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Are there common signaling pathways among highly radioresistant tumors? The examples of high grade gliomas, sarcomas and lung carcinoma.

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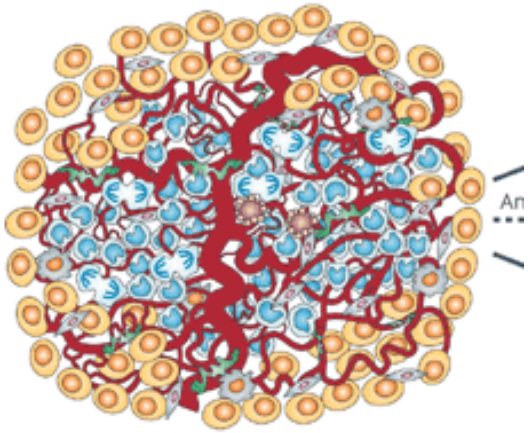
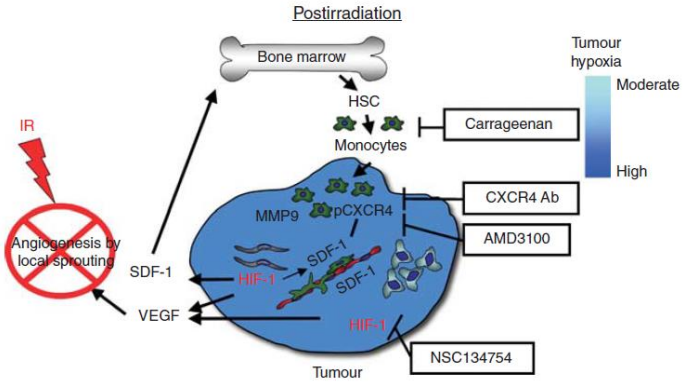
Instituts thématiques Inserm Institut national de la santé et de la recherche médicale

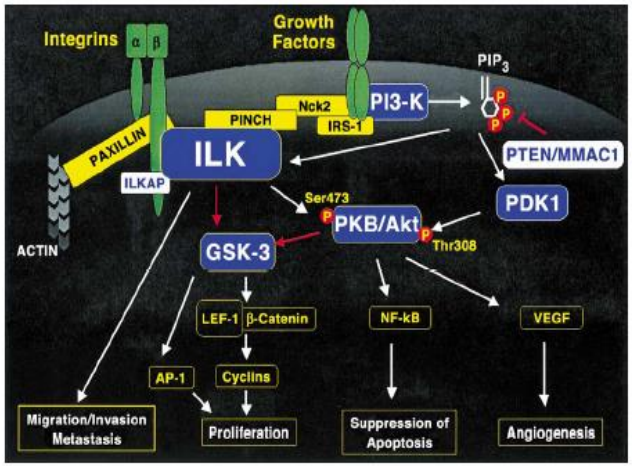
Université Paul Sabatier TOULOUSE III

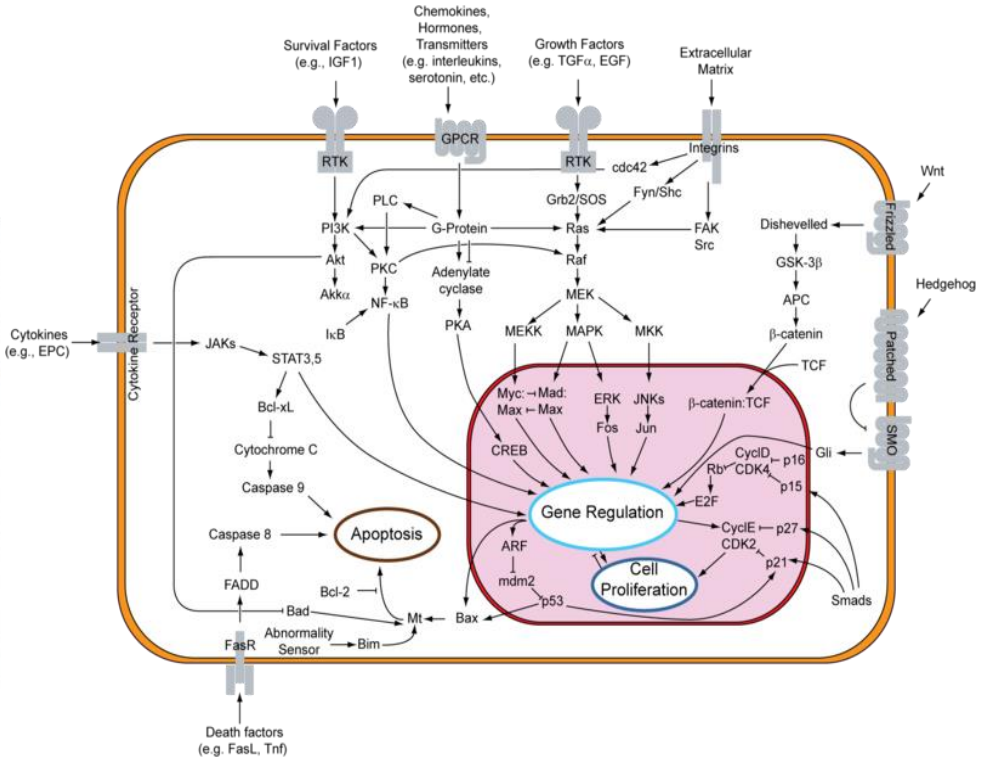
Tumor Radiosensitivity



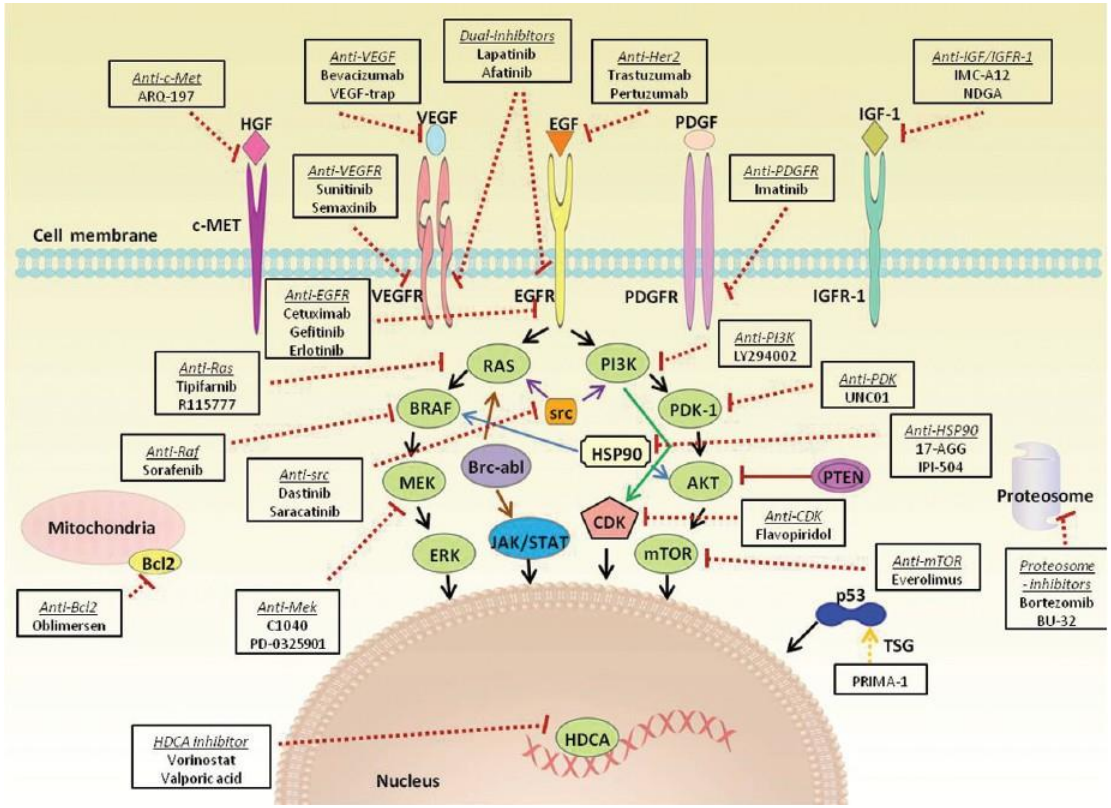
- Intra-cellular radiosensitivity
- Micro-environnement
- Hypoxia
- Tumor Angiogenesis
- Vasculogenesis







Targeting intra-cellular and micro-environnment radioresistance pathway



Radiosensitization

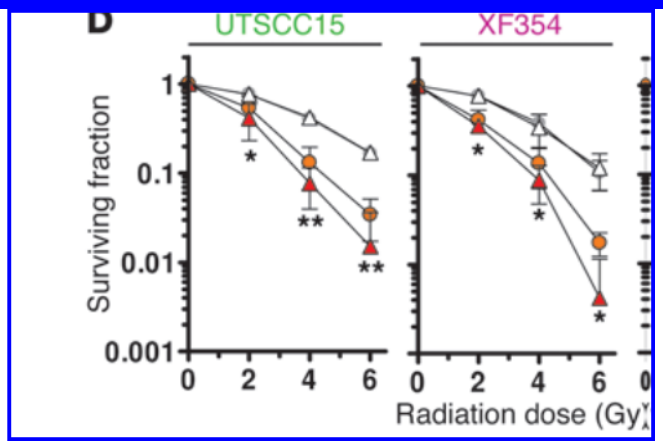
Integrins and Growth factors receptors

α5β1 :

- GBM model : resistance to TMZ
- Expression correlated with worse prognosis in glioma

