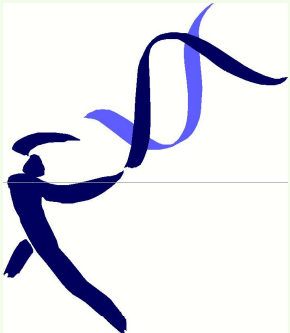


Low microsatellite instability is associated with poor prognosis in stage C colon cancer.

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Definition of MSI-High and MSI-Low

(Dietmaier et al 1997 Cancer Res 57:4749; Boland et al 1998 Cancer Res 58:5248)

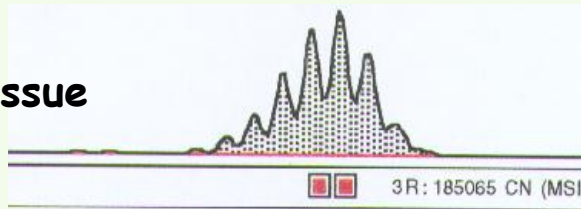
- **Standard microsatellite marker panel for MSI-H**
BAT25/40, BAT26 (mononucleotide)
D2S123, D5S346, D17S250 (dinucleotide repeats)
- **Supplementary panel of 5 markers for MSI-L**

2/5 (40%) MSI-H

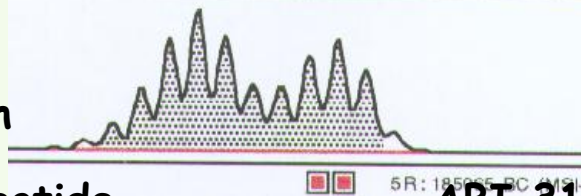
1/5 (20%) MSI-L

0/5 Microsatellite stable MSS

Patient 1
Normal tissue



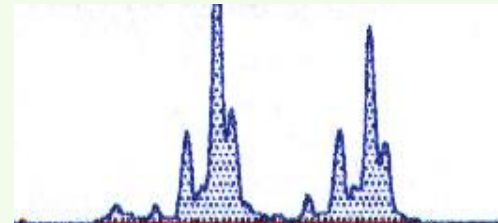
Cancer
MSI-High



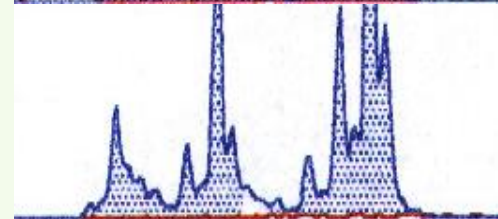
Mononucleotide

ABI 310 Genetic Analyzer

Patient 2
Normal tissue



Cancer
MSI-Low



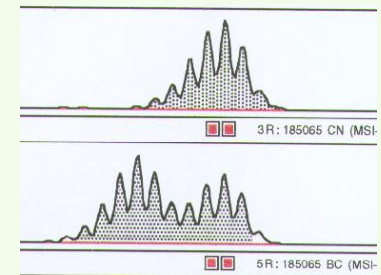
Dinucleotide

Does MSI-Low exist?

- **MSI-H** is due to the loss of mismatch repair function, in sporadic cancer mostly through methylation of the *MLH1* gene
 - **MSI-H** is a subset of the CpG island methylator phenotype (CIMP)

- **MSI-H** tumours have better prognosis but do not respond to chemotherapy as well as **MSS** cancers

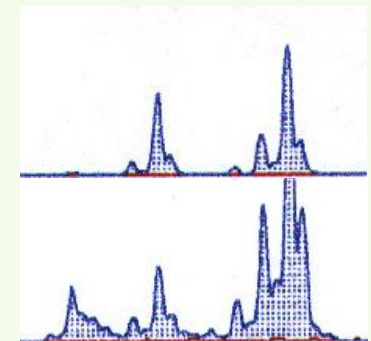
Ribic et al 2003 N Engl J Med 349:247;
Carethers et al Gastroenterol 126:394



- **MSI-L** has no clear cause, and few associations with other markers
- No clinicopathological associations reported for **MSI-L**
- Molecular associations:

