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ORIGINAL ARTICLE

# Clinical relevance of EMT and stem-like gene expression in circulating tumor cells of metastatic colorectal cancer patients

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Using approved methods, circulating tumor cells (CTCs) are only isolated from blood in 30%–50% of metastatic colorectal cancer (mCRC) patients. We previously validated a technique to isolate circulating tumor cells (CTCs) in a cohort of mCRC patients by combining immunomagnetic enrichment of EpCAM$^+$/CD45$^-$ cells with qRT-PCR amplification of *CK20* and *survivin* expression. Here, we examined the prognostic utility of CTC epithelial–mesenchymal transition (EMT) and stem cell gene expression. An 8 ml blood sample was collected from 78 consecutive mCRC patients before treatment with investigational and standard chemotherapeutics. The mRNA expression of EMT (*PI3Kα*, *Akt-2, Twist1*) and stem cell (*ALDH1*) markers was measured. Associations between CTC gene expression and progression-free survival (PFS) and overall survival (OS) were determined using Cox regression models. Among patients without *CK20* or *survivin*-expressing CTCs ($n = 17$), 55% had expression of *ALDH1*, *PI3Kα* and/or *Akt-2*. Patients with positive CTC *Akt-2* expression had a significantly shorter median PFS (3.0 versus 4.0 months) compared with those without CTC *Akt-2* expression in univariable (hazard ratio (HR) = 1.61; log-rank $P = 0.034$) and multivariable analyses (HR = 1.70; adjusted $P = 0.041$). In univariable analysis, CTC *ALDH1* expression was associated with shorter OS (10.0 versus 38.6 months; HR = 2.04, $P = 0.021$). Patients with CTCs expressing *ALDH1*, *PI3Kα* and/or *Akt-2* had a significantly inferior PFS (3.0 versus 7.7 months; HR = 1.88, $P = 0.015$) and OS (10.0 versus 26.8+ months; HR = 2.25, $P = 0.050$) in univariable, but not multivariable, analysis. Conclusions: CTC *Akt-2* expression may serve as a clinically useful prognostic marker in mCRC patients and warrants further evaluation in prospective trials.

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## INTRODUCTION

Circulating tumor cells (CTCs) have been isolated from metastatic colorectal cancer (mCRC) patients,[1,2] and CTC count has been associated with survival.[3–5] However, circulating cancer cells are rare within the bloodstream and exhibit a phenotypic diversity, which may impede their reliable detection. Using the CellSearch assay, CTCs are detected in only 30%–50% of mCRC patients.[1–5] CTCs can undergo the epithelial–mesenchymal transition (EMT)[6–9] and assume a stem cell-like[6] phenotype. Owing to such heterogeneity, current CTC isolation methods that emphasize epithelial traits may not capture their plasticity through activation of EMT and stem cell pathways.[10] Refining the molecular characterization of CTCs may improve the clinical utility of CTC detection and provide insight into mechanisms directing the metastatic process as well as therapeutic resistance. To this end, several CTC markers[3–5,11–17] have been explored in an attempt to improve diagnostic yield and predict outcomes, but the optimal CTC gene expression signature remains to be defined.

We recently validated a technique combining immunomagnetic enrichment with quantitative real-time PCR (qRT-PCR) to isolate EpCAM$^+$/CD45$^-$ CTCs, which express *CK20* and/or *survivin* mRNA in mCRC patients receiving standard and experimental chemotherapy regimens.[18] However, within the cohort of patients with *CK20* and/or *survivin*-expressing CTCs, there was still considerable variability with regards to survival. Furthermore, we hypothesized that a proportion of epithelial–mesenchymal transitioned CTCs may not express *cytokeratin* or *survivin* and, therefore, evade detection. The PI3Kα/Akt-2/mTOR pathway helps regulate the EMT transition by repressing cell adhesion proteins, therefore facilitating cancer cell migration as well as survival and apoptosis.[19] In preclinical studies, Akt-2 has been shown to be critical to the development of colorectal metastasis.[20] Clinically, CTC expression of *PI3Kα*, *Akt-2*, *Twist1* has been significantly associated with outcomes in cancer patients.[8] Similarly, circulating cancer cells may acquire a stem cell phenotype,[19] marked by aldehyde dehydrogenase 1 (ALDH1) upregulation, and CTC *ALDH1* expression has demonstrated prognostic value in patients undergoing chemotherapy.[8,21]

