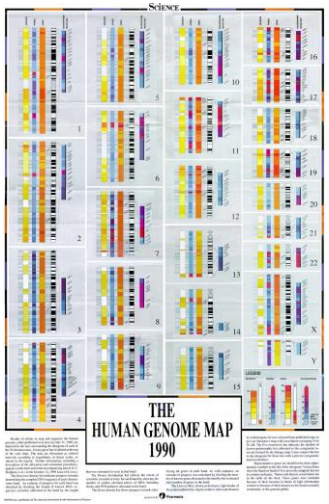
Molecular biology of colorectal cancer

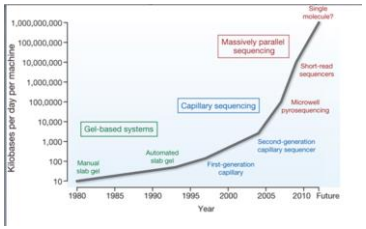
Phil Quirke
Yorkshire Cancer Research
Centenary Professor of Pathology
University of Leeds, UK

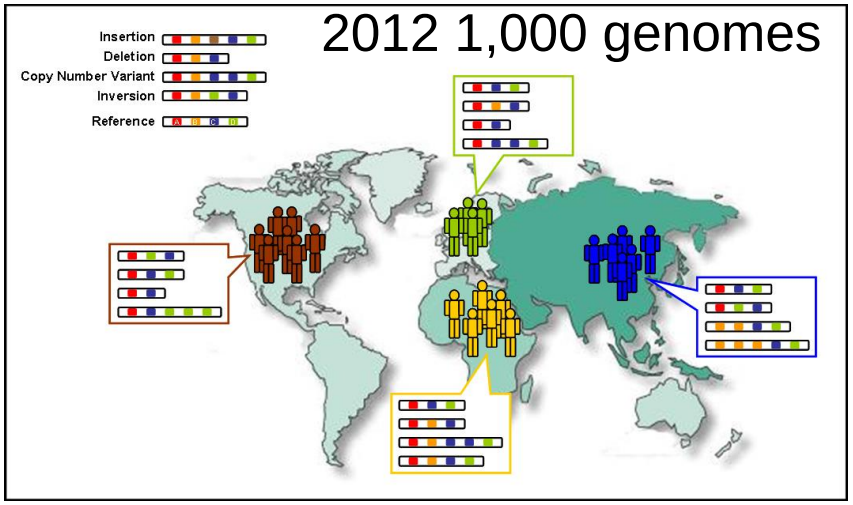
Rapid pace of molecular change

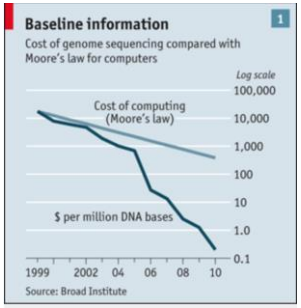
UNIVERSITY OF LEEDS

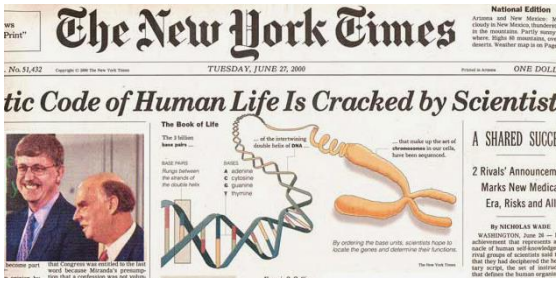


Sequencing changes









2000

100,000 Genomes Project

"It is crucial that we continue to push the boundaries and this new plan will mean we are the first country in the world to use DNA codes in the mainstream of the health service"
The Rt Hon David Cameron MP
The Prime Minister
10 December 2012

Department of Health
NHS England
Genomics england

Genomes Sequenced

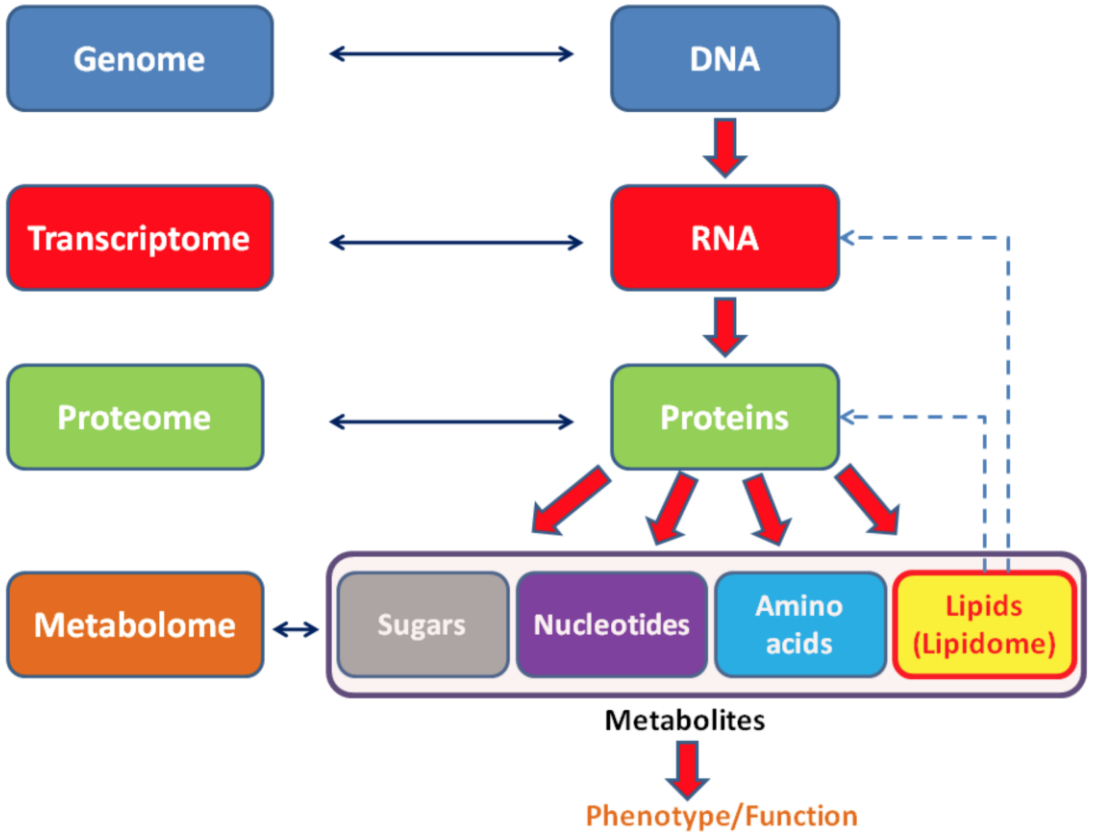
3	4	1	5	1

Which technologies should we use?

UNIVERSITY OF LEEDS

- Genomics- DNA/RNA
- Proteomics
- Metabolome
- Pathways
- Phenotype

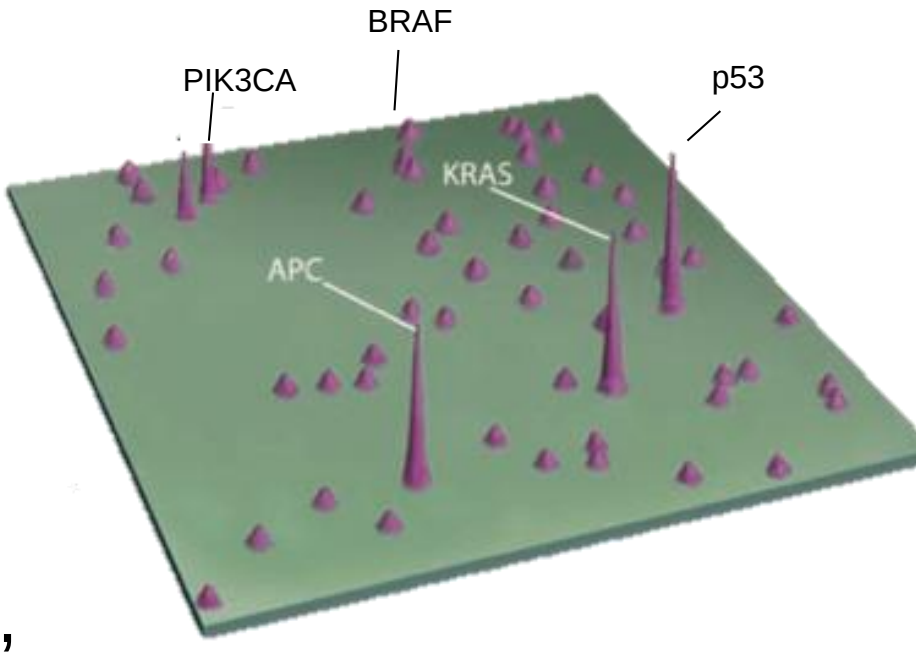
- Test
  - Fit for purpose
  - Cost
  - Changing

