

Molecular Diagnosis of Gastrointestinal Tumors

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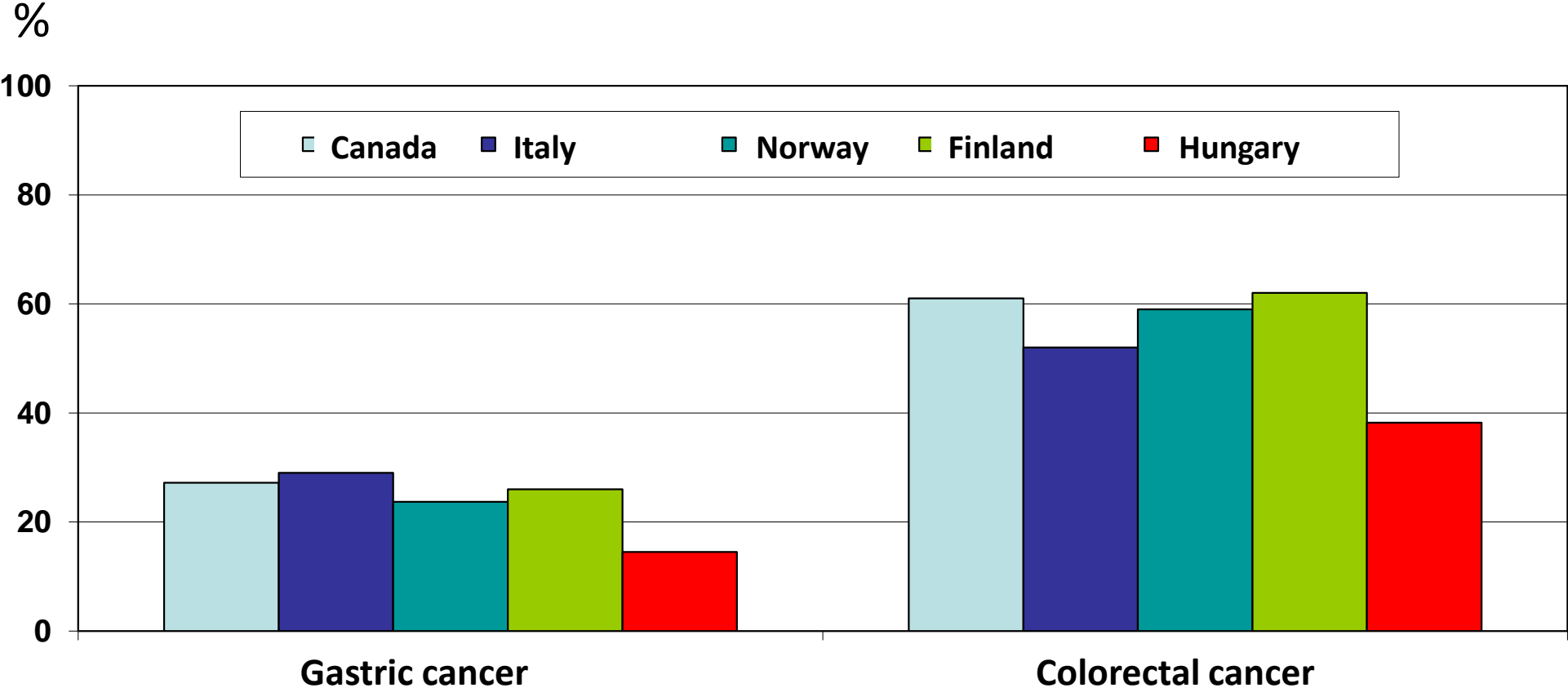
EEA and Norwegian Financial Mechanisms in
Hungary, Development of joint Hungarian
and Norwegian strategy for cancer treatment by
molecular methods. (Prevention, early diagnosis and
therapy)

Colorectal cancers

Patients and Methods

- **Totally 634 surgically resected colorectal cancer samples.**
- **Database construction.**
- **Isolation of DNA from FFPE tumor samples by the assistance of MagNa Pure Compact machine.**
- **PCR-based microsatellite instability test according to Dietmaier and Hofstadter (Lab. Invest., 81:1453-1456, 2001).**
- **Real-time PCR amplification and melting point analysis of KRAS exon 2 and BRAF exon 15 gene regions.**
- **DNA sequence analysis of amplicons.**

Five-year Surviving Rate of Gastic and Colorectal Cancer Based on the National Cancer Registry



Tusnady G, Gaudi I, Rejto L, Kasler M es Szentirmay Z: Survival chances of Hungarian cancer patients in the National Cancer Registry. Magyar Onkol., 52:339-349, 2008

