

# Implementation of 1p/19q testing service for oligodendrogliomas

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A Grade Trainee

# Overview

## # Tumour background

Classification

Oligodendroglioma

1p/19q deletion

## # Testing methods

## # Results

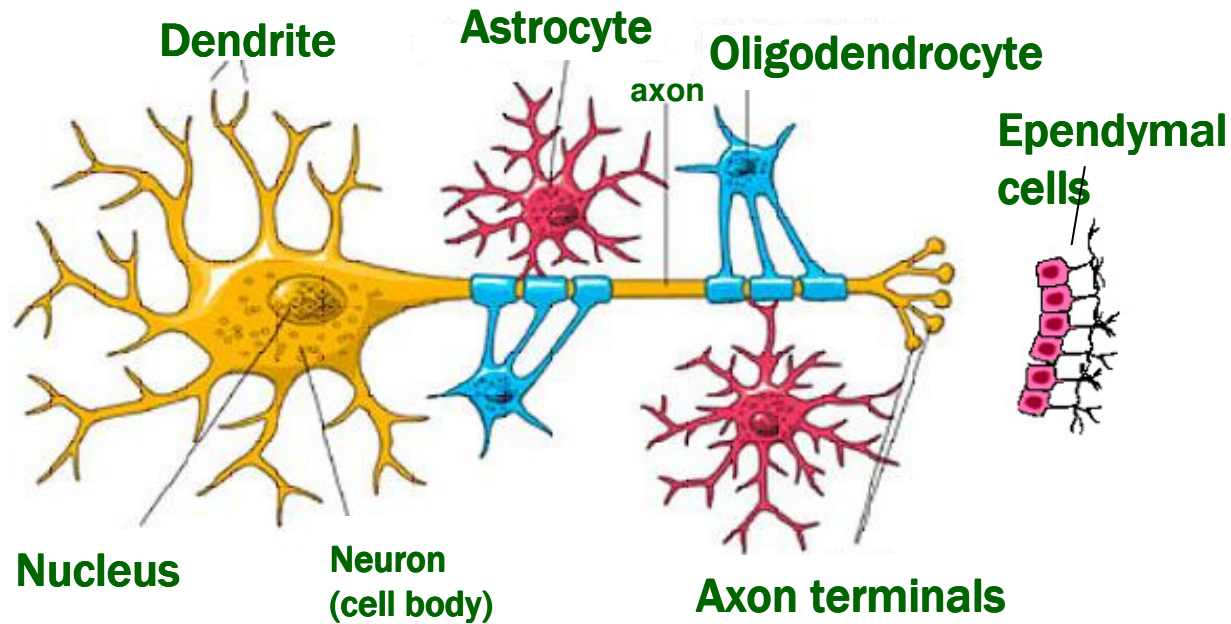
## # Non-concordant cases

## # Discussion

# Gliomas

- ✚ Gliomas are a heterogeneous group of brain tumors derived mainly from glial cells.
- ✚ Glial cells are called ‘supporting cells’, they have four main functions
  - *Supply nutrients and oxygen*
  - *Insulate one neuron from another*
  - *Repair and maintenance*
  - *Help in development of the nervous system*

# Types of Glial cells



✚ Tumors of these cells are called as astrocytomas, oligodendrogliomas, and ependymomas.

✚ Some tumors involve both astrocytes and oligodendrocytes and are known as oligoastrocytomas.

# World Health Organization (WHO) Classification

TUMOUR	GRADE		SURVIVAL	GENETIC CHANGES
Astrocytoma	I	Pilocytic Astrocytoma	(90%) 10 years	-
	II	Diffuse Astrocytoma	(39%) 10 years	Gain 7q, 5, 9 and 19 Mutation TP53 (>60%)
	III	Anaplastic astrocytoma	(22%) 10 Years	Loss of 10q(PTEN), 9p & 1p Gain of 7p(EGFR) Gain of 7p12-13 & loss 10q(together)
	IV	Glioblastoma	(2%) 10Years <b>(29%) 1 Year</b>	TP53 mutation, LOH of 10q(PTEN)-30-90% Gain of 7, Monosomy 10/Trisomy 7 +19, +20, -9, -22, EGFR amplification (35%-40%) <b>Inactivation of MGMT</b>
Oligodendroglioma	II	Oligodendroglioma	10 -15 years	<b>1p deletion</b> <b>1p/19q deletion (90%)</b> EGFR over expression
	III	Anaplastic oligodendroglioma	8 -10 years	<b>1p/19q deletion (50%)</b> Loss of 4q,9p, 10q, 11p and 13q Gain 1q, 6p, and 20q, Loss of 9p, Gain of 8q
Mixed oligoastrocytoma	II	Oligoastrocytoma	?	<b>1p/19q loss(30%-50%oligodendroglial features)</b> <b>TP53 mutation ( 30% astrocytic features)</b> 19q deletion
	III	Anaplastic oligoastrocytoma		