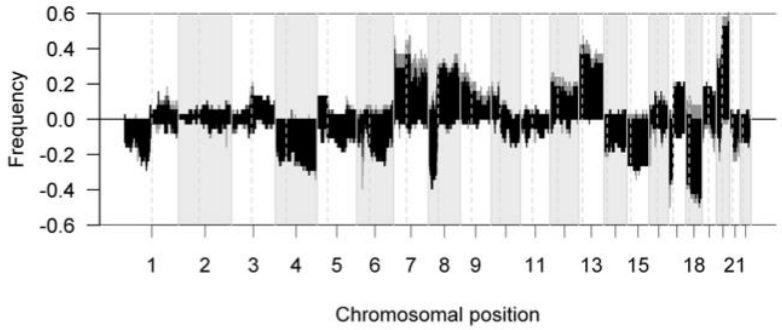


Molecular changes in bowel cancer

UNIVERSITY OF LEEDS

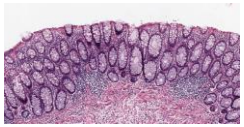
- DNA
  - Mutations – driver and passenger
  - Amplifications
  - Deletions
  - Chromosomal instability,
  - Microsatellite instability
  - Fusion genes
  - Methylation

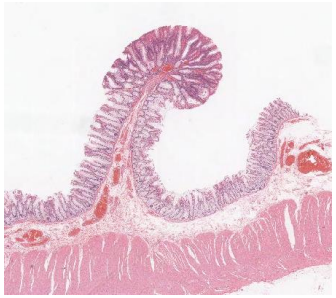


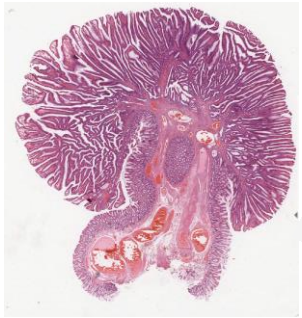


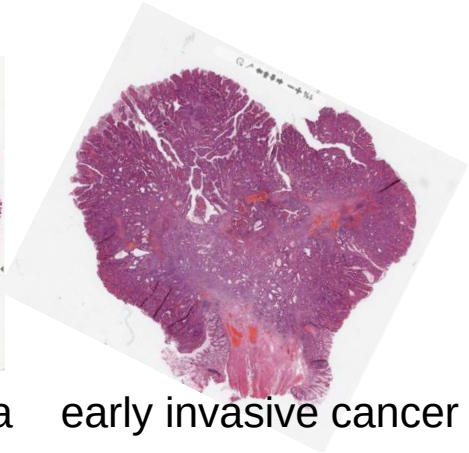
Increasing complexity with time

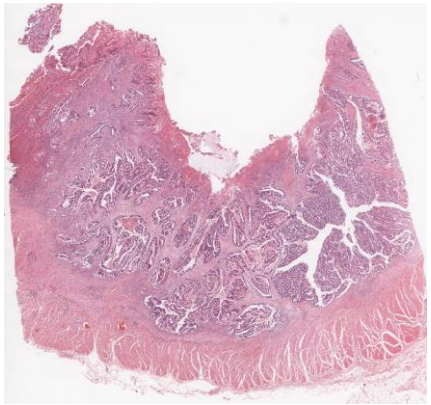
UNIVERSITY OF LEEDS


Normal


early adenoma


later adenoma


early invasive cancer


colorectal cancer

Early

APC, Ras, PIK3CA, BRaf

Later

p53, SMAD4 DNA repair, Copy number

Gradual increase in genomic changes

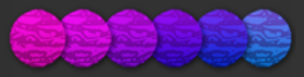
Increasing mutations
Increasing genomic instability
Classical adenomas, Serrated pathway, mismatch repair
Identification of subtypes

Clinical uses

UNIVERSITY OF LEEDS

- Genetic syndromes
- Outcomes
  - DNA
    - Deficient mismatch repair
    - Kiras/Braf/PIK3CA
  - RNA –
    - oncotype Dx ?
    - RNA subtypes?

- Prediction of response
  - Anti-EGFr abs
    - Ras
    - Epiregulin/amphiregulin
    - Her3
  - Braf inhibitor
    - Braf
  - Her2
  - Aspirin PIK3CA
  - Immunotherapy
    - dMMR and anti PD1/PDL1



Genetic syndromes

UNIVERSITY OF LEEDS

HHS Public Access
Author manuscript
Surg Clin North Am. Author manuscript; available in PMC 2016 October 01.

Published in final edited form as:
Surg Clin North Am. 2015 October ; 95(5): 1067–1080. doi:10.1016/j.suc.2015.05.004.

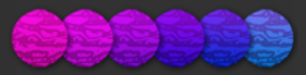
Hereditary Colorectal Cancer: Genetics and Screening

Lodewijk A.A. Brosens, MD, PhD[1,2], G. Johan A. Offerhaus, MD, PhD, MPH[3], and Francis M Giardiello, MD[4]

Colorectal cancer syndromes

Syndrome	Genes	Mode of inheritance	
Lynch syndrome	*MLH1*, *MSH2*, *MSH6*, *PMS2*, or *EpCAM*	Autosomal dominant	3%
(Attenuated) Familial adenomatous polyposis	*APC*	Autosomal dominant	1%
MUTYH-associated polyposis	*MUTYH (MYH)*	Autosomal recessive	
Peutz-Jeghers syndrome	*LKB1 (STK11)*	Autosomal dominant	
Juvenile polyposis syndrome	*SMAD4* (~30%) *BMPR1A* (~20%)	Autosomal dominant	
Hereditary mixed polyposis syndrome	*GREM1*	Autosomal dominant	
Serrated polyposis syndrome	unknown	unknown	

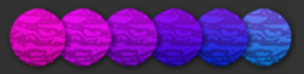
Polymerase proof reading associated polyposis ***POLE & POLD1*** **Autosomal dominant**



HNPCC

UNIVERSITY OF LEEDS

- HNPCC 3%
- Germ line mutations in DNA repair genes leads to hyper mutation
- Inactivates key genes leading to CRC and other cancers
- Truncated proteins appear on cell surface – immune response
- Screening via MSI or immunohiostochemistry
  - dMMR proteins
    - hMSH2 (40%, hMLH1 (40%), hMSH6 (10%), PMS2 (6%) POLD (1%) and POLE (1%)
  - BRAF V600E wild type
- Germ line sequencing



Nice recommendations Feb 2017 Routine testing in England

UNIVERSITY OF LEEDS

Molecular testing strategies for Lynch syndrome in people with colorectal cancer

In development [GID-DG10001] Expected publication date: February 2017 Register as a stakeholder

Project information | Project documents

Status	In progress
Process	DT
Provisional Schedule	
Expected publication	February 2017
Project Team	
Project lead	Robert Fernley

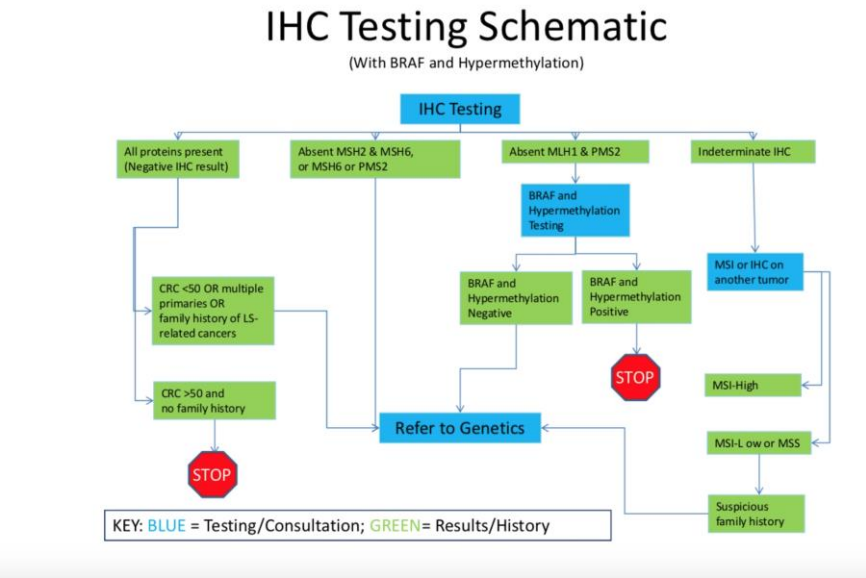
UNIVERSITY OF EXETER | MEDICAL SCHOOL

PenTAG

Molecular testing for Lynch syndrome in people with colorectal cancer

Produced by Peninsula Technology Assessment Group (PenTAG)
University of Exeter Medical School
South Cloisters, St Luke's Campus, Heavitree Road, Exeter, EX1 2LU

Authors Tristan Snowsill, *Research Fellow*[1]
Helen Coelho, *Research Fellow*[1]
Nicola Huxley, *Research Fellow*[1]
Tracey Jones-Hughes, *Research Fellow*[1]
Simon Briscoe, *Information Specialist*[1]
Ian Frayling, *Consultant in Genetic Pathology*[2]
Chris Hyde, *Professor of Public Health and Clinical Epidemiology*[1]



The base case results in the economic evaluation suggest that screening for LS in CRC patients using IHC, *BRAF* V600E and *MLH1* methylation testing would be cost-effective at a threshold of £20,000 per quality-adjusted life year (QALY). The incremental cost-effectiveness ratio for this strategy is £11,008 per QALY compared to no screening. Screening without tumour tests is not predicted to be cost-effective (more costly and less effective than another strategy).

