

New molecular classification(s) of colorectal cancer

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Disclosure slide

- Funding: ROCHE Research grant

Outline

- Prognostic/predictive markers in CRC
- Cancer subtypes
- Colorectal cancer subtypes
- Future directions

Current single predictive/prognostic biomarkers in colorectal cancer

- TNM
- MSI/MS
- RAS
- BRAF
- 18q deletion
- TP53
- TGFBR2
- DCC
- TS
- AREG/EREG

Methods

Single biomarkers
IHC
FISH
qRT-PCR
Sequencing of individual genes
ELISA

Multiplex Signatures
Arrays (miRNA, mRNA, RPPA)
mRNAseq
Exom-seq
WGS
MS/MS proteomics

Why shall we look for subtypes?

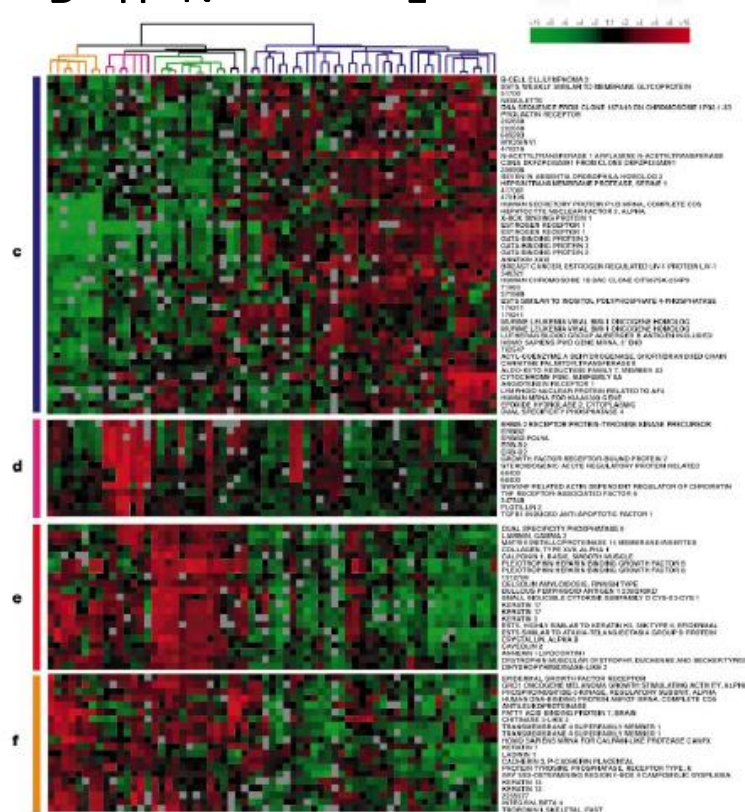
- Single biomarkers are rarely robust enough to be sufficiently predictive or prognostic
- Population tumor heterogeneity can be due to differences in cancer development
- Tumors of similar developmental origin could have similar natural history and respond similarly to therapies
- Other cancers have subtypes (breast) with potential clinical relevance

Subtypes in other cancers

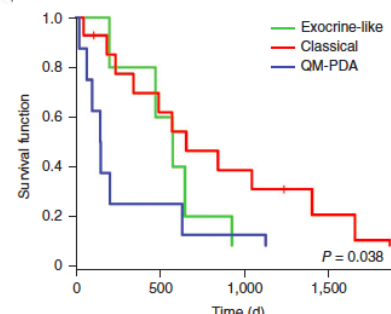
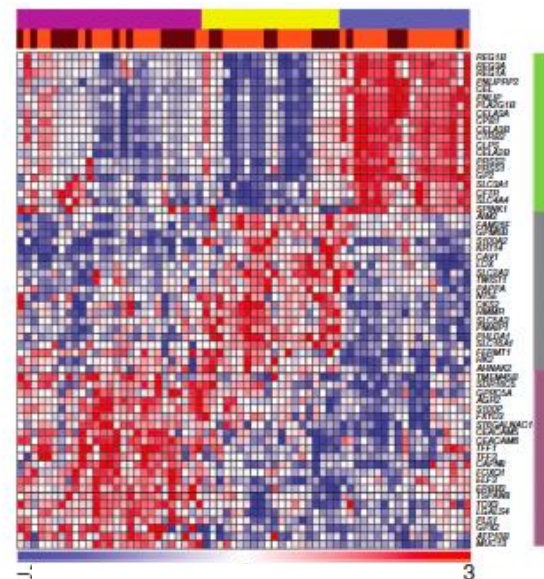
Breast cancer

B H N L

L

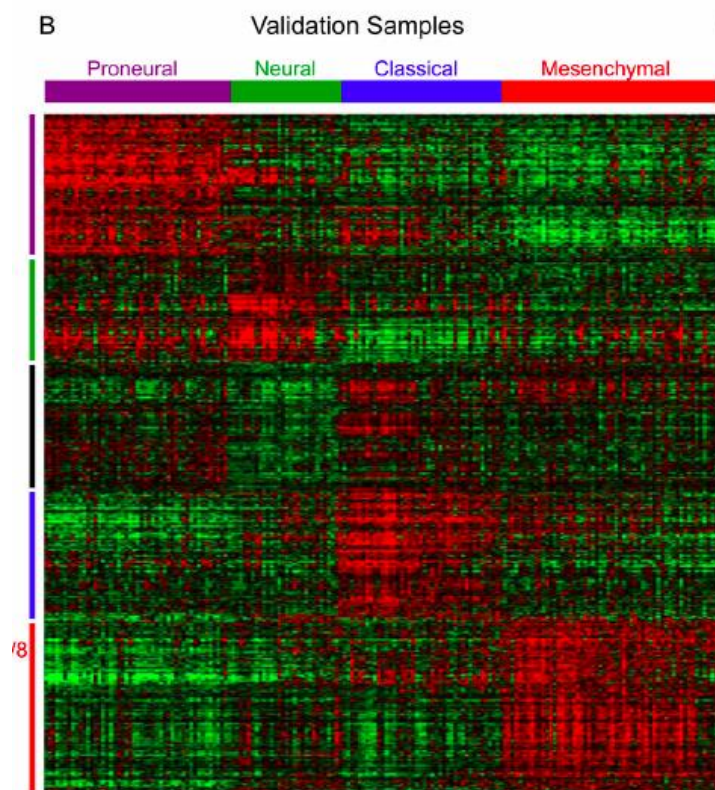


Pancreatic ca. (PDAC)



Subtypes in non-epithelial tumors

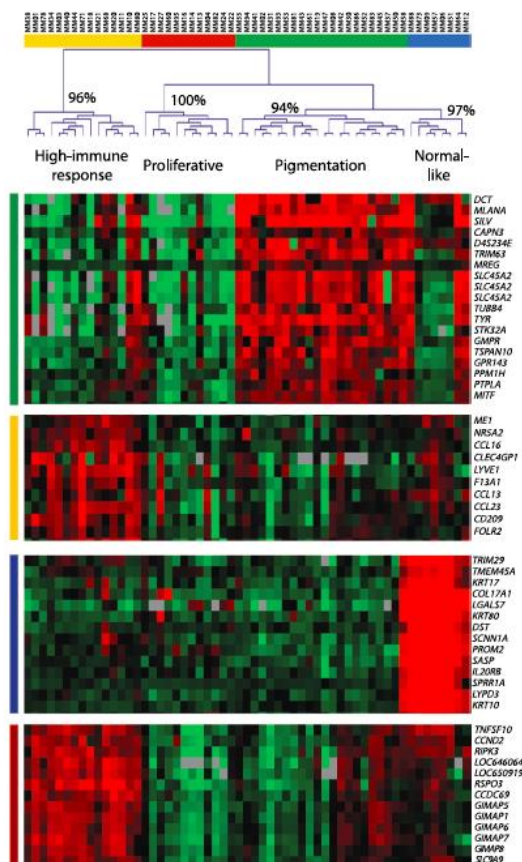
Glioblastoma



Verhaak et al; Cancer Cell, Jan 19, 2010; 17(1): 98

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Cutaneous Melanoma



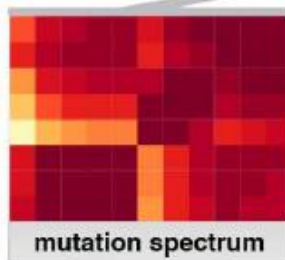
12 TCGA Pan-Cancer types



Sequencing and Analysis



Distribution of mutation rates across the twelve cancer types reveals interesting features, such as clusters in UCEC and COAD/READ that indicate factors other than age in the development of these tumors.

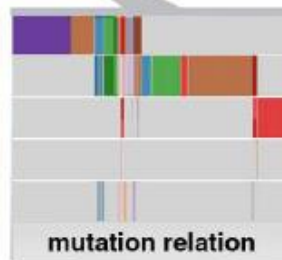


Environmental effects on cancer development can also be observed in mutation spectrum. For instance, lung tumors show higher proportions of C-to-A transversions – a signature of cigarette smoke exposure.

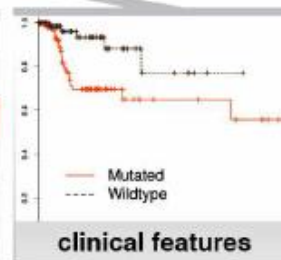
7.5	51.8	79.3	94.6	57.8
0.0	4.4	14.8	0.6	63.2
0.0	2.2	8.1	0.6	63.5
0.0	9.2	4.0	2.2	5.7
0.0	0.0	0.6	0.0	9.9
4.0	5.5	1.2	0.6	20.0
0.5	16.4	15.5	1.3	5.2
0.5	8.8	20.1	0.6	8.3
0.5	6.1	5.3	1.0	36.9
0.0	1.8	3.5	0.3	2.6
0.0	21.5	19.0	1.3	5.2
1.0	11.4	2.9	1.9	1.3

mutated genes

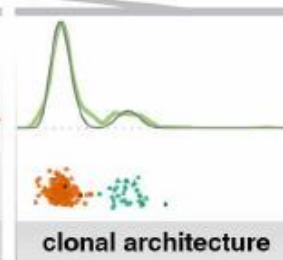
When grouped by mutation, we see that significant mutations fall into several distinct categories: transcription factors & regulators, histone modifiers, genome integrity, *RTK* signaling, cell cycle, and more.



We found 14 significant mutually exclusive pairs and 148 co-occurring pairs. We also identified a set consisting of *TP53*, *PIK3CA*, *PIK3R1*, *SETD2*, and *WT1*.



We found *TP53* to be significant, with mutations being associated with detrimental outcome through joint analysis of 12 tumor types. Mutations in *BAP1* are correlated with detrimental outcome particularly in KIRC and UCEC.



Mutations in *TP53*, *DNMT3A*, and *PIK3CA* play an initiation role in the tumorigenesis. Mutations in *KRAS* and/or *NRAS* largely play a progression role in the tumorigenesis of AML, BRCA, and UCEC.