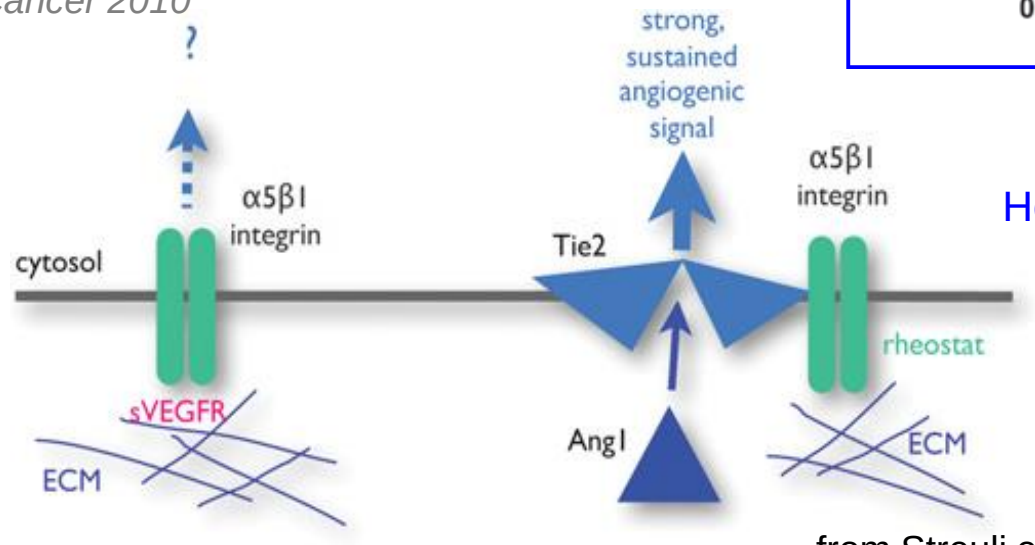
and in small cell lung carcinoma

Janouskova et al, Cancer Res 2012
Lawson et al, Br J Cancer 2010



Eke et al JCI 2012

Head and neck carcinoma



from Streuli et al, Biochem J 2009

Two main integrins involved in radio/ chemo-resistance

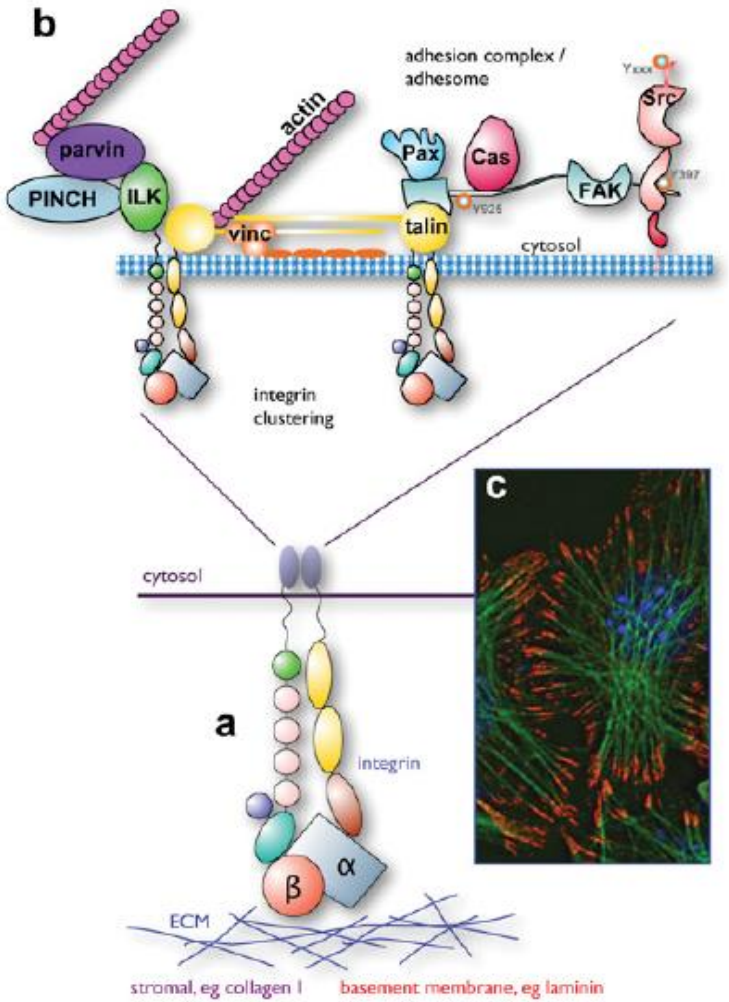
α5β1
αvβ3/ αvβ5

⟷

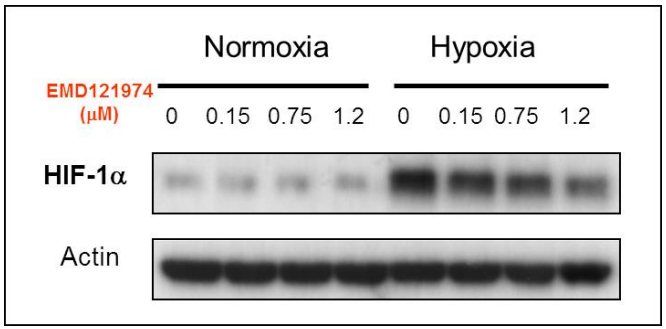
VEGFR
FGFR

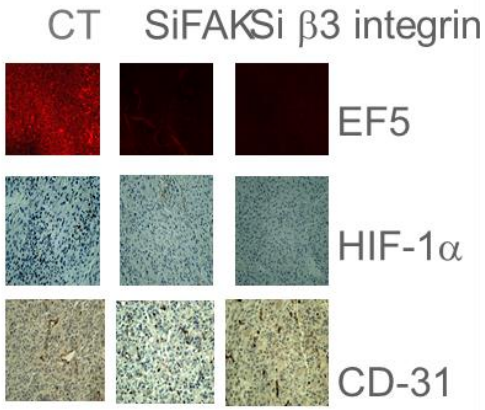
Integrins

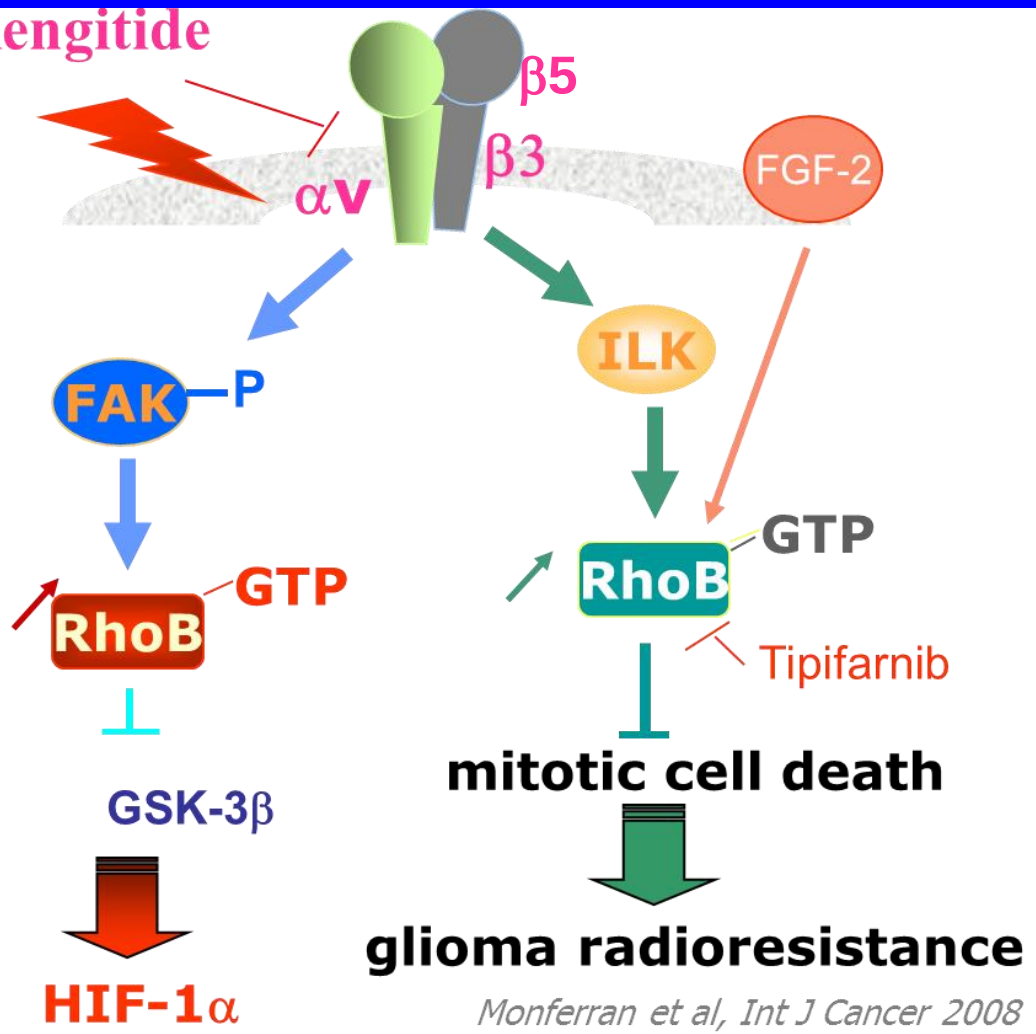
- αvβ3/αvβ5 Integrins
- Express on tumor cells and endothelial cells
- Angiogenesis
- Migration; invasion
- Proliferation
- Survival



Involvement of αvβ3 and avβ5 integrins in the control of radioresistance and of hypoxia pathway







(Skuli Cancer Res 2006 ; Skuli et al, Cancer Res 2009)

Monferran et al, Int J Cancer 2008

Ader et al, Oncogene 2002

Involvement of ILK –HIF1α and survivin in the regulation of radiation induced mitotic cell death in Glioblastoma

(Lanvin et al, Eur J Cancer 2013)

αvβ3

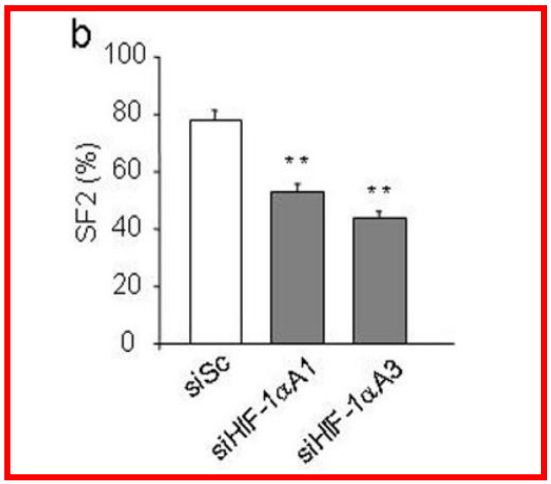
(Gouaze-Andersson, Cancer Res in press)