Classification - DNA

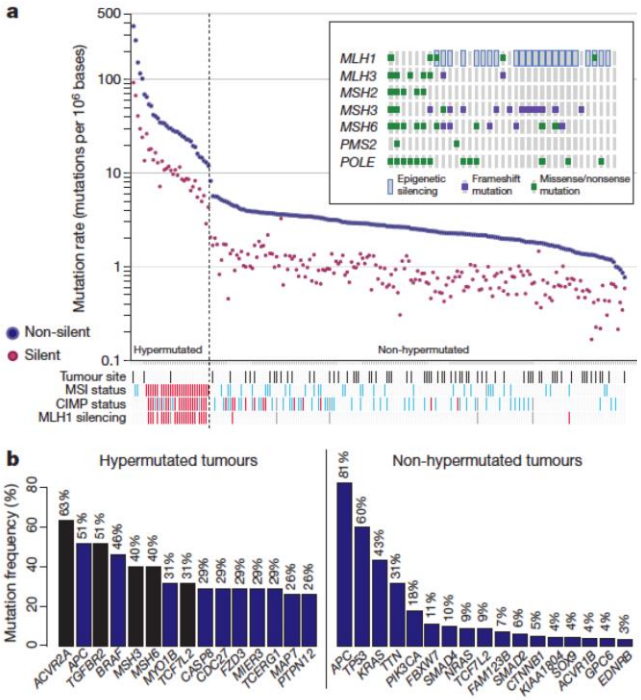
UNIVERSITY OF LEEDS

- Prognosis
  - dMMR/hypermutated 12-15%
  - Genomic instability Rest

Comprehensive molecular characterization of human colon and rectal cancer

The Cancer Genome Atlas Network*

330 | NATURE | VOL 487 | 19 JULY 2012



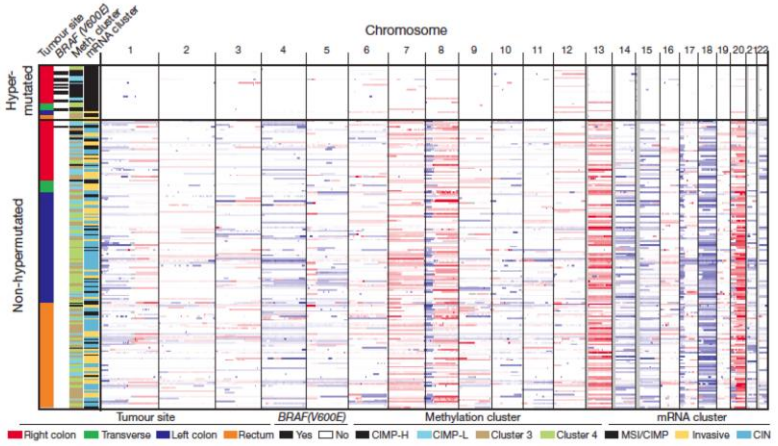


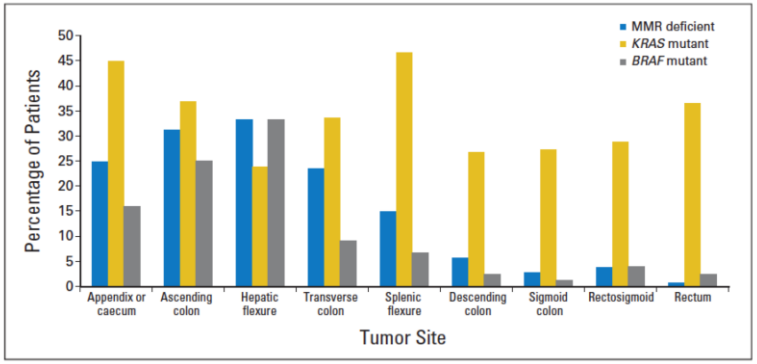
Figure 2 | Integrative analysis of genomic changes in 195 CRCs. Hypermutated tumours have near-diploid genomes and are highly enriched for hypermethylation, CIMP expression phenotype and *BRAF(V600E)* mutations. Non-hypermutated tumours originating from different sites are virtually indistinguishable from each other on the basis of their copy-number alteration patterns, DNA methylation or gene-expression patterns. Copy-number changes of the 22 autosomes are shown in shades of red for copy-number gains and shades of blue for copy-number losses.

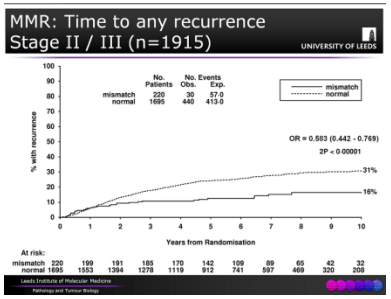
N= 276 with 97 undergoing WGS

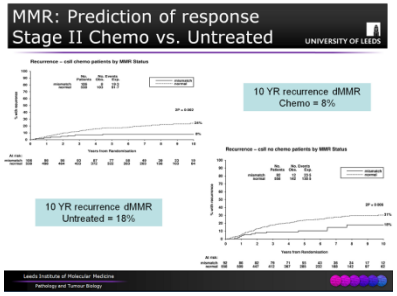
Sporadic deficient Mismatch Repair (dMMR) Hyper mutated tumours

UNIVERSITY OF LEEDS

- Demographics
  - HNPCC dMMR/Braf wild type
  - Sporadic
  - Colon > rectum
  - Females
  - Stage II 12% Stage III 7%, Stage 4 4%
- Prognosis
  - 50% lower recurrence risk in stage II dMMR tumours
  - Prognostic effect dMMR remains in treated group
- Prediction
  - US data suggests less responsive to chemotherapy
  - NO evidence of worse outcome on chemo in Quasar







Other mutations and prognosis

UNIVERSITY OF LEEDS

Recurrence Quasar1 – by KRas 12,13,61

N=789 MRC Focus stage IV

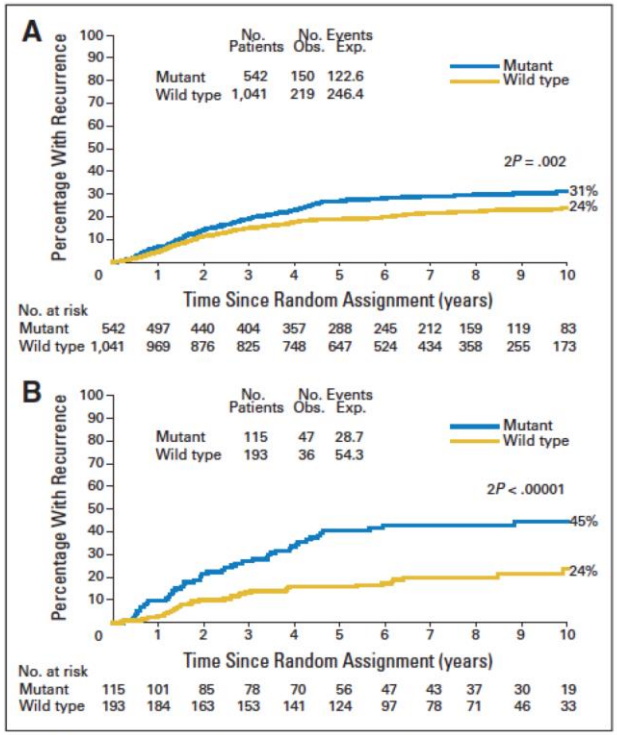
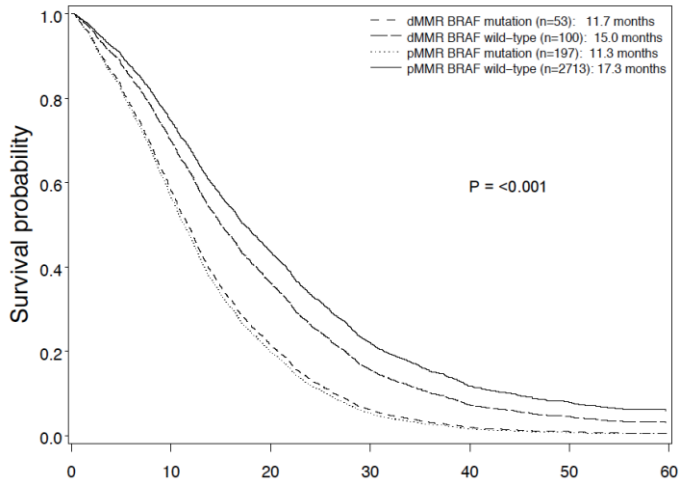


Fig 5. Recurrence by *KRAS* status: (A) all patients, (B) rectum stage II only. Obs., observed number of recurrences; Exp., expected number of recurrences; Var, variance of O-E.

Marker	Endpoint	Group	n	Hazard Ratio* (95% CI)	p-value
KRAS	PFS	W/T KRAS	322	1.0	0.06
		Any KRAS mutation	311	1.17 (1.00, 1.36)	
	OS	W/T KRAS	324	1.0	0.02
		Any KRAS mutation	314	1.23 (1.04, 1.44)	

Marker	Endpoint	group	n	Hazard Ratio* (95% CI)	p-value
BRAF	PFS	W/T BRAF	679	1.0	0.80
		BRAF mutation	54	1.04 (0.78, 1.38)	
	OS	W/T BRAF	684	1.0	<0.0001
		BRAF mutation	54	1.69 (1.26, 2.27)	



BRAF/dMMR
3063 cases
Braf poor prognosis

Braf V600E not prognostic

PIK3CA mutations not prognostic

Multi-Gene RT-PCR Colon Cancer Assay – Oncotype Dx colon

UNIVERSITY OF LEEDS

Colon Cancer Technical Feasibility

Development Studies Surgery Alone
NSABP C-01/C-02 (n=270)
Cleveland Clinic (n = 765)

1035

Development Studies Surgery + 5FU/LV
NSABP C-04 (n=308)
NSABP C-06 (n=508)

816

Selection of Final Gene List & Algorithm

Standardization and Validation of Analytical Methods

Clinical Validation Study – Stage II Colon Cancer
QUASAR (n=1,436)
Test Prognosis and Treatment Benefit

