Serial real-time monitoring of circulating tumour cells and cell-free DNA in blood for prognosis of oesophageal carcinoma: abridged secondary publication

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KEY MESSAGE

Longitudinal serial real-time monitoring of circulating tumour cells and cell-free DNA in blood provides supplementary prognostic information for risk stratification of patients with advanced oesophageal squamous cell carcinoma. It has potential clinical utility for non-invasive monitoring of minimal disease burden to support clinical decision for early switch to next line therapies.

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Introduction

High mortality from cancers results from development of treatment-refractory metastasis. Thus, detecting metastatic disease early is important to improve treatment. Circulating tumour cells (CTCs) are 'seeds' for metastasis. They are shed from primary/metastatic tumours into the bloodstream and comprise only a minute fraction of the total blood cells. Advancements in identification of CTCs enables non-invasive real-time monitoring of tumour molecular heterogeneity and evolving drug resistance.

The incidence of oesophageal squamous cell carcinoma (OSCC) is highest among Chinese populations. OSCC ranks seventh in mortality among Hong Kong men. Patients with OSCC are usually diagnosed at a late stage, often with metastasis. Current treatment involves chemoradiotherapy (CRT) plus surgery. Many patients with distant metastasis die within 1 year of diagnosis. Even with neoadjuvant CRT, patients with locoregionally advanced OSCC die of recurrent disease within 5 years. Treatment for metastatic OSCC involves platinum plus fluoropyrimidine as first-line treatment and docetaxel as second-line treatment.

We isolated rare CTCs from bloods of patients with OSCC to determine their use as predictive biomarkers for cancer recurrence so as to enable timely clinical decisions and improve treatments and outcomes. Clinical significance of CTC enumeration versus computed tomography (CT) and/or positron emission tomography (PET) was examined. Next-generation sequencing (NGS) was used to determine tumour progression and evolution of chemoresistance. Identification of key driver genes for metastasis and druggable targets may provide the rationale for improved diagnosis and targeted treatment of metastatic OSCC.

Methods

Patients with OSCC treated at Queen Mary Hospital between 2017 and 2020 were recruited. Their blood samples were collected for CTC enumeration. Patients with newly diagnosed OSCC underwent endoscopy with ultrasound and CT/PET. Patients with T3/4N+ disease without distant metastasis were treated with neoadjuvant CRT (with carboplatin+paclitaxel). Reassessment was performed after CRT; resectable tumours were surgically removed. Patients with metastasis underwent palliative CT. First-line treatment was platinum+fluoropyrimidine. Patients with responding or stable disease were treated for six cycles, whereas patients with progressive disease underwent alternative CT.

Blood samples were collected at diagnosis, during and after treatment, and at relapse. CTC enumeration was compared with initial/reassessment CT/PET scans. For locoregional disease, specimens taken before treatment were correlated with specimens taken at weeks 9 to 12 to assess early relapse. For metastatic OSCC, serial bloods were collected to determine the efficacy of CTCs as predictive biomarkers of CT response. Their results were correlated with imaging and reassessment results to determine CTC usefulness as predictors for second-line CT. For relapse disease, another CTC sample was taken for analysis. High CTC purity post-treatment was used for comparative NGS mutational profiling with pre-treatment specimens at diagnosis.

CTC enumeration was determined by immunofluorescence staining. The workflow has

been reported in our previous studies.[1-3] NGS was used to determine CTC genomic mutations. Our established bioinformatics pipelines were used for analysis.[3] CTC enumerations were compared between patients and healthy volunteers. Kaplan-Meier and Cox regression analyses were used for survival association. Partial response is defined as ≥30% reduction in the sum of the longest diameter of all target lesions. The pathologic tumour regression grade is predictive of disease-free survival after neoadjuvant CRT in OSCC.[4] Longitudinal data were compared between groups.

Imaging analysis was based on internal references of standardised uptake values. PET parameters (maximum standardised uptake value, metabolic tumour volume, and total lesion glycolysis) were computed. Lesions were categorised with the TNM staging system. Treatment responses were evaluated with RECIST and PRECIST criteria.

Results

In 57 patients (median age, 63 years; 86% were male) with locally advanced stage III-IV OSCC who received palliative platinum-based CT, the median time to progression at interim reassessment, progression-free survival (PFS), and overall survival (OS) were 74.5, 94, and 181 days, respectively, whereas 56%, 90%, and 70% of patients relapsed at interim reassessment, had disease progression, and died, respectively.