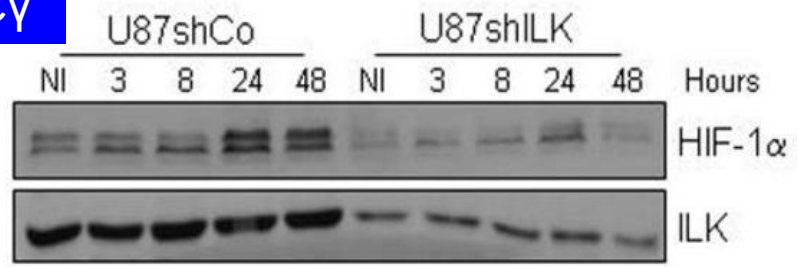
FGFR1

Irradiation

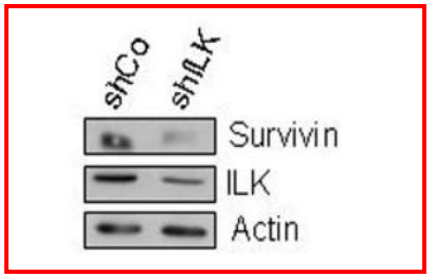
ILK

PLCγ





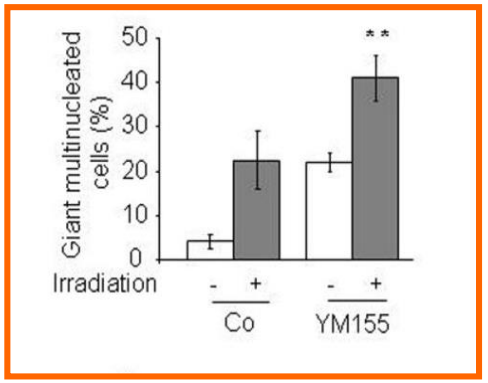
HIF-1α

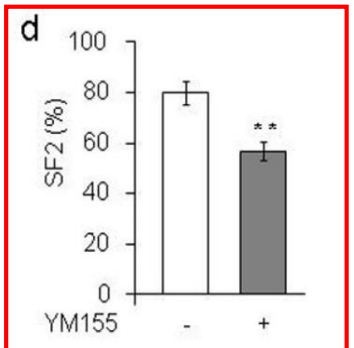


Survivin

Mitotic cell death

Radioresistance





Are these factors predictive of clinical response to radiotherapy.. or to chemotherapy?

Phase I-II Tipifarnib and Radiotherapy for patients with de Novo Glioblastoma

(Ducassou et al, Eur J Cancer 2013)

Tipifarnib: 200mg /day in continuous infusion starting 1 week before and then during 6 weeks of radiotherapy

Median OS : 80.3 weeks (95%CI = [57.8; 102.7]).

Median TTP :
23.1 weeks (95%CI = [15.4; 28.2])

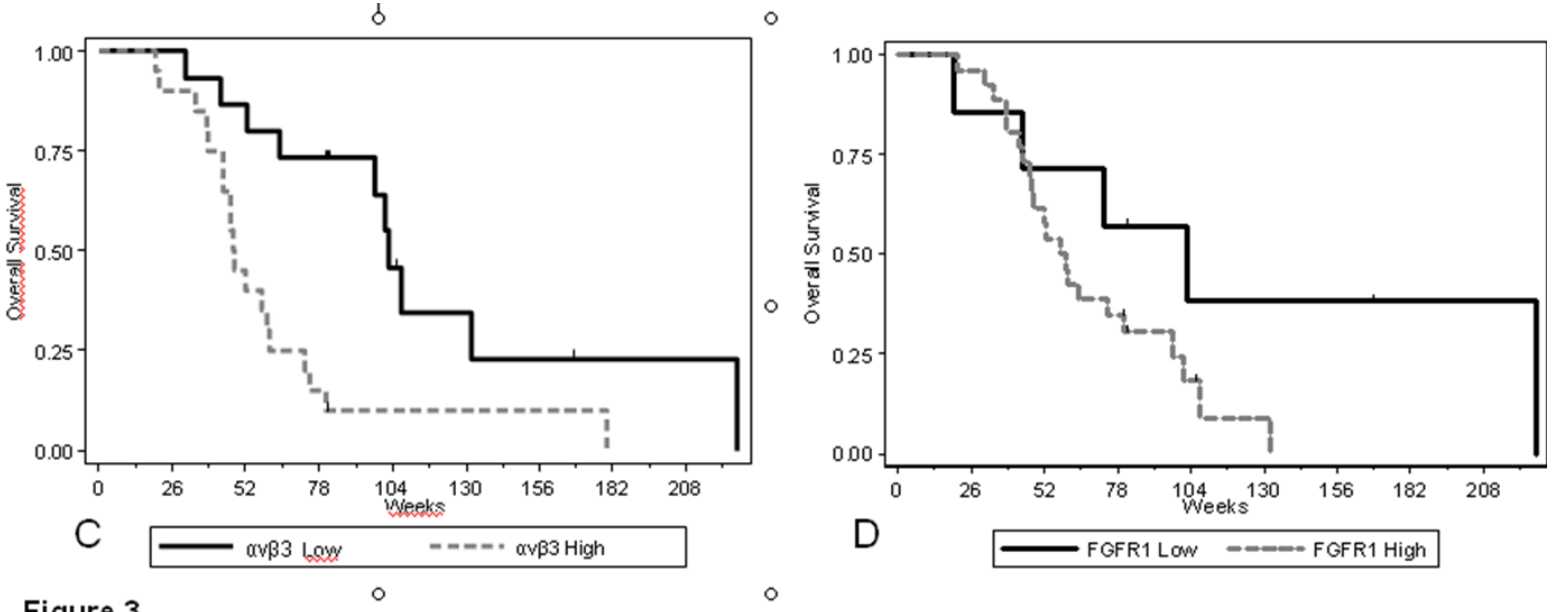


Figure 3

Predictive factor of response to radiotherapy in GBM patients

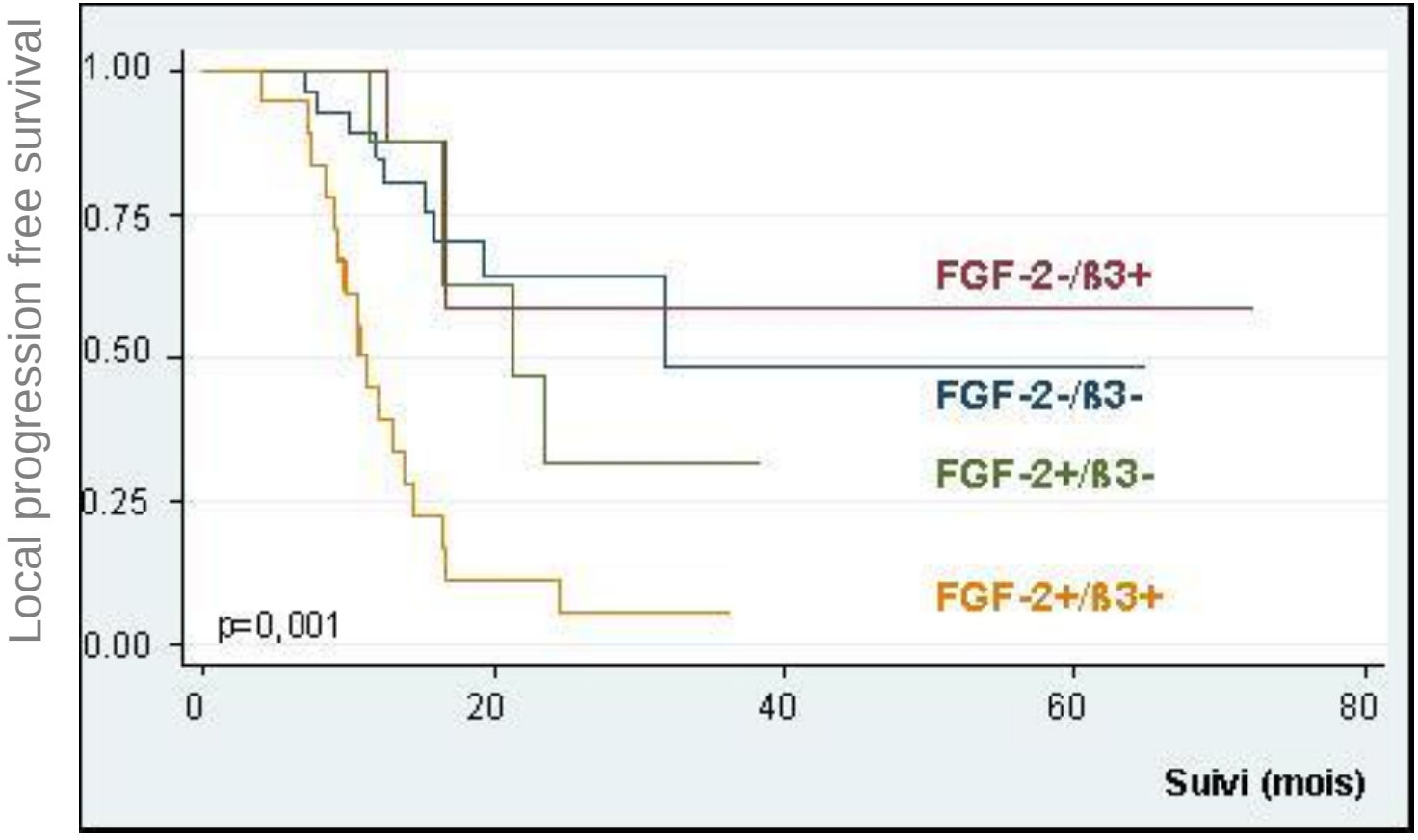
(Ducassou et al, EJC 2013)

Table 6
Multivariate analysis (Cox analysis): Time to progression (TTP) and overall survival (OS) for the patients of the phases I and II according to surgical treatment, age and biological markers. The hazard ratios (HR) are presented with 95% confidence interval.