RECURRENCE SCORE
Calculated from Tumor Gene Expression

STROMAL	CELL CYCLE
FAP	Ki-67
INHBA	C-MYC
BGN	MYBL2

GADD45B

REFERENCE
ATP5E
GPX1
PGK1
UBB
VDAC2

Treatment
core
nes
A 2
BIK
EFN
H E1
AD2L1
RUNX1

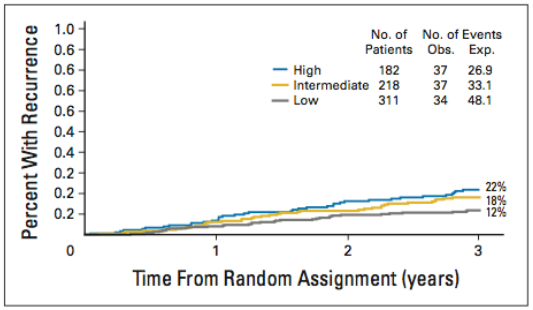
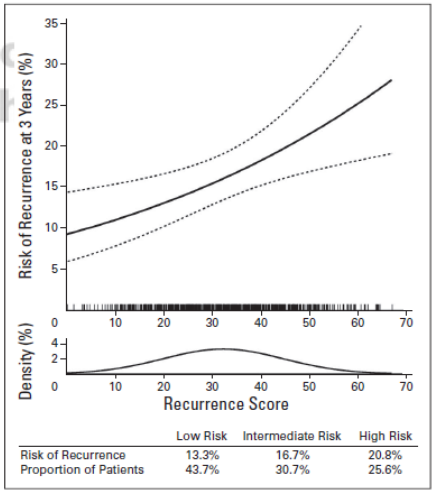


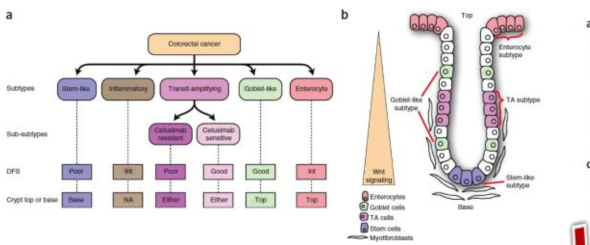
Fig 3. Kaplan-Meier estimates of 3-year recurrence in surgery-alone patients by risk group. Obs, observed; Exp, experienced.

HR = 1.47 (p=0.046)

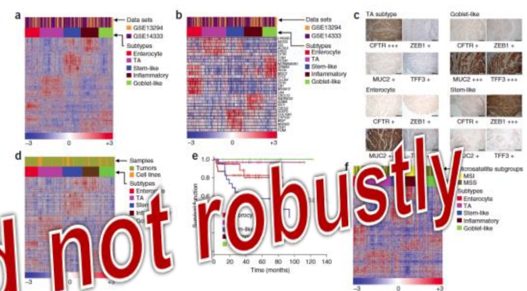


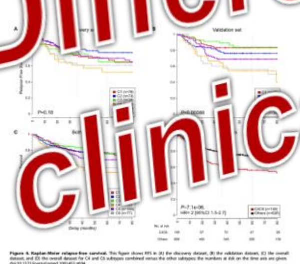
New RNA array patterns

UNIVERSITY OF LEEDS



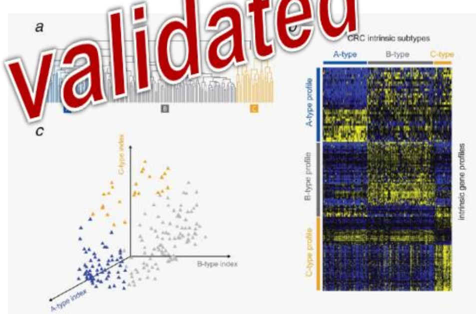
A colorectal cancer classification system that associates cellular phenotype and responses to therapy





Gene Expression Classification of Colon Cancer into Molecular Subtypes: Characterization, Validation, and Prognostic Value

Laetitia Marisa[1], Aurélien de Reyniès[1], Alex Duval[2,3], Janick Selves[4], Marie Pierre Gaub[5,6], Laure Vescovo[1], Marie-Christine Etienne-Grimaldi[7], Renaud Schiappa[1], Dominique Guenot[5], Mira Ayadi[1], Sylvain Kirzin[4], Maurice Chazal[8], Jean-François Fléjou[2,3,9], Daniel Benchimol[10], Anne Berger[11], Arnaud Lagarde[12], Erwan Pencreach[5,6,13], Françoise Piard[14], Dominique Elias[15], Yann Parc[3,16], Sylviane Olschwang[12,17,18,19], Gérard Milano[7], Pierre Laurent-Puig[20*¶], Valérie Boige[15,20¶]



Colorectal cancer intrinsic subtypes predict chemotherapy benefit, deficient mismatch repair and epithelial-to-mesenchymal transition

Paul Roepman[1], Andreas Schlicker[2], Josep Tabernero[3], Ian Majewski[2], Sun Tian[1], Victor Moreno[4,5], Mireille H Snel[1], Christine M Chresta[6], Robert Rosenberg[7], Ulrich Nitsche[7], Teresa Macarulla[3], Gabriel Capella[5], Ramon Salazar[5], George Orphanides[6], Lodewyk FA Wessels[2,8], Rene Bernards[1,2] and Iris M Simon[1]

Different and not robustly clinically validated

The consensus molecular subtypes of colorectal cancer

Justin Guinney[1,21], Rodrigo Dienstmann[1,2,21], Xin Wang[3,4,21], Aurélien de Reyniès[5,21], Andreas Schlicker[6,21], Charlotte Soneson[7,21], Laetitia Marisa[5,21], Paul Roepman[8,21], Gift Nyamundanda[9,21], Paolo Angelino[7], Brian M Bot[1], Jeffrey S Morris[10], Iris M Simon[8], Sarah Gerster[7], Evelyn Fessler[3], Felipe De Sousa E Melo[3], Edoardo Missiaglia[7], Hena Ramay[7], David Barras[7], Krisztian Homicsko[11], Dipen Maru[10], Ganiraju C Manyam[10], Bradley Broom[10], Valerie Boige[12], Beatriz Perez-Villamil[13], Ted Laderas[1], Ramon Salazar[14], Joe W Gray[15], Douglas Hanahan[11], Josep Tabernero[2], Rene Bernards[6], Stephen H Friend[1], Pierre Laurent-Puig[16,17,22], Jan Paul Medema[3,22], Anguraj Sadanandam[9,22], Lodewyk Wessels[6,22], Mauro Delorenzi[7,18,19,22], Scott Kopetz[10,22], Louis Vermeulen[3,22] & Sabine Tejpar[20,22]

CMS1 MSI immune	CMS2 Canonical	CMS3 Metabolic	CMS4 Mesenchymal
14%	37%	13%	23%
MSI, CIMP high, hypermutation	SCNA high	Mixed MSI status, SCNA low, CIMP low	SCNA high
BRAF mutations		*KRAS* mutations	
Immune infiltration and activation	WNT and MYC activation	Metabolic deregulation	Stromal infiltration, TGF-β activation, angiogenesis
Worse survival after relapse			Worse relapse-free and overall survival

Transcriptome/Protein characterisation

UNIVERSITY OF LEEDS

A pathology atlas of the human cancer transcriptome

Mathias Uhlen,[1,2,3]* Cheng Zhang,[1] Sunjae Lee,[1] Evelina Sjöstedt,[1,4] Linn Fagerberg,[1] Gholamreza Bidkhori,[1] Rui Benfeitas,[1] Muhammad Arif,[1] Zhengtao Liu,[1] Fredrik Edfors,[1] Kemal Sanli,[1] Kalle von Feilitzen,[1] Per Oksvold,[1] Emma Lundberg,[1] Sophia Hober,[3] Peter Nilsson,[1] Johanna Mattsson,[4] Jochen M. Schwenk,[1] Hans Brunnström,[5] Bengt Glimelius,[4] Tobias Sjöblom,[4] Per-Henrik Edqvist,[4] Dijana Djureinovic,[4] Patrick Micke,[4] Cecilia Lindskog,[4] Adil Mardinoglu,[1,3,6]† Fredrik Ponten[4]†

Cancer is one of the leading causes of death, and there is great interest in understanding the underlying molecular mechanisms involved in the pathogenesis and progression of individual tumors. We used systems-level approaches to analyze the genome-wide transcriptome of the protein-coding genes of 17 major cancer types with respect to clinical outcome. A general pattern emerged: Shorter patient survival was associated with up-regulation of genes involved in cell growth and with down-regulation of genes involved in cellular differentiation. Using genome-scale metabolic models, we show that cancer patients have widespread metabolic heterogeneity, highlighting the need for precise and personalized medicine for cancer treatment. All data are presented in an interactive open-access database (www.proteinatlas.org/pathology) to allow genome-wide exploration of the impact of individual proteins on clinical outcomes.

