# Transcriptional profiling and consensus molecular subtype (CMS) assignment to understand response and resistance to anti-EGFR therapy in colorectal cancer


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## Background

- Activating mutations in the extended *RAS* (*KRAS, NRAS*, and *BRAF)* genes confer resistance to anti-EGFR Ab (e.g., Cetuximab) therapy of colorectal cancer (CRC) patients[1]
- Left sided $RAS^{WT}$ tumors are incorporated in NCCN guideline as predictor of anti-EGFR Ab therapy[2]
- However, only 40% - 45% $RAS^{WT}$ patients have been found to respond to the therapy[3]
- Identification of novel biomarkers is required for better stratification of the metastatic CRC patients for anti-EGFR Ab therapy

## Objective

- Identification of transcriptomic determinants for predicting and understanding the primary resistance mechanisms to anti-EGFR antibody therapy of colorectal cancer patients

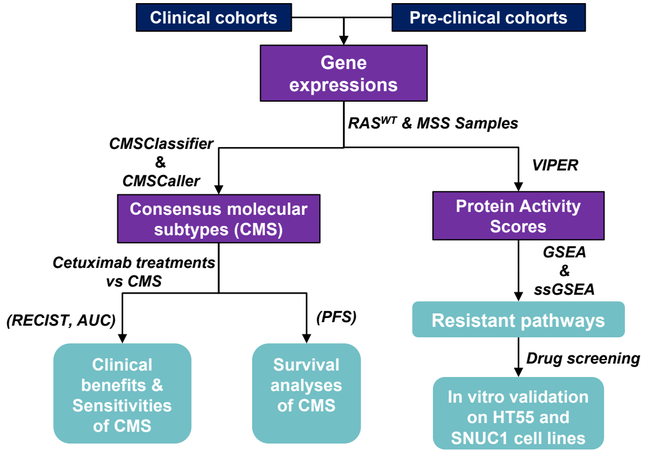
## Materials and Methods

- Gene expression datasets of two retrospective clinical cohorts and two pre-clinical cohorts were downloaded from Gene Expressions Omnibus

**Transcriptomic datasets used in this study**

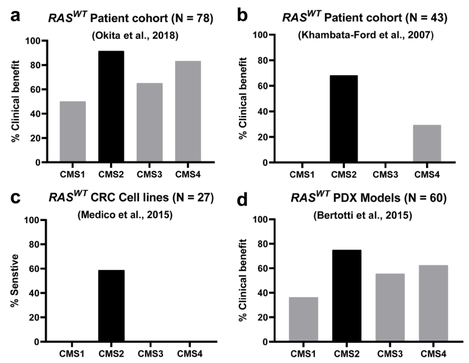
| Datasets | Total Samples | Sample types | Treatment | References |
|---|---|---|---|---|
| **Cell lines** | | | | |
| GSE59857 | 146 | CRC Cell lines | Cetuximab | *Medico et al., Nat Comm, 2015* |
| **PDX Models** | | | | |
| GSE76402 | 216 | PDX Models | Cetuximab | *Bertotti et al., Nature, 2015* |
| **Patient Cohorts** | | | | |
| GSE104645 | 135 | pCRC tissues | FOLFOX/ FOLFIRI & Cetuximab | *Okita et al., Oncotarget,2018* |
| GSE5851 | 68 | pCRC tissues | Cetuximab | *Khambata-Ford et al., J Clin Oncol, 2007* |

- **Workflow:**

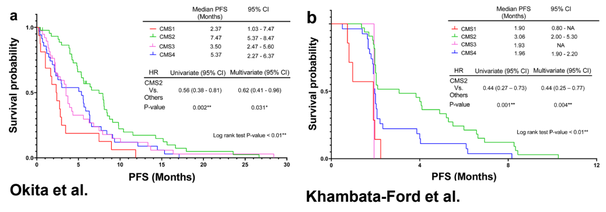



## Results

- Percentages of clinically benefited CMS2 $RAS^{WT}$ patients (92% and 68%), PDX models (84%), and sensitive CRC cell lines (60%) were highest in both clinical and preclinical cohorts