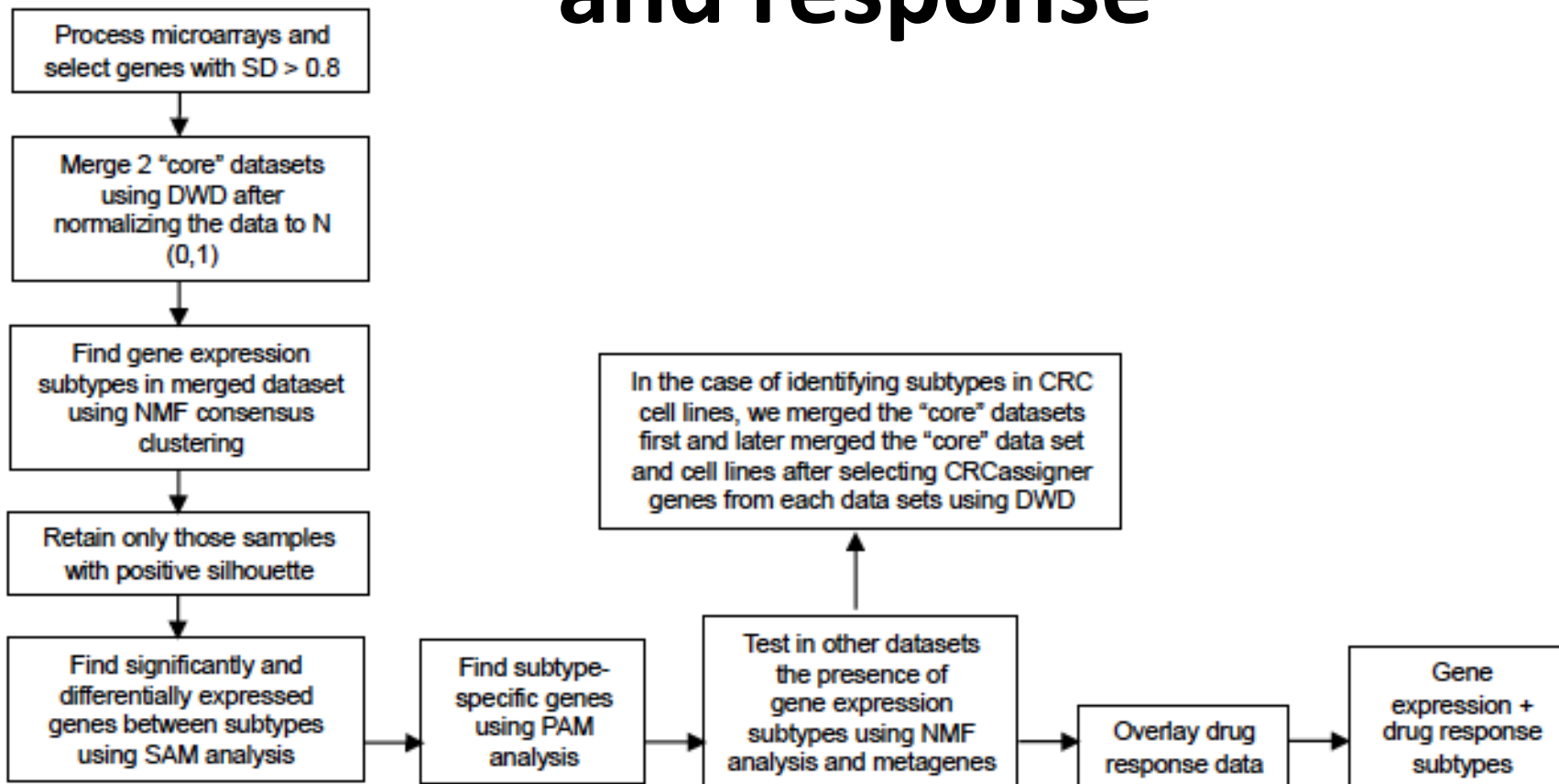
CRC subtypes of multiple team



TCGA

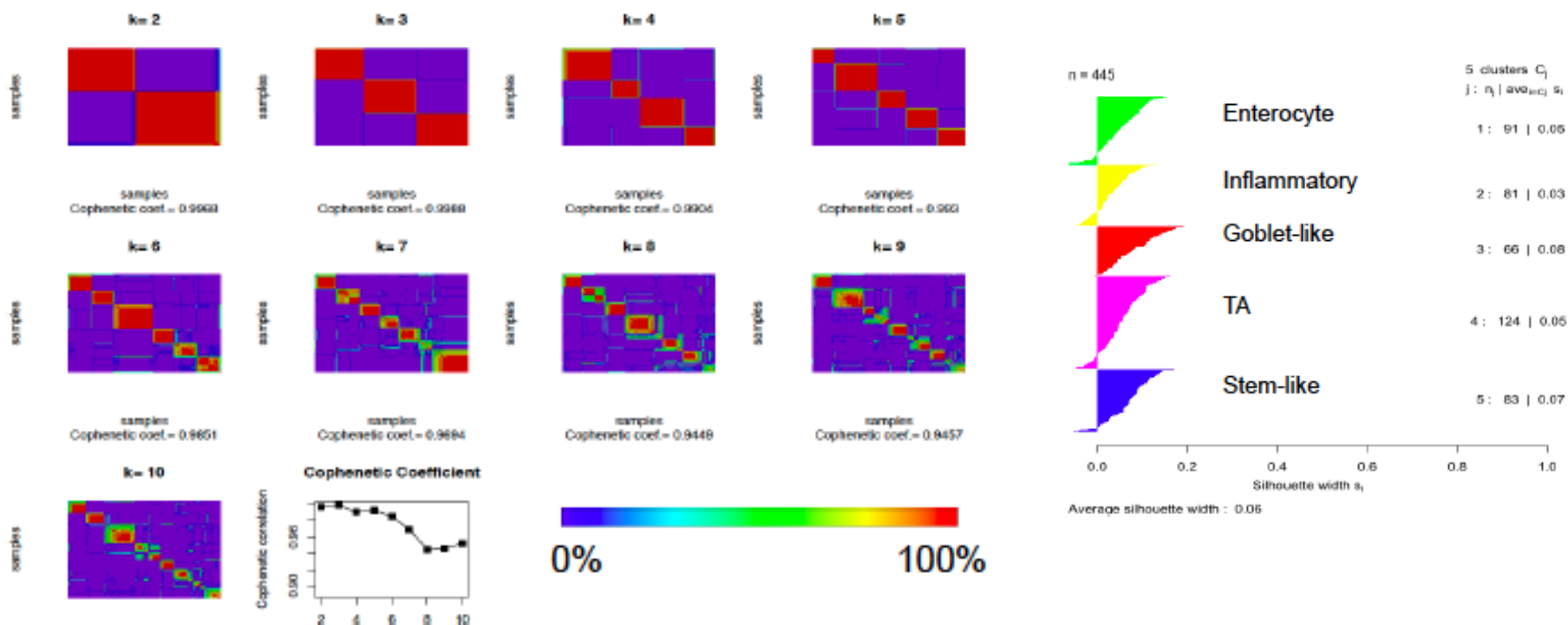
Surface crypt	Lower crypt		CIMP+	Mesenchymal	Mixed
CIN Immune down	dMMR	KRASm	CSC	CIN Wnt up	CIN normal
A type	B type				C type
Inflammatory	Goblet	Transit Amplifying		Stem-like	Enterocyte
CCS1			CCS2	CCS3	
1.1	1.2	1.3	2.1	2.2	
MSI/CIMP		CIN		Invasive	

Methodology to identify subtypes and response



Sadanandam A, Lyssiotis CA, Homicsko K et al. Nat Med. 2013 May;19(5):619-25.

Numbers of subtypes

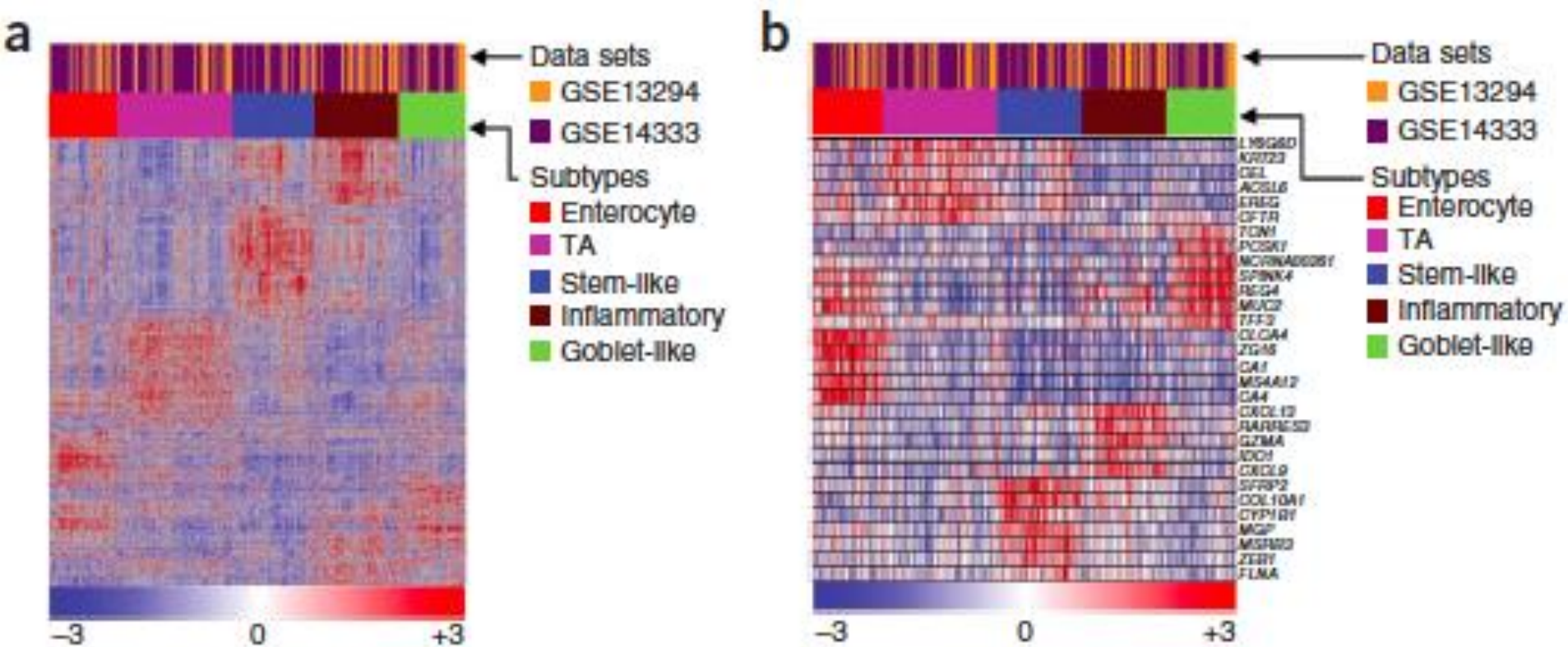


Sadanandam A, Lyssiotis CA, Homiczko K et al. Nat Med. 2013 May;19(5):619-25.

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esmo.org

Subtypes in core dataset

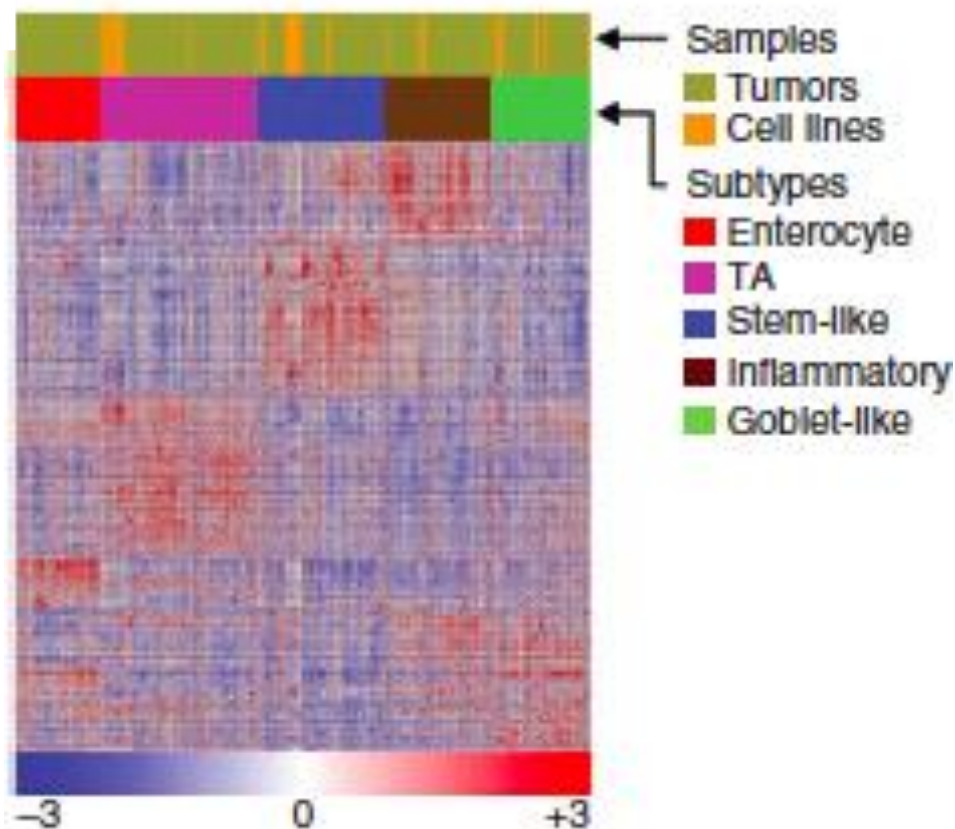


Sadanandam A, Lyssiotis CA, Homicsko K et al. Nat Med. 2013 May;19(5):619-25.