Uhlen *et al.*, *Science* **357**, eaan2507 (2017) 18 August 2017

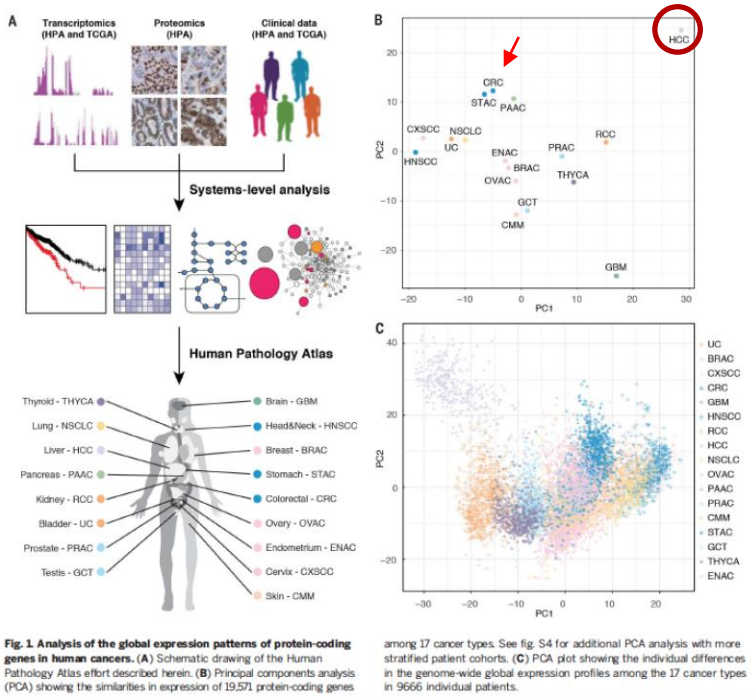
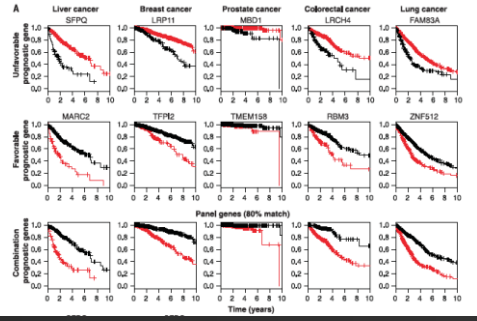


Fig. 1. Analysis of the global expression patterns of protein-coding genes in human cancers. (**A**) Schematic drawing of the Human Pathology Atlas effort described herein. (**B**) Principal components analysis (PCA) showing the similarities in expression of 19,571 protein-coding genes among 17 cancer types. See fig. S4 for additional PCA analysis with more stratified patient cohorts. (**C**) PCA plot showing the individual differences in the genome-wide global expression profiles among the 17 cancer types in 9666 individual patients.



Predictors

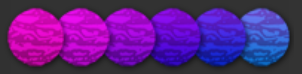
UNIVERSITY OF LEEDS

Table 1. Targeted Therapeutics in Cancer.*

Gene	Genetic Alteration	Tumor Type	Therapeutic Agent
Receptor tyrosine kinase			
EGFR	Mutation, amplification	Lung cancer, glioblastoma	Gefitinib, erlotinib
ERBB2	Amplification	Breast cancer	Lapatinib
FGFR1	Translocation	Chronic myeloid leukemia	PKC412, BIBF-1120
FGFR2	Amplification, mutation	Gastric, breast, endometrial cancer	PKC412, BIBF-1120
FGFR3	Translocation, mutation	Multiple myeloma	PKC412, BIBF-1120
PDGFRA	Mutation	Glioblastoma, gastrointestinal stromal tumor	Sunitinib, sorafenib, imatinib
PDGFRB	Translocation	Chronic myelomonocytic leukemia	Sunitinib, sorafenib, imatinib
ALK	Mutation or amplification	Lung cancer, neuroblastoma, anaplastic large-cell lymphoma	Crizotinib
c-MET	Amplification	Gefitinib-resistant non–small-cell lung cancer, gastric cancer	Crizotinib, XL184, SU11274
IGF1R	Activation by insulin-like growth factor II ligand	Colorectal, pancreatic cancer	CP-751,871, AMG479
c-KIT	Mutation	Gastrointestinal stromal tumor	Sunitinib, imatinib
FLT3	Internal tandem duplication	Acute myeloid leukemia	Lestaurtinib, XL999
RET	Mutation, translocation	Thyroid medullary carcinoma	XL184
Non–receptor tyrosine kinase			
ABL	Translocation (BCR-ABL)	Chronic myeloid leukemia	Imatinib
JAK2	Mutation (V617F), translocation	Chronic myeloid leukemia, myeloproliferative disorders	Lestaurtinib, INCB018424
SRC	Overexpression	Non–small-cell lung cancer; ovarian, breast cancer; sarcoma	KX2–391, dasatinib, AZD0530
Serine–threonine–lipid kinase			
BRAF	Mutation (V600E)	Melanoma; colon, thyroid cancer	SB-590885, PLX-4032, RAF265, XL281
Aurora A and B kinases	Overexpression	Breast, colon cancer; leukemia	MK-5108 (VX-689)
Polo-like kinases	Overexpression	Breast, lung, colon cancer; lymphoma	BI2536, GSK461364
MTOR	Increased activation	Renal-cell carcinoma	Temsirolimus (CCI-779), BEZ235
PI3K	PIK3CA mutations	Colorectal, breast, gastric cancer; glioblastoma	BEZ235
DNA damage or repair			
BRCA1 and *BRCA2*	Mutation (synthetic lethal effect)	Breast, ovarian cancer	Olaparib, MK-4827 (PARP inhibitors)

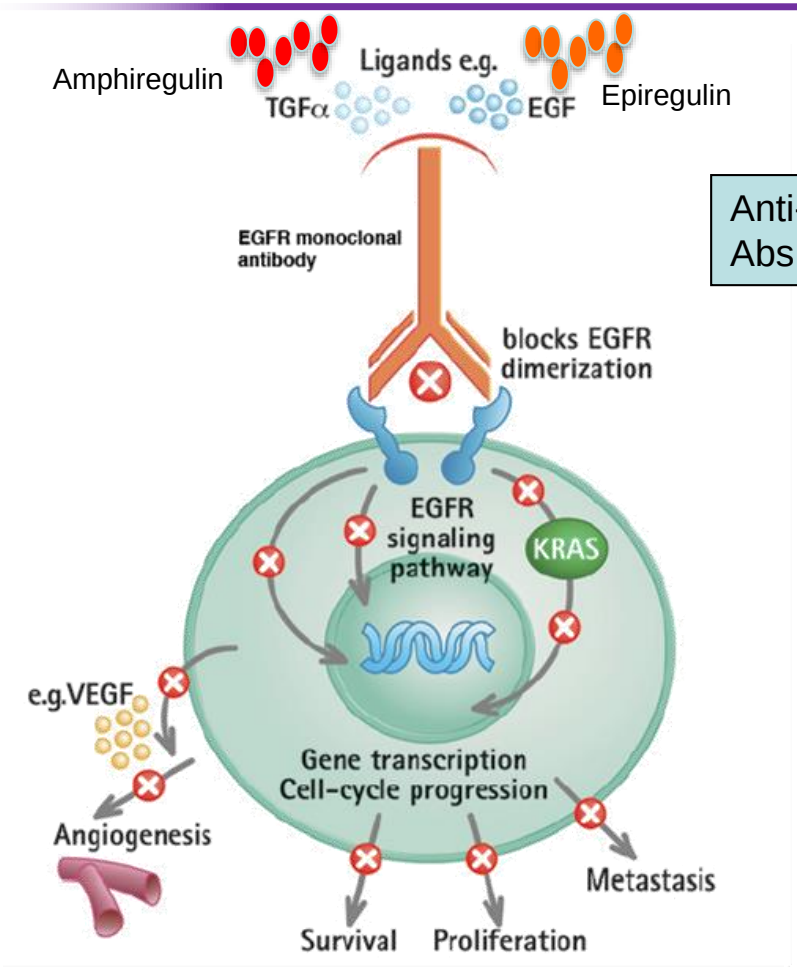
* PARP denotes poly(adenosine diphosphate–ribose) polymerase.

- Targeted therapies CRC
  - Anti EGFr ab's
  - Braf inhibitors
  - Anti PD1 and dMMR
  - Others
    - Herceptin – anti Her2
    - Aspirin – PIK3CA mutations

