Clinical trial design in stratified medicine – an example in colorectal cancer

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What is the problem?

• Every cancer is unique and evolving, so how do we make progress in the clinic?

• Tumour Heterogeneity –
  – Biomarker selection for clinical trials

• Clonal evolution driving inevitable resistance –
  – Combination therapies
  – Earlier intervention

• Validated biomarkers –
  – Intensive characterisation
  – Hypothesis driven

......illustrated from colorectal cancer
Mutation frequencies in human CRC

224 patients: clear separation between hypermutated (16%) and non-hypermutated.

Red: MSI CIMP high or MLH1 silenced, light blue MSI low or CIMP low; black rectum, white colon, grey no data

Pathway alteration in CRC: TCGA

WNT signalling

<table>
<thead>
<tr>
<th>Gene</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>DKK1-A</td>
<td>1.4%</td>
</tr>
<tr>
<td>FZD10</td>
<td>3.3%</td>
</tr>
<tr>
<td>AXIN2</td>
<td>23%</td>
</tr>
<tr>
<td>APC</td>
<td>53%</td>
</tr>
<tr>
<td>CTNNB1</td>
<td>7%</td>
</tr>
<tr>
<td>TCF7</td>
<td>7%</td>
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TGF-β signalling

<table>
<thead>
<tr>
<th>Gene</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGFBR1</td>
<td>17%</td>
</tr>
<tr>
<td>TGFBR2</td>
<td>43%</td>
</tr>
<tr>
<td>ACVR2A</td>
<td>3%</td>
</tr>
<tr>
<td>ACVR1B</td>
<td>20%</td>
</tr>
<tr>
<td>SMAD2</td>
<td>13%</td>
</tr>
<tr>
<td>SMAD3</td>
<td>17%</td>
</tr>
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PI3K signalling

<table>
<thead>
<tr>
<th>Gene</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTEN</td>
<td>20%</td>
</tr>
<tr>
<td>PIK3R1</td>
<td>17%</td>
</tr>
<tr>
<td>PIK3CA</td>
<td>37%</td>
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RTK-RAS signalling

<table>
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<tr>
<th>Gene</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERBB2</td>
<td>13%</td>
</tr>
<tr>
<td>ERBB3</td>
<td>20%</td>
</tr>
<tr>
<td>NRAS</td>
<td>10%</td>
</tr>
<tr>
<td>KRAS</td>
<td>33%</td>
</tr>
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</table>

P53 signalling

<table>
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<th>Gene</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTEN</td>
<td>20%</td>
</tr>
<tr>
<td>PIK3R1</td>
<td>17%</td>
</tr>
<tr>
<td>PIK3CA</td>
<td>37%</td>
</tr>
</tbody>
</table>

Understanding disease biology
Colorectal Cancer Subtyping Consortium > 4000 cases

Integrated analysis by CRCSC of gene expression profiles suggest 4 consensus molecular subtypes in CRC

Overlap between KRAS, NRAS, BRAF, PIK3CA mutant and MMR deficient tumours (n=1947, COIN trial)

- **All wildtype 41.8%**
- **KRAS mutant Samples 823 42.3%**
- **NRAS mutant Samples 69 3.6%**
- **PIK3CA mutant Samples 243 12.8%**
- **BRAF mutant Samples 175 9.0%**

Developing biomarker capability

- Leeds: FOCUS analyses
- Cardiff: COIN analyses
- FOCUS3: collaboration

Smith C, Cheadle J et Clin Cancer Research 2013
A study to determine the feasibility of molecular selection of therapy using KRAS, BRAF and topo-1 in patients with metastatic colorectal cancer

2010 - 11: FOCUS 3

Molecular Type 2

low topo-1 + Either mutation

KRAS or BRAF mutant:
Test addition of bevacizumab

topo-1 low:
Test omit irinotecan

Regimen B:
MdG

Regimen D:
IrMdG + cetuximab

Regimen E:
IrMdG + bevacizumab

Molecular Type 1

low topo-1 + All wildtype

KRAS & BRAF wildtype:
Test addition of cetuximab

Molecular Type 4

high topo-1 + All wildtype

topo-1 high:
Test addition of oxaliplatin

Molecular Type 3

high topo-1 + All wildtype

Molecular Type 3

Regimen A: IrMdG control arm for all randomisations

Molecular Type 1

low topo-1 + All wildtype

KRAS & BRAF wildtype:
Test addition of cetuximab

high topo-1 + All wildtype

topo-1 high:
Test addition of oxaliplatin

Tim Maughan, Mahesh Parmar, Matthew Seymour, Bharat Jasani, Ian Frayling, Julian Sampson, Richard Kaplan, Phil Quirke, Heike Grabsch, Graham Taylor, Geraint Williams, Rachel Butler, Richard Adams, AnnMarie Nelson