- Median PFS (7.47 months and 3.06 months) of CMS2 $RAS^{WT}$ patients in two clinical cohorts were higher than the other CMS subclasses



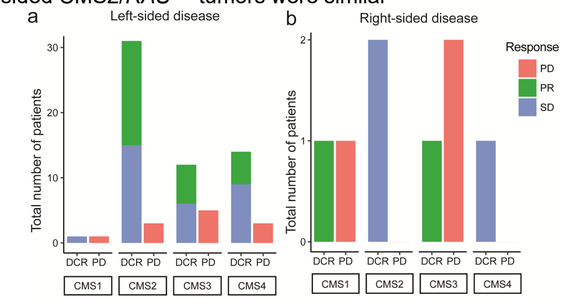

- *RAS* gene mutation and CMS of CRC patients were significantly associated with PFS (p-value < 0.05)
- CMS1 and CMS2 patients showed worst and best prognosis to anti-EGFR treatment, respectively

**Cox proportional hazard analysis of PFS of anti-EGFR treatment in clinical cohorts**

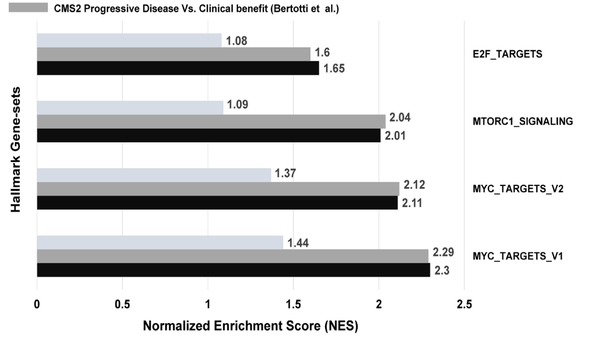
| | Okita et al. cohort | | | | Khambata-Ford et al. cohort | | | |
|---|---|---|---|---|---|---|---|---|
| Variables | Univariate Study | | Multivariate Study | | Univariate Study | | Multivariate Study | |
| | HR | P-value | HR | P-value | HR | P-value | HR | P-value |
| Age | 0.99 | ns | 0.99 | 0.63 | 0.99 | 0.94 | 1.00 | 0.78 |
| Sex | | | | | | | | |
| Female vs Male | 1.14 | n | 0.94 | 0.79 | 1.85 | 0.02* | 1.62 | 0.11 |
| RAS gene mutation | | | | | | | | |
| $RAS^{wt}$ vs $RAS^{mut}$ | **0.34** | **9.44E-07*** | **0.31** | **8.43E-07*** | **0.49** | **1.00E-02*** | **0.61** | **1.40E-01** |
| Tumor sides | | | | | | | | |
| Left vs right | 0.68 | 0.11 | 0.89 | 0.66 | NA | NA | NA | NA |
| CMS | | | | | | | | |
| CMS1 vs CMS2/3/4 | **2.19** | **0.0038*** | 1.44 | 0.33 | **3.47** | **0.002*** | **7.10** | **0.0009*** |
| CMS2 vs CMS1/3/4 | **0.55** | **0.002*** | 0.62 | 0.03 | **0.44** | **0.001*** | **0.44** | **0.003*** |
| CMS3 vs CMS1/2/4 | 1.2 | 0.357 | 1.00 | 0.99 | NA | NA | NA | NA |
| CMS4 vs CMS1/2/3 | 1.22 | 0.32 | 1.57 | 0.05 | 1.6 | 0.06 | 1.59 | 0.09 |

- Age, sex, and primary tumor sidedness (right vs. left colon) were not significantly associated (p-value > 0.05) with progression-free survival (PFS) of cetuximab treatment

