Towards fluorescence molecular in vivo liquid biopsy of circulating tumor cells

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Towards Fluorescence Molecular *in vivo* Liquid Biopsy of Circulating Tumor Cells

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Metastasis is responsible for most (~90%) cancer-related deaths

- **Hematogenous metastasis** mediated by CTCs
- Extremely rare! ~1-100 CTCs/mL
“Liquid Biopsy” Methods Are Field Standard

**Liquid biopsy** of CTCs:

- **Blood sample**
- **+ Analysis**

Implicitly assumes that:

1) The number of CTCs in a (~7.5mL) blood sample is representative of the entire peripheral blood.

2) The number of CTCs in PB is approximately constant in the time around the blood draw.

*Not generally true*
**Diffuse In Vivo Flow Cytometry (DiFC)**

- **Idea:** use *diffuse light* to sample large blood vessels and detect rare *fluorescent* CTCs.
- Large arteries and veins have **hundreds of µL / min** blood flow (~2mL total in mouse)

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**Tail Cross Section**

- Ventral caudal artery ~ 1mm deep
DiFC Instrument

Fiber probe

Probe 1

Probe 2

3mm

Source Fiber

BP-f

LP-f

Detection Fibers (x8)

Asph

0.5X playback speed

Northeastern University
DiFC Data Processing

- Determine direction (+ speed, depth)
- Reduces false-positives (FAR ~1/hour)
Multiple Myeloma Xenograft Model

Inject 5 x 10^6 MM.1s GFP+ Luc+ cells i.v.

N = 2 cohorts x 4 mice each = 8 mice

Allow MM to grow over 5 weeks:

Fluorescence monitoring of rare circulating tumor cell and cluster dissemination in a multiple myeloma xenograft model in vivo
Summary - DiFC

DiFC uses diffuse light to enumerate very rare fluorescently-labeled circulating cells

Advantages:
+ Can sample the entire peripheral blood volume in ~15 min (~100 μL/min)
+ Works on bulk tissue: back-illumination geometry
+ Simple measurement, easy to align

Disadvantages:
- Requires fluorescence cell labeling (e.g. GFP)**
- No images: data is “event detection”
- Does not capture CTCs (phenotyping)
Human Translation of DiFC?

DiFC technology is scalable (in principle*):
- diffuse NIR light
- epi-illumination
- Sensitivity 2-4 mm

Could sample *hundreds of mL blood per min* continuously

Fernando Ivich
BioE-PhD

Signal and measurement considerations for human translation of diffuse *in vivo* flow cytometry
Journal of Biomedical Optics

**BUT** - this would require highly *sensitive* and specific molecularly-targeted CTC contrast agent

*Consider: ~10^6 WBCs vs ~10 CTCs per mL blood*

Good news: these may already exist!

- Major recent advances in intraoperative cancer surgery *fluorescence guided surgery; FGS*
OTL38 Fluorescent Molecular Probe

- FR-α targeted small-molecule molecular probe for guided surgery
- Near-infrared absorption and emission
- FDA approved in 2021 FGS of ovarian and lung cancer ("Cytalux")
OTL-38 Molecular Probe for DiFC?

OTL38 (and analogs) have high reported affinity for CTCs in blood\(^1\)

**R21CA246413**
NIH-NCI IMAT Clinical and Translational Exploratory / Developmental Studies

New NIR DiFC Design*:

- 21 fiber double collection ring
- Low AF lens/filter design

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**References**


**Note**

*OTL* 38 (and analogs) have high reported affinity for CTCs in blood. This new NIR DiFC Design involves a 21 fiber double collection ring and a low AF lens/filter design.
L1210A (FR+) CTCs with OTL38

- Clear detection with OTL38 “pre-labeled” CTCs
- No false positive detections from unlabeled CTCs
L1210A (FR+) CTCs with OTL38

- (At least some) true-positive labeling and detections
- Few false positives (~2/hr) or non-specific labeling with OTL38
Summary - DiFC with OTL38 in Mice

• Fewer detections due lower peak amplitude (binding) and noise from injected / unbound OTL38
• More testing is needed – *but* early data is promising
FGS Contrast Agent Pipeline

Fluorescence Image-Guided Surgery – a Perspective on Contrast Agent Development

Connor W. Barth\textsuperscript{a}, Summer L. Gibbs\textsuperscript{a,b,c}


CTC / CHC Specific Contrast Agents:

Summer Gibbs
OHSU
Melissa Wong
OHSU

Kuni Foundation Grant: “Noninvasive Quantification of Circulating Cancer Cells for Early Detection & Treatment Monitoring”
“But, why not just do liquid biopsy?”

-Reviewer Number 3
CTC Short-Term Dynamics

Lewis Lung Carcinoma xenograft model

5x10^6 LLC Cells s.c. LL/2.Luc.GFP (Imanis Life Sciences)

Scan Time (Minutes)

Detections

= acquisition break
**Very brief summary:**

- Estimating CTC numbers from a small (5%) sample is inaccurate*
- CellSearch uses 0.15% b.v. (7.5 mL of a 5L human b.v.)
To Elaborate Slightly

In general, even with ideal statistics*, sampling more blood is more **accurate** and **sensitive** for rare CTCs:

DiFC shows that CTC numbers may **fluctuate significantly** over minutes, hours, days.

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*Primary axes are logarithmic.
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