Circulating Tumor Cells Predict Prognosis Following First-Generation EGFR-TKI Treatment in EGFR- and TP53-Mutant Non-Small Cell Lung Cancer

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Research

Keywords: Gefitinib, Icotinib, Erlotinib, Circulating tumor cells, NSCLC

Posted Date: November 24th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-107218/v2

Abstract

Background: First-generation EGFR-TKIs have become the first-line standard treatment for advanced non-small cell lung cancer (NSCLC) with EGFR mutations. This study isolates and quantifies circulating tumor cells (CTCs) and evaluates patient prognosis before and after first-line treatment with EGFR-TKIs in advanced NSCLC with EGFR and TP53 mutation.

Methods: Patients with advanced NSCLC with EGFR and TP53 mutation were treated with a first-generation EGFR-TKI using a standard daily dose. Continuous blood samples were collected at baseline (CTCs-d0) and 28 days (CTCs-d28), and the isolation by size of tumor cells (ISET) method was used to detect CTCs.

Results: The CTCs results were divided into favorable (< 5 CTCs) and unfavorable (≥ 5 CTCs) groups. The median progression-free survival (PFS) of patients in the favorable group was significantly longer at baseline compared to those in the unfavorable group (15 vs 7.5 months; p = 0.0055). After 28 days of treatment with first-generation EGFR-TKI, the PFS of patients in the favorable group was 12.5 months, which was significantly longer than the median PFS of 7 months in the unfavorable group (p = 0.0003). After treatment, the PFS of patients with reduced CTCs was significantly better than those with no significant change in CTCs (9 months vs 6 months, p = 0.014). In univariate and multivariate analysis, patients with CTCs-d0 ≥ 5 and CTCs-d28 ≥ 5 had significantly lower PFS when compared to those with CTCs-d0 < 5 and CTCs-d28 < 5, respectively.

Conclusion: This study confirmed for the first time that CTC count is closely correlated with prognosis in EGFR- and TP53-mutant advanced NSCLC following first-line treatment with first-generation EGFR-TKIs.

Background

Lung cancer has the highest incidence and mortality of any malignancy(1). Approximately 85% of lung cancer patients have non-small cell lung cancer (NSCLC), for which traditional chemotherapy has limited efficacy and the 5-year survival rate is less than 15%(2). Molecular therapies targeting different driver genes, especially epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKI), have become standard treatment for advanced NSCLC(3). Almost all patients become resistant to targeted therapy within 1 year(4). Compared with single mutation tumors, NSCLC patients with multiple somatic mutations in the same tumor have a worse prognosis(5). TP53 is the most commonly mutated gene in NSCLC(6, 7). Co-mutation of EGFR and TP53 may be related to EGFR-TKI resistance as well as shorter progression-free survival (PFS) and overall survival (OS)(8-10).

Current approaches to evaluate the efficacy of targeted therapy for NSCLC mainly include imaging examinations and serum tumor markers, and all approaches have limitations. Studies have shown that circulating tumor cells (CTCs) are related to efficacy of EGFR-TKI in advanced NSCLC(11, 12). However, the efficacy of first-generation EGFR-TKIs (gefitinib, erlotinib, and icotinib) in advanced NSCLC with co-mutation of EGFR/TP53 remains unclear.

This study analyzed CTCs counts of 31 cases of advanced NSCLC with EGFR/TP53 co-mutation in which patients received first-line EGFR-TKI treatment. and explored the evaluation of CTCs in the first-line EGFR-TKI treatment of patients with EGFR/TP53 co-mutation in advanced NSCLC.

Methods

Patients and treatments

This research complies with the principles of the Declaration of Helsinki and includes 31 patients with advanced NSCLC who were treated in Jiangsu Province Hospital from October 2012 to May 2019. Inclusion criteria: (1) at least 18 years old, with complete clinical data; (2) non-squamous stage IIIB NSCLC confirmed by histology or cytology with pleural effusion or stage IV NSCLC and no previous treatment history for lung cancer; (3) detection of EGFR and TP53 gene mutations. Exclusion criteria: (1) breastfeeding or pregnant patients; (2) patients with obvious cognitive impairment; (3) patients with uncontrollable infection. The study was approved by institutional ethics committee of Jiangsu Province Hospital.

CTC analysis

A 6 ml blood collection tube was used to collect a blood sample from the median cubital vein. Immediately following blood collection, the tube was gently inverted and mixed eight times to fully mix the blood with anticoagulant. The specimen was stored and further processed within 24h. Based on differences in size and deformability of abnormal cells and blood cells, abnormal cells were enriched by membrane filtration. The blood sample was pre-processed and transferred to a cell filter. The blood in the cell filter was passed through the filter membrane through the abnormal cell separation staining instrument, where abnormal cells gathered on the surface of the filter membrane to enrich CTCs. The Romanowsky staining technique was used to stain the enriched CTCs with an abnormal cell separation staining instrument. The stained filter membrane was removed, fixed on a glass slide, and incubated at 50°C for 30 minutes. After the filter membrane was dry, an appropriate amount of neutral resin mounting tablets were added and the filter was covered with glass. The whole filter was observed under a microscope and counted.

Evaluations of clinical response and progression free survival (PFS)

First-generation EGFR-TKIs were administered for at least 1 month; the longest treatment duration was 31 months; the average treatment duration was 11 months. Dosing was as follows: gefitinib 250 mg per os quaque die (po qd); erlotinib (150 mg po qd); and icotinib [125mg po ter in die (tid)]. CTC number was measured before treatment and again 28 days after treatment with gefitinib, erlotinib, or icotinib. According to the Response Evaluation Criteria in Solid Tumors (RECIST) 1.0, complete response (CR) and partial response (PR) were considered to be effective in imaging examinations; CR, PR and stable disease (SD) were considered to be controlled. PFS was calculated from the first day of treatment to the date of PD.

Statistical analysis

R language (version 4.0.2) and Statistical Package for the Social Sciences (SPSS; version 20.0) were used for data analysis. Chi-square test was used to evaluate categorical variables, and Kaplan-Meier and Cox regression analyses were used for survival analysis. $P < 0.05$ was considered statistically significant.

Results

From October 2012 to May 2019, 31 patients were enrolled in this trial. The baseline status of patients is shown in Table 1. All patients showed disease progression; 16 patients had baseline CTC count (CTCs-d0) ≥ 5 and 15 patients had CTCs-d0 < 5. Male patients and those with curative effects may have CTCs counts ≥ 5, and there was no significant correlation between different baseline CTC counts and other clinical characteristics. Table 1 shows the prevalence of CTCs and the clinical characteristics of patients before treatment.

Patients were divided into a favorable prognostic group (CTCs-d0 = 0–4, n = 15) or an unfavorable prognostic group (CTCs-d0 ≥ 5, n = 16). The median PFS of the favorable group was 15 months, which was significantly longer than the PFS of 7.5 months observed in the unfavorable group [hazard ratio (HR): 0.418, 95% CI: 0.195–0.896, p = 0.0055; Figure 1). Figure 2 summarizes the changes in the number of CTCs from day 0 to day 28 of treatment. Among the 15 patients with CTCs-d0 = 0–4, two patients had CTCs on day 28 (CTS-d28) ≥ 5, and 13 patients had CTCs-d28 = 0–4. Among the 16 patients with CTCs-d0 ≥ 5, six had CTCs-d28 = 0–4, and 10 had CTCs-d28 ≥ 5. Compared with the 10 patients with unchanged CTC counts (6 months, p = 0.014) in the unfavorable group, the median PFS of the six patients showing a decrease in the number of CTCs was 9 months. These patients showed significantly higher CTC counts than the unfavorable group. This suggests that a change in CTC count before and after treatment may predict PFS (9 months vs 6 months, p = 0.014; Figure 3). The CTC count and PFS analysis after treatment suggests that the PFS of the favorable prognosis group (CTCs-d28 = 0–4, n = 20) was significantly higher than that of the poor prognosis group (CTCs-d28 ≥ 5, n = 11) [7 months vs 12.5 months, hazard ratio (HR): 0.317, 95% CI: 0.118-0.853, p = 0.0003; Figure 4).