Baseline, pre-cycle III, post-cycle IV, end of CT, and relapse CTC enumeration data were obtained for 55, 45, 12, 14, and 11 patients with advanced OSCC, respectively. Frequencies of patients with detectable CTCs were 70.9%, 55.6%, 66.7%, 42.9%, and 54.5%, respectively. The median cell-free DNA (cfDNA) levels were 3123, 2176, 3092, 1579, and 3095 copies of haploid genome, respectively.

The mean CTC enumeration was 2.31 and 2.47 cells/5 mL blood at baseline and pre-cycle III, respectively. A cut-off point of ≥3 was chosen to examine its predictive value. Patients with ≥3 CTCs at end of cycle II had significantly higher risk of progression at interim reassessment and worse PFS and OS, compared with those with 0 to 2 CTCs (Table). Patients with 0 to 2 CTCs (low risk) had significant longer time to progression at interim reassessment and longer PFS and OS, compared with those with intermediate risk (other CTC changes) or high risk (≥3 CTCs). Patients with unfavourable changes of both high cfDNA1 (≥3.360) and cfDNA2 (≥3.2817) were categorised as high risk; others were low risk. Patients with favourable changes had significant longer OS, compared with those with unfavourable change.

Patients were categorised into four risk groups based on integration of changes of both CTC1/CTC2 and cfDNA1/cfDNA2 levels from baseline to pre-cycle III. Each specimen was categorised into high (1 mark) and low (0 mark) groups. Patients with two favourable changes were categorised as low risk (0 mark). Patients with two unfavourable changes were categorised as high risk (3 marks) and at risk (2 marks), respectively. Other combinations were categorised as at risk (1 mark). Patients in low-risk group had significantly longer time of progression, PFS, and OS, compared with other groups. CTC enumeration is useful for longitudinal serial monitoring of disease progression. Clinical assessment and PET detected disease progression with a 6-month lag time, after dramatic increase of the cfDNA level and tumour-specific somatic mutations including *TP53* mutation frequency and *MET* amplification.

In multivariate regression analysis, CTC count of ≥3 at pre-cycle III was the independent predictor for shorter time to progression at interim reassessment, whereas CTC count of ≥3 at pre-cycle III and having primary tumour resected at blood collection were independent predictors for shorter PFS, whereas age and combined unfavourable changes of CTC1/2 and cfDNA1/2 were independent predictors for shorter OS (Table).

In 37 patients with locoregionally advanced OSCC who received curative CRT/surgery (n=33) or CRT without surgery (n=4), those with ≥3 CTCs at the end of CRT/pre-operation had higher risk of early progression (hazard ratio=5.859, P=0.031), compared with those with 0-2 CTCs.

In 50 patients with advanced OSCC who received palliative CT, the percentage of usable bases on target for the buffy coat DNAs and CTC samples were 48% and 43% with mean target coverage of 1708 and 1470, respectively. Serial NGS analysis identified seven genes to be associated with poorer OS. Patients with positive mutation signature were associated with shorter OS and PFS.

Conclusion

CTC count at pre-cycle III is the independent predictor for time to progression at interim reassessment and PFS. Combined changes of CTC count and cfDNA level from baseline to pre-cycle III are independent predictors for OS. Baseline and pre-cycle III liquid biopsy can identify patients with advanced OSCC at increased risk of early-disease progression, treatment failure, and death. Prospective serial longitudinal monitoring of CTCs and cfDNAs in patients with advanced OSCC is recommended.

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TABLE. Circulating tumour cells (CTC) counts and cell-free DNA (cfDNA) levels at baseline and two cycles of chemotherapy (pre-cycle III) as predictors of time to progression at interim reassessment, progression-free survival, and overall survival

