Peer Review File
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Comment 1: small typo on page 7 line 6: version 1.1). (21) (citation before the point?)

Reply 1: According to the Vancouver reference style and the reviewer`s advises, we have modified citation numbers to follow behind the previous word before a period or comma (see Page 7, line 3). The same reference was described in Page 8, line 10 with the modified sequence.

Changes in the text: Page 7, line 3 and Page 8, line 10

Comment 2: possibly add reference for the CD-FAST showing validity

Reply 2: In several experimental studies, CD-FAST could achieve highly sensitive, selective, rapid isolation of viable CTCs, and this method could provide uniform, clog-free, ultrafast cell enrichment with pressure drops much less than in conventional size-based filtration (ScreenCell). We added two references in discussion section (see Page 15, line 5-7).

Changes in the text: Page 15, line 5-7

Comment 3: statistical analysis add criteria for the cut off value

Reply 3: We described the criteria for determination of the optimal cut-off values (see Page 10, line 1-2), and also added the name of statistical package used in that analysis (see Page 10, line 12-13).

Changes in the text: Page 10, line 1-2 and line 12-13

Comment 4: DCB was defined as survival without disease progression at six months. How were patients classified who had radiological progression, but clinicians continued to treat, with possible succes? How was this succes defined?

Reply 4: In this study, clinical responses to the treatment were defined according to RECIST version 1.1, but patients in whom the investigator expected further clinical benefit could continue treatment beyond radiological disease progression (see Page 8, line 6-8). Thus, if a patient has been alive for six months and continues to receive ICI, he or she can be classified as DCB, despite the radiologic progression. However, in this study, there was no case who was

treated beyond initial radiologic progression.

Changes in the text: No change

Comment 5: page 11: counts were significantly different at C2-C4 apperently. delta also of importance? Please add at C1 to make clear that at baseline there was no difference.

Reply 5: The median CTC count at C1 was not significantly different based on ICI treatment response (5.0 for DCB vs. 4.6 for NDB; p=0.935), and we described the result in Page 11, line 12.

Changes in the text: No change

Comment 6: missing power analysis. are the 24 patients with cfDNA sufficient? Why so few patients in this group?

Reply 6: As the reviewer commented, the number of patients (n=24) was insufficient to draw out a meaningful outcome. We had planned to collect plasma samples from all patients at every cycle in the early stages of the study. However, cfDNA analysis could not be performed in a considerable number of patients, probably due to shortage in the amount of collected blood to obtain plasma and perform CTC and blood cell analysis at the same time. We add this limitation of the analysis in discussion section (see Page 18, line 7-8).

Changes in the text: Page 18, line 7-8

Comment 7: multivariate=multivariable

Reply 7: We have modified our text as advised in several points of the manuscript.

Changes in the text: Page 10, line 10 / Page 13, line 5,7,16 / Page 14, line 10 / Page 25, line 20,25

Comment 8: besides survival, was difference in response and durable response also tested for the change over time in CTC and ctDNA and dNLR?

Reply 8: Patients with PR had numerically lower CTC counts from C2 to C4, while patients with non-PR had numerically higher CTC from C2 to C4. The results of CTC dynamics showed a similar trend to that of the analysis according to DCB vs NDB, however, there was no

significant difference (Fig. e1, next page). There was no difference in cfDNA amount and ΔcfDNA according to PR vs non-PR or DCB or NDB (Fig. e2 and Table. e1, next page). The values of dNLR were numerically lower at each cycle in patients with PR than those with non-PR, however, there was no significant difference. Patients with PR had numerically lower dNLR from C2 to C4 compared with C1 (Fig. e3, next page).

