



Low CD47 Expression in Specific Populations of Tumor Macrophage Fusion Cells in Blood Correlates with Progressive Disease in Metastatic Cancers



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ABSTRACT

Tumor associated macrophages are known to fuse with cancer cells resulting in the formation of a cellular phenomenon called tumor-macrophage fusion cells (TMFCs). TMFCs are a highly aggressive cell subtype that may move into blood, evading immune surveillance, and possibly aid in seeding of metastatic sites. The formation of TMFCs involves CD47, a ubiquitous cell surface ligand that acts as a “don’t eat me” signal, interacting with the macrophage protein SIRPa, a down regulator of phagocytosis, and syncytin-1, a mediator of hyperploidy fusion in cancer (**Fig. 1A**). In this analysis, we isolated circulating TMFC subtypes from the blood of n=46 metastatic cancer patients (pts) to evaluate the expressions of CD47, SIRPa, and syncytin-1 as it relates to stable, responding, or progressive disease by 30 day imaging by PET scan.

MATERIALS & METHODS

We conducted a prospective pilot study of n=46 pathologically confirmed metastatic cancer pts with breast (n=24), prostate (n=7), pancreas (n=7), or colon (n=8) all being treated with standard of care systemic therapies. We isolated TMFCs from 7.5ml peripheral blood using a CellSieve™ microfilter, followed by staining the cells with CD47, SIRPa, & syncytin-1. TMFCs were identified by their enlarged cell size (>30μm) and a multinucleated structure, which were categorized into 4 distinct subtypes: A) >2 distinct non-contacting nuclei within a TMFC, B) 2 distinct non-contacting nuclei within a TMFC, C) 2 contacting nuclei within a TMFC, D) a single multinucleated nuclei (**Fig. 1A**). CD47, SIRPa, and syncytin-1 expressions were categorized as negative, low, or high. TMFCs subtypes and marker expressions were compared to pts’ clinical status (i.e. progressive, stable or responding) at first available PET/CT scans after blood draw (~30-60 days). All data were analyzed using a Student’s t-test.

RESULTS

- TMFCs were identified in 76% of pts (n=35/46), average 8 cells/sample, and ranging 1-69 cells.
- Of TMFC, 14% were subtype A, 16% B, 16% C, and 54% D.
- CD47 expression was seen in 100% of TMFCs, with low expression in 47% of subtype A, 63% subtype B, 60% subtype C, & 70% subtype D
- Subtype B had significantly lower CD47 (p=0.024) (**Fig. 1B**).
- Expression of syncytin-1 was significantly higher in progressing patients (p<0.001) (**Fig. 3**), and subtype D cells had 75% higher syncytin-1 localized to the nuclear regions (**Fig. 1D**).
- No subtype correlations were found in the SIRPa (**Fig. 1C**).
- Progressing pts had 155% more TMFCs (n=13 TMFCs/sample), 43% more subtype B TMFCs, and significantly lower (p<0.001) CD47 expression than stable/responding pts (**Fig. 2**).

Figure 1. TMFC Subtype Formation and Biomarker Expression from Metastatic Pan-Cancer Patients’ Blood