Genetic classification of colorectal cancers

MMR negative phenotype

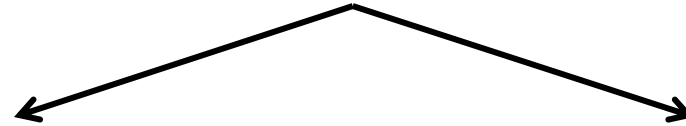


**Sporadic CrC
develops via „polyp-
cancer” sequence**

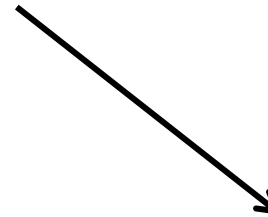


**Microsatellite stability (MSS)
Chromosomal instability**

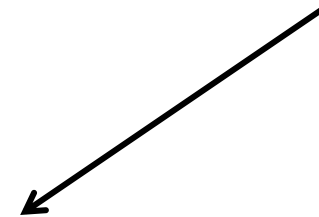
MMR positive phenotype



**Hereditary non-
polyposis CrC
(HNPCC)**

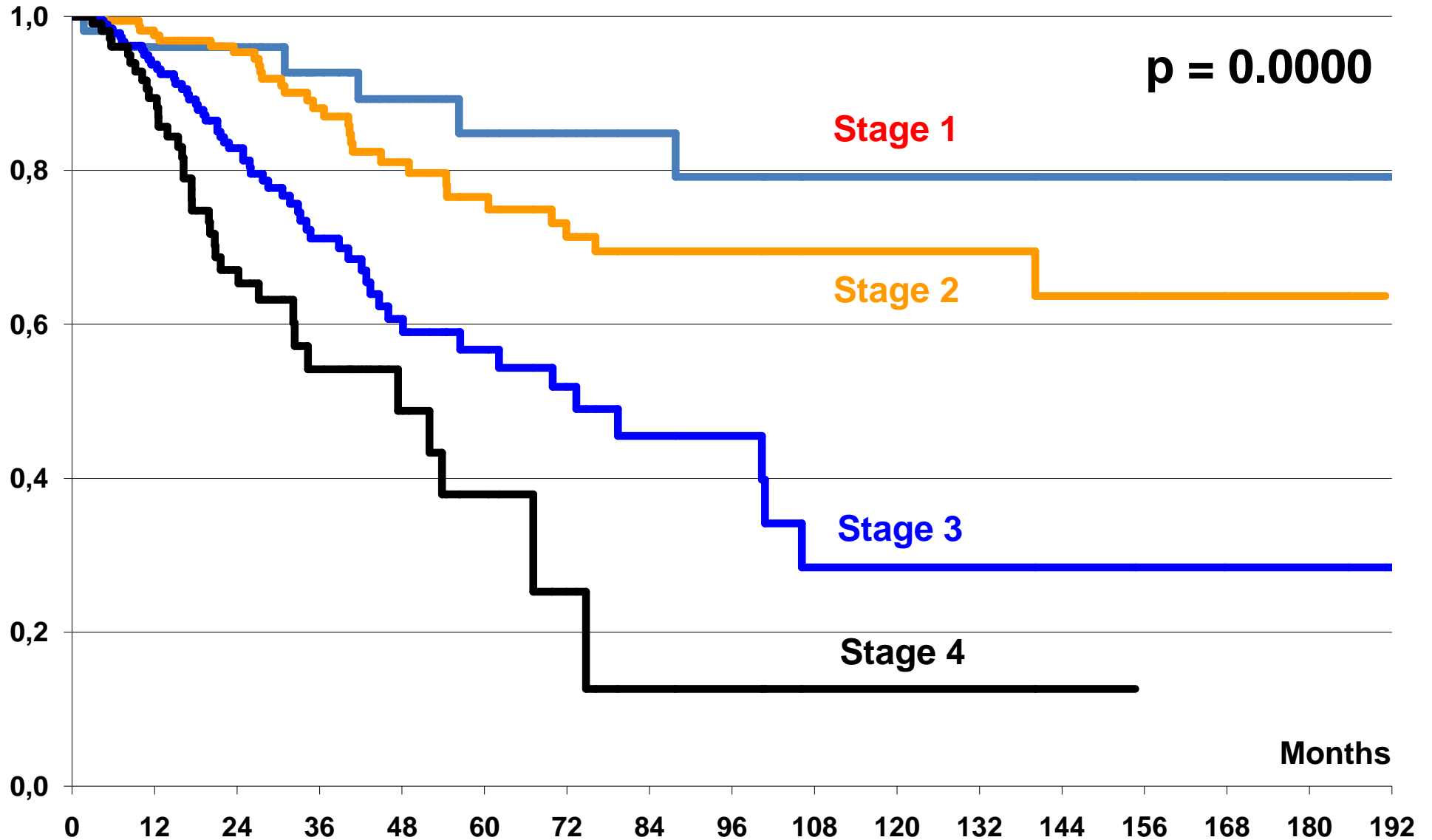


**Sporadic CRC
develops due to the
CpG metilation of
hMLH1 (CIMP+)**



**Microsatellite instability (MSI-H)
Chromosomal stability**

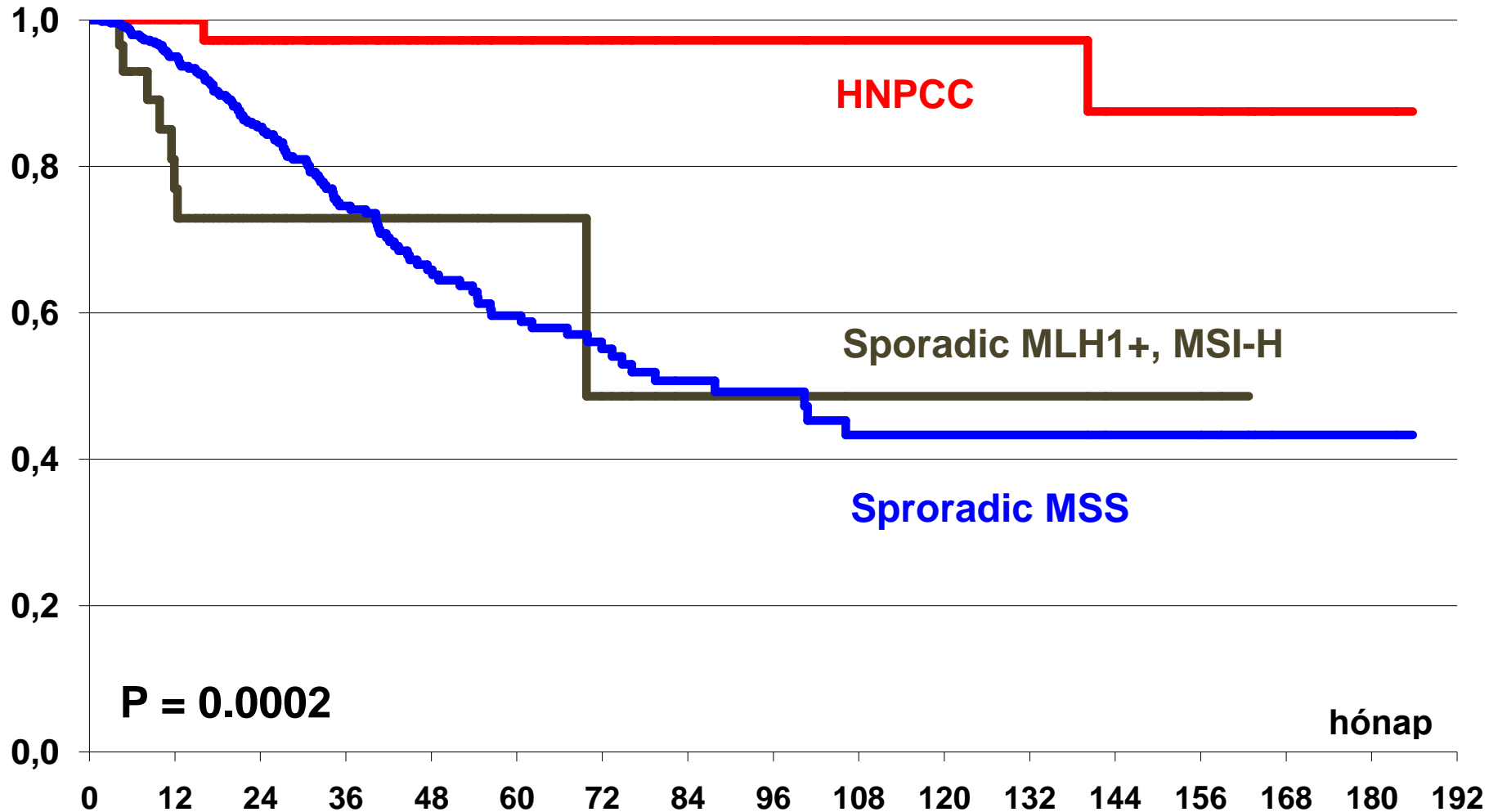
Kaplan-Meier probability survive of CrC according to the TNM-based clinical staging



Correlation of TNM-based clinical stage and the genotype of CrC at the time of diagnosis

Clinical stage	HNPCC MSI-H	CIMP+ MSI-H	Polyp-Cancer MSS
Stage 1 + 2 (%)	69.8	66.7	38.4
Stage 3 + 4 (%)	30.2	33.3	61.6
Total	100	100	100

Kaplan-Meier probability survive of CrC according to the genotype



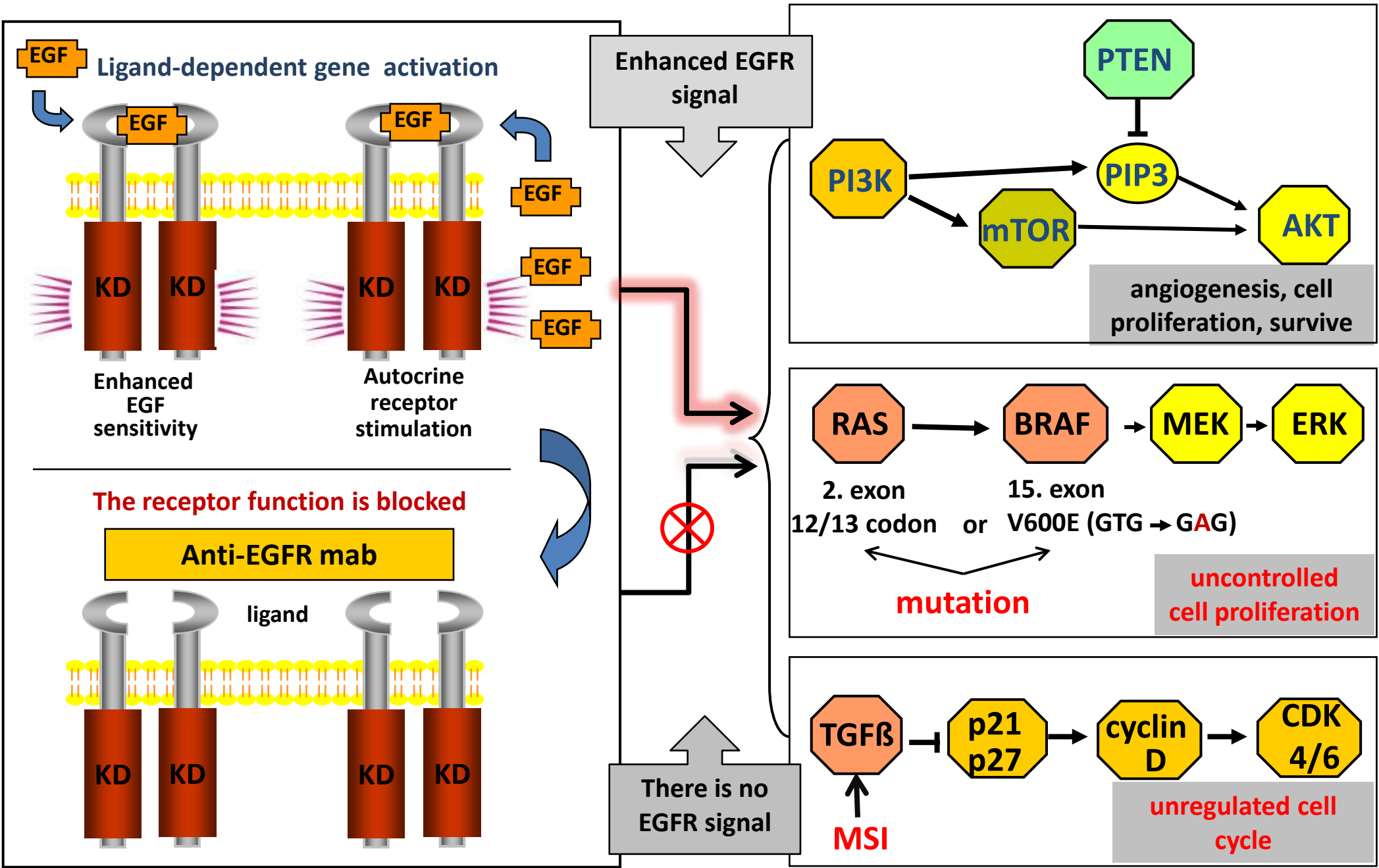
Localization of genetically different CRCs

Colorectal cancer	Localization %		Total %
	Right half-colon	Left half-colon & rectum	
Sporadic CIMP+ MSI-H	100.0	-	100
HNPCC MSI-H	65.1	34.9	100
Sporadic MSS	21.9	78.1	100

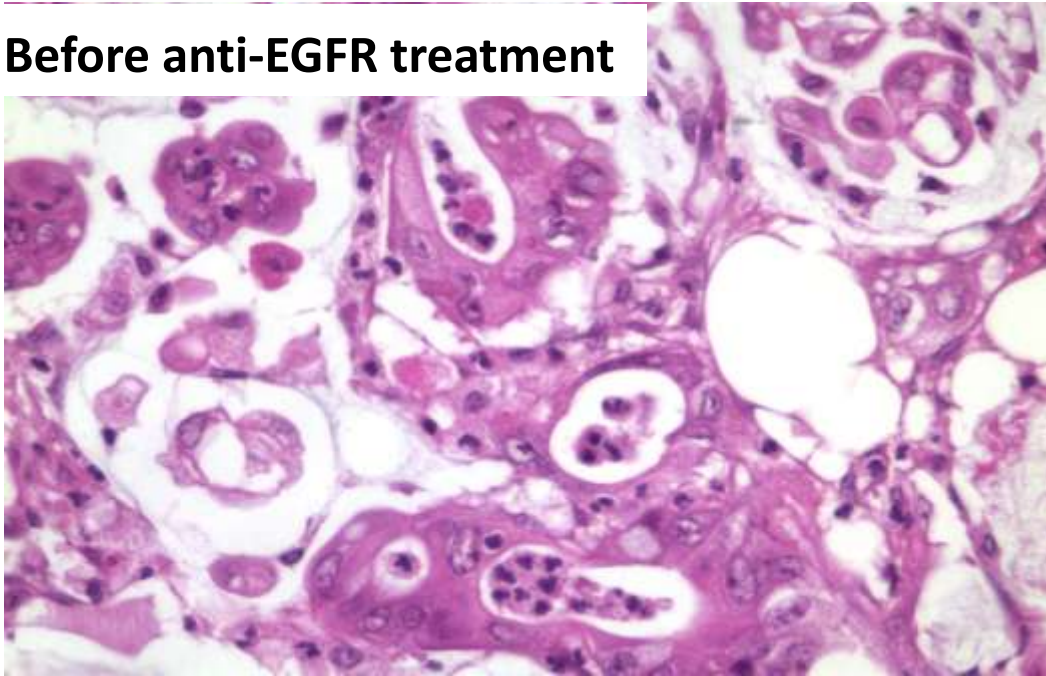
P = 0.000

Significant genetic alterations influencing the therapy response of colorectal cancers

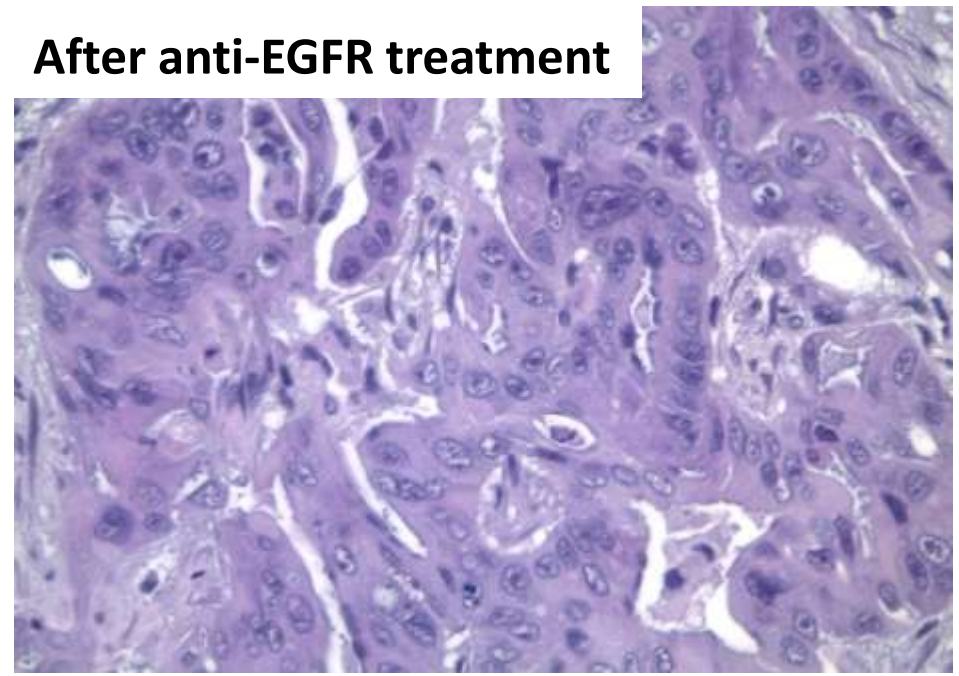
- 1. KRAS or BRAF mutation inhibits the therapeutic effects of anti-EGFR antibodies.**
- 2. The 5-fluorouracil-based chemotherapy has no any effects on microsatellite instable (MSI-H) carcinoma.**