Changes in the text: No change

Fig. e1

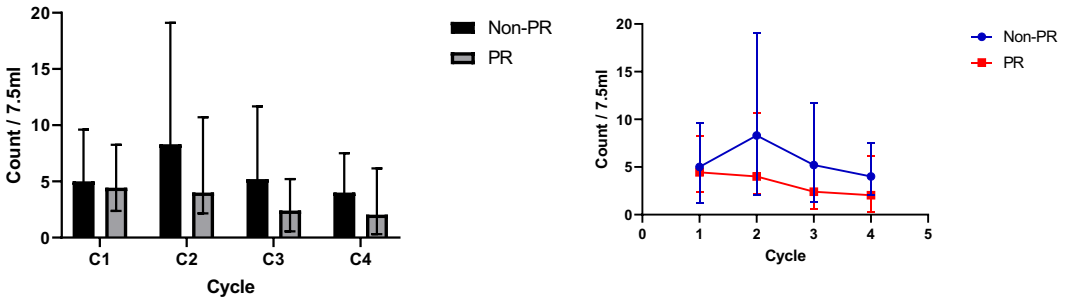


Fig. e2

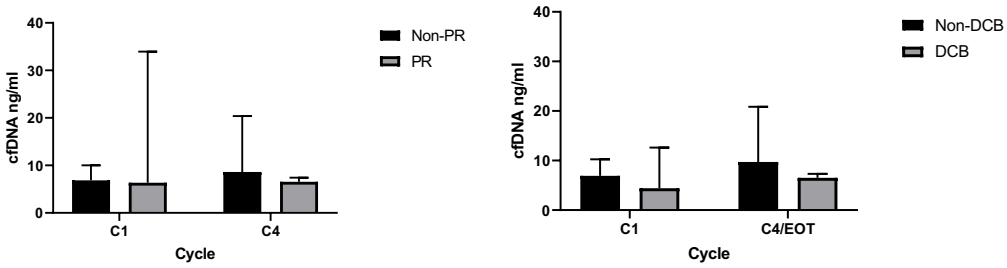
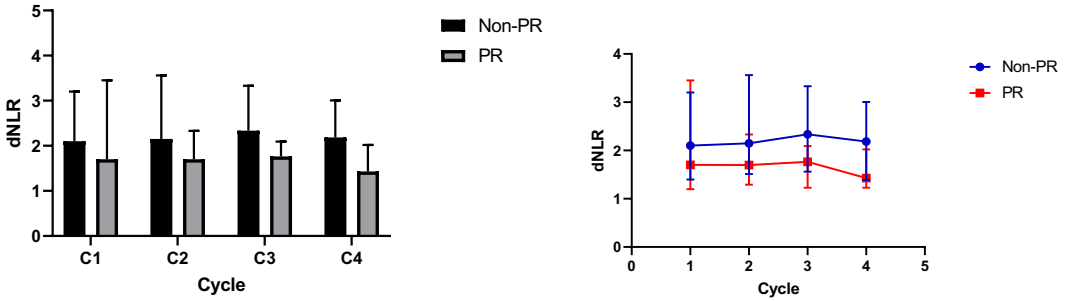


Table e1.

cfDNA % change from C1 to C4, n (%)	Non-PR	PR	*P*	NDB	DCB	*P*
ΔcfDNA >0 (n=16)	13 (72.2)	3 (50.0)	0.302	12 (70.6)	4 (57.1)	0.428
ΔcfDNA ≤0 (n=8)	5 (27.8)	3 (50.0)		5 (29.4)	3 (42.9)	

Fig. e3



Comment 9: CellSearch is cleared (not apprvoed) for several cancers, approved for only one i believe.

Reply 9: The CellSearch system was approved by the FDA in January 2004 for use in a clinical setting to predict outcomes for metastatic breast cancer patients. In November 2007 and February 2008, it was also granted FDA approval to aid in monitoring colorectal and prostate cancer patients.

Changes in the text: No change

Comment 10: refs 26/27 should also be mentioned in the method section

Reply 10: We added several references covering CD-FAST disc in the method section (see Page 8, line 16).

Changes in the text: Page 8, line 16

Comment 11: to my knowledge the proportion of patients at baseline in the study of Tamminga et al was not 88%, but roughly 1/3. I agree witht he statement that measurements of CTC after treatment might be best (delta provides little benefit compared to T1 measurement).

Reply 11: In a study of Tamminga et al., CTCs were present in 33/104 patients (32%) at baseline (see Page 15, line 14). In this study, CTCs were detected in 73/83 patients (88%) at C1 (see Page 15, line 20).

Changes in the text: No change

Comment 12: please show the baseline characteristics of the 83 pts with response and the 24 which were analysed for CTC and cfDNA.

Reply 12: Baseline characteristics of all enrolled patients (n=83) and classification according to DCB and NDB were described in Table 1 (see Page 27-30). The characteristics of patients in whom CTC and cfDNA analyses (n=24) were performed simultaneously were described in next pages (Table e2).

Changes in the text: No change