UPPSALA UNIVERSITET

Molecular analysis of colon cancer

Magnus.Sundstrom@igp.uu.se

UPPSALA UNIVERSITET

Molecular analysis of colon cancer

General aim

Find predictors to identify which patients that need adjuvant chemotherapy

Project specific aim

Evaluate various molecular markers in colon cancer for association to prognosis

Magnus.Sundstrom@igp.uu.se

UPPSALA UNIVERSITET

Molecular analysis of colon cancer

General aim
Find predictors to identify which patients that need adjuvant chemotherapy

Project specific aim
Evaluate various molecular markers in colon cancer for association to prognosis

Patient cohort (n=130)

- Locally and radically resected colon cancer
- Stage II-IV
- Matched case control design
- At least five years follow up
- Fresh frozen tissues available
- Tumors with at least 40% tumor cell content

Magnus.Sundstrom@igp.uu.se

UPPSALA UNIVERSITET

Study design

- Comparison between two groups of patients with different outcome:
  - Those without metastatic disease
  - Those with metastatic disease

Patients with metastases at diagnosis (stage IV) were considered equivalent to those with metachronous metastases appearing within the follow-up period

Rationale

- The primary tumor possess certain traits that leads to both variants of metastasis
- The tumor cell properties should be the same independent upon when metastases appear

UPPSALA UNIVERSITET

Colon cancer patient cohort

	Cases (n)	Controls (n)
Total	72	58
Stage II	16	29
Stage III	30	29
Stage IV	26	-

Cases defined as patients with **metastasis at diagnosis or later**

UPPSALA UNIVERSITET

Methods

- DNA from fresh frozen tumor tissues

- Mutation analysis of hotspot mutations by Pyrosequencing
  - Codons 12, 13 and 61 for **KRAS**
    Benchmarked (n=100) against two commercial IVD kit
  - Codon 600 for **BRAF**
  - Codons 542, 545, 546, 1043 and 1047 for **PIK3CA (PI3K)**

UPPSALA UNIVERSITET

Methods

- DNA from fresh frozen tumor tissues

- Mutation analysis of hotspot mutations by Pyrosequencing
  - Codons 12, 13 and 61 for **KRAS**
    Benchmarked (n=100) against two commercial IVD kit
  - Codon 600 for **BRAF**
  - Codons 542, 545, 546, 1043 and 1047 for **PIK3CA (PI3K))**

- Microsatellite instability (**MSI**)-assay:
  - Commercial PCR assay for five mononucleotide repeat markers
  - MSI-H if instability in two or more markers
  - In statistical analyses, MSS was grouped together with MSI-L

UPPSALA UNIVERSITET

Methods

- DNA from fresh frozen tumor tissues

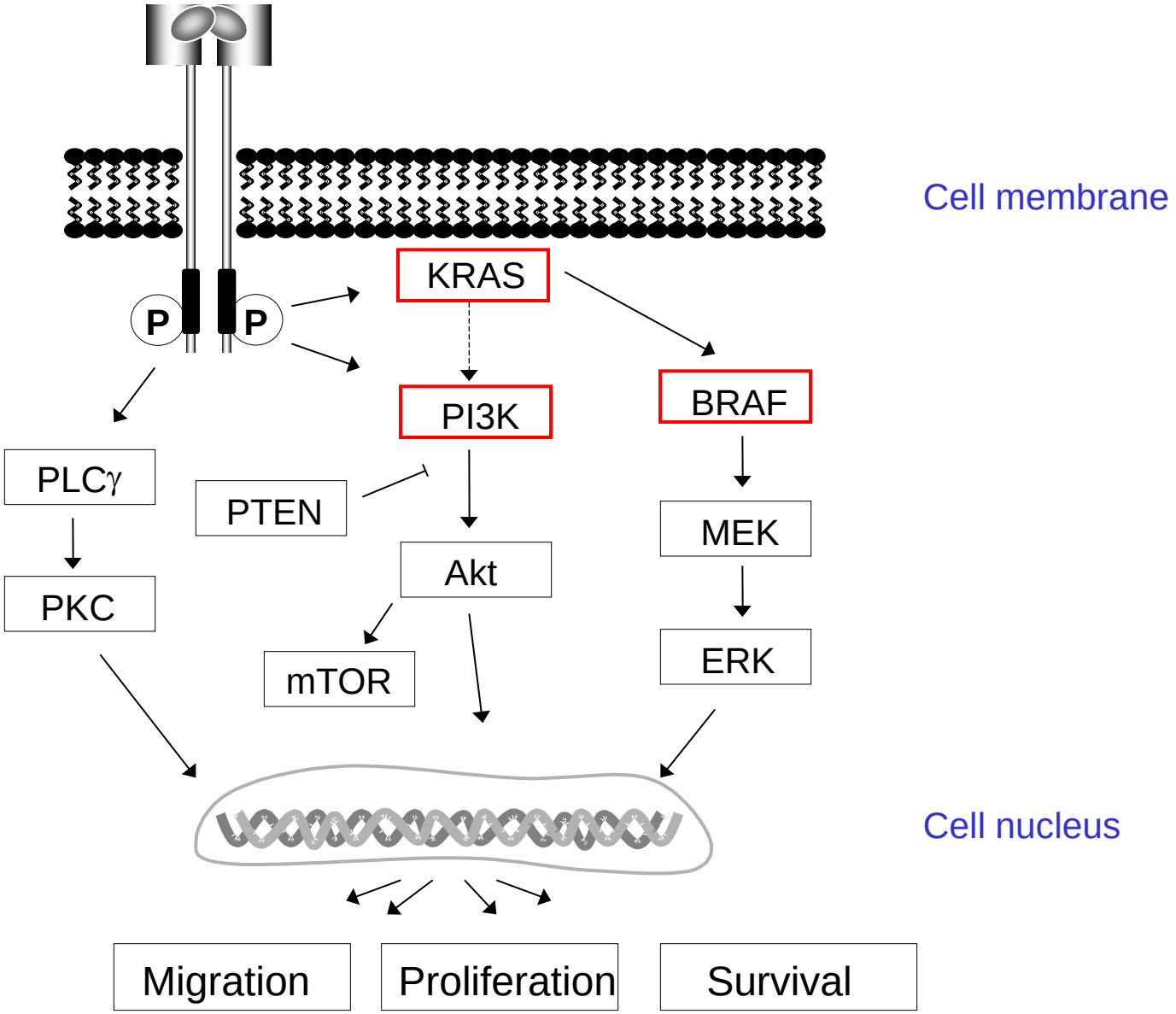
- Mutation analysis of hotspot mutations by Pyrosequencing
  - Codons 12, 13 and 61 for **KRAS**
    Benchmarked (n=100) against two commercial IVD kit
  - Codon 600 for **BRAF**
  - Codons 542, 545, 546, 1043 and 1047 for **PIK3CA (PI3K)**