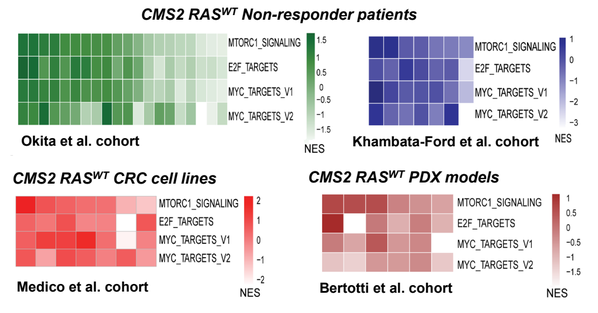
- Response to anti-EGFR antibody therapy was higher for left-sided (DCR: ~83% (58/70) vs 62.5% (5/8) for right-sided) tumors
- However, after controlling for CMS, DCR for right and left sided CMS2/$RAS^{WT}$ tumors were similar




- MTORC1, E2F, and MYC pathway gene-sets were found significantly enriched (FDR < 0.3) in the cetuximab refractory CMS2 $RAS^{WT}$ samples of both clinical and preclinical cohorts




- Single sample GSEA revealed that MTORC1, E2F, and MYC pathways were heterogeneously active (NES > 1) in the cetuximab refractory $RAS^{WT}$ samples



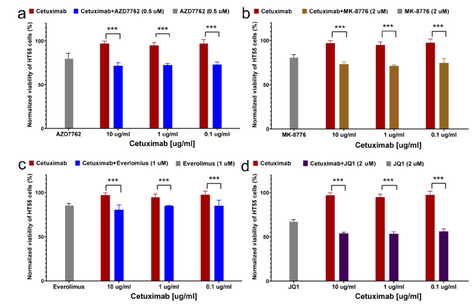

- HT55 and SNUC1 cell lines were resistant to cetuximab (IC50 > 10µg/ml)
- Single agent inhibitors of E2F (AZD7762 and MK-8776), mTOR (Everolimus), and MYC (JQ1) pathways could not inhibit HT55 and SNUC1 cell growth (IC50 > 10µM)

**Pharmacological parameters of cell viability assays of HT55 and SNUC1 lines**

| Cell viability assays | HT55 | | SNUC1 | |
|---|---|---|---|---|
| Single-drug assay | IC50 | AUC | IC50 | AUC |
| Cetuximab (0.01*/0.1$ µg/ml – 100*/50$ µg/ml) | > 10 µg/ml | 383 | > 10 µg/ml | 251 |
| AZD7762 (0.8 µM – 10 µM) | **4.9 µM** | **159** | > 10 µM | 180 |
| MK-8776 (1.25 µM – 20 µM) | > 10 µM | 108 | > 10 µM | 111 |
| JQ1 (0.25 µM – 10 µM) | > 10 µM | 123 | > 10 µM | 136 |
| Everolimus (1 µM – 15 µM) | > 10 µM | 97.1 | > 10 µM | 90.7 |

*HT55 cell line assay; $ SNUC1 cell line assay

- The addition of AZD7762, MK-8776, Everolimus, and JQ1 significantly decreased HT55 cell viability relative to cetuximab alone




- However, combinatorial effect of these drugs with cetuximab was additive, rather than synergistic

## Conclusions

- **CMS may be used as biomarker for classifying $RAS^{WT}$ & MSS CRC patients for anti-EGFR antibody therapy**
- **Both left and right-sided $RAS^{WT}$/MSS/CMS2 tumors may be benefited from cetuximab therapy**
- **RAS-MAPK independent signaling pathways may regulate primary resistance to anti-EGFR antibodies**

## References


1. Therkildsen C, et al., *Acta Oncol,* 2014;53(7):852-64
2. National Comprehensive Cancer Network. Colon Cancer (Version 1.2021)
3. Vidal J, et al., *Ann Oncol,* 2019;30(3):439-46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