Before anti-EGFR treatment



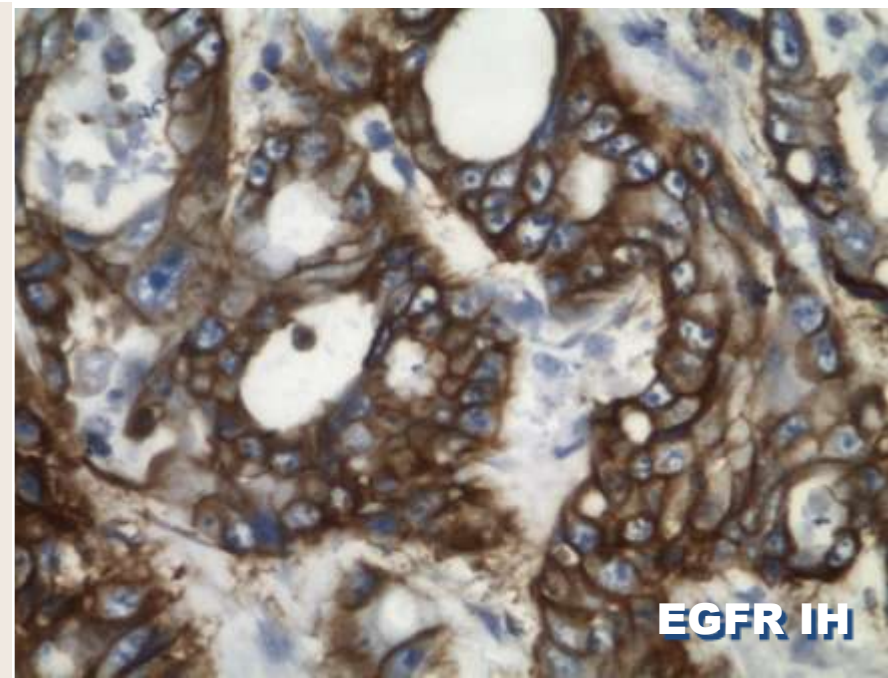
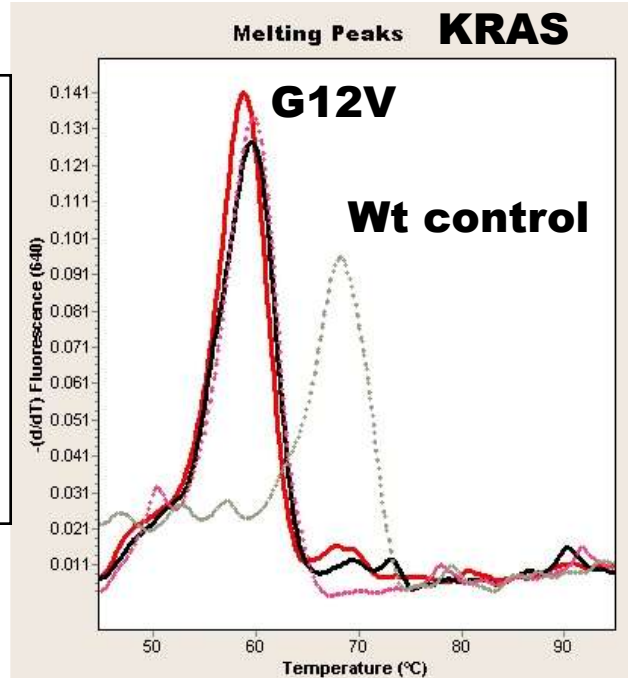
After anti-EGFR treatment



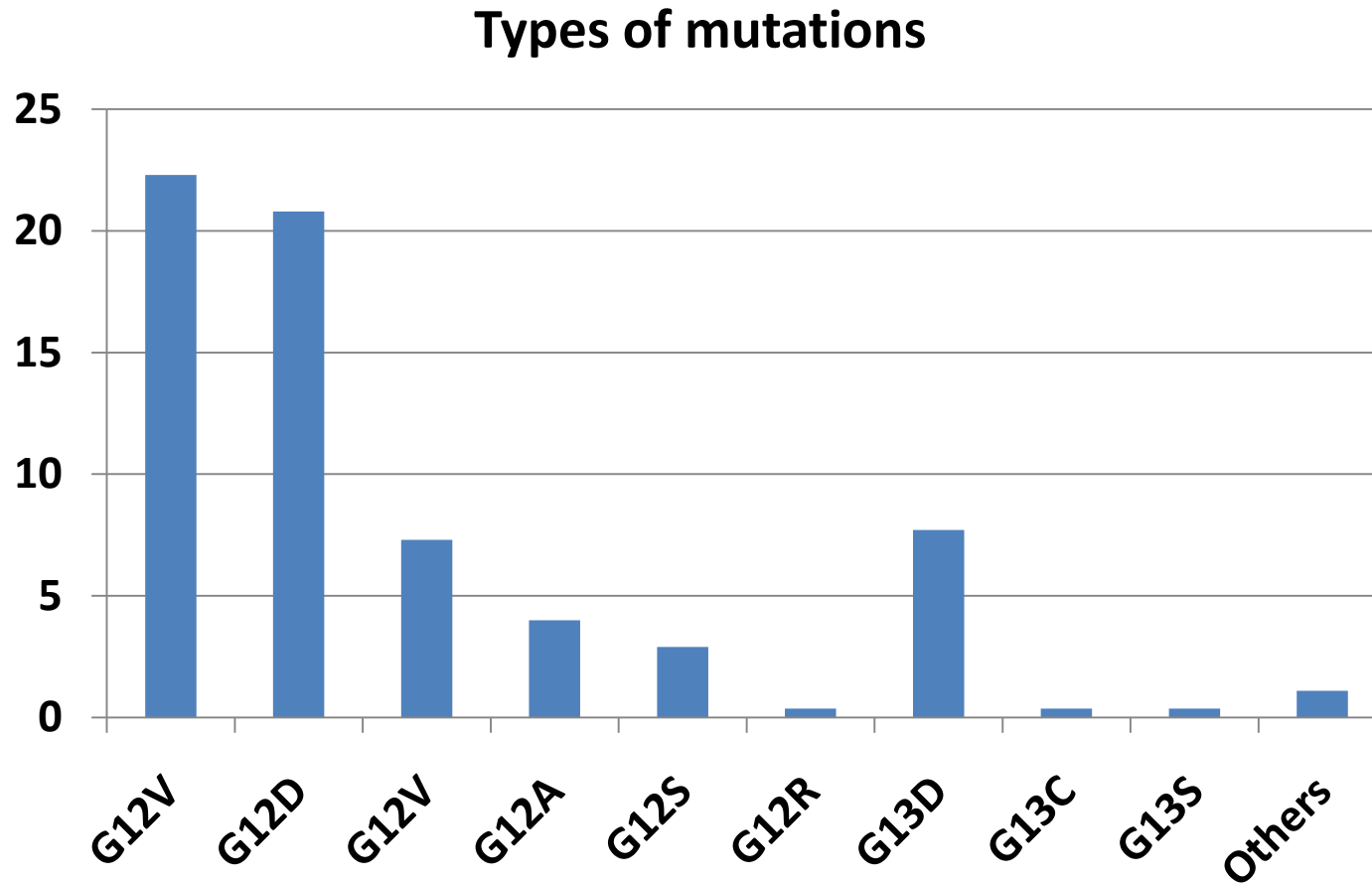
Disease-process:

In spite of the anti-EGFR treatment liver metastasis developed.

The patient died within two years.



Frequency distribution of gain-of-function mutations in codon 12 and codon 13 of KRAS gene (184 tumor samples)



Localization of KRAS and BRAF positive CrCs

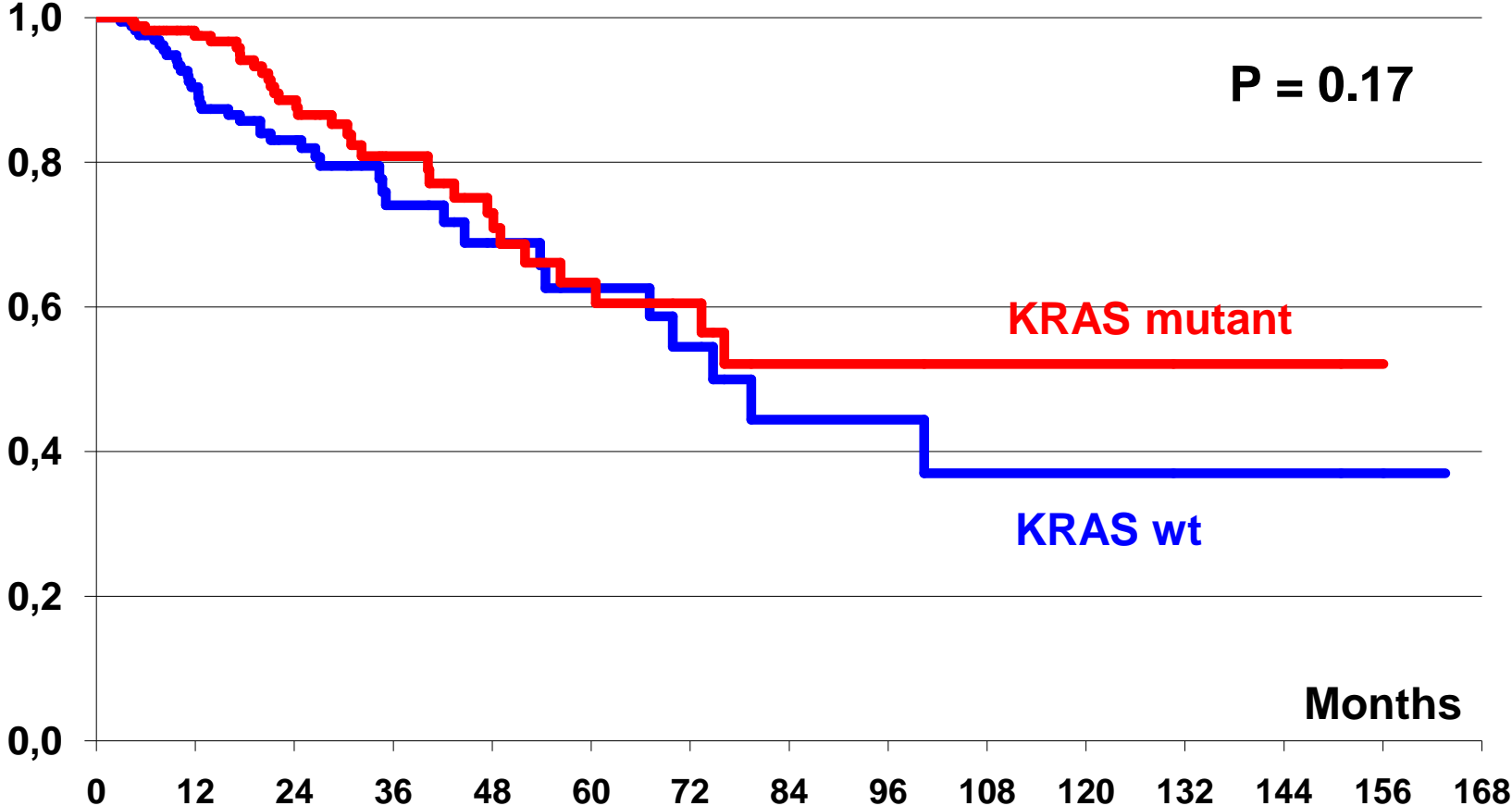
Genetical state	Incidence %		Total %
	Right half-colon	Left half colon & rectum	
KRAS mut	24.2	75.8	100
BRAF mut	66.7	32.3	100

