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## Repurposing EGFR inhibitor utility in colorectal cancer in mutant *APC* and *TP53* subpopulations

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### Abstract

**Background**—EGFR is a major therapeutic target for colorectal cancer (CRC). Currently, extended *RAS/RAF* testing identifies only non-responders to EGFR inhibitors (EGFRi). We aimed to develop a mutation signature that further refines drug-sensitive subpopulations to improve EGFRi outcomes.

**Methods**—A pre-specified, 203-gene expression signature score measuring cetuximab sensitivity (CTX-S) was validated with two independent clinical trial datasets of cetuximab-treated CRC patients (n=44 and n=80) as well as an *in vitro* dataset of 147 cell lines. The CTX-S score was then used to decipher mutated genes that predict EGFRi sensitivity. The predictive value of the identified mutation signature was further validated by additional independent datasets.

**Results**—Here we report the discovery of a 2-gene (*APC+TP53*) mutation signature that was useful in identifying EGFRi-sensitive CRC subpopulations. Mutant *APC+TP53* tumors were more predominant in left- vs right-sided CRCs (52% vs. 21%,  $p=0.0004$ ), in MSS vs MSI cases (47% vs. 2%,  $p<0.0001$ ), and in the consensus molecular subtype 2 vs. others (75% vs. 37%,  $p<0.0001$ ). Moreover, mutant *APC+TP53* tumors had favorable outcomes in two cetuximab-treated PDX datasets ( $p=0.0277$ , n=52;  $p=0.0008$ , n=98).

**Conclusion**—Our findings suggest that the *APC* and *TP53* combination mutation may account for the laterality of EGFRi sensitivity and provide a rationale for refining treated populations. The results also suggest addition of *APC+TP53* sequencing to extended *RAS/RAF* testing that may directly increase the response rates of EGFRi therapy in selected patients.

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**Impact**—These findings, if further validate through clinical trials, could also expand the utility of EGFRi therapies that are currently underutilized.

### Keywords

colorectal cancer; *APC*; *TP53*; mutations; cetuximab

## INTRODUCTION

Two well-characterized EGFR inhibitors (EGFRi) (cetuximab, panitumumab) are FDA approved as first and second line targeted-therapies for metastatic colorectal cancer (CRC) (1–8). Despite approval, utilization has been modest, primarily because of drug restriction to the wild-type *RAS* subpopulation. Early CRC clinical trial studies involving cetuximab/panitumumab, either as monotherapies or as combination therapies, reported that a statistically significant drug response was generally observed in WT *KRAS* patients—but not in MUT *KRAS* patients(5, 8). On the other hand—despite selection—about half of patients with a WT *KRAS* still fail to respond to EGFRi treatments(9, 10), suggesting that additional genes, beyond *KRAS*, may negatively contribute to EGFRi response. Recently, mutations in *NRAS* and *BRAF* were reported to account for EGFRi therapy resistance in some WT *KRAS* CRCs(1–3). More recently, left-sided CRCs have been reported to be more favorably associated with response to cetuximab/panitumumab than right-sided tumors, as indicated by increased response rate (RR), better progression free survival (PFS) and/or overall survival (OS) (6, 11–13). A molecular basis of the laterality of anti-EGFR sensitivity, however, is still poorly understood.

We recently developed a new, robust molecular classification of CRC to help dissect this heterogeneous disease into 5 molecular subpopulations in order to improve treatment strategies(14, 15). This classification complements the recently reported consensus molecular subtypes (CMS) of colorectal cancer that were coalesced from six independent (gene expression) CRC classification systems(16). We performed an integrated analysis targeted gene sequencing for 1321 cancer-related genes, global gene expression, and MSI analyses across a large cohort of human CRC (n = 468). Among a number of mutated genes identified, striking pairwise, statistically significant, correlations were observed between *APC*, *TP53*, *KRAS* and *BRAF* that ultimately suggested a *prognostic* role for *APC*(15). Based on these results, we hypothesized there might also be a *predictive* role for *APC* and other associated genes.

Given the paucity of available clinical trial tissue samples with EGFRi exposure, we elected to use a cetuximab sensitivity (CTX-S) gene expression score as a *surrogate* for cetuximab response data in our CRC cohort, TCGA and other published data. This approach allowed us to develop a 2-gene “mutation signature” that is strongly-correlated with the CTX-S score and can be rapidly translated to the clinic.

## MATERIALS AND METHODS

### Datasets of Patient Samples, Cell Lines, and PDX Models

We previously analyzed 468 stages I-IV colorectal tumors, with global gene expression data from the surgical specimen, MSI status, and targeted gene sequencing of 1321 cancer-related genes(14, 15). A cohort of the 468 colorectal adenocarcinoma patients (including 367 *primary* lesions from stage 1–4 patients and 101 *metastatic* lesions) was accrued between October 2006 and September 2010, and written informed consent was obtained from participating patients as part of the Total Cancer Care® (TCC) project (17). The study was conducted in accordance with recognized ethical guidelines (Declaration of Helsinki, CIOMS, Belmont Report, U.S. Common Rule) and under the approval of the University of South Florida institutional review board (17). Primary and metastatic samples were both included based on our previous work demonstrating a high degree of mutation overlap between matched primary/metastatic samples(18). Here we further used this large, well-curated clinico-genomics/expression database of CRC patient samples to carry out mutation ranking analysis by the CTX-S score and other statistical analyses. We identified *seven* additional independent datasets, from Merck and public resources including Gene Expression Omnibus (GEO) and NCI Genomic Data Commons (GDC), for various validation and correlation analyses. These included WT *KRAS* CRC samples (n=44) selected from the control arm (cetuximab + irinotecan) of a Merck prospective clinical trial (MK0646)(19), a BMS trial cetuximab-treated CRC patient samples (n=80, Khambata-Ford et al.(4)), *in vitro* cetuximab-treated CRC cell lines (n=147, Medico et al.(20)), TCGA CRC patient samples (n=624 including 221 DNA-sequenced samples from TCGA(21)), and an additional set of Stages I-IV CRC patients samples (n=566, Marisa et al.(22)), as well as cetuximab-treated CRC PDX models (n=52, Julien et al.(23) and n=98, Bertotti et al.(24)). A summary of all eight datasets is given in Table 1, and detailed data description is given in Supplementary Methods and Tables S1–8.

### Expression Signatures (see Supplementary Table S9 for gene lists)

**1. The CTX-S score:** A pre-specified gene expression signature score that measures cetuximab sensitivity was initially constructed based on gene expression values from >800 cancer associated genes, each assessed in a set of 44 WT *KRAS* colon tumor samples from patients treated with cetuximab *monotherapy*. 203 genes with *p*-value <0.05 by PFS Cox Regression analysis were identified that included 94 UP and 109 DOWN expressed genes based on their association with response or resistance to cetuximab, respectively. Cetuximab sensitivity score (CTX-S) was defined as the average expression of the genes in the UP arm minus the average expression of genes in the DOWN arm. CTX-S was pre-specified for subsequent analysis of the validation sets. The signature score derivation and validation is summarized in Supplementary Fig. S1 and overall methodology used is similar to as we reported in previous studies(14, 25, 26). **2. The 18-gene RAS pathway score.** This score was developed to measure the MEK functional output in association with RAS pathway activation(27). **3. The 64-gene Wnt pathway score.** We previously adopted a set of 64 “consensus”  $\beta$ -catenin (upregulated) genes from a recent study of Herbst et al.(28) to assess differential activation of Wnt pathway in *APC* subgroups of Moffitt CRCs(15). The 64-gene Wnt pathway scores were calculated from the arithmetic mean expression of the 64 genes. **4.**

**The 24-gene APC mutation-specific Wnt pathway score.** We further selected 24 genes (out of the 64 genes) whose expression was significantly higher in *APC*-mutated tumors than those with wild-type *APC* ( $p < 0.05$  for two-tailed Welch *t* test) (see Supplementary Fig. S2A). The arithmetic mean expression of selected 24 genes is designated as the *APC* mutation-specific Wnt pathway score, which was validated by TCGA CRCs ( $n = 221$ ) (Supplementary Fig. S2B).