K-ras mutations and **MGMT** methylation

Jass et al 1999 J Clin Path 52:455;
Whitehall et al 2001 Cancer Res 61:827



Colon Cancer Study

Aims

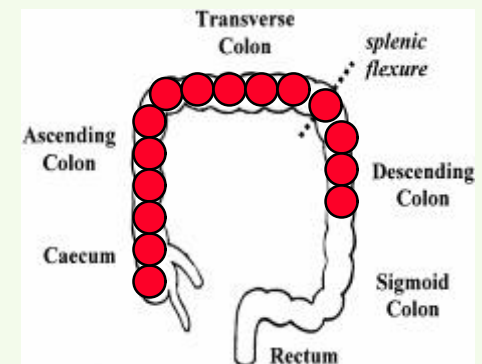
- Examine the MSI-H and -L phenotypes for their association with
- (1) clinicopathological factors
 - (2) MGMT defect
 - (3) p16 gene methylation (representative of CIMP)

Patients

- 183 stage C colon cancer patients operated at the Concord Hospital including 141 proximal cancer patients
- Surgery only, no adjuvant chemotherapy
- Excellent patient follow-up

Key Questions

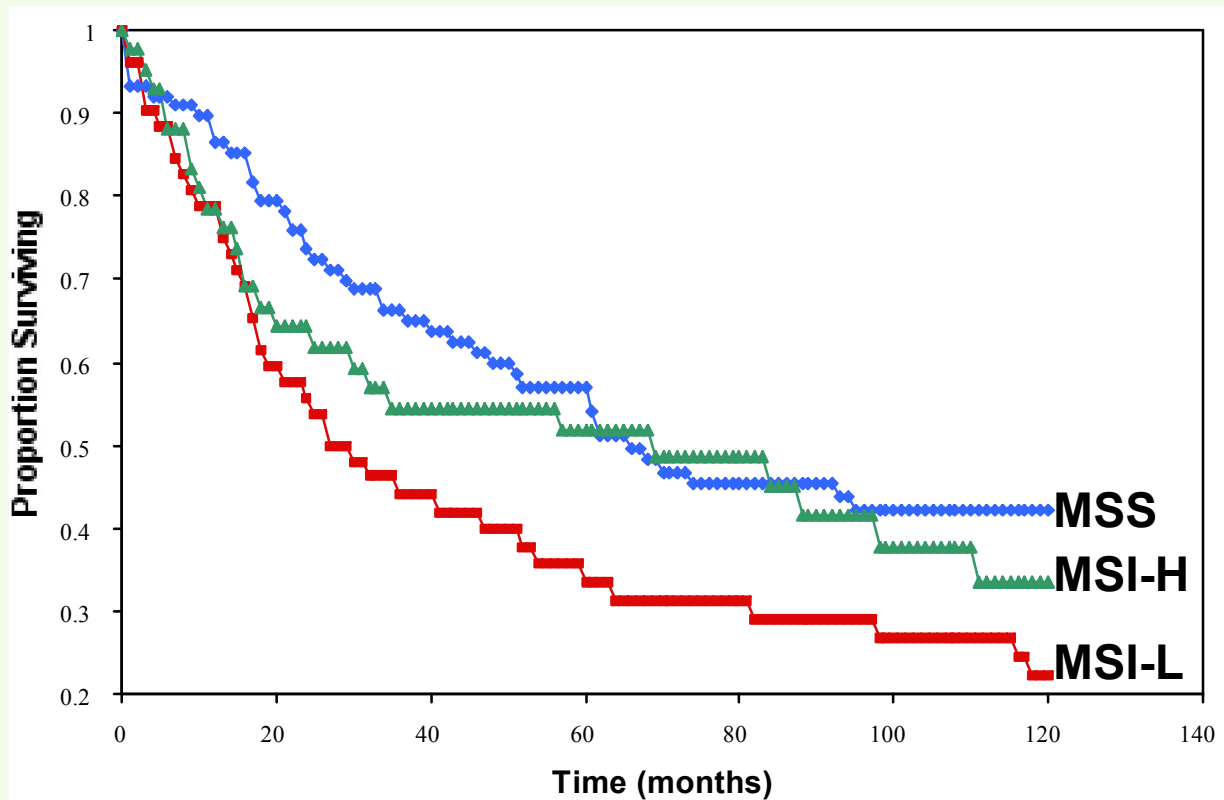
- Is MSI-low a distinct phenotype?
misclassified MSI-high? MSI artefact?



The colon cancer cohort has a higher frequency of MSI-H compared with colorectal cancer

MSI-H	23.0% (42/183)
MSI-L	27.9% (51/183)
MSS	49.1% (90/183)

MSI-L is associated with shorter survival in proximal colon cancer



MSI-H 23.0% (42/183)
MSI-L 27.9% (51/183)
MSS 49.1% (90/183)

$p = 0.026^*$

The same result
obtained for the
141 proximal colon
cancer patients

$p = 0.023^*$

Multivariate Cox regression analysis in stage C colon cancer.