P = 0.000

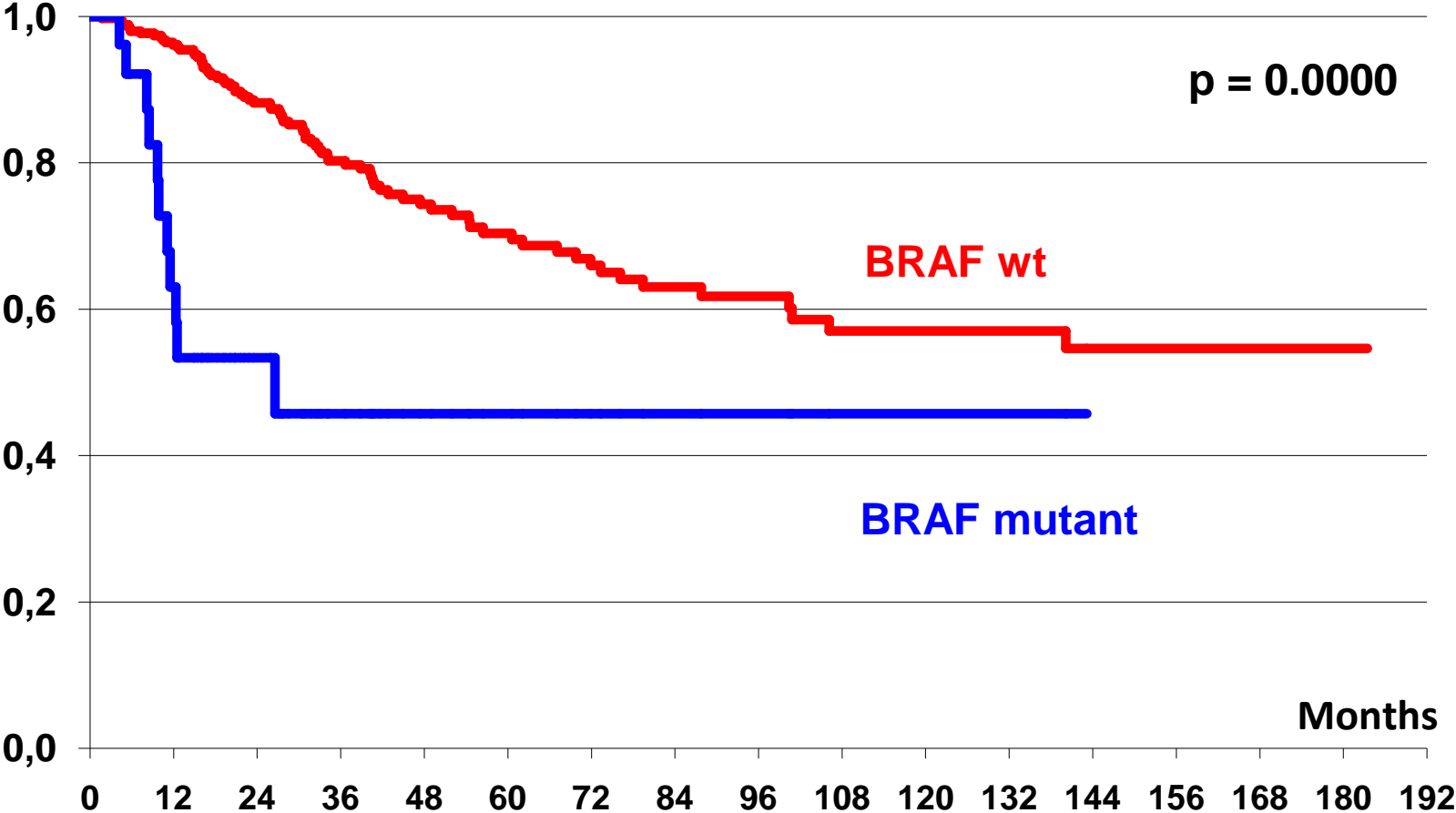
Frequency distribution of KRAS and BRAF mutations in hereditary and sporadic CrC

CrC types	KRAS mut %	BRAF mut %
HNPCC MSI-H	39.5	-
Sporadic CIMP+, MSI-H	8.4	52.8
Sporadic MSS	45.9	4.2

Kaplan-Meier survival function of CrCs according to the KRAS mutation state



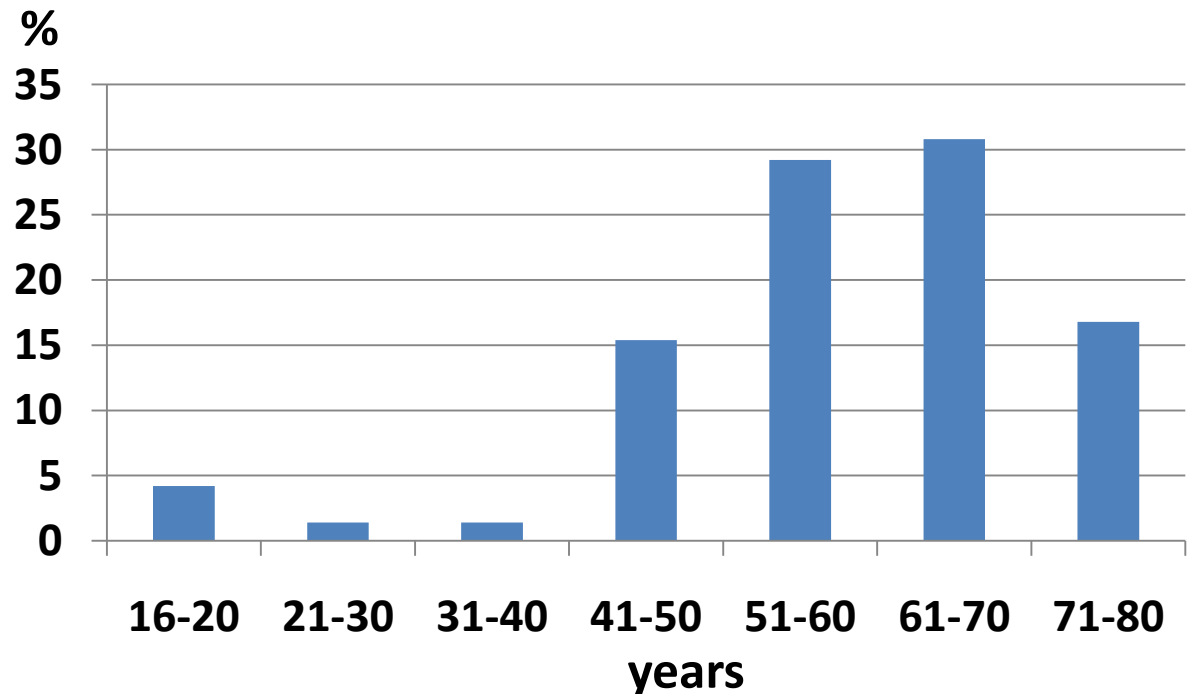
Kaplan-Meier survival function of CrCs according to the BRAF mutation state



Gastrointestinal stromal tumor (GIST)

Study Population

- **No. of tumor samples investigated: 72**
- **Sex: Male 38, Female 34**
- **Patients follow up period: between 17 years and 6 months**
- **Age distribution:**



Methods

- **Collection of formalin-fixed and paraffin-embedded tumor samples and database construction.**
- **CD117 and CD34 immunohistochemistry and pathological assessment of the tumor risk categories.**
- **Isolation of DNA from FFPE tumor samples by the assistance of MagNa Pure Compact machine.**
- **Real-time PCR amplification and melting point analysis of c-kit exon 9, exon 11, and PDGFRA exon 12 and exon 18 gene regions.**
- **High resolution capillary gel electrophoresis analysis of amplicons.**
- **High resolution melting analysis of c-kit exon 11 using LightCycler 480 PCR system to analyze genetic variations in PCR amplicons.**
- **DNA sequence analysis of amplicons.**

High Resolution Melting

Instrument: LC480II / Not Connected

Database: Research Database (Research)



Window: HRM Ckit-11

User: eliza

Experiment

Analyses: Gene Scanning for C-kit 11

Subset Editor

Information: Program: HRM, Color Compensation: Off

Sample Editor

Subset: C-kit 11

Analysis

	1	2	3	4	5	6	7	8	9	10	11
A	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗
B	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗
C	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗
D	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗
E	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗
F	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗
G	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗
H	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗

Report

Scanning results

Sum.

Legend for Results:

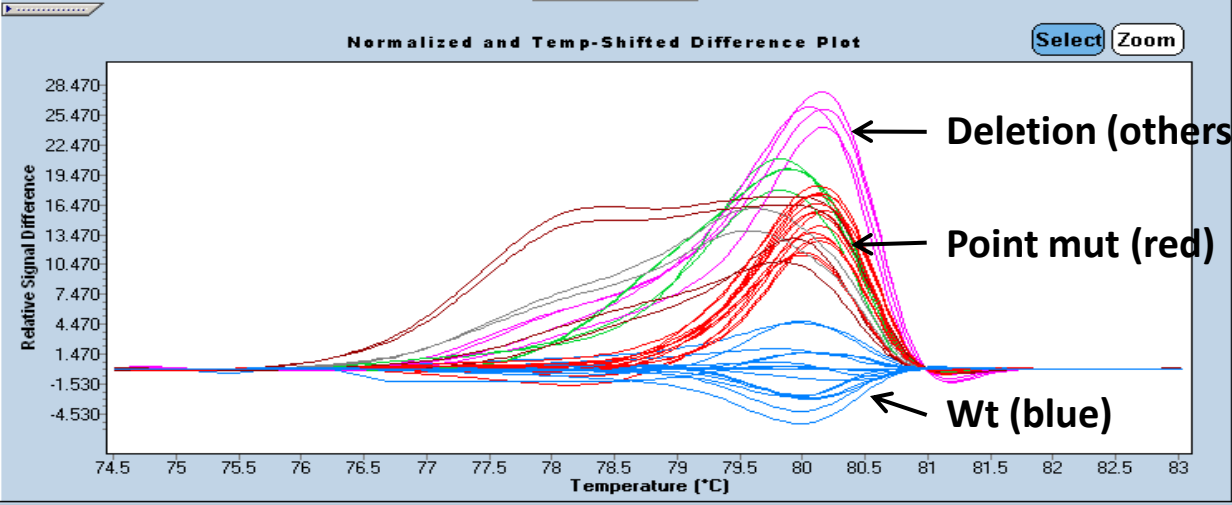
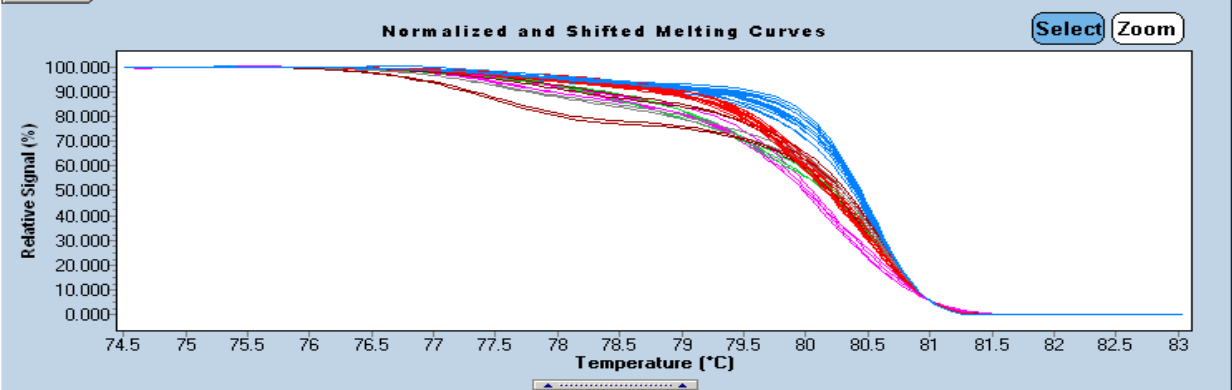
- 1 (Blue)
- 2 (Red)
- 3 (Green)
- 4 (Magenta)
- 5 (Grey)
- 6 (Yellow)
- Unknown (Dark Red)
- Negative (Orange)

