38P - Single molecule localization microscopy for extracellular vesicles detection in cancer

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BACKGROUND

Extracellular vesicles (EV) are nanometric lipid bilayer coated particles constitutively released from cells. EVs are a heterogeneous population and can be sampled as liquid biopsy. Beyond messengers between cells, EVs act as regulators and mediators of many processes underlying cancer evolution: inflammation, proliferation, invasion, immune modulation, angiogenesis, epithelial mesenchymal transition. The aim of the present research is to describe a new method to obtain high resolution images of individual EVs in patients with advanced cancer.

METHODS

Plasma from 10 patients (Table 1) with advanced cancer enrolled in the institutional molecular profiling program STING (NCT04932525) was diluted in PBS 1:50 and loaded on slides for Single Molecule Localization Microscopy (SMLM) imaging. EVs were stained using a mix of anti-tetraspanin Ab (CD9, CD63, CD81) labelled with AF647 fluorophores to only select tetraspanin positive EVs for imaging and analysis. Slides were coated with capture antibodies directed against EV and cancer biomarkers (TSG101, EpCam, CD151, CD9, CD63, CD81). Single molecule imaging (dSTORM) was performed using Abbelight SMART-kit buffer on a SAFe360 Abbelight super-resolution system. Fluorophore labelled EVs were excited with a 500mW 640nm laser at 60% of nominal power over a ROI of 80*80 micrometers, by Abbelight Aster technology for homogeneous laser illumination; for each dataset 10000 frames were collected at 40 FPS, with two-three technical replicates per sample (RAW data). Single molecule localization in 3D was performed on the RAW data using Abbelight Neo Software, and localization clusters corresponding to labelled EVs were extracted using DBSCAN and K-Ripley clustering algorithms.

Birth date	Dec-51	Mar-63	Sep-48	Dec-55	May-69	Mar-81	Aug-49	Sep-63	Mar-73	Aug-49
Gender	F	F	F	M	F	F	F	F	F	F
Collection date	May-21	May-21	May-21	Jun-21	Jul-21	Jul-21	May-21	Jun-21	Jun-21	Jun-21
Primary tumor	lung	lung	lung	lung	sarcoma	breast	breast	breast	breast	breast
Histological type	adenoca	adenoca	SCC	adenoca	PLS	NST	NST	NST	NST	lobular
Grade					3	1	2	2	2	2
ER (%)						90	100	80	100	100
PgR (%)						70	0	0	70	15
Ki-67 (%)						20	20	30	30	20
HER2 (IHC)						0	1+	0	0	1+
PD-L1 (%)	0	0	80	5						
Mutations	no	KRAS exon 2 G12C	no	EGFR exon 20 duplication						
Surgery	no	no	no	no	yes	yes	yes	yes	yes	no
RT met setting	no	yes	no	no	no	no	no	no	no	yes
Neoadj treatment	no	no	no	no	yes	no	no	yes	yes	no
Adjuvant treatment	no	no	no	no	no	yes	yes	no	yes	no
Relapse free interval					4 months	21 months	3 months	4 years	22 months	
Number of lines in met setting	1	3	1	1	1	2	6	5	3	4
Visceral disease	yes	yes	yes	yes	yes	yes	yes	yes	no	no
Organs involved	B, Lu, LN	B, Lu, Br, LN	B, Lu	B, Lu, LN	Lu, soft tissue	B, Li, Lu	B, Li, LN, adrenal gland	B, Lu, LN	B	B, breast, LN

Table 1. Clinical characteristics of patient with advanced cancer. Adenoca adenocarcinoma, B bones, Br brain, EGFR Epidermal growth factor receptor, ER estrogen receptor, F female, HER2 human epidermal growth factor receptor 2, KRAS Kirsten rat sarcoma virus, Li liver, LN lymph nodes, Lu lung, M male, met metastatic, neoadj neoadjuvant, NST no special type, PD-L1 programmed death-ligand 1, PgR progesterone receptor, PLS pleomorphic liposarcoma, RT radiotherapy, SCC squamous cell carcinoma.