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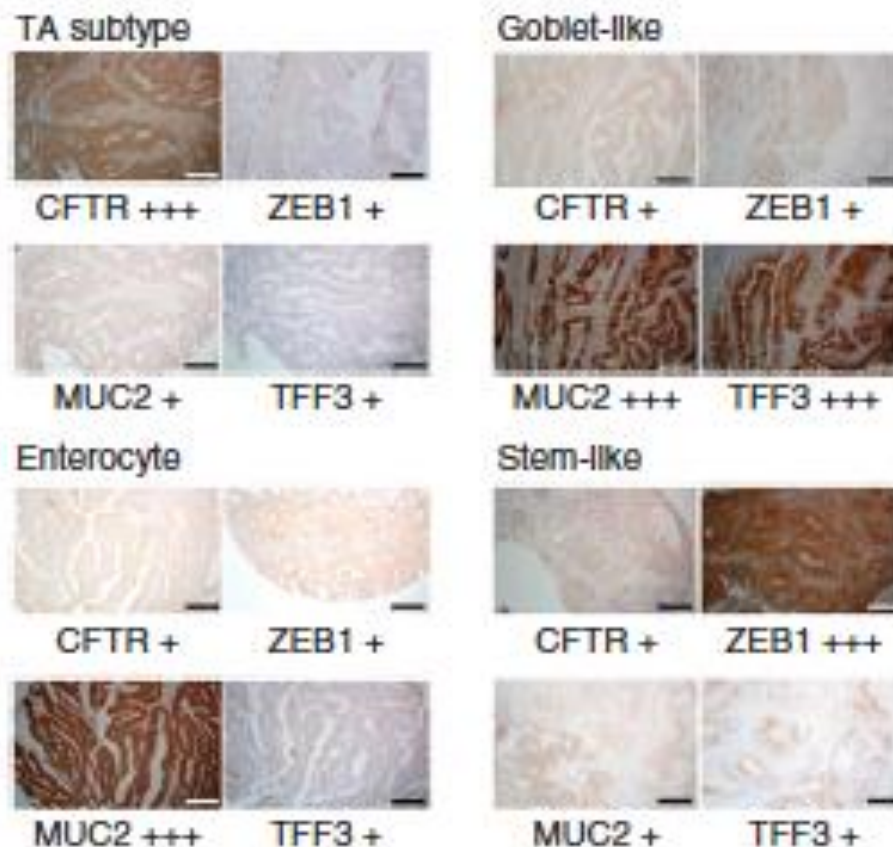
Presence of subtypes in established CRC cell lines



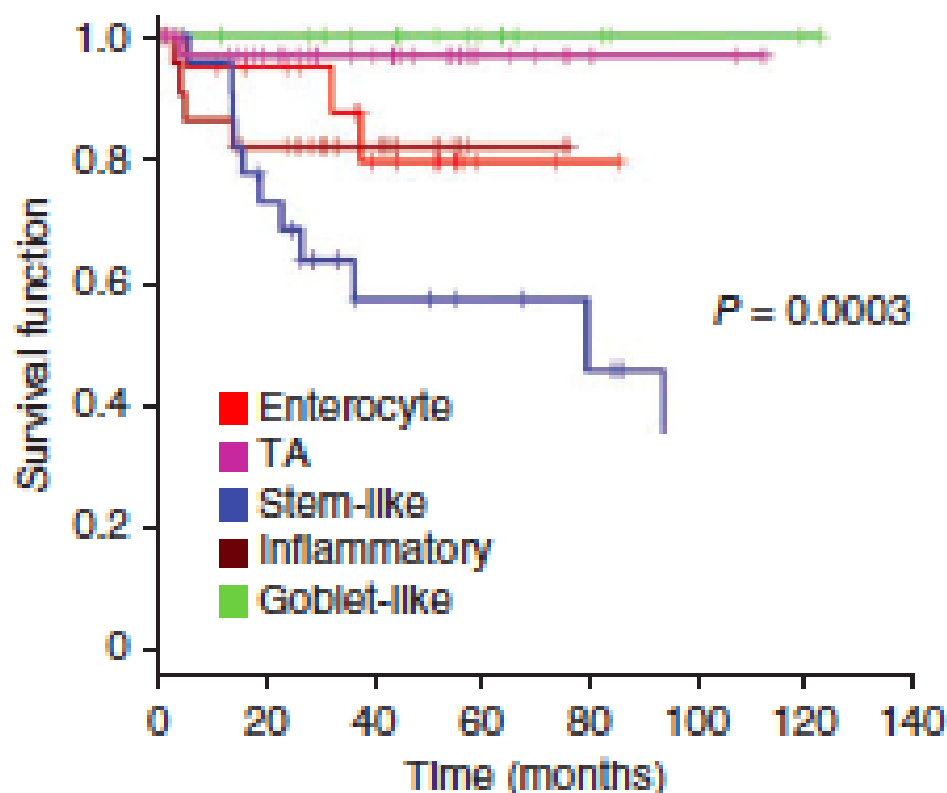
Sadanandam A, Lyssiotis CA, Homicsko K et al. Nat Med. 2013 May;19(5):619-25.

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Potential markers for CRC subtypes



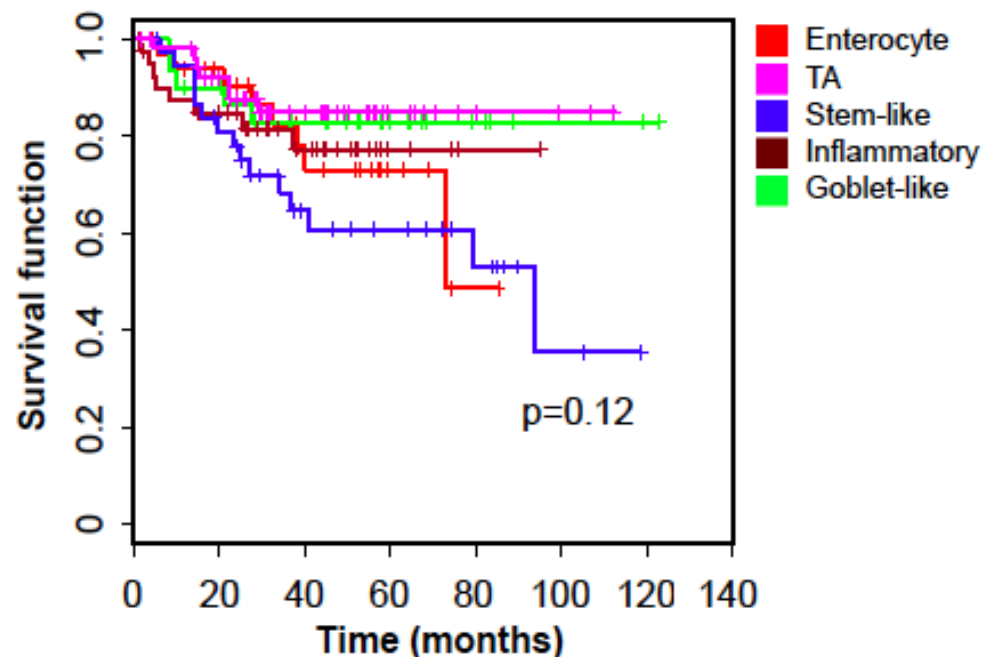
Subtypes could correlate with DFS



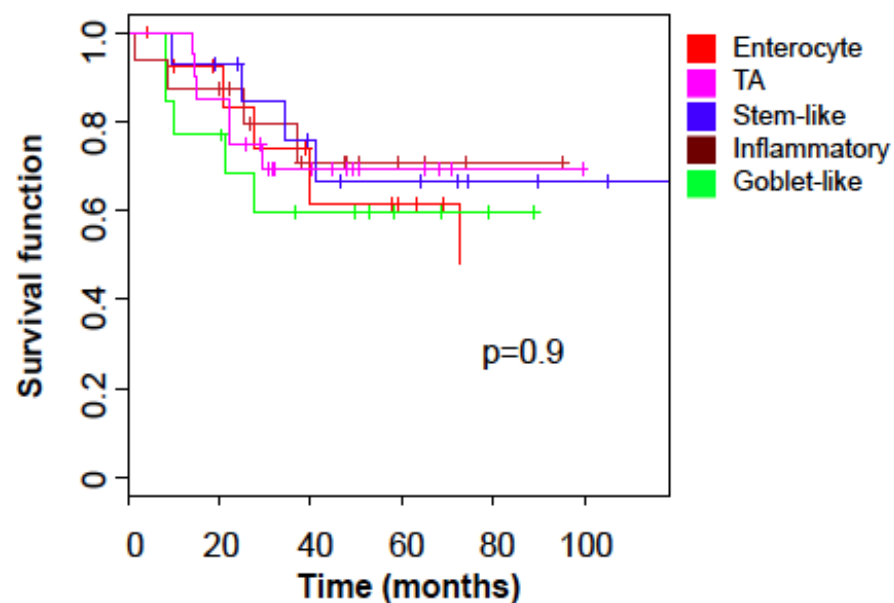
Sadanandam A, Lyssiotis CA, Homicsko K et al. Nat Med. 2013 May;19(5):619-25.