Results Groups Sensitivity

Include	Color	Pos	Name	Results Group
<input checked="" type="checkbox"/>	Red	E9	380/09	2
<input checked="" type="checkbox"/>	Dark Red	F2	508/01	Unknown
<input checked="" type="checkbox"/>	Blue	F3	9/03	1
<input checked="" type="checkbox"/>	Red	F4	5925/04	2
<input checked="" type="checkbox"/>	Grey	F5	641/09	5
<input checked="" type="checkbox"/>	Dark Red	F6	1911/08	Unknown
<input checked="" type="checkbox"/>	Red	F7	6647/08	2
<input checked="" type="checkbox"/>	Blue	F8	9011/08	1
<input checked="" type="checkbox"/>	Dark Red	G2	508/01	Unknown
<input checked="" type="checkbox"/>	Blue	G3	9/03	1
<input checked="" type="checkbox"/>	Red	G4	5925/04	2
<input checked="" type="checkbox"/>	Grey	G5	641/09	5
<input checked="" type="checkbox"/>	Dark Red	G6	1911/08	Unknown
<input checked="" type="checkbox"/>	Red	G7	6647/08	2
<input checked="" type="checkbox"/>	Blue	G8	9011/08	1
<input checked="" type="checkbox"/>	Red	H1	695/09	2

Apply Template [v] Notes Calculate

Negatives Normalization Temperature Shift Difference Plot



New Call [v] Apply [x] Show Standards [x] Select base curve

Filter Comb 465-510 Standards (Auto Group) [v]

C-kit exon 11

Comparison of the Mutation Analysis Methods

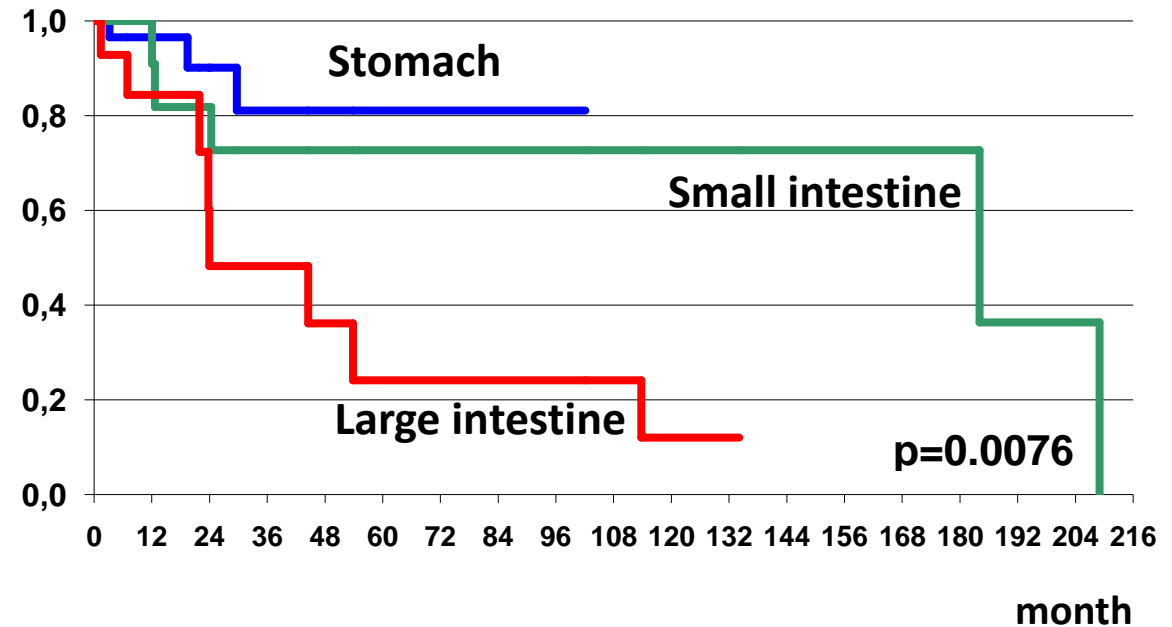
Real-time PCR amplification and melting point analysis & sequencing		High Resolution Melting		
		Wt	Point mut	Deletion
Wt	*****	***** *	*	
Point mut	*****		*****	
Deletion	*****			*****

Asterisks represent tumor samples

Localization of GIST (No = 72)

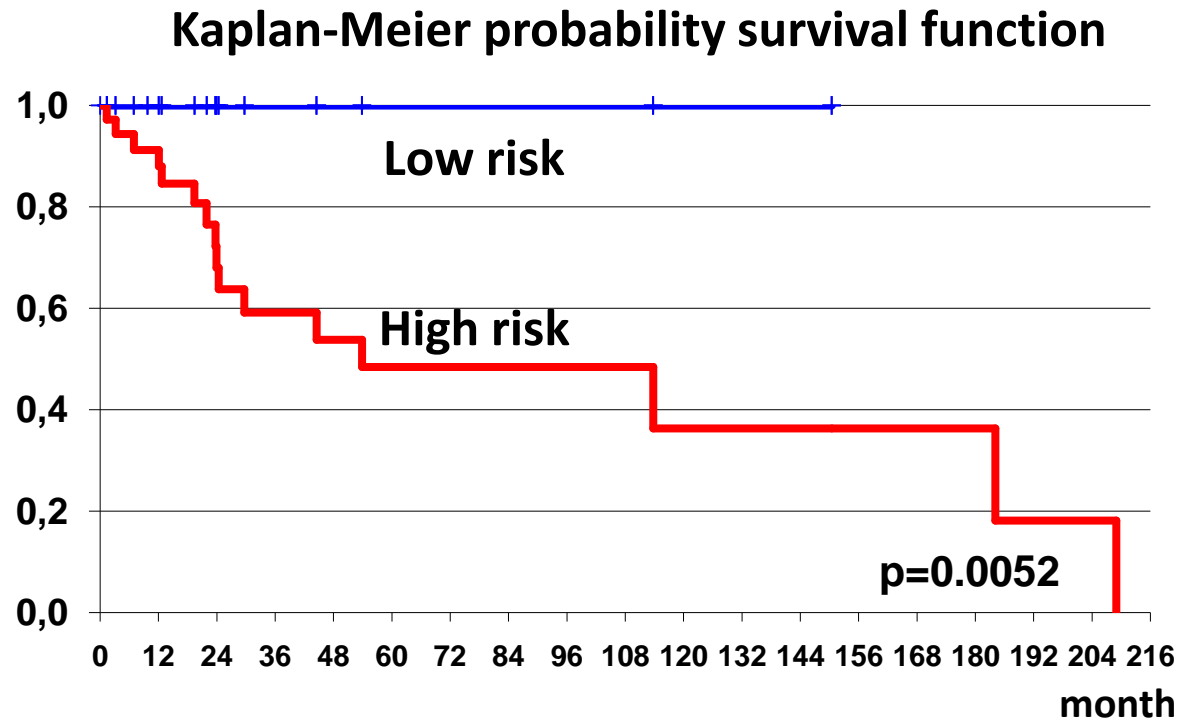
Localization	Frequency (%)
Esophagus	2.8
Stomach	48.6
Small Intestine	26.4
Large Intestine	11.1
Extra-intestinal	6.9
Metastasis	4.2

Kaplan-Meier probability survival function

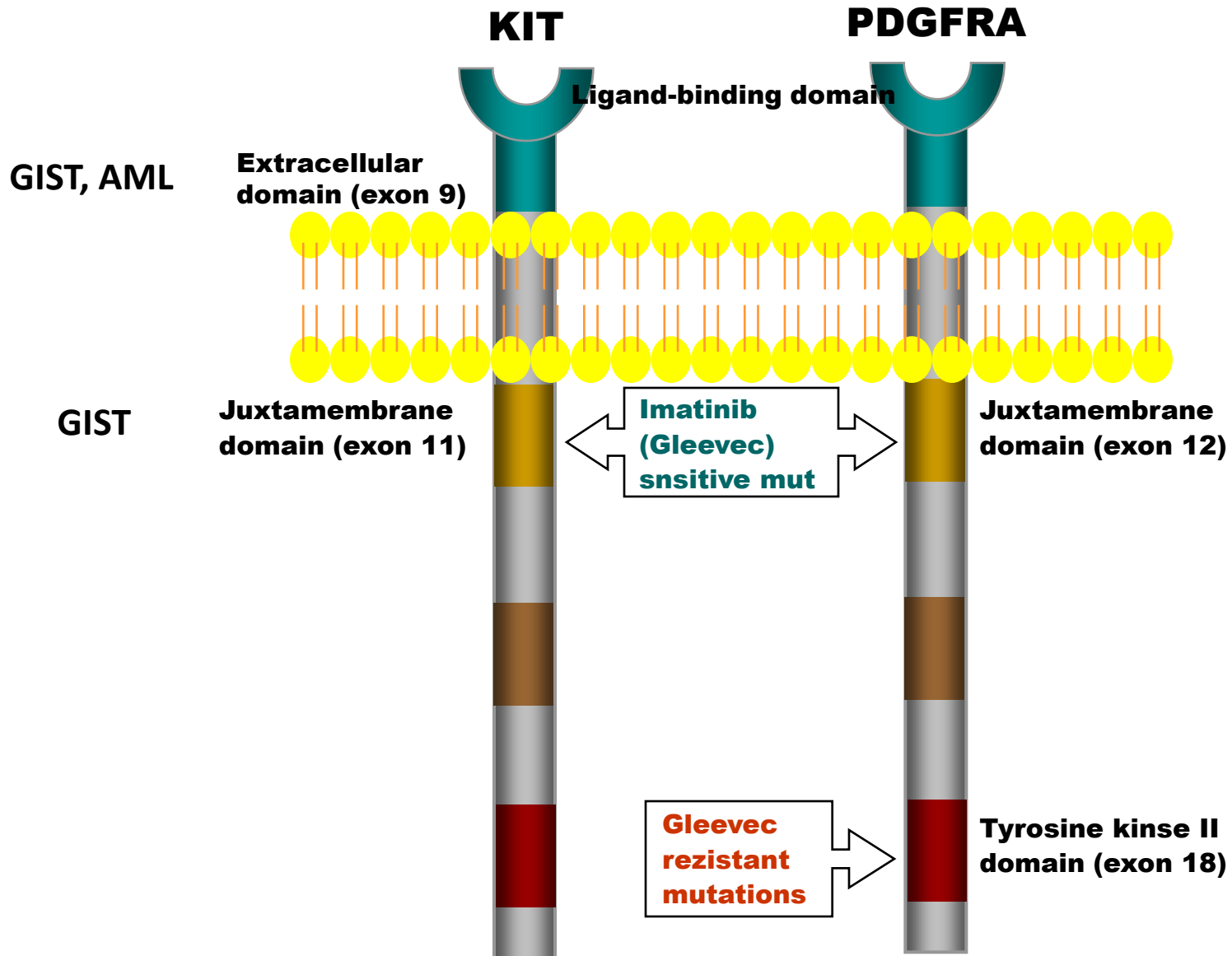


Pathological Risk Grouping of GIST (No = 72)