- Microsatellite instability (**MSI**)-assay:
  - Commercial PCR assay for five mononucleotide repeat markers
  - MSI-H if instability in two or more markers
  - In statistical analyses, MSS was grouped together with MSI-L

- SNP **microarray assay** at core facility
  - Alterations in gene copy numbers, allelic imbalance and average ploidy

UPPSALA UNIVERSITET

Receptor tyrosine kinase signaling



UPPSALA UNIVERSITET

Genomic instability in colorectal cancer

Colorectal cancer tumorigenesis

Two major pathways invoving genomic instability:

Microsatellite instability (MSI-H) in about 15% of sporadic tumors.

• MSI = ”A form of genome instability associated with defects in DNA mismatch repair” i.e. errors in DNA are not corrected.

UPPSALA UNIVERSITET

Genomic instability in colorectal cancer

Colorectal cancer tumorigenesis

Two major pathways involving genomic instability:

Microsatellite instability (MSI-H) in about 15% of sporadic tumors.

- MSI = ”A form of genome instability associated with defects in DNA mismatch repair” i.e. errors in DNA are not corrected.

Chromosome instability (CIN) in 65-70% of colorectal tumors.

- CIN is poorly defined as ”gain or loss of whole chromosomes or fractions of chromosomes”.
- CIN results in aneuploidy.
- CIN tumors have an aggressive clinical behavior.
- Can the reason for this be better understood by detailed SNP array analysis?

UPPSALA UNIVERSITET

Prognostic markers - background

Marker	Prognostic?	Comment
KRAS mut	Controversial	Particular mutations have impact on survival
BRAF mut	Poor prognosis	Especially MSS BRAF mutated patients and in metastatic disease
PIK3CA mut	Controversial	Coexistence of ex 9 and ex 20 mutations is bad
MSI-H	Good prognosis	
CIN	Poor prognosis	“CIN” is poorly defined

UPPSALA UNIVERSITET

Frequency of mutations and MSI-H

	Cohort n=130	CRC in general
KRAS mut	39%	35-45%
BRAF mut	23%	14%
PIK3CA mut	18%	19%
MSI-H	20%	17%

UPPSALA UNIVERSITET

Distribution of mutations and MSI-H according to stage

	Total	Stage II	Stage III	Stage IV	*p*-value (χ^2)
Total	130	45	59	26	
KRAS mut	51	24%	39%	65%	< 0.01
KRAS wt	79	76%	61%	35%	
BRAF mut	30	31%	22%	11%	0.16
BRAF wt	100	69%	78%	89%	
PIK3CA mut	23	13%	17%	27%	0.34
PIK3CA wt	107	87%	83%	73%	
MSI-H	26	33%	15%	8%	
MSS/MSI-L	104	67%	85%	92%	0.02

UPPSALA UNIVERSITET

Distribution of mutations and MSI-H according to stage

	Total	Stage II	Stage III	Stage IV	*p*-value (χ^2)
Total	130	45	59	26	
KRAS mut	51	24%	39%	65%	< 0.01
KRAS wt	79	76%	61%	35%	
BRAF mut	30	31%	22%	11%	0.16
BRAF wt	100	69%	78%	89%	
PIK3CA mut	23	13%	17%	27%	0.34
PIK3CA wt	107	87%	83%	73%	
MSI-H	26	33%	15%	8%	
MSS/MSI-L	104	67%	85%	92%	0.02

