Macrophage Biomarkers CD68 and CD163 Correlate with CRC Patient Survival

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Abstract

Immune cells within the tumor microenvironment (TME) play a vital role in regulating tumor progression, thus, immunotherapies that elicit anti-tumor responses are of great interest for treating various cancers. Macrophages, a current cell type of interest, has the ability to polarize into anti-tumor (M1) and pro-tumor (M2) phenotypes. The density and phenotype of macrophages within the tumor and TME have been linked to prognosis in multiple types of solid tumors. Here, we predicted high immune infiltration and greater amounts of anti-tumor immune cells within the tumor compartment would have increased survival compared to immune excluded or immune desert environments.

Methods: One CRC tumor microarray (containing primary tumors, metastases, and normal tissue) was stained via multiplex immunofluorescence (mIF) for: CD3, CD8, CD56, CD68, CD163, and PD-L1 and analyzed utilizing Flagship Biosciences' proprietary image analysis platform. Machine learning algorithms used cellular features to stratify cells to either the tumoral or stromal compartment. Core level expression data was pulled and represented on a whole-cohort basis. All staining and image analysis outputs were reviewed by a board-certified, MD pathologist.

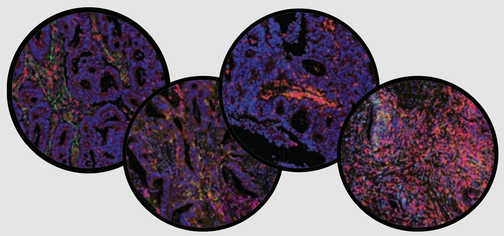


Figure 1. Examples of CRC Cores Stained with Flagship Bioscience's General IO mIF Panel and Image Analysis Markup. One CRC TMA was stained for: CD3, CD8, CD56, CD68, CD163, and PD-L1. Representative images are shown.

Results: There is a correlation between patient survival and the presence or absence of macrophage markers CD68 and CD163 and lack of PD-L1. Specifically, lower levels of $CD68^{+}CD163^{-}$ cells within the tumor and stroma compartments correlate with an increase in patient survival. Similarly, lower PD-L1 expression in the stromal compartment positively correlate with prolonged patient survival in CRC patients.

Here we demonstrated Flagship Biosciences' image analysis platform's ability to analyze CRC patient populations based on immune biomarkers. Our analysis shows a strong relationship between immune cell presence and localization and patient survival. Altering the TME to an anti-tumor immune environment could increase patient survival times. Understanding the immune microenvironments within tumors, though Flagship Biosciences' image analysis software, can provide vital information in diagnosis and treatment decisions for patients.

Conclusion

Lower levels of 'M1' $CD68^{+}$ or $CD68^{+}CD163^{-}$ cells, typically an anti-tumor phenotype, correlate with reduced survival in CRC patients. Lower expression of PD-L1 is associated with slight increase in survival. Higher levels of 'M2' $CD163^{+}$ cells, typically a pro-tumor phenotype, seem to have minimal assocaition with survivalThis suggests that we need additional biomarkers to further phenotype macrophages and determine the pro- or anti-tumor activities of traditionally 'M1' or 'M2' phenotypes. This further highlights the benefits of Flagship Biosciences' proprietary image analysis platform to distinguish these complex immune cell phenotypes in cancer patient populations.

References

1. Edin et al. (2012) PLoS ONE 7(10):e47045.
2. Inagaki et al. (2021) Cancer Sci 112(7):2692-2704.
3. Xue et al. (2021) World J Surgical Oncol 19:186.

CD68 Expression

Lower levels of $CD68^{+}$ cells correlates with increased survival (more pronounced in early stage compared to late stage)