NCRI Colorectal Cancer CSG
Patient Information Sheets

**PIS Stage 1:** (to assess markers)
- Tumour block release: REGISTER

**PIS Stage 2:** (pre marker knowledge)
- Basic 3-arm RCT, toxicity, side effects

**PIS Stage 3:** (post marker knowledge)
- Specific treatments pros and cons; Four different PIS 3
- Consent to randomisation: RANDOMISE

**PIS Stage 4:** (post randomisation)
- Full treatment details of specific therapy allocated
- Five different PIS 4

Thank you to Malcolm and Jan Pope.
Patient Understanding

Q1: Understanding of PIS 2
Q2: Understanding why tumour was tested
Q3: Understanding of different treatments
Q4: Understanding of why you had to wait 2 weeks
Q5: Understanding of how treatment was allocated
Q6: Understanding of what happens during treatment
Q7: Understand give blood, questionnaire, or interview
FOCUS 3 Outcomes

- Acceptance very high, despite the complexity of the study design resulting in rapid accrual.

- Primary endpoint:
  - results to the investigator in 10 working days in 90% of patients was not achieved.
  - 74% of results were available to the investigator within 10 working days and 99% in 21 working days.

- Randomisation delays due to clinical issues had a much greater impact on timelines than biomarker analysis.

- **Proof of principle**: we can undertake complex prospective bio-marker driven randomised trials in the UK.

- Provided evidence of feasibility for MRC FOCUS 4 trial.
Clinical trial design in stratified medicine

What have we learnt? Biomarkers

- Colorectal cancer includes some clearly defined molecular subtypes with differing prognosis and pathway activation (COIN)
- Biomarker characterisation is achievable in multicentre trials but takes 1 month (FOCUS 3)
- Two expert labs working together provides a robust way to provide a national biomarker service in clinical trials (FOCUS 3)
Early intervention: using the interval COIN and COIN B trial designs

**Arm A**
- **N=815**
- 5FU or capecitabine
- oxaliplatin
- CONTINUOUS CT until progression, toxicity or patient choice

**Arm B**
- **N=815**
- 5FU or capecitabine
- oxaliplatin
- cetuximab
- CONTINUOUS CT until progression, toxicity or patient choice

**Arm C**
- **N=815**
- 5FU or cap oxaliplatin
- 5FU or cap oxaliplatin
- 5FU or cap oxaliplatin
- INTERMITTENT CT: Treat for 12 weeks then stop and monitor; restart on progression for a further 12 weeks

**Arm C**
- **N=77**
- 5FU oxaliplatin
- cetuximab
- INTERMITTENT CT + cetuximab:

**Arm E**
- **N=92**
- 5FU or cap oxaliplatin
- 5FU or cap oxaliplatin
- 5FU or cap oxaliplatin
- INTERMITTENT CT + Continuous cetuximab:
Intermittent chemo and Overall Survival

<table>
<thead>
<tr>
<th></th>
<th>N pts</th>
<th>N events</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arm A</td>
<td>467</td>
<td>324</td>
</tr>
<tr>
<td>Arm C</td>
<td>511</td>
<td>371</td>
</tr>
<tr>
<td>Total</td>
<td>978</td>
<td>695</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Arm A</th>
<th>Arm C</th>
<th>Diff.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median survival: months</td>
<td>19.6</td>
<td>18.0</td>
<td>-1.54</td>
</tr>
<tr>
<td>...using one-sided 90% CL*</td>
<td>16.3</td>
<td>16.3</td>
<td>-3.23</td>
</tr>
<tr>
<td>2-year survival rates</td>
<td>35.6%</td>
<td>33.3%</td>
<td>-2.2%</td>
</tr>
<tr>
<td>...using one-sided 90% CL*</td>
<td>29.0%</td>
<td>29.0%</td>
<td>-6.6%</td>
</tr>
</tbody>
</table>

HR point estimate = 1.087
80% CI* = (0.986, 1.198)

* Non-inferiority bound is a one-sided 90% confidence limit (CL), equivalent to the upper limit of an 80% confidence interval (CI)
Subgroup analyses in Arm A v C suggests raised platelets at baseline identify group (28%) needing continuous chemotherapy (test for interaction p 0.003)