	IRS	Time to progression			Overall survival		
		HR	95%CI	*p*	HR	95%CI	*p*
Surgery							
Biopsy		2.66	[0.85; 8.34]	0.093	3.98	[1.37; 11.56]	0.011
Large surgery		1			1		
ILK tumour cells							
	<6	1			1		
	⩾6	1.46	[0.61; 3.52]	0.396	0.69	[0.22; 2.17]	0.530
Fibroblast growth factor receptor 1 (FGFR1) tumour cells							
	<4	1			1		
	⩾4	4.65	[1.02; 21.21]	0.047	4.10	[1.09; 15.40]	0.036
αvβ3 Integrin tumour cells							
	<4	NA			1		
	⩾4	NA			10.38	[2.70; 39.87]	0.001
FAK tumour cells							
	<4	0.96	[0.33; 2.80]	0.947	2.63	[0.90; 7.69]	0.077
	⩾4	1			1		
Age at inclusion							
⩽60 years		3.04	[1.0; 8.82]	0.041	NA		
>60 years		1			NA		

FGFR1 and αvβ3 integrins :
Independent predictive factors of TTP and Overall survival

β3 integrin-FGF-2 :protein expression profile correlated with local control after radio-chemotherapy in locally advanced stage III NSCLC



(Massabeau et al Int J Radiat Bio Phys 2009)

Phase I clinical trial
associating continuous infusion of Cilengitide with radio-chemotherapy

The β 5/ FAK /GSK 3 β integrin pathway in high-grade osteosarcoma: a protein expression profile predictive of response to neoadjuvant chemotherapy

Le Guellec et al, Human Pathol 2013

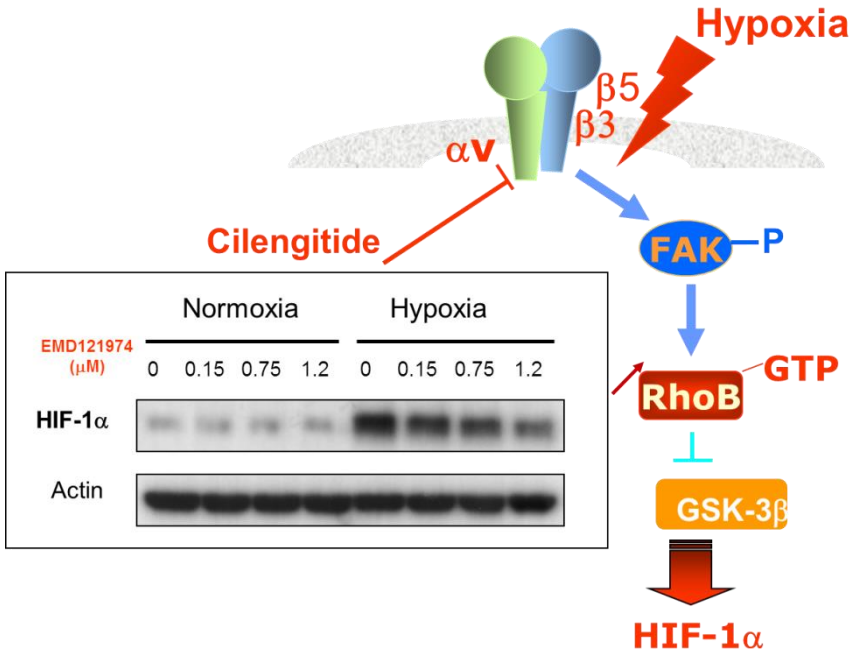


Table 2 Patient and tumor characteristics

Characteristics	n (%)
Age at diagnosis (y)	
Median	18
Range	8-57
Sex	
Female	17 (47.2)
Male	19 (52.8)
High-grade osteosarcoma [a]	
Osteoblastic	8 (22.2)
Chondroblastic	13 (36.1)
Fibroblastic	10 (27.8)
Other	5 (13.9)
Tumor size (cm)	
≤5	5 (14.3)
>5	30 (85.7)
Missing	1
Tumor location	
Long bone	28 (80.0)
Flat bone	5 (14.3)
Other	2 (5.7)
Missing	1
Histological response [b]	
Good responders	20 (55.6)
Poor responders	16 (44.4)

[a] World Health Organization classification, 2002 [1].

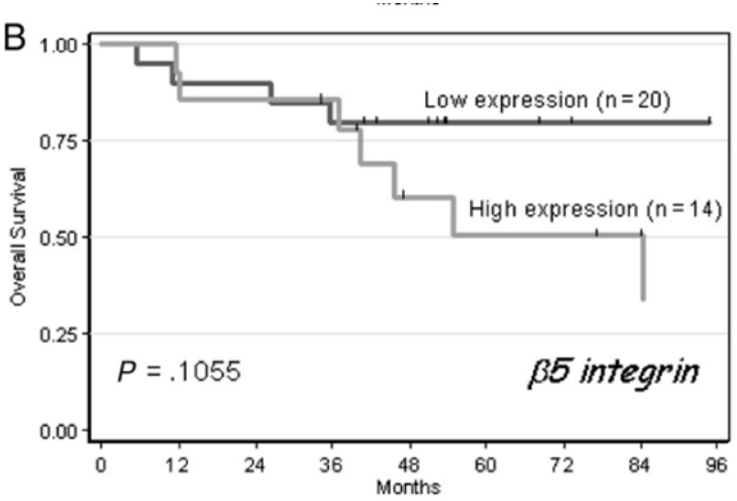
[b] Evaluation performed on the 36 patients who underwent surgery after neoadjuvant chemotherapy. All patients underwent wide conservative surgery with a microscopically complete resection (R0). A *good response* was defined as tumors composed of 10% viable tumor cells or less, and a *poor response* was defined as tumors containing more than 10% viable tumor cells [20,21].

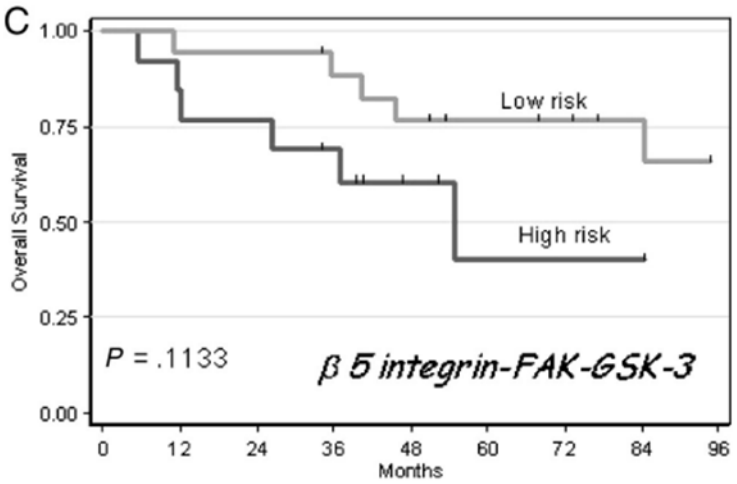
β 5 integrin pathway involved in HIF-1α regulation is associated with a worse OS and predicts response to chemotherapy

Table 4 Univariate analysis: correlation between selected biological marker expressions evaluated by the IRS and response to chemotherapy

Markers	Poor responders	Good responders	*P*	AUC (95% CI)
FAK			.0212*	73.9% (56.1-91.8)
Median (range)	6 (0-9)	3 (0-8)		
Missing	1	3		
GSK3β			.1205	65.6% (45.2-84,0)
Median (range)	6 (2-12)	2.5 (0-9)		
Missing	0	2		
β5 integrin			.1452	64.7% (45.2-84.1)
Median (range)	12 (0-12)	6 (0-12)		
Missing	1	1		
Ezrin			.7015	53.8% (34.2-73.4)
Median (range)	3 (0-12)	4.5 (0-12)		
Missing	2	0		
ILK			.317	60.4% (40.3-80.5)
Median (range)	6 (2-12)	3 (0-12)		
Missing	1	3		
β-catenin			.8149	52.4% (32.3-72.5)
Median (range)	4 (0-12)	4 (0-12)		
Missing	0	3		
β1 integrin			.6908	54% (34-74.1)
Median (range)	6 (3-12)	6 (3-12)		
Missing	1	2		
β3 integrin			.9567	51.1% (33.1-69.1)
Median (range)	4 (0-12)	3.5 (0-12)		
Missing	1	2		
RhoB			.8728	51.7% (30.5-73)
Median (range)	2 (0-6)	2.5 (0-6)		
Missing	3	2		

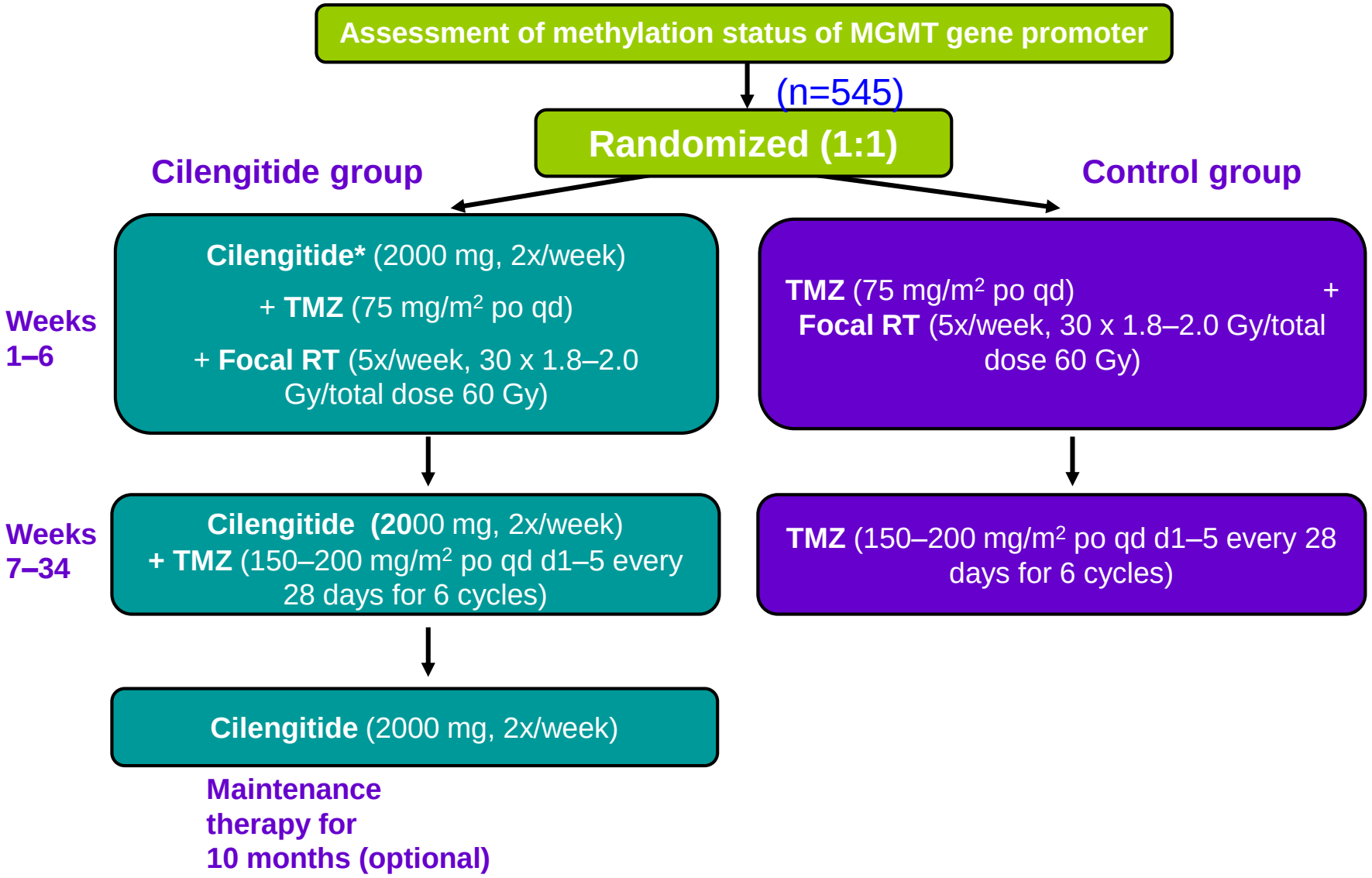
β 5 integrin – FAK- GSK3 β combination: discriminated good and poor responders to chemotherapy, with the highest AUC (89.9%; 95% CI, 77.4-1.00) yielding a sensitivity of 94%, a specificity of 86%, and a diagnostic accuracy of 90%.