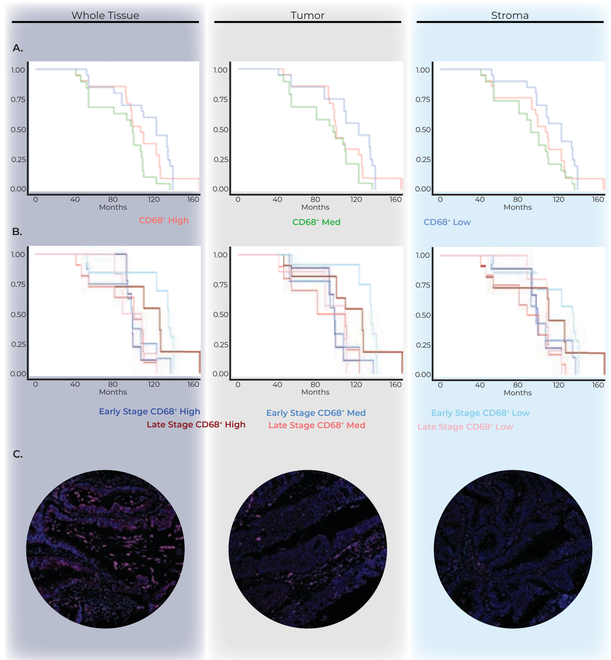


Figure 2. CRC Patient Survival Based on CD68 Expression. Kaplan-Meier survival curves based on High, Medium, or Low CD68 expression (A). Kaplan Meier survival curves stratified on **Early** (stage 1 and 2) or **Late** (stage 3 and 4) disease and based on High, Medium, or Low CD68 expression (B). Representative images of whole tissue CRC tumor cores. CD68 high expression, 126 month survival at late stage (left), CD68 medium expression, 123 month survival at late stage (middle), and CD68 low expression, 134 months survival at early stage (right) (C).

$CD68^{+}CD163^{-}$ Expression

Low levels of CD68+CD163- in the tumor and stromal compartments correlate with increased survival

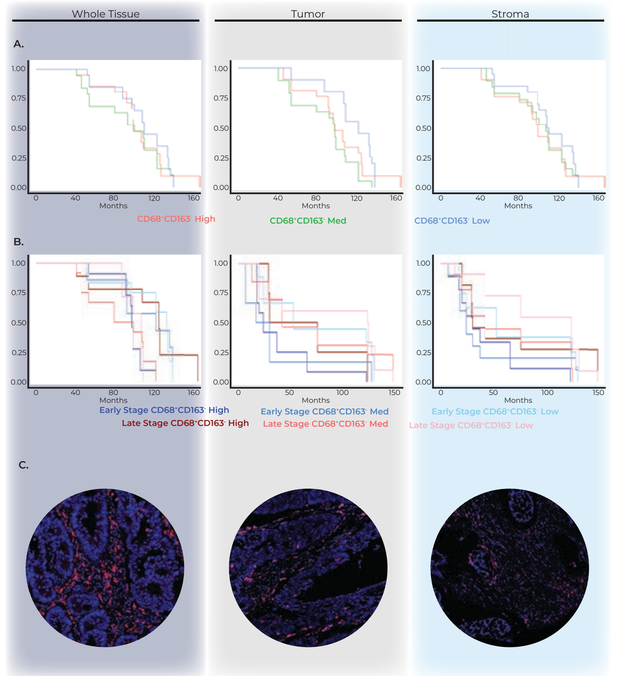
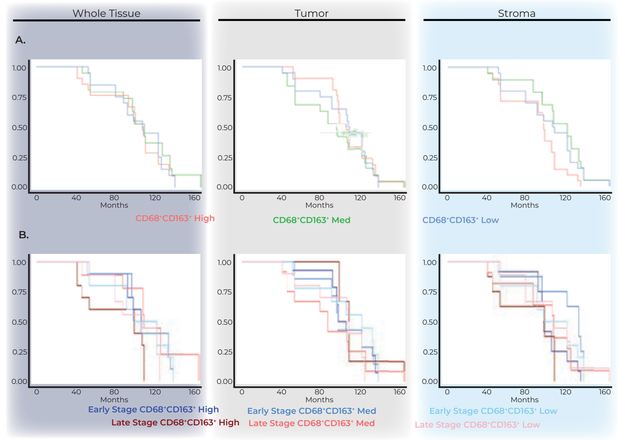


Figure 4. CRC Patient Survival Based on $CD68^{+}CD163^{-}$ Expression. Kaplan-Meier survival curves based on High, Medium, or Low $CD68^{+}CD163^{-}$ expression (A). Kaplan Meier survival curves stratified on **Early** (stage 1 and 2) or **Late** (stage 3 and 4) disease and based on High, Medium, or Low $CD68^{+}CD163^{-}$ expression (B). Representative images of whole tissue CRC tumor cores. $CD68^{+}CD163^{-}$ high expression, 98 month survival at early stage (left), $CD68^{+}CD163^{-}$ medium expression, 123 month survival at late stage (middle), and $CD68^{+}CD163^{-}$ low expression, 123 months survival at early stage (right) (C).

