

U.O. NEUROCHIRURGIA NEURO-ONCOLOGIA



**Istituto Giannina Gaslini
Ospedale Pediatrico IRCCS**



Codevilla, 23/03/2014

Diagnosi molecolare geni coinvolti nei tumori cerebrali che vengono offerti alle famiglie dal 2012 ad oggi

- **INI1/SMARCB1** (Atypical Rhabdoid Tumor): **12 casi (4 casi tra il 2013 e il 2014)**. *Orphanet (<http://www.orpha.net>)*.
- **NF2** (Neurofibromatosi tipo 2): **10 casi (3 casi tra il 2013 e il 2014)**. *Orphanet (<http://www.orpha.net>)*.
- mutazione **V600E** del gene **BRAF** (Gangliogliomi) e verifica della presenza di **fusione** genica tra il locus **BRAF** ed il locus **KIAA1549** (Astrocitomi pilocitici e/o Low Grade Glioma a istologia dubbia): **15 casi (10 casi tra il 2013 e il 2014)**. *A completamento della diagnosi istologica (Dr. P. Nozza)*

*E' in corso un censimento delle attività di genetica dell'anno 2013 richiesto dal **Dipartimento Ligure di Genetica**.*

Publicazioni inerenti l'attività di diagnosi molecolare

Capra V, Mascelli S, Garrè ML, Nozza P, Vaccari C, Bricco L, Sloan-Béna F, Gimelli S, Cuoco C, Gimelli G, Tassano E. **Parental imbalances involving chromosomes 15q and 22q may predispose to the formation of de novo pathogenic microdeletions and microduplications in the offspring.** *PLoS One.* 2013;8(3):e57910. **IF: 4.092**

Samantha Mascelli, Mariasavina Severino, Alessandro Raso, Paolo Nozza, Elisa Tassano, Giovanni Morana, Patrizia De Marco, Elisa Merello, Claudia Milanaccio, Marco Pavanello, Andrea Rossi, Armando Cama, Maria Luisa Garrè, Valeria Capra. **“Constitutional chromosomal abnormalities at 22q11 and 15q26 in a child with a pilocytic astrocytoma of the spinal cord”** *Molecular Cytogenetics* 2013 [under second revision]. **IF: 2.36**

Luca Pio, Samantha Mascelli, Alessandro Raso, Paolo Nozza, Claudia Milanaccio, Angela Rita Sementa, Armando Cama, Piero Buffa, Stefano Avanzini, Marianna Vannati, Valeria Capra, Stefano Giardino, Loredana Amoroso, Manila Antonelli, Salvina Barra, Edoardo Lanino, Andrea Rossi, Giovanni Morana, Gian Michele Magnano, Mariasavina Severino, Maria Luisa Garrè. **Congenital Multifocal Rhabdoid Tumor: a case with peculiar biological behavior and different response to treatment according to location (CNS and Kidney).** *Cancer Genetics* 2014 [under revision]. **IF: 1.915**

Publicazioni del 2013

1: Vecchio D, Daga A, Carra E, Marubbi D, Baio G, Neumaier CE, Vagge S, Corvò R, Pia Brisigotti M, Louis Ravetti J, Zunino A, Poggi A, Mascelli S, Raso A, Frosina G. Predictability, efficacy and safety of radiosensitization of glioblastoma-initiating cells by the ATM inhibitor KU-60019. *Int J Cancer*. 2013 Dec 19. doi: 10.1002/ijc.28680. [Epub ahead of print] PubMed PMID: 24443327.

2: Prato G, Mancardi MM, Baglietto MG, Janis S, Vercellino N, Rossi A, Consales A, Raso A, Garrè ML. Congenital Segmental Lymphedema in Tuberous Sclerosis Complex With Associated Subependymal Giant Cell Astrocytomas Treated with Mammalian Target of Rapamycin Inhibitors. *J Child Neurol*. 2013 Sep 20. [Epub ahead of print] PubMed PMID: 24056156.

3: Mascelli S, Barla A, Raso A, Mosci S, Nozza P, Biassoni R, Morana G, Huber M, Mircean C, Fasulo D, Noy K, Wittemberg G, Pignatelli S, Piatelli G, Cama A, Garrè ML, Capra V, Verri A. Molecular fingerprinting reflects different histotypes and brain region in low grade gliomas. *BMC Cancer*. 2013 Aug 15;13:387. doi: 10.1186/1471-2407-13-387. PubMed PMID: 23947815; PubMed Central PMCID: PMC3765921.

4: Occella C, Bleidl D, Nozza P, Mascelli S, Raso A, Gimelli G, Gimelli S, Tassano E. Identification of an interstitial 18p11.32-p11.31 duplication including the EMILIN2 gene in a family with porokeratosis of Mibelli. *PLoS One*. 2013 Apr 10;8(4):e61311. doi: 10.1371/journal.pone.0061311. Print 2013. PubMed PMID: 23593459; PubMed Central PMCID: PMC3622678.

1: Capra V, Mascelli S, Garrè ML, Nozza P, Vaccari C, Bricco L, Sloan-Béna F, Gimelli S, Cuoco C, Gimelli G, Tassano E. Parental imbalances involving chromosomes 15q and 22q may predispose to the formation of de novo pathogenic microdeletions and microduplications in the offspring. *PLoS One*. 2013;8(3):e57910. doi: 10.1371/journal.pone.0057910. Epub 2013 Mar 6. PubMed PMID: 23483941; PubMed Central PMCID: PMC3590287.

Progetti in corso

AT/RTs

Multi-omics analysis of Atypical Teratoid Rhabdoid Tumors (AT/RTs).

Fondazione Guido Berlucchi **O.N.L.U.S. Associazione per la ricerca sui tumori cerebrali del bambino**

Rhabdoid Tumour (RT): a case report with peculiar biological behaviour and results of treatment (Tx) according to different location (CNS and Kidney).

Milanesi M, M. L., ...

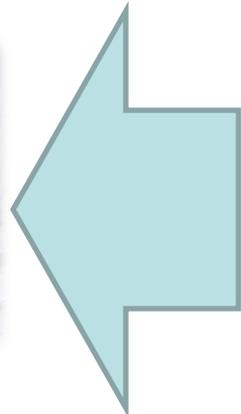
The abstract discusses the genetic and clinical features of Rhabdoid Tumours (RTs). It highlights the presence of the *SMO1* gene and the *SMO1* gene expression profile. The text describes the clinical course of the patient, including the initial diagnosis, treatment, and the subsequent relapse. The abstract also mentions the use of multi-omics analysis to identify the underlying genetic alterations. The diagram shows the *SMO1* gene structure and the expression profile of the *SMO1* gene across different tissues.