### Statistical Methods

The statistical approaches used include (1) Survival Analysis, Correlation Analysis, and the *t* Test; (2) Mutation Ranking Analysis of Moffitt 468 CRCs; (3) Cochran-Mantel-Haenszel (CMH) Test, Barnard Test, and distribution analysis; (4) CMS classification. See Supplementary Methods for detailed description.

The statistical tests used in the article were given unadjusted *p* values for multiple testing, with an  $\alpha = 0.05$  chosen as the significance level, except for the mutation ranking analyses which use the Benjamini and Hochberg false discovery rate method(29). In addition, for the Welch *t* test in comparison among 7 or 5 MSI/MSS subgroups, those unadjusted *p* values remaining significant after adjustments for multi-comparisons by Holm-Bonferroni method(30) were highlighted by a maroon color. All tests were two-sided unless noted otherwise.

## RESULTS

### Development and validation of the CTX-S score

The CTX-S signature score was validated using *two* independent *test* sets of cetuximab-treated patients and a test set of cetuximab-treated CRC cell lines, as summarized in Supplementary Fig. S1. A detailed description of the results is given below:

**Validation Set 1 (*in vivo*)**—The CTX-S score was first validated using 44 CRC control arm samples and data from a Merck prospective clinical trial (MK0646), a randomized phase II/III study of dalotuzumab (IGF-1R inhibitor) in combination with cetuximab and irinotecan in chemo-refractory, *KRAS* wild-type, metastatic CRC(19). In the control arm “C” of MK0646, high IGF-1 expression was shown to be significantly associated with lower response rates to cetuximab + irinotecan and IGF-1 was considered to be a promising biomarker for differential response to anti-IGF1R therapies as well as anti-EGFR therapies(19). Here, we used the MK0646 control arm samples to further test whether the CTX-S signature score could predict positive outcomes to cetuximab + irinotecan therapy. Barnard’s exact test revealed that the CTX-S score was significantly associated with objective responses (OR) (vs. no objective response,  $p = 0.0048$ ; see Fig. 1A and Supplementary Table S10A). Moreover, Kaplan-Meier (KM) survival analysis showed that the CTX-S score was significantly associated with longer progression-free survival (PFS) (208 vs. 83 days, HR = 0.34, 95% CI: 0.07–0.51,  $p = 0.0018$ ) (Fig. 1B) and longer overall survival (OS) (503 vs. 287 days, HR = 0.30, 95% CI: 0.05–0.58,  $p = 0.0052$ ) (Supplementary Fig. S3).

**Validation Set 2 (*in vivo*)**—The CTX-S score was subsequently validated using a second independent, well-characterized, dataset of 80 metastatic CRC patients prospectively treated with cetuximab monotherapy from a BMS clinical trial(4). This dataset was initially used to identify *EREG* and *AREG* as predictive markers whose high expression was significantly associated with longer PFS in cetuximab-treated patients(4), and later used in a variety of other gene expression classification and validation analyses associated with EGFRi treatment prediction(31–33). Notably, *EREG* is a member of the CTX-S score “UP genes”, whereas *AREG* is not included in the gene list of the CTX-S (see Supplementary Table S9). Five of six CR (complete response) + PR (partial response) samples and a majority of SD (stable disease) samples (13/19) had higher CTX-S scores (above the median) (see Supplementary Fig. S4), supporting the CTX-S score as a reasonable measure of cetuximab disease control response (DCR). This notion was supported by Cochran-Mantel-Haenszel (CMH) testing on cetuximab response vs. CTX-S quartile scores (q4 (highest), q3, q2 and q1 (lowest)) (Supplementary Table S10B,C). Moreover, KM analysis found that higher scores were significantly associated with better PFS in patients, *regardless* of *KRAS* mutation status (logrank trend test,  $p=0.0026$ ,  $n=80$ ) (Fig. 1C) as well as in patients with only WT *KRAS* ( $p=0.0320$ ,  $n=43$ ) (Fig. 1D).

**Validation Set 3 (*in vitro*)**—Recently, 147 CRC cell lines with heterogeneous genetic backgrounds were analyzed by Medico et al.(20) for *KRAS*, *NRAS*, and *BRAF* genotypes, MSI (MSI-high) status, as well as global gene expression and *in vitro* cetuximab sensitivity. Our further analysis of these cell lines showed that the CTX-S score was significantly correlated with *in vitro* cetuximab sensitivity (Fig. 1E), supporting the validity of the CTX-S score.

### The CTX-S score was not prognostic

We have previously developed various gene expression signatures that were prognostic for CRC outcomes(14, 26). To assess prognostic potential, a KM analysis of the CTX-S score by quartiles was performed similarly as we did for the PC1.EMT score in the same set of 468 CRCs(14). Results showed that unlike PC1.EMT that predicted poor OS(14), the CTX-S score was *not* prognostic, as shown in either all patients (logrank trend test,  $p=0.969$ ,  $n=468$ ), or in WT RAS patients ( $p=0.273$ ,  $n=264$ ) (Supplementary Fig. S5A,B). This result was confirmed using a second large, independent, set of stage I-IV CRCs ( $n=557$ ) reported by Marisa et al. (2013) for a classification analysis of CRC in association with prognosis (relapse-free survival, RFS)(22) (Supplementary Fig. S6A,B).

### Correlation of the CTX-S score with EREG/AREG, RAS and Wnt signatures

Spearman correlation analysis showed that while the CTX-S score was positively correlated with *EREG* or *AREG* expression in all four patient tumor datasets ( $p<0.0001$ ), it was negatively correlated with 18-gene RAS signature score in the three largest datasets ( $p<0.0001$ ) (Table 2A). Notably, an 18-gene RAS pathway gene expression signature score was previously developed to measure RAS/RAF/MEK pathway activation (27). We recently adapted this signature from use in fresh frozen CRC samples to more clinically-available, FFPE tissues(34) as a means to identify cetuximab non-responders.

The Wnt pathway is another major dysregulated signaling pathway in CRC, ~70% of which have one or two *APC* truncating mutations(15, 21, 35). We found a strong correlation with an *APC mutation-dependent* 24-gene Wnt pathway score across all five datasets tested. In addition, we found that the *APC mutation-dependent* score was significantly correlated with the *in vitro* cetuximab inhibitory effect in 147 cell lines (Supplementary Fig. S7).

These data led us to further investigate a potential association of the CTX-S score with *APC* mutations using Moffitt CRCs. We found that CTX-S scores were significantly higher ( $p<0.0001$  for two-tailed Welch *t* test) in mutant *APC* (n=312, 67%) than wild-type *APC* tumors (n=156, 33%) (Supplementary Fig. S8A). Similar results were obtained when tumors were further divided into WT and MUT *RAS* (*KRAS/NRAS*) or MSI and MSS (Supplementary Fig. S8B,C). It was also true when the effect of MUT *APC* was examined in Moffitt Stage IV patients (n=110) (Supplementary Fig. S8D–F) and in TCGA CRCs (Supplementary Fig. S9A).

