

Supplementary Information

Cell-Free DNA Kinetics in a Pre-Clinical Model of Head and Neck Cancer

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Supplementary Table 1 Muhanna *et al.*

Table S1. Contingency table for ctDNA assay

	Positive	Negative	Total
	<i>True Positive</i>	<i>False Positive</i>	
Positive	37	4	41
	<i>False Negative</i>	<i>True Negative</i>	
Negative	4	24	28
Total	41	28	69
	<i>Sensitivity</i>	<i>Specificity</i>	
	90.24 %	85.71%	
	(76.87% to 97.28%)	(67.33% to 95.97%)	

Supplementary Table 1. Contingency table for ctDNA assay. The specificity and sensitivity of the ctDNA qPCR assay was calculated using data from Supplementary Table 2. See Materials and Methods for definitions of true positive, true negative, false positive and false negative ctDNA results.

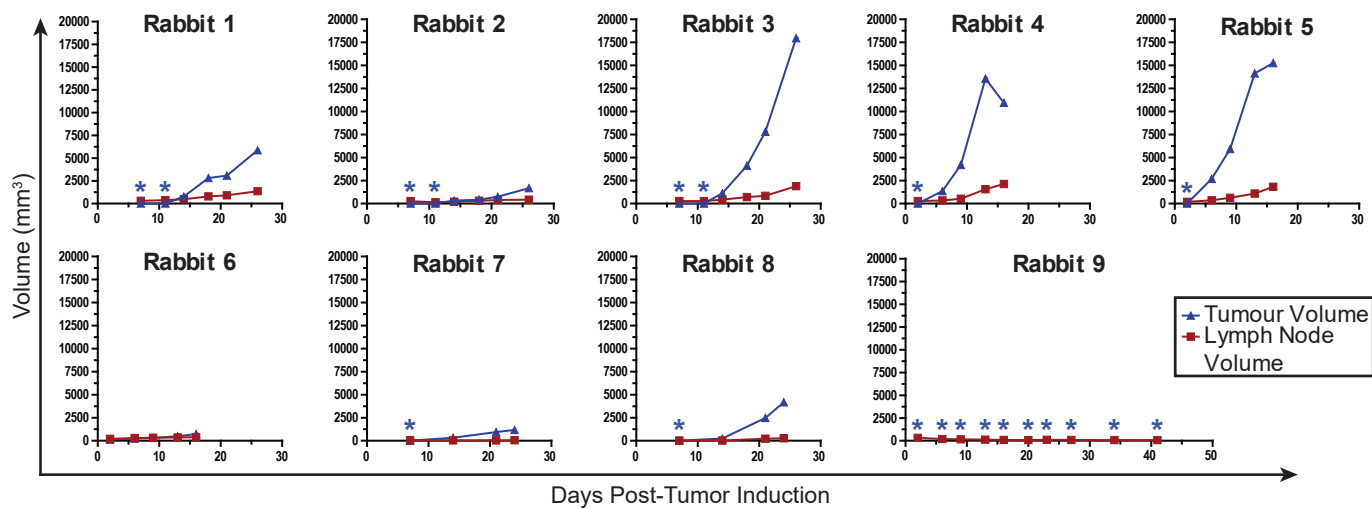
Supplementary Table 2 Muhanna *et al.*

Table S2. Description of data used in Table S1

Rabbit					Pre-Injection	
	True Positive	True Negative	False Positive	False Negative	True Negative	False Positive
1	4	2				
2	3	2		1		
3	4	2				
4	3+1*			1	1	
6	8	1		1	1	
5	4+1*				1	
7	1	6	1	1	1	
8	8	1			1	
9					1	
A					1	
B					1	
C						1
D					1	
E					1	
F						1
G						1
Sub-Total	37	14	1	4	10	3
Total	37	24	4	4		
	<i>True Positive</i>	<i>True Negative</i>	<i>False Positive</i>	<i>False Negative</i>		

