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Extracellular Vesicle Budding from Stromal Macrophages in the Blood of Metastatic Non-Small Cell Lung Cancer Patients Correlates with Poor Survival

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ABSTRACT

Cancer Extracellular Vesicles (EVs) are involved in cellular communication, tumor growth, progression, and metastasis in cancer. The origins of EVs, their formation, and potential clinical use as biomarkers are undefined. Recently, budding of extracellular structures on Cancer Associated Macrophage-Like Cells [CAMLs], a specific subtype of phagocytic circulating stromal cells, has been observed in metastatic non-small cell lung carcinoma (mNSCLC) patients. In this prospective analysis of n=40 mNSCLC samples, we enumerated EV budding on CAMLs to determine if their formation had an effect on clinical outcomes. These preliminary results suggest that EV budding from a specific subtype of circulating tumor associated macrophage prognosticates for worse clinical outcome which may serve as the mechanism for cancer EV formation and spread throughout the body.
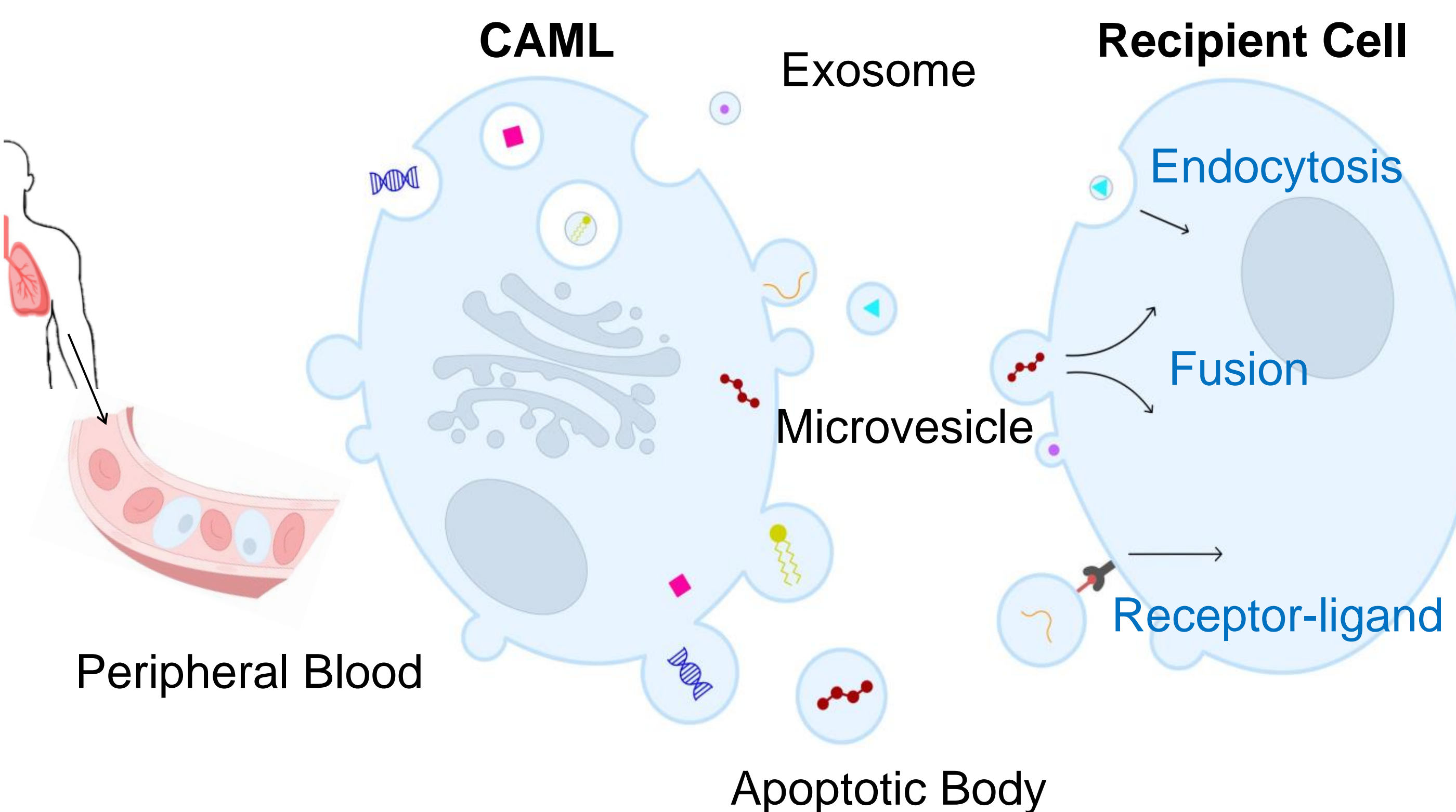
Figure 1. The three subtypes of EVs are separated based on method of formation and size (exosomes [~30-100nm], microvesicles [~100nm-1µm], and apoptotic bodies [~1-5µm]). EVs originate from CAMLs which leave the tumor site and enter circulation.