Kohonen-Corish et al 2005. J Clin Oncol 23: 2318-2324.

Prognostic factor	Hazard Ratio	95% CI	p
Male*	1.5	0.93-2.45	0.095
Age >75 years*	1.6	0.99-2.49	0.051
High histol. grade*	1.3	0.81-2.13	0.274
Free serosal surface involvement*	1.4	0.88-2.35	0.153
Venous invasion*	1.2	0.71-2.13	0.459
Apical node metastasis*	2.2	1.13-4.18	<u>0.020</u>
> 3 nodal metastases	1.9	1.16-3.18	<u>0.011</u>
Tumour size >5cm	0.6	0.38-0.92	<u>0.021</u>
MSI-low	1.9	1.20-2.86	<u>0.005</u>

O⁶-methylguanine methyltransferase (MGMT)

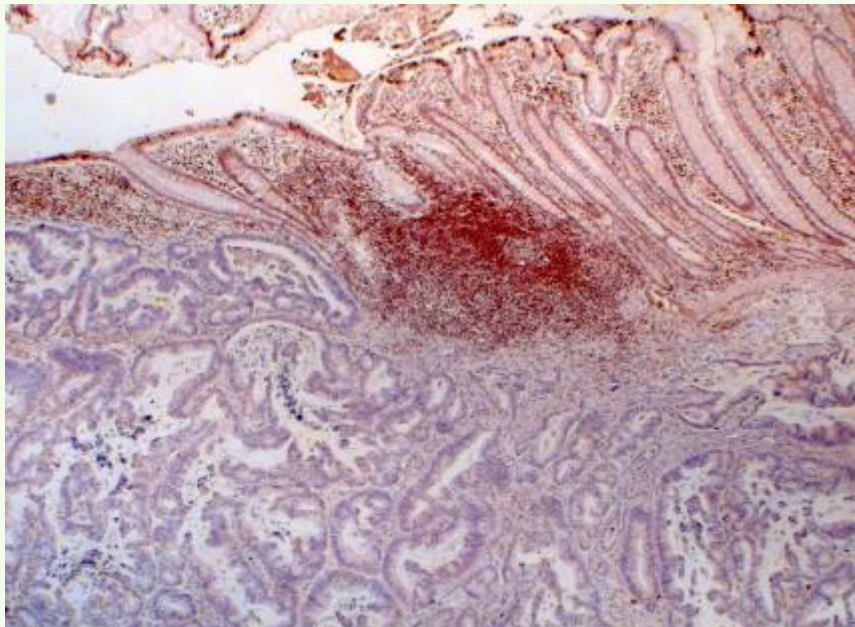
- MGMT is a DNA repair protein that removes O⁶-methylguanine DNA adducts caused by alkylating agents
- MGMT methylation correlates with MSI-L - contributing factor?
Whitehall et al 2001 Cancer Res 61:827
- MGMT methylation is an adverse prognostic marker in lung cancer; prognostic role in colorectal cancer is unknown

p16 cell cycle protein

- Important tumour suppressor gene
- p16 methylation correlates with CIMP in colorectal cancer
- p16 defect is not associated with survival in colorectal cancer

Loss of MGMT protein expression (Immunohistochemistry)

49/178 patients
28%



p16 promoter hypermethylation (Methylation specific PCR)