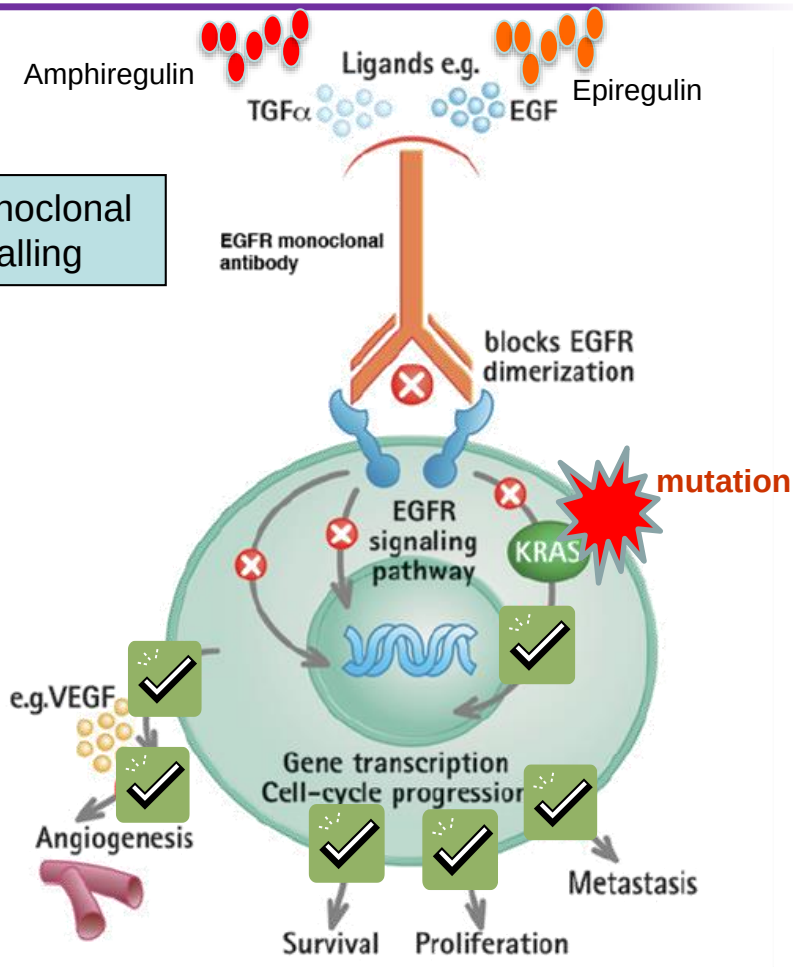


KRAS and the EGFr signalling pathway in KRAS-wild type and KRAS-mutant patients

UNIVERSITY OF LEEDS



Anti-EGFr monoclonal Abs block signalling



KRAS-WT

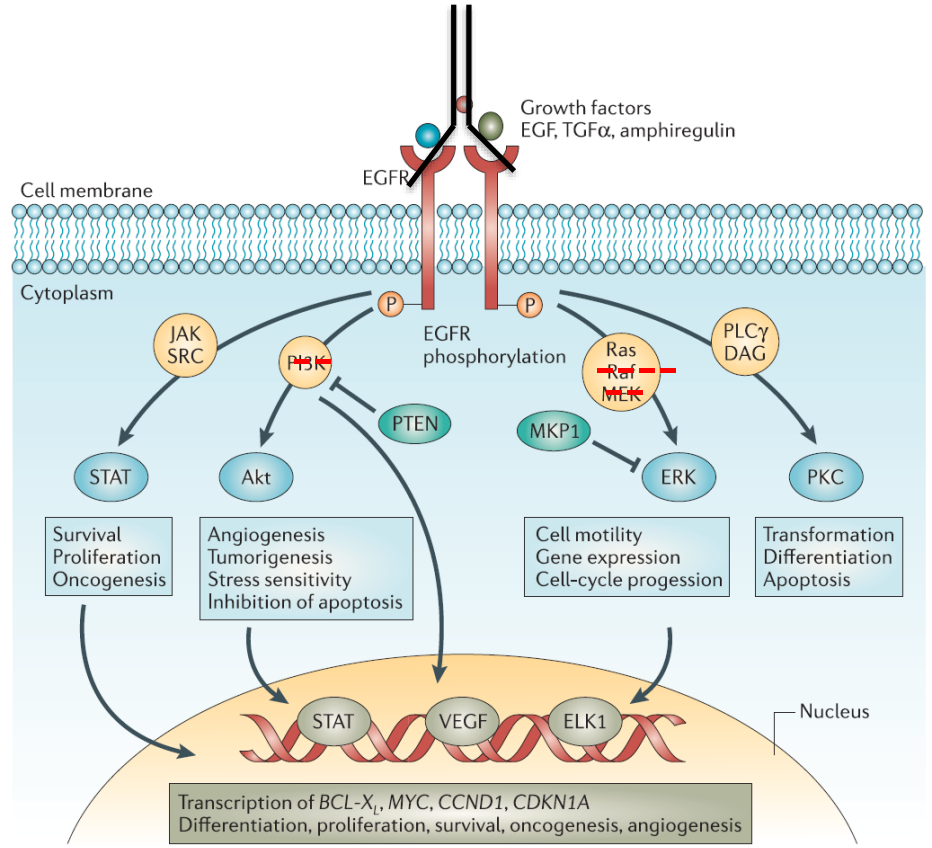
KRAS-MUTANT

BRAF mutants

UNIVERSITY OF LEEDS

- BRAFinhibitors negative
- Block BRAF and others ?
- Early reports
  - No
    - BRAF*i* and MEK
  - Yes 26-35% RR
    - BRAF*i*. anti EGFr and anti PIK3CA
    - BRAF*i*, anti EGFr and Mek inhibitor
    - *BRAFi*, antiEGFr and Irinotecan
- Looks likely that dual inhibition

BRAF*i* and antiEGFr works



CCND1, gene encoding cyclin D1; *CDKN1A*, gene encoding p21; JAK, Janus kinase; TGFα, tranforming growth factor-α.

PD-1 blockade phase 2 data

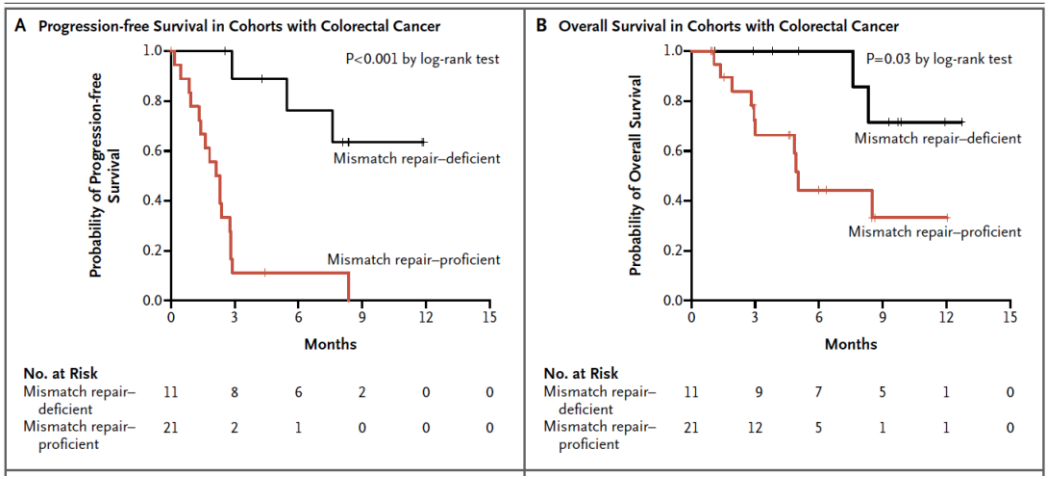
UNIVERSITY OF LEEDS

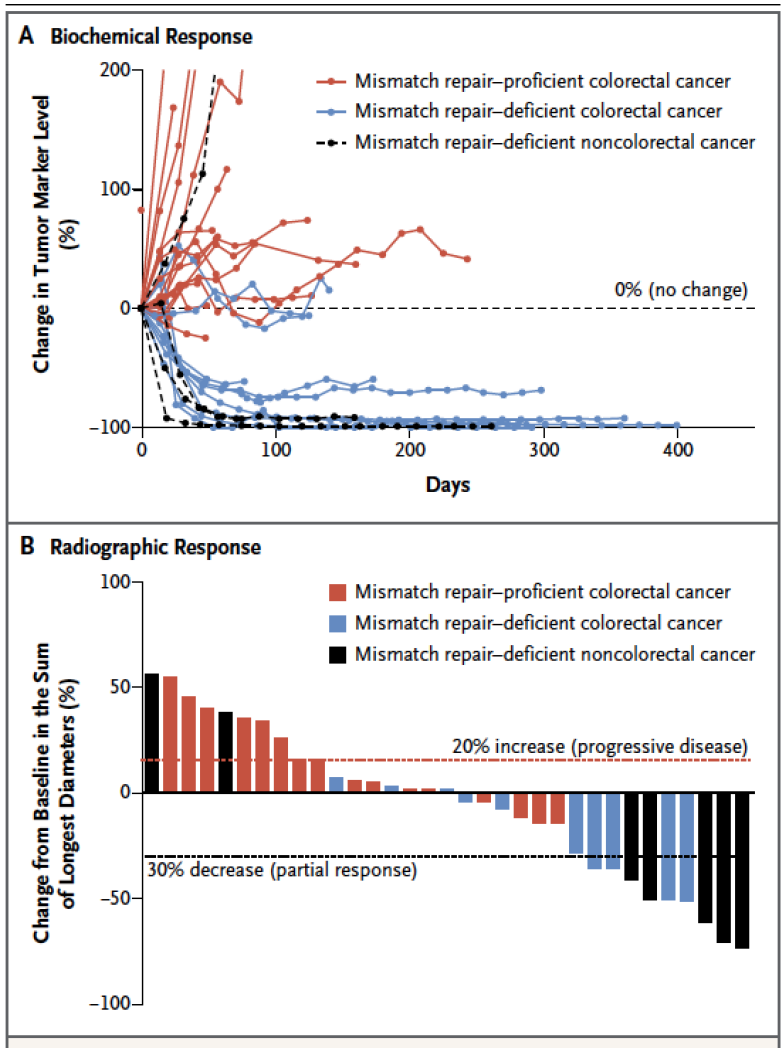
ORIGINAL ARTICLE

PD-1 Blockade in Tumors with Mismatch-Repair Deficiency

D.T. Le, J.N. Uram, H. Wang, B.R. Bartlett, H. Kemberling, A.D. Eyring, A.D. Skora, B.S. Luber, N.S. Azad, D. Laheru, B. Biedrzycki, R.C. Donehower, A. Zaheer, G.A. Fisher, T.S. Crocenzi, J.J. Lee, S.M. Duffy, R.M. Goldberg, A. de la Chapelle, M. Koshiji, F. Bhaijee, T. Huebner, R.H. Hruban, L.D. Wood, N. Cuka, D.M. Pardoll, N. Papadopoulos, K.W. Kinzler, S. Zhou, T.C. Cornish, J.M. Taube, R.A. Anders, J.R. Eshleman, B. Vogelstein, and L.A. Diaz, Jr.