In univariate analysis, CTCs-d0 and CTCs-d28 were significantly correlated with PFS (Table 2). In the multivariate analysis, CTCs-d28 ≥5 was the most important prognostic factor among all poor prognostic indicators, which indicates that the favorable group had a lower risk of disease progression (HR: 0.285, 95% CI: 0.121-0.672, p = 0.004, Table 2).

Discussion

During the proliferation and development of malignant tumors, tumor cells change their cell phenotype through epithelial-mesenchymal transition, fall off from the primary tumor or metastasis, and invade the peripheral circulation to form metastatic CTCs(13).In this study, we used five CTCs per sample as a threshold, and there were fewer patients with CTCs-d0 < 5 compared to those with CTCs-d0 ≥ 5. In 2012,

Krebs et al.(14) used cell search technology to release CTCs from 101 patients with stage III-IV NSCLC, and reported that the number of CTCs in stage IV patients was higher than that in stage III patients. The cut-off value of CTCs ≥ 5 is associated with shorter PFS and OS. In addition, a decrease in the number of CTCs after a cycle of standard chemotherapy may correlate with better PFS and OS. Another study conducted by Tanaka et al. found that patients with metastatic NSCLC had a higher number of CTCs than that non-metastatic patients. These findings indicate that the number of CTCs may be related to cancer stage in NSCLC patients(15). First-generation EGFR-TKIs are the first-line treatment for advanced NSCLC patients with EGFR mutations. However, drug resistance limits the use of first-generation EGFR-TKIs. Studies have shown that CTC count is negatively correlated with the efficacy of EGFR-TKI treatment. With the launch of the next-generation sequencing platform, studies have shown that TP53 mutations in EGFR-mutated NSCLC range from 30% to 60% prevalence(9). When treated with a first-generation EGFR-TKI, patients with EGFR/TP53 double mutations, especially those with missense mutations, exhibit a lower effective rate and PFS compared to advanced NSCLC patients without TP53 mutations(8, 16). Predicting efficacy of first-generation EGFR-TKIs is crucial for the treatment and prognosis of NSCLC patients with EGFR/TP53 co-mutations.

In recent years, CTC count and peripheral blood gene expression data have been used to guide the clinical treatment of NSCLC. Punnoose et al.(17) reported that in EGFR-TKI-treated NSCLC patients, the genome expression of CTCs was highly consistent with that of the primary tumor tissue. Maheswaran et al.(18, 19) reported that EGFR, EGFR T790M, MET, and other gene mutations in CTCs can be used to evaluate the treatment effect and prognosis of NSCLC patients.

Common CTC detection methods include the isolation by size of tumor cells (ISET) method, the Cell SearchTM system, reverse transcription polymerase chain reaction, CTC-chip, and more. These technologies can significantly improve the sensitivity and specificity of CTC detection. ISET is a high-speed cell analysis and sorting technique. Because it is simple, reliable and fast, ISET has been selected as the main method for detecting CTCs(20).

In this study, ISET was used to count CTCs in the peripheral blood of patients with advanced NSCLC. For the first time, this study demonstrated that in NSCLC patients with EGFR/TP53 mutations, low CTC count was correlated with better PFS after first-line treatment with first-generation EGFR-TKIs. The improved PFS was most obvious in patients with reduced CTC count after treatment.

The median PFS of the low CTC group was significantly higher than that of the high CTC group both before and after treatment with a first-generation EGFR-TKI ($P<0.01$). These data indicate that CTC count is closely related to prognosis of advanced NSCLC in patients with EGFR/TP53 mutations following treatment with a first-generation EGFR-TKI. This study is not without shortcomings; the small sample size must be expanded to further confirm these findings.

Conclusion

CTC counts can be used as an index to predict the efficacy of first-line EGFR-TKI treatment in patients with advanced NSCLC with EGFR/TP53 double mutation. Prognosis following treatment with first-generation EGFR-TKIs is closely related to CTC count.

Abbreviations

NSCLC: non-small cell lung cancer

CTCs: circulating tumor cells

EGFR: epidermal growth factor receptor

EGFR-TKI: epidermal growth factor receptor tyrosine kinase inhibitors

PFS: progression-free survival

OS: overall survival

HR: hazard ratio

Declarations

Ethical Approval and Consent to participate

The study was approved by institutional ethics committee of Jiangsu Province Hospital.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are available from the corresponding author on reasonable request.

Competing interests

The authors have no relevant competing interests.

Funding

The National Natural Science Foundation of China (81572262)

The Jiangsu Province's Key provincial Talents Program (ZDRCA2016028)

The 333 high class Talented Man Project (BRA2016516)

The Natural Science Foundation of the Jiangsu Higher Education Institution of China (18KJB320006)

Authors' contributions

Gao Wen and Liu Ping designed research; He Jing, Xu Jing and Zhang Wei performed research and analyzed data; Qian Huizhu and Xu Jing collected the patients' samples; He Jing and Xu Jing wrote the paper.

Acknowledgements

This work was supported by the National Natural Science Foundation of China(81572262), the Jiangsu Province's Key provincial Talents Program (ZDRCA2016028), the 333 high class Talented Man Project (BRA2016516), the Natural Science Foundation of the Jiangsu Higher Education Institution of China(18KJB320006). We would like to thank all the participants recruited for this study.

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Tables

Table 1 Clinicopathological features of 31 patients before EGFR-TKI treatment

Clinicopathological features	n (n=31) n (%)	CTCs-d0 ≥5 (n=16) n (%)	0-4 (n=15) n (%)	p
Age				
≥60y	19（61.3）	9（47.4）	10（52.6）	0.552
<60y	12（38.7）	7（58.3）	5（41.7）	
Gender				
Male	15（48.4）	11（73.3）	4（26.7）	0.019
Female	16（51.6）	5（31.2）	11（68.8）	
Tumor site				
Left	12（38.7）	6（50.0）	6（50.0）	0.886
Right	19（61.3）	10（52.6）	9（47.4）	
EGFR status				
Exon 19del	19（61.3）	10（52.6）	9（47.4）	0.886
Exon 21 L858R	12（38.7）	6（50.0）	6（50.0）	
Treatment response				
PR	15（48.4）	5（33.3）	10（66.7）	0.049
SD	16（51.6）	11（68.8）	5（31.2）	
Lung metastasis				
Yes	17（54.8）	8（47.1）	9（52.9）	0.576
No	14（45.2）	8（57.1）	6（42.9）	
Bone metastasis				
Yes	16（51.6）	10（62.5）	6（37.5）	0.210
No	15（48.4）	6（40.0）	9（60.0）	
Brain metastasis				
Yes	12（38.7）	8（66.7）	4（33.3）	0.183
No	19（61.3）	8（42.1）	11（57.9）	
Others metastasis				
Yes	16（51.6）	10（62.5）	6（37.5）	0.210
No	15（48.4）	6（40.0）	9（60.0）	