International rhabdoid, tumor group meeting; Parigi 12-14 dicembre 2013

Gli aspetti discussi durante il congresso sono stati:

- a) diagnosi patologica e molecolare,
- b) alterazioni genetiche associate alla biologia degli TR,
- c) sviluppo di modelli animali atti a sperimentare nuove terapie,
- d) nuovi protocolli terapeutici e sviluppo di nuovi bersagli terapeutici.

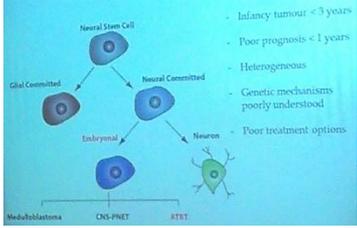
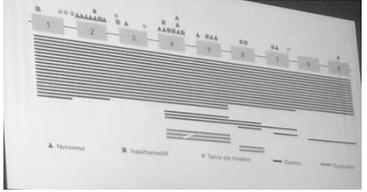


Multi-omics analysis of Atypical Teratoid Rhabdoid Tumors (AT/RTs)

Tale sequenziamento riguarderà:

- 1, analisi epigenetiche (Epigenetic modification analysis);
- 2, analisi del trascrittoma (transcriptome sequencing) e
- 3, analisi dei microRNA (small RNA sequencing).

>80% dei TR origina dall'inattivazione biallelica (entrambe le copie sono mutate e/o perse) del gene *SMARCB1 (INI1)* nelle cellule tumorali.



CNS AT/RT are a heterogeneous entity that comprises subgroups with distinct molecular profiles

- Infancy tumour < 3 years
- Poor prognosis < 1 years
- Heterogeneous
- Genetic mechanisms poorly understood
- Poor treatment options

Gliomi:

Gliomi di alto grado (HGG)

Effetto del KU60019 come radio-sensibilizzante nelle cellule con fenotipo staminale.

In collaborazione con Molecular Mutagenesis & DNA Repair Unit, IRCCS Azienda Ospedaliera Universitaria San Martino—IST, Genova, Italy. (Dr. G.Frosina).

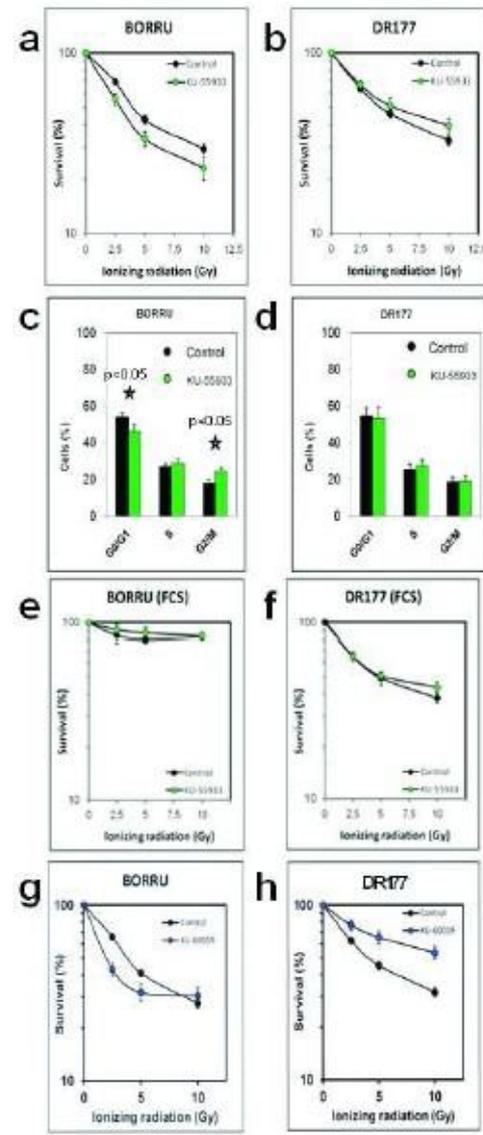
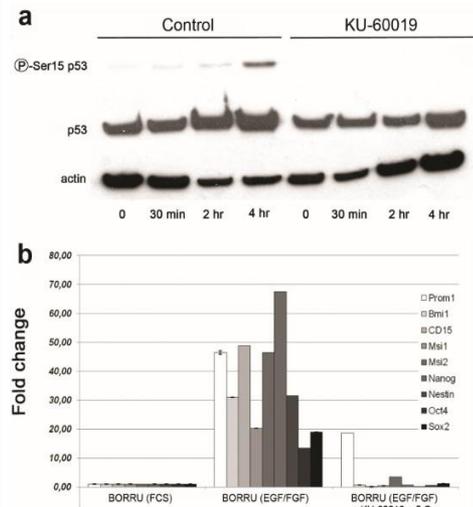
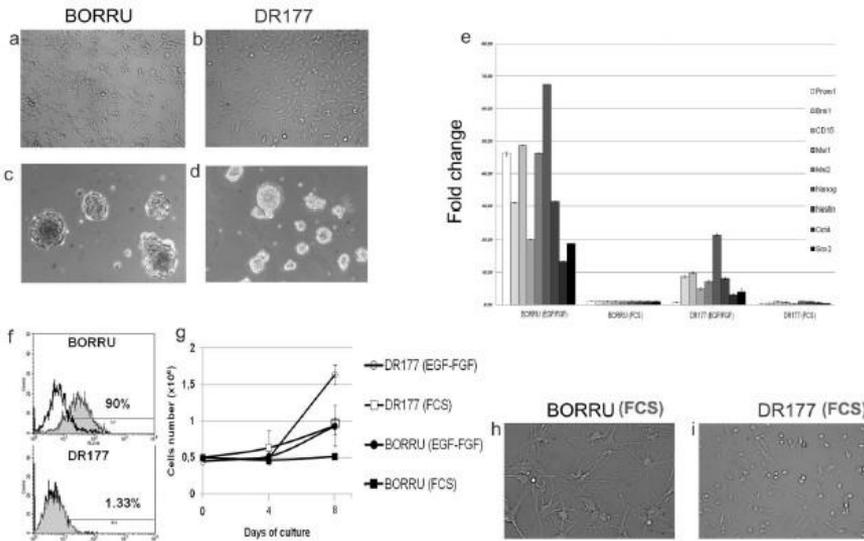
Gliomi (Tutti)

DOPA-PET

Gliomi a basso grado (LGG)

Characterization of Glioma Stem Cells Through Multiple Stem Cell Markers and Their Specific Sensitization to Double-Strand Break-Inducing Agents by Pharmacological Inhibition of Ataxia Telangiectasia Mutated Protein

Alessandro Raso¹; Donatella Vecchio²; Enrico Cappelli³; Monica Ropolo²; Alessandro Poggi⁴; Paolo Nozza⁵; Roberto Biassoni⁶; Samantha Mascelli¹; Valeria Capra¹; Fotios Kalfas⁷; Paolo Severi⁷; Guido Frosina²



Stabilizzazione di nuove linee cellulari da GBL (grado IV) con caratteristiche di staminalità e non.