UPPSALA UNIVERSITET

Distribution of mutations and MSI-H according to stage

	Total	Stage II	Stage III	Stage IV	*p*-value (χ^2)
Total	130	45	59	26	
KRAS mut	51	24%	39%	65%	< 0.01
KRAS wt	79	76%	61%	35%	
BRAF mut	30	31%	22%	11%	0.16
BRAF wt	100	69%	78%	89%	
PIK3CA mut	23	13%	17%	27%	0.34
PIK3CA wt	107	87%	83%	73%	
MSI-H	26	33%	15%	8%	
MSS/MSI-L	104	67%	85%	92%	0.02

UPPSALA UNIVERSITET

Distribution of mutations according to MSI-H status

	Total (n)	MSI-H	MSS/MSI-L	*p*-value (χ^2)
KRAS				
Wild type	79	92%	53%	
Mutation	51	8%	47%	< 0.01
BRAF				
Wild type	100	23%	90%	
Mutation	30	77%	10%	< 0.01
PIK3CA				
Wild type	107	65%	87%	
Mutation	23	35%	13%	0.01

UPPSALA UNIVERSITET

Distribution of mutations according to MSI-H status

	Total (n)	MSI-H	MSS/MSI-L	*p*-value (χ^2)
KRAS				
Wild type	79	92%	53%	
Mutation	51	8%	47%	< 0.01
BRAF				
Wild type	100	23%	90%	
Mutation	30	77%	10%	< 0.01
PIK3CA				
Wild type	107	65%	87%	
Mutation	23	35%	13%	0.01

UPPSALA UNIVERSITET

Aneuploidy and allelic imbalance

- Allele-specific copy number analysis was used to estimate the average ploidy of the samples (Ref. Rasmussen, Genome Biology, 2011)

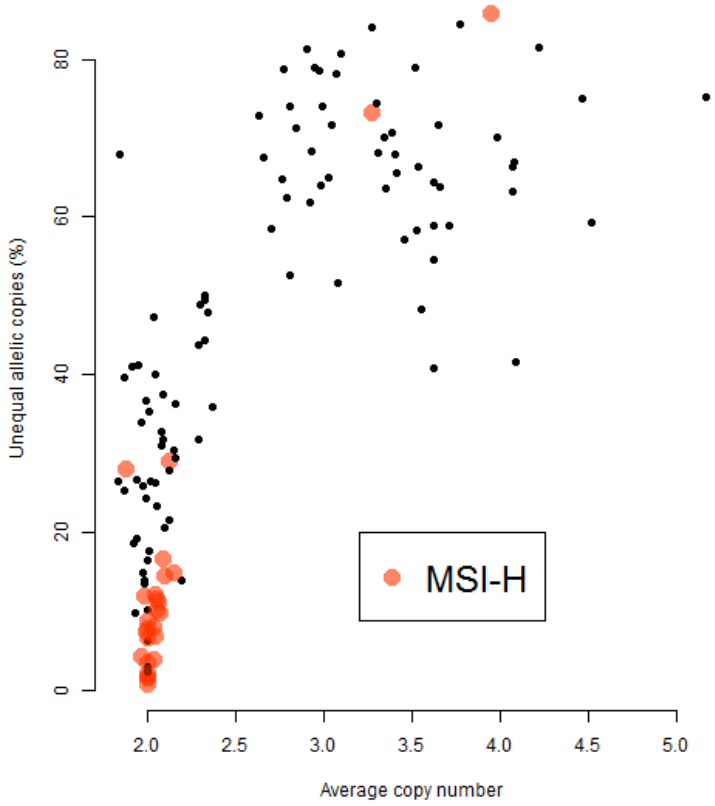
- Average ploidy was plotted against genome-wide extent of allelic-imbalance

UPPSALA UNIVERSITET

Aneuploidy and allelic imbalance

• Allele-specific copy number analysis was used to estimate the average ploidy of the samples (Ref. Rasmussen, Genome Biology, 2011)