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>N</th>
<th>HR (95%)</th>
<th>Interaction p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients</td>
<td>978</td>
<td>1.09 (0.94, 1.26)</td>
<td></td>
</tr>
<tr>
<td>Age ≤65y</td>
<td>577</td>
<td>1.15 (0.95, 1.40)</td>
<td>P=0.376</td>
</tr>
<tr>
<td>Age &gt;65y</td>
<td>401</td>
<td>1.00 (0.80, 1.27)</td>
<td></td>
</tr>
<tr>
<td>Liver mets only</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>763</td>
<td>1.00 (0.85, 1.19)</td>
<td>P=0.066</td>
</tr>
<tr>
<td>Yes</td>
<td>215</td>
<td>1.43 (1.03, 1.97)</td>
<td></td>
</tr>
<tr>
<td>Synchronous</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>296</td>
<td>1.23 (0.93, 1.63)</td>
<td>P=0.245</td>
</tr>
<tr>
<td>Yes</td>
<td>676</td>
<td>1.03 (0.87, 1.24)</td>
<td></td>
</tr>
<tr>
<td>WHO PS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>484</td>
<td>1.18 (0.95, 1.46)</td>
<td></td>
</tr>
<tr>
<td>1+</td>
<td>494</td>
<td>1.02 (0.83, 1.26)</td>
<td></td>
</tr>
<tr>
<td>Restart compliance ≤60%</td>
<td>469</td>
<td>1.14 (0.92, 1.41)</td>
<td></td>
</tr>
<tr>
<td>&gt;60%</td>
<td>509</td>
<td>1.05 (0.85, 1.29)</td>
<td></td>
</tr>
<tr>
<td>WB &lt;10,000/l</td>
<td>719</td>
<td>1.06 (0.89, 1.27)</td>
<td>P=0.496</td>
</tr>
<tr>
<td>≥10,000/l</td>
<td>259</td>
<td>1.19 (0.90, 1.58)</td>
<td></td>
</tr>
<tr>
<td>CEA &lt;100g/l</td>
<td>432</td>
<td>0.94 (0.75, 1.19)</td>
<td>P=0.110</td>
</tr>
<tr>
<td>≥100g/l</td>
<td>343</td>
<td>1.25 (0.99, 1.58)</td>
<td></td>
</tr>
<tr>
<td>Alk. phos.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;300 U/l</td>
<td>836</td>
<td>1.08 (0.92, 1.27)</td>
<td>P=0.679</td>
</tr>
<tr>
<td>≥300 U/l</td>
<td>142</td>
<td>1.21 (0.83, 1.76)</td>
<td></td>
</tr>
<tr>
<td>Platelets</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;400,000/µl</td>
<td>703</td>
<td>0.96 (0.80, 1.15)</td>
<td>P=0.003</td>
</tr>
<tr>
<td>≥400,000/µl</td>
<td>271</td>
<td>1.54 (1.17, 2.02)</td>
<td></td>
</tr>
<tr>
<td>KRAS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild-type</td>
<td>481</td>
<td>1.23 (0.99, 1.54)</td>
<td></td>
</tr>
<tr>
<td>Mutant</td>
<td>310</td>
<td>0.90 (0.70, 1.16)</td>
<td></td>
</tr>
<tr>
<td>12-week response</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>653</td>
<td>1.17 (0.97, 1.42)</td>
<td>P=0.171</td>
</tr>
<tr>
<td>N</td>
<td>325</td>
<td>0.96 (0.75, 1.23)</td>
<td></td>
</tr>
</tbody>
</table>
COIN B: testing a targeted therapy in the interval in a biomarker defined cohort

PFS from start of first Chemotherapy-Free Interval

Median PFS (months):
- Arm D: 3.1 (IQR 2.1 to 8.1)
- Arm E: 6.0 (IQR 2.9 to 10.9)

Hazard ratio (Arm E vs Arm D): 0.67 (95% CI 0.46 to 0.98); p=0.039

Wasan H et al, Lancet Oncology 2014
What have we learnt? Using the interval

- 74% of pts with metastatic CRC have no deficit from an interval in chemotherapy (platelets normal, COIN A v C)
- Testing an agent in the interval in a molecularly defined cohort is a viable way of showing efficacy with HR c 0.65 (COIN-B)
- We can negotiate the use of novel agents for use in this setting (cetuximab COIN B, AZ 8931, GSK BRAFi, MEKi)
FOCUS4: an umbrella trial programme