52/149 patients
35%

Cell line Tumour 1 Tumour 2

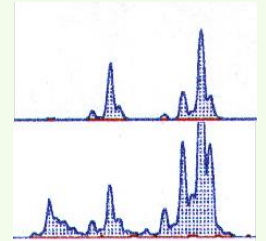
U M M U M U



Tumour 1
Unmethylated

Tumour 2
Methylated

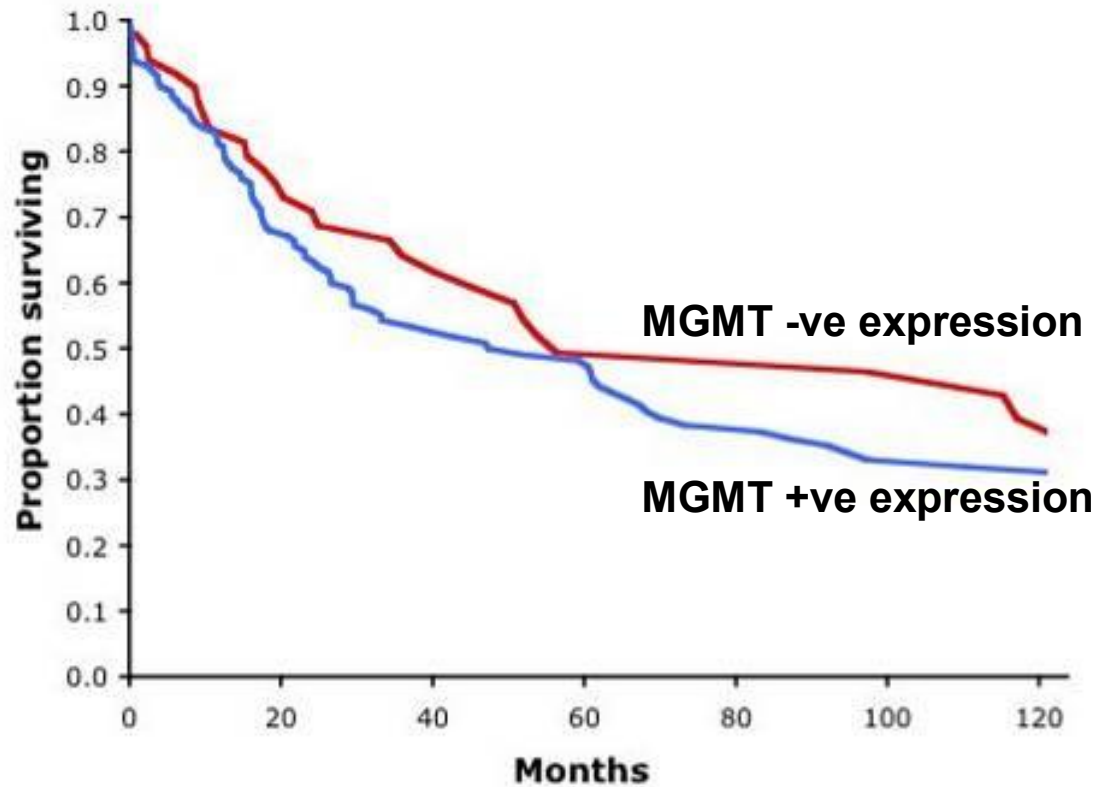
MGMT defect is increased and p16 methylation is decreased in MSI-L



	MSI-H	MSI-L	MSS	
MGMT	30.0%	42.0% ↑	19.0%	MSI-L vs MSS p= 0.004*
p16	53.0%	16.0% ↓	36.0%	p= 0.029*

- Confirmation of MGMT association with MSI-L
However, less than half MSI-L tumours have MGMT defect
- New finding: p16 methylation is inversely associated with MSI-L
- what about other CIMP genes?

Loss of MGMT protein expression is not associated with survival in stage C colon cancer



MGMT loss
27.5% (49/178)

MGMT positive
72.5% (129/178)

p = 0.548 NS

Summary - MSI and MGMT in colon cancer

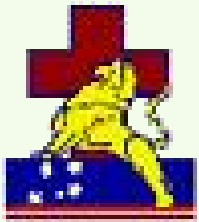
- MSI-L is a marker for poorer survival in stage C colon cancer
 - a parallel study in colorectal cancer did not find as strong association (Wright et al 2005 Gut 54:103)
- MGMT loss of expression is associated with MSI-L
- MGMT loss does not appear to be associated with shorter survival in contrast to lung cancer
- p16 methylation was inversely associated with MSI-L

Conclusions

- MSI-L is a distinct phenotype but more reliable gene markers are needed to characterise it and resolve why it is associated with poorer survival
- MSI-L may be a stronger adverse prognostic factor in proximal colon cancer than in colorectal cancer
- Relationship between MSI-L and the CpG island methylator phenotype CIMP needs further characterisation

Acknowledgments

Concord Hospital



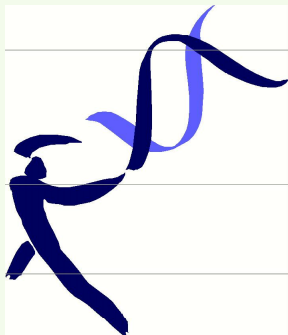
Les Bokey
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Betty Lin
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Royal Prince Alfred Hospital

Ron Trent



Garvan Institute of Medical Research



Joe Daniel

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