$CD68^{+}CD163^{+}$ Expression

Presence of $CD68^{+}CD163^{+}$ cells have minimal correlation with survival overall
Late stage patients had reduced survivial times with high levels of $CD68^{+}CD163^{+}$ cell in whole tissue and stroma



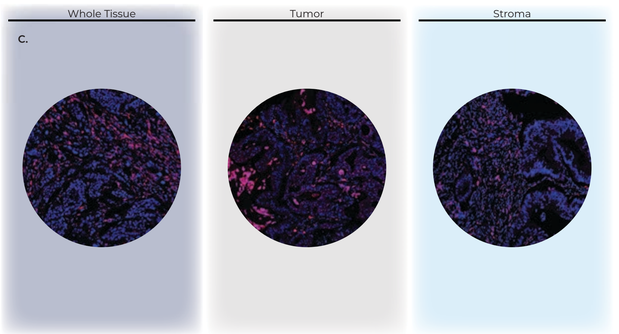


Figure 5. CRC Patient Survival Based on $CD68^{+}CD163^{+}$ Expression. Kaplan-Meier survival curves based on High, Medium, or Low $CD68^{+}CD163^{+}$ expression (A). Kaplan Meier survival curves stratified on **Early** (stage 1 and 2) or **Late** (stage 3 and 4) disease and based on High, Medium, or Low $CD68^{+}CD163^{+}$ expression (B). Representative images of whole tissue CRC tumor cores. $CD68^{+}CD163^{+}$ high expression, 110 month survival at late stage (left), $CD68^{+}CD163^{+}$ medium expression, 97 month survival at early stage (middle), and $CD68^{+}CD163^{+}$ low expression, 92 months survival at early stage (right) (C).

PD-L1 Expression

Low or Medium expression of PD-L1 correlates with slight increase in survival in any compartment (specifically early stage)

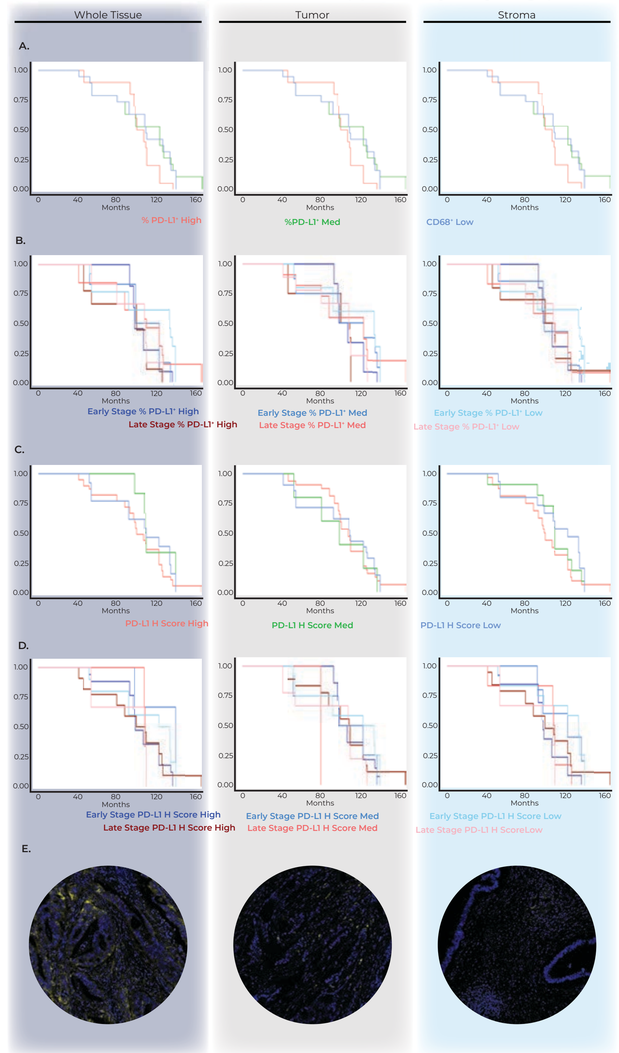


Figure 6. CRC Patient Survival Based on PD-L1 Expression. Kaplan-Meier survival curves based on High, Medium, or Low % PD-$L1^{+}$ (A). Kaplan Meier survival curves stratified on **Early** (stage 1 and 2) or **Late** (stage 3 and 4) disease and based on High, Medium, or Low % PD-$L1^{+}$ (B). Kaplan-Meier survival curves based on High, Medium, or Low PD-L1 H score (C). Kaplan Meier survival curves stratified on **Early** (stage 1 and 2) or **Late** (stage 3 and 4) disease and based on High, Medium, or Low PD-L1 H score (D).Representative images of whole tissue CRC tumor cores. PD-L1 high expression, 93 month survival at early stage (left), PD-L1 medium expression, 41 month survival at late stage (middle), and PD-L1 low expression, 135 months survival at early stage (right) (E).

