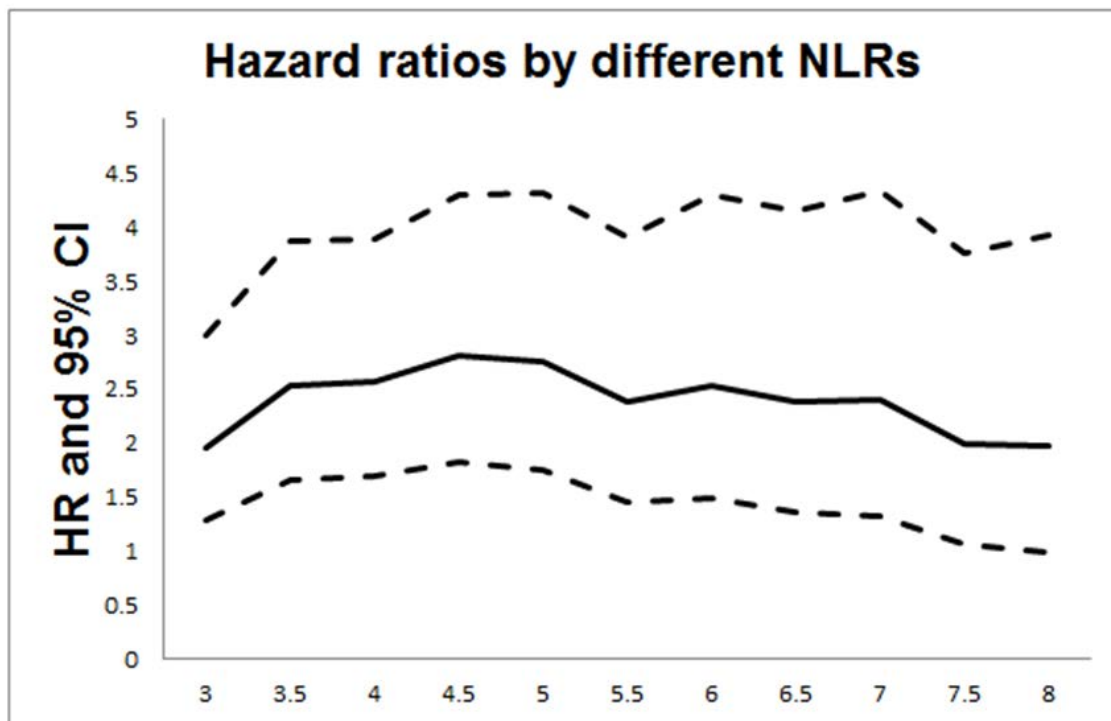


## SUPPLEMENTAL DATA

**ATTACC Protocol:**

The ATTACC or Assessment of Targeted Therapies Against Colorectal Cancer protocol is a novel enrichment framework which is in place at M D Anderson Cancer Center. This clinical trial platform provides one protocol for enrollment, tissue ascertainment, and biomarker screening of patients (termed the “umbrella” protocol) and several separate protocols (termed the “companion” protocols) for each of the treatment trials. Each of the “companion” protocols requires the presence of a biomarker predicted to identify a subset of patients with a higher likelihood of response. General eligibility includes 5-FU refractory metastatic colorectal cancer, adequate performance status, and ability to obtain tumor tissue for testing. Patients consent to the “umbrella” protocol, undergo real-time assessment of the relevant biomarkers using CLIA-certified assay, and then are assigned to companion protocol. Patients would subsequently be consented and enroll in the particular companion protocol, which are stand-alone protocols with independent regulatory oversight. ATTACC is an ongoing program with more than 300 patients enrolled to date. Enrollment has been consistently been at approximately 15 patients per month. These patients represent a subset of patients seen at MD Anderson and reflect the trial eligible population that would be available for therapeutic clinical trials. Current biomarker in a CLIA environment testing includes: 46 gene Ion Torrent NGS panel (including PIK3CA, KRAS, NRAS, BRAF, PTEN, APC, and AKT), PTEN IHC, and a CpG Island methylation panel.

**Supplemental Figure 1.** HR for death (solid line) and 95 % CI (dashed lines) for survival derived from different NLRs in patients with mCRC.



