these issues will be reflected in the database. An audit trail within the system will track all changes made to the data.

13 QUALITY ASSURANCE - MONITORING

In order to guarantee the authenticity and the credibility of the data in conformity with good clinical practices, the Sponsor has installed a quality assurance system which includes:

- Trial management in accordance with the procedures at Gustave Roussy,
- Quality control of data at the investigating site by the Clinical Research Assistant (CRA),
- Possible auditing of investigating centres.

13.1 On-site monitoring (source data verification)

Quality control on the site will be ensured by the CRA.

The CRA must check that the investigator's file exists and that it is updated.

The CRA must verify the consent forms, that subjects fulfil eligibility criteria, the validity of evaluation criteria and treatment toxicity with the help of source documents (and others to be specified and adapted according to the study).

The CRA will work in collaboration with the data manager to help to resolve discrepancies.

The CRA will check drug accountability and ensure that the drug accountability forms are validated and signed by the in-house pharmacist before any request for destruction.

13.2 Central monitoring

As detailed in the section 12, collected data in the eCRF will be verified through use of programmed edit checks specified by the data centre.

13.3 Independent Data Monitoring Committee

An Independent Data Monitoring Committee (IDMC) will be responsible for the data and safety monitoring of trial. The IDMC will be constituted of two expert physicians, one pharmacovigilance representative and one statistician. They will review annual reports describing inclusion and safety data, as well as any decision to perform an additional interim analysis, and its relative results. They will make recommendations to the trial steering committee. The IDMC will work in accordance with DAMOCLES guidelines (Lancet 2005).

14 DATA OWNERSHIP / PUBLICATION POLICY

The investigator promises, on his/her behalf as well as that of all the persons involved in the conduct of the trial, to guarantee the confidentiality of all the information provided by Gustave Roussy until the publication of the results of the trial.

All publications, abstracts or presentations including the results of the trial require prior approval of the Sponsor (Gustave Roussy).

All oral presentations, manuscripts must include a rubric mentioning the Sponsor, the investigators / institutions that participated in the trial, the cooperative groups, learned societies which contributed to the conduct of the trial and the bodies which funded the research.

The Study Investigator-Coordinator will write an article reporting on the results as soon as possible after the final analysis and will be the first author of the publication.

The list of authors and their order will be determined in conformity with « Uniform requirements for manuscripts submitted to biomedical journal » (http://www.icmje.org/). Patient enrolment only will not justify authorship by itself.

Publications of additional results (biological study, imaging…) will be scheduled after publication of the main report, after approval of the Sponsor and the trial committee.

Methodological papers may be published before the main report if their content does not jeopardize the trial conduct.

15 DATA PROTECTION

15.1 Confidentiality

Investigator agrees that the collection, processing and disclosure of personal data and medical information related to the Subject, and personal data related to Investigator and any investigational staff is subject to compliance with applicable personal data protection and security laws and regulations.

Investigator agrees to adhere to the principles of medical confidentiality in relation to Clinical Trial Subjects.

Investigator shall not disclose the identity of Clinical Trial Subjects to third parties without prior written consent of the Sponsor.

15.2 Investigator's personal data

Investigator hereby expressly consents to the processing of Investigator's personal data collected by Sponsor. Such consent shall authorize the transfer of personal data to countries other than the Institution's own country, for the following purposes:

a) the conduct and interpretation of the Clinical Trial;

b) review by governmental or regulatory agencies, Sponsor, and its agents, affiliates and collaborators;

c) satisfying legal or regulatory requirements;

d) publication on national and international public websites and other websites and databases that serve a comparable purpose;

e) upon request of individual patients and doctors provision to individual patients and doctors who may be interested in participating in a clinical trial at Institution;

f) storage in Sponsor's databases for use in selecting sites in future clinical trials.

16 REFERENCES

Albright, A L, R J Packer, R Zimmerman, L B Rorke, J Boyett, et G D Hammond. 1993. « Magnetic Resonance Scans Should Replace Biopsies for the Diagnosis of Diffuse Brain Stem Gliomas: A Report from the Children's Cancer Group ». Neurosurgery 33 (6): 1026□1029; discussion 1029□1030.

Aplenc, Richard, Susan M Blaney, Lewis C Strauss, Frank M Balis, Suzanne Shusterman, Ashish Mark Ingle, Shruti Agrawal, Junfeng Sun, John J Wright, et Peter C Adamson. 2011. « Pediatric Phase I Trial and Pharmacokinetic Study of Dasatinib: A Report from the Children's Oncology Group Phase I Consortium ». Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology 29 (7): 839□844. doi:10.1200/JCO.2010.30.7231.

Barrow, Jennifer, Martyna Adamowicz-Brice, Maria Cartmill, Donald MacArthur, James Lowe, Keith Robson, Marie-Anne Brundler, David A Walker, Beth Coyle, et Richard Grundy. 2011. « Homozygous Loss of ADAM3A Revealed by Genome-Wide Analysis of Pediatric High-Grade Glioma and Diffuse Intrinsic Pontine Gliomas ». Neuro-Oncology 13 (2): 212□222. doi:10.1093/neuonc/noq158.

Bilaniuk, L T, P T Molloy, R A Zimmerman, P C Phillips, S N Vaughan, G T Liu, L N Sutton, et M Needle. 1997. « Neurofibromatosis Type 1: Brain Stem Tumours ». Neuroradiology 39 (9): 642□653.

Blauwblomme, Thomas, Pascale Varlet, John R Goodden, Marie Laure Cuny, Helene Piana, Thomas Roujeau, Federico Dirocco, et al. 2009. « Forniceal Glioma in Children. Clinical Article ». Journal of Neurosurgery. Pediatrics 4 (3): 249□253. doi:10.3171/2009.4.PEDS08472.

Bouffet, E, M Raquin, F Doz, J C Gentet, C Rodary, F Demeocq, P Chastagner, P Lutz, O Hartmann, et C Kalifa. 2000. « Radiotherapy Followed by High Dose Busulfan and Thiotepa: A Prospective Assessment of High Dose Chemotherapy in Children with Diffuse Pontine Gliomas ». Cancer 88 (3): 685□692.

Broniscer, Alberto, Justin N Baker, Michael Tagen, Arzu Onar-Thomas, Richard J Gilbertson, Andrew M Davidoff, Atmaram S Pai Panandiker, et al. 2010. « Phase I Study of Vandetanib during and after Radiotherapy in Children with Diffuse Intrinsic Pontine Glioma ». Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology 28 (31): 4762□4768. doi:10.1200/JCO.2010.30.3545.

Broniscer, Alberto, Sharyn D Baker, Cynthia Wetmore, Atmaram S Pai Panandiker, Jie Huang, Andrew M Davidoff, Arzu Onar-Thomas, et al. 2013. « Phase I Trial, Pharmacokinetics, and Pharmacodynamics of Vandetanib and Dasatinib in Children with Newly Diagnosed Diffuse Intrinsic Pontine Glioma ». Clinical Cancer Research: An Official Journal of the American Association for Cancer Research 19 (11): 3050□3058. doi:10.1158/1078-0432.CCR-13-0306.