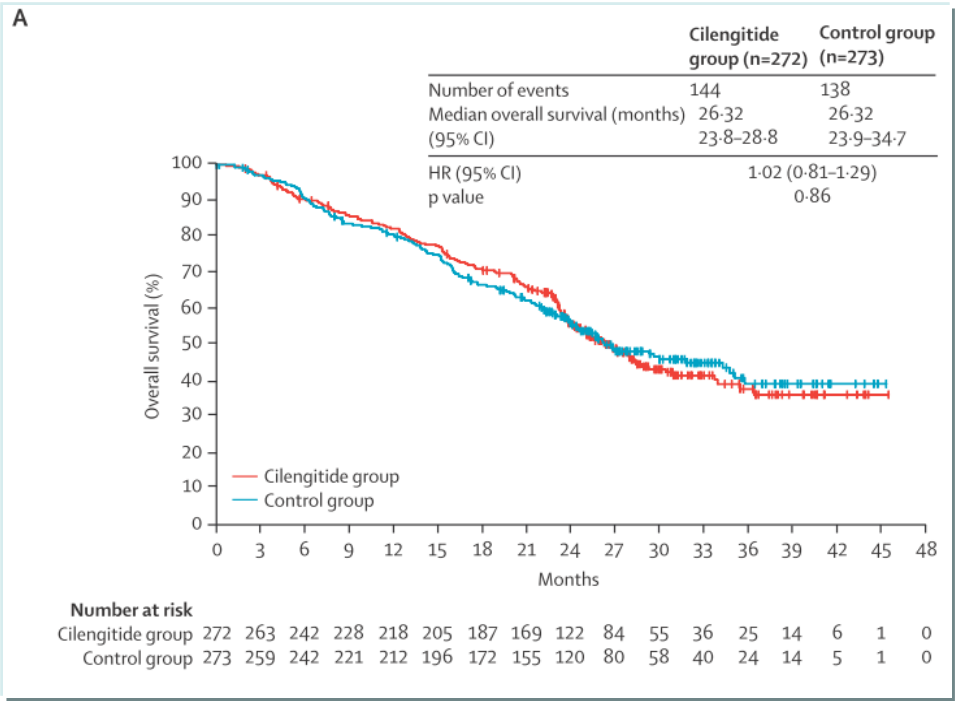
Inhibition of integrin’s pathways with radio-chemotherapy and clinical trials : disappointment and hopes!

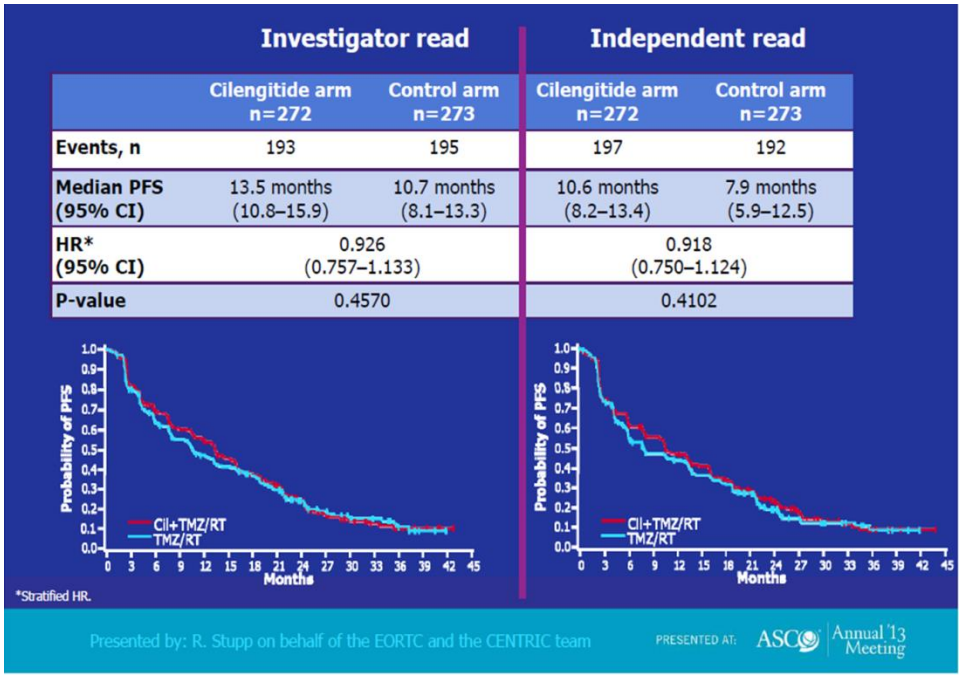
Study 011 : Centric Merck-EORTC



Results

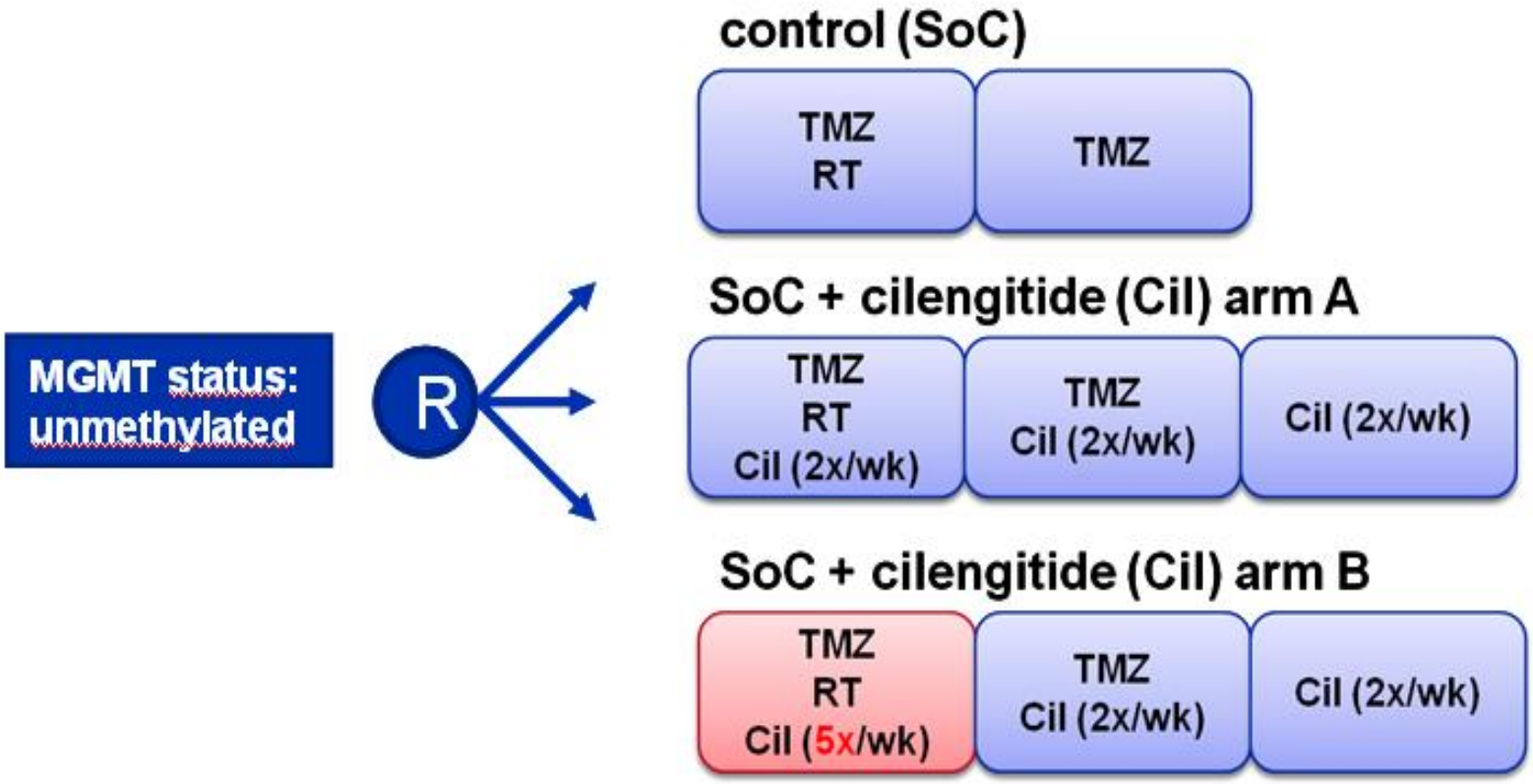
(Stupp et al, Lancet Oncol 2014)





Yes but : short high life of Cilengitide

GBM: Phase II CORE



n=265

Primary endpoint: OS

Potential radiosensitizing effect of β5/ β3 integrin inhibition when administred during each fraction of radiotherapy in GBM (5 f/ week)

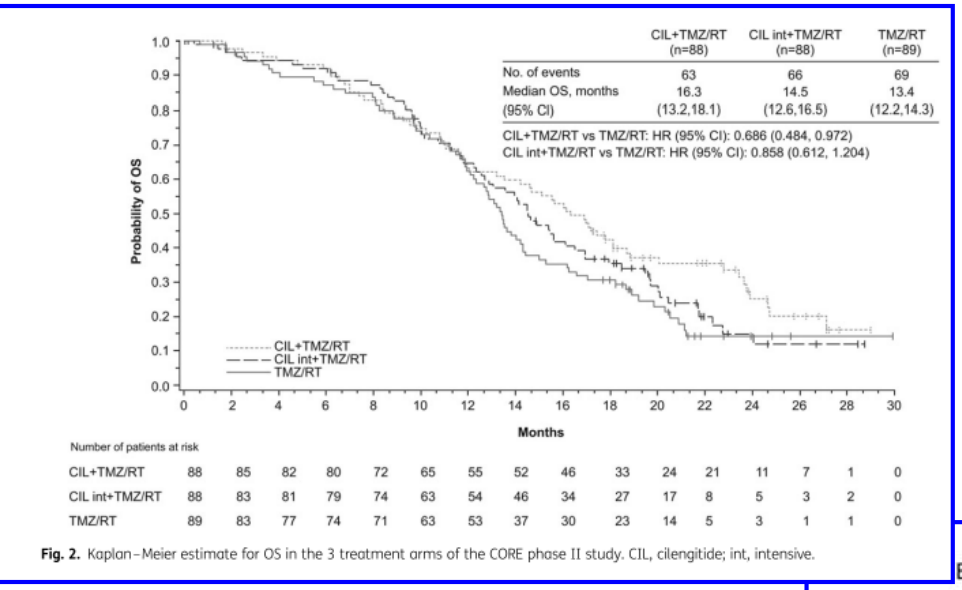


Fig. 2. Kaplan–Meier estimate for OS in the 3 treatment arms of the CORE phase II study. CIL, cilengitide; int, intensive.