EGFR-TKI				
Gefitinib	23（74.2）	12（52.2）	11（47.8）	0.561
Erlotinib	7（22.6）	4（57.1）	3（42.9）	
Icotinib	1（3.2）	0（0.0）	1（100.0）	

Table 2. Univariate and multivariate analysis for PFS

Covariates	Univariate analysis			Multivariate analysis		
	HR	95% CI	p value	HR	95% CI	p value
CTCs-d0	0.350	0.163-0.754	0.007	0.405	0.180-0.912	0.029
CTCs-d28	0.246	0.108-0.561	<0.001	0.285	0.121-0.672	0.004
EGFR status	1.081	0.512-2.280	0.839			
EGFR-TKI	0.860	0.432-1.712	0.667			
Age	0.775	0.370-1.627	0.501			
Gender	0.742	0.360-1.528	0.418			
Tumor site	0.981	0.466-2.065	0.959			
Treatment response	1.313	0.621-2.775	0.476			
Lung metastasis	0.917	0.442-1.901	0.815			
Bone metastasis	0.647	0.303-1.379	0.259			
Brain metastasis	0.800	0.381-1.680	0.556			
Others metastasis	0.738	0.357-1.525	0.412			

Figures

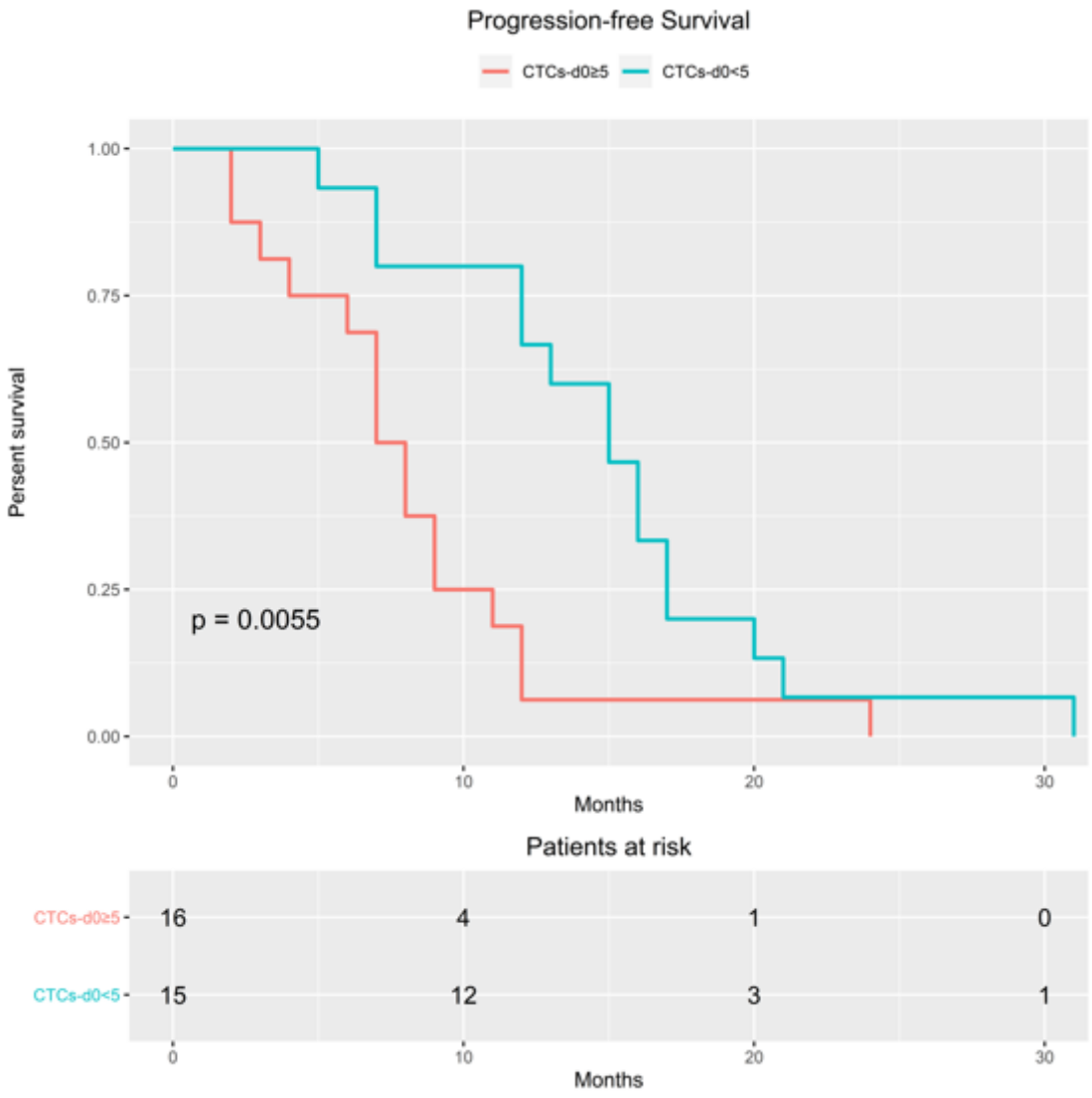
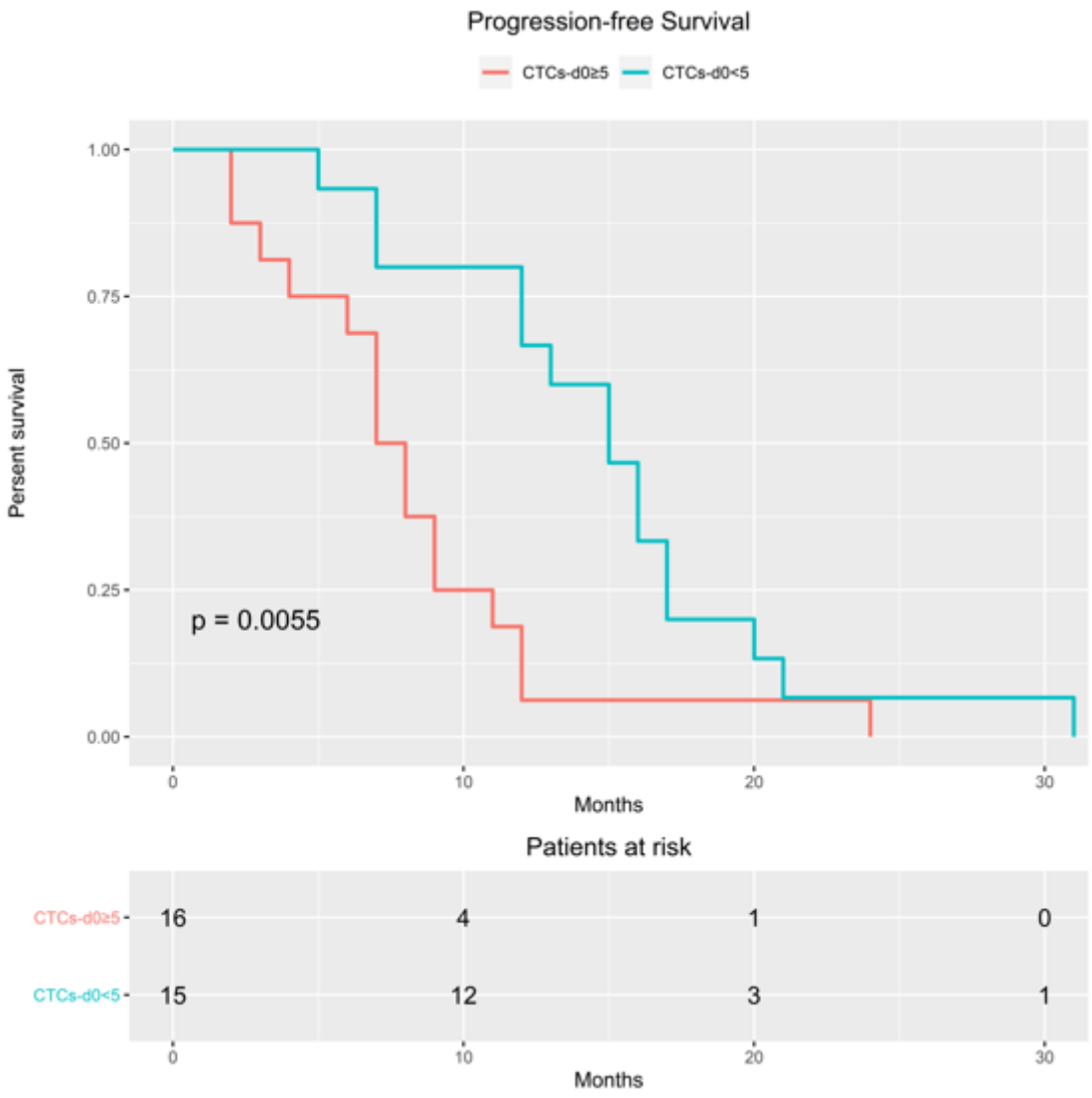