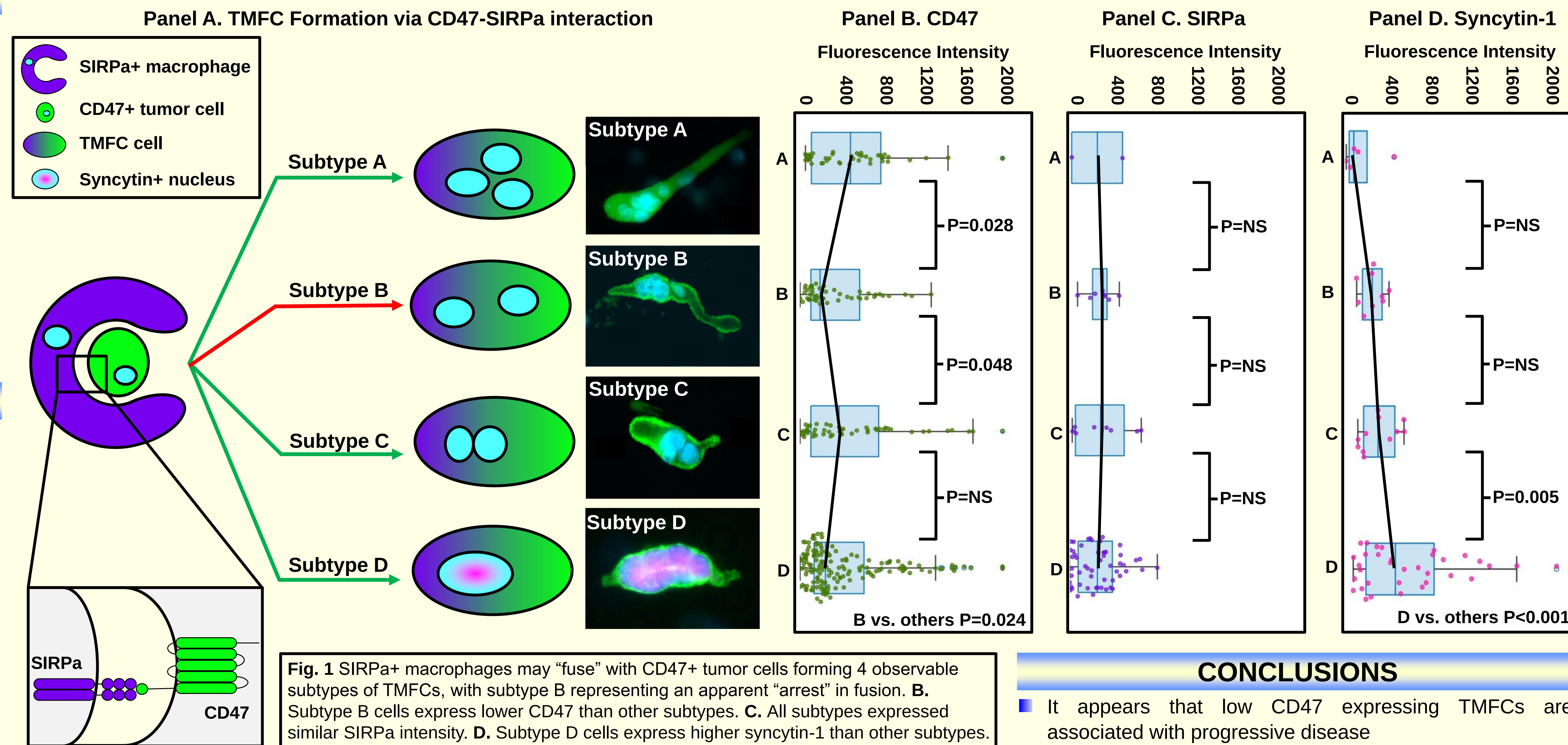


Figure 2. TMFC CD47 Expression in Metastatic Pan-Cancer Patients

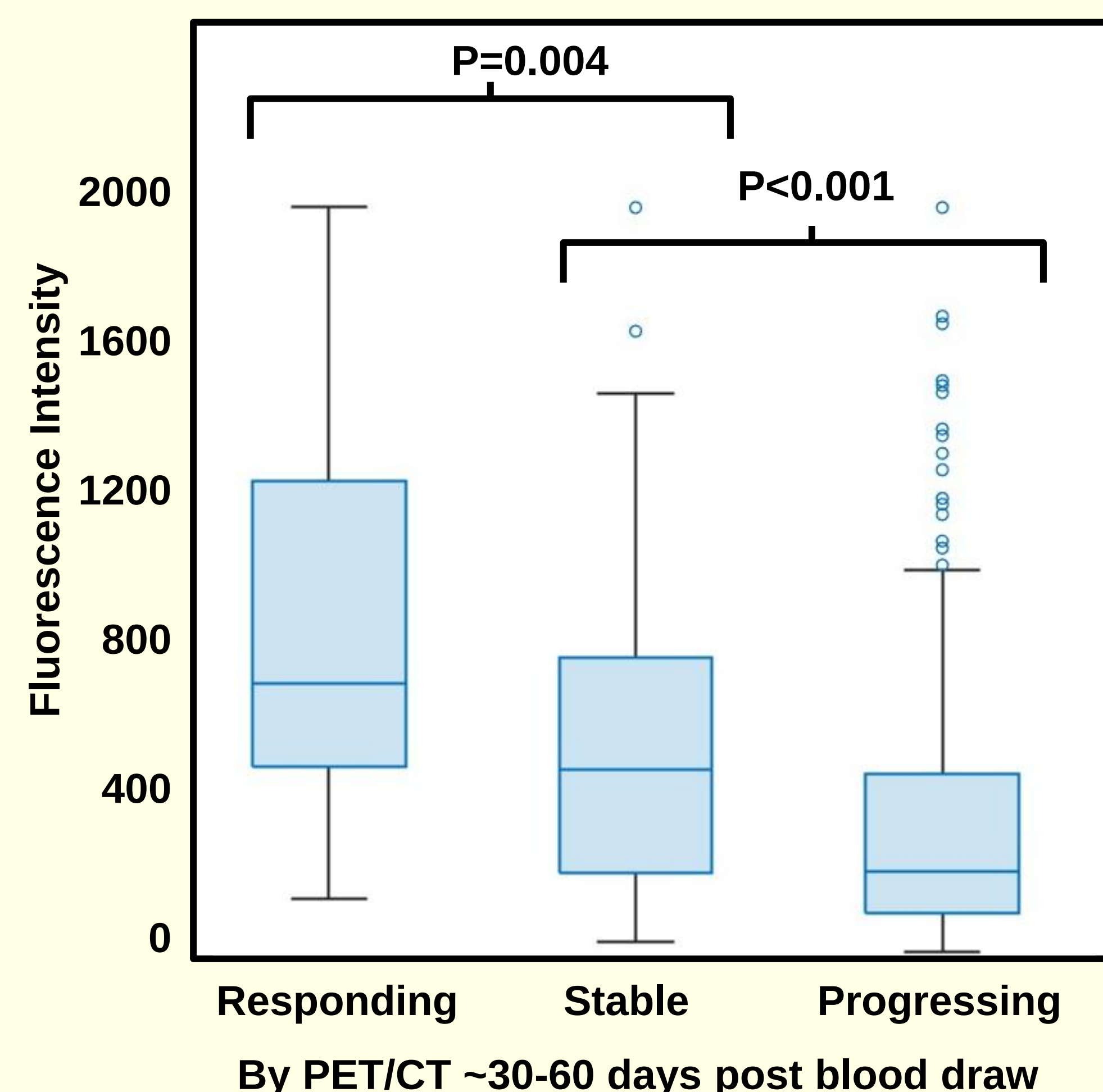
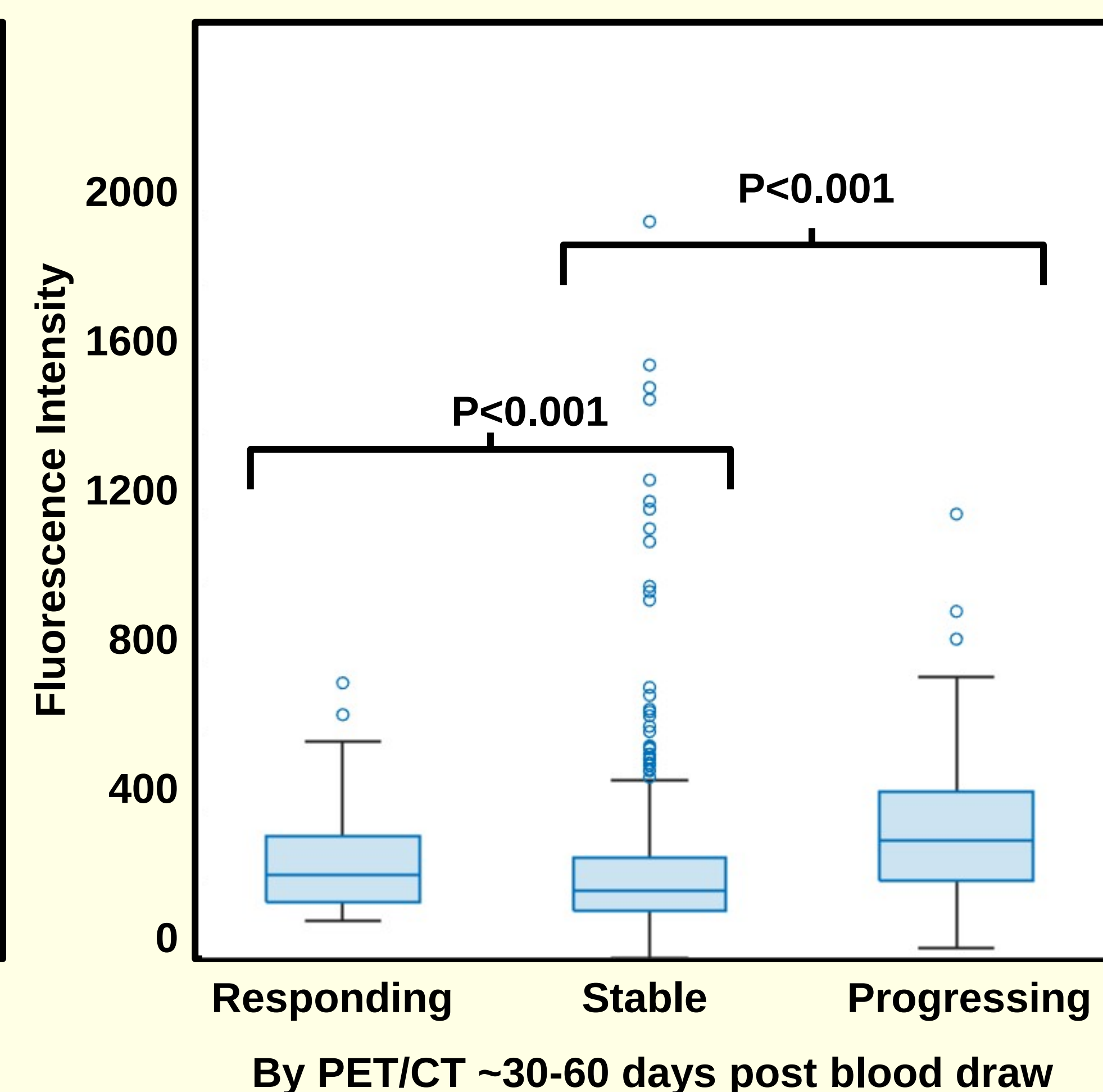


Figure 3. TMFC Syncytin-1 Expression in Metastatic Pan-Cancer Patients



CONCLUSIONS

- It appears that low CD47 expressing TMFCs are associated with progressive disease
- This finding contrasts existing studies on primary tumors which correlate high CD47 with more aggressive cancers.
- These data suggest that highly aggressive low CD47 cancer cell subtypes circulate the blood and may be pertinent to efforts targeting CD47 in cancer.

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