Extracellular vesicles (EVs) are lipid membrane-enclosed, nanosized particles secreted by cells to regulate intercellular communication. EVs released by cancer cells are valuable carriers of tumor information and are considered as having promising potential as biomarkers in cancer diagnosis, progression, and surveillance. In our ongoing study, we are investigating the diagnostic and prognostic value of blood circulating EVs expressing cancer stem cell markers in a cohort of patients with advanced cancer.

**METHODS**

This prospective observational study enrolled patients with histologically or cytologically confirmed diagnosis of advanced cancer. 195 patients, recruited from January 2016 to February 2021, were included in this analysis. Identification, enumeration, and phenotypic characterization of EVs from whole fresh blood samples were obtained by applying a recently patented simplified polychromic flow cytometry method based on EV staining with a lipophilic cationic dye (LCD). EV subpopulations were identified based on their positivity to selected cancer stem cell markers, including CD133, EPICAM, CD90 and CD29. EV subtype concentration was compared between cancer patients and healthy controls. The relationship between overall survival and blood concentration of circulating CD133+ EVs was explored in a subgroup of patients with advanced colorectal cancer.

**RESULTS**

Figure 2. Comparisons of CD133+ EPICAM: EVs between cancer patients and HCs. A total of 183 cancer patients (91 WSCC advanced cancer, 65 melanoma, 29 pancreatic cancer, 8 breast cancer, 1 ovarian cancer and 5 acute myeloid leukemia) were included in this analysis. Median blood concentration of circulating CD133+ EVs was significantly higher in the cancer patient population (median EVs/µl = 19.62; 5% to 74.22; 75.94) compared to HCs (median EVs/µl = 1.04; 5% to 4.40; 25.00) (p < 0.000001).

Figure 3. Survival analysis according to blood concentration of CD133+ EVs in patients with advanced colorectal cancer. Survival analysis revealed a correlation between high CD133+ EV-containing EVs (EV concentration > 74 EVs/µl) and poor survival in the colorectal cancer cohort. Median OS in patients with higher CD133+ EV concentration was 20.9 (95% CI 17.9–26.0) months vs 36.2 (95% CI 27.5–43.7) months in patients with lower blood concentrations of CD133+ EVs (EV concentration ≤ 74 EVs/µl) (p = 0.000012). In the multivariate analysis, EVs were an independent poor survival factor (HR = 0.95; 95% CI 0.86–1.06) vs EV concentration ≤ 74 EVs/µl. The median survival was 36.2 versus 43.7 months in patients with EV concentration ≤ 74 EVs/µl versus >74 EVs/µl, respectively (p = 0.000012).

**CONCLUSIONS**

- Blood concentration of CD133+CD326+ EVs were significantly higher in cancer patients than healthy controls.
- A difference in overall survival according to blood level of CD133+CD326+ EVs was observed in patients with advanced colorectal cancer.
- Overall, our results suggest a potential role of blood circulating EVs with a cancer stem cell-phenotype as a diagnostic and prognostic tool in cancer patients.

**REFERENCES**