1. Introduction

- Colorectal cancer (CRC) is the 3rd most common cancer diagnosed worldwide in both men and women [1].
- Only 39% are diagnosed at localized stage. 5-year survival rates decrease rapidly for patients with advanced and metastasized disease (stage III 61%, stage IV 8%).
- Better markers for detection of CRC progression, therapeutic resistance and minimal residual disease are still needed. Liquid biopsies (CTCs, ctDNA), are promising biomarkers shed by the tumor into the blood [2].
- Analysis of KRAS, BRAF, and PIK3CA gene mutations on CTCs would be a key tool to guide CRC therapy in real-time [1].

2. Methods and Workflow

- **CTC Workflow**
  - CTC Isolation using a label-free microfluidic device that utilizes inertia and laminar microvortices (Vortex technology). Cancer cells (>1um) larger and more deformable, are trapped in vortices while smaller cells (red and white blood cells) pass through [3].
  - Cells were collected in a well-plate, fixed with PFA, stained for DAPI, EpCAM, CD45, and enumerated using standardized classification criteria [3].
  - DNA was extracted & subjected to targeted mutational analysis using Sanger sequencing.

- **ctDNA Workflow**
  - ctDNA isolation by a multiplexed mutation and enrichment technology (Boreal Genomics) [4].

3. Results

- **Cell Immunostaining & Classification**
  - Gallery of CTCs and WBC stained with EpCam, CD45, and DAPI. Scale bar represents 20 μm.
  - 81.4% of CTCs were EpCam+. 18.4% EpCAM- CTCs would have been missed with EpCam-based approaches.

- **CTC Enumeration**
  - 80% of CRC samples ‘positive’ for CTCs using age-matched healthy threshold (0.4 CTCs/mL).
  - Patient #2: 99 CTCs/mL (mean: 3.4 CTCs/mL)
  - Age-matched healthy donors:
    - 0 to 0.4 CTCs/mL (mean: 0.1 CTC/mL)

- **CTCs, ctDNA, and CEA marker**
  - CTC numbers & ctDNA levels showed a similar dynamic and seem more reliable compared to CEA (N=2).

4. Conclusion & Future Directions

- CTCs and ctDNA are complementary and both may reveal disease progression or recurrence earlier than imaging and seem more reliable than CEA marker (more experiments needed). CTC molecular analysis may give additional information and will potentially help promote the development of tailored therapies for every individual patient.

- **Next?** Current WGA methods perform well on fresh cells but have variable bias on fixed cells (data not shown). WGA and NGS (Next-Generation Sequencing) - including target sequencing and whole genome sequencing - are currently evaluated on fresh CTCs.

5. Acknowledgements & References

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