- An integrated trial programme of parallel, molecularly stratified randomised comparisons for patients with advanced or metastatic colorectal cancer who are fit for 1\textsuperscript{st} line chemotherapy

- The trial design exploits a ‘window of opportunity’ to test clinical efficacy of targeted novel agent(s) in an interval after 1\textsuperscript{st} line chemotherapy but \textit{before} resistance to standard agents occurs in prespecified biomarker defined subgroups

- It is derived from a multi arm multi stage (MAMs) design to be cost and time efficient and adaptable to new biomarker and clinical data as the trials proceed
**Principle 1:** Evaluate multiple treatments and biomarkers in the same protocol

**Principle 2:** Investigate new treatments in the earliest and most likely responsive settings that are clinically feasible

- **Diagnostic biopsy**
- **Biomarker analysis**
- **mCRC**
  - First line chemo 16 wks
  - Stable/responding
- **REGISTER**
- **on FFPE tumour block**
  - BRAF, PIK3CA, KRAS, NRAS mutation;
  - mRNA EREG;
  - IHC MMR, PTEN
- **ALLOCATE**
- **RANDOMISE**
- **Primary endpoint**
  - PFS in the interval
- **Restart first line chemo on progression**
  - rebiopsy

- **C**
  - KRAS
  - N
  - Novel oral
  - CAP
- **D**
  - All WT
  - P
  - Novel oral
- **N**
  - NONE
  - N
  - rebiopsy

- **A**
  - P
  - Novel oral
- **B**
  - P
  - Novel oral

- **FOCUS4**
Principle 3: Use randomised evidence with a control group for each biomarker/treatment cohort evaluation

mCRC First line chemo 16 wks Stable/responding

Biomarker analysis

Diagnostic biopsy

on FFPE tumour block BRAF, PIK3CA, KRAS, NRAS mutation; mRNA EREG; IHC MMR, PTEN

REGISTER

ALLOCATE

RANDOMISE

rebiopsy

Primary endpoint
PFS in the interval

Restart first line chemo on progression
Prognostic Effect of KRAS, NRAS and BRAF mutations on overall survival in metastatic CRC

Maughan TS et al, Lancet 2011
FOCUS4 Adaptive Multistage Design

**RANDOMISATION**

**Stage I**
Interim analysis for safety and LSA* (PFS)

**Stage II**
Interim analysis for LSA* (PFS)

**Stage III**
Interim analysis for efficacy (PFS)

**Stage IV**
Interim analysis for efficacy (OS)

Consider testing new hypotheses biomarkers cohorts and agents

Principle 4:
In initial stages, assess each treatment in the presumptive biomarker-enriched subset but without assuming in the design that this association will be confirmed in later stages

Design: Parmar, Royston MRC CTU; tested in Stampede
**Principle 5:** Ensure rapid evaluation of each new treatment, which involves:

a) the flexibility of a phase II and phase III component to each trial; and b) targeting a reasonably large treatment effect, with discontinuation if no activity shown.