Adjuvant treatment effect on subtype specific DFS I.

b. DFS – all samples (GSE14333)

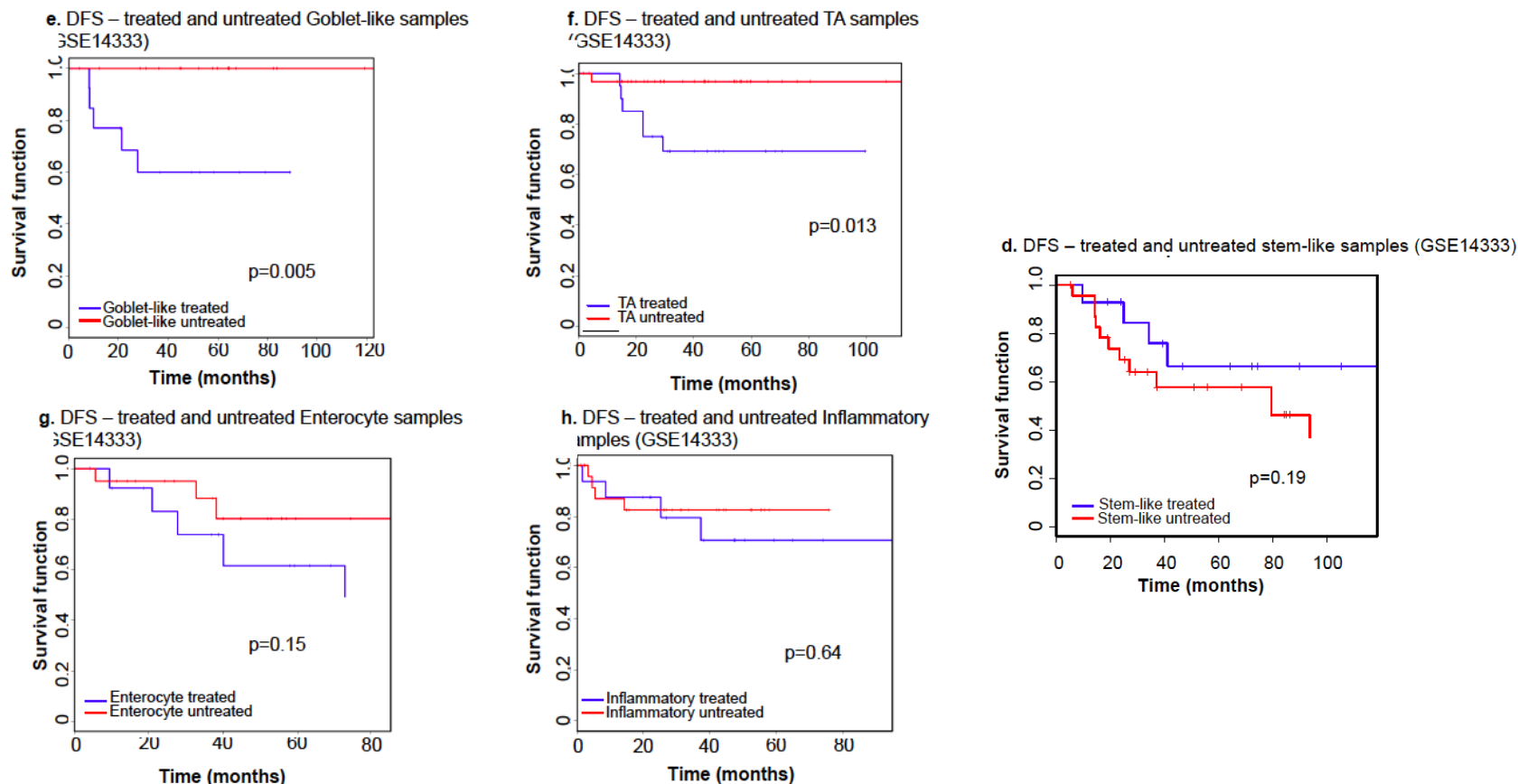


c. DFS – only treated samples (GSE14333)



Sadanandam A, Lyssiotis CA, Homicsko K et al. Nat Med. 2013 May;19(5):619-25.

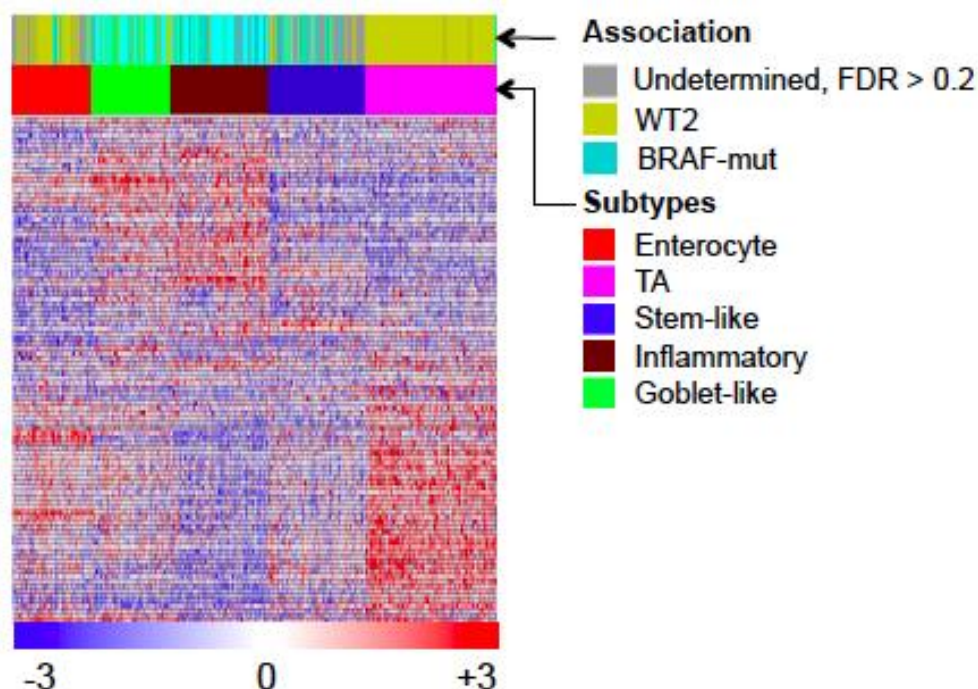
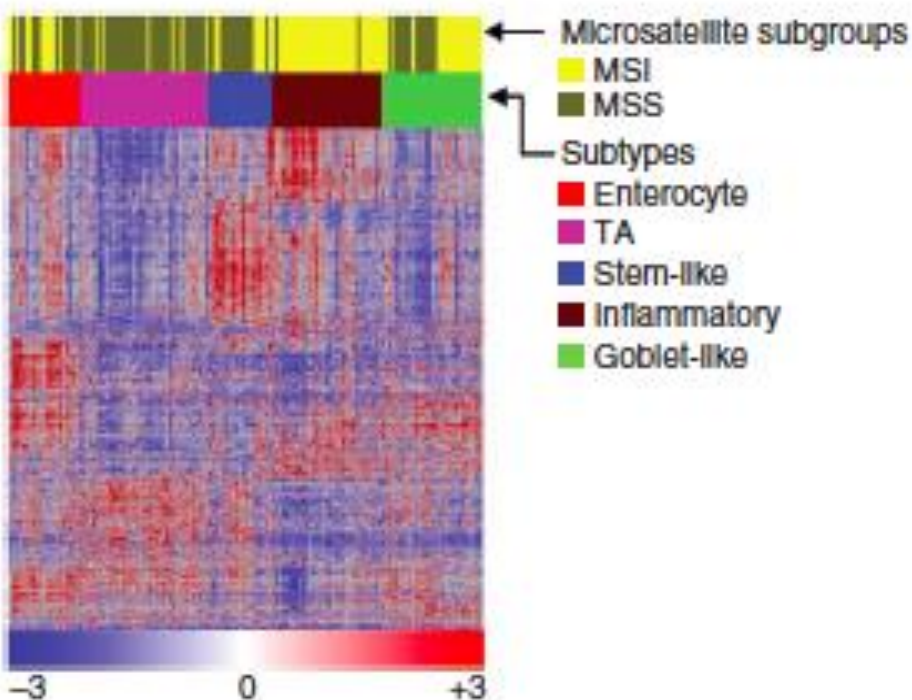
Adjuvant treatment effect on subtype specific DFS II.



Sadanandam A, Lyssiotis CA, Homicsko K et al. Nat Med. 2013 May;19(5):619-25.

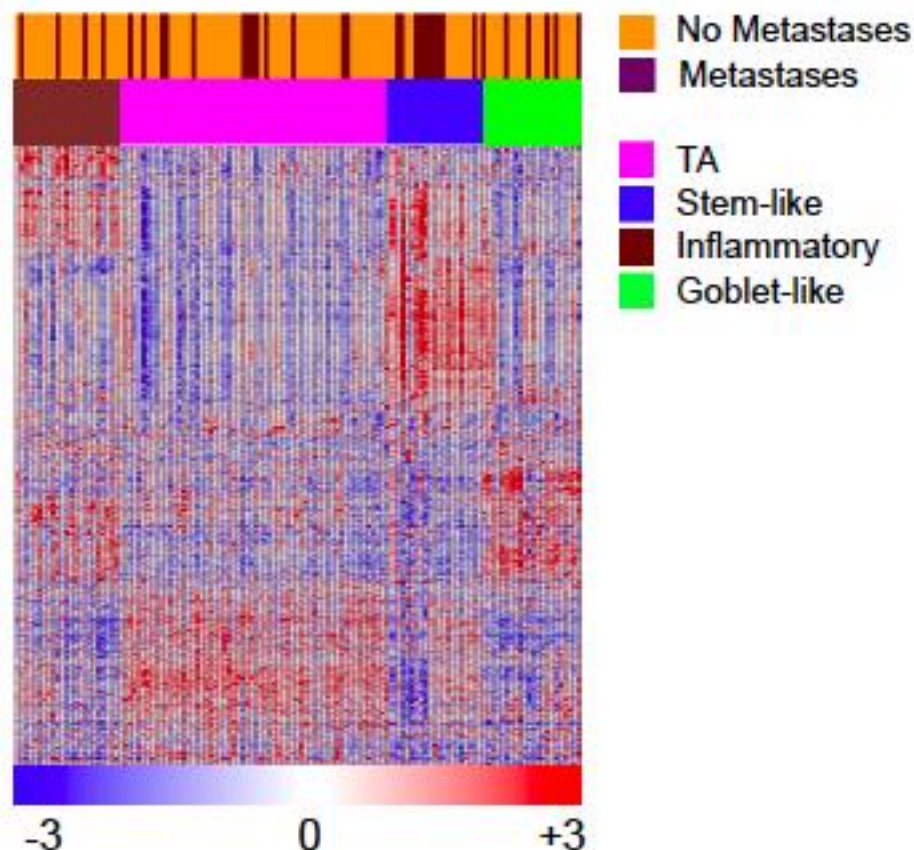
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CRC subtypes and correlation with MSI/MSS status or BRAF-mut signature



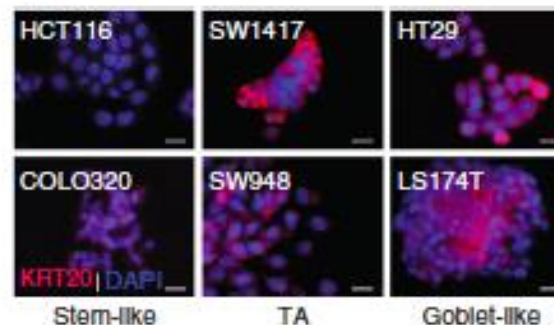
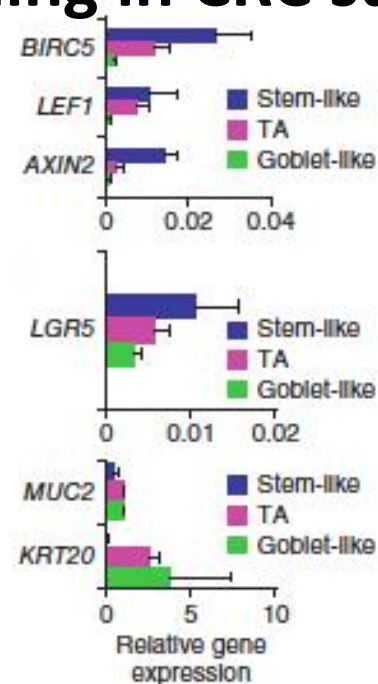
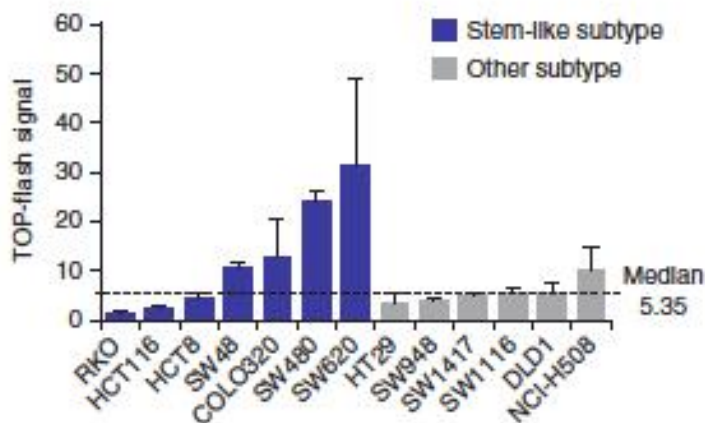
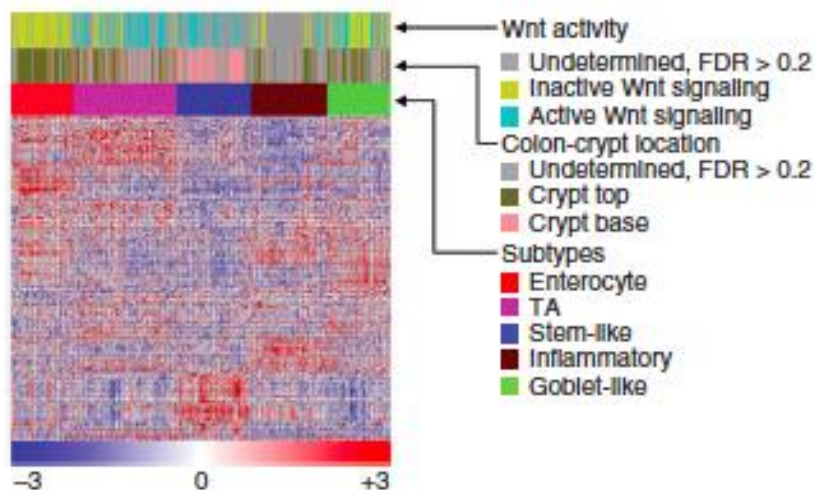
Sadanandam A, Lyssiotis CA, Homicsko K et al. Nat Med. 2013 May;19(5):619-25.