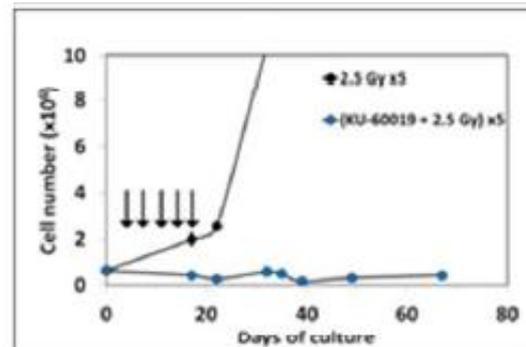
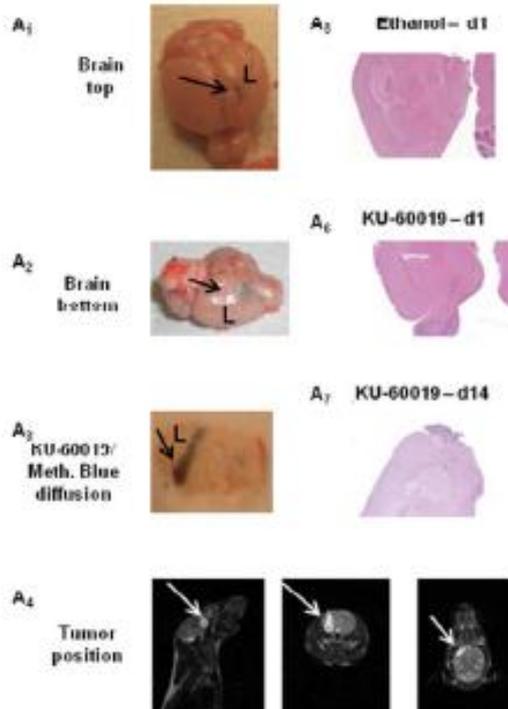
Verificato l'effetto del **KU60019** come radio-sensibilizzante nelle cellule con fenotipo staminale.
L'inibizione del pathway di ATM

Predictability, efficacy and safety of radiosensitization of glioblastoma-initiating cells by the ATM inhibitor KU-60019

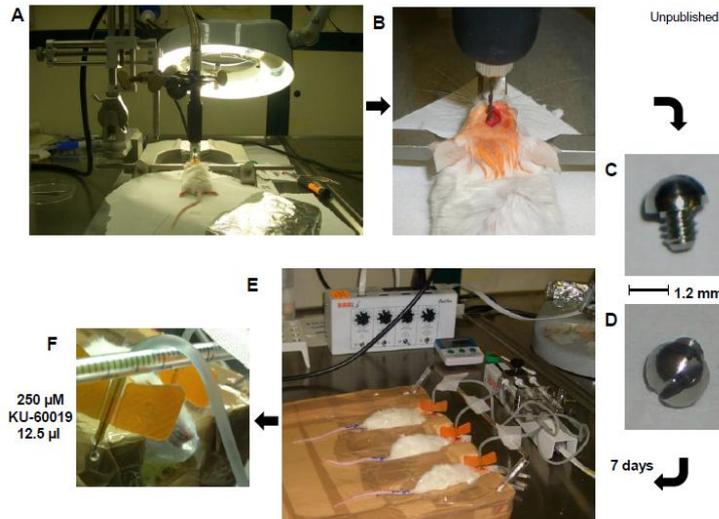
Donatella Vecchio¹, Antonio Daga², Elisa Carra², Daniela Marubbi^{2,3}, Gabriella Baio⁴, Carlo E. Neumaier⁴, Stefano Vagge⁵, Renzo Corvò⁵, Maria Pia Brisigotti⁶, Jean Louis Ravetti⁶, Annalisa Zunino⁶, Alessandro Poggi⁷, Samantha Mascelli⁸, Alessandro Raso⁸ and Guido Frosina¹

Table 1. Determinants of the response to KU-60019¹

GSC line	D ₃₇ Ethanol (Gy)	D ₃₇ KU-60019 (Gy)	DER	Response to KU-60019	ROC analysis	Gene expression							Proliferative activity PDT (h)	
						DDR			Stem		PI3K/Akt			
						TP53	ATM	MDM2	PROM1	NES	PI3K	Akt		PTEN
BORRU	6.20	3.25	1.91	+		1.16	2.56	1.47	8.64	1.16	1.00	0.98	1.96	53.9
VIPI	8.6	4.8	1.79	+		0.08	0.04	0.12	0.43	0.46	7.57	1.92	0.14	18.0
COMI	3.70	2.10	1.76	+		0.19	1.14	1.38	1.43	2.36	3.16	2.05	2.38	60.0
MPM176	6.40	3.75	1.71	+		0.00	0.58	0.13	0.47	0.33	2.27	0.65	2.95	31.5
DEMI	>10	>10	1	-		1.56	1.06	0.25	3.08	2.41	0.07	2.07	0.48	56.5
PERU	>10	>10	1	-		7.67	9.61	6.62	1.76	1.94	0.42	1.14	1.00	58.9
2.11	1.60	2.50	0.64	-		3.52	2.40	3.36	1.06	1.63	0.36	1.00	0.81	63.5
DR177	7.80	19.7	0.39	-		1.13	0.58	5.86	0.06	0.30	0.31	0.61	0.01	32.9
P						<0.0001	ns	<0.01	ns	ns	<0.0001	ns	ns	ns
Cut Off						≤0.19	-	≤1.47	-	-	>0.42	-	-	-