Collins V P. J Neurol Neurosurg Psychiatry 2004; Heim S et al., Cancer Cytogenetics, 2008,

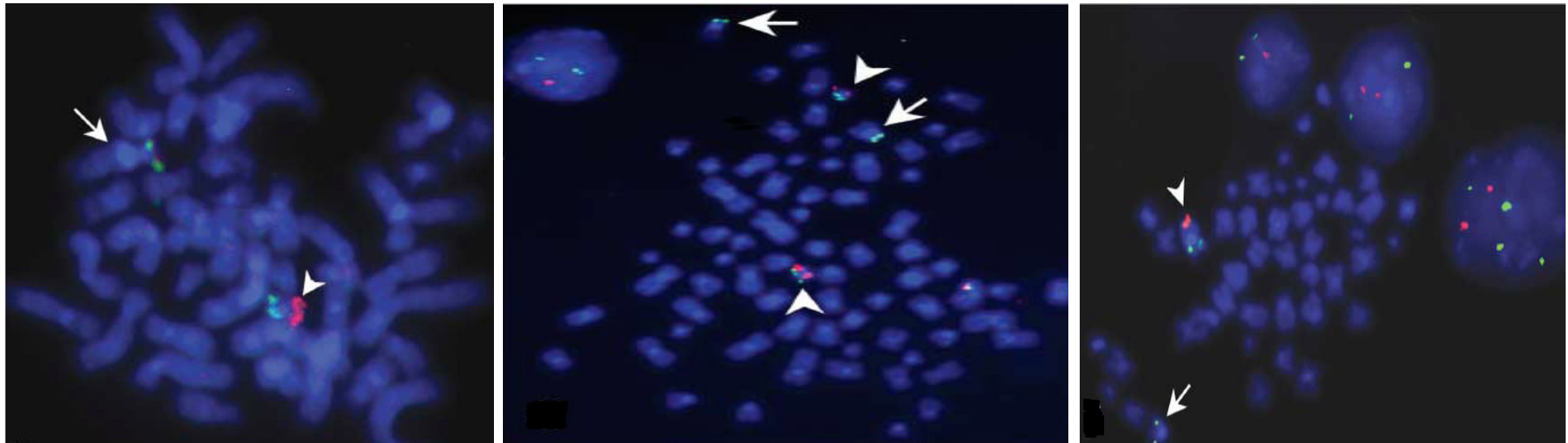
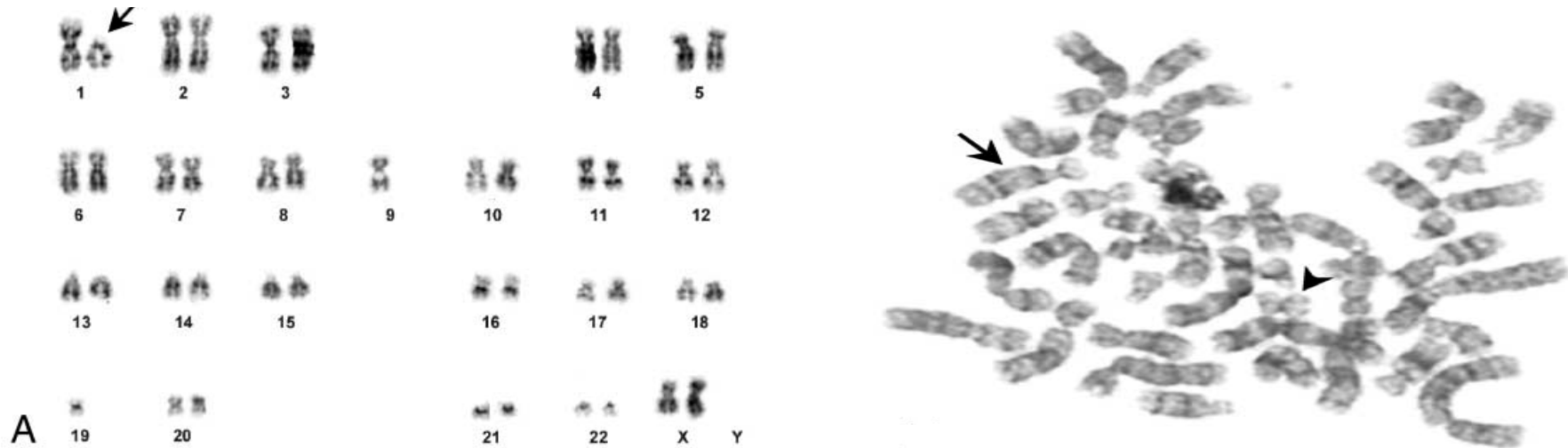
# Oligodendroglioma