<table>
<thead>
<tr>
<th>Molecular cohort</th>
<th>Randomised allocation ratio</th>
<th>Phase</th>
<th>Outcome and stage</th>
<th>Target HR</th>
<th>Max number of events required: total (control arm)</th>
<th>Estimated cumulative analysis time (months)</th>
<th>Max number of pts required</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRAF mutation</td>
<td>2:1</td>
<td>2</td>
<td>PFS - I</td>
<td>0.5</td>
<td>41 (16)</td>
<td>20.4</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>PFS - II</td>
<td>0.5</td>
<td>76 (28)</td>
<td>32.5</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>PFS - III</td>
<td>0.5</td>
<td>118 (42)</td>
<td>46.5</td>
<td>139</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>OS - IV (potential)</td>
<td>0.65</td>
<td>217 (79)</td>
<td>100.4</td>
<td>301</td>
</tr>
<tr>
<td>PIK3CA mutation and/or PTEN loss</td>
<td>2:1</td>
<td>2</td>
<td>PFS - I</td>
<td>0.65</td>
<td>107 (40)</td>
<td>17.0</td>
<td>170</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>PFS - II</td>
<td>0.65</td>
<td>197 (71)</td>
<td>26.5</td>
<td>264</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>PFS - III</td>
<td>0.65</td>
<td>303 (107)</td>
<td>37.2</td>
<td>373</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>OS - IV (potential)</td>
<td>0.7</td>
<td>289 (109)</td>
<td>54.6</td>
<td>546</td>
</tr>
<tr>
<td>KRAS or NRAS mutation</td>
<td>2:1</td>
<td>2</td>
<td>PFS - I</td>
<td>0.65</td>
<td>109 (41)</td>
<td>16.1</td>
<td>177</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>PFS - II</td>
<td>0.65</td>
<td>198 (72)</td>
<td>22.8</td>
<td>273</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>PFS - III</td>
<td>0.65</td>
<td>302 (107)</td>
<td>31.4</td>
<td>378</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>OS - IV (potential)</td>
<td>0.7</td>
<td>287 (109)</td>
<td>50.6</td>
<td>574</td>
</tr>
<tr>
<td>All wild type</td>
<td>2:1</td>
<td>2</td>
<td>PFS - I</td>
<td>0.65</td>
<td>109 (41)</td>
<td>20.0</td>
<td>180</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>PFS - II</td>
<td>0.65</td>
<td>198 (72)</td>
<td>30.6</td>
<td>275</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>PFS - III</td>
<td>0.65</td>
<td>301 (107)</td>
<td>42.3</td>
<td>381</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>OS - IV (potential)</td>
<td>0.7</td>
<td>289 (109)</td>
<td>60.8</td>
<td>547</td>
</tr>
</tbody>
</table>
Overcoming resistance with drug combination

**BRAF p.V600E mutant (FOCUS4-A)**

- 8%
  - Tumours with p.V600E mutation demonstrate sensitivity to BRAF inhibition – but not in CRC.
  - Evidence of increased efficacy and less toxicity with BRAFi-MEKi in p.V600E mutant melanomas.
  - Preclinical CRC models demonstrate EGFR inhibition also required to overcome feedback resistance.
  - Current status:
    - GSK phase 1 trial of BRAFi + panitumumab + MEKi shows combination is safe
    - For inclusion in FOCUS4 A summer 2015
Overcoming resistance with drug combinations
Summary of preliminary activity in studies of BRAFi-based therapy in BRAFmut CRC

<table>
<thead>
<tr>
<th>Regimen</th>
<th>N=</th>
<th>PR/CR</th>
<th>DCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>D or V mono</td>
<td></td>
<td>5%</td>
<td></td>
</tr>
<tr>
<td>D + T</td>
<td>43</td>
<td>12%</td>
<td>63%</td>
</tr>
<tr>
<td>D + P</td>
<td>15</td>
<td>13.3%</td>
<td>86.6%</td>
</tr>
<tr>
<td>V + C</td>
<td>11</td>
<td>-</td>
<td>36.3%</td>
</tr>
<tr>
<td>E + C</td>
<td>24</td>
<td>29.2%</td>
<td>79.2%</td>
</tr>
<tr>
<td>D + T + P</td>
<td>15</td>
<td>40%</td>
<td>80%</td>
</tr>
<tr>
<td>V + C + Ir</td>
<td>8</td>
<td>50%</td>
<td>100%</td>
</tr>
<tr>
<td>E + C + BYL</td>
<td>20</td>
<td>30%</td>
<td>90%</td>
</tr>
</tbody>
</table>

D = dabrafenib, T = trametinib, P = panitumumab, V = vemurafenib, C = cetuximab, E = encorafenib, Ir = irinotecan, BYL = BYL719

Adapted from slide presented by Gary Middleton
Biomarker Cohort Stratification

Cardiff and Leeds laboratories working together
Agreed protocols; mutual QA; tested in FOCUS3

Principle 6: Allow the possibility to refine any biomarkers through the course of the trial

Leeds: Quirke, Richman, Seymour, Chambers
Cardiff: Williams, Jasani, Sampson, Cheadle, Butler, Adams
Biomarker validation between labs