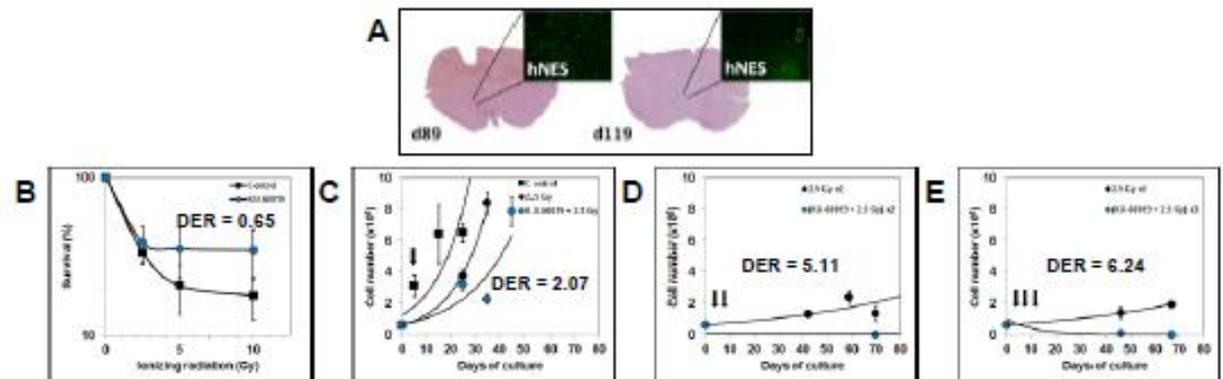


Pharmacokinetics, pharmacodynamics and efficacy on pediatric tumors of the glioma radiosensitizer KU60019



Both adult and pediatric GIC were radiosensitized after exposure to KU60019. The results indicate that ATM inhibition may be a radiosensitizing procedure effective on a wide range of adult and pediatric GBM.

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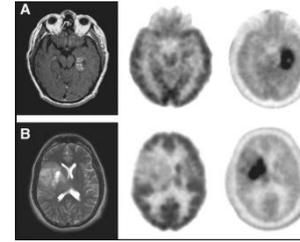


18F-DOPA PET: Fluorine-18-L-dihydroxyphenylalanine

Alto Grado (III, IV)
Grado II
Basso Grado (I)



Captazione del tracciante



RAZIONALE

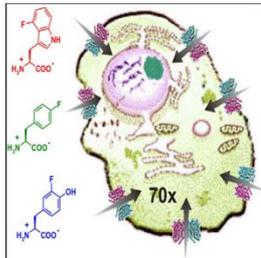
Proliferative Activity in Human Brain Tumors:
Comparison of Histopathology and
L-[1-¹¹C]Tyrosine PET

J Neurooncol
DOI 10.1007/s11060-012-0986-1

LABORATORY INVESTIGATION

The role of LAT1 in ¹⁸F-DOPA uptake in malignant gliomas

Ryan S. Youland · Gaspar J. Kitange · Timothy E. Peterson · Deanna H. Pafundi ·
Judith A. Ramiscal · Jenny L. Pokorny · Caterina Giannini · Nadia N. Laueck ·
Ian F. Parney · Val J. Lowe · Debra H. Brinkman · Jann N. Sarkaria



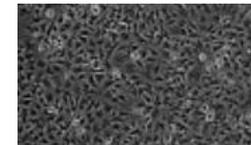
Systems L: LAT1 Complex

Systems ASC: ASCT1/2 Complex

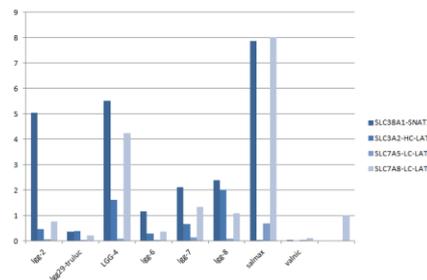
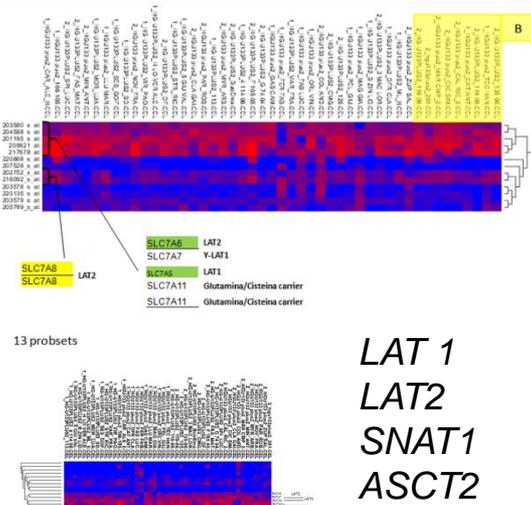
System A: SNAT1

RISULTATI PRELIMINARI

Analisi espressione su tutta la casistica >qPCR
Analisi dell'immunofenotipo su paraffinato



*cell transfection with
mi interference*



- 1 Potenziale marcatore di progressione
- 2 Potenziale Target terapeutico

Pediatric Low grade gliomas (LGGs):

- The P72 TP53 may predict long survivals in subtotal or partial resected low grade glioma.
- Gene signature related to site of lesion: prognostic value.

In collaborazione con Asper Biotechnology, Ltd, Tartu, Estonia

Epidemiology and Biostatistics Unit, G. Gaslini Institute (Dott.ssa A. Pistorio).

Molecular Medicine Unit, G. Gaslini Institute (Dott. R. Biassoni).

Pathology Unit, G. Gaslini Institute (Dr. P. Nozza)

Pre-Clinical working group, SIOP. Heidelberg group (Dr. S. Pfister)

Computational Biology research group in Florence, "Careggi" University-Hospital (Dott. A. Magi).

- A distinct gene-expression profile in paediatric Ganglioglioma.

In collaborazione con Pathology Unit, G. Gaslini Institute (Dr. P. Nozza)

(David Eisenstat, MD, MA, FRCPC). Division Director, Pediatric Hematology/Oncology/Palliative Care Professor, Departments of Pediatrics and Medical Genetics, Adjunct Professor, Department of Oncology. Muriel & Ada Hole Kids with Cancer Society Chair in Pediatric Oncology Faculty of Medicine and Dentistry University of Alberta.