Broniscer, Alberto, Murali Chintagumpala, Maryam Fouladi, Matthew J Krasin, Mehmet Kocak, Daniel C Bowers, Lisa C Iacono, et al. 2006. « Temozolomide after Radiotherapy for Newly Diagnosed High-Grade Glioma and Unfavorable Low-Grade Glioma in Children ». Journal of Neuro-Oncology 76 (3): 313□319. doi:10.1007/s11060-005-7409-5.

Cao, Carolyn, Jeffrey M Albert, Ling Geng, Percy S Ivy, Alan Sandler, David H Johnson, et Bo Lu. 2006. « Vascular Endothelial Growth Factor Tyrosine Kinase Inhibitor AZD2171 and Fractionated Radiotherapy in Mouse Models of Lung Cancer ». Cancer Research 66 (23): 11409□11415. doi:10.1158/0008-5472.CAN-06-2414.

Cappellano, Andrea Maria, Eric Bouffet, Sergio Cavalheiro, Maria T Seixas, et Nasjla Saba da Silva. 2011. « Diffuse Intrinsic Brainstem Tumor in an Infant: A Case of Therapeutic Efficacy with Vinorelbine ». Journal of Pediatric Hematology/oncology 33 (2): 116□118. doi:10.1097/MPH.0b013e3181fe68e0.

Chassot, Andrea, Sandra Canale, Pascale Varlet, Stephanie Puget, Thomas Roujeau, Laura Negretti, Frederic Dhermain, et al. 2012. « Radiotherapy with Concurrent and Adjuvant Temozolomide in Children with Newly Diagnosed Diffuse Intrinsic Pontine Glioma ». Journal of Neuro-Oncology 106 (2): 399□407. doi:10.1007/s11060-011-0681-7.

Chawla, Sheema, David N Korones, Michael T Milano, Ali Hussain, Abdel R Hussien, Ann G Muhs, Manisha Mangla, Howard Silberstein, Sven Ekholm, et Louis S Constine. 2012. « Spurious Progression in Pediatric Brain Tumors ». Journal of Neuro-Oncology 107 (3): 651□657. doi:10.1007/s11060-011-0794-z.

Chinnaiyan, Prakash, Minhee Won, Patrick Y Wen, Amyn M Rojiani, Merideth Wendland, Thomas A Dipetrillo, Benjamin W Corn, et Minesh P Mehta. 2013. « RTOG 0913: A Phase 1 Study of Daily Everolimus (RAD001) in Combination with Radiation Therapy and Temozolomide in Patients with Newly Diagnosed Glioblastoma ». International Journal of Radiation Oncology, Biology, Physics 86 (5): 880□884. doi:10.1016/j.ijrobp.2013.04.036.

Choi, Seung Joon, Hyung-Sik Kim, Su-Joa Ahn, Yu Mi Jeong, et Hye-Young Choi. 2013. « Evaluation of the Growth Pattern of Carcinoma of Colon and Rectum by MDCT ». Acta Radiologica (Stockholm, Sweden: 1987), février. doi:10.1177/0284185113475923.

Chung, Hye Won, Jing Wen, Jong-Baeck Lim, SeungMin Bang, Seung Woo Park, et Si Young Song. 2009. « Radiosensitization Effect of STI-571 on Pancreatic Cancer Cells in Vitro ». International Journal of Radiation Oncology, Biology, Physics 75 (3): 862□869. doi:10.1016/j.ijrobp.2009.06.021.

Dhermain, Frederic G, Peter Hau, Heinrich Lanfermann, Andreas H Jacobs, et Martin J van den Bent. 2010. « Advanced MRI and PET Imaging for Assessment of Treatment Response in Patients with Gliomas ». Lancet Neurology 9 (9): 906□920. doi:10.1016/S1474-4422(10)70181-2.

Donahue, B, J Allen, J Siffert, M Rosovsky, et R Pinto. 1998. « Patterns of Recurrence in Brain Stem Gliomas: Evidence for Craniospinal Dissemination ». International Journal of Radiation Oncology, Biology, Physics 40 (3): 677□680.

Finkelstein, Dianne M, Alona Muzikansky, et David A Schoenfeld. 2003. « Comparing Survival of a Sample to that of a Standard Population ». Journal of the National Cancer Institute 95 (19): 1434□1439.

Fontanilla, Hiral P, Chelsea C Pinnix, Leena M Ketonen, Shiao Y Woo, Tribhawan S Vats, Michael E Rytting, Johannes E Wolff, et Anita Mahajan. 2012. « Palliative Reirradiation for Progressive Diffuse Intrinsic Pontine Glioma ». American Journal of Clinical Oncology 35 (1): 51□57. doi:10.1097/COC.0b013e318201a2b7.

Fouladi, Maryam, Fred Laningham, Jianrong Wu, Melinda A O'Shaughnessy, Kristen Molina, Alberto Broniscer, Sheri L Spunt, et al. 2007. « Phase I Study of Everolimus in Pediatric Patients with Refractory Solid Tumors ». Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology 25 (30): 4806□4812. doi:10.1200/JCO.2007.11.4017.

Frappaz, Didier, Matthias Schell, Philippe Thiesse, Perrine Marec-Bérard, Carmine Mottolese, David Perol, Christophe Bergeron, et al. 2008. « Preradiation Chemotherapy May Improve Survival in Pediatric Diffuse Intrinsic Brainstem Gliomas: Final Results of BSG 98 Prospective Trial ». Neuro-Oncology 10 (4): 599□607. doi:10.1215/15228517-2008-029.

Freidlin, Boris, Edward L Korn, Robert Gray, et Alison Martin. 2008. « Multi-Arm Clinical Trials of New Agents: Some Design Considerations ». Clinical Cancer Research: An Official Journal of the American Association for Cancer Research 14 (14): 4368□4371. doi:10.1158/1078-0432.CCR-08-0325.

Geoerger, Birgit, Edward J Estlin, Isabelle Aerts, Pamela Kearns, Brenda Gibson, Nadège Corradini, François Doz, et al. 2012. « A Phase I and Pharmacokinetic Study of Plitidepsin in Children with Advanced Solid Tumours: An Innovative Therapies for Children with Cancer (ITCC) Study ». European Journal of Cancer (Oxford, England: 1990) 48 (3): 289□296. doi:10.1016/j.ejca.2011.10.036.

Geoerger, Birgit, Darren Hargrave, Fabienne Thomas, Anna Ndiaye, Didier Frappaz, Felipe Andreiuolo, Pascale Varlet, et al. 2011. « Innovative Therapies for Children with Cancer Pediatric Phase I Study of Erlotinib in Brainstem Glioma and Relapsing/refractory Brain Tumors ». Neuro-Oncology 13 (1): 109□118. doi:10.1093/neuonc/noq141.