• Average ploidy was plotted against genome-wide extent of allelic-imbalance



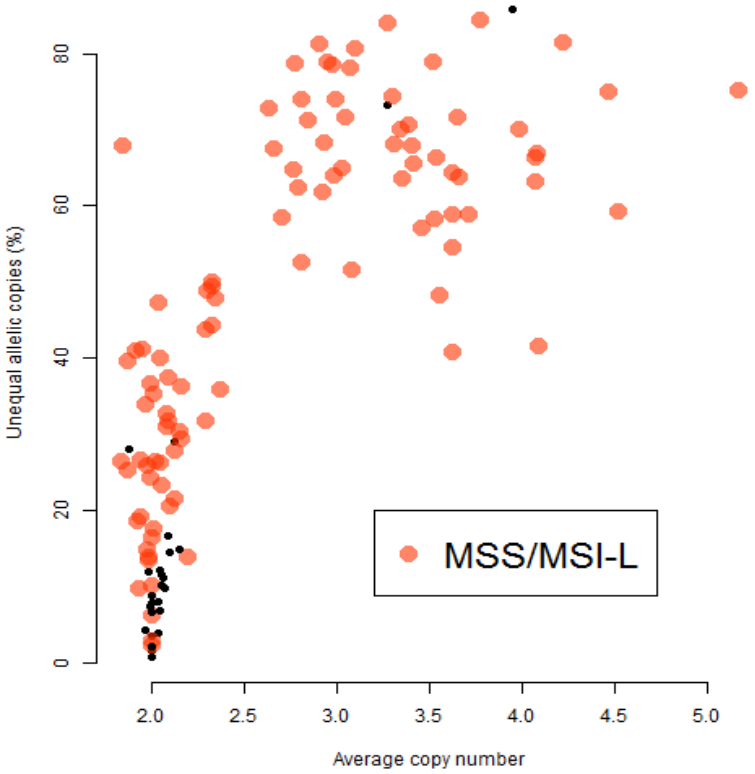
MSI-H tumors were diploid or near-diploid and had low levels of allelic-imbalance

UPPSALA UNIVERSITET

Aneuploidy and allelic imbalance

- Allele-specific copy number analysis was used to estimate the average ploidy of the samples (Ref. Rasmussen, Genome Biology, 2011)

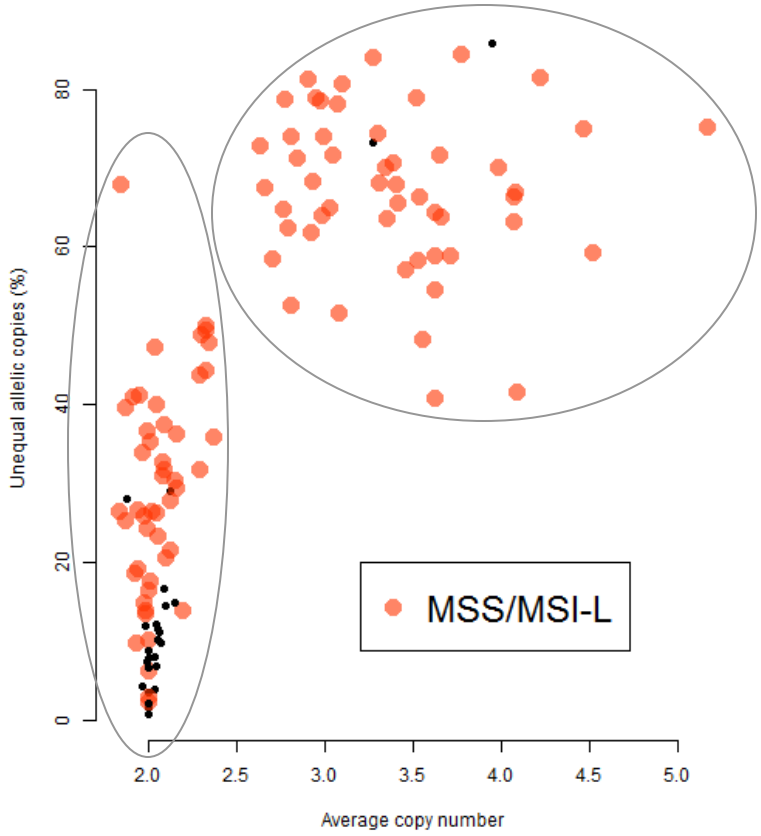
- Average ploidy was plotted against genome-wide extent of allelic-imbalance