Confronto tra low grade gliomas in rapida progressione *versus* spontanea stabilizzazione della malattia

**SIOP-LGG
Preclinical Working Group**

Second Scientific Workshop, December 4-5, 2011
 Location: Heidelberg, Haus Buhl (Hauptstraße 234)
 Hosts: Olaf Witt & Stefan Pfister
 German Cancer Research Center and University Hospital Heidelberg

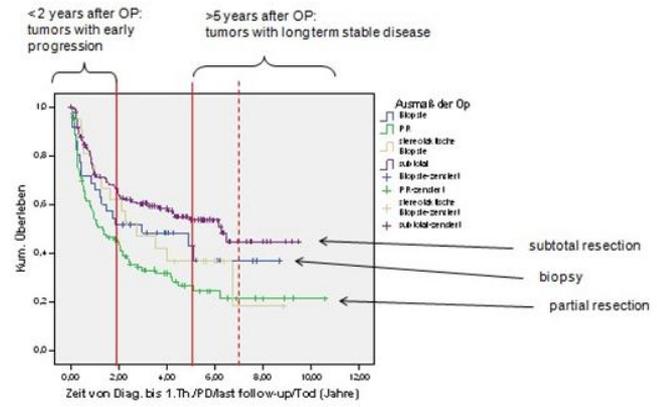
Biological studies on LGGs,
 on behalf of the Neuro-oncology group
 G. Gaslini Children's Research Institute
 Genoa-Italy.

SIOP-LGGs Pre-Clinical Working Group Meeting Heidelberg

ARTUCEBA.ORG

Associazione per la ricerca sui tumori cerebrali del bambino

HIT-LGG 1996
 EFS of incompletely resected PA WHO ¹ without therapy at diagnosis (n= 293)



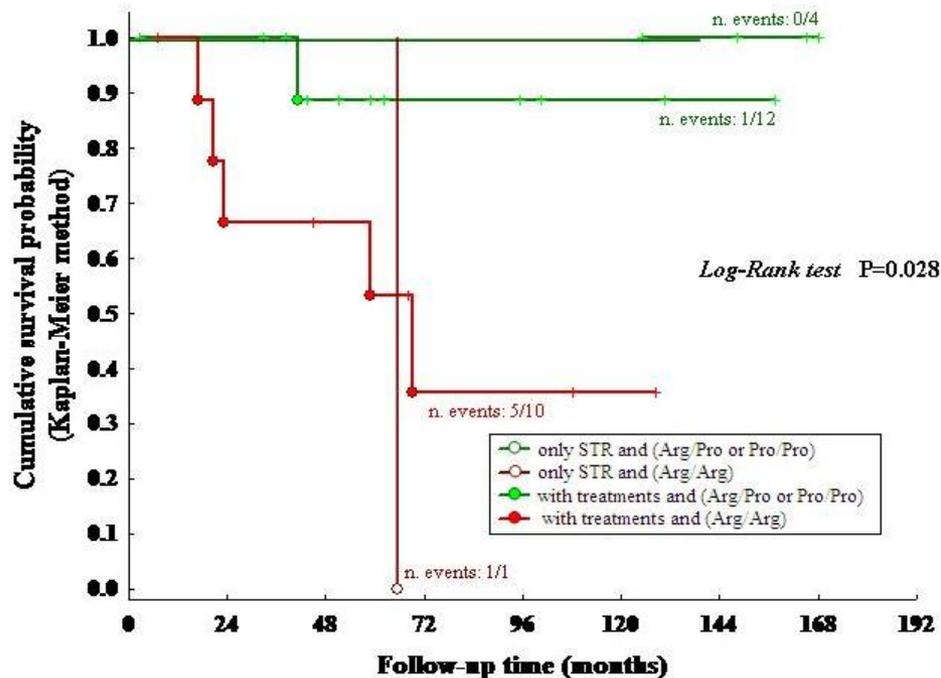
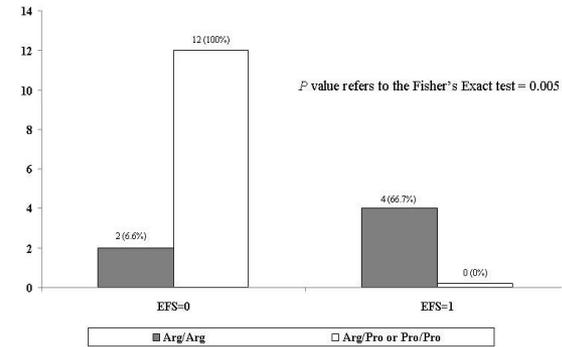
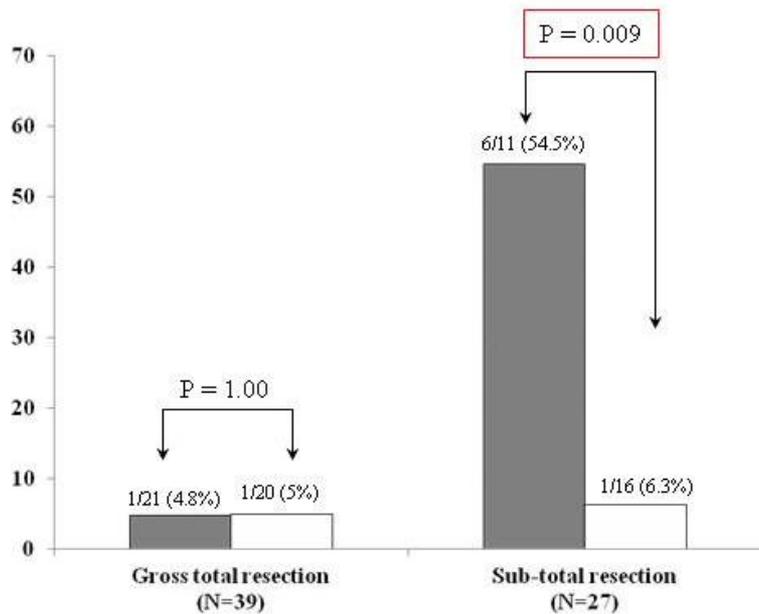
- CUT-OFFs:**
- progressing tumors <2 years after diagnosis as Early Progressors
 - stable tumors > 5 (6/7) years as Long Term Stable Disease.

8-9 Ottobre 2013, Padova

2014, Marsiglia

Esistono specifici marcatori molecolari predittivi per i LGGs in rapida progressione di malattia ?

p53
Guardiano del genoma
Pro72 TP53

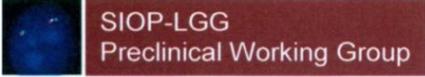


Nei pazienti in cui la resezione totale del tumore non è possibile, occorre valutare lo stato del polimorfismo (R72P) del gene TP53.