MATERIALS & METHODS

We initiated a single blind prospective pilot study to evaluate extracellular budding on the CAMLs of mNSCLC patients from blood samples obtained prior to therapy to determine their prevalence and clinical utility. Anonymized blood was procured and filtered to isolate CAMLs and stained for cytokeratin, CD45, CD31 and PD-L1. EV budding was observed as small (≤1 µm) bulbous protrusions from the cell periphery. EVs were quantified and compared against patient progression free survival (PFS) and overall survival (OS) with hazard ratios (HRs) at 24 months by censored univariate analysis. The imaged EVs were also characterized by their PD-L1 biomarker expression.

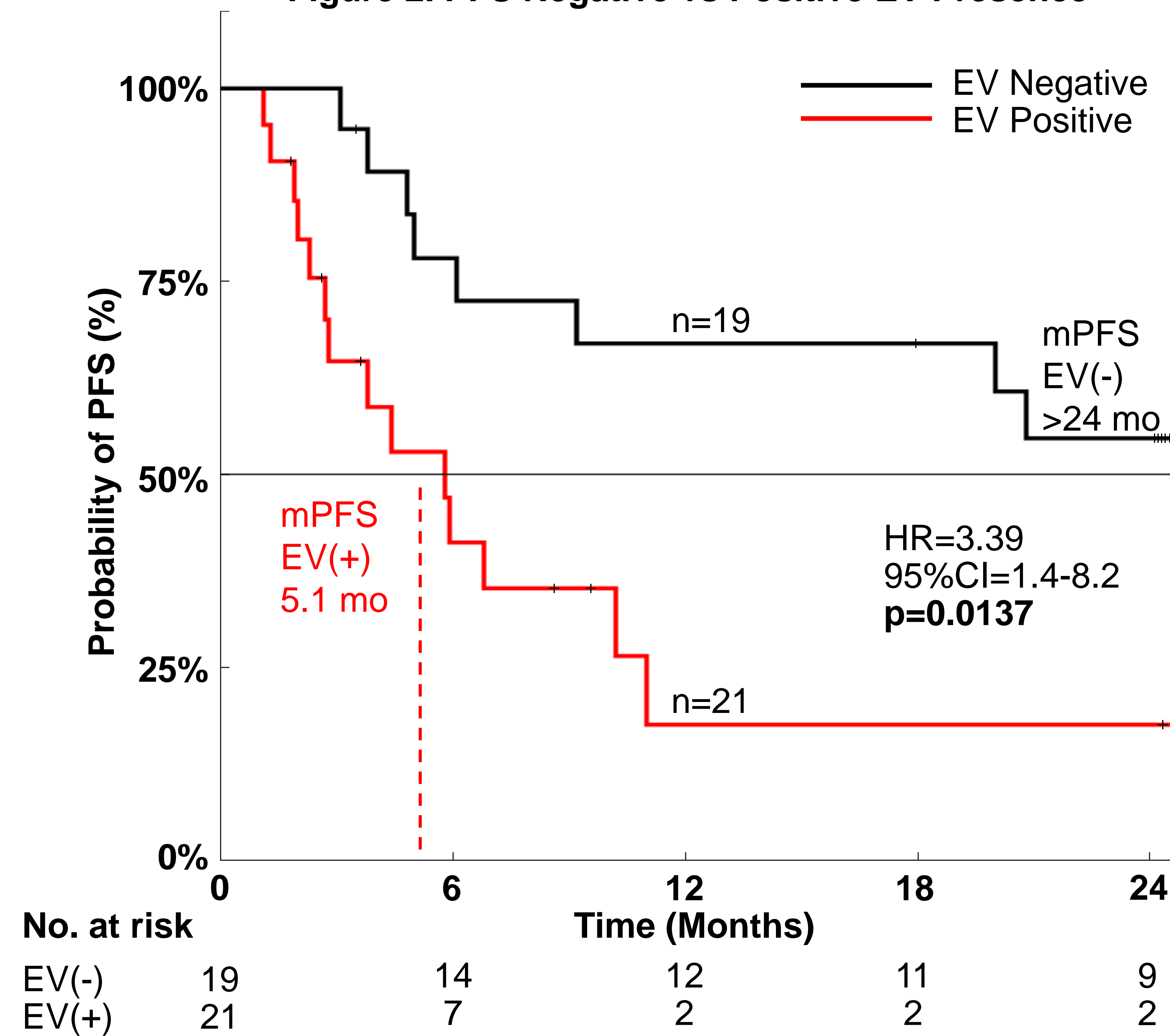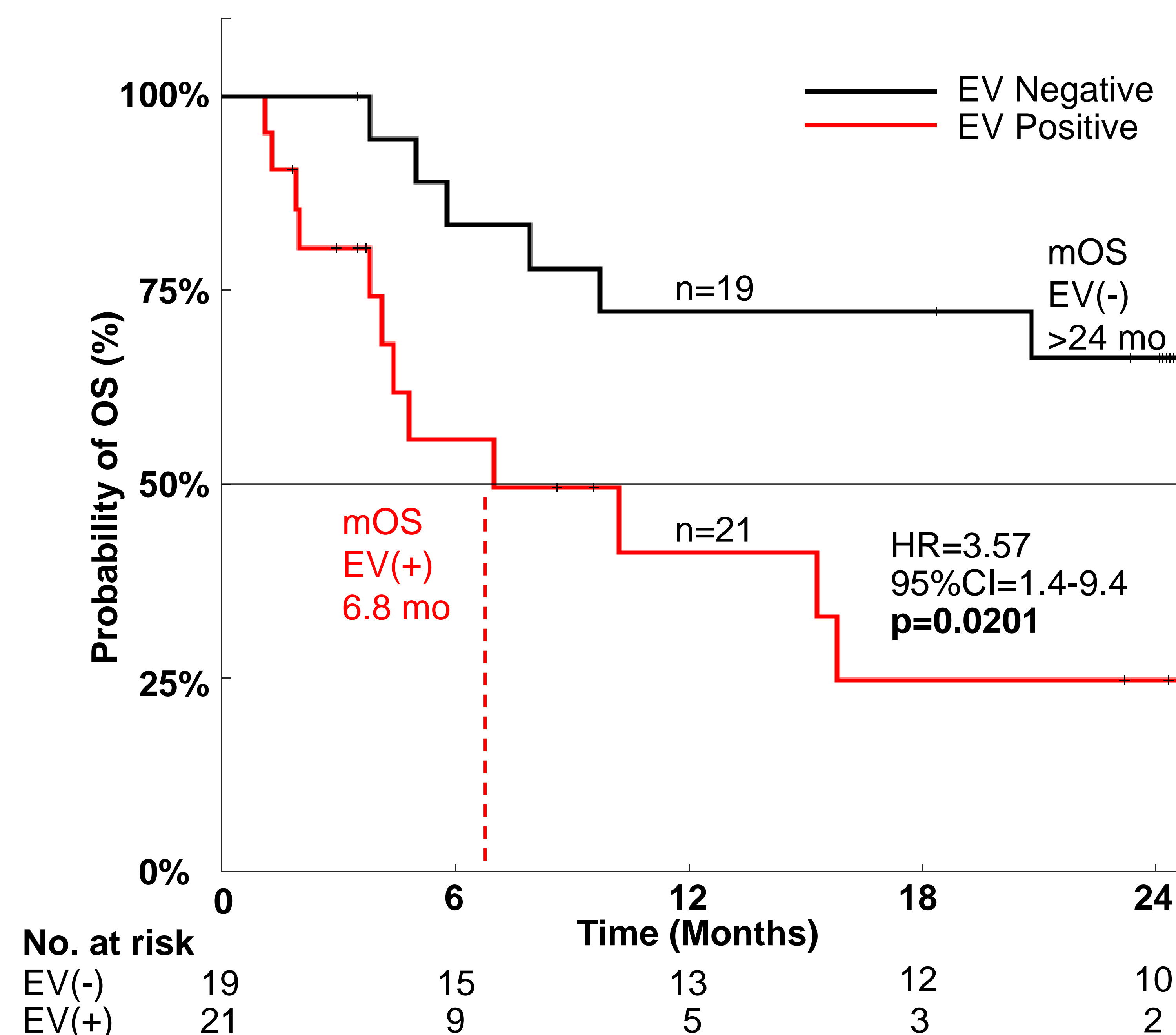### Figure 2. PFS Negative vs Positive EV Presence