Figure 1

PFS according to CTCs count at baseline.



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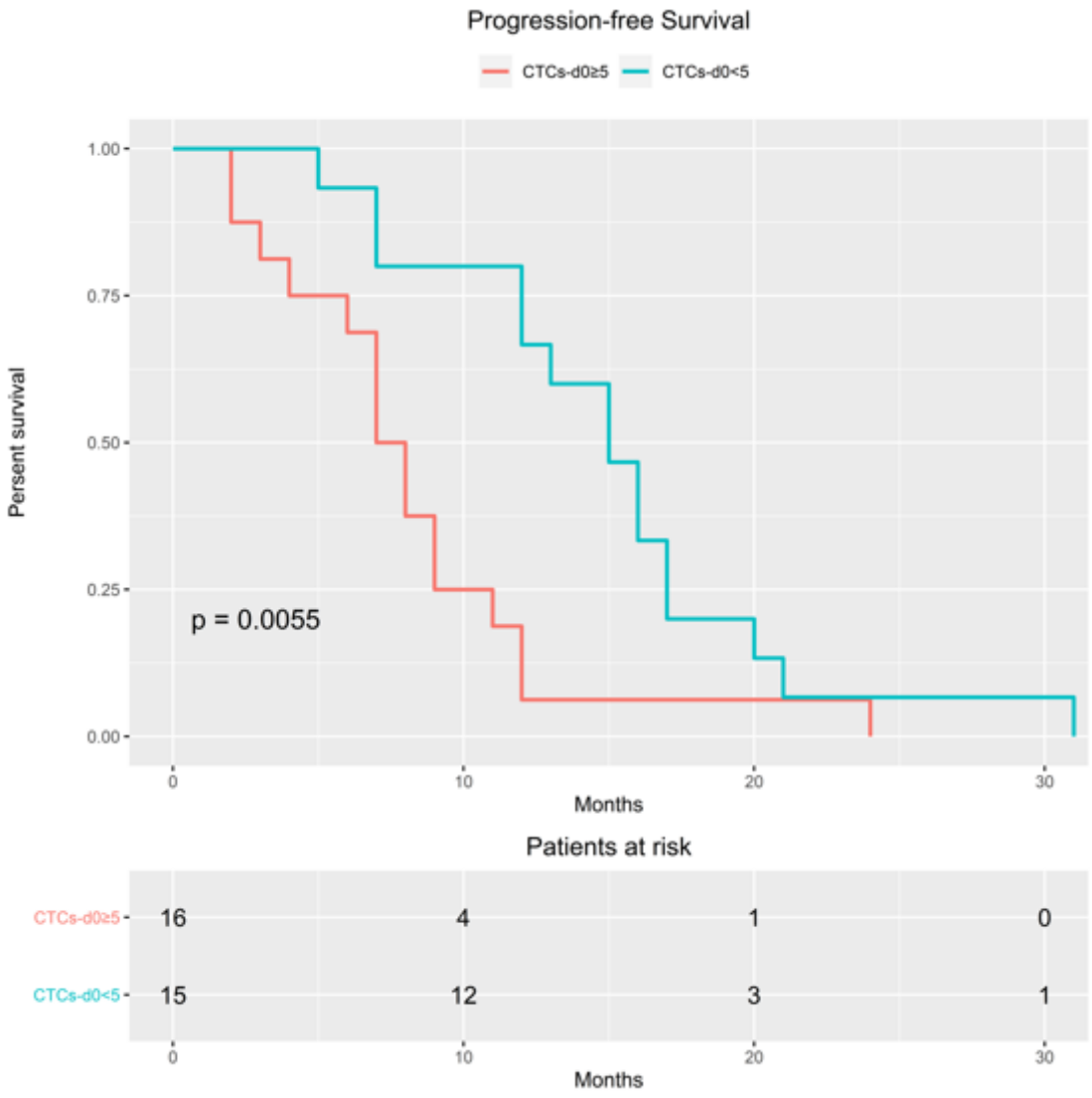

