Extracellular Vesicles Budding from Stromal Macrophages from the Blood Circulating in Metastatic Non-Small Cell Lung Cancer Patients Predict Clinical Outcome

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ABSTRACT

Extracellular vesicles (EVs) which includes exosomes, microvesicles, and apoptotic bodies are involved in cellular communication, tumor growth, and metastasis in cancer (**Fig 1**). Recently, extracellular budding structures on Cancer Associated Macrophage-Like Cells [CAMLs], a subtype of phagocytic circulating stromal cells found in circulation, was observed in patients with metastatic non-small cell lung carcinoma (mNSCLC). In this prospective analysis of n=104 mNSCLC patients, we enumerated EV budding on CAMLs to determine their clinical significance on Progression Free Survival (PFS) & Overall Survival (OS), further subtyping based on treatment with or without PD-L1 Immunotherapy (IMT) based on standard of care treatment. These preliminary data suggests that EV positive (EV+) CAMLs prognosticates for worse outcomes in mNSCLC.

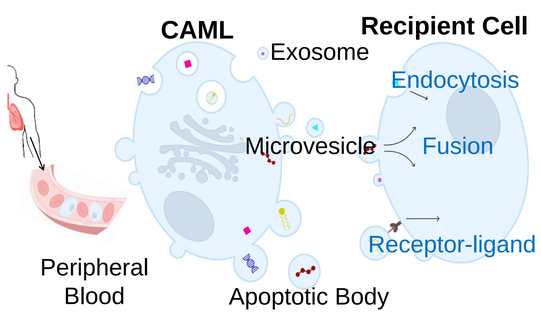
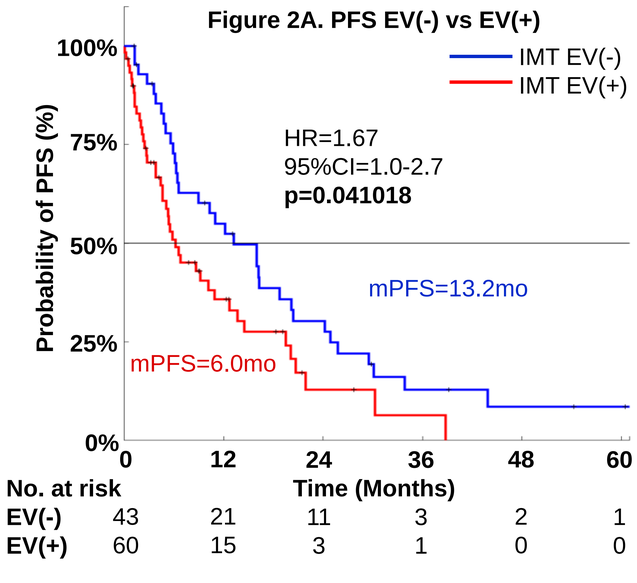


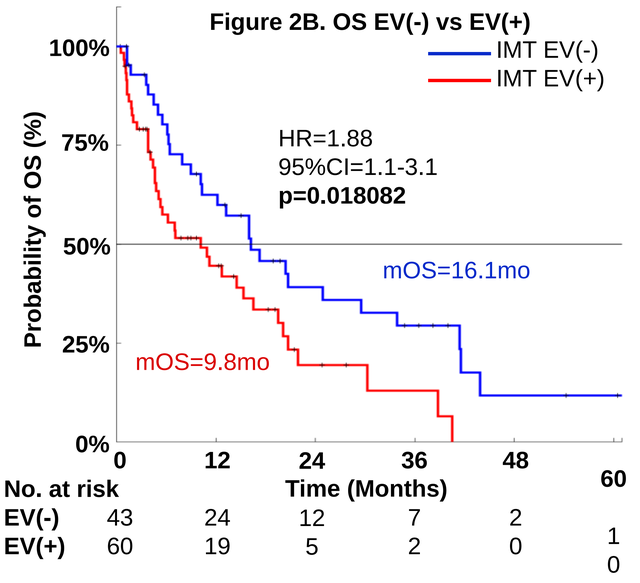
Figure 1. Three subtypes of EVs are defined by method of formation and size (exosomes [~30-100nm], microvesicles [~100nm-1µm], and apoptotic bodies [~1-5µm]).

MATERIALS & METHODS

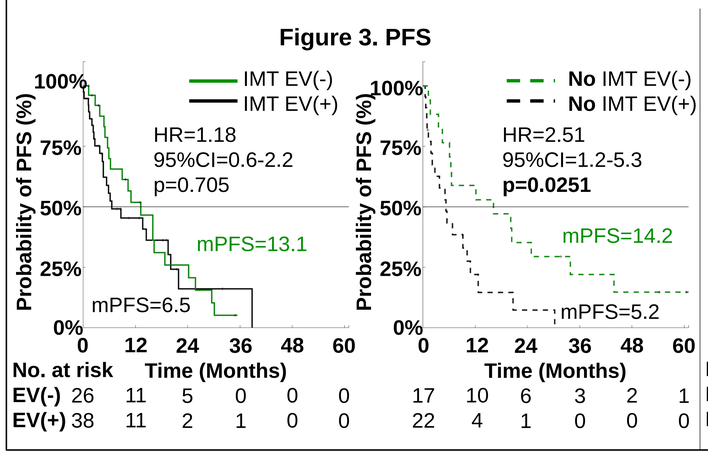
We initiated a single blind multi-year prospective study to investigate the relationship between EV budding in CAMLs to PFS & OS prior to start of new treatment lines for mNSCLC. Anonymized blood (7.5 mL) was procured from n=104 pathologically confirmed mNSCLC patients & filtered to isolate CAMLs to measure EV budding using tumor/EV markers (i.e. cytokeratin, CD63 or CD81) and immune specific marker (PD-L1). Blood was filtered by CellSieve™ microfiltration and EV budding characterized as small (≤5µm) bulbous protrusions from the cell cytoplasm. EVs were quantified by presence (EV+) or absence (EV-) to compare PFS & OS with hazard ratios (HRs) at 60 months by censored univariate and multivariate analyses.