What about continous infusion of Cilengitide during radiotherapy?

Radiosensitizing effect ?

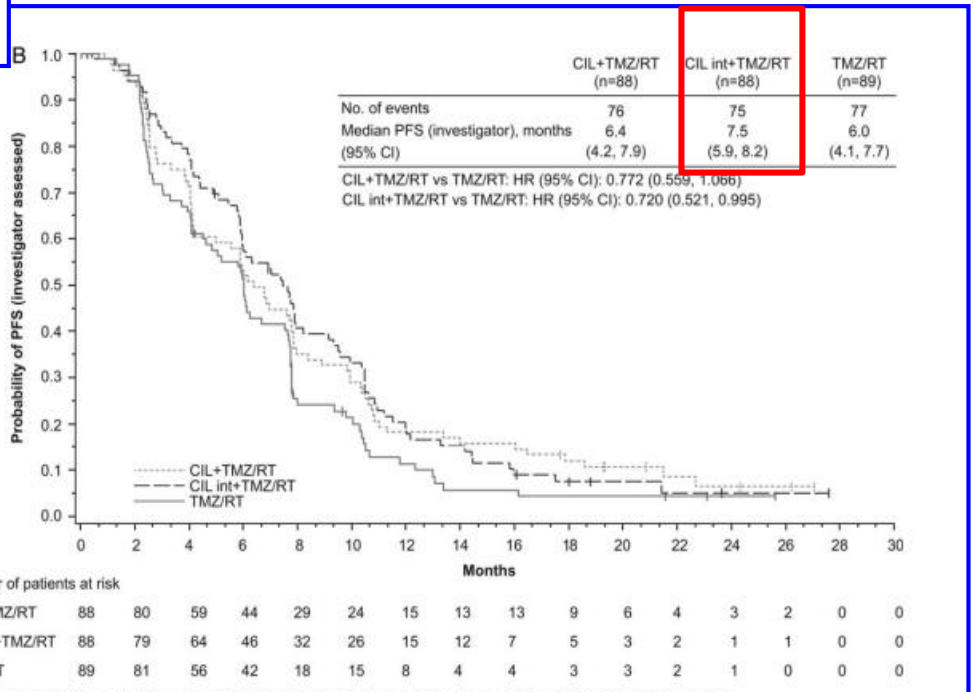
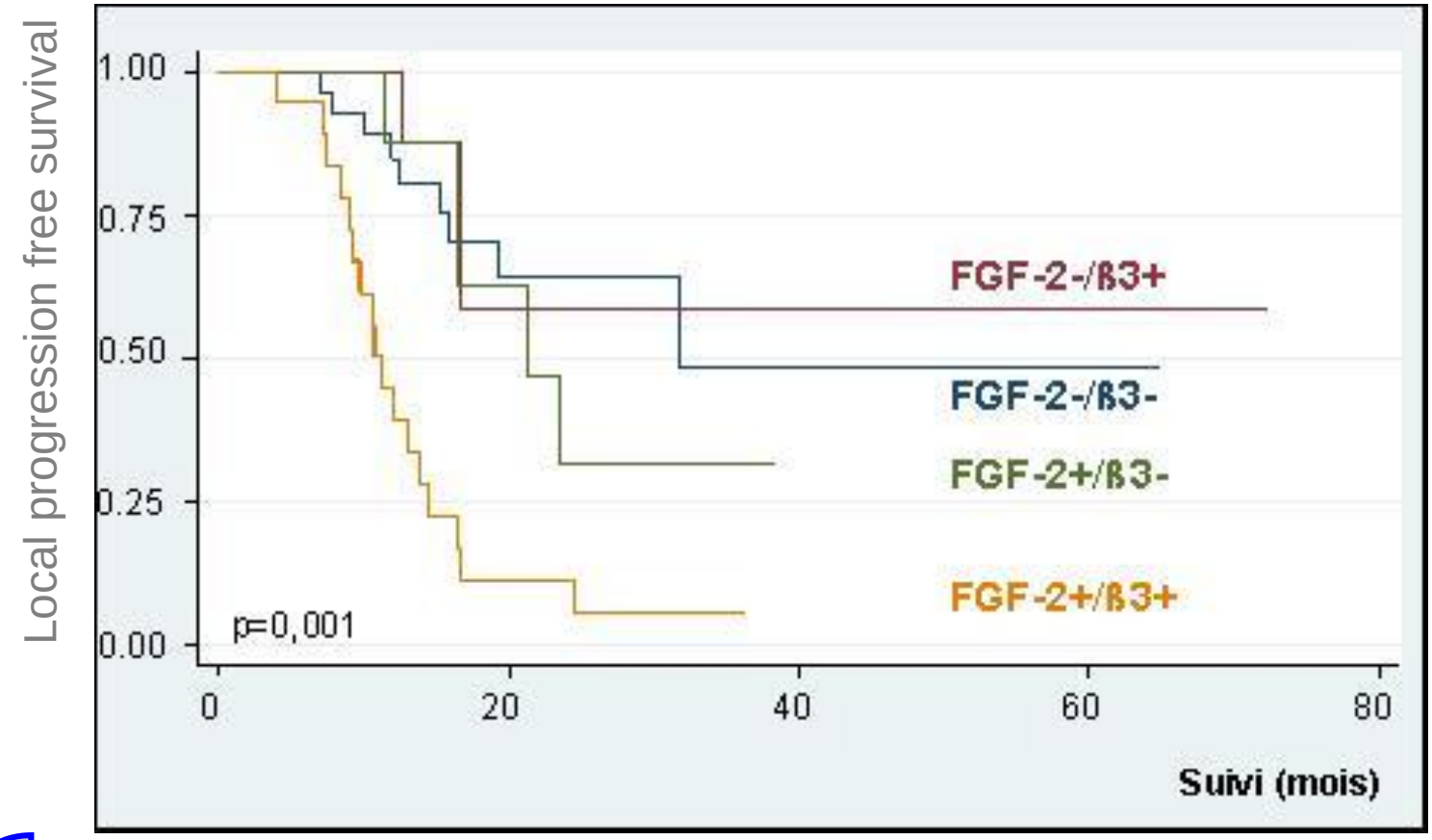


Fig. 3. Kaplan–Meier estimate for PFS assessed (A) by IRC and (B) by investigators. CIL, cilengitide; int, intensive.

(Nabors et al, Neuro-Oncology 2015)

β3 integrin-FGF-2 :protein expression profile correlated with local control after radio-chemotherapy in locally advanced NSCLC



(Massabeau et al Int J Radiat Biol Phys 2009)

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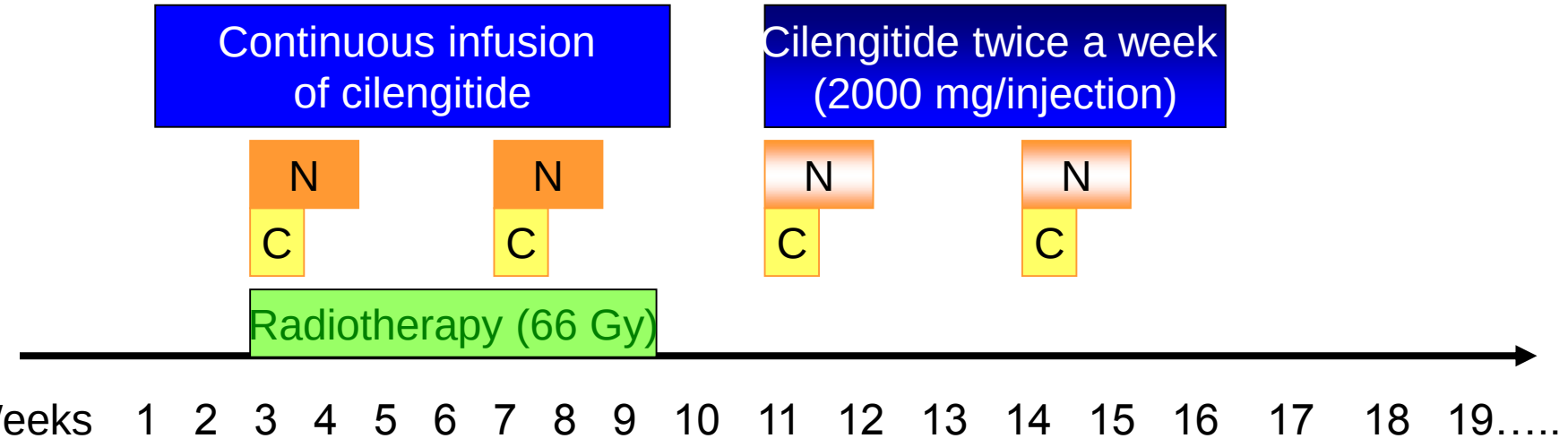


Dose Level	Schedule	Cilengitide dosing
-1	Continuous infusion (7 days pump) starting 2 weeks before radiotherapy until the end of radiotherapy	8 mg/h
Starting dose		**12mg/h**
+1		18 mg/h
+2		27mg/h
+3		40 mg/h

