1796P - CXCL12-loaded-hydrogel (CLG) based 'pseudo niche': a new device for CTCs capturing and characterization

Luigi Portella*[1], Giuseppe Guardascione*[1], Giulia Bertolini[2], Caterina Ieranò[1], Crescenzo D'Alterio[1], Giuseppina Rea[1], Sara Santagata[1], Anna Maria Trotta[1], Rosalba Camerlingo[3], Emilia Scarpa[4], Sabrina Cecere[4], Alessandro Ottaiano[5], Giuliano Palumbo[6], Alessandro Morabito[6], Roberto Pacelli[7], Sandro Pignata[4] and Stefania Scala[1]

[1]Microenvironment Molecular Targets, [3]Cell Biology and Biotherapy, [4]Gynecology Oncology, Abdominal Oncology[5], Thoracic Medical Oncology[6]-Istituto Nazionale Tumori IRCCS - Fondazione G Pascale, Napoli, Naples, ITALY; [2]Tumor Genomic Unit Istituto Nazionale Tumori di Milano, Milan, ITALY; [7]Department of Advanced Biomedical Sciences, School of Medicine, University of Naples Federico II, Naples, ITALY.

*These authors equally contributed and declare no conflict of interest; mail to: l.portella@istitutotumori.na.it

Background

Circulating tumor cells (CTCs) respond to chemokine gradients and "home" toward niche microenvironments at a target organ via extravasation. Few CTCs, only 0.01% or less, develop metastasis but their molecular background is unknown. The pre-metastatic niches (PMN) is a complex and permissive microenvironment that favours metastasis formation. The chemokine CXCL12, CXCR4 ligand, expressed in PMN, mobilizes and attracts CXCR4-expressing bone marrow derived cells (BMDCs), immunosuppressive immune cells and CTCs at the metastatic site promoting metastasis. CXCR4 expressing CTCs are the most probable candidates to generate metastases and CXCR4 inhibition impairs metastatic dissemination targeting cancer cell "seeding" and suppressing PMN-formation (CTC "soil")[1]. A CXCL12-loaded hydrogel (Belotero®) (CLG) able to mimic a PMN was developed to isolate and characterize CTCs with infiltrating capability[2]. TRAP4MET is a monocentric biological clinical trial (EC n. 50/20) in which 50 metastatic patients are enrolled to evaluate feasibility of CLG-dependent CTCs isolation and characterization.

Aims and Medical Need

- Current enrichment strategies fail to identify CTCs endowed with the capability to extravasate and metastasize.
- Implanted biomaterials are able to create a 'fake niche', rerouting and trapping extravasating CTCs, thus enabling cells enumeration and molecular characterization[2-3].
- The aim is to select and trap CTCs with infiltrating and migratory capabilities from metastatic cancer patients to enumerate and characterize the specific, invasive CTC population.

Materials & Methods

Gel preparation: Hyaluronic Acid based gel Belotero Intense® was purchased from Merz Pharma. CXCL12 (R&D System) (300 ng/ml) was dropped onto the sterile gel, gently mixed and immediately used. **EG/CLG Infiltration assay:** 150ul Empty Gels (EG) or CLG were placed on a well of an 8-well chamber slide. 100-500-1000 A498 (renal cancer), HT29 (colon cancer) or OVCAR8 (ovarian cancer) cells were seeded over the gels in 150ul Serum Free (SF) media and allowed to infiltrate ON, then cells were fixed with 400ul 2%FA+Hyaluronidase (HAase) washed in PBS and stained with DAPI for enumeration. **EG/CLG Recovery assay:** 200 HT29 were suspended in 150ul SF media, seeded on 150ul EG or CLG, allowed to migrate ON. Cells were recovered by gel digestion with HAase and grown in complete media.

Results

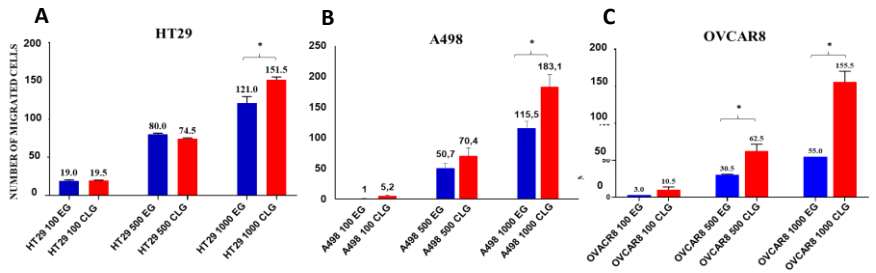


Figure 1. Human cancer cells efficiently infiltrate CLG in vitro. Cancer cells were seeded over the EG/CLG, allowed to infiltrate ON and then fixed with 2%FA+HAase and stained with DAPI.