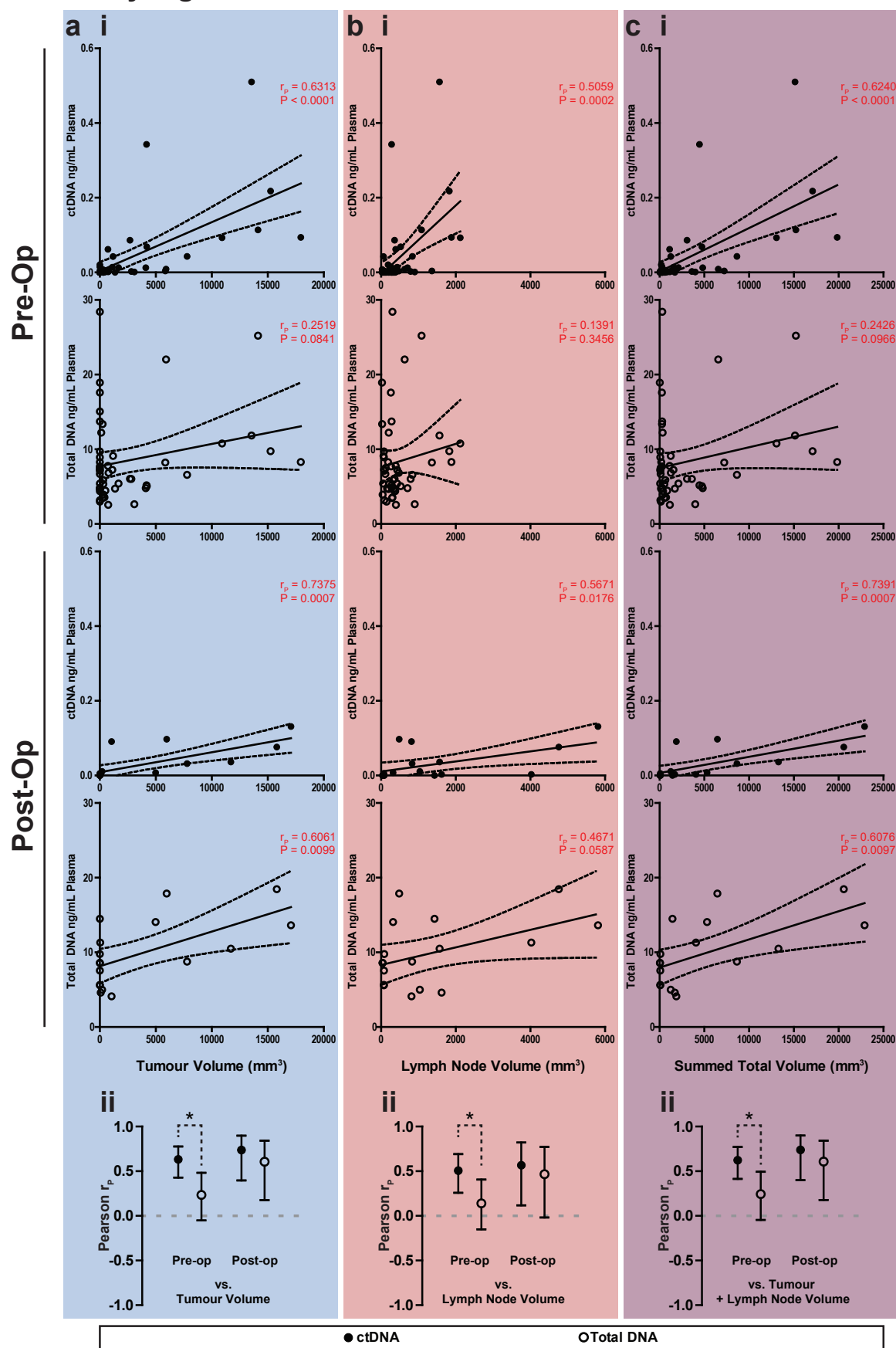
Supplementary Table 2. Description of data used in Table S1. The number of true positive, true negative, false positive, and false negative ctDNA results are shown for each of the rabbits injected with the VX2 cell lines in the study. The number of true negative and false positive ctDNA results are shown (gray box) for pre-injection blood from rabbits 4-9 and for seven additional uninjected rabbits designated A-G. Asterisks denote instances where ctDNA was detected for non-detectable tumour that subsequently developed.