Geoerger, Birgit, Bruce Morland, Anna Ndiaye, Francois Doz, Gabriel Kalifa, Anne Geoffray, Fabienne Pichon, et al. 2009. « Target-Driven Exploratory Study of Imatinib Mesylate in Children with Solid Malignancies by the Innovative Therapies for Children with Cancer (ITCC) European Consortium ». European Journal of Cancer (Oxford, England: 1990) 45 (13): 2342□2351. doi:10.1016/j.ejca.2009.03.007.

Gilbertson, Richard J, D Ashley Hill, Roberto Hernan, Mehmet Kocak, Russell Geyer, Jim Olson, Amar Gajjar, et al. 2003. « ERBB1 Is Amplified and Overexpressed in High-Grade Diffusely Infiltrative Pediatric Brain Stem Glioma ». Clinical Cancer Research: An Official Journal of the American Association for Cancer Research 9 (10 Pt 1): 3620□3624.

Giussani, Carlo, Andrew Poliakov, Raymond T Ferri, Lauren L Plawner, Samuel R Browd, Dennis W W Shaw, Tanya Z Filardi, et al. 2010. « DTI Fiber Tracking to Differentiate Demyelinating Diseases from Diffuse Brain Stem Glioma ». NeuroImage 52 (1): 217□223. doi:10.1016/j.neuroimage.2010.03.079.

Grill, Jacques, Stephanie Puget, Felipe Andreiuolo, Cathy Philippe, Laura MacConaill, et Mark W Kieran. 2012. « Critical Oncogenic Mutations in Newly Diagnosed Pediatric Diffuse Intrinsic Pontine Glioma ». Pediatric Blood & Cancer 58 (4): 489□491. doi:10.1002/pbc.24060.

Gururangan, Sridharan, Colleen A McLaughlin, James Brashears, Melody A Watral, James Provenzale, R Edward Coleman, Edward C Halperin, et al. 2006. « Incidence and Patterns of Neuraxis Metastases in Children with Diffuse Pontine Glioma ». Journal of Neuro-Oncology 77 (2): 207□212. doi:10.1007/s11060-005-9029-5.

Haas-Kogan, Daphne A, Anuradha Banerjee, Tina Young Poussaint, Mehmet Kocak, Michael D Prados, J Russell Geyer, Maryam Fouladi, et al. 2011. « Phase II Trial of Tipifarnib and Radiation in Children with Newly Diagnosed Diffuse Intrinsic Pontine Gliomas ». Neuro-Oncology 13 (3): 298□306. doi:10.1093/neuonc/noq202.

Hankinson, Todd C, Elizabeth J Campagna, Nicholas K Foreman, et Michael H Handler. 2011. « Interpretation of Magnetic Resonance Images in Diffuse Intrinsic Pontine Glioma: A Survey of Pediatric Neurosurgeons ». Journal of Neurosurgery. Pediatrics 8 (1): 97□102. doi:10.3171/2011.4.PEDS1180.

Hayward, Robert M, Nicolas Patronas, Eva H Baker, Gilbert Vézina, Paul S Albert, et Katherine E Warren. 2008. « Inter-Observer Variability in the Measurement of Diffuse Intrinsic Pontine Gliomas ». Journal of Neuro-Oncology 90 (1): 57□61. doi:10.1007/s11060-008-9631-4.

Hipp, Sean J, Emilie A Steffen-Smith, Nicholas Patronas, Peter Herscovitch, Jeffrey M Solomon, Robyn S Bent, Seth M Steinberg, et Katherine E Warren. 2012. « Molecular Imaging of Pediatric Brain Tumors: Comparison of Tumor Metabolism Using 18F-FDG-PET and MRSI ». Journal of Neuro-Oncology 109 (3): 521□527. doi:10.1007/s11060-012-0918-0.

Hipp, Sean J, Emilie Steffen-Smith, Dima Hammoud, Joanna H Shih, Robyn Bent, et Katherine E Warren. 2011. « Predicting Outcome of Children with Diffuse Intrinsic Pontine Gliomas Using Multiparametric Imaging ». Neuro-Oncology 13 (8): 904□909. doi:10.1093/neuonc/nor076.

Janssens, Geert O, Marc H Jansen, Selmer J Lauwers, Peter J Nowak, Foppe R Oldenburger, Eric Bouffet, Frank Saran, et al. 2013. « Hypofractionation vs Conventional Radiation Therapy for Newly Diagnosed Diffuse Intrinsic Pontine Glioma: A Matched-Cohort Analysis ». International Journal of Radiation Oncology, Biology, Physics 85 (2): 315□320. doi:10.1016/j.ijrobp.2012.04.006.

Kaste, Sue C, Robert A Kaufman, Amar Gajjar, et Alberto Broniscer. 2013. « Magnetic Resonance Imaging Is the Preferred Method to Assess Treatment-Related Skeletal Changes in Children With Brain Tumors ». Pediatric Blood & Cancer, mars. doi:10.1002/pbc.24536.

Khuong-Quang, Dong-Anh, Pawel Buczkowicz, Patricia Rakopoulos, Xiao-Yang Liu, Adam M Fontebasso, Eric Bouffet, Ute Bartels, et al. 2012. « K27M Mutation in Histone H3.3 Defines Clinically and Biologically Distinct Subgroups of Pediatric Diffuse Intrinsic Pontine Gliomas ». Acta Neuropathologica 124 (3): 439□447. doi:10.1007/s00401-012-0998-0.

Krueger, Darcy A, Marguerite M Care, Katherine Holland, Karen Agricola, Cynthia Tudor, Prajakta Mangeshkar, Kimberly A Wilson, Anna Byars, Tarek Sahmoud, et David Neal Franz. 2010. « Everolimus for Subependymal Giant-Cell Astrocytomas in Tuberous Sclerosis ». The New England Journal of Medicine 363 (19): 1801□1811. doi:10.1056/NEJMoa1001671.

Laprie, Anne, Andrea Pirzkall, Daphne A Haas-Kogan, Soonmee Cha, Anuradha Banerjee, Terry P Le, Ying Lu, Sarah Nelson, et Tracy R McKnight. 2005. « Longitudinal Multivoxel MR Spectroscopy Study of Pediatric Diffuse Brainstem Gliomas Treated with Radiotherapy ». International Journal of Radiation Oncology, Biology, Physics 62 (1): 20□31. doi:10.1016/j.ijrobp.2004.09.027.

Levy, Antonin, Jean Menard, Laurence Albiges, Yohann Loriot, Mario Di Palma, Karim Fizazi, et Bernard Escudier. 2013. « Second Line Treatment of Metastatic Renal Cell Carcinoma: The Institut Gustave Roussy Experience with Targeted Therapies in 251 Consecutive Patients ». European Journal of Cancer (Oxford, England: 1990) 49 (8): 1898□1904. doi:10.1016/j.ejca.2013.02.003.