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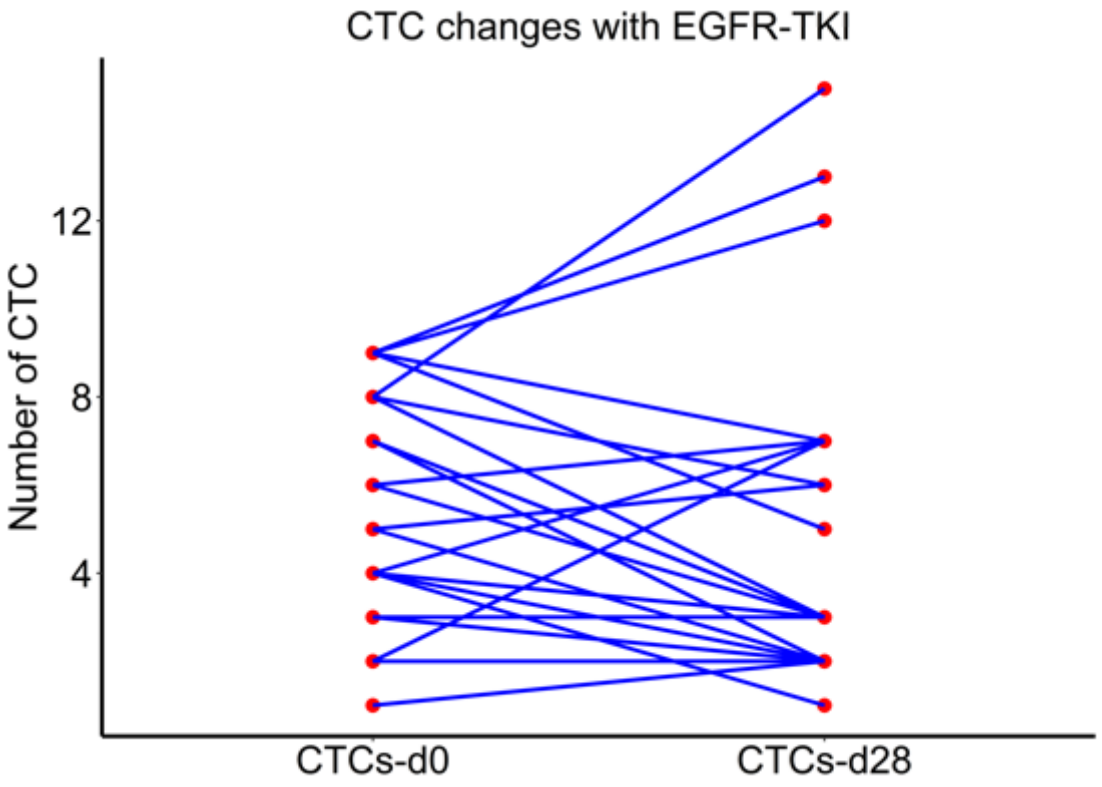
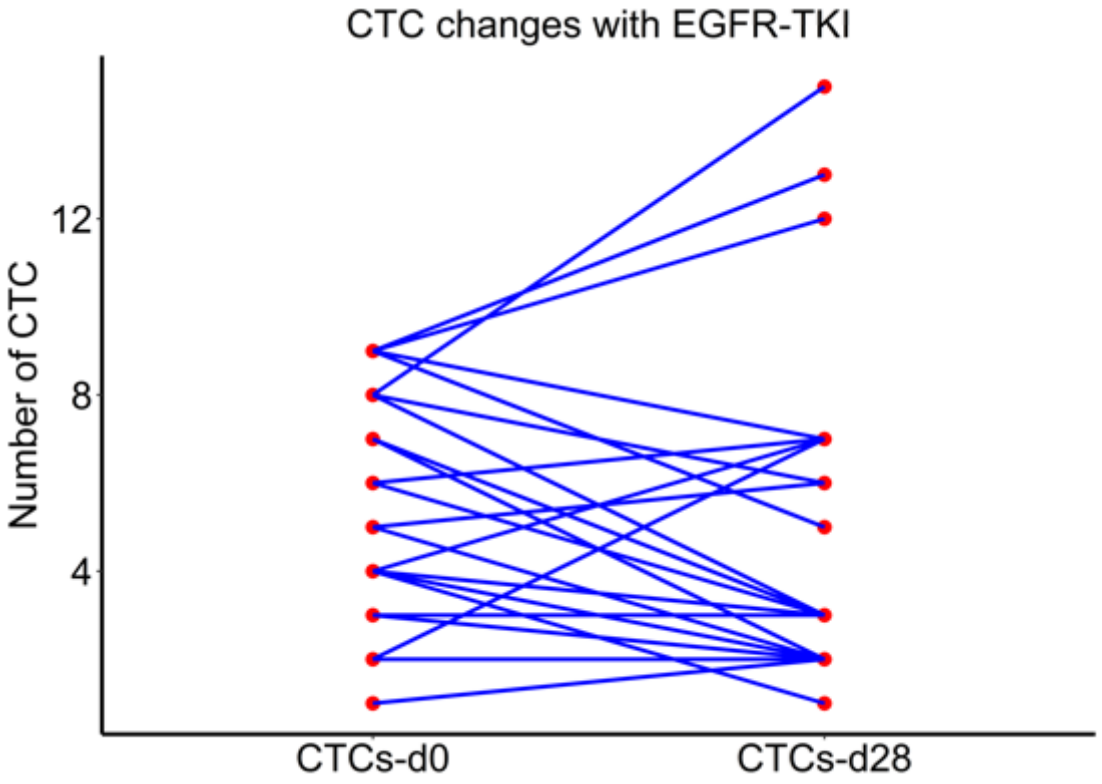


Figure 2

CTCs changes with EGFR treatment for 28 days.



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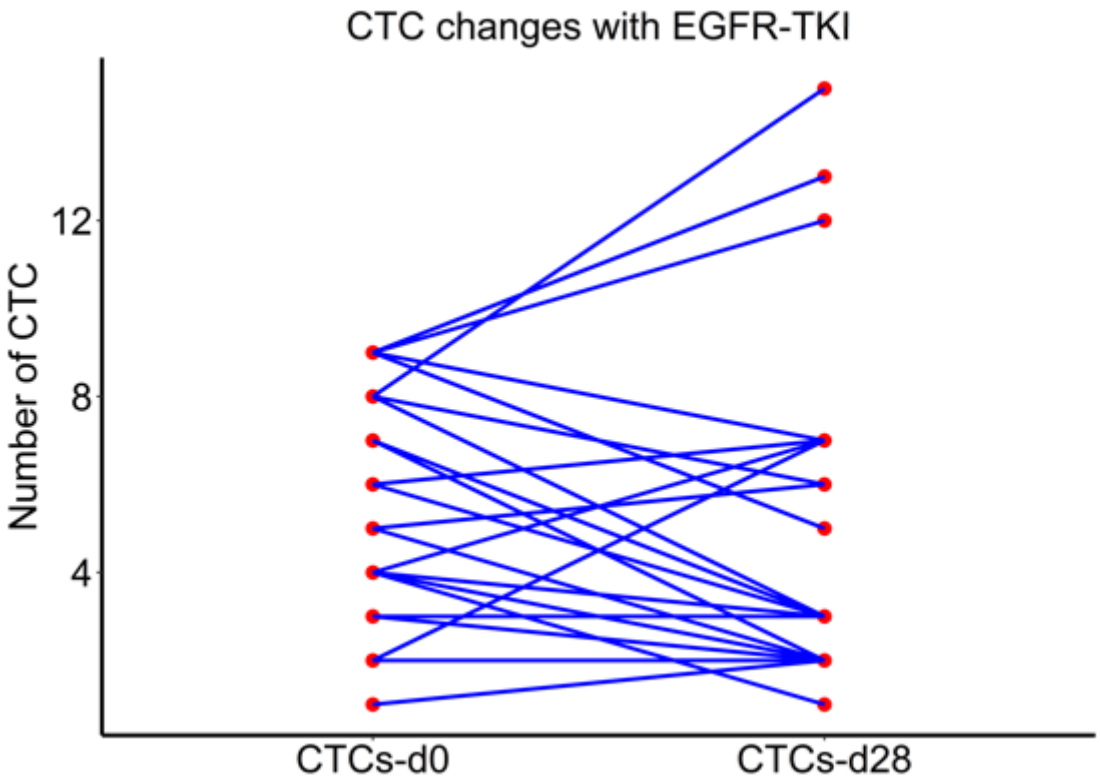