### Identification of *APC* and *TP53* as the highest-ranked CTX-S associated mutated genes

The finding of a potentially significant role of mutant *APC* in predicting cetuximab sensitivity prompted us examine additional genes. We applied an analytical approach fusing RNA-based gene expression signatures with DNA mutations to rank mutated genes in Moffitt CRCs (see Methods). The top 20 ranked mutated genes are shown in Table 2B, and a full rank list of mutated genes is given in Supplementary Table S11. While MSI status, *BRAF* (i.e. *BRAF*(V600E)) and *TGFBR2* were the most negatively correlated with the CTX-S score, *TP53* and *APC* were the highest ranked mutated genes that were strongly (positively) correlated with the scores (Table 2B, left). Here we treated the MSI status as “a mutated gene”. Notably, many negatively correlated genes, such as *BRAF* and *TGFBR2*, were strongly associated with MSI(15, 21, 35). After MSI tumors were removed, all the negatively correlated mutated genes had statistically non-significant, adjusted *P* values while *TP53* and *APC* remained the only statistically significant *positively* correlated genes (Table 2B, right and Supplementary Table S12). This is supported by an analysis showing striking trends by multiple *APC* genotypes and *TP53* mutations (Supplementary Table S13).

### *APC+TP53* doubly-mutated (*AP*) tumors had the highest CTX-S scores

Since *APC* and *TP53* were frequently *co-mutated* in CRC tumors(15, 21, 35), we examined whether mutant *APC+TP53* together might *cooperatively* predict cetuximab sensitivity. For this purpose, Moffitt CRCs were divided into four subgroups: (1) MUT *APC* + MUT *TP53* (*AP*); (2) MUT *APC* + WT *TP53* (*A*); (3) WT *APC* + MUT *TP53* (*P*); and (4) WT *APC* + WT *TP53* (WT *AP*). Analysis indicates that doubly-mutated *AP* tumors had significantly higher CTX-S scores than all other three subgroups including the *A* and *P* mutant groups ( $p<0.0001$  for two-tailed Welch *t* test) (Fig. 2A left panel). Notably, *A* and *P* mutant groups were not significantly different from each other, but had significantly higher scores than WT *AP* tumors ( $p<0.0001$ ). The same pattern was observed in the 110 Stage IV tumors (Fig. 2A right panel). Similar results were obtained in Moffitt WT *RAS* tumors for all stages (n=264), and for Stage IV (n=54) patients (Fig. 2B). The significant association was validated using the TCGA CRC dataset (Supplementary Fig. S9B).

### **APK\* triply-mutated tumors were associated with higher CTX-S scores than other RAS-mutated tumors**

We found that the presence of MUT *AP* also had a striking *positive* effect on the CTX-S scores of *KRAS/NRAS*-mutated (*K/N*)-tumors in Moffitt CRCs. Note that because the great majority of *RAS* mutations in CRCs are *KRAS* mutations (~40%) and the frequency of *NRAS* mutations is much lower (~5%), for simplicity, we used *K\** to represent both *KRAS* and *NRAS* mutations (*K/N*). Although WT *RAS* tumors (n=264) had significantly higher CTX-S scores ( $p < 0.0001$  for two-tailed Welch *t* test) than MUT *RAS* tumors (n=111) in which the mutant *APK\** subpopulation was excluded, the *APK\** triply-mutated tumors (n=91) had even higher scores ( $p < 0.0001$ ) (Fig. 2C left panel).

When restricted to Moffitt Stage IV tumors (n=110), a striking difference remained for mutated *K\** tumors between the *APK\** and non-*APK\** subpopulations (Fig. 2C right panel). In the TCGA CRCs, the mutant *APK\** tumors and WT *RAS* tumors had no significant difference in CTX-S scores but both had significantly higher scores than other *RAS*-mutated tumors (Supplementary Fig. S9C). These data suggest that some *APK\** patients (heretofore not treated) may be sensitive to CTX treatment.

### **Comparison of the CTX-S scores among seven MSI and MSS subgroups**

We next examined the association of *APC+TP53* doubly-mutated (*AP*) tumors with the MSI/MSS status. In the 468 Moffitt CRCs, very strikingly, there were only two mutant *AP* tumors (1 *APK* and 1 *APB*) identified out of 61 MSI-H tumors (Fig. 3D). All 197 other *AP* tumors were MSS tumors. Similarly, the TCGA dataset had only one mutant *AP* tumor (*APK*) out of 28 MSI cases (Supplementary Fig. S9D). The statistical significances of these associations are given using Barnard's exact test (Supplementary Table S14).

Our data led us to postulate that *AP* mutations might *cooperatively* play a role in modulating cetuximab sensitivity in MSS tumors. To more specifically examine this, we divided the Moffitt CRCs into 1 MSI and 6 MSS subgroups. The groups can be statistically ordered with decreasing CTX-S scores as: *AP* > [*APK\**, *A\_P*] > *AK\*\_PK\** > *K\*\_MUT BRAF* > [*WT AP*, MSI], where the groups inside the brackets are not significantly different from each other (Fig. 2E). Similar results were also obtained in 220 TCGA CRCs (Supplementary Fig. S10A) and in Moffitt Stage IV patients (n=110) (Supplementary Fig. S10B). Taken together, these data suggest the clinically relevant, provocative possibility that some *APK\* RAS-mutant* MSS tumors might benefit from EGFRi therapies.

### **Mutant AP genotype association with left-sided, MSS tumors explains biology and left-sided CTX sensitivity**

We assessed if the effect of mutant *AP* might be associated with the tumor sidedness. A plot of the CTX-S scores (high to low) of individual tumors with sidedness was produced to illustrate frequencies of the seven MSI and MSS subgroups (Left vs. Right) (see Fig. 3 (A) Moffitt and (B) TCGA, respectively). The CTX-S score was significantly higher in left-sided tumors than in right-sided tumors in all Moffitt patients (n=464,  $p < 0.0001$ , two-tailed Welch *t* test), WT *RAS* only patients (n=262,  $p < 0.0001$ ), and WT *RAS/RAF* patients (n=209,  $p < 0.0001$ ) (Fig. 3C). Similar results were obtained for the TCGA dataset (Fig. 3D). The

CMH test or Barnard's exact test were performed on the frequencies of MSI and mutant *AP* by the sidedness (Left vs Right) in Moffitt and TCGA CRCs (Supplementary Tables S15 and S16). As expected, MSI was strongly associated with right-sided tumors ( $p < 0.0001$ ) in both datasets. Moreover, in close association with significantly differing CTX-S scores, the percentage of *AP* tumors was significantly higher in left-sided than right-sided cases in both datasets (Fig. 3C, D). We further performed the distribution analysis and CHM tests of the CTX-S score by quartiles for different A/P/K\*/B subgroups (Left vs. Right) in the Moffitt 464 patients (see Supplementary Table S17 A–E). The quartiles with higher scores (Q4 or Q3) are predicted to be more responsive to cetuximab. Briefly, this subset analysis shows while LEFT-sided *AP* tumors have 92% (77/84) of cases with cetuximab scores greater than the median, 82% (14/17) of RIGHT-sided *AP* tumors also exceed the median. Moreover, LEFT- and RIGHT-sided *APK\** tumors have cetuximab sensitivity scores greater than the median in 76% (37/49) and 73% (30/41) of cases, respectively. These data compare to all other tumors *without AP* mutations, where only 26% (72/276) of cases greater than the median, including only 41% (44/108) when restricted to *KRAS/NRAS/BRAF* wildtype tumors. Thus it appears that cetuximab sensitivity is driven by *APC + TP53* mutations that may overcome sidedness and/or *KRAS/NRAS* mutation status to some extent. Consequently, some right and left sided tumors not currently considered for therapy may benefit. In addition, analysis using the two-tailed Welch *t* test showed no significant left vs. right CTX-S score difference in any particular subgroup in Moffitt dataset (Supplementary Fig. S11). These data suggest that the mutation frequencies, rather than the CTX-S scores, may be responsible for the sidedness effect.