Here, we aim to refine the utility of our approach by measuring the mRNA expression of EMT (*PI3Kα*, *Akt-2, Twist1*) and stem cell (*ALDH1*) markers in circulating cancer cells. We found a proportion of *CK20*$^-$ and *survivin*$^-$ cells express *PI3Kα*, *Akt-2* or *ALDH1*, and that this gene expression panel may prognostically stratify subgroups of mCRC patients receiving chemotherapy.

## MATERIALS AND METHODS

### Patient population and study design

Patients consented for peripheral blood collection and received standard FDA-approved therapies (including fluoropyrimidines, oxaliplatin, irinotecan, bevacizumab, cetuximab, panitumumab, regorafenib), or received experimental agents being tested in three phase I or II clinical

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trials examining 5-FU plus brivanib (NCT01046864), PRI-724 (NCT01302405) and celecoxib plus EPO906 (NCT00159484). Patients were enrolled at the Norris Comprehensive Cancer Center-University of Southern California or the Los Angeles County-University of Southern California Medical Center, between June 2009 and April 2014.[18] The Institutional Review Board at the University of Southern California approved the study. Twenty healthy blood donors (age $\geqslant$18 years), who had no known medical illness or history of malignant disease, served as control subjects. All the CTC studies were performed without knowledge of patients' clinical status.

### Sample collection and tumor cell enrichment

The sample collection and tumor cell enrichment were performed as previously described.[18] An 8 ml blood sample was drawn from each patient into one Vacutainer CPT Tube with Sodium Citrate (BD, Franklin Lakes, NJ, USA). Negative immunomagnetic selection using anti-CD45 specific antibodies (Dynabeads M-450 CD45 pan Leukocyte, Invitrogen, Waltham, MA, USA) was performed to enrich for tumor cells following the manufacturer's instructions. The CD45-negative ($CD45^-$) supernatant was transferred to 15 ml tubes for immune separation using Dynabeads (Dynabeads Epithelial Enrich, #161.02, Invitrogen). Using Dynabeads coated with a monoclonal antibody towards human EpCAM, tumor cell selection was performed following the manufacturer's instructions.

### qRT-PCR and multiplex-PCR

*CK20* and *survivin* mRNA expression levels were analyzed by the iTaq Fast SYBR Green Supermix (Bio-Rad #172-5101; Bio-Rad, Hercules, CA, USA) and an Applied Biosystems 7500 PCR Detection System (Applied Biosystems, Foster City, CA, USA). *ALDH1*, *Akt-2* and *PI3Kα* mRNA expression levels were analyzed using the HotStarTaq Master Mix (Qiagen, Qiagen GmbH, Germany, #203443) and a thermocycler, and determined by using the Agilent 2100 Bioanalyzer on a DNA LabChip (Agilent Technologies, Santa Clara, CA, USA).

### Analysis of mRNA expression

Primers for EMT markers and stem cell markers were provided by AdnaGen (AdnaGen, Langenhagen, Germany). The analysis of tumor cell-derived mRNA was performed by qRT-PCR for the following transcripts: PI3Kα, Akt-2, Twist1 and ALDH1. Thermal profiles were used as per the recommendations of the supplier (AdnaGen). The AdnaTest EMT-2/StemCell Detect Kit (AdnaGen), containing oligo$(dT)_{25}$-coated beads, was used to isolate mRNA from tumor cells. The PrimerMix StemCell was used to amplify ALDH1, and the PrimerMix EMT-2 was used to amplify three EMT-related genes (*Akt-2*, *PI3Kα*, *Twist1*) and one control gene (Actin). Visualization of PCR fragments was carried out with the Agilent 2100 Bioanalyzer, with a limit of detection of $\geqslant$0.15 ng $\mu l^{-1}$.

