Liquid biopsy for Head and Neck Cancers

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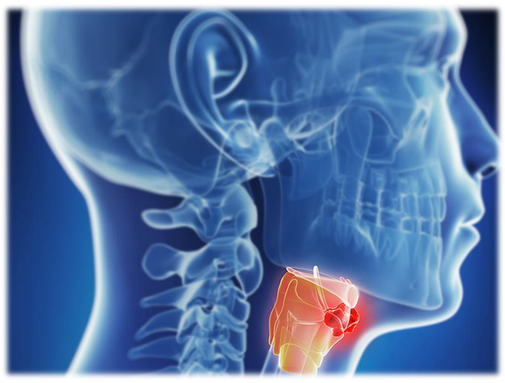
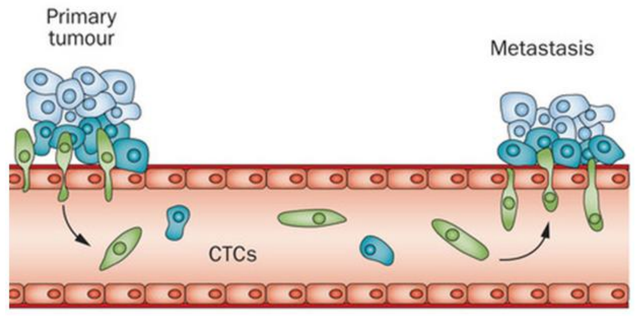
Head and Neck Cancer (HNC) : Circulating Tumour Cells



* 7th most common cancer, 900 000 new cases, 300 000 deaths (1)
* Less than 50% survive beyond 5 years
* Metastatic disease is responsible for 88% of HNSCC patient deaths within 12 months of diagnosis
* Tumour cells are shed by primary and metastatic cancers.
* Circulating tumour cells are a hallmark of invasive cancer cells and key to metastasis.

Advantages of blood to determine tumour burden

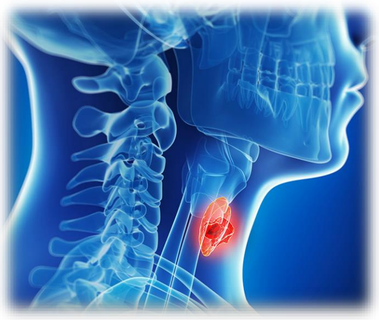
- **Minimally invasive** blood test vs multiple tumour biopsies.
- **Serial sampling** (intratumour heterogeneity & tumour evolution).
- **Real time monitoring** (metastatic progression & treatment response).

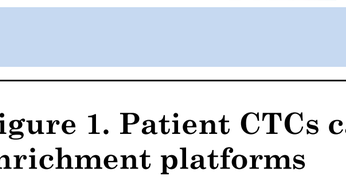


AIMS

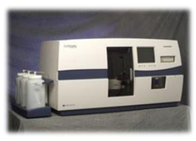
1. Compare CTC enrichment platforms (CellSearch®, ScreenCell®, RosetteSep™, Miltenyl Beads®, Microfluidic Technologies)
2. Characterize patient CTCs (IHC, Immunofluorescnce, DNA FISH)
3. Expand patient CTCs ex-vivo in MSK (2D) media and Happy Cell (3D)
4. Perform drug sensitivity testing on cultured CTCs
5. Develop a single CTC picking strategy for sequencing

MATERIALS & METHODS

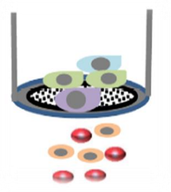




Enrichment

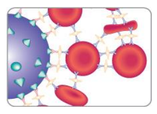


CellSearch
EpCAM



ScreenCell
Filtration

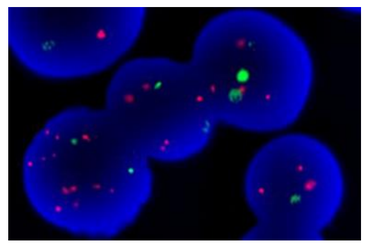
Microfluidic
Spiral Chip



RosetteSep ab
(CD45 depletion)