RISK GROUPS	FREQUENCY (%)
Low risk GIST	40.3
High risk GIST	59.7



Summary of Significant Genetik Alterations in GIST



Significant mutations in GIST 72 tumors

Gleevec sensitive mutations, c-kit			Gleevec resistance mutation, PDGFRA	Total
Exon 9 point m. & dupl	Exon 11 del	Exon 11 point mut	Exon 18 point mut & delins	
5 (6.9 %)	31 (43.1%)	15 (20.8 %)	8 (11.1 %)	59 (81.9%)

C-Kit exon 11

Highly Gleevec sensitive deletion mutations

Types of deletion in 14 tumor samples

cDNA

1650 1730

ACCCATGTATGAAGTACAGT**GGAAGGTTGT**TGAGGAGATA**AATGGAACA**ATTATGTTT**AC**CATAGACCCA**A**CACA**ACTTCC**
A---A

ACCCATGTAT**GAA**-----GAGGAGATA**AATGGAACA**ATTATGTTT**AC**CATAGACCCA**A**CACA**ACTTCC**

ACCCATGTATGAAGTACA-----TGT**TGAGGAGATA****AATGGAACA**ATTATGTTT**AC**CATAGACCCA**A**CACA**ACTTCC**

ACCCATGTATGAAGTACAG-----GTTGT**TGAGGAGATA****AATGGAACA**ATTATGTTT**AC**CATAGACCCA**A**CACA**ACTTCC**

ACCCATGTATGAAGTACAG-----GTTGT**TGAGGAGATA****AATGGAACA**ATTATGTTT**AC**CATAGACCCA**A**CACA**ACTTCC**

ACCCATGTATGAAGTACAG-----GTTGT**TGAGGAGATA****AATGGAACA**ATTATGTTT**AC**CATAGACCCA**A**CACA**ACTTCC**

ACCCATGTATGAAGTACAG-----GTTGT**TGAGGAGATA****AATGGAACA**ATTATGTTT**AC**CATAGACCCA**A**CACA**ACTTCC**

ACCCATGTATGAAGTACAGT**GG**-----GG

ACCCATGTATGAAGTACAGT**GG**-----CC**A**CACA**ACTTCC**

ACCCATGTATGAAGTACAGT**GGAAG**-----GACCCA**A**CACA**ACTTCC**

ACCCATGTATGAAGTACAGT**GGAAGGTT**-----CC

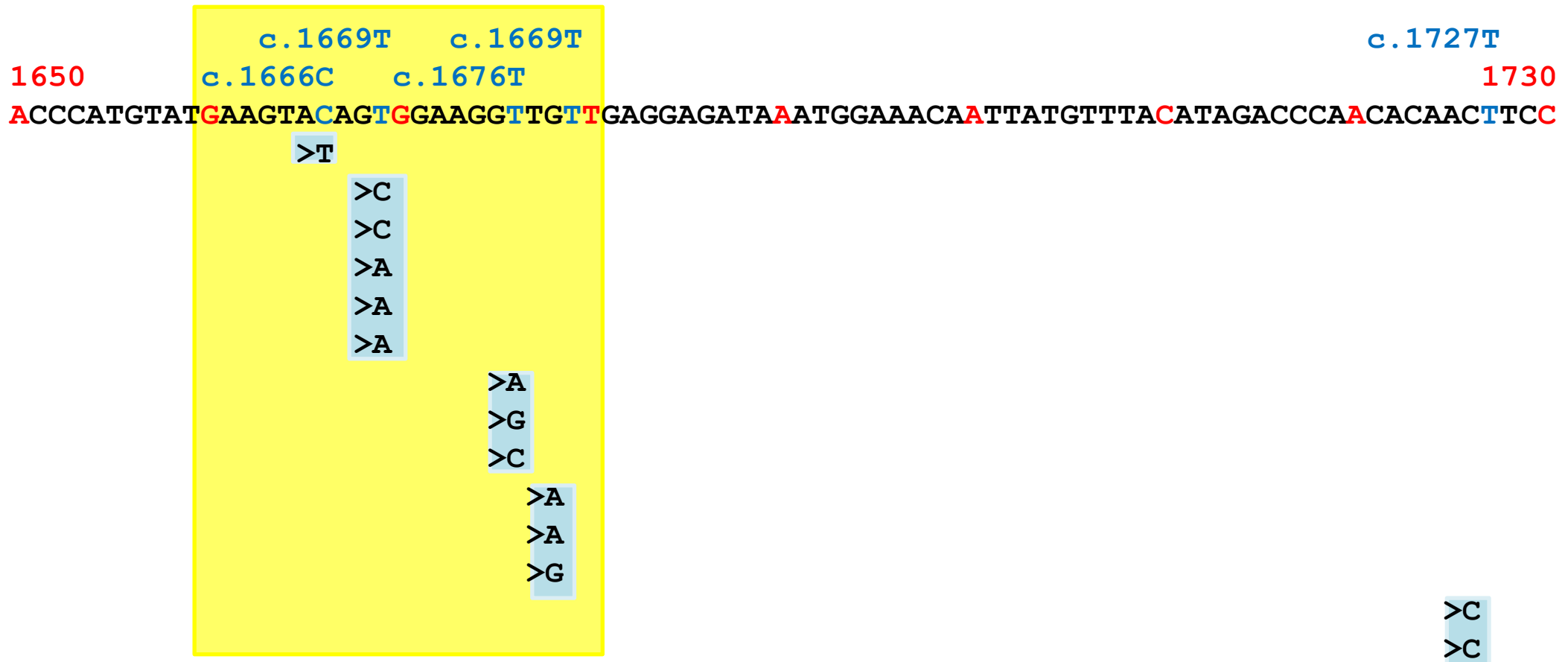
ACCCATGTATGAAGTACAGT**GGAAGGTTGT**TGAGGAGATA**AATGGAACA**ATTATGTTT**AC**CATAGACCCA**A**CACA**ACTTCC**

c.1735-1737

C-Kit exon 11

Highly Gleevec sensitive point mutations

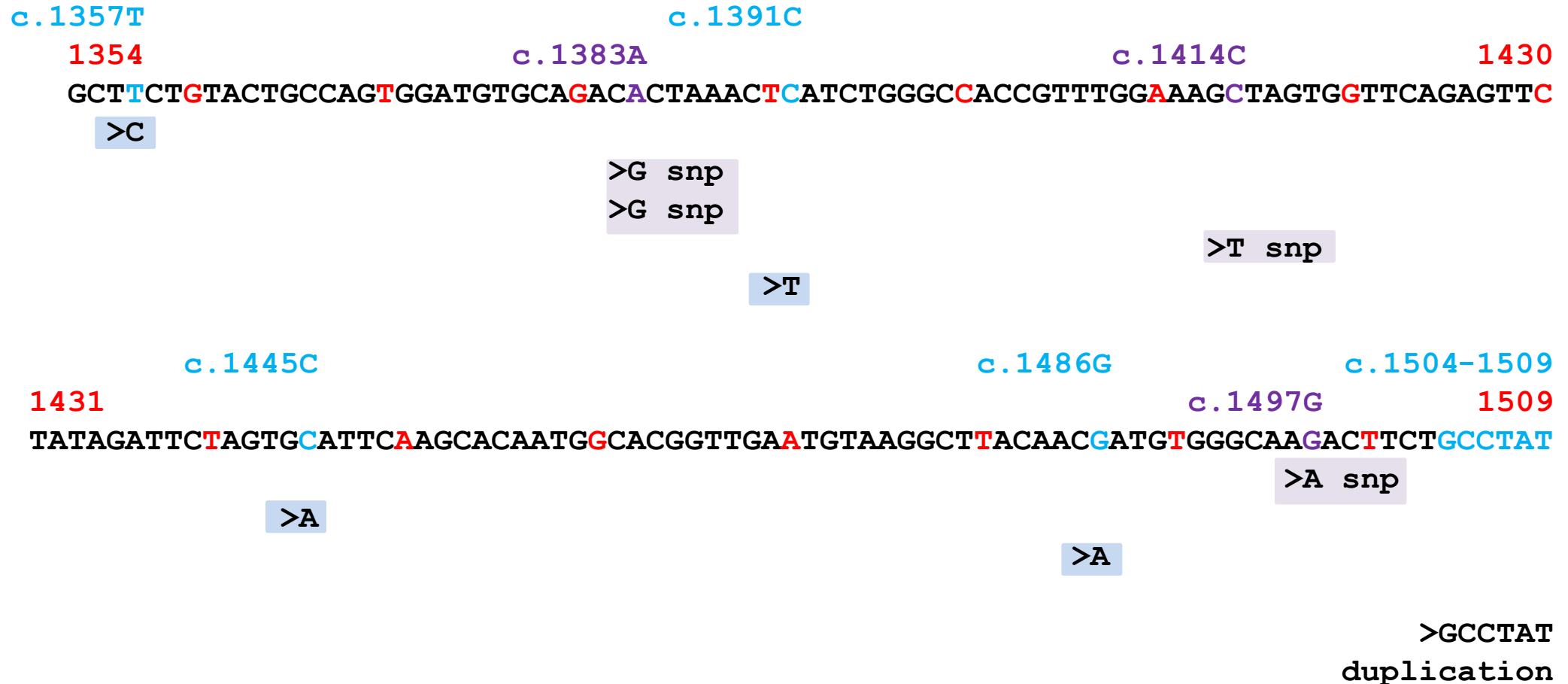
Mutation pattern (14 tumors)