- ✚ Oligodendroglioma is a well-differentiated, diffusely infiltrating tumour of adults, typically located in the white matter and cortex of the cerebral hemispheres and composed predominantly of cells morphologically resembling oligodendrocytes.
- ✚ About 5~20% intracranial gliomas are oligodendrogliomas and oligoastrocytomas, affecting about 15,000 people every year, representing the second most common primary parenchymal brain lesions.
- ✚ Patients usually present with seizures in low-grade gliomas. The other symptoms including headache, and mental and cognitive changes, vertigo/nausea, and visual problems which are generally more associated with malignant forms and also the location of the tumour in the brain.

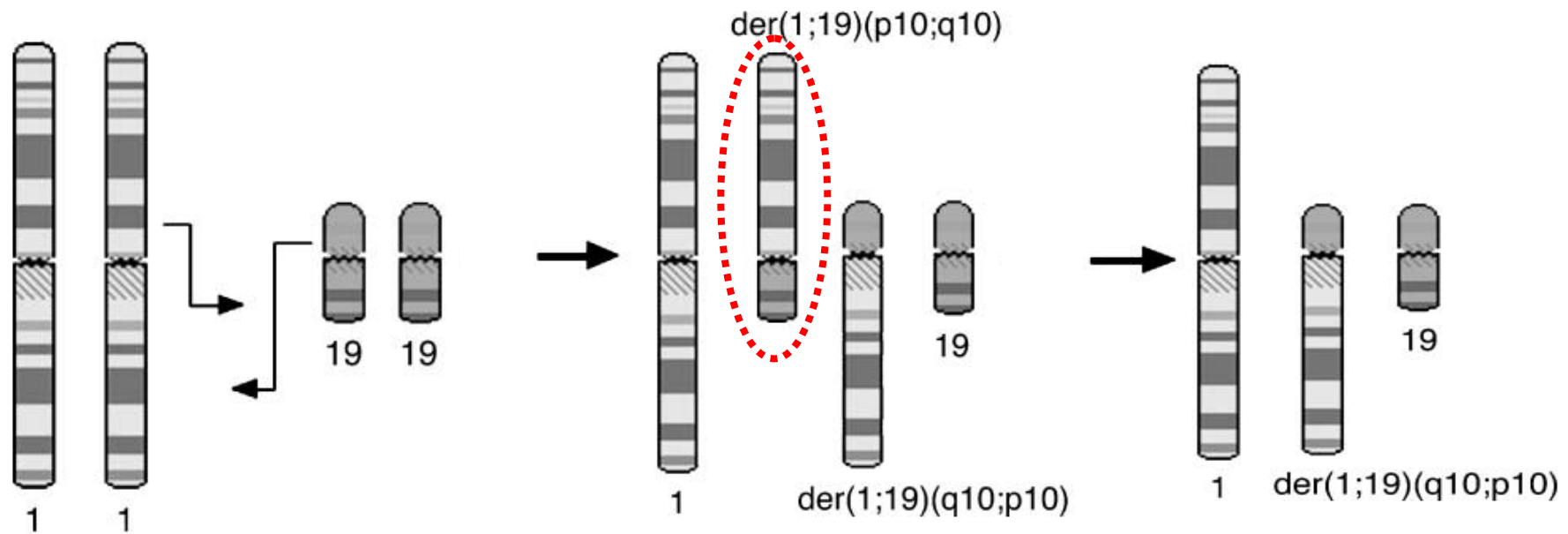
ORIGINAL ARTICLE

## Identification of der(1;19)(q10;p10) in Five Oligodendrogliomas Suggests Mechanism of Concurrent 1p and 19q Loss

Constance A. Griffin, MD, Peter Burger, MD, Laura Morsberger, BA, Raluca Yonescu, MD, Sharon Swierczynski, MD, PhD, Jon D. Weingart, MD, and Kathleen M. Murphy, PhD