MSS/MSI-L tumors had extensive genomic aberrations

UPPSALA UNIVERSITET

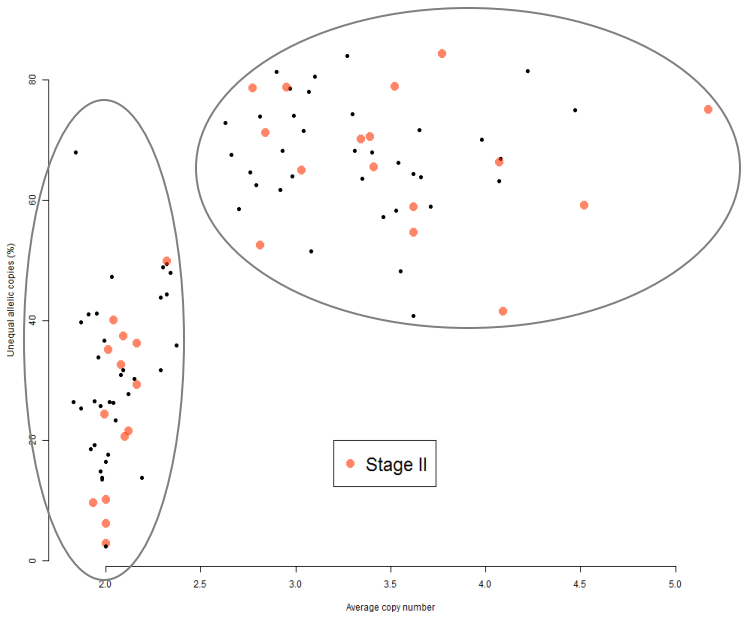
Aneuploidy and allelic imbalance

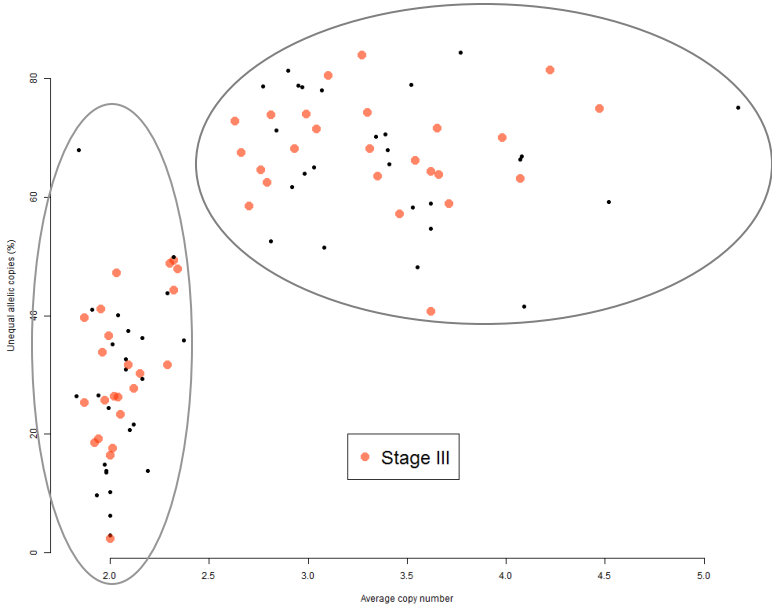


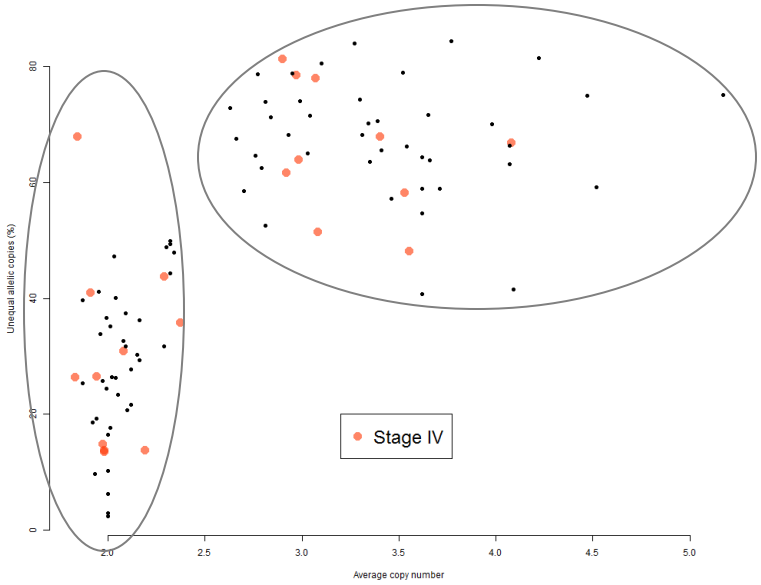
Two subgroups of MSS/MSI-L tumors