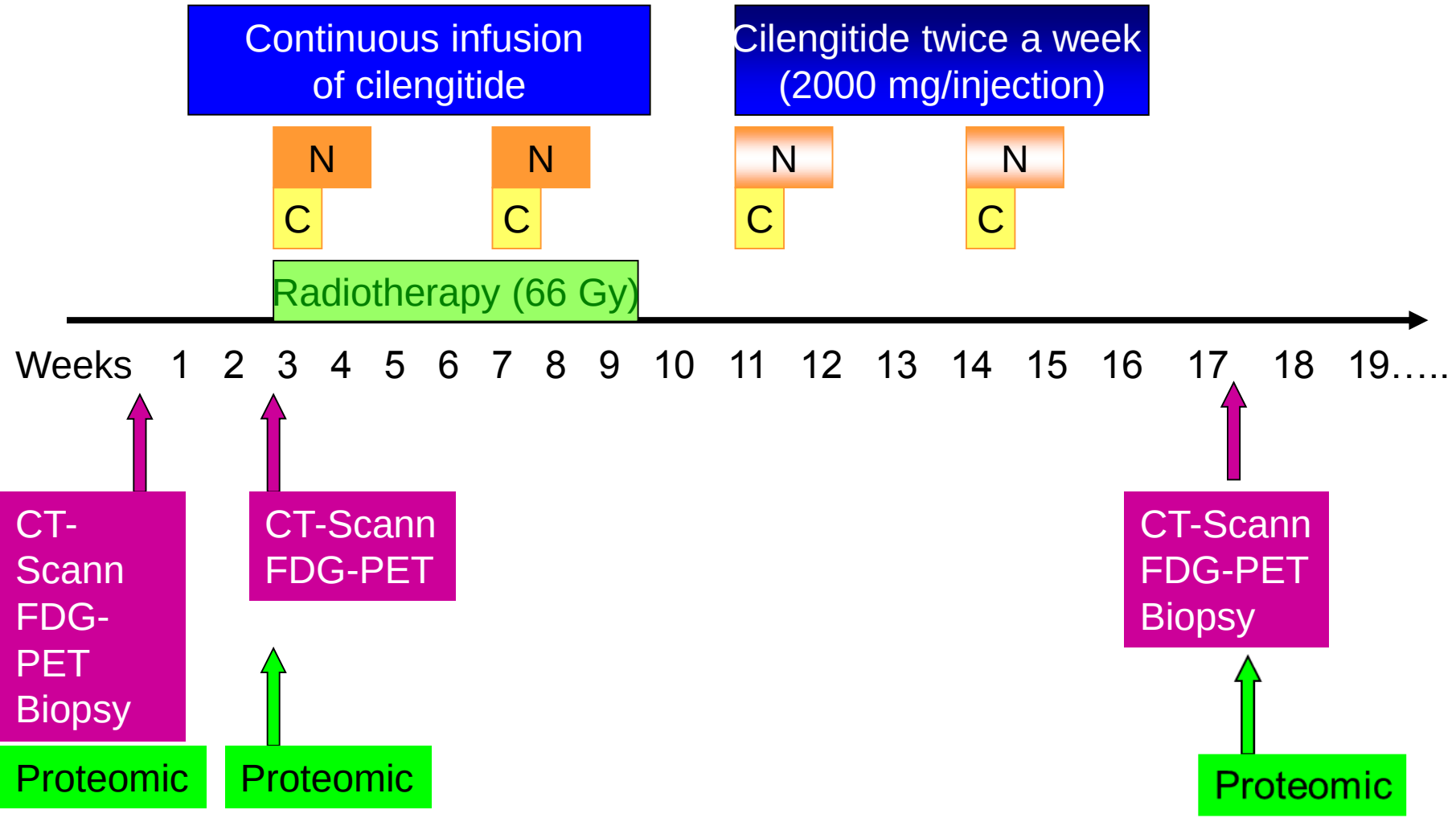
DLT period observation :
From week 3 to one month after the end of radio-chemotherapy

C — CDDP 80 mg/m^2

N — Navelbine 25 mg/m^2

N — Navelbine 15 mg/m^2

Translational research



Patients characteristics

Age (years) (range)	62.5 (43-75)
ECOG Performance status (%)	
0	7 (50%)
1	7 (50%)
Location	
Right lung	7 (50%)
Left Lung	6 (43%)
Mediastinum	1 (7%)
Histological type	
Adenocarcinoma	8 (57%)
Squamous cell carcinoma	5 (36%)
Undifferenciated carcinoma	1 (7%)
Overall stage	
IIIA	3 (21%)
IIIB	11 (79%)
T stage	
T0	1 (7%)
T2	2 (14%)
T3	3 (21%)
T4	8 (57%)
N stage	
N1	1 (7%)
N2	8 (57%)
N3	5 (36%)

- Fourteen patients were included between March 2010 and July 2013.
- Eleven patients were evaluable for DLT.

- Three patients were considered not evaluable for DLT and efficacy due to early withdrawn (Patient 1 and 14 for metastatic progressive disease on the imaging evaluation performed after 2 weeks of exclusive Cilengitide treatment while Patient 2 was withdrawn because of a cholestatic hepatitis in a context of pulmonary infection at week 4

Dose Level	Schedule	Cilengitide dosing	N patients
-1	Continuous infusion (7 days pump) starting 2 weeks before radiotherapy until the end of radiotherapy	8 mg/h	
Starting dose		**12mg/h**	5 (2 replaced)
+1		18 mg/h	3
+2		27mg/h	3
+3		40 mg/h	3

→ No DLT

→ **DLT : Tracheo-bronchial fistula in the radiotherapy fields**

After inclusion of the third patient at level 3, development of Cilengitide was interrupted by Merck KGa

Response

Response evaluated on TDM (RECIST)

	N	%
Best Response		
Partial response	9	81.8
Stable disease	2	18.2
Progression Before 6 months post RT		
No	9	81.8
yes	2	18.2

Response evaluated on PET (PERCIST)

	N	(%)
Response Week 3		
PET Evaluation		
Not Done	3	27
Done	8	72.7
Stable disease	7	87.5
Progression	1	12.5
Response 2 months post-RT		
Not Done	2	18.2
Done	9	81.8
Complete response	4	44.4
Partial response	4	44.4
Stable disease	1	11.1

Estimation TTP	N	%
Progression No	**5 (45,5)**	
Yes	6 (54,5)	

Median TTP : 14.4 m (95%CI=[8.4 ; Not Reach]

Estimation PFS	N	%
Progression or Death No	**4 (36.4)**	
Yes	7 (63.6)	

Median PFS : 14.4 m (95%CI=[8.4 ; Not Reach]

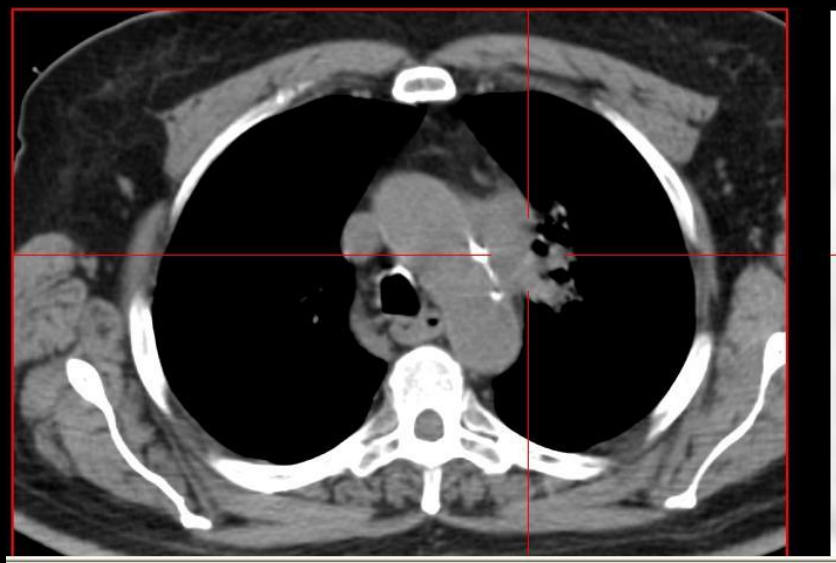
Estimation OS	N	%
Alive	**5**	**(45.5)**
Dead	**6**	**(54.5)**

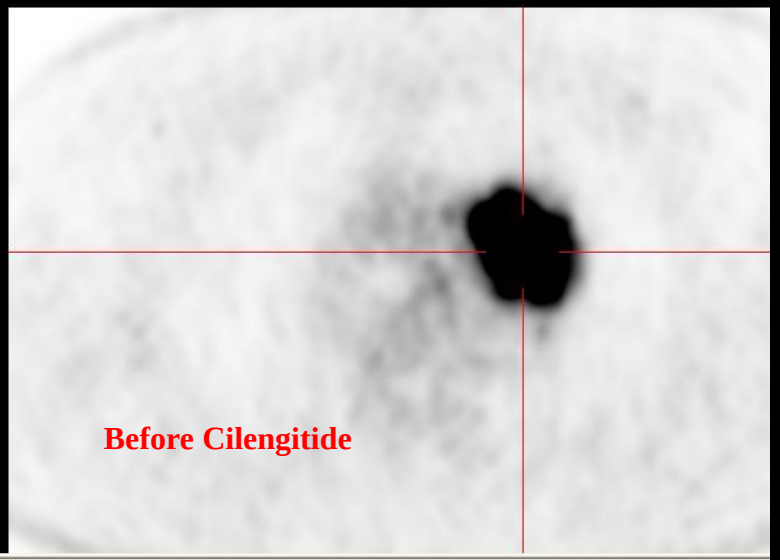
Median Overal Survival : 29.4 m
95%CI =[11.73; not reached]

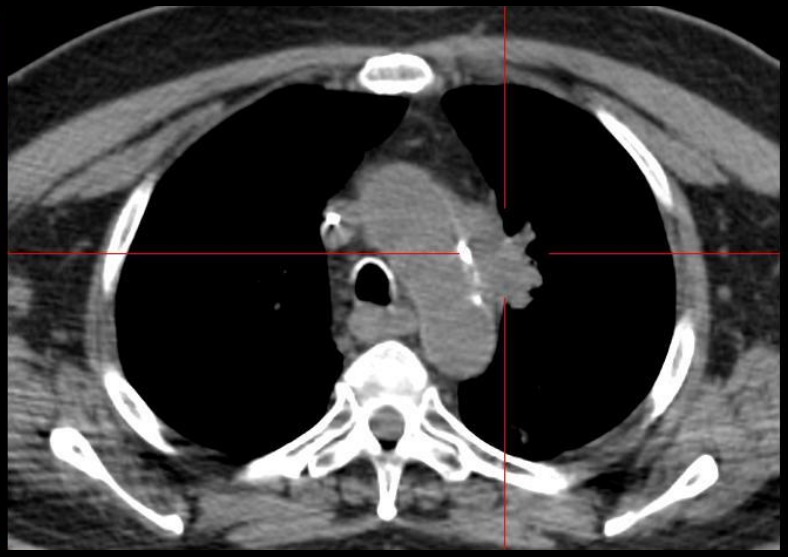
Dose Level	Best response	TEP 3 weeks	TEP 18 weeks	Delay (m)	Local progression	Progression or death
0	PR	Stable	PR	38.18		yes
0	PR	Stable	CR	52.99	No	No
0	PR	PD	Stable	10.61		yes
1	PR	Stable	CR	45.24	No	No
1	PR		CR	9.89	No	Yes
1	Stable		PR	8.87		Yes
2	PR	Stable	PR	14.36		yes
2	PR	Stable	PR	8.38		Yes
2	Stable			3.06		Yes
3	PR	Stable	CR	23.46	No	No
3	PR	Stable		13.31	No	No