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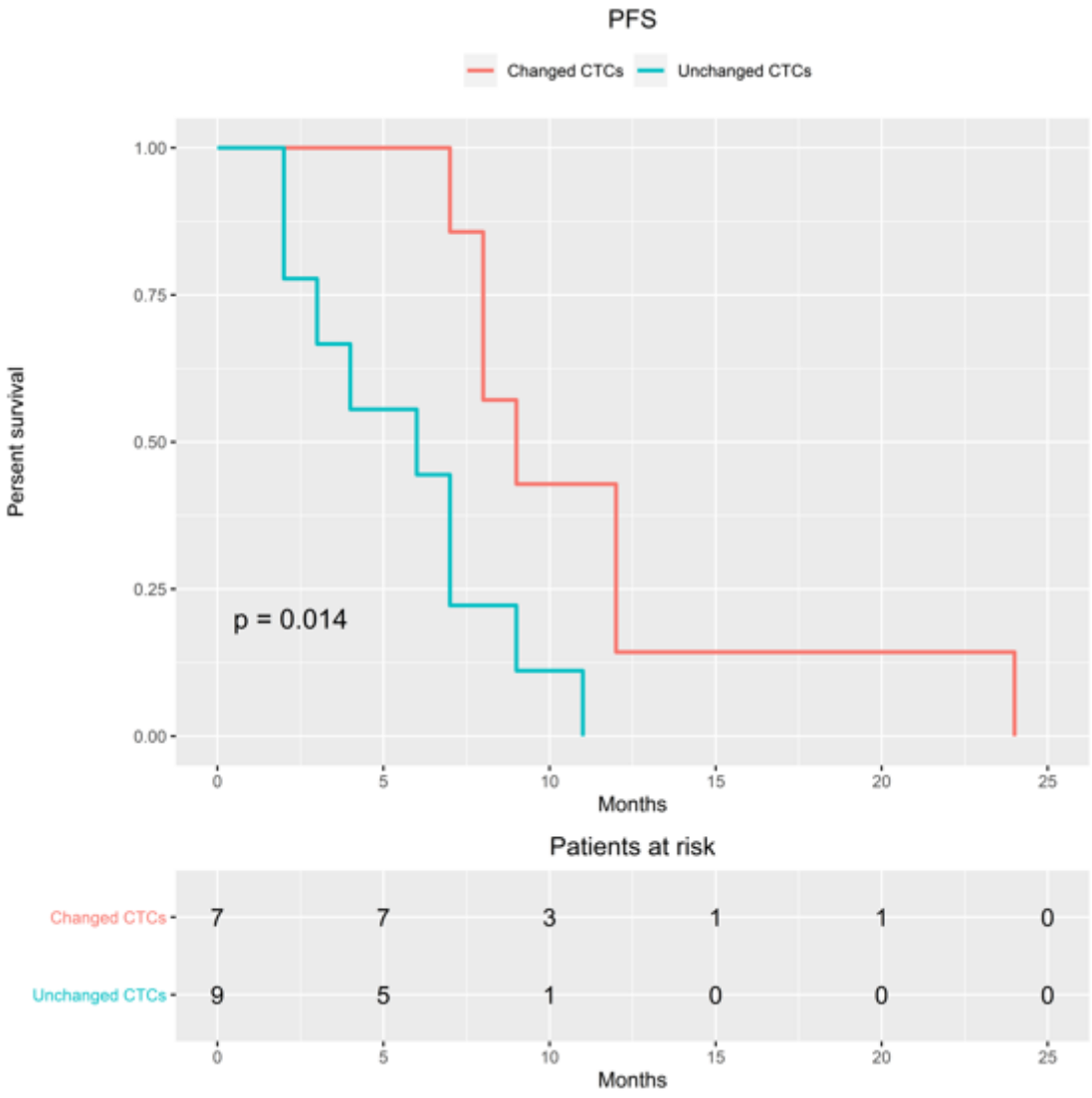
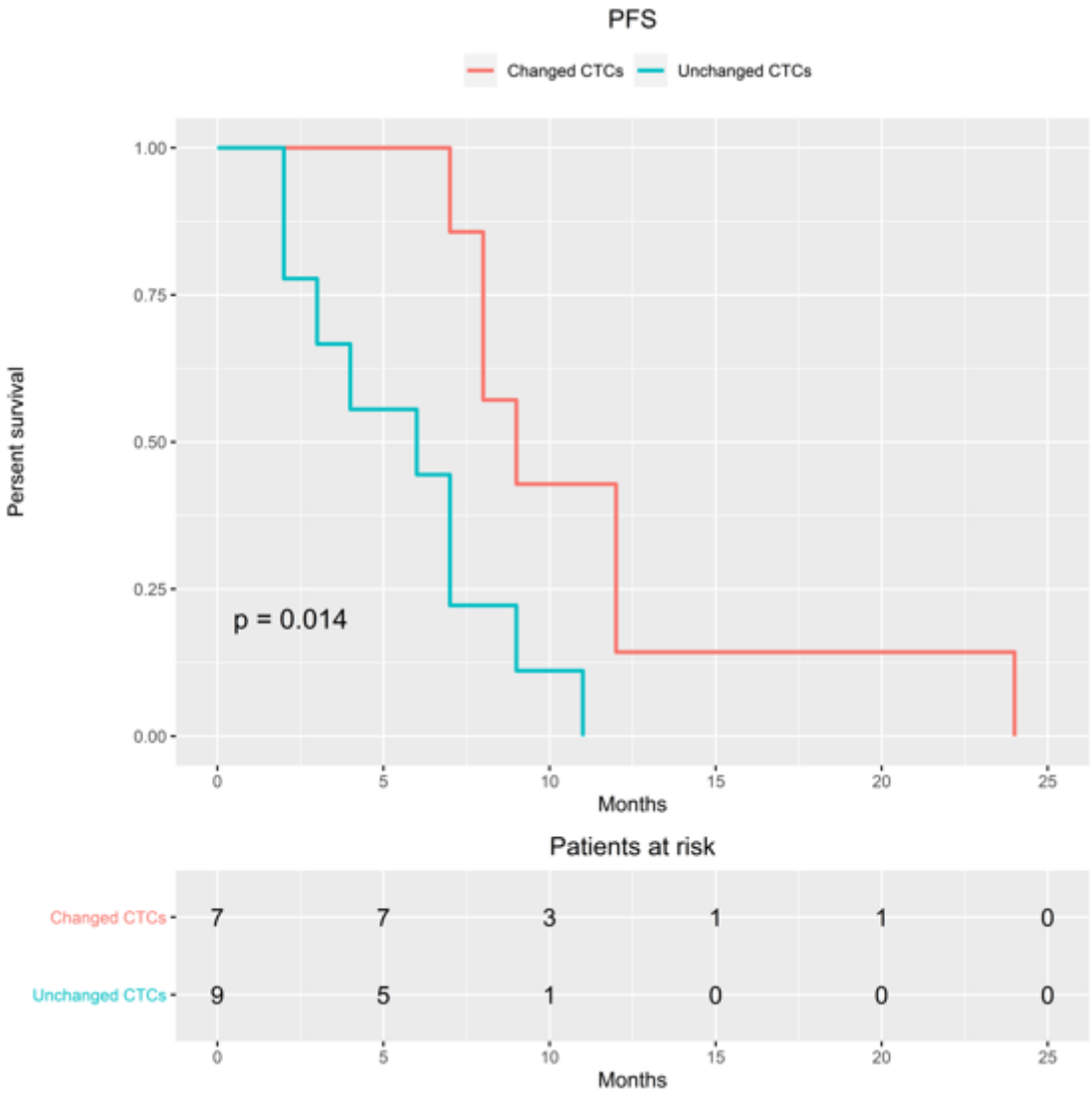


Figure 3

CTCs changes and PFS after EGFR-TKI treatment.