UPPSALA UNIVERSITET

MSS/MSI-L: aneuploidy and allelic imbalance



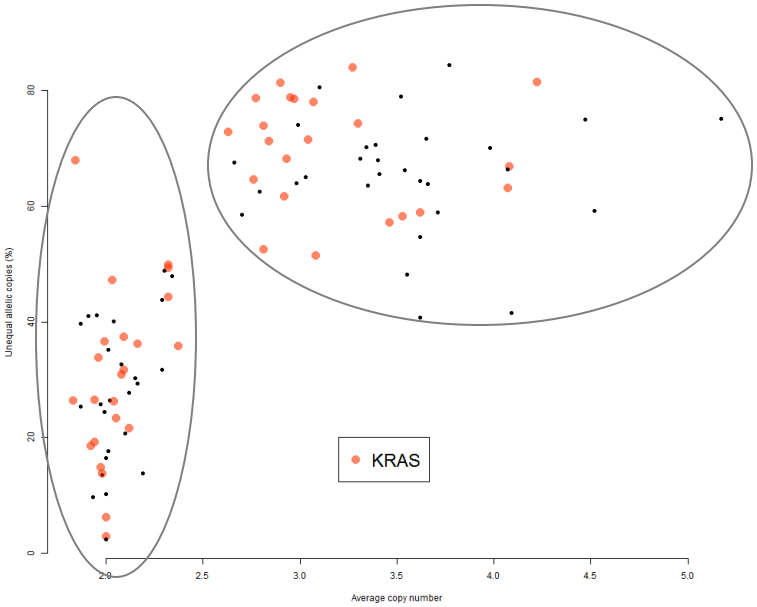


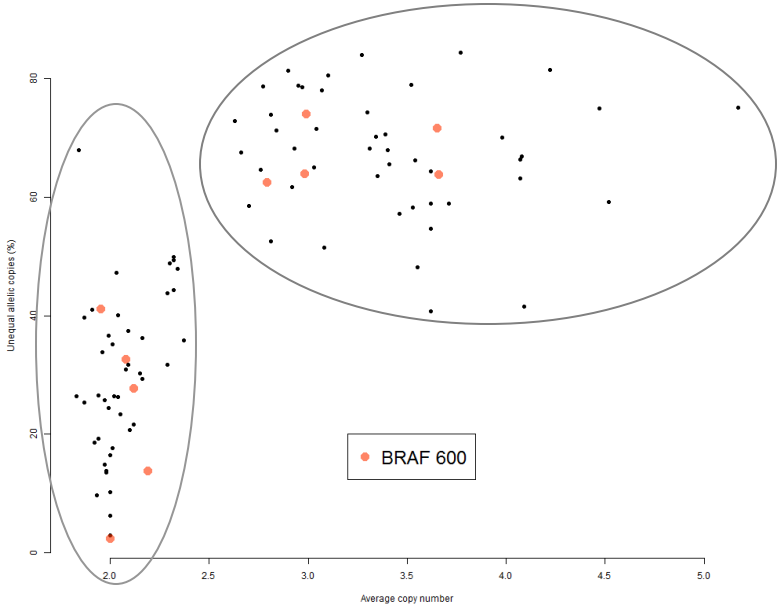


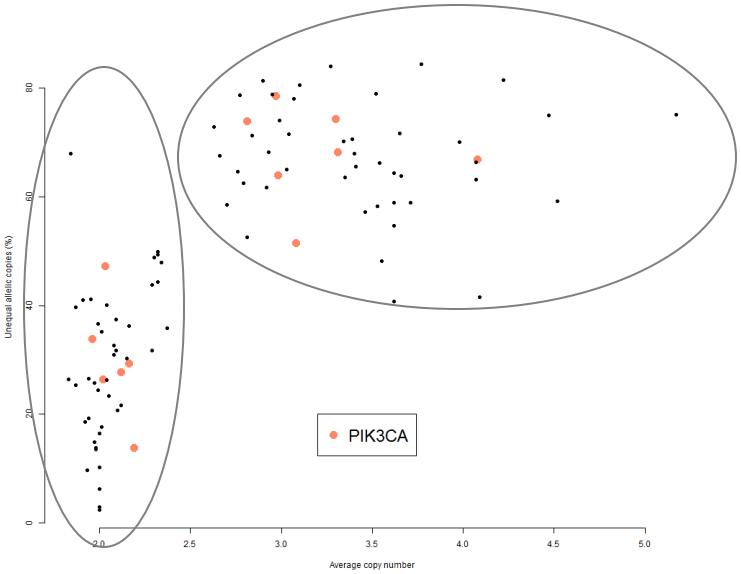
Two subgroups of MSS/MSI-L patients but no association to **stage**