# Proposed mechanism



Griffin et al

J Neuropathol Exp Neurol • Volume 65, Number 10, October 2006

TABLE 1. G-Band Karyotypes

Case

1	39–44,XX, <u>der(1;19)</u> (q10;p10),-9,inc[cp3]/61–85<4n>,XXX, <u>der(1;19)</u> (q10;p10)x2,-7,-9,inc[cp4]
2	36–43,X,-X, <u>der(1;19)</u> (q10;p10),add(3)(p21),add(4)(p14),del(6)(q16),add(8)(q13),add(9)(p13),add(12)(p11.2),-12,-13,-15,+2mar[cp7]
3	45,X,-Y,[10]/45,XY, <u>der(1;19)</u> (q10;p10),del(4)(q21q31),del(14)(q22q32)[5]/46,XY[2]
4	45,X,-Y[6]/46,X,-Y,+7[5]/48,XY,t(2;13)(q21;q22),+7,+21[3]/76–103,XXY, <u>der(1;19)</u> (q10;p10)x3,inc[cp9]
5	45,XX, <u>der(1;19)</u> (q10;p10)[2]

# 1p/19q deletion analysis

- ✚ PCR-based investigations  
(Microsatellite analysis, real-time PCR)
- ✚ MLPA (Multiplex ligation probe dependent amplification )
- ✚ Comparative genomic hybridization  
(Metaphase, array)
- ✚ Fluorescent *in situ* hybridization (FISH)

# Specimens

✚ Frozen tissues

✚ Formalin fixed paraffin-embedded specimens (FFPE)

FFPE -requires pre-treatment

- To remove DNA-protein cross linkages- DNA denaturation
- Removal of membrane proteins, cytoplasmic proteins (enhance signal intensity , reduce background)

**Slide 10**

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**M1**

MALAVAST, 12/02/2011

# Method

**Joint project by cytogenetic and molecular trainees.**

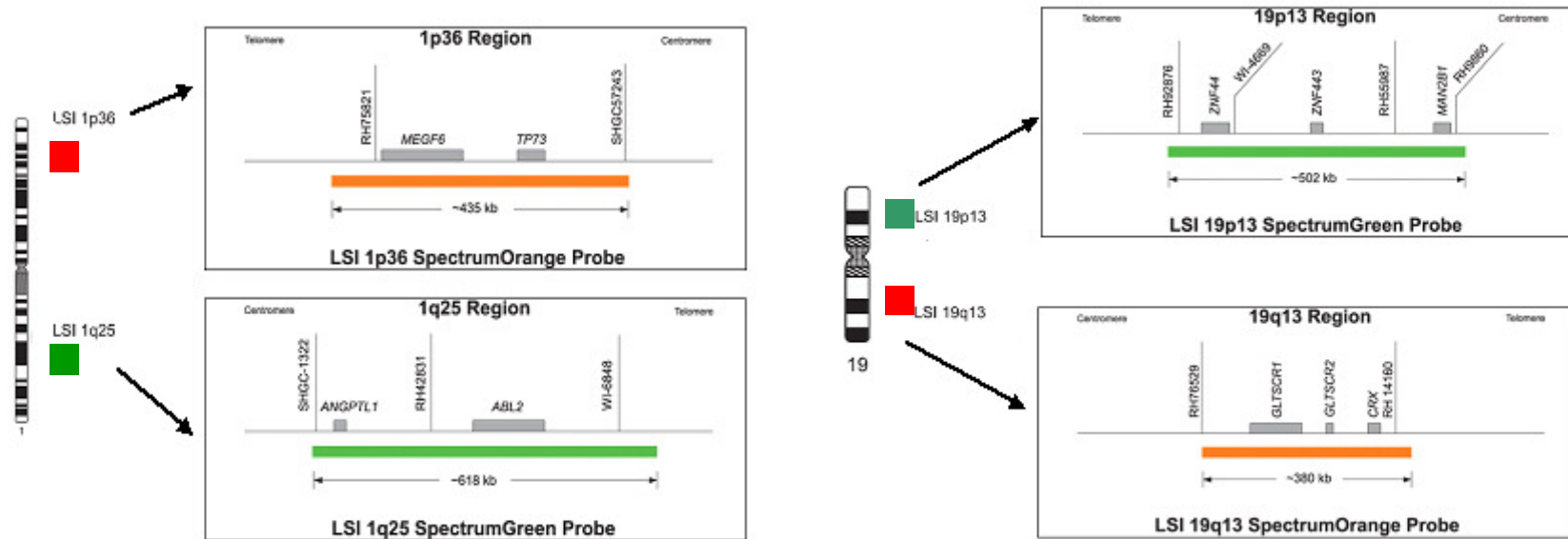
FISH and MLPA on 28 gliomas (10 previously analyzed by FISH)