CRC subtypes and correlation with metastases



Sadanandam A, Lyssiotis CA, Homicsko K et al. Nat Med. 2013 May;19(5):619-25.

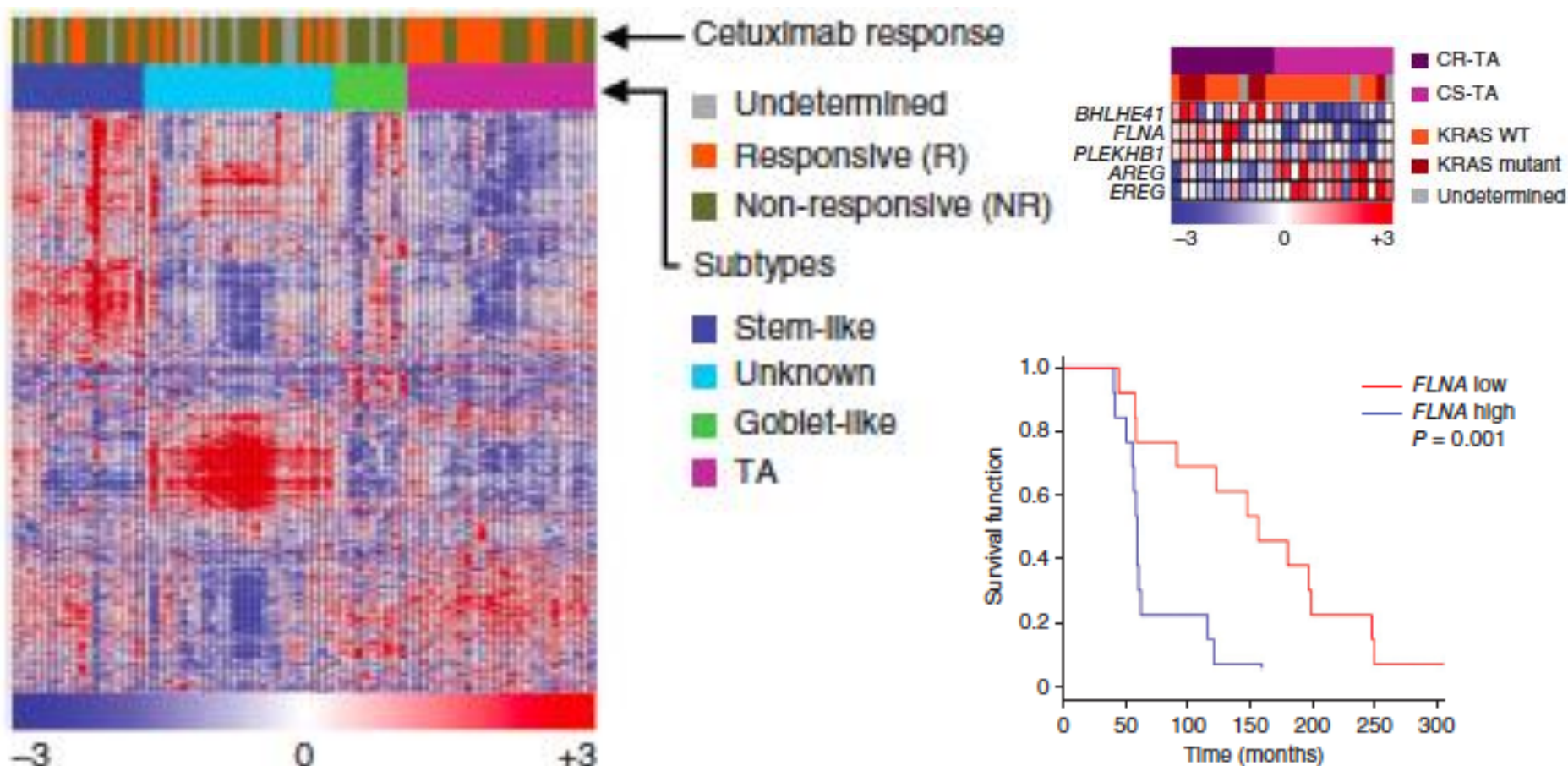
Cellular phenotype and Wnt signalling in CRC subtypes



Sadanandam A, Lyssiotis CA, Homicsko K et al. Nat Med. 2013 May;19(5):619-25.

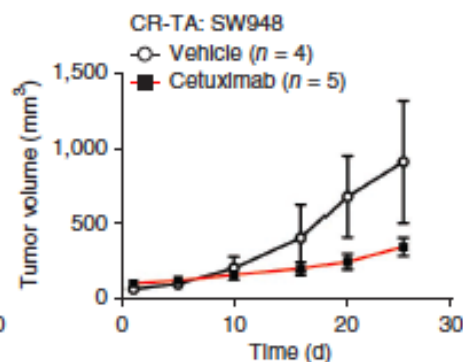
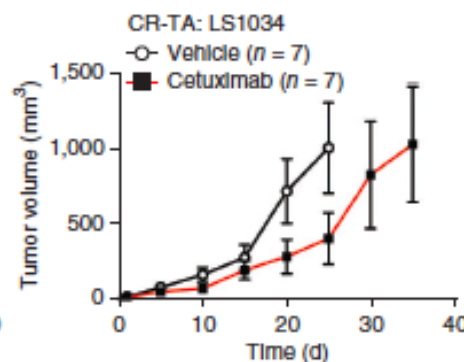
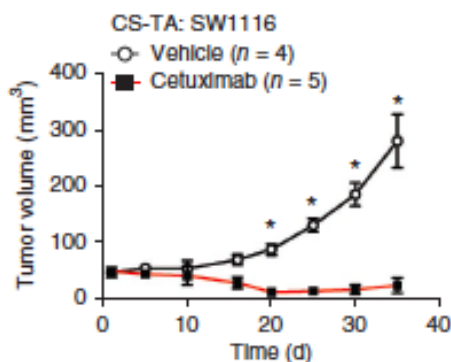
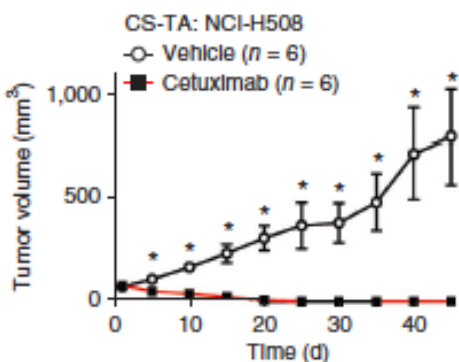
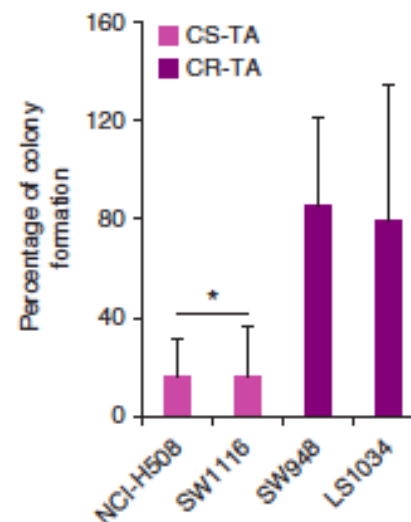
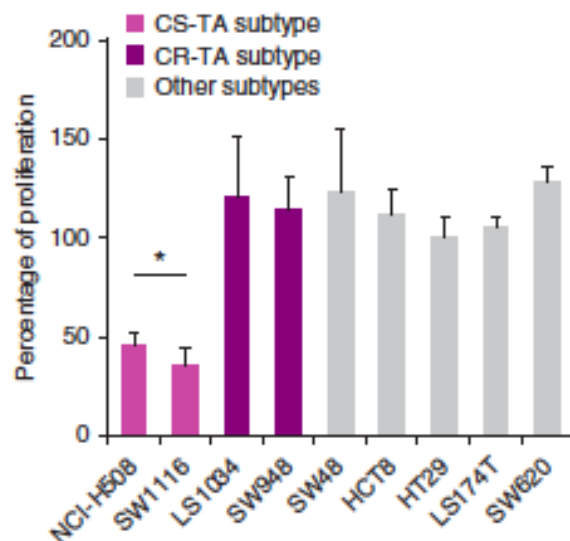
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Cetuximab sensitivity and CRC subtypes I.



Sadanandam A, Lyssiotis CA, Homicsko K et al. Nat Med. 2013 May;19(5):619-25.