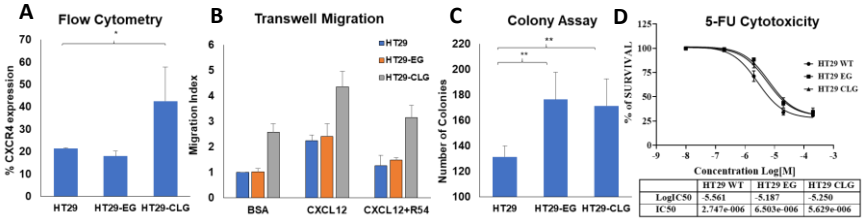
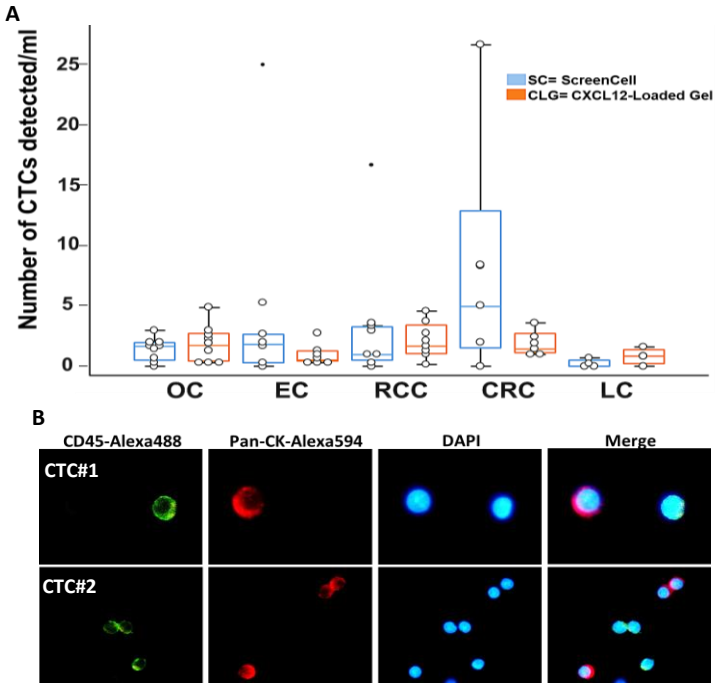


Figure 2. HT29-CLG overexpress CXCR4, form more colonies, migrate toward CXCL12 and are more resistant to 5-FU. Compared to HT29 and HT29-EG, HT29-CLG overexpress CXCR4 (21.5±0.3/18±2.2/42.5%±15 respectively) and highly migrates toward CXCL12 (migration index: 2,5/3,1 and 4,3 respectively). Moreover, gel recovered cells, develop higher number of colonies (131±8/176±21/171±21 respectively) and are more resistant to 5-FU (IC_{50} 2.7/6.5/5.6µM respectively) as compared to parental cells.

TRAP4MET: CTCs from 29 patients (8 Ovarian Cancer (OC), 7 Renal Cancer (RCC), 6 Endometrial Cancer (EC), 5 Colorectal cancer (CRC) and 3 Lung Cancer (LC)) were isolated from CLG vs SC.



	#Patients	CTCs SC/cc	ST.ERR	CTCs CLG/cc	ST.ERR
OC	8	1,42	0,35	1,89	0,56
RCC	7	3,62	2,23	2,16	0,60
EC	6	5,28	3,97	0,98	0,38
CRC	5	8,40	4,78	1,91	0,48
LC	3	0,22	0,22	0,81	0,45
TOT	29				

Figure 3 and table 1. CTCs from metastatic patients were successfully isolated using CLG. Average CTC/cc number with CLG were: 1.89±0.56 in OC, 2.16±0.60 in RCC, 0.98±0.38 in EC, 1.91±0.48 in CRC and 0.81±0.45 in LC.

Conclusion

- **CLG-derived cancer cells showed a more aggressive phenotype compared to the parental cell line.**
- **CLG-device allowed the capturing of CTCs capturing, enumeration and characterization.**
- **TRAP4MET clinical trial evaluated CLG vs Screen Cell (SC) CTCs in 29 patients with solid cancer (8 OC, 7 RCC, 6 EC, 5 CRC and 3 LC).**
- **CLG average CTC/cc was higher for OC (CLG: 1.89±0.56 vs SC: 1.42±0.35), lower in RCC (CLG: 2.16±0.60 vs SC: 3.62±2.23) lower in EC (CLG: 0.98±0.38 vs SC: 28±9.97 in EC), lower in CRC (CLG: 1.91±0.48 vs SC:8.40±4.78); higher in LC (CLG: 0.81±0.45 vs SC: 0.22±0.22).**
- **CLG is a feasible tool to isolate, count and characterize patients derived CTCs. Potentially CLG isolated CTCs display higher metastatic capability. The molecular characterization of CLG-isolated CTCs is ongoing.**

References

1. Zhou M, et al: Improving anti-PD-L1 therapy in triple negative breast cancer by polymer-enhanced immunogenic cell death and CXCR4 blockade. Journal of controlled release 2021;334:248-262.
2. Ierano C et al CXCL12 loaded-dermal filler captures CXCR4 expressing melanoma circulating tumor cells. Cell death & disease 2019;10:562
3.Huang Y, et al: Chemokine releasing particle implants for trapping circulating prostate cancer cells. Scientific reports 2020;10:4433.