- 10 Oligodendroglioma II
- 14 Anaplastic oligodendroglioma III
- 1 Oligoastrocytoma
- 2 Glioblastoma multiforme
- 1 Unknown

## FISH protocol

- Pre-treatment (*Dako histology FISH accessory Kit*)
- FISH (Vysis dual color probes )
- Analysis (200 nuclei)

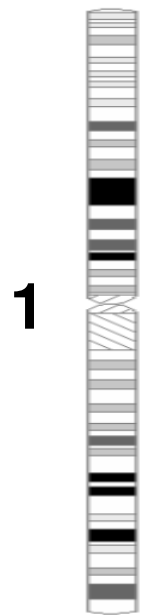
# Vysis LSI 1p36/ 1q25 and 19q13/19p13 Dual- Color probe



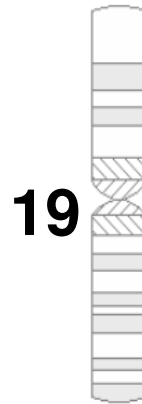
## MLPA

- ✚ SALSA MLPA KIT P088-B1 Oligodendroglioma 1p-19q
- ✚ 43 MLPA probes with amplification products between 130 and 481 nt
  - 15 probes on 1p (1p11.2-1p36.33) and 3 probes on 1q(1q21.2-1q32.1)
  - 8 probes on 19q(19q12-19q13.43 ) and 2 probes on 19p(19p13.2)

# FISH and MLPA probes location

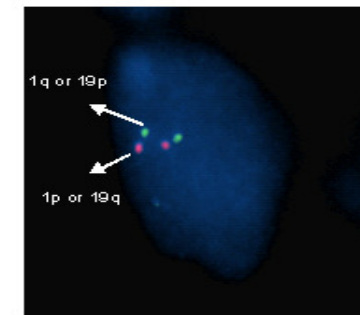


FISH and MLPA PROBES	CHROMOSOMAL POSITION	GENE	PROBE SIZE
<b>1</b>			
3062-L01761	1p36.33	TNFRSF4	391nt
2890-L07968	1p36.33	GNB1	178nt
4693-L04071	1p36.33	TNFRSF14	346nt
<b>FISH probe</b> <b>FISH-MLPA probe/1682-L01262</b>	<b>1p36.32</b> <b>1p36.32</b>	<b>MEGF6</b> <b>TP73</b>	<b>435kb (FISH)</b> <b>328nt (MLPA)</b>
2189-L02365	1p36.23	PARK7	454nt
4885-L04269	1p36.22	MFN2	400nt
1866-L01425	1p35.3	PTAFR	320nt
2877-L02344	1p33	FAF1	283nt
2876-L02343	1p32.2	PPAP2B	160nt
2875-L02342	1p32.1	CYP2J2	337nt
2872-L02339	1p31.1	LPHN2	274nt
2871-L02338	1p22.2	GTF2B	256nt
2870-L02337	1p21.3	DPYD	373nt
1032-L00604	1p13.2	NRAS	472nt
5745-L05183	1p11.2	NOTCH2	130nt
<b>CENTROMERE</b>			
1917-L01461	1q21.2	LMNA	310nt
<b>FISH probe</b> <b>FISH probe</b>	<b>1q25.2</b> <b>1q25.2</b>	<b>ABL2</b> <b>ANGPTL1</b>	<b>618kb</b>
6961-L06541	1q31.3	CRB1	427nt
6557-L06115	1q32.1	TNNT2	190nt
<b>19</b>			
2488-L02236	19p13.2	SMARCA4	142nt
2314-L01805	19p13.2	LDLR	211nt
<b>FISH probe</b> <b>FISH probe</b> <b>FISH probe</b>	<b>19p13.2</b> <b>19p13.2</b> <b>19p13.2</b>	<b>ZNF44</b> <b>ZNF443</b> <b>MAN2B1</b>	<b>502kb</b>
<b>CENTROMERE</b>			
2881-L02348	19q12	CCNE1	166nt
2882-L02349	19q13.11	PDCD5	184nt
2883-L02350	19q13.12	UPK1A	238nt
28889-L02356	19q13.2	TGFB1	364nt
3221-L02651	19q13.32	ZNF342	409nt
<b>FISH probe</b> <b>FISH probe</b> <b>FISH probe</b>	<b>19q13.33</b> <b>19q13.33</b> <b>19q13.33</b>	<b>GLTSCR1</b> <b>GLTSCR2</b> <b>CRX</b>	<b>380kb</b>
2887-L02354	19q13.33	PPP1R15A	220nt
0348-L00174	19q13.33	BAX	301nt
2705-L0283	19q13.43	BC-2	266nt