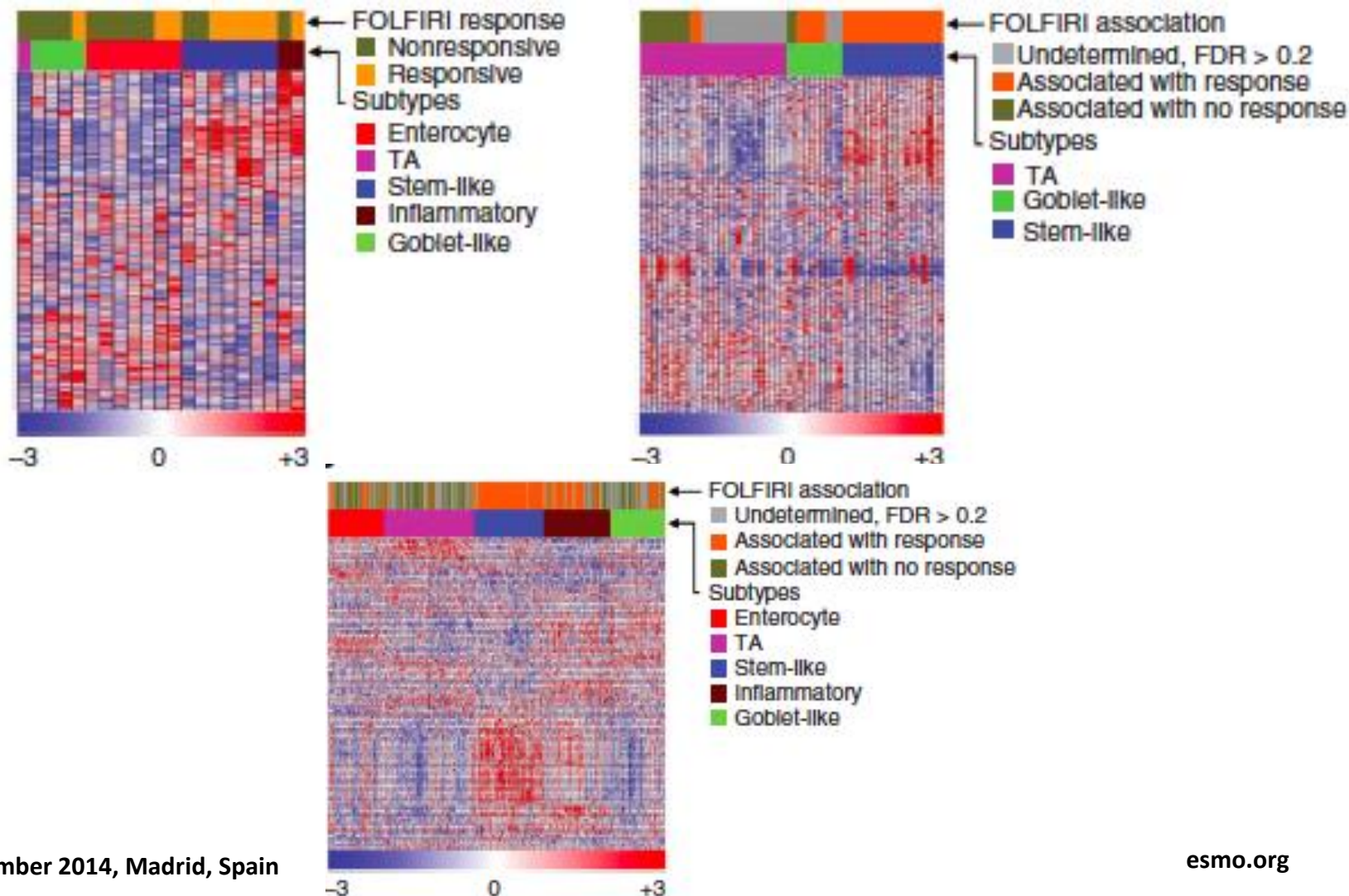
Cetuximab sensitivity and CRC subtypes III.



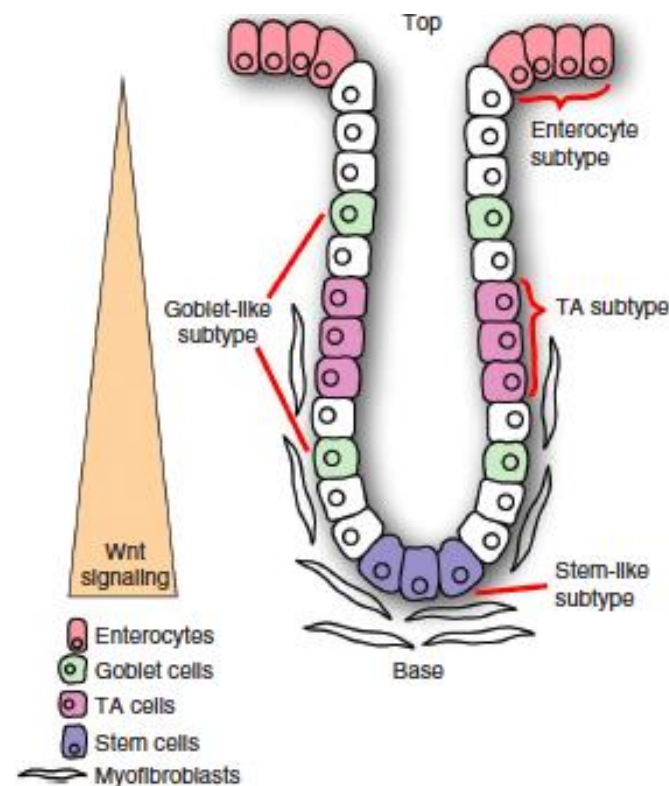
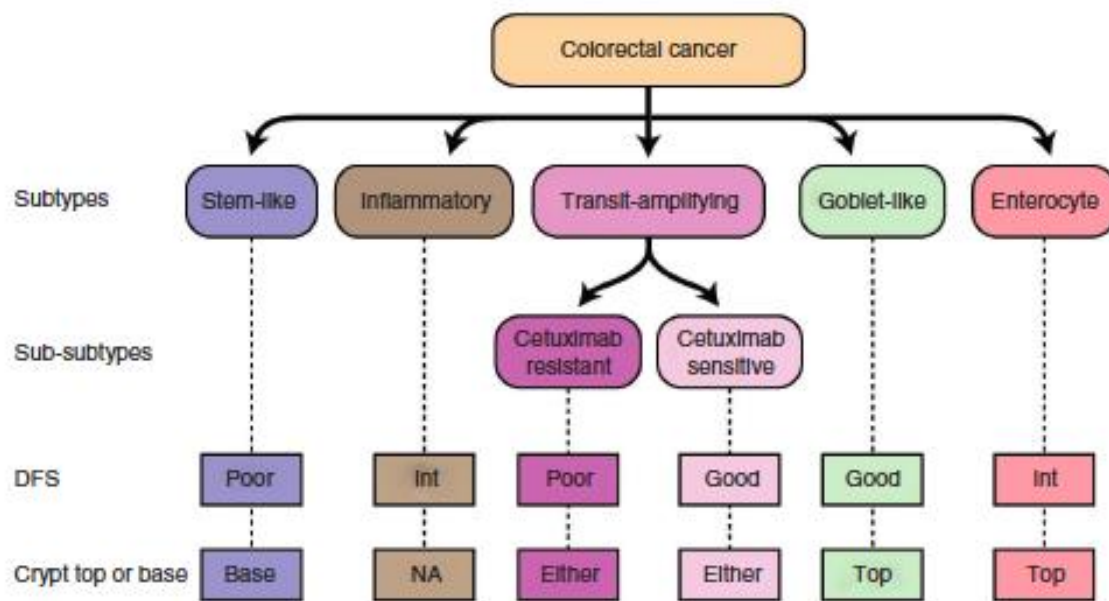
Sadanandam A, Lyssiotis CA, Homicsko K et al. Nat Med. 2013 May;19(5):619-25.

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FOLFIRI sensitivity



Summary of CRC subtypes I.



Sadanandam A, Lyssiotis CA, Homicsko K et al. Nat Med. 2013 May;19(5):619-25.

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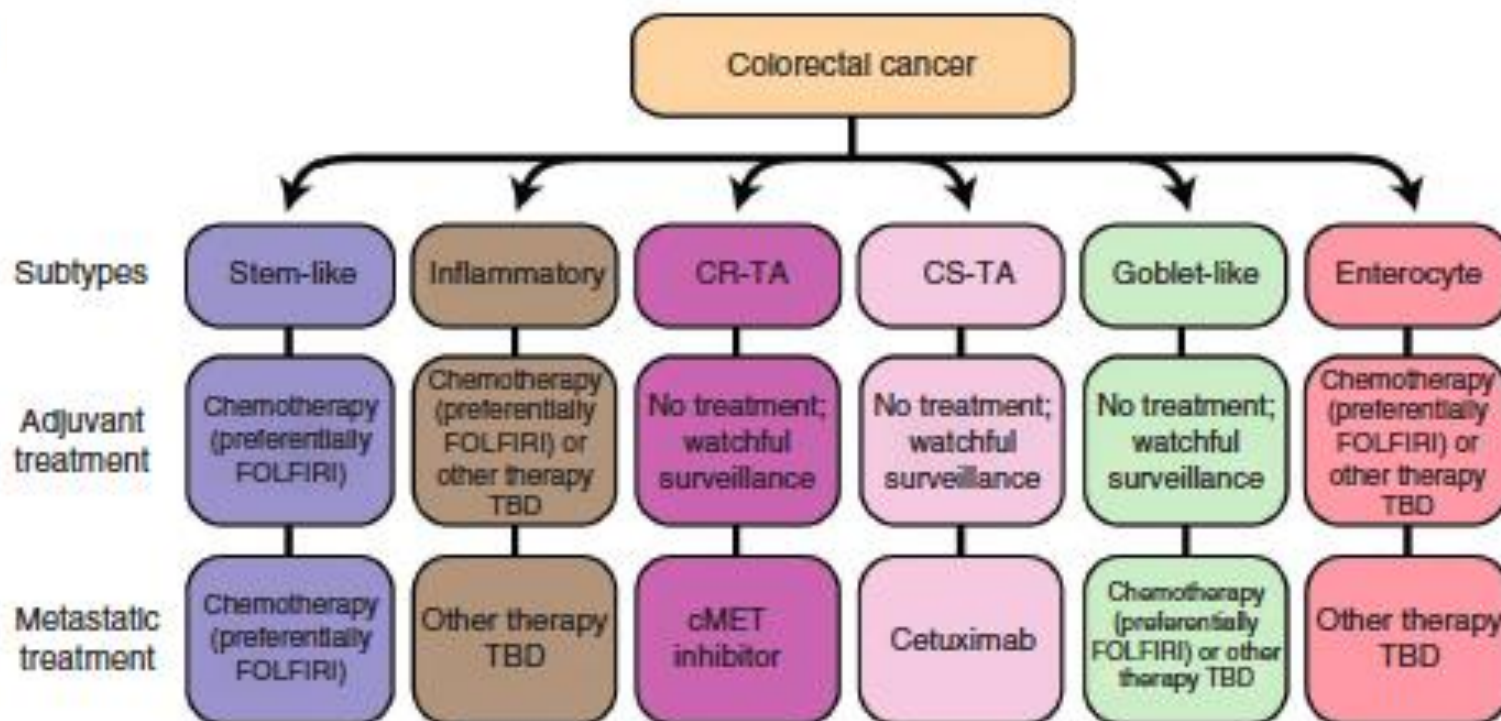
Summary of CRC subtypes II.

CRC subtypes	Signature genes	Blomarkers for qRT-PCR assay	Blomarkers for IHC
Stem-like	<i>SFRP2, ZEB1</i>	<i>SFRP2</i> ⁺	<i>ZEB1</i> ⁺
Inflammatory	<i>RARRES3</i>	<i>RARRES3</i> ⁺	[<i>RARRES3</i> TBD]
CR-TA	<i>CFTR, FLNA</i>	<i>CFTR</i> ⁺ , <i>FLNA</i> ⁺	<i>CFTR</i> ⁺ [<i>FLNA</i> TBD]
CS-TA	<i>CFTR, (FLNA)</i>	<i>CFTR</i> ⁺ , (<i>FLNA</i>) ⁻	<i>CFTR</i> ⁺ [<i>FLNA</i> TBD]
Goblet-like	<i>MUC2, TFF3</i>	<i>MUC2</i> ⁺ , <i>TFF3</i> ⁺	<i>MUC2</i> ⁺ , <i>TFF3</i> ⁺
Enterocyte	<i>MUC2, (TFF3)</i>	<i>MUC2</i> ⁺ , (<i>TFF3</i>) ⁻	<i>MUC2</i> ⁺ , (<i>TFF3</i>) ⁻

Sadanandam A, Lyssiotis CA, Homicsko K et al. Nat Med. 2013 May;19(5):619-25.

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Summary of CRC subtypes III.



Sadanandam A, Lyssiotis CA, Homicsko K et al. Nat Med. 2013 May;19(5):619-25.

