Healthcare Innovations
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Circulating tumor cells in patients with glioblastoma

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INTRODUCTION

- Glioblastoma (GBM) is an aggressive type of tumor arising from the central nervous system (1).
- GBM remains an incurable disease despite current standard of care, with survival rate of approximately 15 months from diagnosis (2).
- GBM can release tumoral content which crosses the blood-brain barrier (BBB) and can be detected in patients' blood, such as circulating tumor cells (CTCs) (3).
- CTCs carry tumor information and have shown promise as prognostic and predictive biomarkers in other cancer types (4).
- CTCs have been detected in GBM patients and characterized using an astrocytic marker, glial fibrillary acidic protein (GFAP). EGFR amplifications are observed in <50% of GBMs (4).
- Currently, the prognostic utility of CTCs in GBM is not well understood (5).
- We hypothesized that CTCs could predict clinical outcomes in newly diagnosed GBM patients.
- Here, we aim to isolate CTCs from GBM patients using a label free technology and characterize them using GFAP, cell surface vimentin and EGFR to study their prognostic value.

METHODS

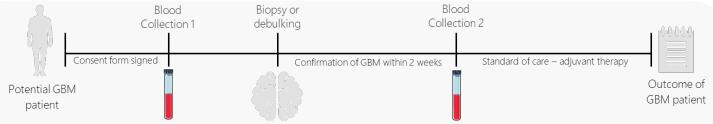

Figure 1. Scheme for sample collection. Blood was collected from GBM patients (n=20) in two timepoints (before and after surgery).

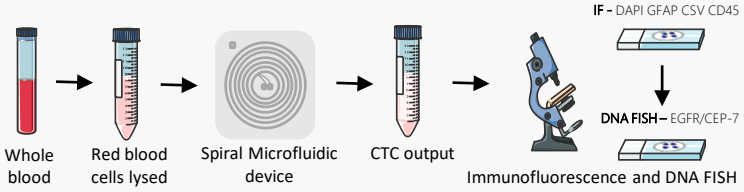

Figure 2. Scheme for CTC isolation and characterization. Red blood cells were lysed and samples were loaded in a syringe and pumped through the spiral chip. The CTC outlet was cytospun for Immunofluorescence staining using (DAPI (nuclei), anti-CD45 (leukocyte common antigen), anti-GFAP (Glial fibrillary acidic protein) and CSV (cell surface vimentin). CTC positive criteria: diameter larger than 9µm and GFAP or CSV positive and CD45 negative.

RESULTS

CTC enrichment and characterization

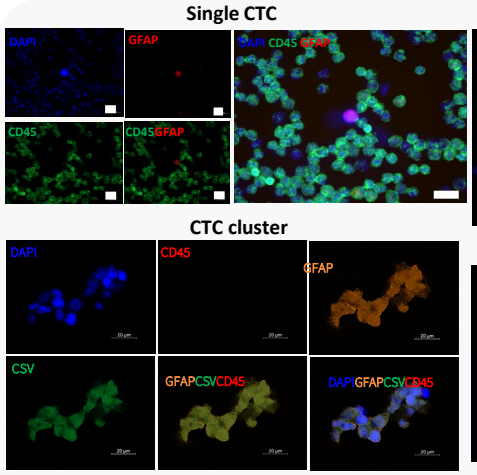

Fig 3. Representative image of CTC characterization using immunofluorescence targeting GFAP (red), CD45 (green) and DAPI (blue), scale bar = 20µm.

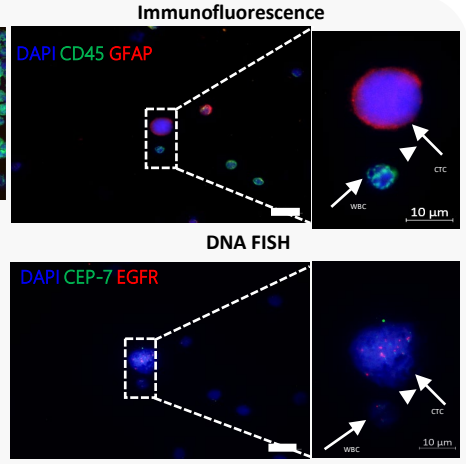

Fig 4. Characterization of putative CTC at a molecular level using DNA FISH to detect EGFR (red) copies and CEP-7 (green).

CTC counts and clinical outcome correlation

Table 1. CTCs counts and clinical data from GBM patients

Pt	Tumour volume (cm3)	Extent of resection*	CTC counts before and after surgery		Outcome
01	20.0	Biopsy	1	2	Deceased
02	14.4	Near total	1	3	Deceased
03	1.7	Complete	1	0	No recurrence
04	26.3	Complete	0	0	No recurrence
05	38.7	Near total	17#	3#	Recurrence
06	83.2	Near total	5	5	Deceased
07	24.8	Debulking	0	0	Recurrence
08	4.4	Near total	1	0	Deceased
09	53.8	Debulking	0	2	Deceased
10	44.9	Near total	3	2	Deceased
11	40.5	Biopsy	0	3	No recurrence
12	33.0	Near total	0	1	Recurrence
13	39.9	Complete	0	0	No recurrence
14	63.8	Near total	0	1	Recurrence
15	39.5	Complete resection	24#	3	Deceased
16	14.3	Complete resection	0	0	N/A
17	50.0	Biopsy	0	N/A	N/A
18	19.0	Debulking	0	0	N/A
19	30.2	Debulking	2	1	N/A
20	57.6	Complete	0	0	N/A

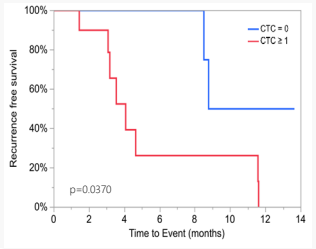

Fig 5. Kaplan-Meier curve showing recurrence-free mean survival time of CTC = 0 group and CTC ≥1 group **after surgery**.

CONCLUSIONS

- We isolated CTCs in 13 out of 20 patients (65%) (9/20 before surgery (45%) and 11/19 after surgery (57.9%).
- Patients with CTC counts ≥ 1 after surgery had a significant shorter recurrence-free survival (p=0.0370).
- CTCs and CTC clusters have potential prognostic value as biomarkers for GBM management.

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