***Ex vivo* culture**
MSK (2D/3D),
Hypoxia (1-2% O_2)

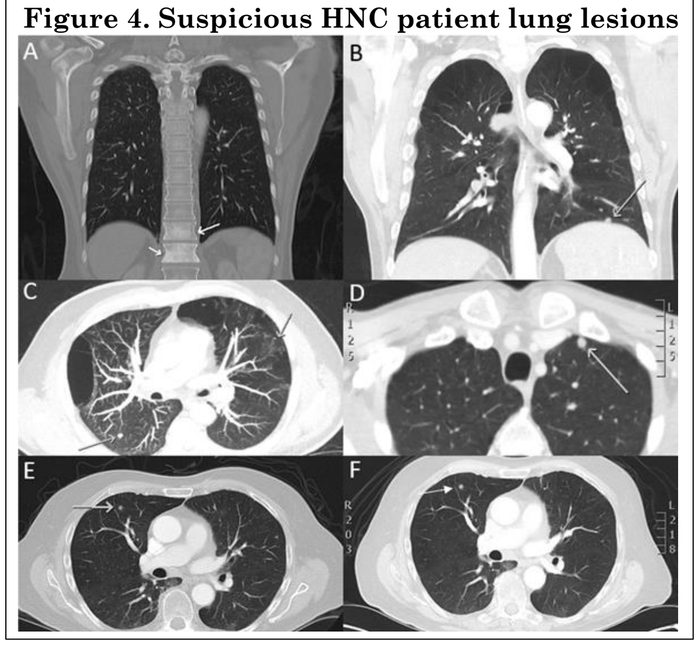
Drug sensitivity testing
- Repurposed drugs



Characterization

IF: (CK 8,18,19+, EGFR+, CD45-, DAPI+)
DNA FISH: (EGFR, FGFR1, MYC, CCND1, PIK3CA)
(Cover over 60% of known amplifications in HNC (TCGA))

Figure 4. Suspicious HNC patient lung lesions



RESULTS

Figure 1. Patient CTCs captured using : (A) CellSearch® (18.6%) (B) ScreenCell® (46.4%) and (C) RosetteSep™ (64.0%) enrichment platforms

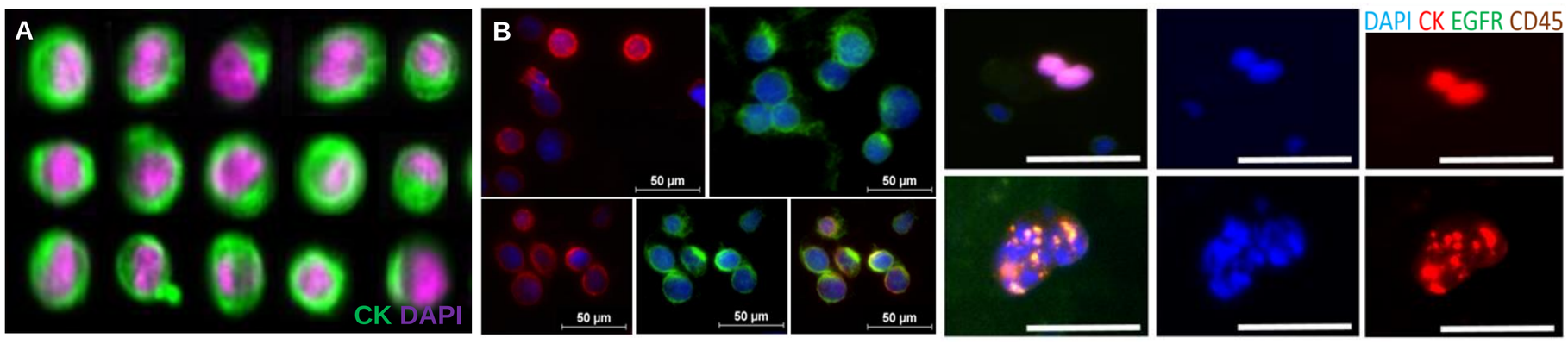


Figure 2. Short term ex-vivo CTC culture (2D/3D)