Future directions

CRCSC consortium



VALL D'HEBRON
Institute of Oncology



Swiss Institute of
Bioinformatics



THE UNIVERSITY OF TEXAS

~~MD~~ Anderson
Cancer Center



CRC subtypes of multiple team



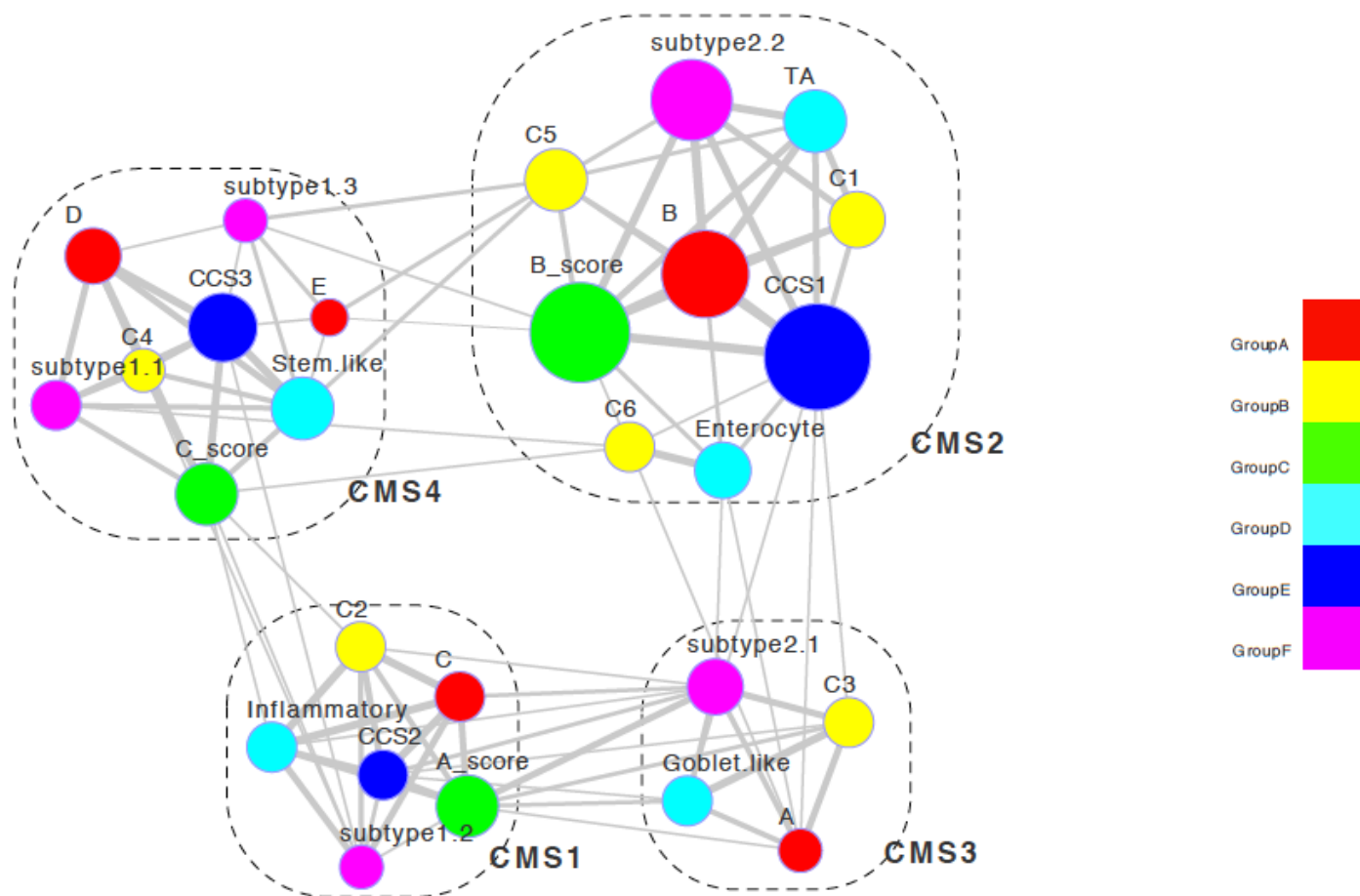
TCGA

Surface crypt	Lower crypt		CIMP+	Mesenchymal	Mixed
CIN Immune down	dMMR	KRAS sm	CSC	CIN Wnt up	CIN normal
A type	B type				C type
Inflammatory	Goblet	Transit Amplifying		Stem-like	Enterocyte
CCS1			CCS2	CCS3	
1.1	1.2	1.3	2.1	2.2	
MSI/CIMP		CIN		Invasive	

Results from the CRCSC consortium, SAGE, presented at ASCO 2014, **Abstract No: 3511**

26-30 September 2014, Madrid, Spain

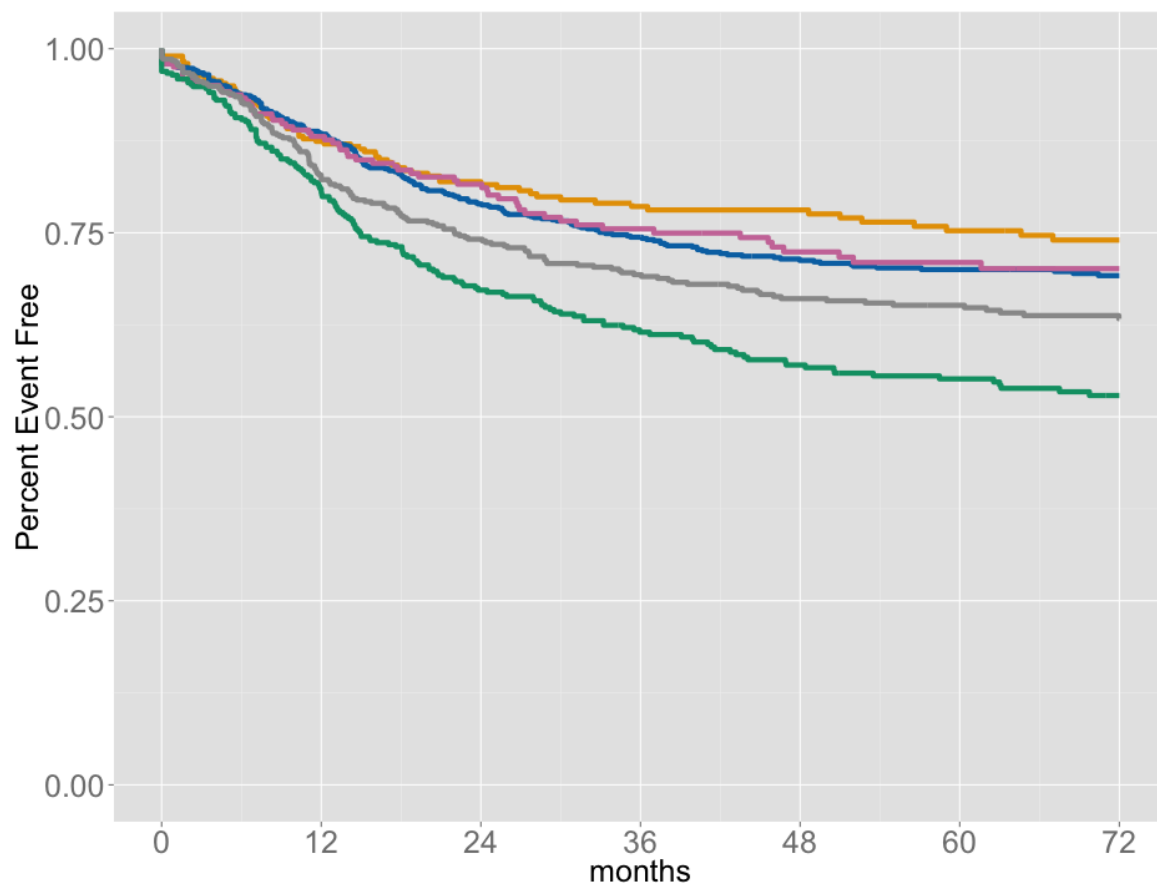
Network of samples



Results from the CRCSC consortium, SAGE, presented at ASCO 2014, **Abstract No: 3511**

26-30 September 2014, Madrid, Spain

Relapse-free Survival



Overall logrank $p = 0.00342^*$

CMS4 vs. **CMS1**

HR = 1.8 (1.1 – 2.9)

$p = 0.023^*$

CMS4 vs. **CMS2**

HR = 1.7 (1.3 – 2.2)

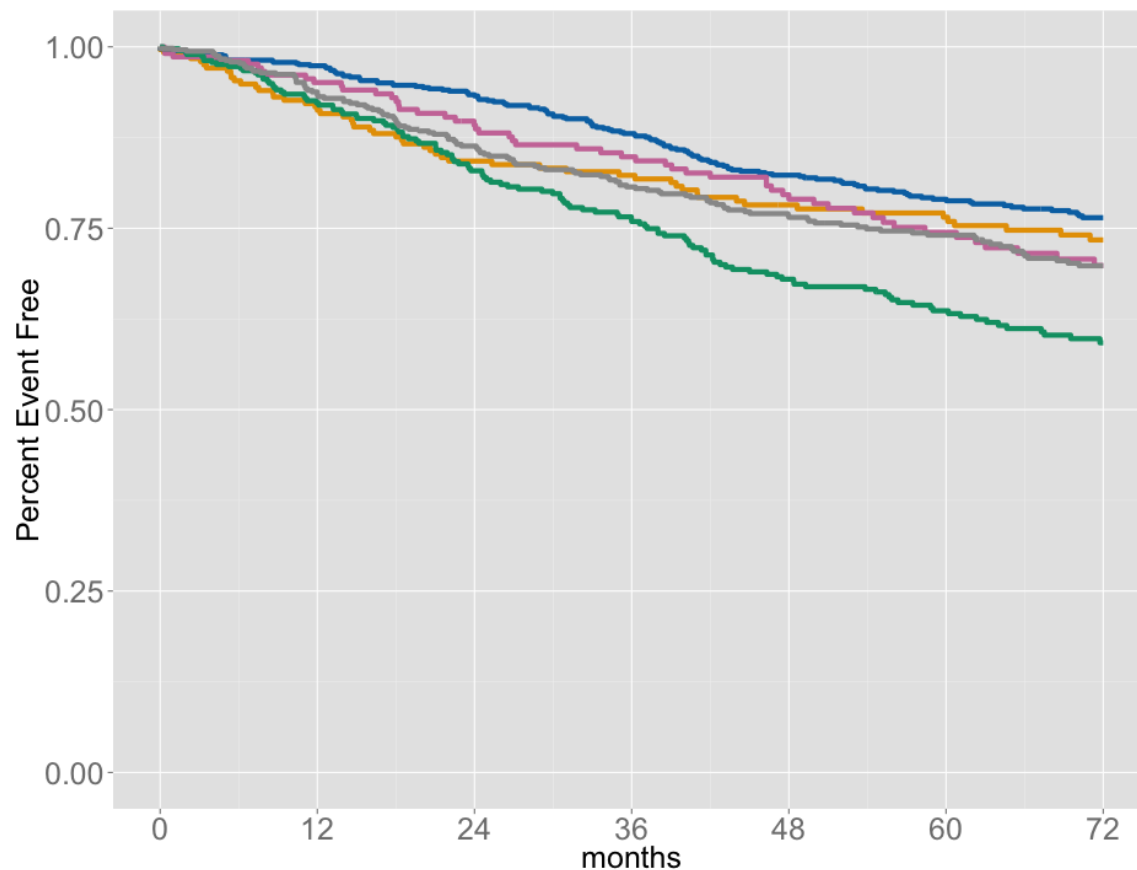
$p = 0.00024^*$

* Adjusted for stage, adjuvant chemotherapy, MSI, *BRAF* mut, and stratified by dataset.

Results from the CRCSC consortium, SAGE, presented at ASCO 2014, **Abstract No: 3511**

26-30 September 2014, Madrid, Spain

Overall Survival



Overall logrank $p = 0.00105^*$

CMS4 vs. **CMS2**

HR = 2.0 (1.5 - 2.8)

$p = 0.00012^*$

* Adjusted for stage, MSI, *BRAF* mut, and stratified by dataset.