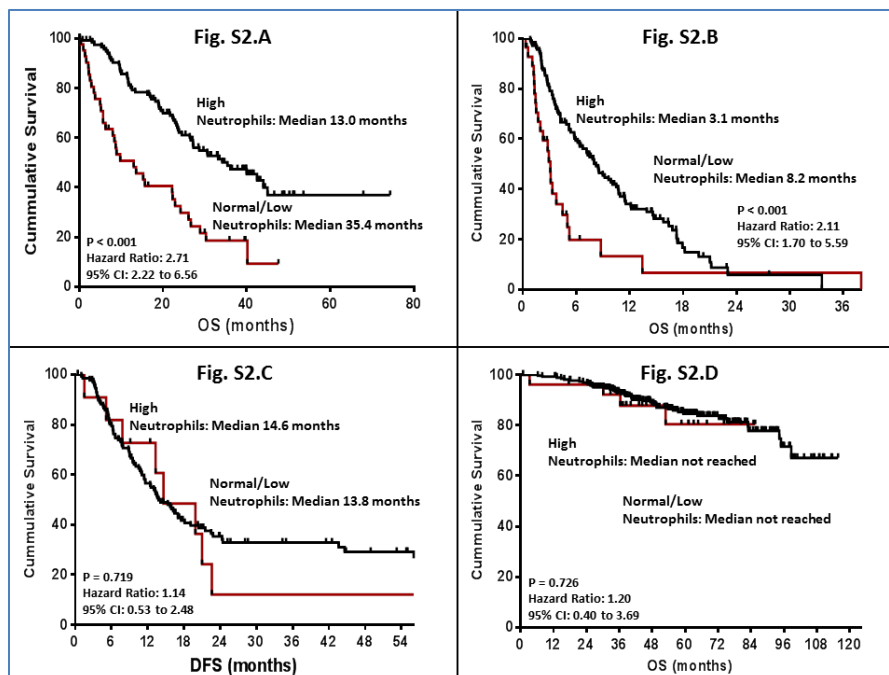
**Supplemental Table 1.** Patient cohorts included in the study and survival estimates

	Cohort 1		Cohort 2		Cohort 3		Cohort 4		Cohort 5	
Clinical setting	Previously untreated mCRC (Exploratory)		Previously untreated mCRC (Validation)		Refractory mCRC		Liver-limited mCRC after hepatectomy		Stage II/III Resected CRC	
Total patients	39		166		161		198		274	
NLR class*	High NLR	Low NLR	High NLR	Low NLR	High NLR	Low NLR	High NLR	Low NLR	High NLR	Low NLR
Patients, <i>N</i>	12	27	40	126	60	101	25	173	29	245
Median OS, m	-	-	15.3	34.2	3.7	9.0	-	-	NR	NR
Median DFS, m	-	-	-	-	-	-	9.5	13.3	-	-
3-year OS	-	-	-	-	-	-	8	38	75	90
HR (95% CI)	-	-	2.7 (1.8 – 4.3)		2.2 (1.5 – 3.1)		1.8 (1.1 – 2.9)		2.4 (1.1 – 5.1)	
<i>P</i> value	-	-	< 0.001		< 0.001		0.010		0.020	

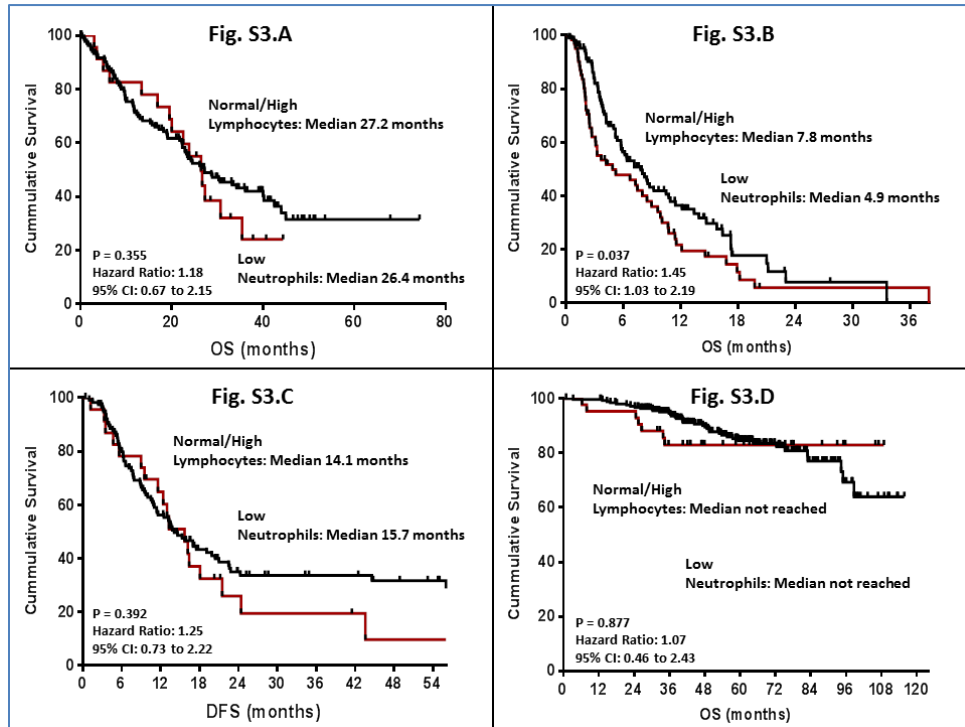
*Abbreviations:* mCRC, metastatic colorectal cancer; NLR, neutrophil-lymphocyte ratio; OS, overall survival; m, months; NR, not reached; DFS, disease-free survival; HR, hazards ratio; CI, confidence interval