Variable	Univariate analysis		Multivariate analysis	
	Hazard ratio (95% confidence interval)	P value	Hazard ratio (95% confidence interval)	P value
Time to progression at interim reassessment (n=52)				
Age	1.003 (0.95-1.06)	0.906	-	-
Stage at blood collection: IV vs III (ref)	0.262 (0.11-0.62)	**0.002**	-	-
Lymph node metastasis: yes vs no (ref)	0.392 (0.18-0.84)	**0.016**	-	-
Baseline CTC count: ≥3 vs 0-2 (ref) [n=50]	2.072 (0.87-4.95)	0.101	-	-
Pre-cycle III CTC count: ≥3 vs 0-2 (ref) [n=42]	3.426 (1.32-8.87)	**0.011**	3.426 (1.32-8.87)	**0.011**
Baseline log cfDNA (n=39)	4.980 (0.91-27.22)	0.064	-	-
Pre-cycle III log cfDNA (n=35)	2.241 (0.77-6.53)	0.139	-	-
Progression-free survival (n=54)				
Age	0.956 (0.91-1.00)	0.062	-	-
Previous treatment: yes vs no (ref)	0.561 (0.30-1.05)	0.071	-	-
Stage at blood collection: IV vs III (ref)	0.430 (0.22-0.85)	**0.016**	-	-
Primary tumour resected at blood collection: yes vs. no (ref) [n=51]	0.373 (0.19-0.75)	**0.005**	0.402 (0.19-0.87)	**0.02**
Metastasis: yes vs no (ref) [n=40]	0.517 (0.25-1.08)	0.080	-	-
Liver metastasis: yes vs no (ref)	0.449 (0.21-0.98)	**0.043**	-	-
Baseline CTC count: ≥3 vs 0-2 (ref) [n=52]	1.349 (0.69-2.63)	0.380	-	-
Pre-cycle III CTC count: ≥3 vs 0-2 (ref) [n=43]	3.680 (1.73-7.81)	**0.001**	4.014 (1.81-8.88)	**0.001**
Baseline log cfDNA (n=47)	1.959 (0.67-5.76)	0.222	-	-
Pre-cycle III log cfDNA (n=40)	1.681 (0.70-4.06)	0.249	-	-
Overall survival (n=57)				
Age	0.940 (0.89-0.99)	**0.027**	0.932 (0.87-0.99)	**0.032**
Previous treatment: yes vs no (ref)	0.534 (0.28-1.02)	0.056	-	-
Baseline CTC count: ≥3 vs 0-2 (ref) [n=55]	0.973 (0.44-2.14)	0.946	-	-
Pre-cycle III CTC count: ≥3 vs 0-2 (ref) [n=45]	3.576 (1.63-7.84)	**0.001**	-	-
Baseline log cfDNA (n=48)	8.338 (2.42-28.7)	**0.001**	-	-
Pre-cycle III log cfDNA (n=41)	5.451 (1.74-17.1)	**0.004**	-	-
Change of cfDNA1/2 (n=40)				
Two favourable changes with 0-1 mark (other combinations) [n=21]	Reference	-	-	
One favourable change with 1-3 marks (high cfDNA1 & cfDNA2) [n=19]	4.444 (1.85-10.68)	**0.001**		
Change of CTC1/2 (n=43)				
Two favourable changes with 0-1 mark (low CTC1 & CTC2) [n=22]	Reference	**0.012**	-	-
One favourable change with 1-3 marks (other combinations) [n=16]	3.103 (1.11-8.65)	0.089		
Two unfavourable changes with 3 marks (high CTC1 & CTC2) [n=5]	6.178 (1.87-20.4)	**0.004**		
Combined changes of CTC and cfDNA (n=44)				
Two favourable changes with 0-1 mark (n=12)	Reference	**0.001**		**0.002**
One favourable change with 1-3 marks (n=22)	3.103 (1.11-8.65)	**0.030**	6.008 (1.27-28.5)	**0.024**
Two unfavourable changes with 3 marks (n=8)	6.178 (1.87-20.4)	**0.003**	9.520 (1.81-50.0)	**0.008**
Two unfavourable changes with 4 marks (n=2)	53.07 (7.02-401)	**1.2×10^{-4}**	81.958 (7.95-845)	**2.2×10^{-4}**

Disclosure

The results of this research have been previously published in:

1. Ko JMY, Ng HY, Lam KO, et al. Liquid biopsy serial monitoring of treatment responses and relapse in advanced esophageal squamous cell carcinoma. Cancers (Basel) 2020;12:1352.
2. Ko JMY, Lam KO, Kwong DLW, et al. Circulating tumor cell enumeration for serial monitoring of treatment outcomes for locally advanced esophageal squamous cell carcinoma. Cancers 2023;15:832.

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