Supplementary Figure 1 Muhanna *et al.*



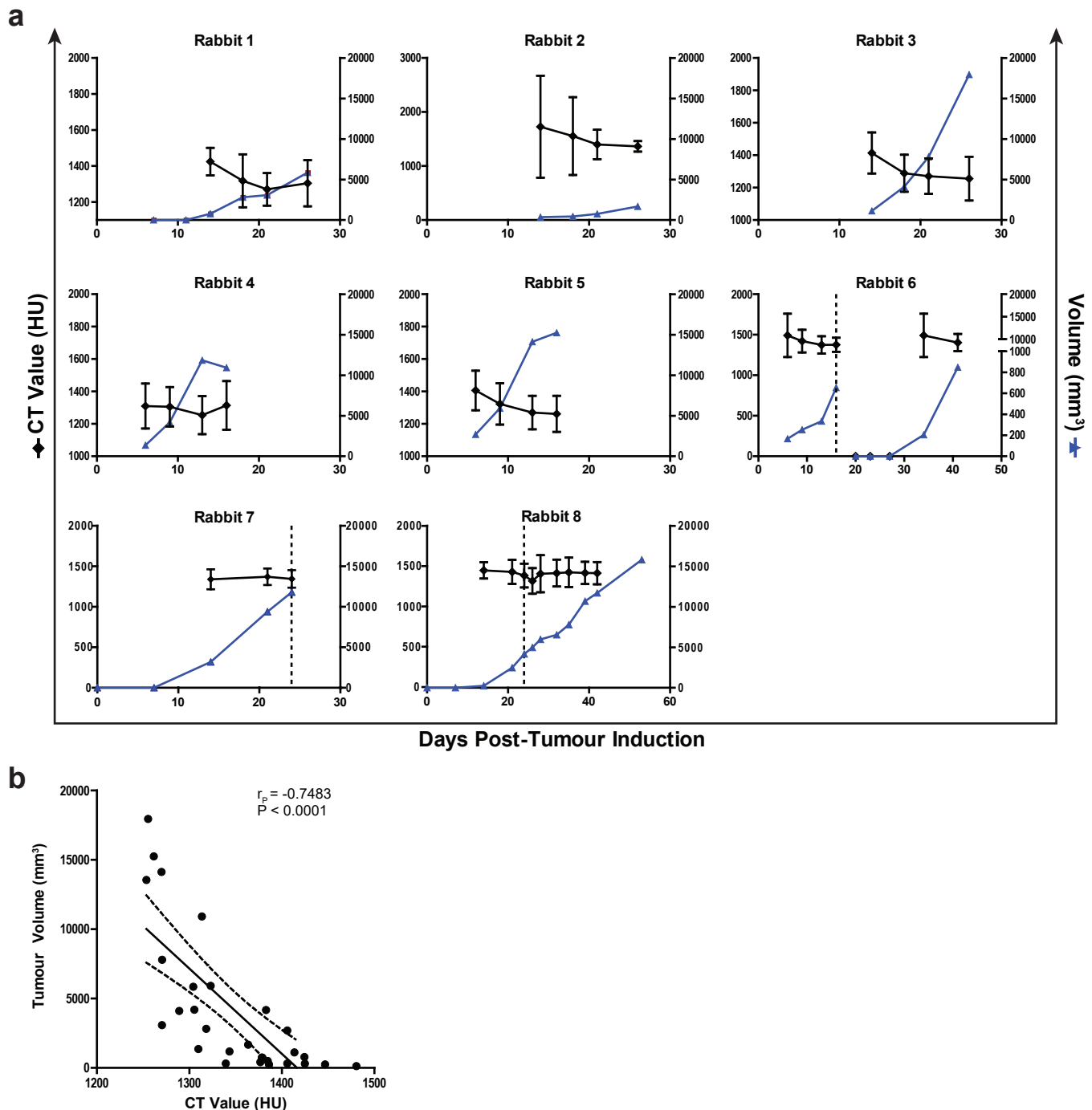
Supplementary Figure 1: Volume of primary tumour (blue triangles) and lymph node (red squares) vs. time as measured by CT for all rabbits in this study. Asterisks denote non-detect-able tumour size.

Supplementary Figure 2 Muhanna et al.



Supplementary Figure 2: Correlation of ctDNA or total plasma DNA vs. tumour volume (a), lymph node volume (b) or tumour + lymph node volume (c) in both the pre-operative and post-operative setting, as indicated. For each comparison, individual scatter plots with linear regression trend lines (solid) and 95% confidence intervals (dashed) are shown in (i). Summary plots of correlation coefficients (Pearson r_p , mean $\pm$ 95% confidence interval) are shown in (ii). Asterisks indicate $P < 0.05$.

Supplementary Figure 3 Muhanna *et al.*



Supplementary Figure 3: (a) Correlation between average CT values (black) (Hounsfield Units, HU) and tumour volume (blue) of each tumour. Dotted vertical line represents the day of tumour resection surgery for Rabbits 6, 7, and 8. Error bars represent SD. (b) Correlation between average CT values (Hounsfield Units, HU) and tumour volume for all tumours in the study prior to operation. Linear regression trend line (solid) and 95% confidence intervals (dashed) are shown.