#### Unlike the *AP*, single driver mutations were not consistently associated with the sidedness

Single driver mutations such as *KRAS*, *NRAS* and *BRAF* mutations have been used in predicting EGFRi responses (5, 6, 8). We performed Barnard's test to examine if there is a potential association of the frequencies of four single drivers *APC*, *TP53*, *KRAS/NRAS* and *BRAF* with the sidedness in the Moffitt dataset (Supplementary Table S18) and the TCGA dataset (Supplementary Table S19). We observed a few, but scattering, significant associations that (i) MUT *APC* was significantly more left-sided in TCGA among all patients ( $n=217$ ,  $p=0.0002$ ), but not for MSS patients ( $n=190$ ,  $p=0.23$ ) and (ii) the same is also true for MUT *TP53*; (iii) MUT *KRAS/NRAS* was only significantly associated with right-sided tumors in Moffitt MSS patients; (iv) As expected, MUT *BRAF* (that was strongly associated with MSI tumors) was significantly associated with right-sided tumors in both Moffitt and TCGA all patients. However, when MSI cases were removed, the association of *BRAF* mutations became insignificant in MSS patients of both datasets, with very low counts of *BRAF*-mutated tumors.

#### *APK\** patients were more often distant metastatic whereas WT *AP* MSS tumors were associated with mucinous histotypes

We also examined the distribution of age, stages and histotypes as well as other clinic-pathological parameters in the MSI and 6 MSS subgroups of Moffitt CRCs (see Supplementary Table S20). MSI was significantly associated with Stage I-II (60%,  $p < 0.01$ , individual chi-square ( $\Gamma^2$ ) contribution) and less associated with Stage IV (10%,  $p < 0.05$ ) and distant metastasis (11%,  $p < 0.01$ ). By contrast, the *APK\** patients were more often Stage III



(45%,  $p < 0.05$ ) and distantly metastatic (49%,  $p < 0.05$ ). Interestingly, the WT *AP* subgroup (having low CTX-S scores) was significantly more associated with the mucinous histotype (32%,  $p < 0.001$ ), whereas the “cetuximab-sensitive” *AP* tumors appeared to be least mucinous (3%,  $p < 0.05$ ).

### ***AP* mutations were predominant in the CMS2 subtype linked to Wnt and MYC activity**

The consensus molecular subtypes (CMS) were recently created from a comprehensive molecular analysis of thousands ( $n=4151$ ) of human tumors to best define CRC(16). We performed CMS classification in Moffitt CRCs (see Supplementary Methods and Table S21). Results show that the majority of *AP* MSS tumors (57%) were the CMS2, whereas *APK\** tumors were also more associated with the CMS2 subtype than all other CMS classes, whereas most of the 59 MSI tumors were the CMS1 subtype (Fig. 3E). The strong association of the *AP/APK\** MSS tumors with the CMS2 was confirmed by Barnard’s exact test (Fig. 3F left panel). Furthermore, we found that the CTX-S scores of the CMS2 type were significantly higher ( $p < 0.0001$ ) than all other CMS classes (Fig. 3F right panel).

### ***AP* mutations associated with better outcomes in PDX models treated with cetuximab**

To test our hypothesis, we identified two published cetuximab-treated CRC Patient-derived tumor xenograft (PDX) datasets (Julien et al.,  $n=52$  PDX models(23); Bertotti et al.  $n=98$  PDX models(24)) which also had *APC* and *TP53* mutation data (see Supplementary Tables S7 and S8). We performed the CMH trend test on cetuximab response by frequencies of *A* and/or *P* mutations in these two datasets. (i) For the Julien dataset, when the frequencies of *AP* mutations vs. *A* or *P* mutations vs. WT *AP* were compared between CR/PR/SD vs. PD tumors, the CMH trend *P* value was 0.0277 in 52 all PDX models or 0.0592 (marginally significant) in 25 WT *RAS* models (Table 3). (ii) For the larger Bertotti dataset, however, when compared between PR & SD vs. PD tumors, the CMH trend *P* value was 0.0008 in 98 PDX models that had WT *KRAS*, *NRAS*, *BRAF* and *PIK3CA* (Table 3). In addition, the CMH test was also performed when PR ( $n=23$ ) and SD ( $n=50$ ) cases were separated, with a *p* value of 0.0133 (Supplementary Table S22). Notably, the frequency of *AP* mutations had no difference between PR and SD cases (83% vs. 84%). These data again support a cooperative role of *AP* mutations in positively predicting cetuximab response (CR/PR and SD).

## **DISCUSSION**

We have developed and validated a cetuximab sensitivity (CTX-S) signature score, using outcomes data from two independent, prospective clinical trials, as well as by an *in vitro* cetuximab-treated cell line dataset. The robustness of the CTX-S score is also supported by the findings that: (1) the score was not prognostic; (2) the score had a strong correlation with *EREG* and *AREG*, the predictive biomarkers of cetuximab response(4, 6, 36, 37); (3) the score was significantly associated with the CMS2 subtypes, which were recently reported to be associated with better cetuximab response(38, 39).

Due to the limited availability of clinical trial tissue samples with cetuximab exposure (especially mutant *RAS* patients), clinical outcomes, and deep molecular analysis beyond

*RAS/RAF* testing, we used the CTX-S score as a “proxy” for clinical response. Using an integrated analytic approach we previously described(40), with the “lens” of gene expression, we identified a 2-gene mutation signature (*APC+TP53*)(*AP*) to predict cetuximab sensitivity, which was subsequently validated using two independent PDX datasets.

Identification of the 2-gene mutation signature (*AP*) has also provided new molecular insight into the recent observation that patients with MSI (often right-sided) almost uniformly lack *AP* mutations, and thus are resistant to EGFRi treatment, whereas patients with left-sided tumors more commonly harbor *AP* mutations, and are thus more responsive(6, 12, 13, 41, 42). Notably, it is the combination of *AP* mutations, rather than single driver mutations (*APC*, *TP53*, *KRAS/NRAS*, or *BRAF*), that was consistently and significantly associated with the sidedness. Currently, only left-sided CRCs with a WT *KRAS/NRAS/BRAF* status are considered eligible for EGFRi therapy(6). However, our further analysis suggests that mutations---rather than sidedness---may ultimately determine sensitivity to cetuximab. This is supported by the distribution analysis of the CTX-S score by quartiles for Left vs. Right *A/P/K\*/B* subgroups in the Moffitt464 patients (see Supplementary Table S17 A–E). In either-sided tumors, *AP* and *APK\** were the most “sensitive” subgroups, whereas WT *AP* (that also had WT *RAS/RAF* and often had mucinous histotypes) was one of the highly “resistant” subgroups.

These data have two important clinical implications that may facilitate achieving the goal of precision cancer care. First, they could re-define the current clinical strategy guided by extended *RAS/RAF* testing and sidedness by *excluding* currently eligible, *left*-sided WT *AP* tumors, but *including* currently ineligible, *right*-sided MUT *AP* tumors. Second, the unexpected finding of a strong association of *APK\** with predicted CTX sensitivity suggests a potential therapeutic opportunity ---requiring further *clinical* validation--- to a subpopulation of previously excluded *RAS* patients with a poor prognosis who harbor *APK\** mutations(15). For example, a recent analysis of gene expression markers on CALGB 80203 (Alliance) trial data reported that high expression of CD73 was associated with longer PFS from cetuximab both in *wild-type KRAS* patients (chemo + cetuximab: HR (hazard ratio)=0.91 versus chemo only: HR=1.57,  $P_{\text{interaction}}=0.026$ ) as well as in *mutant KRAS* patients (chemo + cetuximab: HR=0.80 versus chemo only: HR=1.29,  $P_{\text{interaction}}=0.025$ ) (43), suggesting an intriguing and *provocative* hypothesis that some fraction of mutant *KRAS* patients may actually benefit from EGFRi therapies. Moreover, a number of clinical trial analyses have indicated that a substantial percentage (10–60%) of *mutant KRAS* patients treated with cetuximab/panitumumab have achieved stable disease (SD)(4, 36, 37, 44–51). Furthermore, analysis of the FIRE-3 (AIO KRK-0306) study showed that for the FOLFIRI + cetuximab treatment, compared to the wild-type *KRAS* exon 2 patients (n=297) who had 13 (4.4%) CR, 171 (57.6%) PR and 52 (17.5%) SD), *RAS* mutant patients (n=97) achieved 1 (1.0%) CR, 36 (37.1%) PR and significantly higher SD (31, 32.0%)(51). Finally, preclinical studies have indicated that EGFRi resistance can be reversed in some mutant *KRAS* CRC cell lines(52), and some *KRAS*-mutated CRC tumors may be decoupled from *RAS* pathway activation(25).