$PD\text{-}L1^{+}CD68^{+}CD163^{-/+}$ Expression

Low PD-L1 expression on either $CD68^{+}CD163^{+}$ or $CD68^{+}CD163^{-}$ positively correlates with increased survival in early and late stage

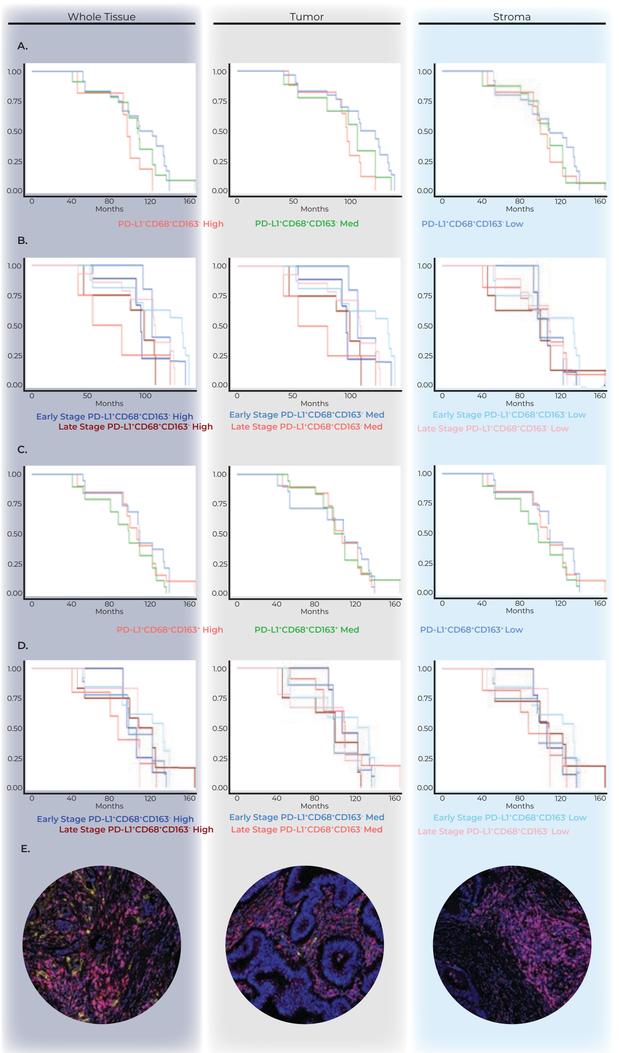


Figure 7. CRC Patient Survival Based on $PD\text{-}L1^{+}CD68^{+}CD163^{+}$ or $PD\text{-}L1^{+}CD68^{+}CD163^{-}$ Expression. Kaplan-Meier survival curves based on High, Medium, or Low $PD\text{-}L1^{+}CD68^{+}CD163^{+}$ H score (A). Kaplan Meier survival curves stratified on **Early** (stage 1 and 2) or **Late** (stage 3 and 4) disease and based on High, Medium, or Low $PD\text{-}L1^{+}CD68^{+}CD163^{-}$ H score (B). Kaplan-Meier survival curves based on High, Medium, or Low $PD\text{-}L1^{+}CD68^{+}CD163^{+}$ H score (C). Kaplan Meier survival curves stratified on **Early** (stage 1 and 2) or **Late** (stage 3 and 4) disease and based on High, Medium, or Low $PD\text{-}L1^{+}CD68^{+}CD163^{-}$ H score (D).Representative images of whole tissue CRC tumor cores. $PD\text{-}L1^{+}CD68^{+}CD163^{+}$ high expression, 46 month survival at late stage (left), $PD\text{-}L1^{+}CD68^{+}CD163^{+}$ medium expression, 107 month survival at early stage (middle), and $PD\text{-}L1^{+}CD68^{+}CD163^{-}$ low expression, 52 months survival at early stage (right) (E).