N Engl J Med 2015;372:2509-20.

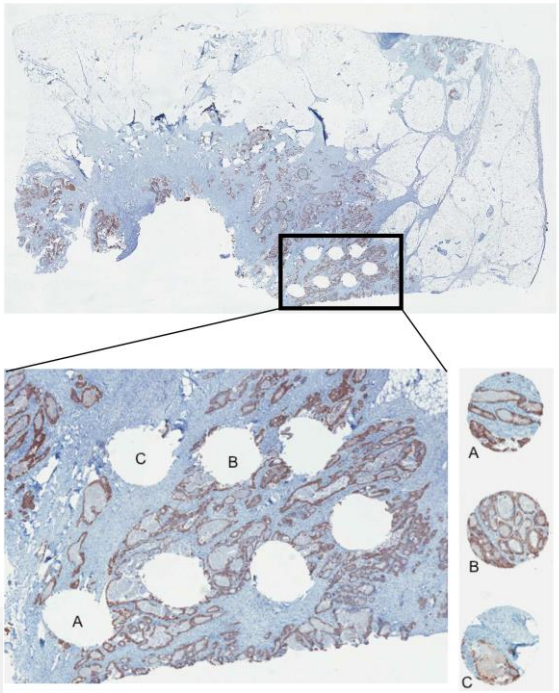


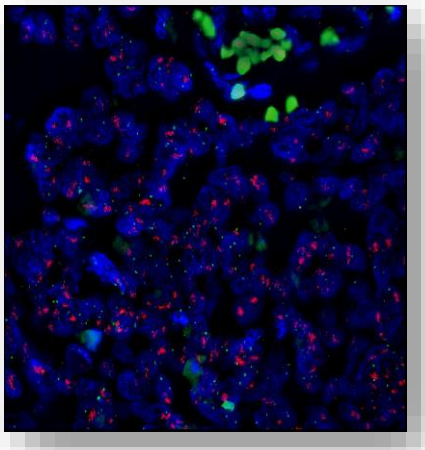


Reapplying drugs from other sites - small populations

UNIVERSITY OF LEEDS

- Challenge
  - Agents positive in other cancers
    - Her-2
    - Stage II Quasar 25/1767 (1.4%) all pMMR
    - Stage IV CRC 29/1340 (2.2%)
      - Ki ras/Braf wild type 24/461 (5.2%)
      - Ki ras/Braf mutant 5/527 (0.95%)
    - 96.4% amplified
- ? Others –fusion genes



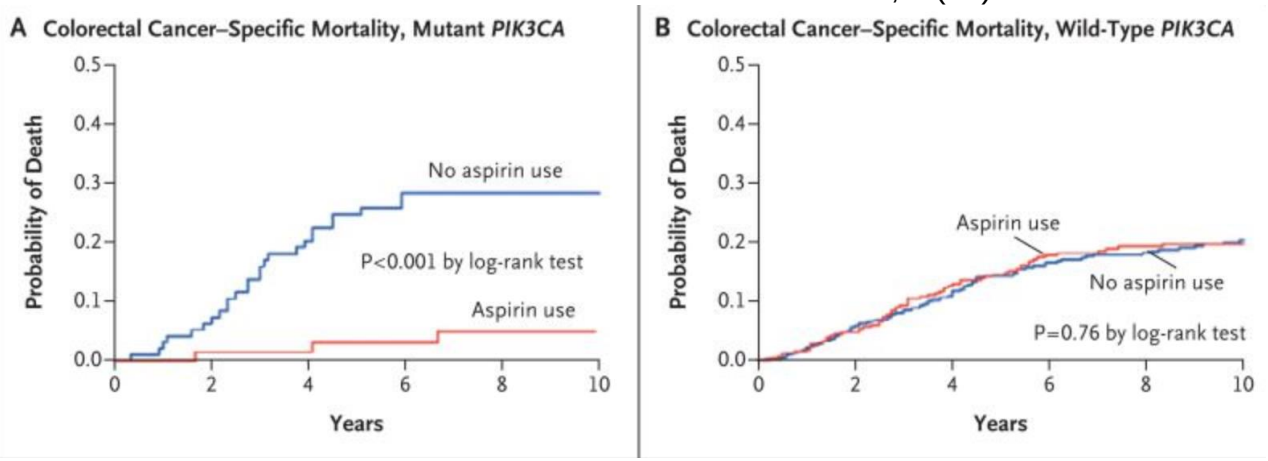


Susan Richman
Katie Southward
Gemma Hemmings

PIK3CA mutations

UNIVERSITY OF LEEDS

- Quasar1
  - 874 cases PIK3CA mutations 13.9 %. 83 exon 9 (9.5%) exon 20 (4.4%).
  - No prognostic effect in stage II
- Aspirin
  - PIK3CA mutations reduced recurrence
    - NEJM HR 0.18 New Engl J Med. 2012 Oct 25;367(17):1596-606.
    - Victor HR 0.11 J Clin Oncol. 2013 Dec 1;31(34):4297-305.



Add Aspirin trials

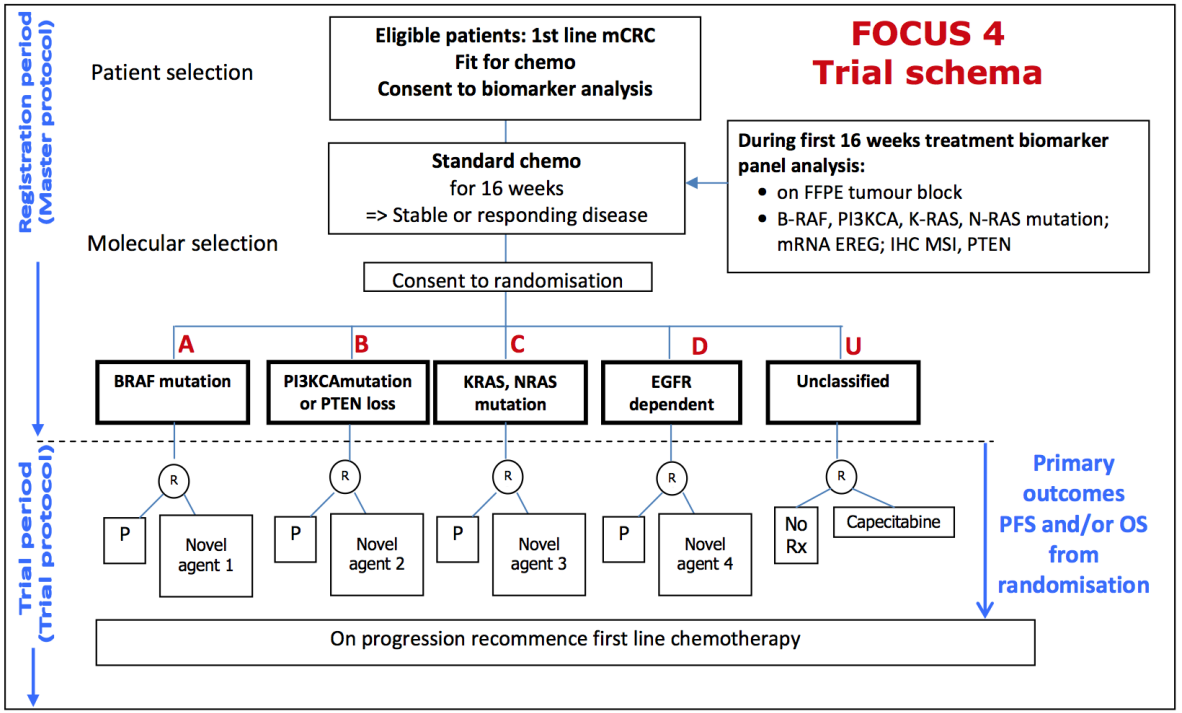
Changing clinical trials – Focus 4

UNIVERSITY OF LEEDS

EME/CRUK Focus 4
n = 3,500

Testing NGS
Kras 12,13, 59, 61,146
Nras 12,13, 61
PIK3CA exon 9 & 20
Braf V600E
p53