Our data suggest a hypothesis by which mutations in *APC+TP53* might enable Wnt and p53 pathway “crosstalk” to transactivate the EGFR pathway, essentially addicting tumors to EGF ligands or enhance antibody-dependent cellular cytotoxicity (ADCC). Activation of the EGFR-PI3K-AKT signaling pathway has been clearly demonstrated in the *APC<sup>Min/+</sup>* mouse by a mechanism involving upregulation of PGE2(53, 54). Similar to WNT, the p53 pathway has crosstalk with the EGFR pathway. Specifically, mutant *TP53* has been shown to induce ERG1 transcription that is driven by p-ERK(55, 56). We found that the *APC+TP53* double-mutated tumors were predominantly the CMS2 subtype that was associated with WNT and MYC activation and frequent mutations in either *APC* or *TP53*(16).

In conclusion, we have identified a 2-gene signature in identifying cetuximab-sensitive subpopulations. The signature may be useful in refining the appropriate subpopulation of patients for EGFRi treatment. Moreover, our findings provide a rationale for further prospective clinical studies that add sequencing of *APC+TP53* to extended *RAS/RAF* testing to improve and expand the clinical utility of EGFRi---even potentially to some previously excluded patients harboring mutant RAS.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## ACKNOWLEDGEMENTS

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## Abbreviations

<b>CRC</b>	colorectal cancer
<b>EGFRi</b>	EGFR inhibitor
<b>CTX</b>	cetuximab
<b>PFS</b>	progression free survival
<b>OS</b>	overall survival
<b>RFS</b>	relapse free survival
<b>CR</b>	complete response
<b>PR</b>	partial response
<b>SD</b>	stable disease
<b>PD</b>	progressed disease
<b>ORR</b>	objective response rate

## PDX Patient-derived tumor xenografts

For *APC*, *TP53*, *KRAS*, *NRAS* and *BRAF* mutations, single **bolded** letters *exclusively* refer to specific individual gene mutations: **A** = *APC* mutation; **P** = *TP53* mutation; **K** = *KRAS* mutation; **N** = *NRAS* mutation; **K\*** = K or N; **B** = *BRAF* mutation (e.g. **AP** = *APC* mutation + *TP53* mutation; **APK\*** = *APC* mutation + *TP53* mutation + *KRAS/NRAS* mutation). WT **AP** = WT *APC* + WT *TP53*. When HUGO gene names are identified, the identified gene may not be exclusive (e.g. MUT *APC* = any tumor harboring *APC* mutations that could include *KRAS*, *BRAF*, *NRAS*, *TP53*). WT = wild-type; MUT = mutant

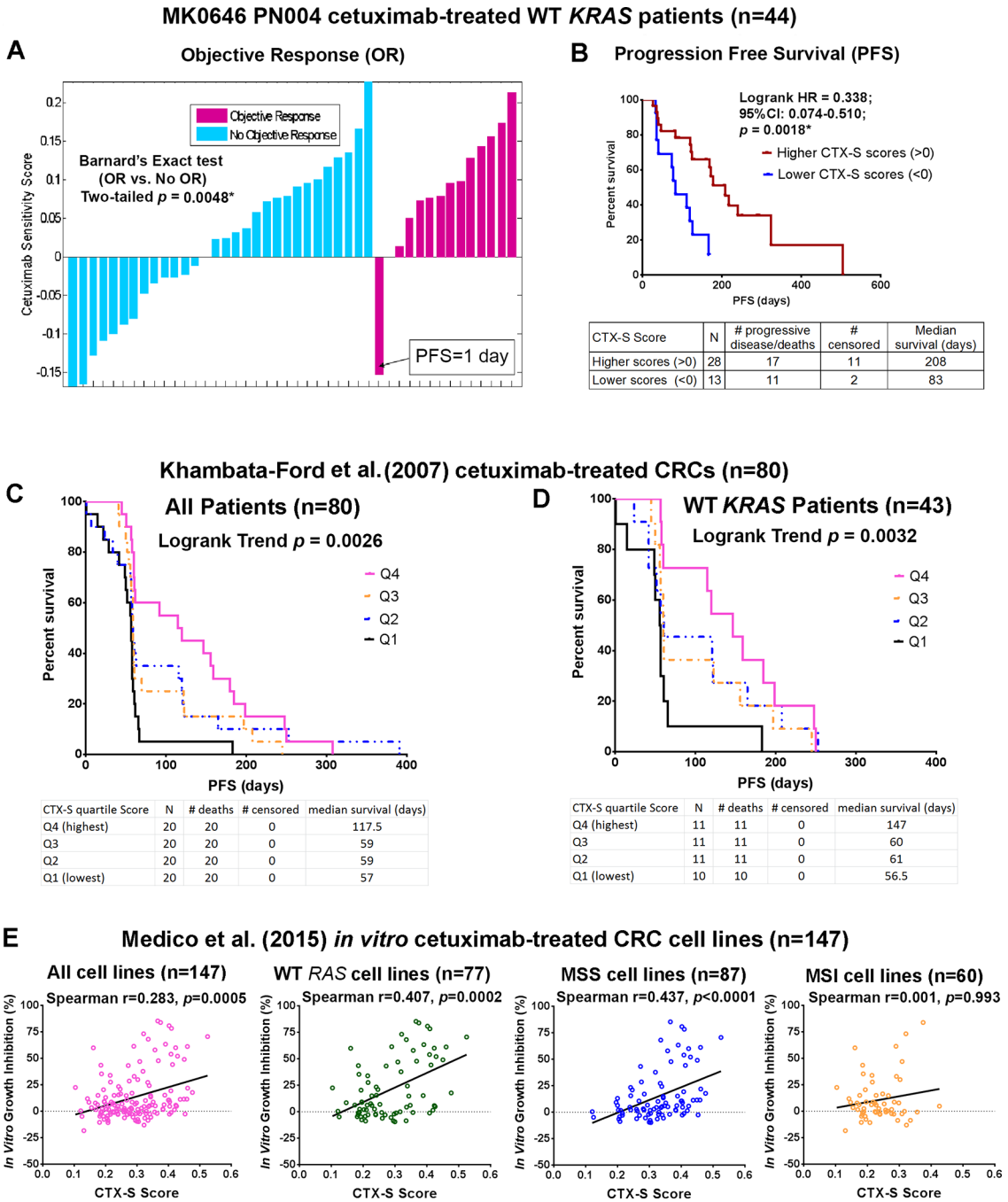
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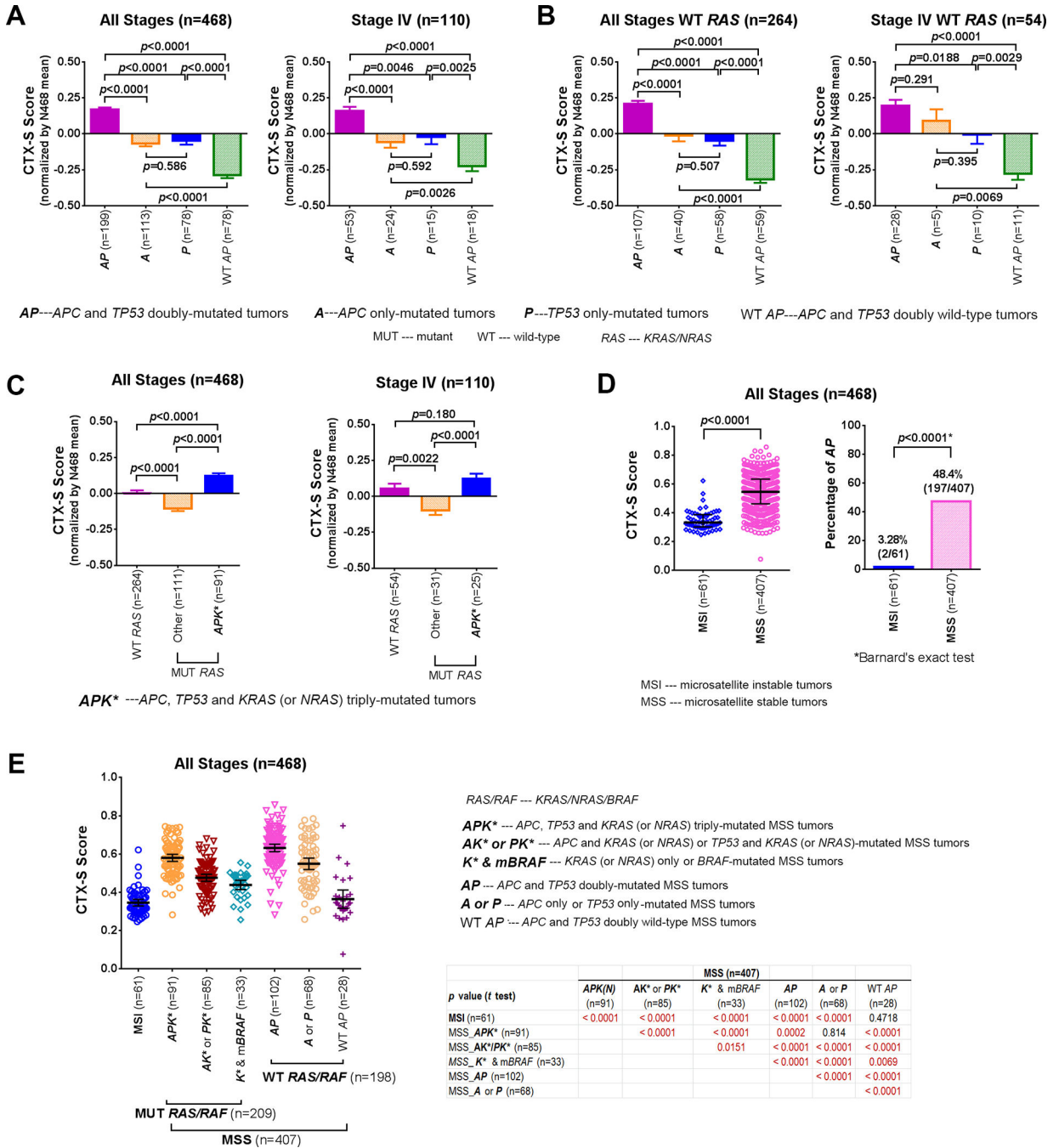