Li, Minglun, Gong Ping, Christian Plathow, Thuy Trinh, Kenneth E Lipson, Kai Hauser, Robert Krempien, Juergen Debus, Amir Abdollahi, et Peter E Huber. 2006. « Small Molecule Receptor Tyrosine Kinase Inhibitor of Platelet-Derived Growth Factor Signaling (SU9518) Modifies Radiation Response in Fibroblasts and Endothelial Cells ». BMC Cancer 6: 79. doi:10.1186/1471-2407-6-79.

Löbel, U, J Sedlacik, W E Reddick, M Kocak, Q Ji, A Broniscer, C M Hillenbrand, et Z Patay. 2011. « Quantitative Diffusion-Weighted and Dynamic Susceptibility-Weighted Contrast-Enhanced Perfusion MR Imaging Analysis of T2 Hypointense Lesion Components in Pediatric Diffuse Intrinsic Pontine Glioma ». AJNR. American Journal of Neuroradiology 32 (2): 315□322. doi:10.3174/ajnr.A2277.

Löbel, Ulrike, Jan Sedlacik, Noah D Sabin, Mehmet Kocak, Alberto Broniscer, Claudia M Hillenbrand, et Zoltán Patay. 2010. « Three-Dimensional Susceptibility-Weighted Imaging and Two-Dimensional T2*-Weighted Gradient-Echo Imaging of Intratumoral Hemorrhages in Pediatric Diffuse Intrinsic Pontine Glioma ». Neuroradiology 52 (12): 1167□1177. doi:10.1007/s00234-010-0771-9.

Louis, D N, M P Rubio, K M Correa, J F Gusella, et A von Deimling. 1993. « Molecular Genetics of Pediatric Brain Stem Gliomas. Application of PCR Techniques to Small and Archival Brain Tumor Specimens ». Journal of Neuropathology and Experimental Neurology 52 (5): 507□515.

Mandell, L R, N Wollner, et Z Fuks. 1987. « Is Cranial Radiation Necessary for CNS Prophylaxis in Pediatric NHL? » International Journal of Radiation Oncology, Biology, Physics 13 (3): 359□363.

McGirt, Matthew J, Khoi D Than, Jon D Weingart, Kaisorn L Chaichana, Frank J Attenello, Alessandro Olivi, John Laterra, et al. 2009. « Gliadel (BCNU) Wafer plus Concomitant Temozolomide Therapy after Primary Resection of Glioblastoma Multiforme ». Journal of Neurosurgery 110 (3): 583□588. doi:10.3171/2008.5.17557.

McGirt, Matthew J, Graeme Woodworth, Alexander L Coon, George Thomas, Michael A Williams, et Daniele Rigamonti. 2005. « Diagnosis, Treatment, and Analysis of Long-Term Outcomes in Idiopathic Normal-Pressure Hydrocephalus ». Neurosurgery 57 (4): 699□705; discussion 699□705.

Mehta, Vivek K. 2012. « Radiotherapy and Erlotinib Combined: Review of the Preclinical and Clinical Evidence ». Frontiers in Oncology 2: 31. doi:10.3389/fonc.2012.00031.

Meyzer, Candice, Frédéric Dhermain, Denis Ducreux, Jean-Louis Habrand, Pascale Varlet, Christian Sainte-Rose, Christelle Dufour, et Jacques Grill. 2010. « A Case Report of Pseudoprogression Followed by Complete Remission after Proton-Beam Irradiation for a Low-Grade Glioma in a Teenager: The Value of Dynamic Contrast-Enhanced MRI ». Radiation Oncology (London, England) 5: 9. doi:10.1186/1748-717X-5-9.

Monje, Michelle, Siddhartha S Mitra, Morgan E Freret, Tal B Raveh, James Kim, Marilyn Masek, Joanne L Attema, et al. 2011. « Hedgehog-Responsive Candidate Cell of Origin for Diffuse Intrinsic Pontine Glioma ». Proceedings of the National Academy of Sciences of the United States of America 108 (11): 4453□4458. doi:10.1073/pnas.1101657108.

Negretti, Laura, Karim Bouchireb, Christine Levy-Piedbois, Jean Louis Habrand, Frederic Dhermain, Chantal Kalifa, Jacques Grill, et Christelle Dufour. 2011. « Hypofractionated Radiotherapy in the Treatment of Diffuse Intrinsic Pontine Glioma in Children: A Single Institution's Experience ». Journal of Neuro-Oncology 104 (3): 773□777. doi:10.1007/s11060-011-0542-4.

Niyazi, Maximilian, Ute Ganswindt, Silke Birgit Schwarz, Friedrich-Wilhelm Kreth, Jörg-Christian Tonn, Julia Geisler, Christian la Fougère, et al. 2012. « Irradiation and Bevacizumab in High-Grade Glioma Retreatment Settings ». International Journal of Radiation Oncology, Biology, Physics 82 (1): 67□76. doi:10.1016/j.ijrobp.2010.09.002.

Packer, R J, J M Boyett, R A Zimmerman, A L Albright, A M Kaplan, L B Rorke, M T Selch, J M Cherlow, J L Finlay, et W M Wara. 1994. « Outcome of Children with Brain Stem Gliomas after Treatment with 7800 cGy of Hyperfractionated Radiotherapy. A Childrens Cancer Group Phase I/II Trial ». Cancer 74 (6): 1827□1834.

Packer, R J, L N Sutton, R Elterman, B Lange, J Goldwein, H S Nicholson, L Mulne, J Boyett, G D'Angio, et K Wechsler-Jentzsch. 1994. « Outcome for Children with Medulloblastoma Treated with Radiation and Cisplatin, CCNU, and Vincristine Chemotherapy ». Journal of Neurosurgery 81 (5): 690□698. doi:10.3171/jns.1994.81.5.0690.

Panigrahy, Ashok, Marvin D Nelson Jr, Jonathan L Finlay, Richard Sposto, Mark D Krieger, Floyd H Gilles, et Stefan Blüml. 2008. « Metabolism of Diffuse Intrinsic Brainstem Gliomas in Children ». Neuro-Oncology 10 (1): 32□44. doi:10.1215/15228517-2007-042.

Paugh, Barbara S, Alberto Broniscer, Chunxu Qu, Claudia P Miller, Junyuan Zhang, Ruth G Tatevossian, James M Olson, et al. 2011. « Genome-Wide Analyses Identify Recurrent Amplifications of Receptor Tyrosine Kinases and Cell-Cycle Regulatory Genes in Diffuse Intrinsic Pontine Glioma ». Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology 29 (30): 3999□4006. doi:10.1200/JCO.2011.35.5677.

Pollack, Ian F, Regina I Jakacki, Susan M Blaney, Michael L Hancock, Mark W Kieran, Peter Phillips, Larry E Kun, et al. 2007. « Phase I Trial of Imatinib in Children with Newly Diagnosed Brainstem and Recurrent Malignant Gliomas: A Pediatric Brain Tumor Consortium Report ». Neuro-Oncology 9 (2): 145□160. doi:10.1215/15228517-2006-031.