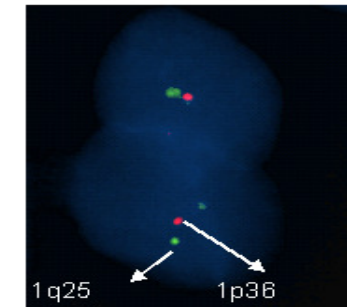
# Assessment and interpretation of FISH results

Assessment and interpretation of FISH results	
<b>NORMAL</b>	
2/2	A signal pattern with equal number of red signal for 1p36/19q13 and green signal for 1q25/19p13
3/3	
4/4	
<b>DELETION</b>	
1/2	A signal pattern with less red signal for 1p36/19q13 than green signal for 1q25/19p13
2/4	
3/6	
<b>IMBALANCE</b>	
0/1	A signal pattern with red for 1p36/19q13 and green 1q25/19p13 ratio is not 1:2
1/3	
2/3	
1/4	
5/3	
<b>REVERSE IMBALANCE</b>	
2/1	A signal pattern with red signal for 1p36/19q13 is more than green signal for 19q13/1p25
3/1	
4/2	
A total of 100 nuclei are counted by two experience cytogeneticist, with additional 100 cells for border line cases.	
<b>INTERPRETATION</b>	
From total nuclei counted one with deletion/imbalance were added and expressed as a percentage of the total. The number of nuclei with imbalance only were added and expressed separately.	
<b>1p or 19q deletion:</b> percentage of nuclei with deletion or imbalance is 30% or greater. Additional count is required for percentage of nuclei between 26% and 30%.	
<b>No 1p or 19q deletion:</b> Percentage of nuclei with deletion/imbalance less than 26%	
<b>CALCULATION OF MARKER AND REFERENCE RATIO</b>	
Loss of heterozygosity: <i>Ratio of red :green signal</i> <0.85	