C-Kit exon 9

Moderately Gleevec sensitive point mutations and SNPs

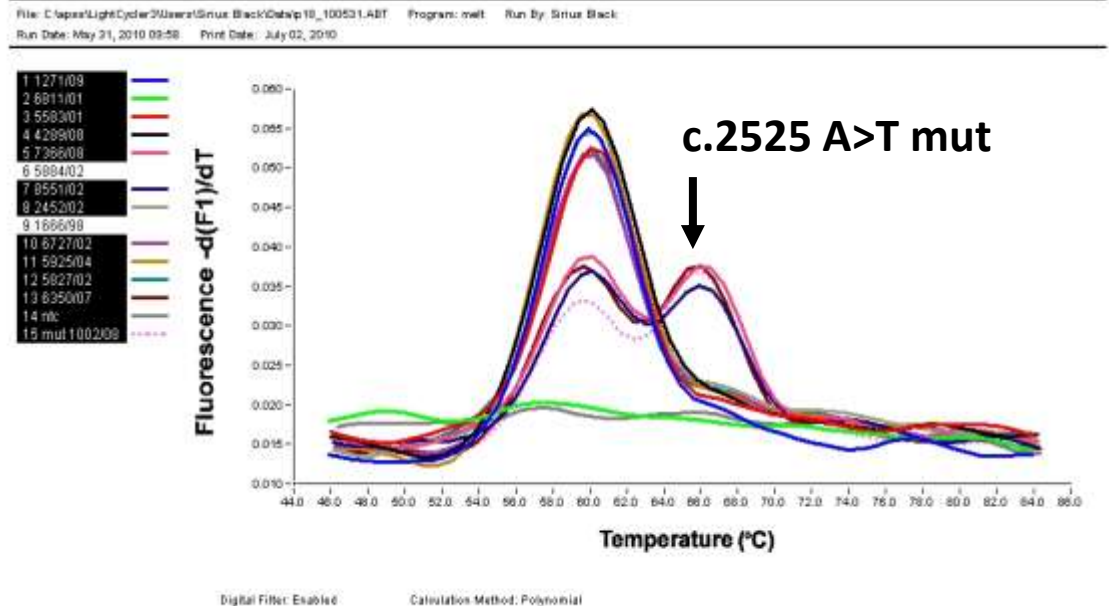
9 tumors (5 mutations, 4 SNPs)



Detection and Frequency of Gene Alterations in exon 18 of PDGFRA

Detection of p.D842V mutation by
real-time PCR and melting point analysis

GENE ALTERATION	FREQUENCY %
c.2525A>T (p.D842V)	9.7
Delins	1.4
c.2472C>T snp	11.1
c.2442T>C snp	2.8
c.2526C>T snp	1.4
c.2538T>C snp	1.4



Correlation Between the Mutation Status of c-kit exon 9 & 11 and the Metastatic Potential of GIST

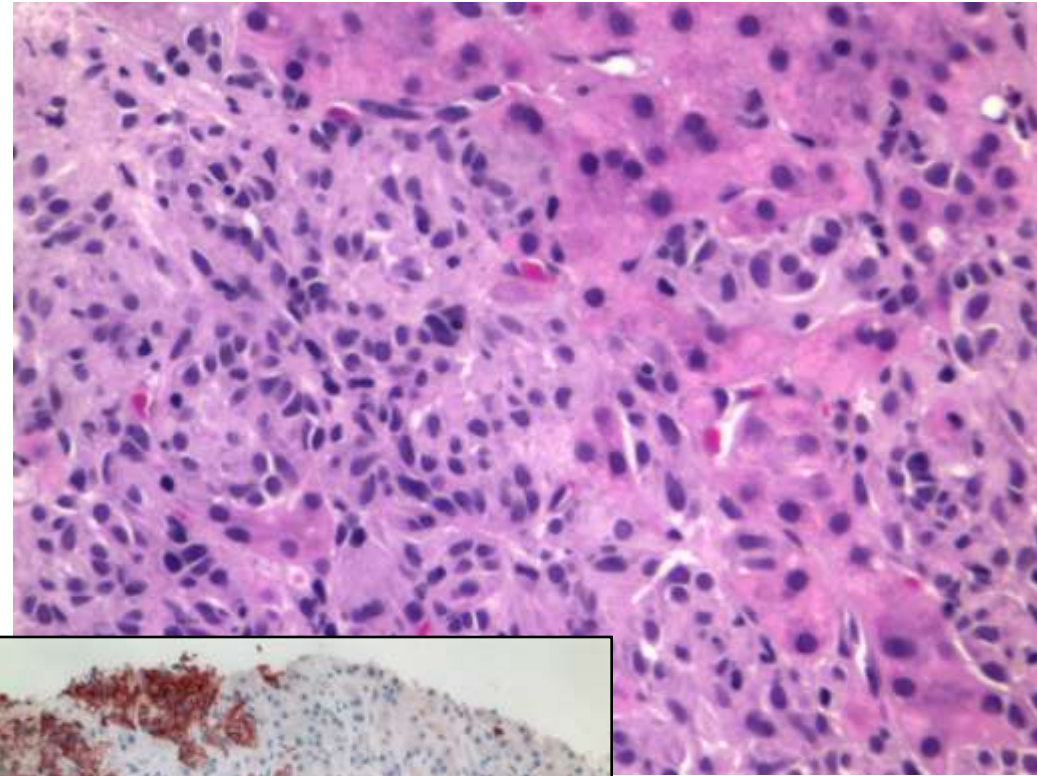
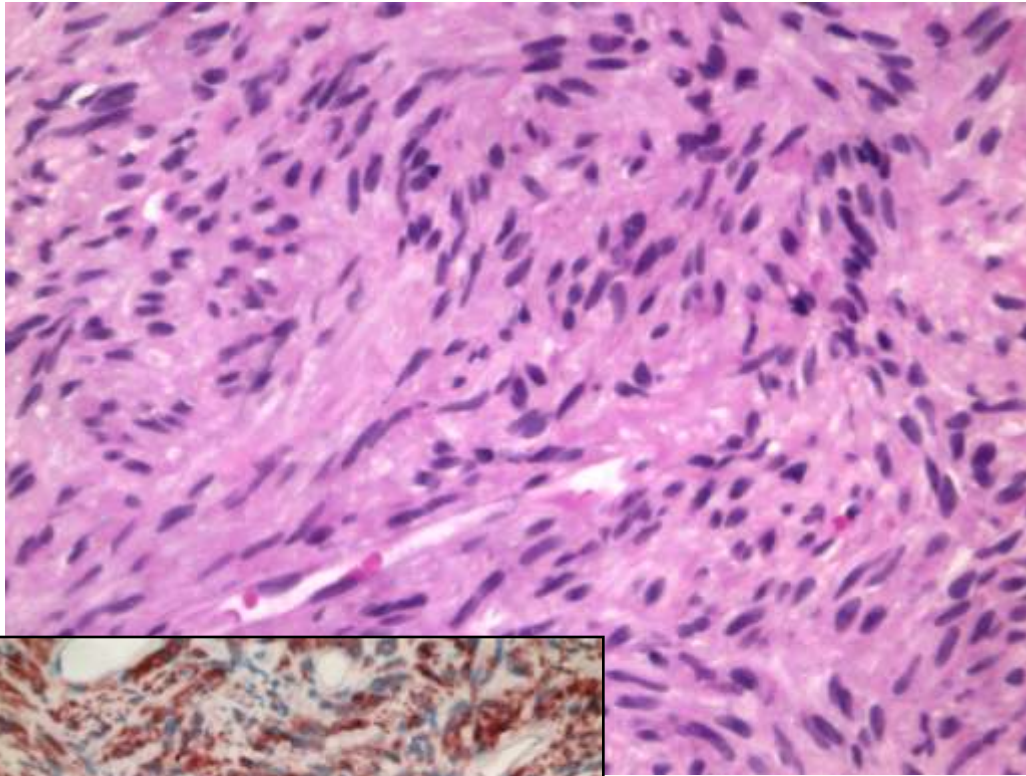
C-kit	Metastasis		Total %
	Absent	Present	
Wild type	76.2	23.8	100
Exon 9 & 11 point mut	60.0	40.0	100
Exon 11 deletion	25.8	74.2	100

Pearson chi-square

P = 0.0010

The c-kit mutations promote the metastatic tendency of GIST

MP487/08, 55-year-old male with a 42x35x31 mm abdominal mass and a 22 mm in diameter nodule in the 5th segment of liver. Core biopsy from the abdominal mass and liver nodule.

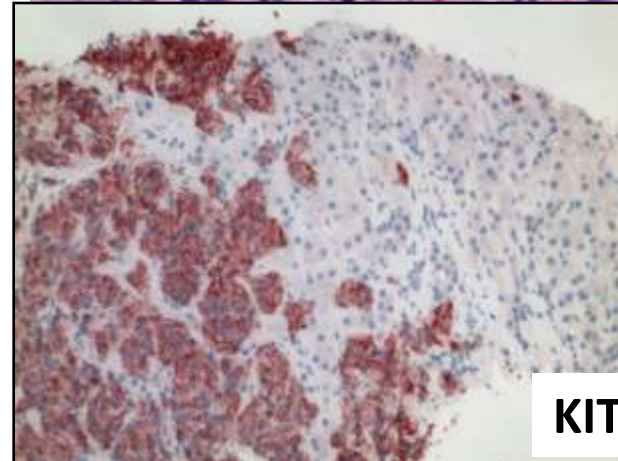
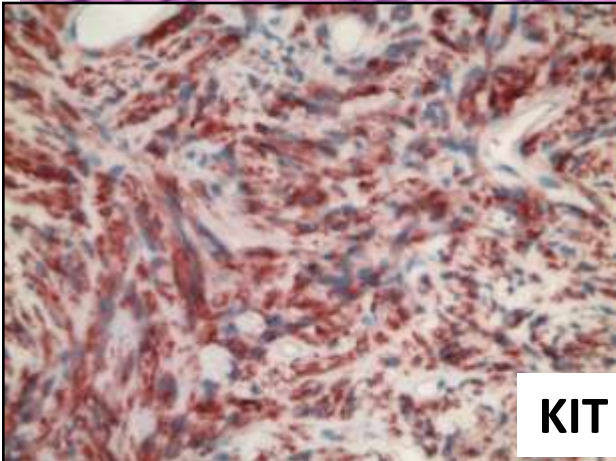


Primary tumor

Liver metastasis

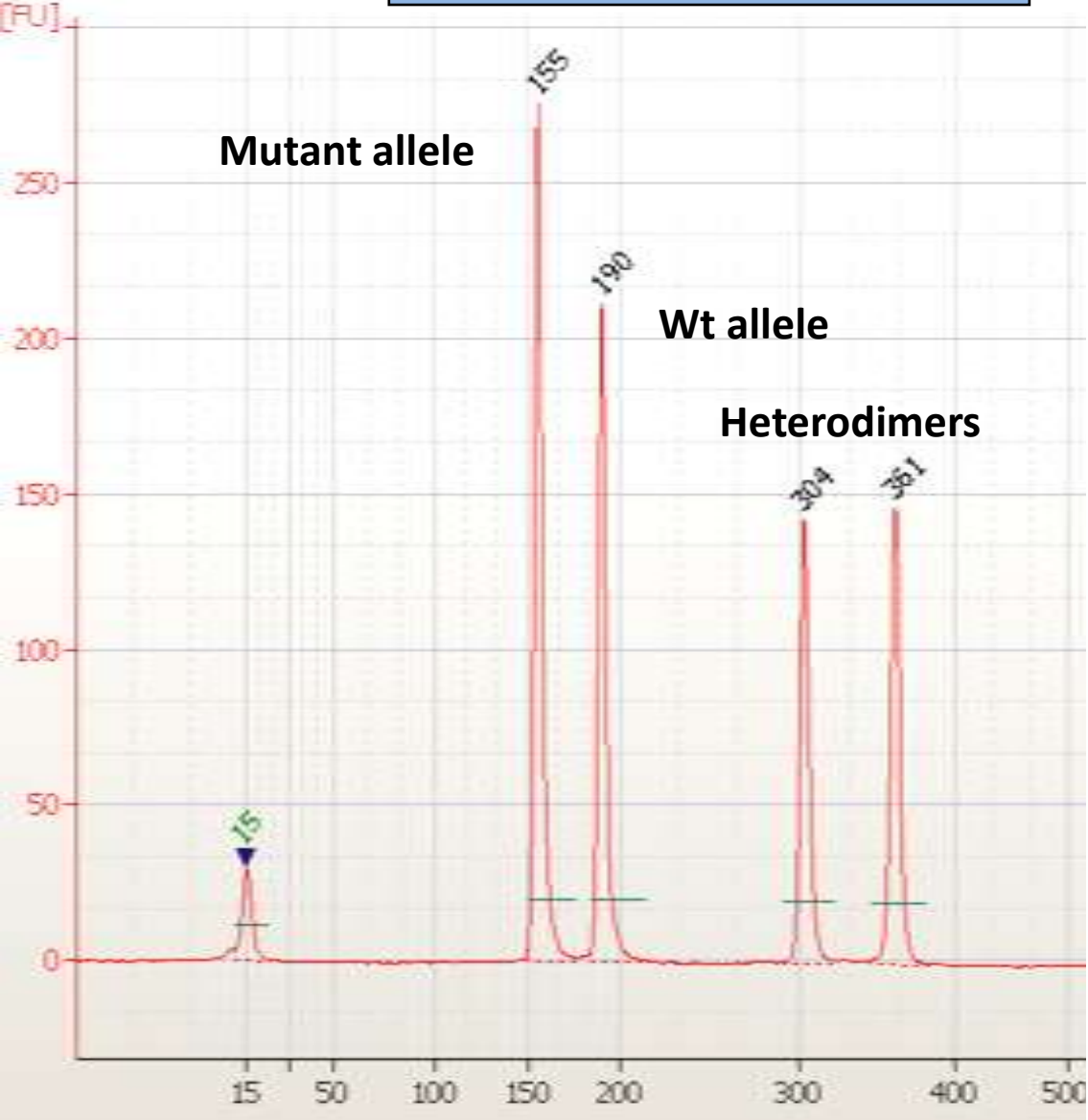
KIT (CD117)