Pollack, Ian F, Clinton F Stewart, Mehmet Kocak, Tina Young Poussaint, Alberto Broniscer, Anu Banerjee, James G Douglas, Larry E Kun, James M Boyett, et J Russell Geyer. 2011. « A Phase II Study of Gefitinib and Irradiation in Children with Newly Diagnosed Brainstem Gliomas: A Report from the Pediatric Brain Tumor Consortium ». Neuro-Oncology 13 (3): 290□297. doi:10.1093/neuonc/noq199.

Poussaint, Tina Young, Mehmet Kocak, Sridhar Vajapeyam, Roger I Packer, Richard L Robertson, Russell Geyer, Daphne Haas-Kogan, et al. 2011. « MRI as a Central Component of Clinical Trials Analysis in Brainstem Glioma: A Report from the Pediatric Brain Tumor Consortium (PBTC) ». Neuro-Oncology 13 (4): 417□427. doi:10.1093/neuonc/noq200.

Puget, Stephanie, Cathy Philippe, Dorine A Bax, Bastien Job, Pascale Varlet, Marie-Pierre Junier, Felipe Andreiuolo, et al. 2012. « Mesenchymal Transition and PDGFRA Amplification/mutation Are Key Distinct Oncogenic Events in Pediatric Diffuse Intrinsic Pontine Gliomas ». PloS One 7 (2): e30313. doi:10.1371/journal.pone.0030313.

Roujeau, Thomas, Federico Di Rocco, Christelle Dufour, Franck Bourdeaut, Stephanie Puget, Christian Sainte Rose, et Michel Zerah. 2011. « Shall We Treat Hydrocephalus Associated to Brain Stem Glioma in Children? » Child's Nervous System: ChNS: Official Journal of the International Society for Pediatric Neurosurgery 27 (10): 1735□1739. doi:10.1007/s00381-011-1538-2.

Roujeau, Thomas, Guilherme Machado, Matthew R Garnett, Catherine Miquel, Stephanie Puget, Birgit Geoerger, Jacques Grill, et al. 2007. « Stereotactic Biopsy of Diffuse Pontine Lesions in Children ». Journal of Neurosurgery 107 (1 Suppl): 1□4. doi:10.3171/PED-07/07/001.

Royston P1, Parmar MK. « The use of restricted mean survival time to estimate the treatment effect in randomized clinical trials when the proportional hazards assumption is in doubt » Stat Med. 2011 Aug 30;30(19):2409-21. doi: 10.1002/sim.4274. Epub 2011 May 25.

Samadani, Uzma, et Kevin D Judy. 2003. « Stereotactic Brainstem Biopsy Is Indicated for the Diagnosis of a Vast Array of Brainstem Pathology ». Stereotactic and Functional Neurosurgery 81 (1-4): 5□9. doi:75097.

Sanvitale, Caroline E, Georgina Kerr, Apirat Chaikuad, Marie-Christine Ramel, Agustin H Mohedas, Sabine Reichert, You Wang, et al. 2013. « A New Class of Small Molecule Inhibitor of BMP Signaling ». PloS One 8 (4): e62721. doi:10.1371/journal.pone.0062721.

Saratsis, Amanda M, Madhuri Kambhampati, Kendall Snyder, Sridevi Yadavilli, Joseph M Devaney, Brennan Harmon, Jordan Hall, et al. 2013. « Comparative Multidimensional Molecular Analyses of Pediatric Diffuse Intrinsic Pontine Glioma Reveals Distinct Molecular Subtypes ». Acta Neuropathologica, décembre. doi:10.1007/s00401-013-1218-2.

Sarkaria, Jann N, Eva Galanis, Wenting Wu, Allan B Dietz, Timothy J Kaufmann, Michael P Gustafson, Paul D Brown, et al. 2010. « Combination of Temsirolimus (CCI-779) with Chemoradiation in Newly Diagnosed Glioblastoma Multiforme (GBM) (NCCTG Trial N027D) Is Associated with Increased Infectious Risks ». Clinical Cancer Research: An Official Journal of the American Association for Cancer Research 16 (22): 5573□5580. doi:10.1158/1078-0432.CCR-10-1453.

Sarkaria, Jann N, Evanthia Galanis, Wenting Wu, Patrick J Peller, Caterina Giannini, Paul D Brown, Joon H Uhm, Steven McGraw, Kurt A Jaeckle, et Jan C Buckner. 2011. « North Central Cancer Treatment Group Phase I Trial N057K of Everolimus (RAD001) and Temozolomide in Combination with Radiation Therapy in Patients with Newly Diagnosed Glioblastoma Multiforme ». International Journal of Radiation Oncology, Biology, Physics 81 (2): 468□475. doi:10.1016/j.ijrobp.2010.05.064.

Schwartzentruber, Jeremy, Andrey Korshunov, Xiao-Yang Liu, David T W Jones, Elke Pfaff, Karine Jacob, Dominik Sturm, et al. 2012. « Driver Mutations in Histone H3.3 and Chromatin Remodelling Genes in Paediatric Glioblastoma ». Nature 482 (7384): 226□231. doi:10.1038/nature10833.

Sethi, Rajni, Jeffrey Allen, Bernadine Donahue, Matthias Karajannis, Sharon Gardner, Jeffrey Wisoff, Saroj Kunnakkat, et al. 2011. « Prospective Neuraxis MRI Surveillance Reveals a High Risk of Leptomeningeal Dissemination in Diffuse Intrinsic Pontine Glioma ». Journal of Neuro-Oncology 102 (1): 121□127. doi:10.1007/s11060-010-0301-y.

Shah, Niketa C, Amit Ray, Ute Bartels, James Rutka, Eric Bouffet, James Drake, Cynthia E Hawkins, et Annie Huang. 2008. « Diffuse Intrinsic Brainstem Tumors in Neonates. Report of Two Cases ». Journal of Neurosurgery. Pediatrics 1 (5): 382□385. doi:10.3171/PED/2008/1/5/382.

Steffen-Smith, Emilie A, David J Venzon, Robyn S Bent, Sean J Hipp, et Katherine E Warren. 2012. « Single- and Multivoxel Proton Spectroscopy in Pediatric Patients with Diffuse Intrinsic Pontine Glioma ». International Journal of Radiation Oncology, Biology, Physics 84 (3): 774□779. doi:10.1016/j.ijrobp.2012.01.032.

Sturm, Dominik, Hendrik Witt, Volker Hovestadt, Dong-Anh Khuong-Quang, David T W Jones, Carolin Konermann, Elke Pfaff, et al. 2012. « Hotspot Mutations in H3F3A and IDH1 Define Distinct Epigenetic and Biological Subgroups of Glioblastoma ». Cancer Cell 22 (4): 425□437. doi:10.1016/j.ccr.2012.08.024.