<table>
<thead>
<tr>
<th>Assay</th>
<th>Leeds</th>
<th>Cardiff</th>
</tr>
</thead>
<tbody>
<tr>
<td>KRAS codons 12/13</td>
<td>32/97 (33%)</td>
<td>31/94 (33%)</td>
</tr>
<tr>
<td>KRAS codon 61</td>
<td>3/96 (3.1%)</td>
<td>3/95 (3.2%)</td>
</tr>
<tr>
<td>KRAS codon 146</td>
<td>1/95 (1.1%)</td>
<td>1/87 (1.1%)</td>
</tr>
<tr>
<td>BRAF codon 600</td>
<td>12/96 (12.5%)</td>
<td>12/93 (12.9%)</td>
</tr>
<tr>
<td>NRAS codons 12/13</td>
<td>2/95 (2.1%)</td>
<td>2/90 (2.2%)</td>
</tr>
<tr>
<td>NRAS codon 61</td>
<td>2/95 (2.1%)</td>
<td>2/95 (2.1%)</td>
</tr>
<tr>
<td>PIK3CA exon 9</td>
<td>10/95 (10.5%)</td>
<td>9/94 (9.6%)</td>
</tr>
<tr>
<td>PIK3CA exon 20</td>
<td>1/96 (1.0%)</td>
<td>2/87 (2.3%)</td>
</tr>
</tbody>
</table>

The percentage of mutations found at each mutation hotspot shown for the labs in Leeds and Cardiff. The percentages reflect the number of samples which yielded a mutation in a testable sample.

pTEN protein expression

A; negative, B; grade 1-minimal cytoplasmic staining, C; grade 2-moderate cytoplasmic staining, where staining intensity is less than the adjacent stromal staining and D; grade 3- strong cytoplasmic staining, where staining intensity is equal to the adjacent stromal staining.

(x200 magnification)

Richman et al unpublished
Principle 7:
Allow the possibility to introduce a new biomarker + treatment pairing into the overall trial programme
Translational research opportunities
Intensive characterisation

mCRC
First line chemo 16 wks
Stable/responding

Diagnostic biopsy
Biomarker analysis

REGISTER

Novel oral P
Novel oral P
Novel oral P
Novel oral P
CAP

BRAF, PIK3CA, KRAS, NRAS mutation; mRNA EREG; IHC MMR, PTEN mutation

Diagnostic biopsy

ALLOCATE

RANDOMISE

S-CORT MRC stratified medicine consortium

Primary endpoint
PFS in the interval

Restart first line chemo on progression

Germline DNA GEL pilot
Circulating free DNA Rosenfeld Inivata

BRAF PIK3CA KRAS ALL WT NONE

rebiopsy

rebiopsy
Circulating tumour DNA integrates heterogeneity

Cancer evolution can be monitored noninvasively via plasma DNA

Murtaza, ..Rosenfeld, 2013
Seven Key principles underpinning FOCUS 4 trial design

1. Evaluate multiple treatments and biomarkers in the same protocol
2. Investigate new treatments in the earliest and most likely responsive settings that are clinically feasible
3. Use randomised evidence with a control group for each biomarker/treatment cohort evaluation
4. In initial stages, assess each treatment in the presumptive biomarker-enriched subset but without assuming in the design that this association will be confirmed in later stages
5. Ensure rapid evaluation of each new treatment, which involves:
   a) the flexibility of a phase II and phase III component to each trial; and
   b) targeting a reasonably large treatment effect, with discontinuation if no activity shown
6. Allow the possibility to refine any biomarkers through the course of the trial
7. Allow the possibility to introduce a new biomarker + treatment pairing into the overall trial programme

Working together in biomarker driven clinical trials: colorectal cancer

Clinical Investigators
Tim Maughan & Richard Wilson (Chairs)
Gary Middleton (A), Harpreet Wasan (B), Richard Wilson (C),
Richard Adams (D), Tim Maughan (N), Will Steward (safety), Les Samuel (Scotland)

Patient reps:
Malcolm & Jan Pope

Biomarker Specialists
Cardiff: Bharat Jasani, Rachel Butler
Leeds: Phil Quirke, Susan Richman

MRC CTU
MRC Programme Leads: Rick Kaplan, Max Parmar

Miscellaneous
Research Networks
NCRI Colorectal Clinical Studies Group
ECMC network for experimental treatments
National Clinical Research Networks

Funders
Cancer Research UK (CTAAC)
NIHR Efficacy and Mechanisms Evaluation Programme
CR-UK Science committee (translational work tba)

Pharma
Astrazeneca
Glaxo Smith Klein
Others

HEIs
Oxford, Cardiff, Leeds, Belfast,
Birmingham, Leicester, Imperial, UCL
Translational science collaboration (tbc)
Oxford (Maughan, Tomlinson),
Cambridge (MacDermott, Rosenfeld),
Cardiff (Adams, Clarke),
Belfast (Wilson, Johnston),
Glasgow (Sampson),
Leuven (Tejpar)
human gene names
proteins in complexes indicated with "•"
Phosphatidylinositol-3, 4, 5-trisphosphate