mPFS EV(-) >24 mo
mPFS EV(+) 5.1 mo
n=19
n=21
HR=3.39
95%CI=1.4-8.2
p=0.0137

No. at risk					
EV(-)	19	14	12	11	9
EV(+)	21	7	2	2	2

Figure 3. OS Negative vs Positive EV Presence

mOS EV(-) >24 mo
mOS EV(+) 6.8 mo
n=19
n=21
HR=3.57
95%CI=1.4-9.4
p=0.0201

No. at risk					
EV(-)	19	15	13	12	10
EV(+)	21	9	5	3	2

RESULTS

- CAMLs were identified in 88% (n=35/40) of all samples
- EV budding was identified in 60% (n=21/35) of CAMLs
- Presence of EV budding in CAMLs was associated with significantly worse PFS (HR=3.39, 95%CI=1.4-8.2, **p=0.0137**)
- Presence of EV budding in CAMLs was associated with significantly worse OS (HR=3.57, 95%CI=1.4-9.4, **p=0.0201**)
- EVs were found to have positive PD-L1 expression in 57% (n=12/21) of CAMLs
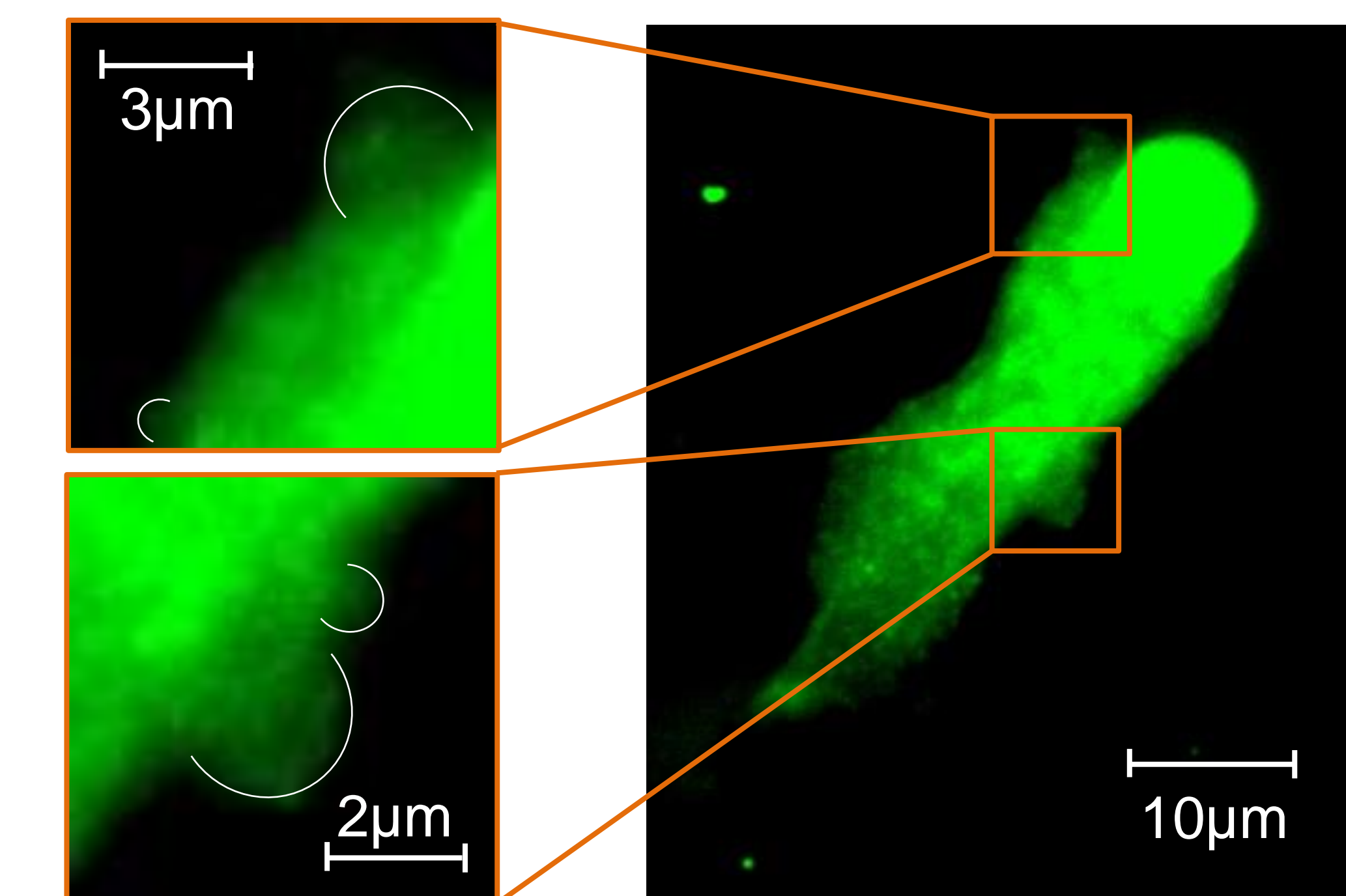

Figure 4. EV positive CAML (45µm in diameter) stained for PD-L1. Multiple PD-L1 positive EVs ranging from sizes of <1µm to 3µm.

CONCLUSIONS

- EV budding found on phagocytic stromal cells found in the blood appear with tumor positive biomarkers, predict faster disease progression and poor survival.
- These findings suggest that CAMLs are an origin cell for some cancer EVs and can be evaluated through liquid biopsy analysis.
- Larger validation studies and cross comparison of PD-L1 on EVs as it related to immunotherapy response is ongoing.

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FUNDING SOURCES