\* NLR class is defined as high if NLR > 5 and low if NLR ≤ 5.

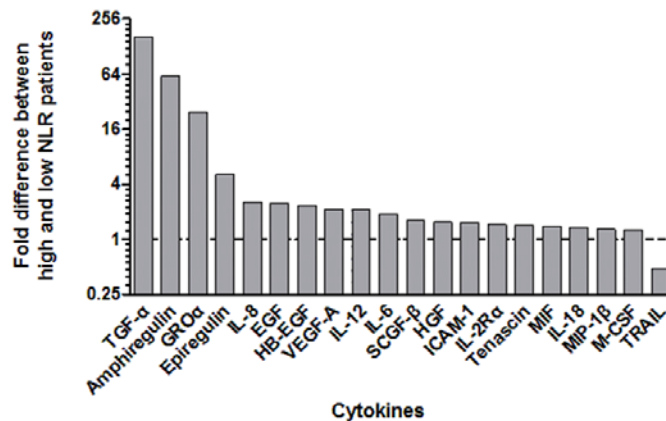
**Supplemental Figure 2.** Kaplan-Meier survival curves illustrating OS and DFS in different settings of CRC, compared between patients with a high neutrophil count (black line), defined as greater than upper limit of normal (ULN), and those with a normal/low neutrophil count (red line). **(A)** OS in cohort 2 (previously untreated mCRC). **(B)** OS in cohort 3 (refractory mCRC). **(C)** DFS in cohort 4 (liver metastatic disease after hepatectomy). **(D)** OS in cohort 5 (stage II/III CRC after surgery).



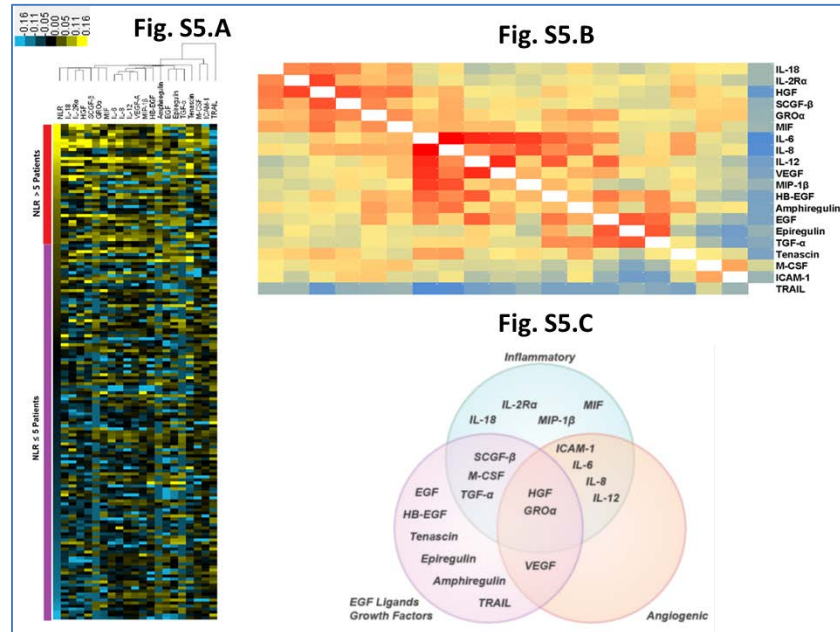
**Supplemental Figure 3.** Kaplan-Meier survival curves illustrating OS and DFS in different settings of CRC, compared between patients with low lymphocyte count (red line), defined as less than lower limit of normal (LLN), and those with a normal/high lymphocyte count (black line). **(A)** OS in cohort 2 (previously untreated mCRC). **(B)** OS in cohort 3 (refractory mCRC). **(C)** DFS in cohort 4 (liver metastatic disease after hepatectomy). **(D)** OS in cohort 5 (stage II/III CRC after surgery).