### Statistical analysis

The mRNA levels of CTC *ALDH1*, *PI3K* and *Akt-2* were categorized into low and high values at the cutoff values provided by AdnaGen. The associations between the three markers and progression-free survival (PFS) and overall survival (OS) were analyzed by Kaplan–Meier curves and log-rank test in the univariable analysis and Cox regression model in the multivariable model, adjusting for baseline characteristics and treatment administered after CTC collection that were associated with PFS or OS at a significance level of 0.1.

## RESULTS

### Patient and tumor characteristics

Patient and tumor characteristics are described in Table 1. Among 78 mCRC patients, the median duration of follow-up was 23.5 months (range: 2.0, 38.6 months), median PFS was 3.1 months (95% confidence interval: 2.1, 5.0 months) and median OS was 10.4 months (95% confidence interval: 8.9, 17.2 months). Patients received a median of three prior lines of therapy (range 0–5). Most patients had received fluoropyrimidines (97.4%), oxaliplatin (89.7%), irinotecan (75.6%) and bevacizumab (88.5%) before CTC collection. After CTC collection, 62.8% of patients received experimental therapies on clinical trials. There was an even distribution of primary tumor site across all the patients, and most did not have liver-limited metastases.

**Table 1.** Metastatic colorectal cancer patient baseline characteristics ($n = 78$)

| *Characteristic* | n *(%)* |
|---|---|
| *Age, years* | |
| Median (range) | 57.5 (28-81) |
| *Sex* | |
| Male | 42 (53.8) |
| Female | 36 (46.2) |
| *Race* | |
| White | 51 (65.4) |
| African American | 3 (3.8) |
| Asian | 9 (11.5) |
| Hispanic | 15 (19.2) |
| *Primary tumor site*[a] | |
| Right colon | 21 (26.9) |
| Left colon | 31 (39.7) |
| Rectum | 23 (29.5) |
| Unspecified CRC | 3 (3.8) |
| *Liver-only metastases* | |
| Yes | 14 (18.0) |
| No | 64 (82.0) |
| *ECOG performance status* | |
| 0 | 24 (30.8) |
| 1 | 48 (61.5) |
| 2 | 6 (7.7) |
| *Number of prior lines of therapy* | |
| 0–1 | 17 (21.8) |
| 2 | 20 (25.6) |
| 3 | 23 (29.5) |
| 4–5 | 18 (23.1) |
| *Prior lines of therapy* | |
| Fluoropyrimidine | 76 (97.4) |
| Oxaliplatin | 70 (89.7) |
| Irinotecan | 59 (75.6) |
| Bevacizumab | 69 (88.5) |
| Cetuximab, Panitumumab | 28 (35.9) |
| *Therapy initiated after CTC collection* | |
| Fluoropyrimidine | 28 (35.9) |
| Oxaliplatin | 14 (17.9) |
| Irinotecan | 12 (15.4) |
| Bevacizumab | 20 (25.6) |
| Cetuximab, Panitumumab | 2 (2.6) |
| Experimental Agents | 49 (62.8) |
| *Baseline CEA (ng $ml^{-1}$)* (N = *75*) | |
| Median (range) | 35.9 (0.9–16 300) |
| *Baseline LDH (units $l^{-1}$)* (N = *61*) | |
| Median (range) | 199 (120–1871) |

Abbreviations: CEA, carcinoembryonic antigen; CRC, colorectal cancer; CTC, circulating tumor cell; ECOG, Eastern Cooperative Oncology Group; LDH, lactate dehydrogenase. [a]Left- and right-sided tumors were demarcated by the splenic flexure; transverse colon tumors were considered right-sided.