**Fig. 1. Validation analysis of the cetuximab sensitivity (CTX-S) signature score in two independent sets of CRC patient samples derived from clinical trials and one set of *in vitro* cetuximab-treated CRC cell lines.**

**A.** A waterfall plot of objective response (OR) vs. adjusted CTX-S score in MK0646 PN004 wild-type (WT) *KRAS* CRCs (n=44) (see Supplementary Table S1 for detailed data description). The  $p$  value is for Barnard's exact test (OR: No OR) (see Supplementary Table S10A). **B.** Kaplan Meier (KM) survival (PFS) analysis by higher (>0) vs. lower (<0) CTX-S scores was also performed the PN004 CRCs (n=41). Note: for **A, B**, of 44 CRCs, one sample with PFS of 1 day and two samples with CTX-S scores near 0.00 as shown in **Fig.**



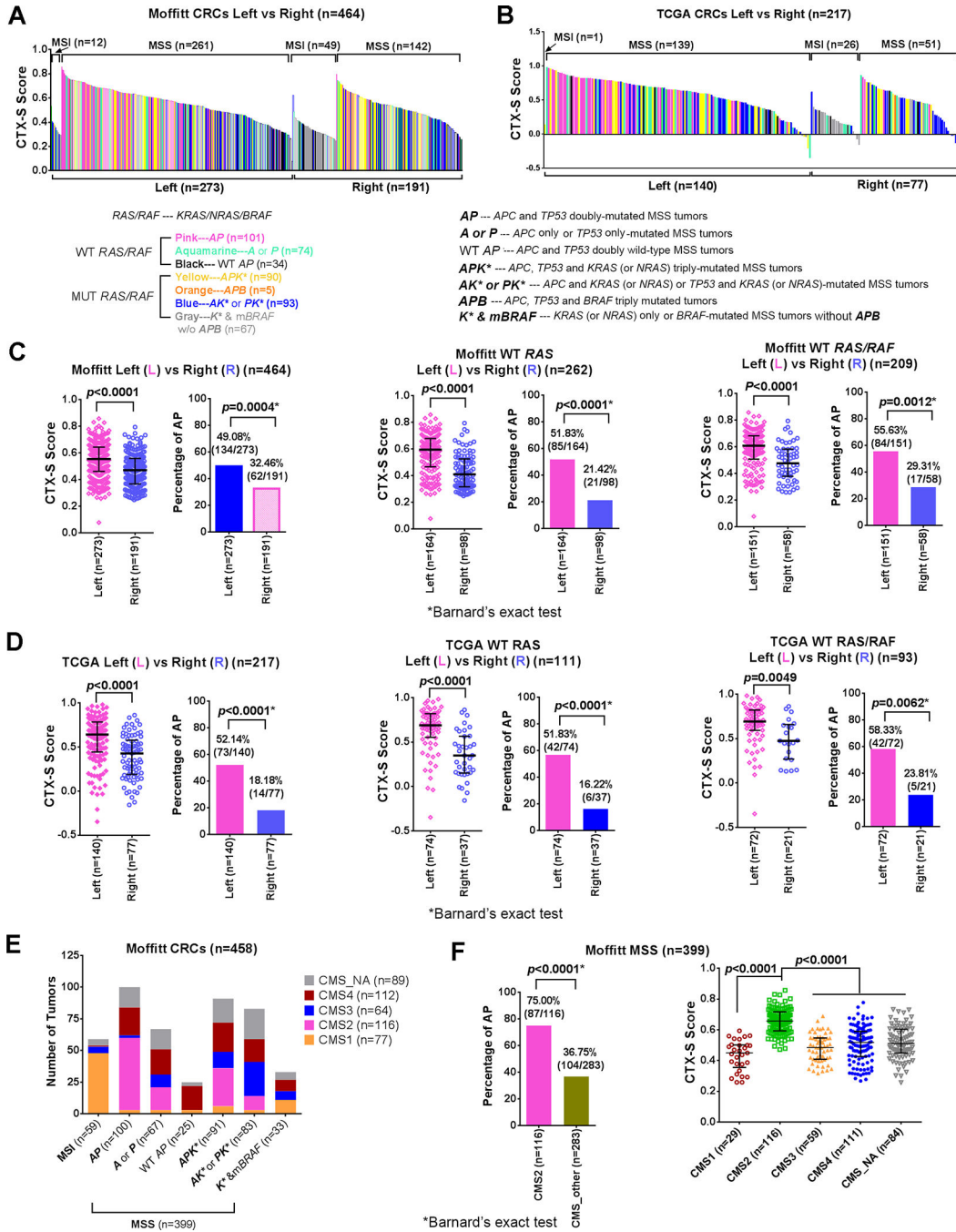
**1A** were excluded from Barnard test and KM analysis. Also see Supplementary Fig. S3 for similar KM analysis on OS. **C.** KM survival (PFS) analysis by the CTX-S quartile scores was performed in Khambata-Ford et al. (2007) cetuximab-treated CRCs (n=80)(4). **D.** KM PFS analysis in Khambata-Ford WT *KRAS* patients (n=43). Also see Supplementary Table S10B,C for the Cochran-Mantel-Haenszel test showing the significant association of the CTX-S score with improved response (CR/PR and SD). **E.** Spearman correlation analysis of the CTX-S score with *in vitro* growth inhibition (%) by 10 µg/ml of cetuximab in Medico et al. (2015) cetuximab treated CRC cell lines (20). The analysis was performed in all cell lines (n=147) and WT *RAS* (*KRAS/NRAS*) cell lines (n=77) as well as MSS (n=87) and MSI cell lines (n=60), respectively (see detailed data description in Supplementary Table S3A). Note that the results for the other doses of cetuximab (1, 25, 50 and 100µg/ml) were similar, as 10 µg/ml had a 0.945 or higher (Pearson) correlation with these doses for *in vitro* growth inhibition (Supplementary Table S3B).