UPPSALA UNIVERSITET

MSS/MSI-L: aneuploidy and allelic imbalance



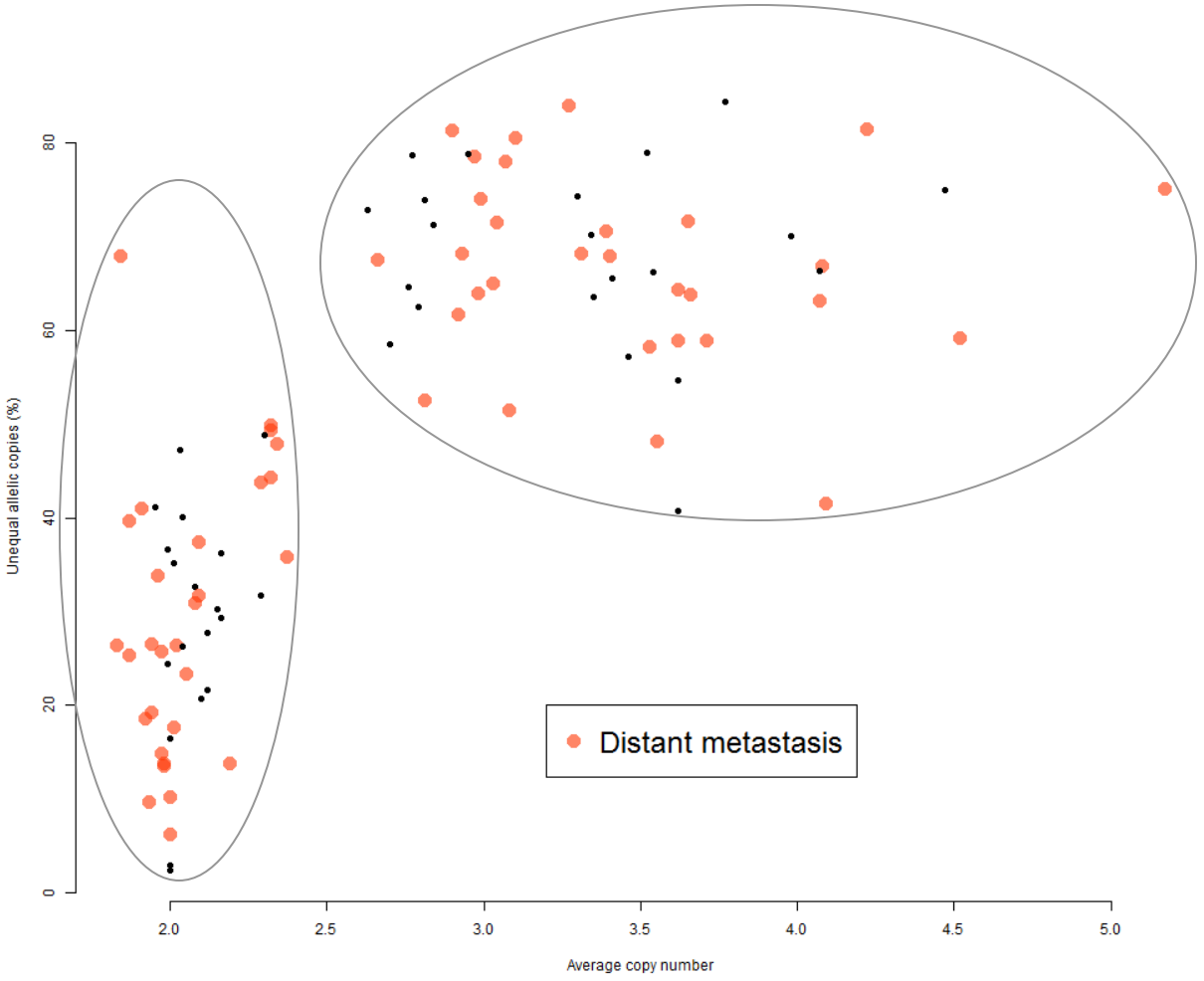




Two subgroups of MSS/MSI-L patients but no association to **mutations**

UPPSALA UNIVERSITET

Molecular markers for distant metastasis?



No association of the MSS/MSI-L subgroups to **distant metastasis**

UPPSALA UNIVERSITET

Molecular markers for distant metastasis

		Distant metastasis		
	Total	**Yes**	**No**	***p*-value (χ^2)**
MSI-H	26	12%	29%	
MSS/MSI-L	104	88%	71%	0.02
KRAS mut	51	50%	26%	
KRAS wt	79	50%	74%	<0.01
BRAF mut	30	17%	31%	
BRAF wt	100	83%	69%	0.05
PIK3CA mut	23	18%	17%	
PIK3CA wt	107	82%	83%	0.87

UPPSALA UNIVERSITET

Molecular markers for distant metastasis

		Distant metastasis		
	Total	Yes	No	*p*-value (χ^2)
MSI-H	26	12%	29%	
MSS/MSI-L	104	88%	71%	0.02
KRAS mut	51	50%	26%	
KRAS wt	79	50%	74%	<0.01
BRAF mut	30	17%	31%	
BRAF wt	100	83%	69%	0.05
PIK3CA mut	23	18%	17%	
PIK3CA wt	107	82%	83%	0.87

UPPSALA UNIVERSITET

Molecular markers for distant metastasis

		Distant metastasis		
	Total	Yes	No	*p*-value (χ^2)
MSI-H	26	12%	29%	
MSS/MSI-L	104	88%	71%	0.02
KRAS mut	51	50%	26%	
KRAS wt	79	50%	74%	<0.01
BRAF mut	30	17%	31%	
BRAF wt	100	83%	69%	0.05
PIK3CA mut	23	18%	17%	
PIK3CA wt	107	82%	83%	0.87

UPPSALA UNIVERSITET

Molecular markers for distant metastasis

		Distant metastasis		
	Total	**Yes**	**No**	***p*-value (χ^2)**
KRAS wt + MSI-H	24	**11%**	28%	
KRAS wt + MSS/MSI-L	55	39%	47%	
KRAS mut + MSS/MSI-L	49	**49%**	24%	**<0.01**