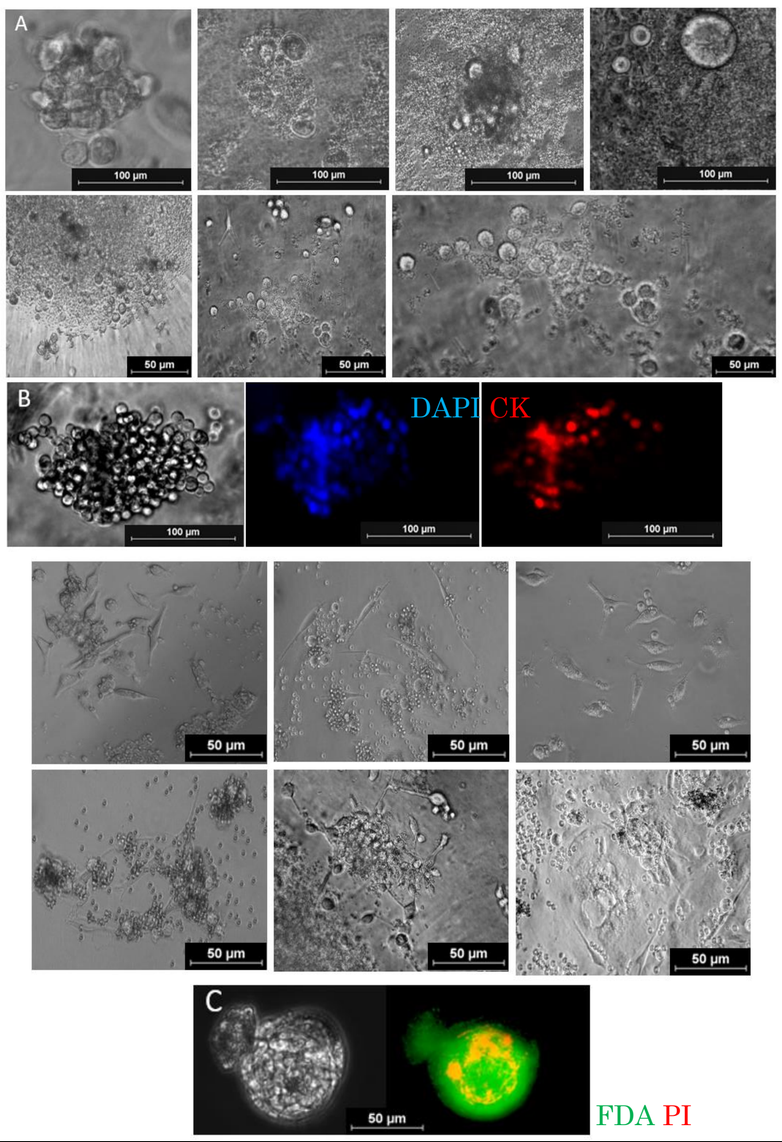


Figure 3a. CTC count at baseline vs short term culture success samples, *P*=0.0002

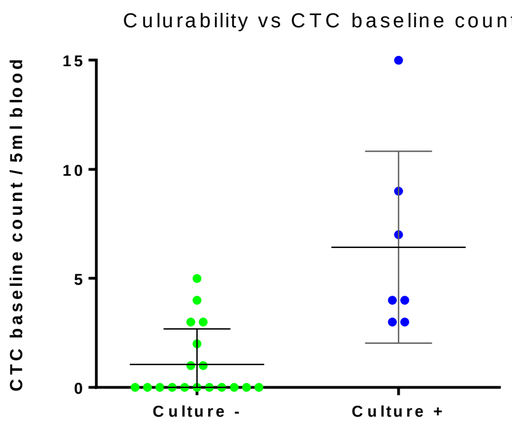


Figure 3b. Correlation between HPV status and short term culture success, *P*=0.007

	Culture negative	Culture positive
HPV-negative	16	2
HPV-positive	2	5

Discussion

- In a paired HNC patient cohort, CTCs were detected in 8/43 (18.6%) by CellSearch®, 13/28 (46.4%) by ScreenCell® and 16/25 (64.0%) by RosetteSep™ (including CTC clusters). Patients were clinically and radiographically M0. In a few patients, suspicious lesions and metastasis were found in the lungs after 6 months (Figure 4).
- Low numbers of CTCs remains a bottleneck in the field of HNC
- Ex-vivo culture allows for the expansion of CTCs in the short term in defined MSK media + Happy Cell (2D/3D formats)
- Short term CTC cultures were successfully generated in 7/25 HNC patients (5/7 of these cultures were from HPV-positive patients). Cultures remained more viable in 3D formats than in 2D (63 days vs 50 days).
- Blood samples with higher CTC counts had a higher success rate of culture (p=0.0002; Mann-Whitney test, Figure 3a), as did those from HPV+ patients (p=0.007; Fisher's exact test, Figure 3b)
- **There are currently no methods to predict which patients with a higher disease burden will develop metastases. The ability to do so would lend itself to escalation at diagnosis.**

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