**Fig. 2.** The “MUT APC + MUT TP53” (AP) doubly-mutated tumors had significantly higher cetuximab sensitivity (CTX-S) scores than other tumors with “MUT APC only” (A), “MUT TP53 only” (P) or WT APC + WT TP53” (WT AP) in Moffitt CRCs.

**A.** Comparison for all stage patients (n=468) and Stage IV patients (n=110), respectively, regardless of RAS mutation status; **B.** Comparison for all stage WT RAS patients (n=266) and Stage IV WT RAS patients (n=55), respectively; **C.** Comparison of the CTX-S scores were also performed between WT RAS, APK\* (MUT APC + MUT TP53 + MUT KRAS(or NRAS)) and other MUT RAS tumors without APK\*. MUT – mutant; WT – wild-type; WT RAS – patients with wild-type KRAS/NRAS. The CTX-S scores were normalized by the

mean of 468 CRCs, and bars represent Mean with standard errors (SEM). *p* values for two-tailed Welch *t* test are shown. **D.** The CTX-S scores were compared between MSI (i.e. MSI-high) and MSS tumors (left panel) in all patients (n=468). Bars represent Median with interquartile range. The comparison was also made for the percentage of “MUT *APC* + MUT *TP53*” (*AP*), with “\*” representing two-tailed *p* values for Barnard’s exact test (right panel) (see Supplementary Table S14A). **E.** Comparison of CTX-S scores among 1 MSI and 6 MSS subgroups in Moffitt CRCs (n=468). Bars represent Median with interquartile range. Unadjusted *p* values for two-tailed Welch *t* test are shown and those remaining being significant after adjustments for multi-comparisons by Holm-Bonferroni method are highlighted by maroon color.



**Fig. 3. Both the CTX-S score and the percentage of “MUT APC + MUT TP53” (AP) tumors were significantly higher in left-sided tumors in Moffitt and TCGA CRCs.**

A diagram of the CTX-S scores (high to low) vs. 7 MUT/WT APC/TP53/RAS(KRAS/NRAS)/BRAF subgroups in MSI and MSS tumors was displayed for Moffitt (n=464) (A) or TCGA (n=217) (B) datasets. MUT – mutant; WT – wild-type. Note: Of 468 CRCs, 4 samples without tumor location information were excluded; Of 221 CRCs, 3 samples without tumor location information and 1 sample with MSI status information were excluded. Comparison of the CTX-S scores between “Left” and “Right” CRCs was made in

all (available-data) patients, WT *RAS* patients, and WT *RAS/RAF* patients on Moffitt (C) and TCGA (D) CRCs, respectively. Note: WT *RAS*--patients with wild-type *KRAS/NRAS*; WT *RAS/RAF*--patients with wild-type *KRAS/NRAS/BRAF*; Bars represent Median with interquartile range. *p* values are for two-tailed Welch *t* test. The comparison was also made for the percentage of “MUT *APC* + MUT *TP53*” (AP) (Left vs. Right). \* two-tailed *p* values for Barnard’s exact test (see Supplementary Table S15 and S16). **The AP tumors were predominantly CMS2 subtype.** E. The plot of the number of tumors of 1 MSI and 6 MSS subgroups (similarly as defined in Fig. 2E) vs. CMS1–4 and CMS\_NA in Moffitt CRCs (n=458). Note: Of 468 CRCs, 10 samples without appropriate microarray data for the consensus molecular subtypes (CMS) classification analysis(16) were excluded as described previously(15). F. Left panel, the comparison of the percentage of “MUT *APC* + MUT *TP53*” (AP) (regardless of RAS mutation status) between CMS2 and other CMS subclasses in Moffitt MSS CRCs (n=399), with “\*” representing a two-tailed *p* value of Barnard’s exact test. CMS\_other includes CMS1, CMS3, CMS4 and CMS\_NA (indeterminate). Right panel, comparison of the CTX-S scores among CMS1–4 and CMS\_NA subclasses in MSS CRCs (n=399). Bars represent Median with interquartile range. *p* values are for two-tailed Welch *t* test.

List of eight colorectal cancer datasets used

Table 1.

Datasets	Samples	N of samples	Stage	Cetuximab response	Prognosis	Gene Expression	MSI status	DNA sequencing	Driver Mutations	Primary tumor location
1. MK0646-PN004 CRCs(19)	patient tumors	44	IV	PFS, OS OR		Aflymetrix		targeted-sequencing <i>KRAS</i>	<i>KRAS</i>	
2. Khambata-Ford et al. (2007), <i>J Clin Oncol</i> (4)	patient tumors	80	IV	PFS, CR/PR, SD, PD		Aflymetrix		targeted-sequencing <i>EGFR, KRAS, BRAF</i>	<i>KRAS</i>	
3. Medico et al. (2015) <i>Nat Commun</i> (20)	cell lines	147		<i>In vitro</i> growth inhibition		Aflymetrix	MSI/MSS	targeted-sequencing <i>KRAS, NRAS, BRAF</i>	<i>KRAS, NRAS, BRAF</i>	
4. Moffitt CRCs---Schell et al. (2016) <i>Nat Commun</i> (15); Schell et al. (2016) <i>Clin Cancer Res</i> (14)	patient tumors	468	I, II, III, IV		OS	Aflymetrix	MSI/MSS	targeted-sequencing (1321 genes)	<i>APC, TP53, KRAS, NRAS, BRAF</i>	Left/Right
5. TCGA CRCs---TCGA <i>Nature</i> (2012) (21); NCI GDC	patient tumors	624	I, II, III, IV			RNAseq	MSI/MSS	whole exome sequencing on 221 tumors	<i>APC, TP53, KRAS, NRAS, BRAF</i>	Left/Right
6. Marisa et al. (2013) <i>PLoS Med</i> (22)	patient tumors	566	I, II, III, IV		RFS	Aflymetrix		targeted-sequencing <i>KRAS, BRAF, TP53</i>	<i>TP53, KRAS, BRAF</i>	
7. Julien et al. (2012) <i>Clin Cancer Res</i> (23)	PDX models	52	I, II, III, IV	PR (+++), SD (++), PD (+, -)			MSI/MSS	targeted-sequencing 13 genes	<i>APC, TP53, KRAS, BRAF</i>	
8. Bettoni et al. (2015) <i>Nature</i> (24)	PDX models	98	I, II, III, IV	PR, SD, PD				whole exome sequencing	<i>APC, TP53, KRAS, NRAS, BRAF</i>	

**Note:** PFS---progression free survival; OS--- overall survival; OR--- objective survival; CR---complete response; PR---partial response; SD---stable disease; PD---progressed disease; MSS---microsatellite stable tumors. Also see Supplementary Tables S1–S8 for detailed data description of individual datasets, respectively.