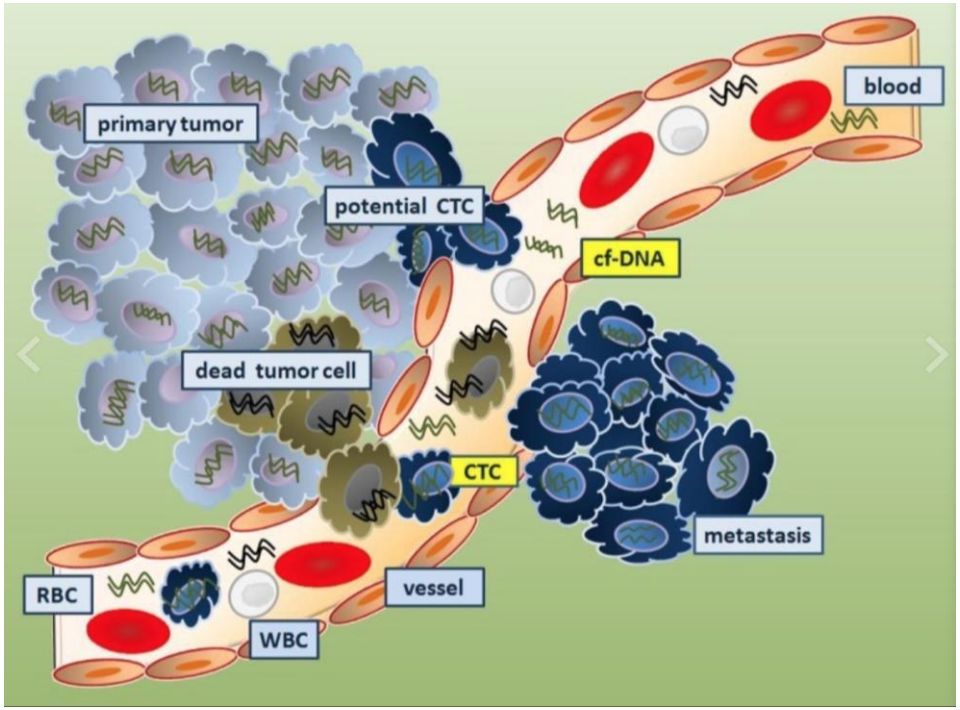
PTEN immuno
dMMR immuno

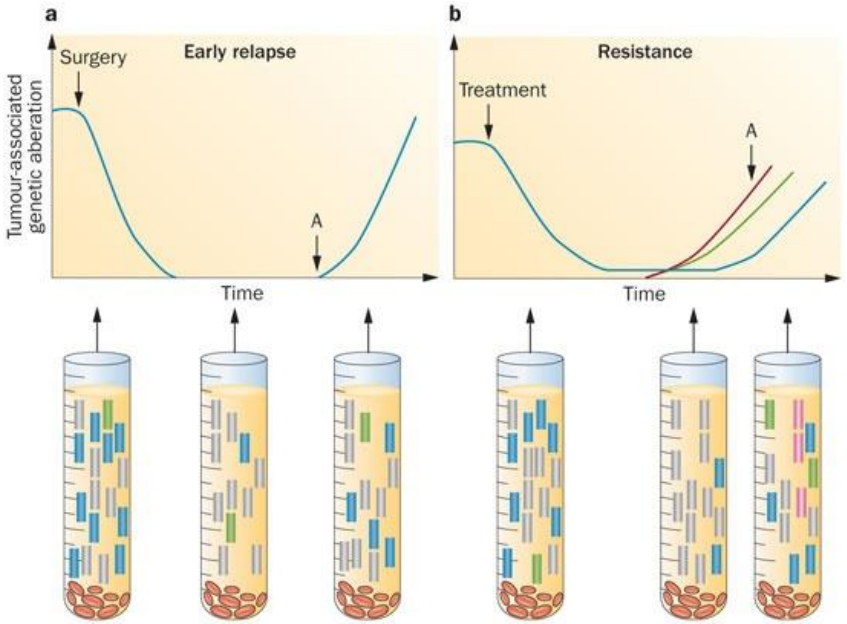


aspirin
oral Her 1,2,3 inhibitor (AZD8931)
WEE-1 inhibitor

Labs
Leeds and Cardiff
Susan Richman
Gemma Hemmings

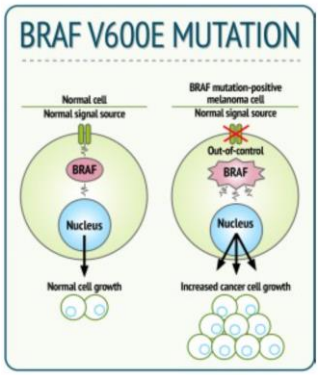
Following disease



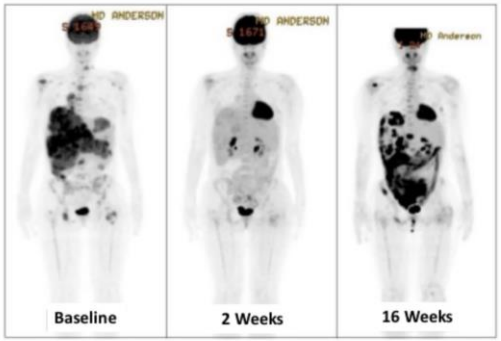


Resistance develops on therapy

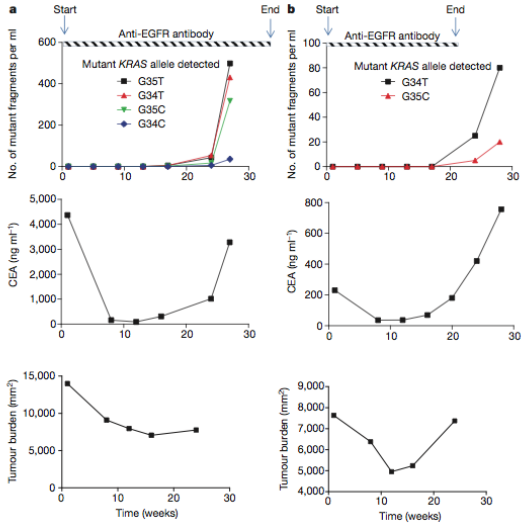
UNIVERSITY OF LEEDS



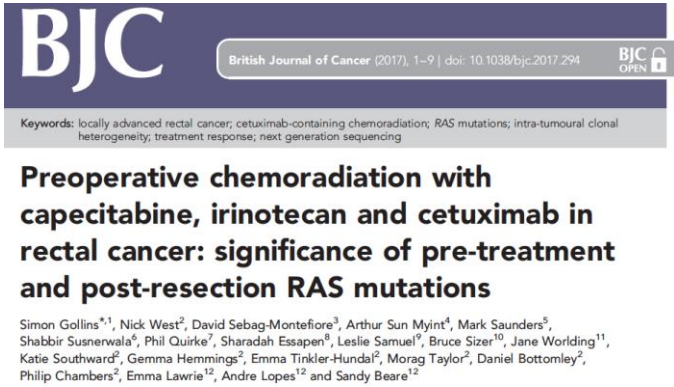
Resistance to BRAF Inhibitors



Average response duration ~6 months



Emerging mutational Resistance Ras in CRC with anti-EGFr Rx



BJC

British Journal of Cancer (2017), 1–9 | doi: 10.1038/bjc.2017.294

BJC OPEN

Keywords: locally advanced rectal cancer; cetuximab-containing chemoradiation; *RAS* mutations; intra-tumoural clonal heterogeneity; treatment response; next generation sequencing

Preoperative chemoradiation with capecitabine, irinotecan and cetuximab in rectal cancer: significance of pre-treatment and post-resection RAS mutations

Simon Gollins[*,1], Nick West[2], David Sebag-Montefiore[3], Arthur Sun Myint[4], Mark Saunders[5], Shabbir Susnerwala[6], Phil Quirke[7], Sharadah Essapen[8], Leslie Samuel[9], Bruce Sizer[10], Jane Worlding[11], Katie Southward[2], Gemma Hemmings[2], Emma Tinkler-Hundal[2], Morag Taylor[2], Daniel Bottomley[2], Philip Chambers[2], Emma Lawrie[12], Andre Lopes[12] and Sandy Beare[12]

Excite phase 2
Rectal cancer
19% gained mutations
35% lost mutations
after treatment

Screening - DNA

- Screening/diagnosis
- Cologuard
- DNA mutations in stool

Imperiale TF, Ransohoff DF, Itzkowitz SH, et al. Multitarget stool DNA testing for colorectal-cancer screening. N Engl J Med. 2014;370(14):1987-97.

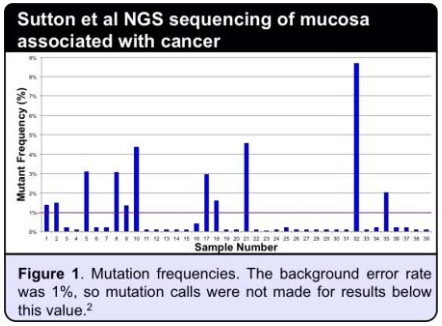


Figure 1. Mutation frequencies. The background error rate was 1%, so mutation calls were not made for results below this value.[2]

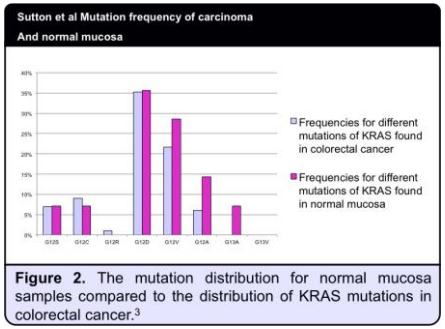


Figure 2. The mutation distribution for normal mucosa samples compared to the distribution of KRAS mutations in colorectal cancer.[3]

Kate Sutton

Summary

UNIVERSITY OF LEEDS

- **Diagnosis of hereditary disease**
- **Routine screening for HNPCC**
- **DNA subtypes**
  - **Hypermutators/dMMR are important 12%**
  - **Worse prognosis RAS mutants and BRAF in stage 4**
  - RNA profiles not yet clinically usable
- **Predictors**
  - **K and NRas mutations present insensitive to an**
    - Anti PD1/PDL1 and dMMR story developing
    - Braf - ?anti EGFr and Braf*i*
    - ? Her-2 and anti-Her2 therapy
    - ? PIK3CA,
- **New trial methods**

Thanks to:

UNIVERSITY OF LEEDS

- Yorkshire Cancer Research
- Quasar1 Richard Gray, Kelly Handley, Gordon Hutchins
- Focus 1-4, Piccolo, Susan Richman, Matt Seymour, Jenny Barrett, Gemma Hemmings
- Tim Palmer, Susan Richman, Morag Taylor
- Trialists, collaborators and colleagues
- MRC, CRUK and Yorkshire Trials Units