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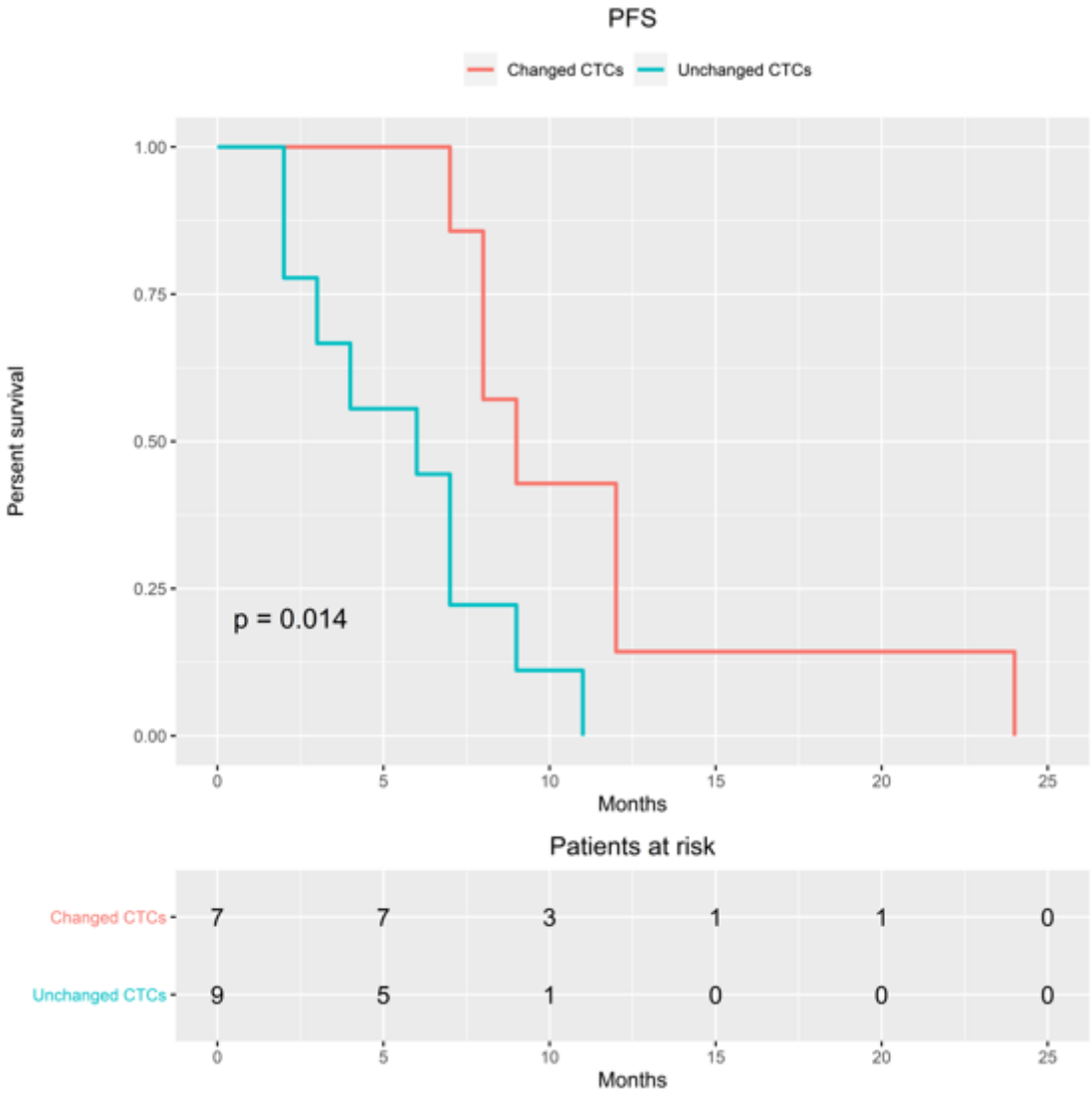

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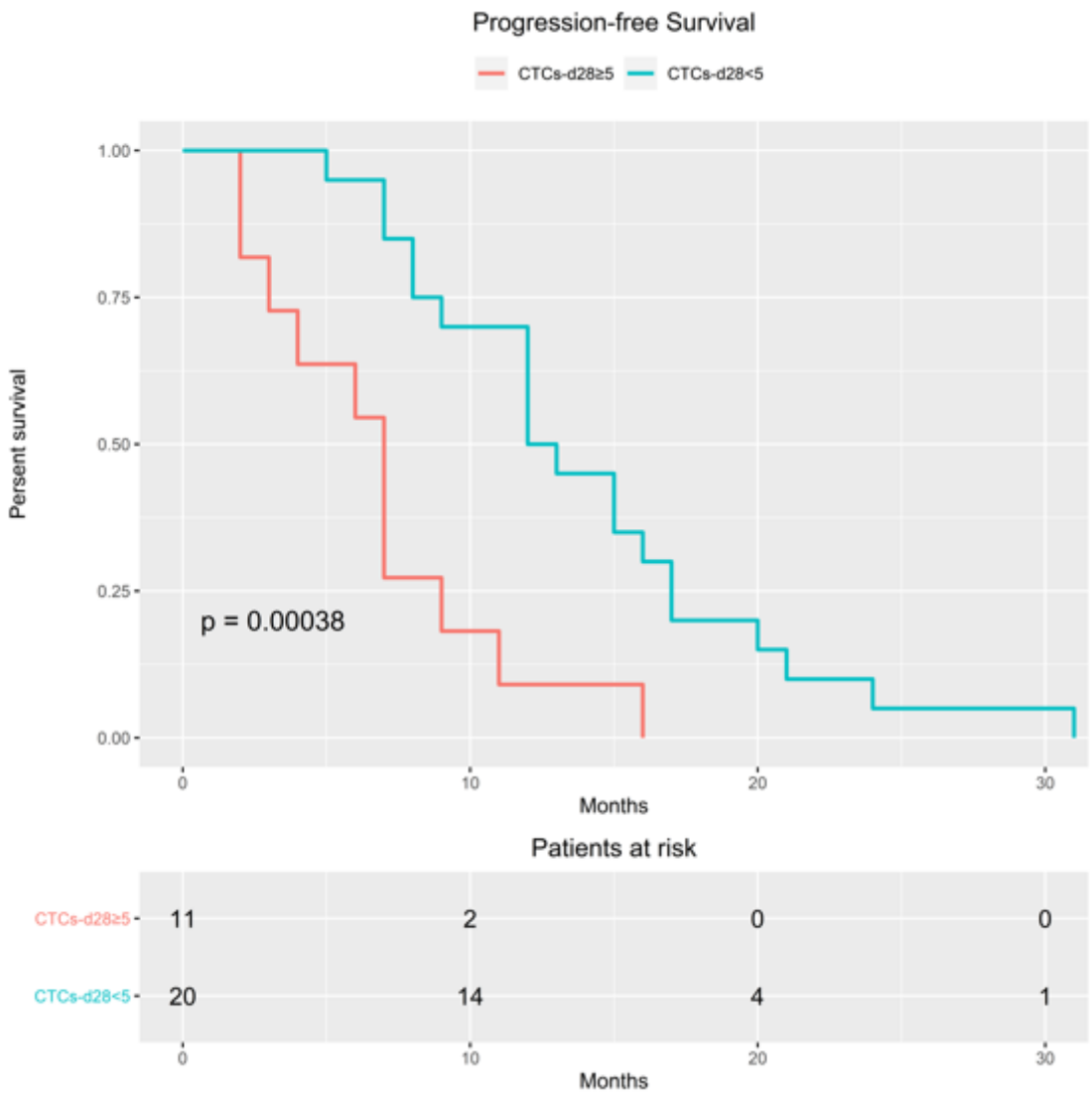
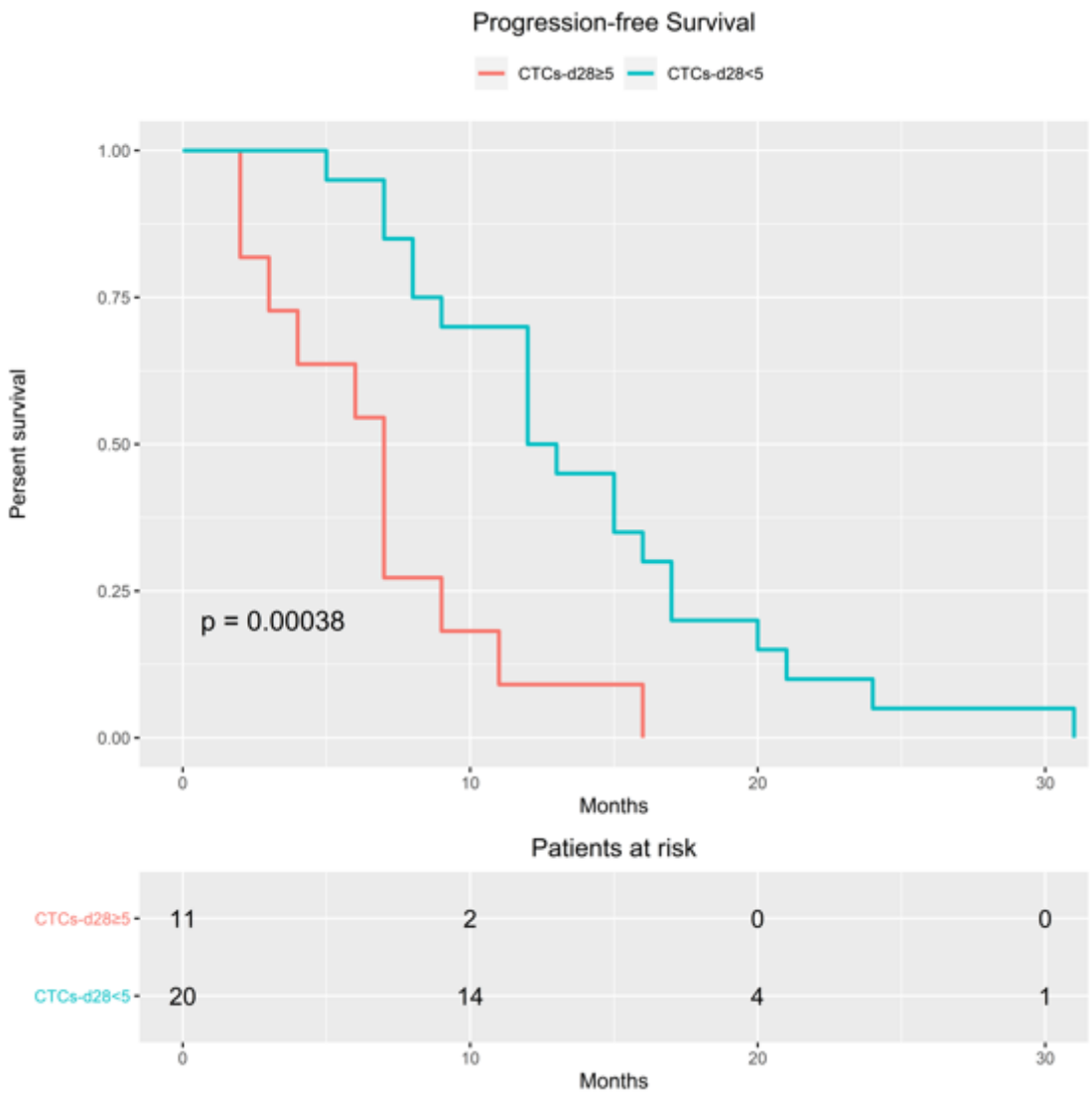