**Supplemental Figure 4.** Proportional changes in the concentration of 20 cytokines between high- and low-NLR subgroups in cohort 2.



**Supplemental Figure 5. (A)** Expression heatmap showing expression of cytokines, with “yellow” representing high expression and “blue” representing low expression. **(B)** Coherence heatmap, with “red” showing positive correlation with NLR and “blue” showing negative correlation. **(C)** Venn diagram illustrating coherence matrix segregation into separate functional overlapping orders.



**Supplemental Table 2.** Coefficients of Spearman’s correlation of NLR and cytokines

Cytokines	Correlation with NLR		Cluster
	Coefficient	P value	
HGF	0.511	<0.0001*	Inflammatory
IL-8	0.374	<0.0001*	Angiogenic
GRO $\alpha$	0.373	<0.0001*	Inflammatory
IL-18	0.311	<0.0001*	-
IL-2R $\alpha$	0.307	<0.0001*	Inflammatory
ICAM-1	0.299	<0.0001*	-
SCGF- $\beta$	0.297	0.0001*	Inflammatory
Tenascin	0.288	0.0002*	-
Amphiregulin	0.287	0.0002*	EGF Ligands
IL-6	0.282	0.0002*	Angiogenic
MIP-1 $\beta$	0.247	0.001*	-
VEGF	0.228	0.003	Angiogenic
EGF	0.221	0.004	EGF Ligands
HB-EGF	0.210	0.007	EGF Ligands
MIF	0.206	0.008	Inflammatory
Epiregulin	0.170	0.028	EGF Ligands
IL-12	0.150	0.054	Angiogenic
M-CSF	0.149	0.055	-
TGF- $\alpha$	0.099	0.205	EGF Ligands
TRAIL	-0.408	<0.0001*	-

*Abbreviations:* NLR, neutrophil-lymphocyte ratio; IL, interleukin; HGF, hepatocyte growth factor; M-CSF, macrophage-colony stimulating factor; VEGF-A, vascular epidermal growth factor A; MIP-1 $\beta$ , macrophage inflammatory protein-1 $\beta$ ; GRO $\alpha$ , chemokine (C-X-C motif) ligand 1 (CXCL1); MIF, macrophage migration inhibitory factor; SCGF- $\beta$ , stem cell growth factor- $\beta$ ; TRAIL, TNF-related apoptosis-inducing ligand; EGF, epidermal growth factor; HB-EGF, heparin-binding EGF-like growth factor; TGF- $\alpha$ , transforming growth factor- $\alpha$ ; ICAM-1, intercellular adhesion molecule-1.

\* P-values reflect significance based on Bonferroni adjustment.

**Supplemental Table 3.** Mean and standard deviation of cytokines selected for generating cytokine scores.

Cytokines	Mean	Standard Deviation
HGF	8.08	0.76
IL-8	4.46	1.75
GRO $\alpha$	2.14	4.02
IL-18	6.52	0.85
IL-2R $\alpha$	7.67	0.77
ICAM-1	16.86	1.19
SCGF- $\beta$	13.53	0.85
Tenascin	10.30	0.86
Amphiregulin	4.63	2.88
IL-6	3.60	1.27
MIP-1 $\beta$	5.88	0.93
TRAIL	5.44	0.95

*Abbreviations:* IL, interleukin; HGF, hepatocyte growth factor; MIP-1 $\beta$ , macrophage inflammatory protein-1 $\beta$ ; GRO $\alpha$ , SCGF- $\beta$ , stem cell growth factor- $\beta$ ; TRAIL, TNF-related apoptosis-inducing ligand; ICAM-1, intercellular adhesion molecule-1.