UPPSALA UNIVERSITET

Molecular markers for distant metastasis

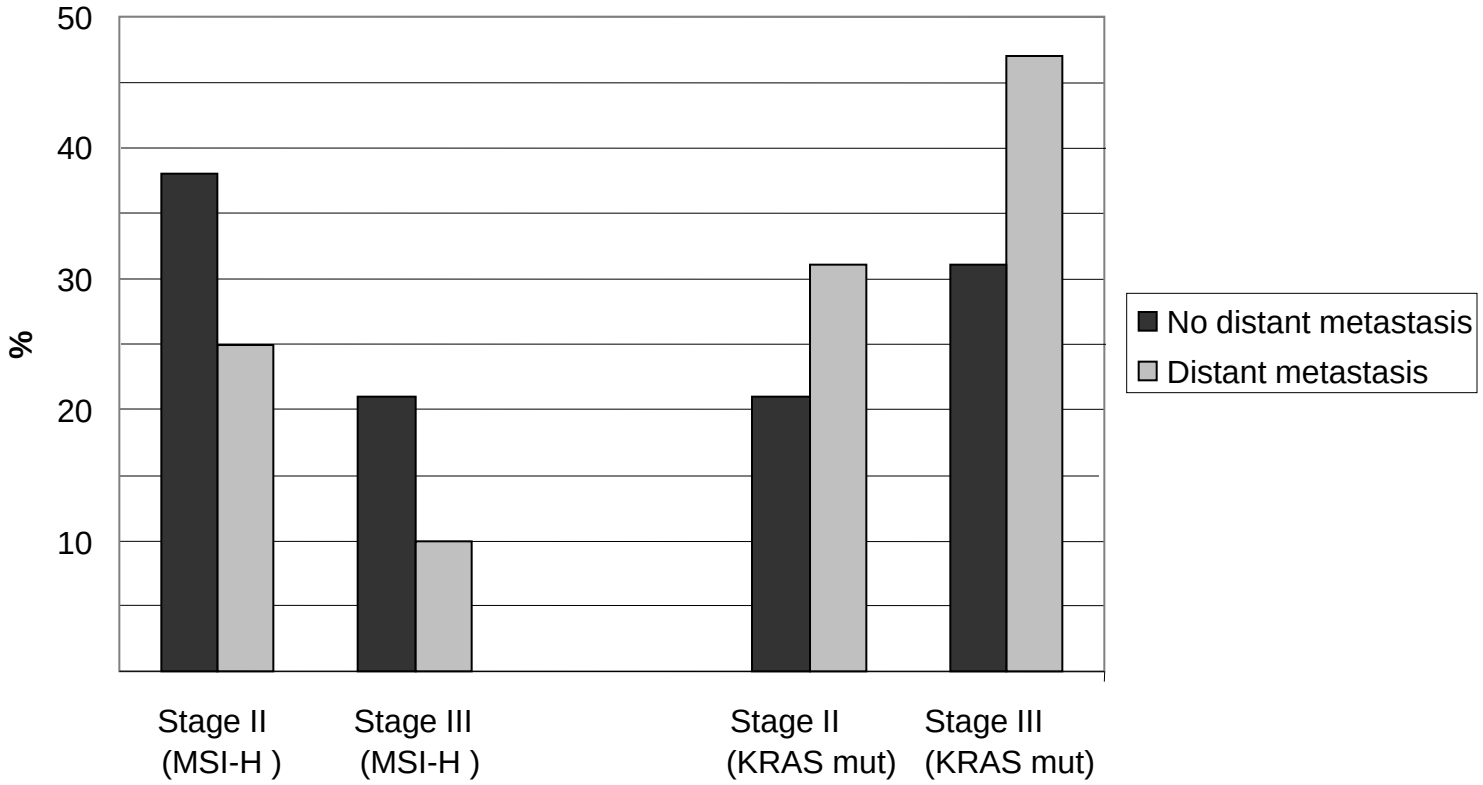
		Distant metastasis		
	Total	**Yes**	**No**	***p*-value (χ^2)**
KRAS wt + MSI-H	24	**11%**	28%	
KRAS wt + MSS/MSI-L	55	39%	47%	
KRAS mut + MSS/MSI-L	49	**49%**	24%	**<0.01**

Conclusion:

- Confirmation of favorable prognosis for MSI-H patients
- Prognostic value of KRAS mutations
- ”Good genotype” = ”KRAS wt + MSI-H”
- ”Bad genotype” = ”KRAS mutated + MSS/MSI-L”
- KRAS and MSI status can help estimate risk for metastatic progression
- Is this true also for individual stages?

UPPSALA UNIVERSITET

Frequencies of MSI-H and KRAS mutations after stratification by stage and distant metastasis



Next

- Statistical analysis of genotyping data together with additional clinical data
- Survival data, time to distant metastasis etc.
- More bioinformatic analysis of microarray data

UPPSALA UNIVERSITET

Tack!

- **Karolina Edlund**
- **Johan Botling**
- **Patrick Micke**
- **Simin Tahmasebpoor**
- **Niklas Nordquist** - Swedish University of Agricultural Sciences
- **Helgi Birgisson** - Department of Surgery, Uppsala University
- **Lars Påhlman** - Department of Surgery, Uppsala University
- **Ulrik Wallin** - Department of Surgery, Uppsala University
- **Bengt Glimelius** - Department of Radiology, Oncology and Radiation Science, UU
- **Markus Mayrhofer** - Department of Medical Sciences, Uppsala Universitet
- **Hanna Göransson Kultima** - Department of Medical Sciences, Uppsala Universitet
- **Anders Isaksson** - Department of Medical Sciences, Uppsala Universitet

- **This project was supported by:**

The Swedish Cancer Society, Selanders stiftelse, Regionala forskningsrådet i Uppsala- och Örebroregionen, ALF Uppsala University/Akademiska Sjukhuset and Lions Cancerforskningsfond Uppsala