### CTC *ALDH1*, *PI3Kα* and *Akt-2* expression in mCRC patients and healthy donors

We first compared biomarker expression in 78 mCRC patients and 20 healthy donors. As shown in Figure 1a, the expression of *ALDH1* (69 versus 0%), *PI3Kα* (4 versus 0%) and *Akt-2* (49 versus 0%) was significantly higher in the mCRC patients compared with the

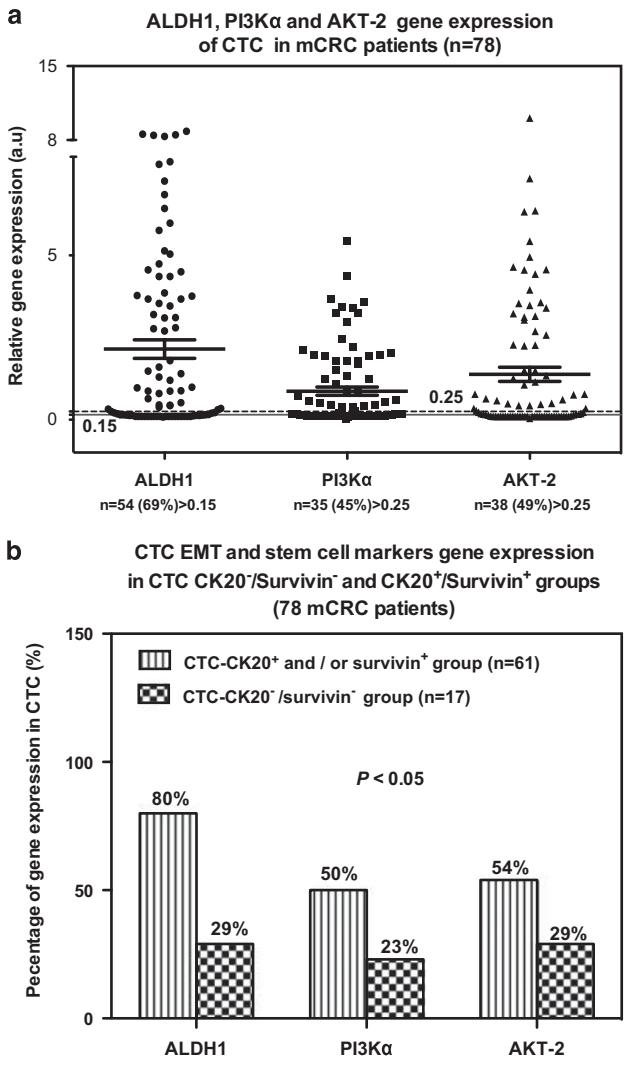


**Figure 1.** (**a**) CTC *ALDH1*, *PI3ka*, *Akt-2* gene expression levels in 78 mCRC patients. (**b**) Comparison of EMT markers, ALDH1 in mCRC patients. CTC, circulating tumor cell; EMT, epithelial–mesenchymal transition; mCRC, metastatic colorectal cancer.

healthy donors ($P < 0.001$ for all comparisons). Cutoff values for detection were provided by AdnaGen: 0.25 ng $\mu l^{-1}$ for *PI3Kα*, *Akt-2*, *Twist1* and 0.15 ng $\mu l^{-1}$ for *ALDH1*. On the basis of these cutoff points, the sensitivity and specificity for detecting CTCs by the presence of either *ALDH1*, *PI3Kα* or *Akt-2* expression was 83% and 100%, respectively. There was no significant *Twist1* gene expression detected in mCRC patients or in healthy donors (data not shown).

### CTC *ALDH1*, *PI3Kα* and *Akt-2* expression in mCRC patients

Next, we compared the expression of *ALDH1*, *PI3Kα* and *Akt-2* in patients with and without *CK20/survivin*-expressing CTCs using the AdnaGen cutoff values. Seventy-eight percent of patients ($n = 61$) had *CK20* and/or *survivin*-expressing CTCs. Among patients without *CK20* or *survivin*-expressing CTCs ($n = 17$), 29%, 23% and 29% had expression of *ALDH1*, *PI3Kα* and/or *Akt-2* markers, respectively; 55% had expression of any of three markers. Among patients without *CK20*-expressing CTCs ($n = 21$), 14, 33, 29 and 29% had expression of *survivin*, *ALDH1*, *PI3Kα* and *Akt-2*, respectively; 62% had expression of any of four markers. The expression of *ALDH1* (80 versus 29%), *PI3Kα* (50 versus 23%) and *Akt-2* (54 versus 29%) was significantly higher in patients with *CK20* and/or *survivin*-expressing CTCs relative to those with CTCs negative for both *CK20* and *survivin* expression ($P < 0.05$ for all comparisons; Figure 1b).