KIT (CD117)

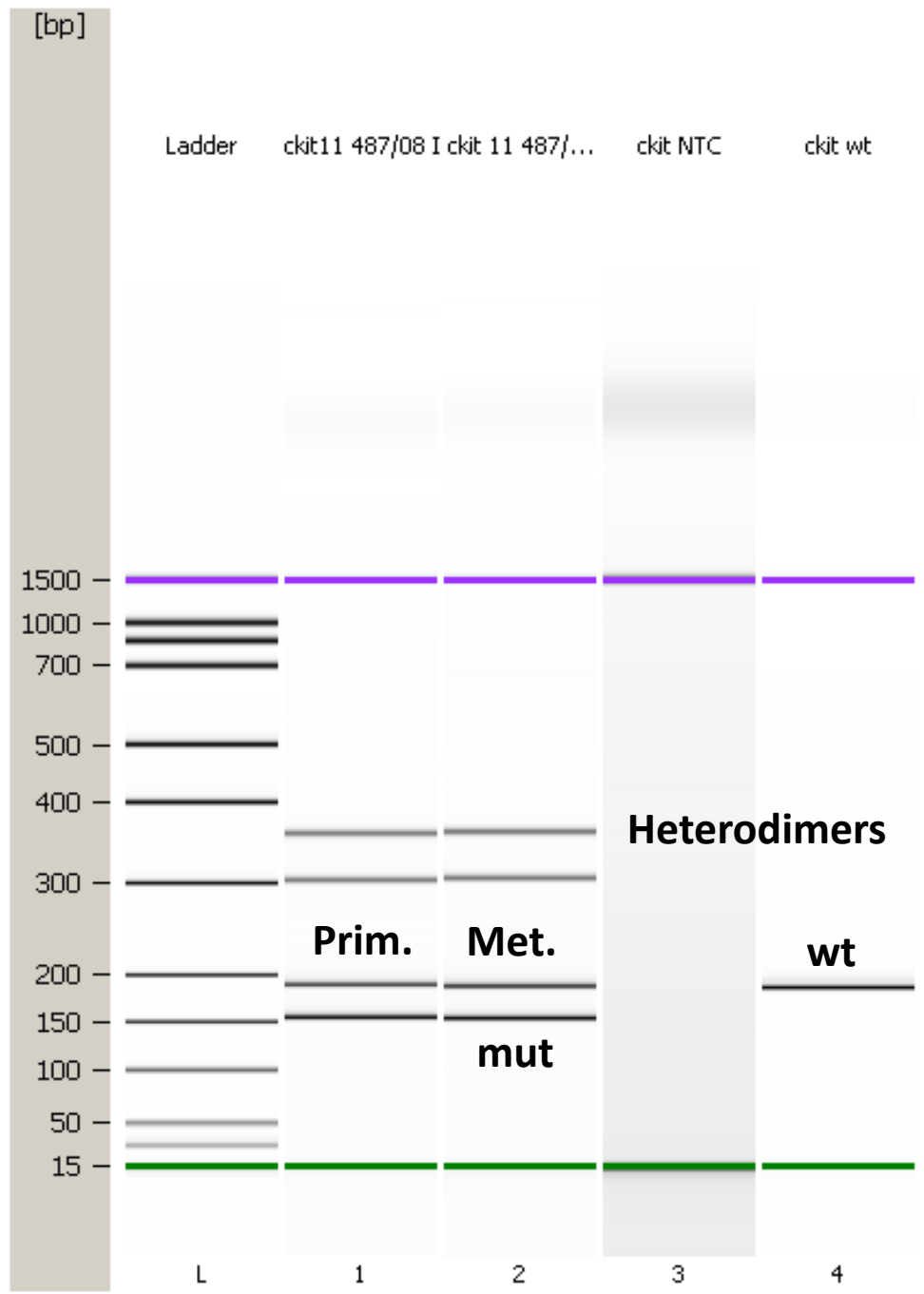


MP487/08

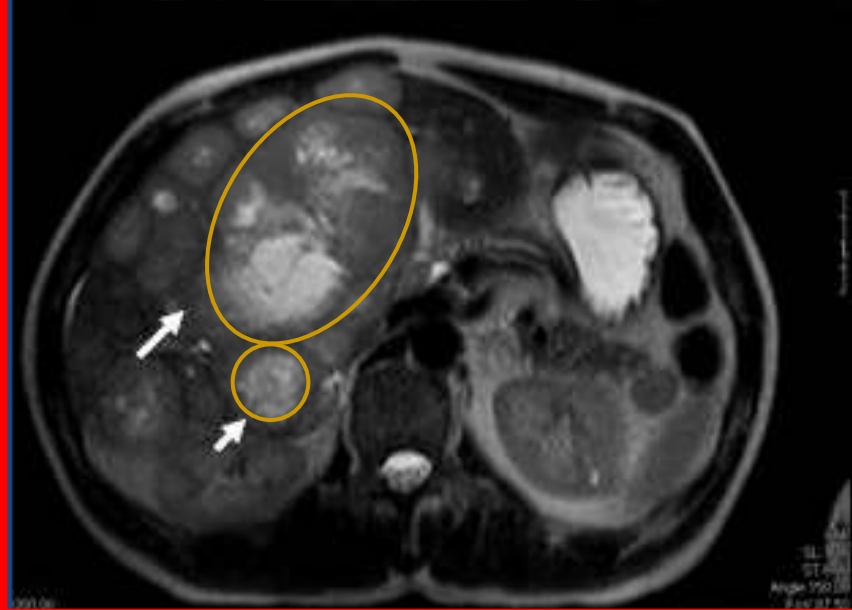
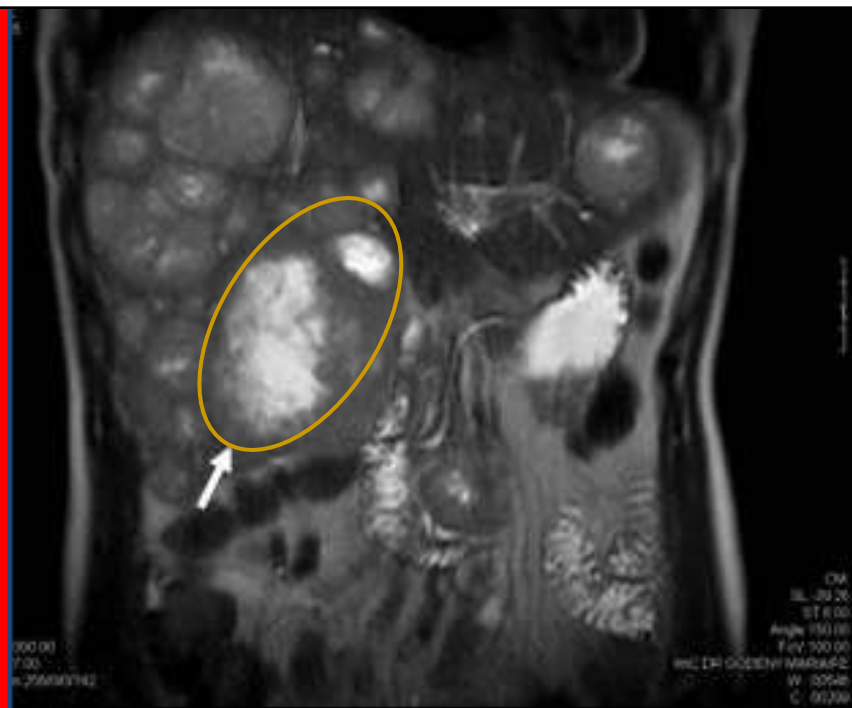
cKIT exon 11: 35 bp deletion



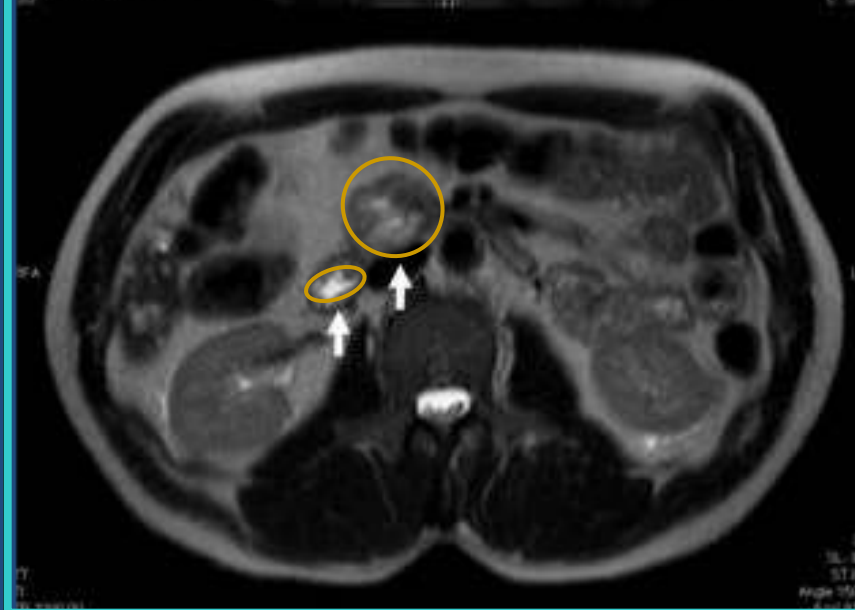
High resolution capillary gel electrophoresis



55 year-old male, at the time of diagnosis



8 moths after Gleevec treatment



Kaplan-Meier Probability Function of Gleevec Sensitive Mutation Bearing GIST Versus other Subtype of GIST