**Table 2. A.**

Spearman Correlations with the cetuximab sensitivity (CTX-S) score

All CRC tumors/cell lines (regardless of RAS status)										
Spearman Corr. with CTX-S score	Moffitt CRCs (n=458)*		TCGA CRCs (n=624)		Marisa et al (2013) CRCs (n=566)		Khambata-Ford et al (2007) CRCs (n=80)		Medico et al (2015) CRC cell lines (n=147)	
	r	p value	r	p value	r	p value	r	p value	r	p value
Gene expression/ Signature scores										
EREG	0.562	<0.0001	0.592	<0.0001	0.655	<0.0001	0.711	<0.0001	0.266	<b>0.001</b>
AREG	0.463	<0.0001	0.469	<0.0001	0.542	<0.0001	0.624	<0.0001	-0.173	<b>0.036</b>
18-gene RAS pathway score	-0.361	<0.0001	-0.209	<0.0001	-0.491	<0.0001	0.123	0.277	-0.106	0.201
64-gene Wnt pathway score	0.069	0.140	0.023	0.573	0.036	0.655	0.276	<b>0.013</b>	0.311	<b>0.0001</b>
24-gene Wnt pathway score	0.553	<0.0001	0.457	<0.0001	0.558	<0.0001	0.666	<0.0001	0.488	<0.0001

Note

\* Of Moffitt 468 CRCs, 10 samples lacking appropriate gene probe values for some signature scores were excluded from correlation analysis.

**Table 2. B.**

Ranking of cetuximab sensitivity score-associated mutated genes using Moffitt CRCs

All Patients (n=468)						MSS Patients (n=407)					
gene	p value	mutation		Asso Dir (+/-)	Adjusted p	gene	p value	mutation		Asso Dir (+/-)	Adjusted p
		N	PCT					N	PCT		
<i>TP53</i>	<b>1.89E-24</b>	<b>277</b>	<b>59.2%</b>	+	<b>5.8E-22</b>	<i>TP53</i>	<b>4.64E-22</b>	<b>261</b>	<b>64.1%</b>	+	<b>4.09E-19</b>
MSI_high*	6.74E-24	61	13.0%	-	2.06E-21	<i>APC</i>	<b>8.55E-12</b>	<b>296</b>	<b>72.7%</b>	+	<b>7.52E-09</b>
<i>TGFBR2</i>	1.93E-23	62	13.2%	-	5.9E-21	<i>BRAF</i>	0.000709	18	4.4%	-	0.6227
<i>APC</i>	<b>7.96E-21</b>	<b>312</b>	<b>66.7%</b>	+	<b>2.44E-18</b>	<i>KRAS</i>	0.001858	177	43.5%	-	0.998439
<i>BRAF</i>	5.67E-17	53	11.3%	-	1.74E-14	<i>SMAD3</i>	0.00204	14	3.4%	-	0.998439
<i>CELSR1</i>	1.56E-06	72	15.4%	-	0.000478	<i>BARD1</i>	0.002188	7	1.7%	-	0.998439
<i>HDLBP</i>	4.03E-06	23	4.9%	-	0.001234	<i>CTNNB1</i>	0.003312	13	3.2%	-	0.998439
<i>ITGB4</i>	6.05E-06	45	9.6%	-	0.001852	<i>BRCA1</i>	0.005571	25	6.1%	-	0.998439
<i>PML</i>	6.3E-06	22	4.7%	-	0.001926	<i>IDH1</i>	0.006636	5	1.2%	-	0.998439
<i>HSPA2</i>	1.23E-05	14	3.0%	-	0.003749	<i>RAD18</i>	0.006847	6	1.5%	-	0.998439
<i>MLL2</i>	2.29E-05	84	17.9%	-	0.006995	<i>SMAD2</i>	0.006852	12	2.9%	-	0.998439
<i>MICAL1</i>	2.36E-05	26	5.6%	-	0.007217	<i>TLR7</i>	0.011293	5	1.2%	-	0.998439
<i>PTPRS</i>	2.58E-05	47	10.0%	-	0.007885	<i>HECW1</i>	0.012886	24	5.9%	+	0.998439
<i>MAP3K9</i>	2.72E-05	21	4.5%	-	0.008312	<i>TRIB3</i>	0.013672	8	2.0%	-	0.998439
<i>ITPR1</i>	4.23E-05	34	7.3%	-	0.012936	<i>NRP2</i>	0.016995	15	3.7%	-	0.998439
<i>HDAC4</i>	5.35E-05	20	4.3%	-	0.016377	<i>TAF15</i>	0.02442	5	1.2%	-	0.998439
<i>DOT1L</i>	5.87E-05	24	5.1%	-	0.017956	<i>GRM1</i>	0.02554	18	4.4%	-	0.998439
<i>RPS6KA2</i>	5.95E-05	12	2.6%	-	0.018212	<i>AFF4</i>	0.026074	7	1.7%	-	0.998439
<i>CHD5</i>	5.96E-05	23	4.9%	-	0.018239	<i>FLT4</i>	0.027436	18	4.4%	+	0.998439
<i>MLL4</i>	7.03E-05	54	11.5%	-	0.021519	<i>EGFR</i>	0.0279	11	2.7%	+	0.998439

**Note:** p value from normal scores test for comparing CTX-S scores of mutated and wild-type tumors for the given gene; N (PCT) – the number (percentage) of mutated tumors; *APC* – *APC* truncated mutation; *BRAF* – *BRAF*(V600E)

\* patients with MSI-high status. “Asso Dir” – the directionality of the association (+/-); Adjusted p value was calculated using the Hochberg and Benjamini method(29). Here top 20 genes are listed according to their adjusted p values. See a full rank list of 1321 cancer-associated genes in Supplementary Tables S11 and S12.



**Table 4.**

Cochran-Mantel-Haenszel (CMH) trend test on cetuximab (CTX) response by frequencies of MUT *APC* (*A*) and/or MUT *TP53* (*P*) in cetuximab-treated CRC PDX models

Response		Julien all PDX models (n=52)				Julien WT <i>KRAS</i> (n=25)				Bertotti PDX models (n=98)*			
		Mutation				Mutation				Mutation			
Frequency Expected Cell Chi-Square Col PCT		<i>AP</i>	<i>A or P</i>	WT <i>AP</i>	Total	<i>AP</i>	<i>A or P</i>	WT <i>AP</i>	Total	<i>AP</i>	<i>A or P</i>	WT <i>AP</i>	Total
<b>CR/PR &amp; SD</b>		10 6.92 1.37 50.00	8 9.35 0.19 29.63	0 1.73 1.73 0.00	18	7 4.8 1.00 70.00	5 6.72 0.44 35.71	0 0.48 0.48 0.00	12	61 54.38 0.81 83.56	6 8.94 0.97 50.00	6 9.68 1.40 46.15	73
<b>PD</b>		10 13.08 0.72 50.00	19 17.65 0.10 70.37	5 3.27 1.92 100.00	34	3 5.2 0.93 30.00	9 7.28 0.41 64.29	1 0.52 0.44 100.00	13	12 18.62 2.36 16.44	6 3.06 2.82 50.00	7 3.32 4.09 53.85	25
Total		20	27	5	52	10	14	1	25	73	12	13	98
<b>CMH</b> (nonzero corr.)	DF	1				1				1			
	value	<b>4.85</b>				3.56				<b>11.23</b>			
	<i>P</i>	<b>0.0277</b>				0.0592				<b>0.0008</b>			

**Note:** MUT – mutant; WT – wild-type; *A* – *APC* mutation; *P* – *TP53* mutation; *AP* – *APC* mutation + *TP53* mutation; WT *AP* – WT *APC* + WT *TP53*; CR – complete response; PR – partial response; SD – stable disease; PD – progressed disease. For illustration purposes, the antitumor activities “+++”, “++” and “+/-” in Julien et al. PDX models were re-expressed as “CR/PR”, “SD” and “PD”, respectively, according to the pharmacological annotation as described (23). See Supplementary Tables S7 and S8 for detailed data description of Julien et al. (23) and Bertotti et al. (24) CTX-CRC PDX models, respectively.

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