### Prognostic utility of CTC *PI3Kα*, *Akt-2* and *ALDH1* expression

We examined the prognostic relevance of CTC *ALDH1*, *PI3Kα* and *Akt-2* expression in the mCRC patients with and without *CK20/survivin*-expressing CTCs.

In univariate analysis, patients with positive CTC *Akt-2* expression had a significantly shorter PFS (3.0 versus 4.0 months, hazard ratio (HR) = 1.63, log-rank $P = 0.034$) compared with those without CTC *Akt-2* expression (Table 2, Figure 2a). This association maintained significance in a multivariate model accounting for baseline performance status and therapy received (standard versus experimental) after CTC measurement (HR = 1.70, log-rank $P = 0.041$). There was no significant association between CTC *Akt-2* expression and OS (Table 3). Patients with positive CTC *ALDH1* expression had a significantly shorter OS (10.0 versus 38.6 months, HR = 2.04, log-rank $P = 0.021$; Figure 2b and Table 3) and trend towards inferior PFS (3.0 versus 4.5 months, HR = 1.50, log-rank $P = 0.079$; Table 2) compared with CTC *ALDH1*-negative patients in

**Table 2.** CTC marker expression and progression-free survival (PFS) in mCRC patients

| *Gene* | n | *Median (95% CI), months* | *HR (95% CI), univariable analysis* | P-*value*[a] | *HR (95% CI), multivariable analysis*[b] | P-*value*[b] |
|---|---|---|---|---|---|---|
| *ALDH1* | | | | 0.079 | | 0.72 |
| − | 24 | 4.5 (0.9, 9.1) | 1 (reference) | | 1 (reference) | |
| + | 54 | 3.0 (2.1, 4.0) | 1.50 (0.89, 2.55) | | 0.90 (0.49, 1.63) | |
| *PI3ka* | | | | 0.10 | | 0.38 |
| − | 43 | 3.3 (2.1, 5.7) | 1 (reference) | | 1 (reference) | |
| + | 35 | 2.9 (1.5, 4.4) | 1.46 (0.90, 2.37) | | 1.25 (0.76, 2.05) | |
| *Akt-2* | | | | **0.034** | | **0.041** |
| − | 40 | 4.0 (2.1, 6.4) | 1 (reference) | | 1 (reference) | |
| + | 38 | 3.0 (1.5, 3.3) | 1.61 (0.98, 2.64) | | 1.70 (1.02, 2.84) | |
| *ALDH1/PI3ka/Akt2* | | | | **0.015** | | 0.34 |
| − | 15 | 7.7 (0.9, 14.4) | 1 (reference) | | 1 (reference) | |
| + | 63 | 3.0 (2.1, 4.0) | 1.88 (1.01, 3.51) | | 1.40 (0.70, 2.81) | |

Abbreviations: CI, confidence interval; CTC, circulating tumor cell; HR, hazard ratio; mCRC, metastatic colorectal cancer. [a]*P*-value was based on the log-rank. [b]Based on multivariable Cox regression model adjusting for ECOG (Eastern Cooperative Oncology Group) at baseline and therapy received after CTC measurement. Bold entries mean patients with positive Akt-2 expression ($P = 0.034$) or any positive CTC marker ($P = 0.015$) had a significant shorter PFS compared with those without CTC Akt-2 expression or no elevated CTC markers.

univariate analysis. However, CTC *ALDH1* expression was not significantly associated with survival in multivariate analysis. Patients with positive CTC *PI3Kα* expression trended towards a shorter PFS, but this difference was not statistically significant (2.9 versus 3.3 months, HR = 1.46, log-rank $P = 0.10$; Table 2) and did not withstand correction in multivariable analysis. There was no significant association between CTC *PI3Kα* expression and OS (Table 3).