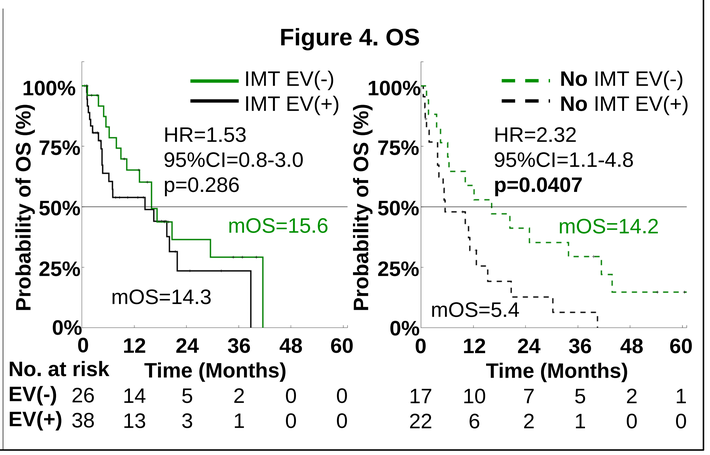
Figure 2. Survival analysis of mNSCLC patients with EV(-) CAMLs vs EV(+) CAMLs for PFS (2A) or OS (2B).





Figures 3 & 4. PFS & OS of mNSCLC patients treated with IMT or without IMT by EV(-) CAMLs vs EV(+) CAMLs.





CONCLUSIONS

- EV budding on phagocytic stromal cells found in the blood of mNSCLC patients appears to predict for poorer PFS and OS (**Figs 5 and 6**)
- Poorer PFS and OS caused by EV presence in CAMLs is reduced with the addition of PD-L1 immunotherapy (**Figs 3 and 4**)
- Larger validation studies are ongoing in both mNSCLC and stage III NSCLC patients

FUNDING SOURCES

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RESULTS

- CAMLs were identified in 93% (n=97/104) of all samples
- EV budding was identified in 62% (n=60/97) of samples with CAMLs
- EV(+) CAMLs were associated with significantly worse PFS (HR=1.67, **p=0.0410**) and OS (HR=1.88, **p=0.0108**) (**Fig 2**)
- EV(+) patients NOT treated with IMT had significantly worse PFS (HR=2.51, **p=0.0251**) and OS (HR=2.32, **p=0.0407**) (**Figs 3 and 4**)
- EV(+) patients appeared to benefit from additional IMT with longer mPFS and mOS (**Figs 3 and 4**)

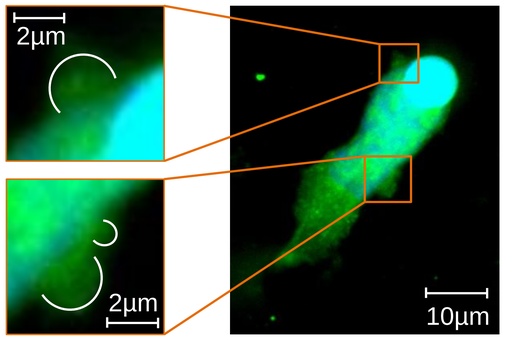

Figure 5. EV positive CAML (45µm) stained for PD-L1 with PD-L1 positive EVs ranging <1µm-3µm.

Figure 6. Patient demographic table.

Median Age (Range)		**66 (42-82)**
Sex (M/F)		**63 (61%) / 40 (39%)**
Race	White	**79 (77%)**
	Black	**18 (17%)**
	Other	**6 (6%)**
Histology	Adeno	**59 (58%)**
	NSCLC/Unknown	**23 (22%)**
	Squamous	**21 (20%)**
Smoker History	Never	**16 (16%)**
	Light (<50pks/yr)	**47 (46%)**
	Heavy (≥50pks/yr)	**33 (32%)**
	Unknown	**7 (6%)**
ECOG	0	**48 (47%)**
	≥1	**47 (46%)**
	Unknown	**8 (7%)**
Recurrence	Localized	**29 (28%)**
	Distant	**74 (72%)**
Immunotherapy	Pembro	**25 (24%)**
	Durva	**12 (12%)**
	Atezo	**11 (11%)**
	Other	**18 (17%)**
EV Presence	EV(-)	**43 (42%)**
	EV(+)	**60 (58%)**