La variante Arg/Arg (R72P) è un fattore prognostico avverso per cui si manifestano casi di Early Progression.



U.O.C. Neurochirurgia, Istituto Giannina Gaslini,
Genova-Italia



SIOP-LGG
Preclinical Working Group

Samantha Mascelli/Alessandro Raso (Genoa, Italy)

- Molecular predictors for early progression in children affected by LGGs

on behalf of the Neuro-Oncology group
G. Gaslini Institute
Genoa-Italy.

October 8 & 9, 2013, Padova-Italia



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O.N.L.U.S. Associazione per la ricerca sui tumori cerebrali del bambino

Estensione dell'analisi a 140 casi di LGGs

Prof Dominique Figarella-Branger

Pathologique et Neuropathologie, Hôpital de la Timone, Marseille, Francia

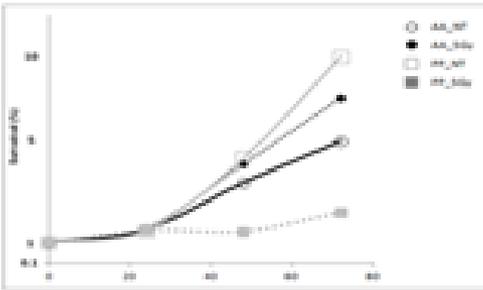
Prof Uri Tabori

Haematology/Oncology, The Hospital for Sick Children, Toronto, Canada

Test funzionali eseguiti su linee cellulari primarie di LGGs

Linee cellulari di gliomi WHO grado I Radio-trattate con dosi da 2.5 Gy, 5 Gy, 7.5 Gy, 10 Gy e 20 Gy.

N	CODICE	Prelievo	Diag. istol.	Sede	Sindr. genet.
1	1/02	12/06/2002	Na	FCP	
2	4/02	03/07/2002	Na	FCP	
3	28/03	13/02/2003	GG	temporale	
6	49/03	mar-03	Na	FCP	
7	65/04	4/07/2004	GG	FCP	
8	71/04	09/04/2004	Na		
12	89/03	30/3/03	Na	FCP	
13	109/03	11/10/2003	Na	TE	
16	111/03	23/11/03	Na	FCP	
17	113/03	05/11/2003	Na	parasellare	
20	133/03	02/08/2007	GG	fronto-mesiale dr.	



MTT test, Curve di crescita: la variante R72 sembra essere radio-resistente mentre, la variante P72 si mostra radio-sensibile.

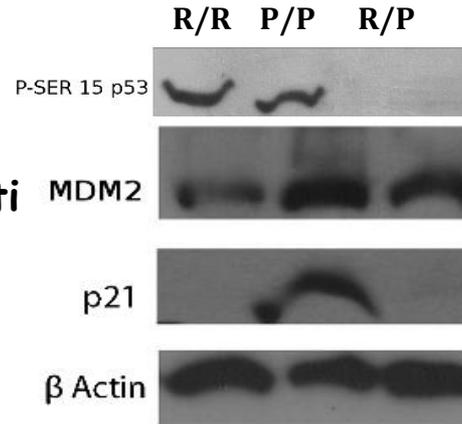
Radio-resistenti

Radio-sensibili

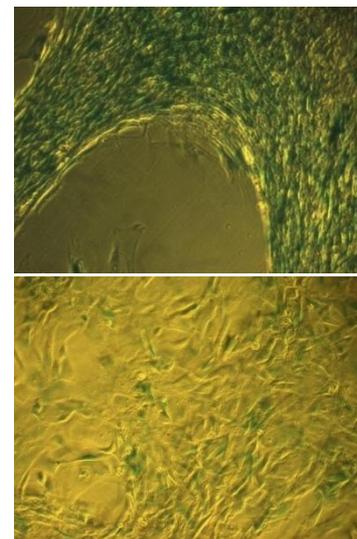
Senescenti

Non senescenti

Saggio apoptotico dell'annexina V



Saggio per la determinazione dell'attivazione del pathway indotto da p53



P/P
24h

Test di senescenza:
Saggio della β -galattosidasi

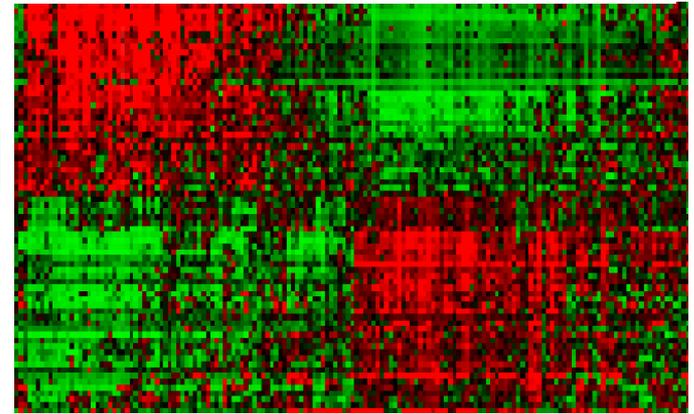
R/R
24h

Pathway alterati ??

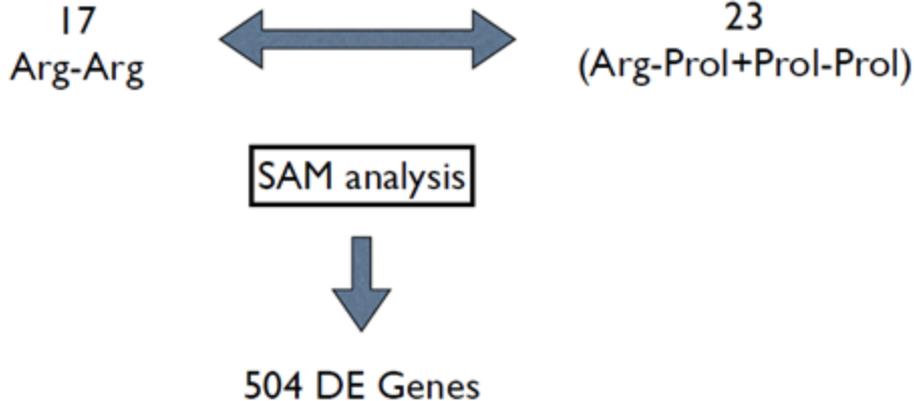


Computer Science
(Dipartimento di Area Critica
Medico Chirurgica)
Università degli Studi di Firenze,
Firenze, Italy