In univariate analysis, patients with any positive CTC marker (*ALDH1*, *PI3Kα* or *Akt-2*) had a significantly inferior median PFS (3.0 versus 7.7 months, HR = 1.88, log-rank $P = 0.015$; Table 2) and OS (10.0 versus 26.8+ months, HR = 2.25, log-rank $P = 0.050$; Table 3) compared with those with no elevated CTC markers (Figure 3). These associations, however, were not significant in multivariate analysis.

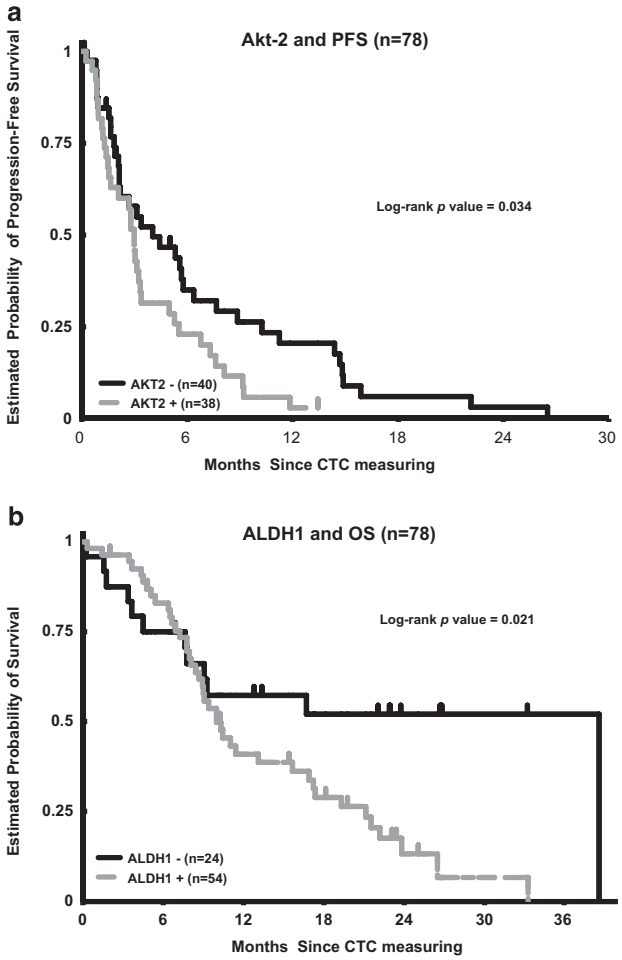


**Figure 2.** Kaplan–Meier cumulative probability of survival based on (**a**) CTC *Akt-2* and (**b**) CTC *ALDH1* gene expression. CTC, circulating tumor cell; OS, overall survival; PFS, progression-free survival.

## DISCUSSION

We evaluated the clinical relevance of *PI3Kα*, *Akt-2* and *ALDH1* gene expression in the colorectal circulating cancer cells. To our knowledge, we are the first to report a significant association between CTC *Akt-2* expression and PFS in mCRC patients receiving different standard and experimental therapeutics. Moreover, we found that patients whose CTCs do not express *CK20* or *survivin* may express *PI3Kα*, *Akt-2* and/or *ALDH1* and that this gene expression signature may serve as a clinically useful prognostic marker.

Although the EMT program is thought to be integral to the ability of CTCs to form metastases, CTCs may also revert back to an epithelial state through the mesenchymal to epithelial transition.[22] The position along which a CTC resides within the epithelial–mesenchymal axis is malleable, influenced by disease progression and cancer-directed therapy.[10] Furthermore, it has been postulated that a subpopulation of CTCs exists in a semi-mesenchymal state, exhibiting both epithelial and mesenchymal traits.[23]

On the basis of these observations, we hypothesized that incorporating both epithelial and mesenchymal genes into a multimarker model would optimize the prognostic value of CTC detection. In our cohort, 62% of patients without *CK20*-expressing CTCs were found to have expression of *survivin*, *PI3Kα*, *Akt-2* or *ALDH1*. Over half of patients whose CTCs did not express *CK20* or *survivin* expressed *PI3Kα*, *Akt-2* or *ALDH1*.

