Epidermal Growth Factor Receptor (EGFR) Mutational Detection in VTX-1 Isolated CTCs, ctDNA from the Same Tube of Blood, and Comparison to Tumor Tissue in Non-Small-Cell Lung-Cancer (NSCLC) Patients

Haiyan E. Liu¹, Meghah Vuppalapaty², Clementine Lemaire¹, Charles L. Willkerson¹, Michael Chiu¹, Steve Crouse¹, Nasim Barzanian¹, James Carroll¹, Jonathan W. Goldman², Elodie Sollier-Christen².

1. Vortex Biosciences, Inc., Menlo Park, CA, USA; 2. David Geffen School of Medicine at UCLA, Los Angeles, CA, USA.

1. INTRODUCTION

1. Lung Cancer and EGFR Tyrosine Kinase Inhibitor (TKI) Treatment

   - Lung Cancer is the leading cause of cancer-related mortality worldwide.
   - 85% cases are Non Small Cell Lung Cancer (NSCLC).
   - EGFR mutations occur in 10-30% of NSCLC patients.
   - There are dramatic clinical benefits of TKI treatment in the presence of EGFR mutations.
   - Resistance develops when a new mutation occurs so ongoing monitoring by non-invasive liquid biopsy has a substantial clinical benefit.

2. EGFR Mutation Profiling and Liquid Biopsy

   - Tumor biopsies: risky, painful, expensive, not always successful.
   - Liquid biopsy: cheaper, less invasive, less risky, can better facilitate continuous monitoring.
   - Two promising liquid biopsies: Circulating Tumor Cells (CTCs) & Circulating Tumor DNA (ctDNA).

3. Vortex Technology, VTX-1

   - Microfluidic label-free CTC isolation system.
   - Meets the needs of liquid biopsy with high CTC recovery and purity, and a simple, fully automated process.

4. Antiocoagulants and Patients Samples

   - Capture Performance
     - 4 blood collection tubes (BCTs) were tested side-by-side: EDTA, CellSave, LBGard and Streck.
     - Spiking of H1975 cells and HCC27 DNA into healthy blood to mimic patient samples.
     - Comparison of: DNA quantity (ctDNA, CTC), capture efficiency, WBC viability and EGFR mutations.

5. EGFR Mutation Comparison

   - ctDNA: high spiking in blood vs PDB
     - ctDNA: high spiking in blood vs PDB
     - ctDNA: low spiking in blood vs PDB

6. NSCLC Patient Samples: In Progress

   - Patient samples are being collected from UCLA David Geffen School of Medicine in LBGard tubes.
   - Blood samples were sent to Vortex. Plasma was collected for ctDNA EGFR mutations.
   - CTCs were collected from plasma depleted blood by VTX-1, enumerated and analyzed for EGFR mutations.
   - Three Cohorts of patients:
     - TKI untreated, biopsy analyzed
     - TKI ongoing, progressing
     - TKI ongoing, doing well

7. Mutational Profiling of EGFR

   - ctDNA: ctEGFR kit from EntroGen
     - Kit designed and validated for ctDNA with low input.
     - Simple PCR workflow detects 3 main mutations in single PCR reaction (4 fluorescent probes).
     - Ultra-sensitive: 0.4% Limit of Detection (LOD) in 5ng of input DNA.

8. Cell Lines: 3 NSCLC cell lines (A549, H1975, HCC27) with known EGFR mutations were used to characterize the assay for CTC workflow. CTC mutations of each cell line were confirmed by Sanger sequencing.

9. DNA Input: Mutant DNA can be identified for DNA quantities as low as 0.2 ng (~33 cells).

10. Sensitivity: 0.1% to 2% for a total DNA varying from 25ng (~4 CTCs among 4000 WBCs) to 1ng (~4 CTCs among 200 WBCs).

11. Table: 3. WORKFLOW AND VERIFICATION ON SPIKED CELLS

   - Workflow: for both spiked experiments and patient samples.
     - Plasma was extracted from blood samples by centrifugation.
     - CTCs were isolated from the plasma-depleted blood (PDB) by VTX-1.
     - CTCs were immunostained (CK, Vimentin, CD45) and enumerated.
     - EGFR mutation assay was performed for DNA from CTCs and ctDNA from plasma.

   - Assay feasibility on ctDNA & CTC from the same blood tube spiked with cells and DNA

12. ctDNA EGFR assay on blood vs. plasma-depleted blood (PDB):

   - Exp. 1: DNA: spiking in plasma
     - Plasma N/A
     - No VTX-1 processing involved

   - Exp. 2: Cells high spiking in blood
     - Blood 250/mL
     - No VTX-1

   - Exp. 3: Cells high spiking in blood vs PDB
     - Blood 250/mL
     - No VTX-1

   - Exp. 4: Cells + DNA: low spiking in blood vs PDB
     - Blood 50/mL
     - No VTX-1

13. Conclusions & Future Directions

   - ctEGFR assay works well on both ctDNA and Vortex-enriched CTCs.
   - VTX-1 processing of plasma-depleted blood enables CTC and ctDNA EGFR mutation detection from a single blood of tube.
   - Workflow can support ctDNA and CTC DNA mixing after extraction to perform a single molecular assay.
   - LBGard, CellSave and Streck perform similarly for EGFR assay, but VTX-1 capture performance is optimal for LBGard. Thus, LBGard was selected for patient samples processing in this study.
   - This non-invasive EGFR mutation analysis will be potentially a useful tool for monitoring treatment and medication guidance of NSCLC patients.

14. Acknowledgements & References

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