Results from the CRCSC consortium, SAGE, presented at ASCO 2014, **Abstract No: 3511**

26-30 September 2014, Madrid, Spain

Summary of CRCSC results

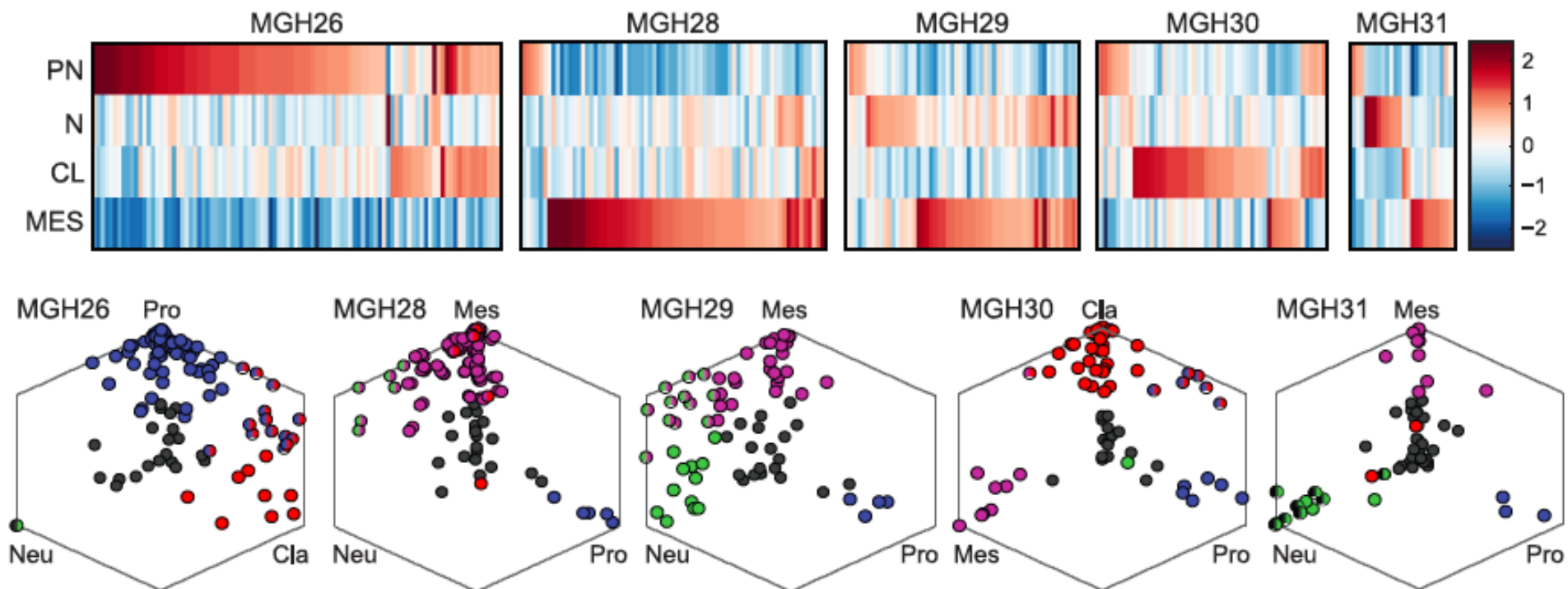
CMS1	13%	Females, older age, right colon, MSI, hypermutation, <i>BRAF</i> mut, immune activation	Better RFS, intermediate OS, worse SaR
CMS2	35%	Left colon, epithelial, MSS, high CIN, <i>TP53</i> mut, WNT/MYC pathway activation	Intermediate RFS, better OS, better SaR
CMS3	11%	Epithelial, CIN/MSI, <i>KRAS</i> mut, <i>MYC</i> ampl, IGFBP2 overexpression	Intermediate RFS, OS and SaR
CMS4	20%	Younger age, stage III/IV, mesenchymal, CIN/MSI, TGF β /VEGF activation, NOTCH3 overexpression	Worse RFS, worse OS Intermediate SaR
Unclassified	21%	Mixed subtype with variable epithelial-mesenchymal activation?	Intermediate RFS, OS and SaR

Results from the CRCSC consortium, SAGE, presented at ASCO 2014, **Abstract No: 3511**

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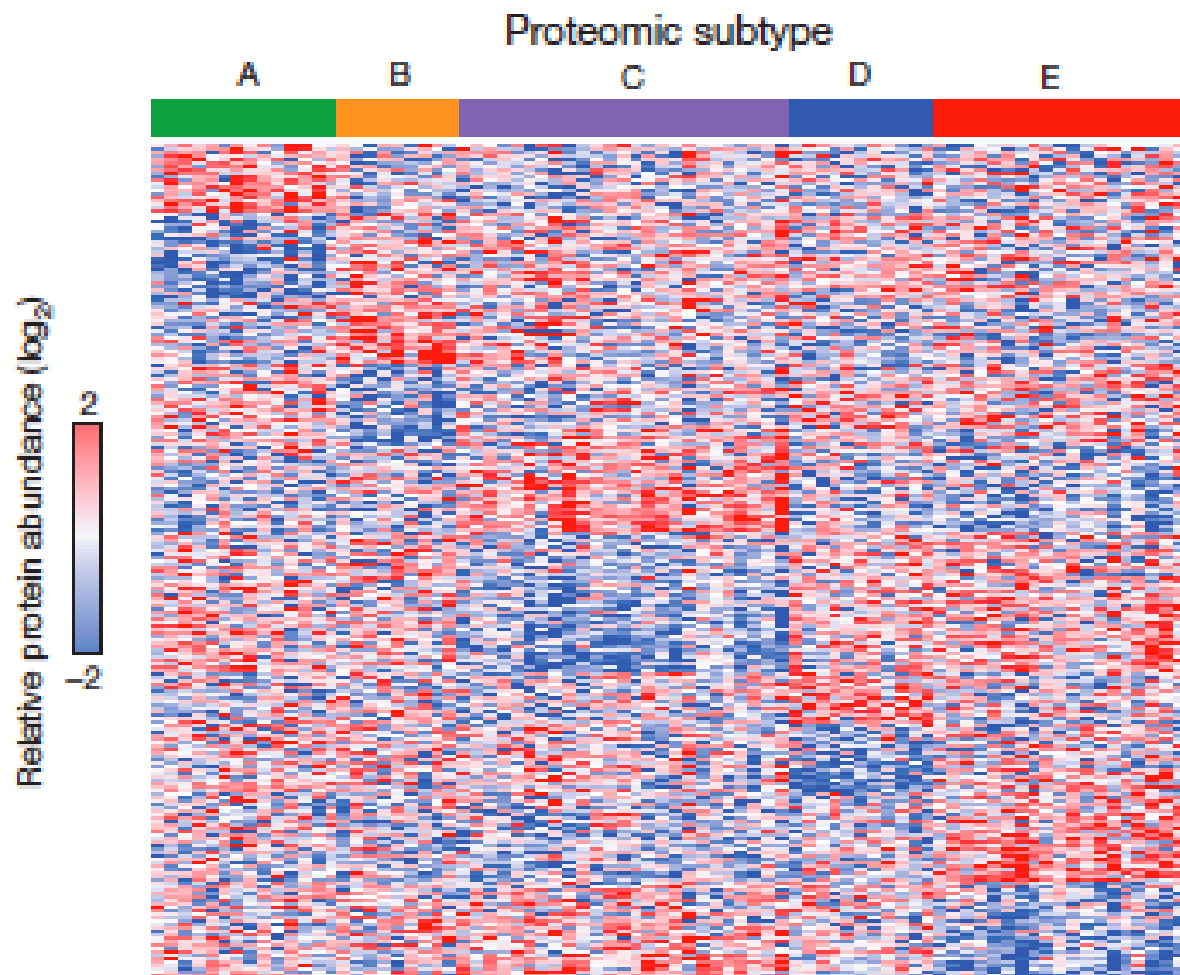
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Subtype plasticity at single cell level (in Glioblastoma)

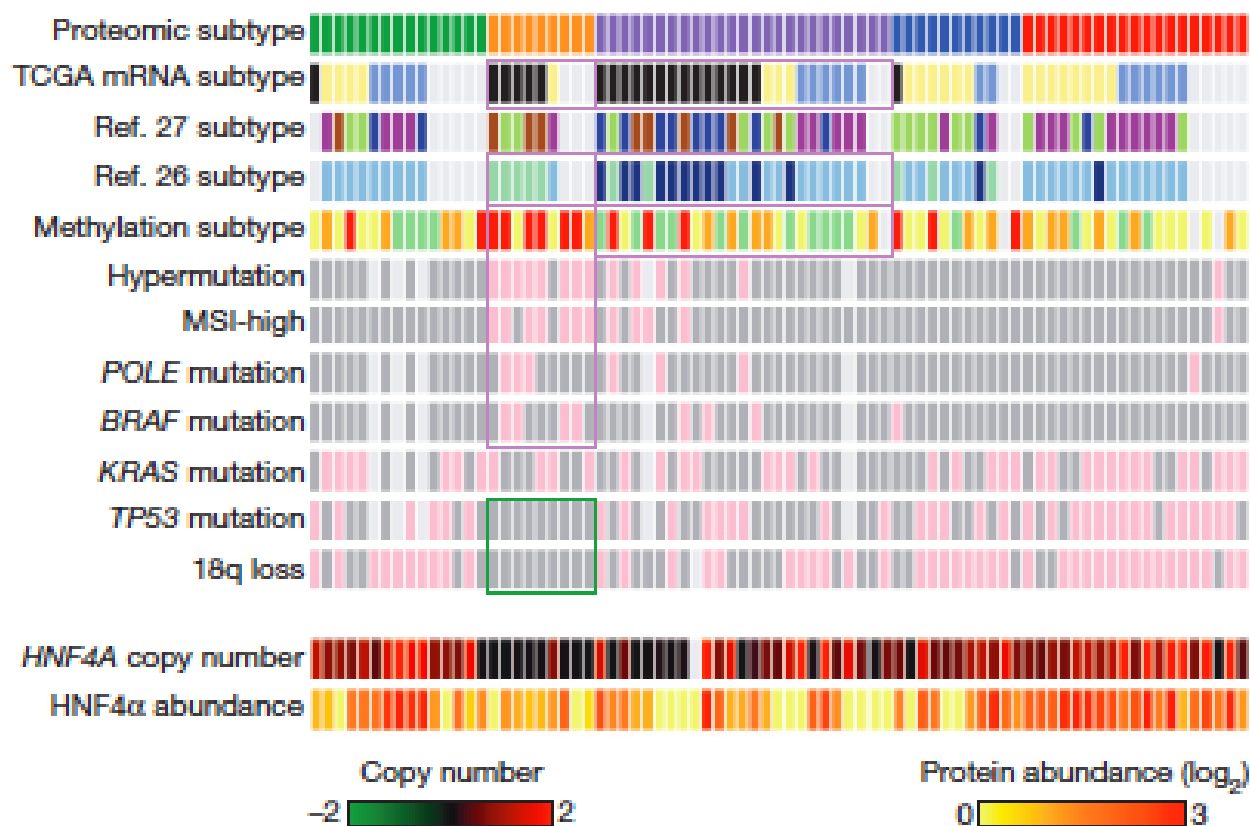


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Proteomic meets gene signatures. Lost in translation?



Proteomic meets gene signatures. Lost in translation?



Conclusions

- Subtypes do exist in CRC
- CRC subtypes might correlate with treatment sensitivity
- More large scale retrospective and prospective studies are needed for validation
- Tumor heterogeneity/plasticity should be addressed and followed upon disease progression (primary vs. metastatic biopsy)
- Gene expression signatures might be overtaken by proteomics based signatures in the future