Sufit, Alexandra, Andrew M Donson, Diane K Birks, Jeffrey A Knipstein, Laura Z Fenton, Paul Jedlicka, Todd C Hankinson, Michael H Handler, et Nicholas K Foreman. 2012. « Diffuse Intrinsic Pontine Tumors: A Study of Primitive Neuroectodermal Tumors versus the More Common Diffuse Intrinsic Pontine Gliomas ». Journal of Neurosurgery. Pediatrics 10 (2): 81□88. doi:10.3171/2012.3.PEDS11316.

Sydes, Matthew R, Mahesh K B Parmar, Malcolm D Mason, Noel W Clarke, Claire Amos, John Anderson, Johann de Bono, et al. 2012. « Flexible Trial Design in Practice - Stopping Arms for Lack-of-Benefit and Adding Research Arms Mid-Trial in STAMPEDE: A Multi-Arm Multi-Stage Randomized Controlled Trial ». Trials 13: 168. doi:10.1186/1745-6215-13-168.

Thakur, S B, S Karimi, I J Dunkel, J A Koutcher, et W Huang. 2006. « Longitudinal MR Spectroscopic Imaging of Pediatric Diffuse Pontine Tumors to Assess Tumor Aggression and Progression ». AJNR. American Journal of Neuroradiology 27 (4): 806□809.

Tillema, Jan-Mendelt, James L Leach, Darcy A Krueger, et David Neal Franz. 2012. « Everolimus Alters White Matter Diffusion in Tuberous Sclerosis Complex ». Neurology 78 (8): 526□531. doi:10.1212/WNL.0b013e318247ca8d.

Wagner, S, M Benesch, F Berthold, A K Gnekow, S Rutkowski, R Sträter, M Warmuth-Metz, R-D Kortmann, T Pietsch, et J E A Wolff. 2006. « Secondary Dissemination in Children with High-Grade Malignant Gliomas and Diffuse Intrinsic Pontine Gliomas ». British Journal of Cancer 95 (8): 991□997. doi:10.1038/sj.bjc.6603402.

Warren, Katherine E. 2012. « Diffuse Intrinsic Pontine Glioma: Poised for Progress ». Frontiers in Oncology 2: 205. doi:10.3389/fonc.2012.00205.

Warren, Katherine E, Keith Killian, Miia Suuriniemi, Yonghong Wang, Martha Quezado, et Paul S Meltzer. 2012. « Genomic Aberrations in Pediatric Diffuse Intrinsic Pontine Gliomas ». Neuro-Oncology 14 (3): 326□332. doi:10.1093/neuonc/nor190.

Weiler, M, P-N Pfenning, A-L Thiepold, J Blaes, L Jestaedt, J Gronych, L M Dittmann, et al. 2013. « Suppression of Proinvasive RGS4 by mTOR Inhibition Optimizes Glioma Treatment ». Oncogene 32 (9): 1099□1109. doi:10.1038/onc.2012.137.

Yu, Paul B, Donna Y Deng, Carol S Lai, Charles C Hong, Gregory D Cuny, Mary L Bouxsein, Deborah W Hong, et al. 2008. « BMP Type I Receptor Inhibition Reduces Heterotopic [corrected] Ossification ». Nature Medicine 14 (12): 1363□1369. doi:10.1038/nm.1888.

Zagzag, D, D C Miller, E Knopp, J P Farmer, M Lee, S Biria, A Pellicer, F J Epstein, et J C Allen. 2000. « Primitive Neuroectodermal Tumors of the Brainstem: Investigation of Seven Cases ». Pediatrics 106 (5): 1045□1053.

Zarghooni, Maryam, Ute Bartels, Eric Lee, Pawel Buczkowicz, Andrew Morrison, Annie Huang, Eric Bouffet, et Cynthia Hawkins. 2010. « Whole-Genome Profiling of Pediatric Diffuse Intrinsic Pontine Gliomas Highlights Platelet-Derived Growth Factor Receptor Alpha and Poly (ADP-Ribose) Polymerase as Potential Therapeutic Targets ». Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology 28 (8): 1337□1344. doi:10.1200/JCO.2009.25.5463.

APPENDIX 1. PERFORMANCE STATUS SCALES

Karnofsky Performance Status Scale (Patients > 12 Years) and Lansky-Play Scale (Patients ≤ 12 Years)

Karnofsky Performance Status Scale		Lansky-Play Scale	
100 %	Normal; no complaints	100%	Fully active, normal
90%	Able to carry on normal activity; minor signs or symptoms of disease	90%	Minor restrictions in physically strenuous activity
80%	Normal activity with effort; some signs or symptoms of disease	80%	Active, but tires more quickly
70%	Cares for self; unable to carry on normal activity or work	70%	Both greater restriction of, and less time
60%	Requires occasional assistance; able to care for most personal needs	60%	Up and around, but minimal active play; keeps busy with quieter activities
50%	Requires considerable assistance and frequent medical care	50%	Gets dressed, but lies around much of the day; no active play; able to participate in all quiet play and activities
40%	Disabled; requires special care and assistance	40%	Mostly in bed; participates in quiet activities
30%	Severely disabled; hospitalization is indicated though death not imminent	30%	In bed; needs assistance even for quiet play
20%	Very sick; hospitalization necessary; active supportive treatment necessary	20%	Often sleeping; play entirely limited to very passive actualities
10%	Moribund; fatal processes progressing rapidly	10%	No play; does not get out of bed
0%	Dead	0%	Unresponsive

APPENDIX 2. NCI - CTCAE

National Cancer Institute - Common Terminology Criteria for Adverse Events

(NCI-CTCAE, Version 4.0)

http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40

http://ctep.cancer.gov/

APPENDIX 3. BRAIN IMAGING PROTOCOL

Guideline imaging parameters are provided below. Each manufacturer will provide different parameters, which however should conform in general to those shown below. The imaging studies for both the standard and functional MR imaging will be required from each center prior to acceptance. The images should be supplied in DICOM format, native DSC, DWI, and DTI series should be included. Spectroscopy data are to be exported as raw data.

Centers may participate in the study undertaking MRI at either 1.5 or 3.0T field strength, but the same field strength must be employed for all subsequent examinations of the enrolled subject. Centers may opt to undertake the functional MR imaging according to local priorities and experience.

The functional imaging parameters are advisory guidelines; sites wishing to participate in this arm will be required to provide details of their site and system specific parameters.