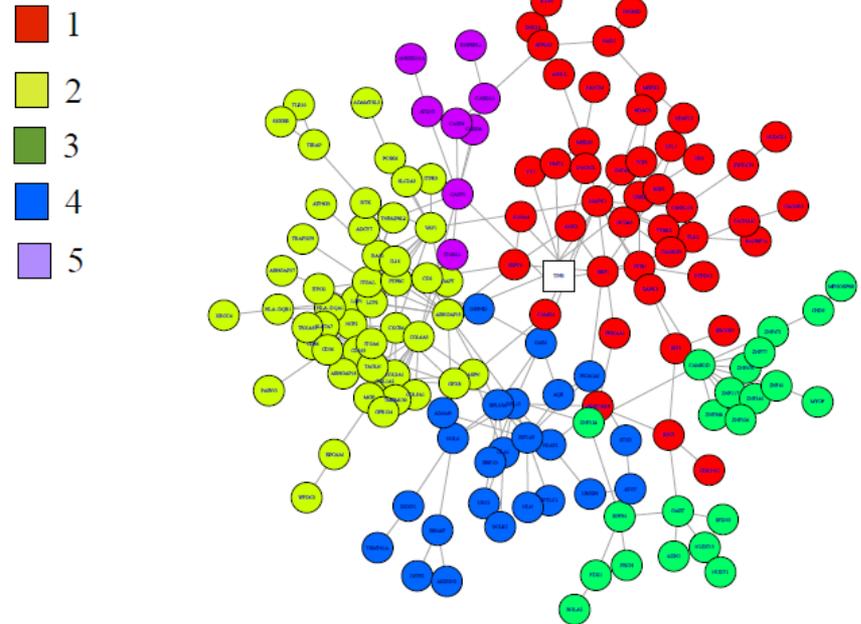
Microarray technology
(GeneChip® H.G. U133 Plus 2.0 Array Affymetrix)



Arg-Arg vs (Arg-Prol + Prol-Prol)



148 DE genes Network - Communities



RESEARCH ARTICLE

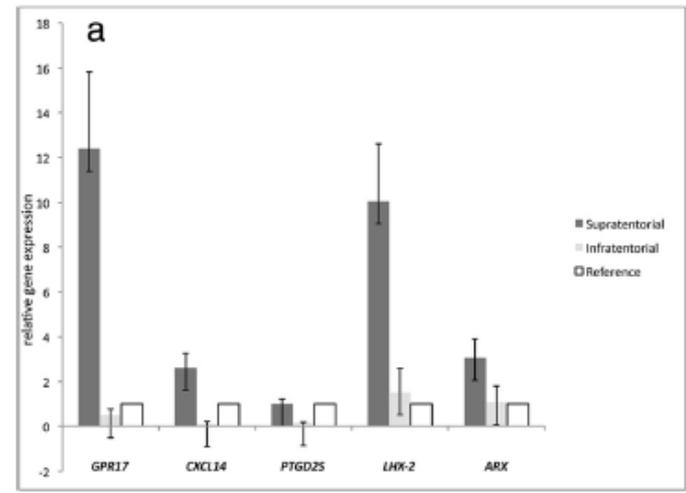
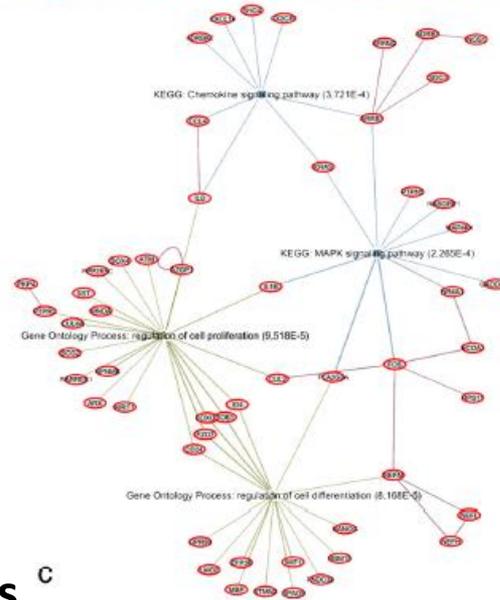
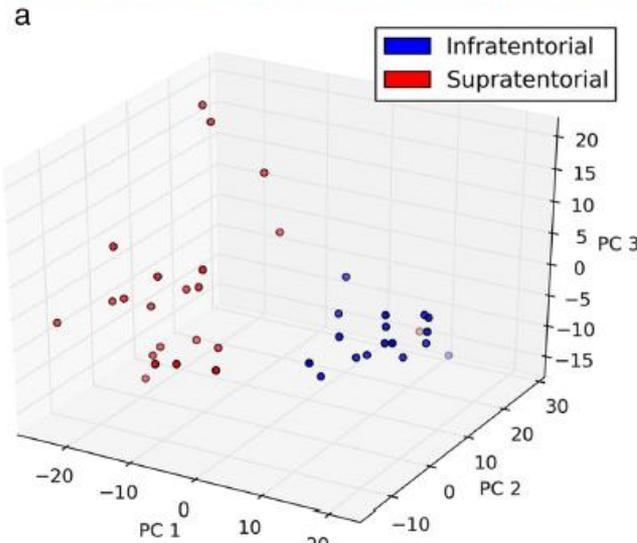
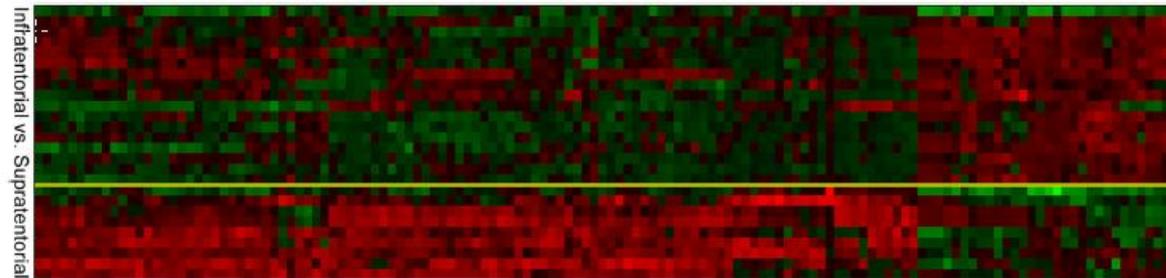
Open Access

Molecular fingerprinting reflects different histotypes and brain region in low grade gliomas