*PI3Kα* and *Akt-2* expression have been implicated in EMT and stem cell renewal[24] and have been reliably detected in CTCs.[8] In cancer cell lines, PI3Kα-Akt-2 signaling has been shown to promote cell motility, invasiveness and metastatic potential,[25] partly through its interaction with Twist1 and downstream effectors. In a landmark study by Ryachahou *et al*.,[20] the Akt-2 isoform, in particular, was found to have sustained overexpression across all stages of CRC. In Akt-2 knockout CRC mouse models, the formation of liver tumors was greatly repressed, further

**Table 3.** CTC marker expression and overall survival (OS) in mCRC patients

| *Gene* | n | *Median (95% CI), months* | *HR (95% CI), univariable analysis* | P-*value*[a] | *HR (95% CI), multivariable analysis*[b] | P-*value*[b] |
|---|---|---|---|---|---|---|
| *ALDH1* | | | | **0.021** | | 0.30 |
| − | 24 | 38.6 (7.6, 38.6) | Reference | | Reference | |
| + | 54 | 10.0 (8.4, 15.6) | 2.04 (1.05, 3.97) | | 1.45 (0.72, 2.92) | |
| *PI3kα* | | | | 0.25 | | 0.74 |
| − | 43 | 15.6 (9.4, 23.8) | Reference | | Reference | |
| + | 35 | 8.9 (7.7, 17.4) | 1.36 (0.78, 2.34) | | 1.10 (0.63, 1.93) | |
| *Akt-2* | | | | 0.72 | | 0.69 |
| − | 40 | 13.1 (8.4, 19.3) | Reference | | Reference | |
| + | 38 | 10.3 (7.8, 21.1) | 1.10 (0.64, 1.89) | | 0.88 (0.48, 1.62) | |
| *ALDH1/ PI3kα/Akt2* | | | | **0.050** | | 0.41 |
| − | 15 | 26.8+ | Reference | | Reference | |
| + | 63 | 10.0 (8.4, 16.9) | 2.25 (0.96, 5.27) | | 1.46 (0.60, 3.55) | |

Abbreviations: CI, confidence interval; CTC, circulating tumor cell; HR, hazard ratio; mCRC, metastatic colorectal cancer. [a]*P*-value was based on the log-rank test for the positive (+) or negative (−) groups of CTC ALDH1, PI3kα and Akt-2 expression. [b]Based on multivariable Cox regression model adjusting for ECOG (Eastern Cooperative Oncology Group) at baseline and therapy received after CTC measurement. Bold entries mean patients with positive ALDH1 expression ($P = 0.021$) or any positive CTC marker ($P = 0.05$) had a significant shorter OS compared with those without CTC ALDH1 expression or no elevated CTC markers.