	Parameter Guideline for Structural Brain MRI (1.5T)					
	Axial T1	**Sagittal T1**	**Axial T2**	**Coronal T2 FLAIR**	**Axial T1 + Gad**	**Sagittal T1 + Gad**
TR (ms)	< 600	< 600	> 5000	> 9000	< 600	< 600
TE (ms)	minimum	minimum	100	140	minimum	minimum
TI (ms)	-	-	-	2200-2800	-	-
Maximum Slice Thickness (mm)	4.0	4.0	4.0	4.0	4.0	4.0
Maximum Slice Gap (mm)	0.4	0.4	0.4	0.4	0.4	0.4
FOV (mm)	240	240	240	240	240	240
Matrix	256 x 256	220 x 180	256 x 256	256 x 220	256 x 256	220 x 180
Contrast	No	No	No	No	Yes 0.1mmol/kg	Yes 0.1mmol/kg

	Parameter Guideline for Structural Brain MRI (3.0T)					
	Axial T1	**Sagittal T1**	**Axial T2**	**Coronal T2 FLAIR**	**Axial T1 + Gad**	**Sagittal T1 + Gad[§]**
TR (ms)	< 650	< 650	> 4000	> 9000	< 650	< 650
TE (ms)	minimum	minimum	85	120	minimum	minimum
TI (ms)	-	-	-	2800	-	-
Maximum Slice Thickness (mm)	4.0	4.0	4.0	4.0	4.0	4.0
Maximum Slice Gap (mm)	0.4	0.4	0.4	0.4	0.4	0.4
FOV (mm)	240	240	240	240	240	240
Matrix	256 x 256	220 x 180	256 x 256	256 x 220	256 x 256	220 x 180
Contrast	No	No	No	No	Yes 0.1mmol/kg	Yes 0.1mmol/kg

[§]May be replaced by a 3D gradient echo volume

FUNCTIONAL MULTIMODAL MR IMAGING AT 1.5T

	DWI (EPI)		DTI (EPI)		DSC (EPI)ǂ		ASL	
	1.5 T	3.0T	1.5 T	3.0T	1.5 T	3.0T	1.5 T	3.0T
TR (ms)	> 5000	> 4000	Minimum	Minimum	< 2000	< 2000	Minimum	Minimum
TE (ms)	Minimum	Minimum	Minimum	Minimum	30 - 60	20 - 50	Minimum	Minimum
TI (s)							1.0 - 1.5	1.0 - 2.0
Maximum Slice Thickness (mm)	4.0	4.0	4.0	3.0	5.0	4.0	4.0	4.0
Maximum Slice Gap (mm)	0.4	0.4	1.0	1.0	1.0	1.0	0.5	0.5
FOV (mm)	250	250	250	250	250	250	250	250
Matrix	128x128	128x128	128x128	128x128	128x128	128x128	128x128	128x128

ǂPhilips Presto T2* perfusion TR: 16ms TE: 20-30ms

SPECTROSCOPY

The voxel volume is adapted to the lesion size respecting a minimum volume of 3.4cm^3 (15 x 15 x 15mm). The pulse sequence can be either PRESS or SE, the suggested parameters are:

at **1.5T**: TR 1500ms, TE 30 ms, vector length 2000, number of averages 192, phase cycling 8-16, bandwidth superior to 2kHz.

at **3.0T**: TR 2000ms, TE 30 ms, vector length 2000, number of averages 128, phase cycling 8-16, bandwidth superior to 2kHz.

APPENDIX 4. RANO CRITERIA FOR LOW-GRADE GLIOMA

Complete response requires all the following criteria compared with the baseline scan: (1) Complete disappearance of the lesion on T2 or FLAIR imaging (if enhancement had been present, it must have resolved completely); (2) no new lesions, no new T2 or FLAIR abnormalities apart from those consistent with radiation effects, and no new or increased enhancement; (3) patients must be off corticosteroids or only on physiological replacement doses; and (4) patients should be stable or improved clinically.

Partial response requires all of the following criteria compared with the baseline scan: (1) greater than or equal to 50% decrease in the product of perpendicular diameters of the lesion on T2 or FLAIR imaging sustained for at least 4 weeks compared with baseline; (2) no new lesions, no new T2 or FLAIR abnormalities apart from those consistent with radiation effects, and no new or increased enhancement; and (3) patients should be on a corticosteroid dose that should not be greater than the dose at time of baseline scan, and should be stable or improved clinically.

Minor response requires the following criteria compared with baseline: (1) a decrease of the area of non-enhancing lesion on T2 or FLAIR MR imaging between 25% and 50% compared with baseline; (2) no new lesions, no new T2 or FLAIR abnormalities apart from those consistent with radiation effect, and no new or increased enhancement; and (3) patients should be on a corticosteroid dose that should not be greater than the dose at time of baseline scan, and should be stable or improved clinically.

Stable disease is present if the changes do not qualify for complete, partial, or minor response, or progression and requires: (1) stable area of non-enhancing abnormalities on T2 or FLAIR imaging; (2) no new lesions, no new T2 or FLAIR abnormalities apart from those consistent with radiation effect, and no new or increased enhancement; and (3) patients should be on a corticosteroid dose that should not be greater than the dose at time of baseline scan, and should be stable or improved clinically.

Progression is defined by any of the following: (1) development of new lesions or increase of enhancement (radiological evidence of malignant transformation); (2) a 25% increase of the T2 or FLAIR non-enhancing lesion on stable or increasing doses of corticosteroids compared with baseline scan or best response after initiation of therapy, not attributable to radiation effect or to comorbid events; (3) definite clinical deterioration not attributable to other causes apart from the tumour, or decrease in corticosteroid dose; or (4) failure to return for evaluation because of death or deteriorating condition, unless caused by documented non-related disorders.

APPENDIX 5. MEDICATIONS THAT CAN INDUCE OR INHIBIT CYP3A4

CYP3A4	CYP3A4 INHIBITORS		
INDUCERS	Strong inhibitors	Moderate inhibitors	Weak inhibitors
barbiturates	clarithromycin	aprepitant	alprazolam
carbamazepine	conivaptan	atazanavir	amlodipine
Efavirenz	indinavir	cimetidine	azithromycin
glucocorticoids	itraconazole	ciprofloxacin	bicalutamide
modafinil	ketoconazole	darunavir	chlorzoxazone
nevirapine	lopinavir	diltiazem	cilostazol
oxcarbazepine	mibefradil	erythromycin	cyclosporine
phenobarbital	nefazodone	fluconazole	fluvoxamine
phenytoin	nelfinavir	grapefruit juice	ginkgo
pioglitazone	posaconazole	imatinib	goldenseal
Rifabutin	ritonavir	tofisopam	isoniazid
Rifampin	saquinavir	verapamil	lacidipine
St. John’s wort	telithromycin		oral contraceptives
topiramate	troleandomycin		peppermint oil
troglitazone	voriconazole		propiverine
			ranitidine
			ranolazine
			roxithromycin
			Seville orange juice
			tabimorelin

Note: FDA classification of CYP3A4 inhibitors can be found at http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm093664.htm

APPENDIX 6. DRUG MONITORING PROCEDURES

Drug Reconciliation Procedures

The drug formulation, dose, number of bottles/blister dispensed, received, and returned must be recorded. The investigator/pharmacist must maintain an accurate record of the shipment and dispensing of the study drug in a drug accountability ledger, a copy of which must be given to Gustave Roussy at the end of the study. An accurate record of the date and amount of study drug dispensed to each patient must be available for inspection at any time. All drug supplies are to be used only for this protocol and not for any other purpose.