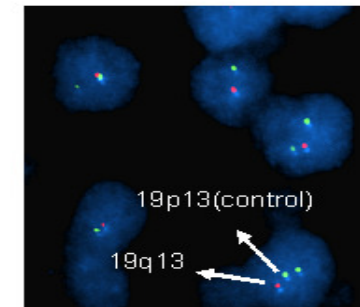
1R/1G  
2R/2G  
4R/4G

Normal



**Deletion**  
1R/2G  
2R/4G  
3R/6G

1p deletion



**Imbalance**  
1R/3G  
2R/3G  
1R/4G

19q deletion

# Results

Glioma type	*FISH (other lab)	FISH	MLPA
Oligodendroglioma-II	No deletion	No deletion	No deletion
Oligodendroglioma-II		1p/19q deletion	1p/19q deletion
Oligodendroglioma-II		1p/19q deletion	1p/19q deletion
Oligodendroglioma-II		1p/19q deletion	1p/19q deletion
Oligodendroglioma-II		1p/19q deletion	1p/19q deletion
Oligodendroglioma-II		1p/19q deletion	1p/19q deletion
Oligodendroglioma-II		1p/19q deletion	1p/19q deletion
Oligodendroglioma-II		1p/19q deletion	1p/19q deletion
Oligodendroglioma-II		1p/19q deletion	1p/19q deletion
Oligodendroglioma-II		1p/19q deletion	1p/19q deletion
Oligodendroglioma-II		1p/19q deletion	1p/19q deletion
Anaplastic OLG-III	No deletion	No deletion	No deletion
Anaplastic OLG-III	1p/19q deletion	1p/19q deletion	1p/19q deletion
?Anaplastic OLG-III		1p/19q deletion	1p/19q deletion
?anaplastic OLG-III		1p/19q deletion	1p/19q deletion
?anaplastic OLG-III (recurrent irradiated OD)		1p/19q deletion	1p/19q deletion
Anaplastic OLG-III	1p deletion	No deletion	No deletion
Anaplastic OLG-III	1p/19q deletion	1p/19q deletion	1p/19q deletion
Anaplastic OLG-III	1p/19q deletion	1p/19q deletion	1p/19q deletion
Anaplastic OLG-III		1p/19q deletion	1p/19q deletion
Anaplastic OLG-III		1p/19q deletion	1p/19q deletion
Anaplastic OLG-III		1p/19q deletion	1p/19q deletion
Anaplastic OLG-III		1p/19q deletion	1p/19q deletion
Anaplastic OLG-III	1p/19q deletion	1p/19q deletion	1p/19q deletion
Anaplastic OLG-III (recurrent OD)		1p/19q deletion	1p/19q deletion
anaplastic oligodendroglioma	No deletion	No deletion	No deletion
Oligoastrocytoma-Grade II	1p/19q deletion	1p deletion	No deletion
Glioblastoma multiform -IV		19q deletion	19q duplication
Glioblastoma with OLG component (GBMO)	No deletion	No deletion	No deletion

1p/19q deletion  
Oligodendroglioma 9/10 (90%)

1p/19q deletion  
Anaplastic oligodendroglioma  
12/15 (80%)

3 non-concordant cases  
1 previous FISH  
2 MLPA/FISH  
Array CGH performed on these cases

# Non-concordant cases

Case/ Grade	Genotype	FISH*	FISH	MLPA	ARRAY CGH
OA II	1p	Deletion 57% (ratio 0.67)	Deletion 34% *(ratio 0.83)	No deletion Borderline deletion probe ratio	Partial deletion 1p34-1p36.3
	19q	Deletion 57% (ratio 0.62)	No deletion	No deletion	Loss of 19
AOD III	1p	Deletion 48% (ratio 0.82)	No deletion	No deletion	No deletion
	19q	No deletion	No deletion ? Additional green signal	No deletion	Gain of 19p
GBM IV	1p	---	No deletion	No deletion	No deletion
	19q	---	Deletion 53% (ratio 0.76) ? Additional green signal gain of 19p	Duplication	Gain of 19p Gain of 19q with partial deletion 19q13.31-19q13.33

	<b>LOH</b>	<b>FISH</b>	<b>MLPA</b>	<b>A CGH</b>
<b>SAMPLES</b>	Tumour and constitutional DNA	Tumour	Tumour	Tumour
<b>NUMBER OF PROBES OR MARKERS</b>	4-10	1	8-10	Thousands
<b>% OF TUMOUR CELLS</b>	>70%	<30%	50-70%	? 50%
<b>LABOUR</b>	Easy	Labour intensive	Easy	Easy
<b>TURN AROUND</b>	One week	One week	One week	One week
<b>ADDITIONAL INFORMATION</b>	Duplication	none	Duplication	Whole genome screen (gain and loss)
<b>COST</b>	Cheap	Expensive	Cheap	Expensive

# Discussion

- ✚ FISH and MLPA showed a 1p/19q loss in 90% of low grade oligodendroglioma and 85% of anaplastic oligodendroglioma which is similar to that reported in the literature.
- ✚ FISH and MLPA were concordant in 25/28 (89%) cases which suggests that MLPA is an alternative effective screening technique for 1p/19q deletion.
- ✚ FISH was more efficient at detecting deletions in heterogeneous tumour samples , but MLPA was more effective at detecting duplications.
- ✚ Array CGH on the three non-concordant cases revealed further chromosomal information from that obtained by either MLPA or FISH.

# Acknowledgements

Thank you to.....

- ☺ .....St Georges Pathology Department - Dr. Leslie Bridges, Kay Elderfield
- ☺ .....South West Thames Molecular Genetic Laboratory- Mariana Grobler, Samantha Butler, Rohan Taylor
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