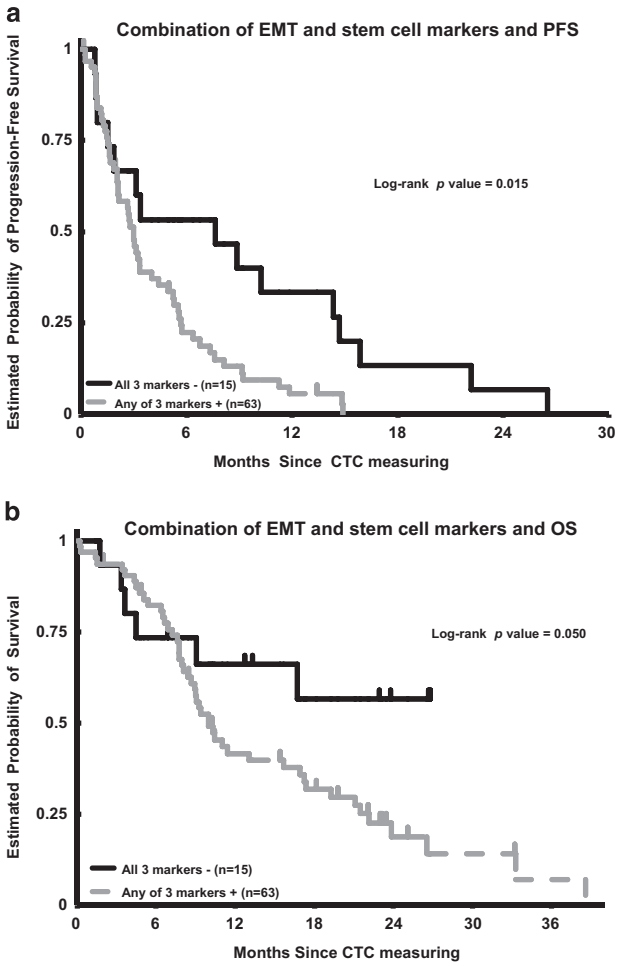


**Figure 3.** Kaplan–Meier cumulative probability of (**a**) progression-free survival (PFS) and (**b**) overall survival (OS) based on circulating tumor cell (CTC) *ALDH1*, *PI3ka*, *Akt-2* gene expression. EMT, epithelial–mesenchymal transition.

supporting an essential role for Akt-2, in conjunction with PI3K, in the development and growth of CRC metastases.[20]

Clinically, evidence from breast cancer patients supports the use of *PI3Ka*, *Akt-2* and *Twist1* as sensitive markers for CTC identification,[8] as well as predictors of response to endocrine and cytotoxic chemotherapy.[8] In our study, 47%–51% of mCRC ($n = 78$) and 52%–57% patients ($n = 70$) with *CK20/survivin*-expressing CTCs also overexpressed *PI3Ka* and/or *Akt-2*, respectively, which is comparable to findings in other metastatic cancer patients.[8] CTCs also frequently overexpress stem cell markers[24] and acquire many of their stem cell properties during the EMT, allowing them to re-seed distant organs. In CRC patients, the stem cell marker, *ALDH1*, has been associated with EMT signaling.[26] In our study, 70% of mCRC patients ($n = 78$) and 80% patients ($n = 70$) with *CK20/survivin*-expressing CTCs had *ALDH1* overexpression.

The evidence for a prognostic effect of CTC EMT-related and stem-like markers has been inconsistent, with some[8,27,28] but not all studies[29,30] demonstrating a significant relationship with disease recurrence or survival. The prognostic utility of adding stem cell (for example, CD133) to epithelial markers in advanced CRC patients was demonstrated by Linuma *et al.*,[17] though this study did not use a negative enrichment step, which may have limited assay sensitivity. In our study, patients whose CTCs expressed at least one elevated marker (*ALDH1*, *PI3Ka*, *Akt-2*) had inferior survival, suggesting that an increasingly mesenchymal or stem-like CTC phenotype may serve as a surrogate for tumor invasiveness and refractory disease to affect survival.

Our study has several limitations, the first of which is the small sample size of our retrospective cohort. Furthermore, there was heterogeneity with respect to the type of treatment received and the time point at which blood was drawn from patients, which may have limited the power of our analysis. Notably, we did not detect *Twist1* expression in any of our patients, and this may be due to *Twist1* downregulation in CD45-depleted cells. However, the expression of *PI3Ka*, *Akt-2,* or *ALDH1* was able to distinguish cancer patients from healthy controls and patients with or without *CK20/survivin*-expressing CTCs with high sensitivity and specificity. Importantly, a proportion of CTCs may lose their EpCAM expression during the EMT[30,31] and may, therefore, not be detected by our method.

In summary, we examined CTC *ALDH1*, *PI3Ka* and *Akt-2* mRNA expression on CTC and found CTC *Akt-2* expression to predict PFS in mCRC patients receiving different standard and experimental regimens. Larger prospective studies are needed to validate this gene expression model and assess its utility to predict disease progression and response to cytotoxic and targeted therapeutics.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## ACKNOWLEDGMENTS

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