Figure 4

PFS according to CTCs on day 28.



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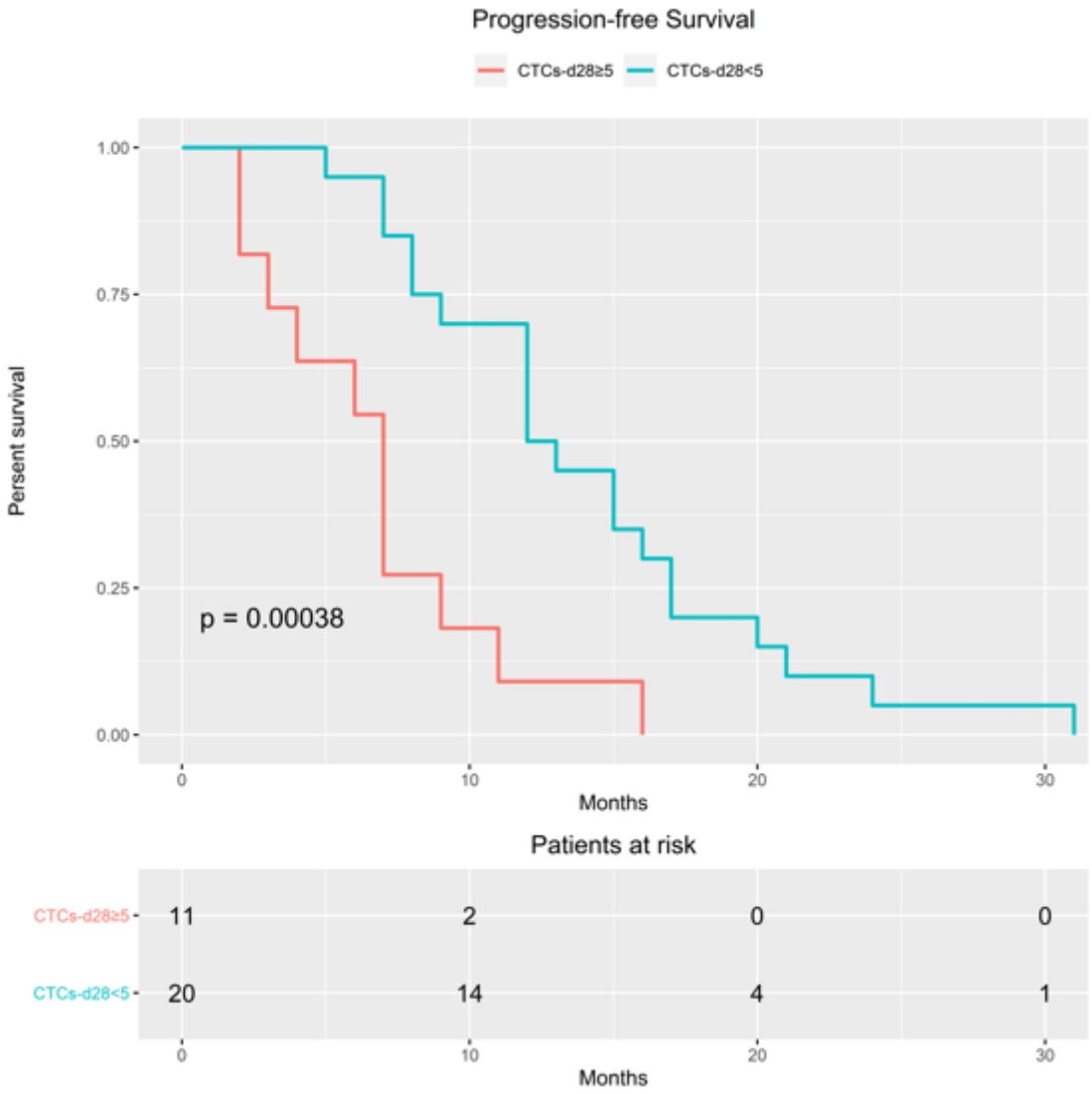

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