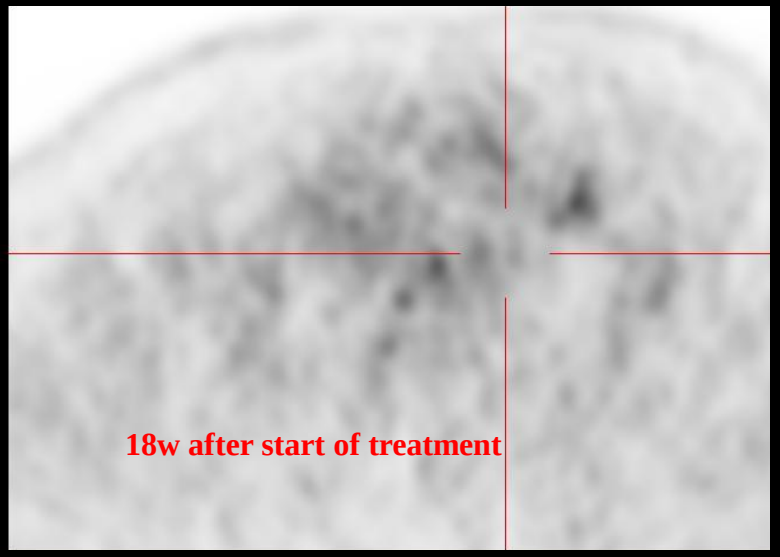
Proteomic analysis before and after Cilengitide for patients CR vs PR

TEP-TDM patient 4 (Dose level 0) : CR

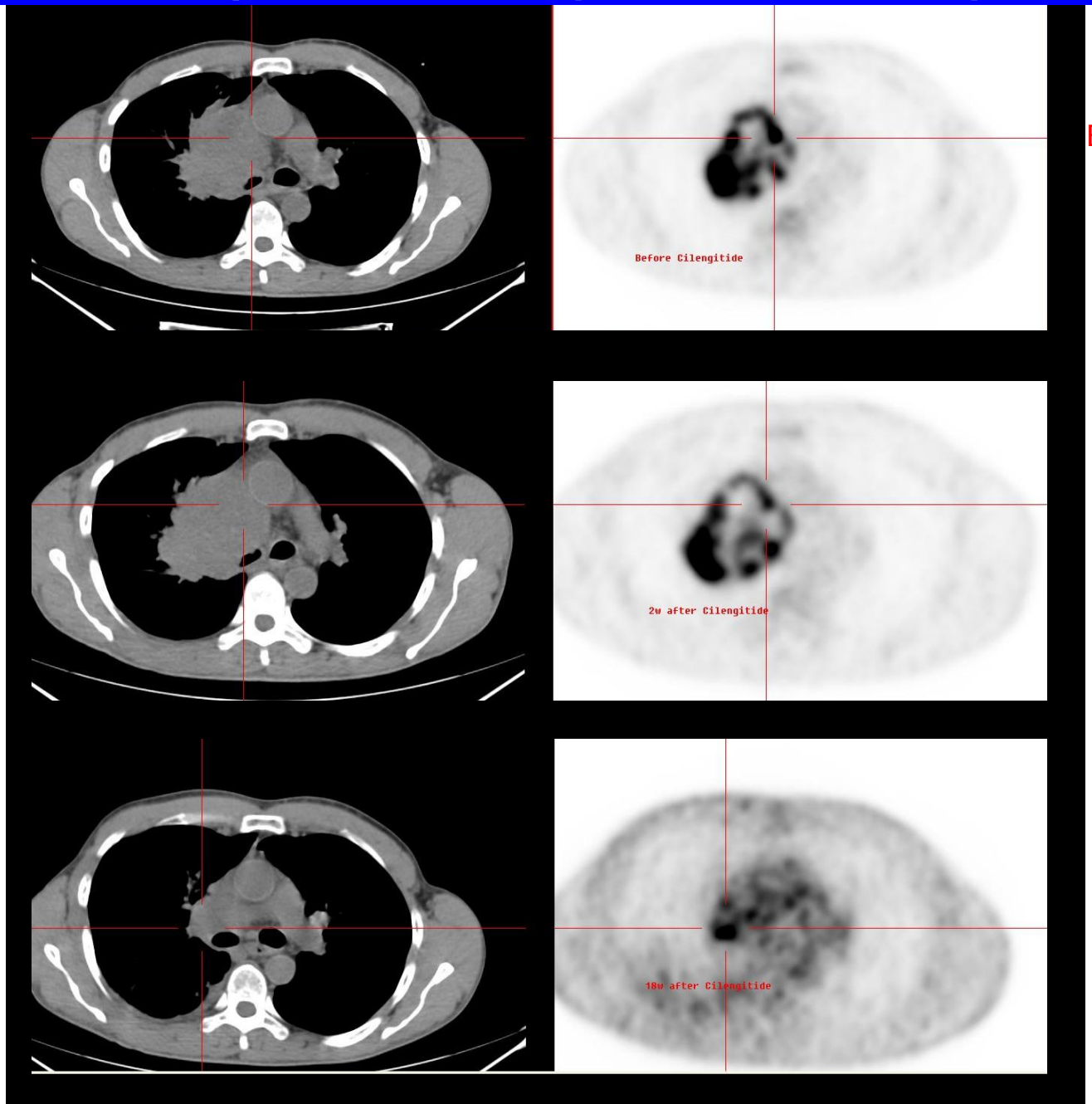








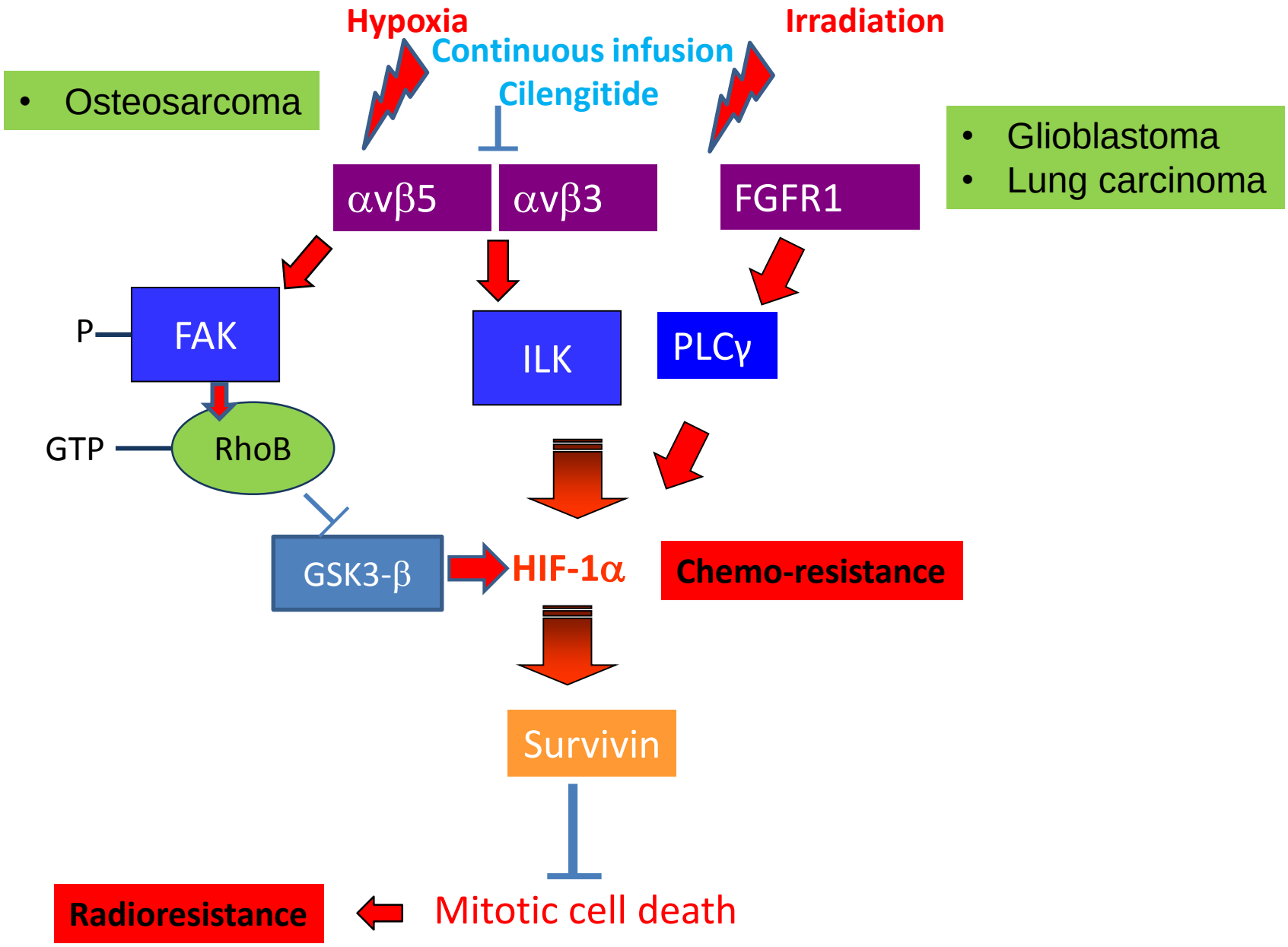
TEP-TDM patient 10 (Dose level 2) : PR



Before treatment

After 2 weeks cilengitide

After 18 weeks



Proteomic analysis is currently performed to predict good responders to this treatment and to bring to light new targets

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from signalling pathway to clinical trial »
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