Samantha Mascelli^{1†}, Annalisa Barla^{2†}, Alessandro Raso¹, Sofia Mosci², Paolo Nozza³, Roberto Biassoni⁴, Giovanni Morana⁵, Martin Huber⁶, Cristian Mircean⁶, Daniel Fasulo⁷, Karin Noy⁷, Gayle Wittemberg⁷, Sara Pignatelli⁸, Gianluca Piatelli¹, Armando Cama¹, Maria Luisa Garré⁸, Valeria Capra¹ and Alessandro Verri²

Table 4 qPCR expression values for the selected 15 genes

Gene name	Supratentorial		Infratentorial		ΔCt median	I. vs. S. p-value
	ΔCt	2 ^{-ΔΔCt}	ΔCt	2 ^{-ΔΔCt}		
ABBA1	2.19	1.46	2.88	0.9	2.72	-
APOD	-1.33	0.69	-2.46	1.6	-1.87	-
ARX	6.03	2.46	8.25	0.53	7.33	0.03
CXCL14	0.84	12.61	7.17	0.18	4.5	0.009
FOSB	4.15	0.98	3.84	1.27	4.11	-
FOXC1	4.65	0.7	3.38	1.69	4.14	-
GPR17	2.85	1.52	8.18	0.04	3.43	0.0049
LHX2	4.6	7.59	9.04	0.35	7.32	0.01
NRXN2	6.28	1.18	6.52	0.99	6.51	-
PTGD2S	1.34	1.66	5.4	0.1	2.08	0.0049
SDC3	2.9	0.94	1.79	2.03	2.81	-
SNX22	6.98	0.73	6.34	1.11	6.5	-
SPOCK1	3.99	0.88	3.06	1.69	3.81	-
TIMP4	2.62	0.92	2.42	1.05	2.48	-
ZFHX4	-0.43	0.85	-0.84	1	-0.84	-

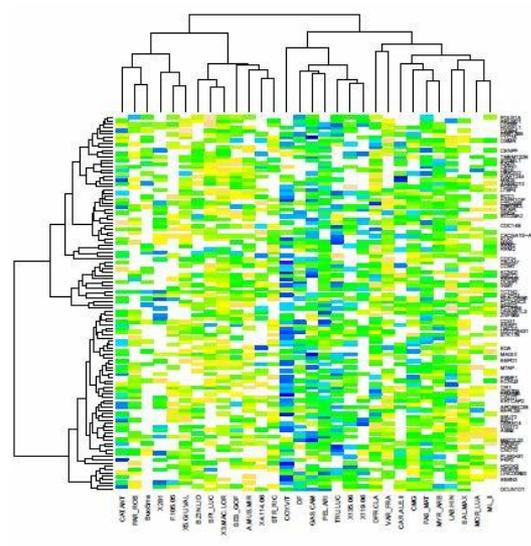


206 DE genes

“Gene signature related to site of lesion”: ha anche un valore prognostico ?

Best fitted Poisson Regression model

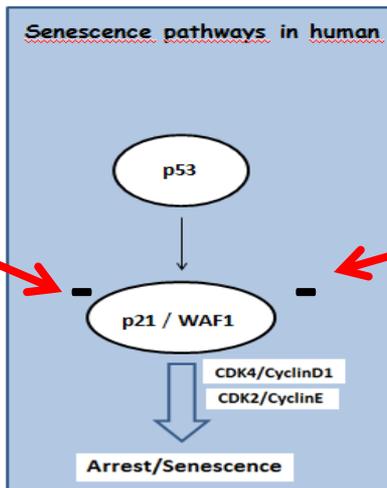
Patients WHO I	Hazard Ratio (IRR)	95% CI [#]	p*
EFS (No of events=9/27, 33.3%)			
KIAA0101 expression > 0.248	6.5	1.7 - 24.2	0.007



114 DE genes

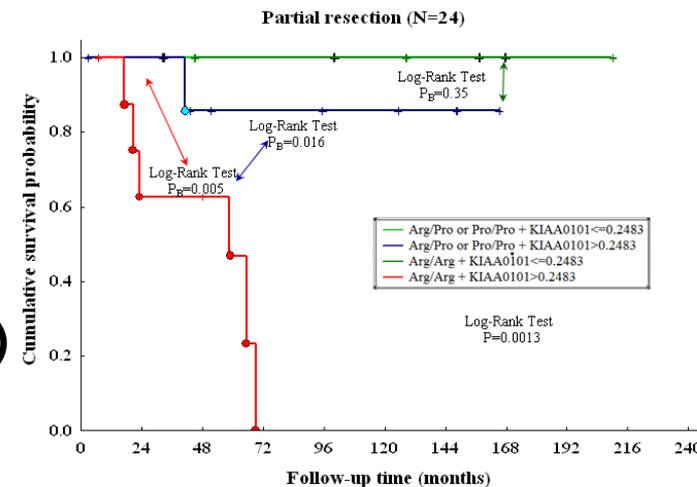
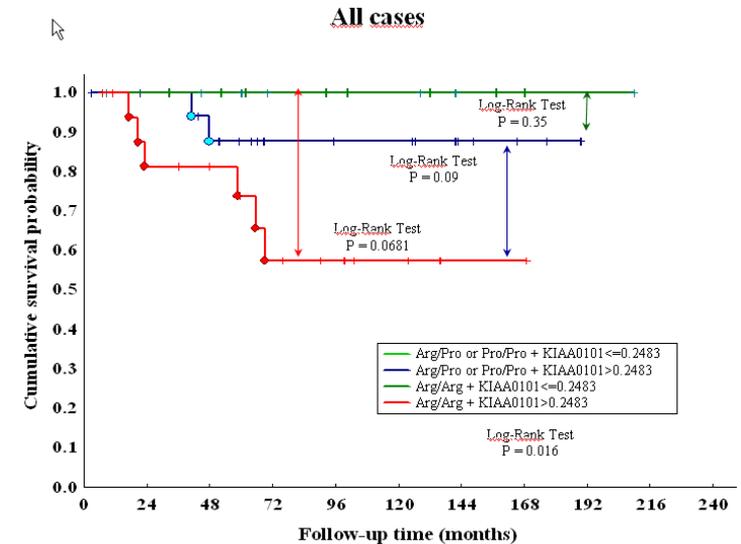


KIAA0101



R72 TP53

Deplezione di p15(PAF)/KIAA0101 risulta nella *upregulation* di p21



Si confermano i casi di early progression per il gruppo di casi con genotipo **R/R + KIAA0101 > 0.2483**

Un sentito ringraziamento