RESULTS

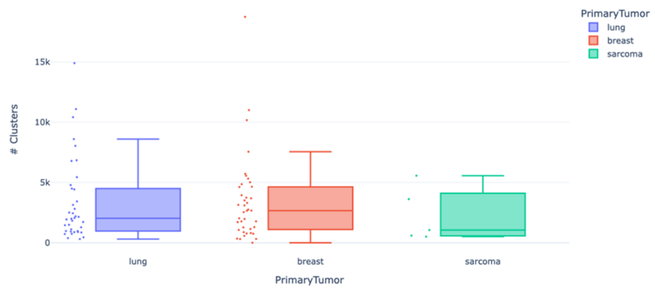

Figure 1. EV count per patient and primary tumor type (each data point = 1 captured antibody for 1 patient).

Figure 2. Median EV size (nm) per patient and primary tumor type (each data point = 1 captured antibody for 1 patient).

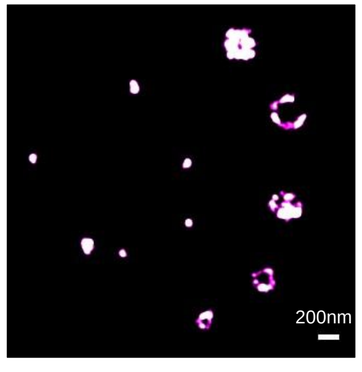

Fig. 3: Super resolution imaging of CD81 capture spot enabling to see individual tetraspanin positive EVs in patient with sarcoma.

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Presenting author has no conflict of interest to declare.

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High resolution images of EVs were obtained with SMLM. Number and size distribution of clusters were quantified for each Ab allowing to observe differences between patient samples.
Median number of EV clusters was 2665.5, 2030 and 1055 for breast cancer, lung cancer and sarcoma respectively.
Median size of EVs was 62.44nm, 56.095nm and 50.46nm for breast cancer, lung cancer and sarcoma respectively.
Thus, SMLM can be considered as an additional technique to detect and characterize individual EVs in clinical samples.

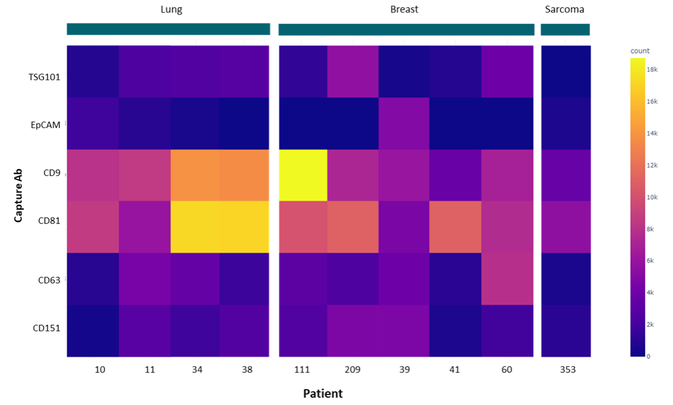

Fig. 4: Total number of EVs imaged per capture Ab per patient per cancer type

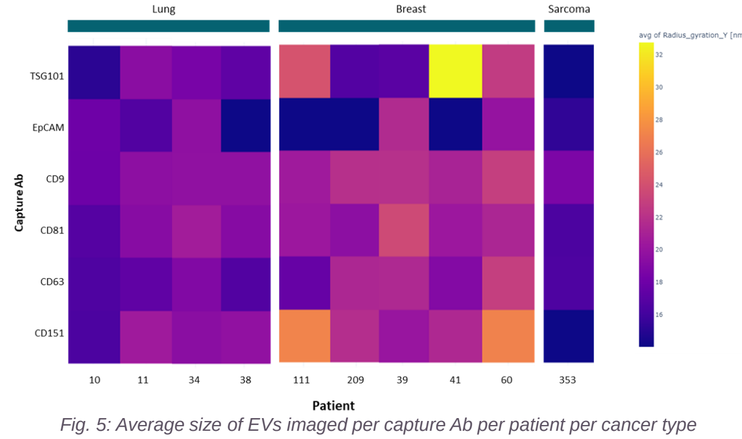

Fig. 5: Average size of EVs imaged per capture Ab per patient per cancer type

CONCLUSION

SMLM imaging with tetraspanin labeling is able to detect differences in EV clusters quantity and size distributions in plasma from patients with advanced cancer. Additional investigations are ongoing to further develop the technique and make it applicable in clinical practice.