Molecular subgroups from the AGITG MAX trial; Right (R) or Left (L) primary site of colorectal cancer and outcomes for metastatic colorectal cancer (mCRC).

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Background

- The Phase III MAX trial (capecitabine (C) v C/bevacizumab (CB) v CB/mitomycin C (CBM)) confirmed improved PFS with the addition of B to C
- Previous reports have described differences in biology and outcome based on whether the primary is right or left sided.
- Possible differences in response to biological agents have also been reported based on side of primary lesion.[1,2]
- We have previously published molecular markers from the MAX trial;
  - Extended RAS
  - BRAF
  - PTEN
  - PIK3CA
  - VEGF, IL-6, IL-8, BFGF and PDGFBB
- Here we have assessed the panel of markers based on right or left primary site to assess if primary site impacts on outcome with bevacizumab when combined with capecitabine.

Methods

- DNA was macrodissected from archival formalin fixed paraffin embedded tumour tissue.
- Mutation status for extended RAS was determined using pyrosequencing and confirmed with Sanger sequencing.
- BRAF mutations were determined by high resolution melt analysis, and confirmed by Sanger sequencing.
- Pro-angiogenic markers were assessed by Bioplex® (Bio-rad). Protein was extracted in 2% w/v lysis buffer (2% SDS, 200 mM DTT, 20 mM Tris-HCL, pH 8.8), at 100ºC for 20 min, then at 80C for 2 hr with agitation. Protein was solubilised in Bioplex® lysis buffer via 2D Cleanup kit (Bio-rad) (Bruhn et al. 2014).
- PTEN status was assessed by Taqman copy number PCR.
- Biological/molecular marker status was correlated with efficacy outcomes (RR, PFS & OS).
- Predictive analyses were undertaken using a test for interaction involving both C vs CB + CBM.

Patients and Results

- 440 patients had primary site documented and were analysed for baseline characteristics.
- 298 patients had molecular results.
- 28% had right sided primary.
- Major differences between right versus left respectively are as follows;
  - female 49% v 33% (p<0.01),
  - prior history of diabetes 13% v 27% (p=0.02),
  - lung involvement 28% v 44% (p<0.01),
  - BRAF MT 16% v 3.5% (p=<0.001),
  - PTEN loss 27.6% v 53% (p=0.01).
  - Survival was higher for left sided primary (Fig 1)
  - Multivariate analysis confirmed side of primary as an independent prognostic factor (table 1)
  - All RAS mutation was not statistically higher in R primary, 45% v 37% (p=0.27).
  - There was no difference in rate of PIK3CA mutation,
  - No difference in high v low expression of assessed angiogenic markers (VEGF, IL-6, IL-8, BFGF, PDGFBB).
  - When comparing right v left, right sided primary predicted for poor outcome for OS: median right 13.2 v left 20 months, p=0.001 (HR 0.67, 95% CI 0.53-0.85), but not for PFS (HR 0.96, 95% CI 0.78-1.20).
  - There was no signal that bevacizumab effect differed between right and left primary, PFS for right primary HR 0.82 (95% CI 0.54-1.22) and left primary HR 0.51 (95% CI 0.4-0.63), test for interaction p=0.10, (Figure 2)

References
[1]SY Brule et al, J Clin Oncol 31, 2013 (supp #3528)
[2]MK Boisen et al, Ann Oncol 24, 2554-59

Fig 1: Overall survival Left v Right primary site

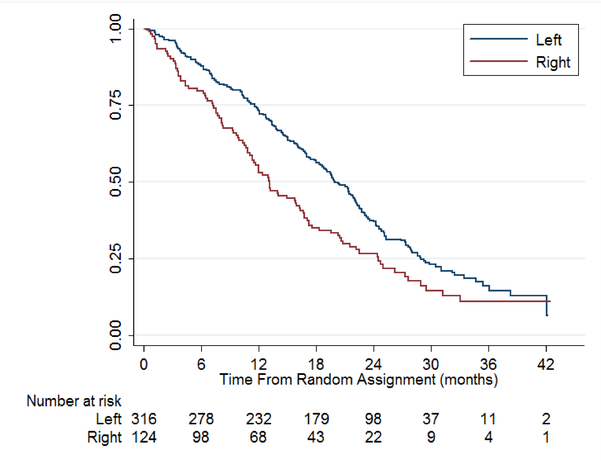
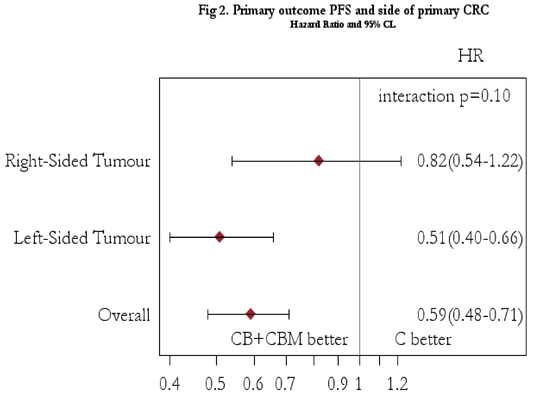


Table 1: Multivariate analysis for overall survival

Variable	HR	95% CI		P-value
C	1.00			
CB	0.87	0.67	1.14	0.319
CBM	0.89	0.68	1.16	0.389
ECOG≥1	1.98	1.59	2.47	<0.001
Neutrophils ≥8x109 /L	1.56	1.14	2.13	0.005
Alkaline phosphatase ≥140 U/L	1.70	1.35	2.14	<0.001
Prior radiotherapy	1.63	1.17	2.25	0.003
Primary tumour resected	0.67	0.52	0.87	0.003
Left sided tumour	0.60	0.47	0.77	<0.001

Fig 2. Primary outcome PFS and side of primary CRC



Conclusions

- There are more negative prognostic factors in patients with right sided primary, in particular high BRAF MT, and patients with right sided primary have inferior overall survival when compared to those with a left sided primary.
- We did not demonstrate any additional significant molecular difference between left and right colon in this patient group.
- There was no suggestion that site of primary had any impact on bevacizumab effect on PFS.

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