Destruction of the Investigational Medicinal Product

Written authorization must be obtained from the Sponsor before destruction. Written documentation of any IMP that has been destroyed must contain the following:

- Identity (batch numbers and patient numbers) of IMP destroyed
- Quantity of IMP destroyed
- Date of destruction
- Method of destruction
- Name and signature of responsible person (or company) who destroyed IMP

APPENDIX 7. BIRTH CONTROL METHODS

1. Birth control methods which may be considered as highly effective:

For the purpose of this guidance, methods that can achieve a failure rate of less than 1% per year when used consistently and correctly are considered as highly effective birth control methods. Such methods include:

- combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation [1]
    - o oral
    - o intravaginal
    - o transdermal
- progestogen-only hormonal contraception associated with inhibition of ovulation [1] :
    - o oral
    - o injectable
    - o implantable [2]
- intrauterine device (IUD) [2]
- intrauterine hormone-releasing system (IUS) [2]
- bilateral tubal occlusion [2]
- vasectomised partner [2,3]
- sexual abstinence [4]

2. Acceptable birth control methods which may not be considered as highly effective Acceptable birth control methods that result in a failure rate of more than 1% per year include:

- progestogen-only oral hormonal contraception, where inhibition of ovulation is not the primary mode of action
- male or female condom with or without spermicide [5]
- cap, diaphragm or sponge with spermicide [5]

3. Birth control methods which are considered unacceptable in clinical trials

Periodic abstinence (calendar, symptothermal, post-ovulation methods), withdrawal (coitus interruptus), spermicides only, and lactational amenorrhoea method (LAM) are not acceptable methods of contraception. Female condom and male condom should not be used together.

[1] Hormonal contraception may be susceptible to interaction with the IMP, which may reduce the efficacy of the contraception method.

[2] Contraception methods that in the context of this guidance are considered to have low user dependency.

[3] Vasectomised partner is a highly effective birth control method provided that partner is the sole sexual partner of the WOCBP trial participant and that the vasectomised partner has received medical assessment of the surgical success.

[4] In the context of this guidance sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatments. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the subject.

[5] A combination of male condom with either cap, diaphragm or sponge with spermicide (double barrier methods) are also considered acceptable, but not highly effective, birth control methods.

For more information, please refer to the CTFG Guidelines "recommendations related to contraception and pregnancy testing in clinical trials"

APPENDIX 8. DIAGNOSIS AND MANAGEMENT OF PSEUDOPROGRESSION IN PATIENTS WITH DIPG AND DIFFUSE MIDLINE GLIOMAS

Imaging follow-up in children with DIPG after radiotherapy faces several challenges. Response assessment criteria defined in adult glioblastoma can not be applied strictly. During the 6 month period following radiotherapy, gliomas frequently develop new areas of contrast enhancement, which are due to treatment effect rather than tumor progression. This phenomenon has also been described in DIPG (Chassot et al, J Neurooncol 2012 ; Carceller et al, J Neurooncol 2016). The frequency of this complication (15-20% of cases) may be increased by the concomitant use of specific radiosensitizers ; with erlotinib for example, the rate of radiologic pseudoprogression was as high as 50% (Yvert et al, Neurooncol 2016). Dasatinib and vandetanib combination has also been associated with radiation-induced changes (Bronsicer et al, Clin Cancer Res 2013).

Increase of brainstem perfusion has been observed in DIPG shortly after irradiation, ie within the first two months after irradiation (Sedlacik et al, Am J Neuroradiol 2013 ; Calmon et al, Int J Radiat Oncol Biol Phys 2017). This phenomenon, frequently associated with new contrast-enhancing lesions, has been correlated with improved survival ; in the most recent publication, the median progression-free survival was 4.6 months longer in patients with relative Cerebral Blood Volume (rCBV) in the tumor above 2.6 (Calmon et al, Int J Radiat Oncol Biol Phys 2017). This increase in brainstem perfusion may be linked to the decrease of oedema and intersticial pressure since Apparent Diffusion Coefficient (ADC) is decreasing immediately after radiotherapy (Calmon et al, Neuro-Oncol 2018).

Authors consider that any increase in tumor size with new contrast enhancement occuring during the first six months after irradiation of an adult glioblastoma should be considered as a pseudoprogression unless true progression can be acertained (new lesions outside the radiation field, increase in the size of the infiltration beyond structures already involved). Often, it is only the evolution of the patient that can confirm or not the pseudoprogression. Several studies have examined the value of perfusion MRI for distinguishing glioma recurrence from pseudoprogression, but the results were inconsistent. A recent meta-analysis showed that patients with a rCBV higher than the cutoff are 3 times more likely to have a glioma recurrence than a pseudoprogression (Wan et al, Medicine 2017).

In the case of DIPG, pseudoprogression has not been studied extensively. On the contrary to adult glioblastoma, the increase in perfusion shortly after radiotherapy should not be mistaken as a progression since it is associated with longer PFS (Calmon et al, Int J Radiat Oncol Biol Phys 2017). Among the sequences able to discriminate pseudoprogression from progression, Arterial Spin Labelling (ASL), a non invasive method to measure perfusion, was the most sensitive to discriminate pseudoprogression from true progression. (Calmon et al, Neuro-Oncol 2018)

Bevacizumab has been proposed as a treatment for symptomatic pseudoprogression and its efficacy has been observed in many cases-series. It is currently tested in a prospective trial in the USA.

DEFINITION

Any MRI change occuring inside the radiation field, typically in case of ring contrast enhancement, during the first six months after radiotherapy can be a pseudoprogression. Patients should therefore not be removed from the study before an advice of the Central Radiology Review Committee.

CLINICAL AND MRI CHARACTERISTICS

Increased perfusion (rCBV or ASL).

No increase of infiltration outside the fields initially involved.

Decrease of the enhancement on follow-up MRI that should be repeated within two months (this condition is not mandatory to consider pseudoprogression but will be confirmatory).

Patients can be symptomatic (ie worsening of clinical condition typically occurring after a phase of clinical improvement) but usually the symptoms are less important than the imaging changes. This should not exclude the possibility of a pseudoprogression.

DIAGNOSTIC PROCEDURE FOR BIOMEDE

Central Radiology Review Committee expeditate review of any suspicion of pseudoprogression. Images should be sent to Study Monitor at Gustave Roussy (Villejuif).

Relevant clinical information should be also sent for central review.

THERAPEUTIC MEASURES ALLOWED IN THE BIOMEDE TRIAL

They should be limited to patient with worsening or no improvement of clinical signs.

Corticosteroids up to 2mg/kg/day of prednisone or equivalent.

Bevacizumab 10 mg/kg/dose every two weeks. A maximum of 4 doses is advised. Response is usually observed within a couple of weeks.

Shunting may be necessary and is allowed but bevacizumab should be started earlier than two weeks after the procedure.

Coordinating investigator available anytime for discussion about the management of this complication.

Specific treatment of the BIOMEDE trial should not be stopped.