Evidence for Early Detection Of Metastatic Variants and Tumour Evolution in ctDNA of Patients with Oesophageal Adenocarcinoma

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INTRODUCTION

Oesophageal adenocarcinoma (OA) has a dismal survival rate[1] and no routine blood-borne biomarker. Circulating tumour DNA (ctDNA) detection in plasma DNA is an emerging non-invasive biomarker[2,3].

OA ctDNA analysis has previously been shown to track disease relapse and progression[4]. However, little is known about how well ctDNA reflects genetic tumour evolution over time.

Two patients with OA that had previously undergone tumour sampling at diagnosis and longitudinal plasma DNA collection were recruited to the PEACE (Posthumous Evaluation of Advanced Cancer Environment) study; providing a unique opportunity to compare tumour- and ct-DNA from diagnosis to death

AIM

Study tumour genetic heterogeneity at time of death and compare it to longitudinal plasma DNA samples.

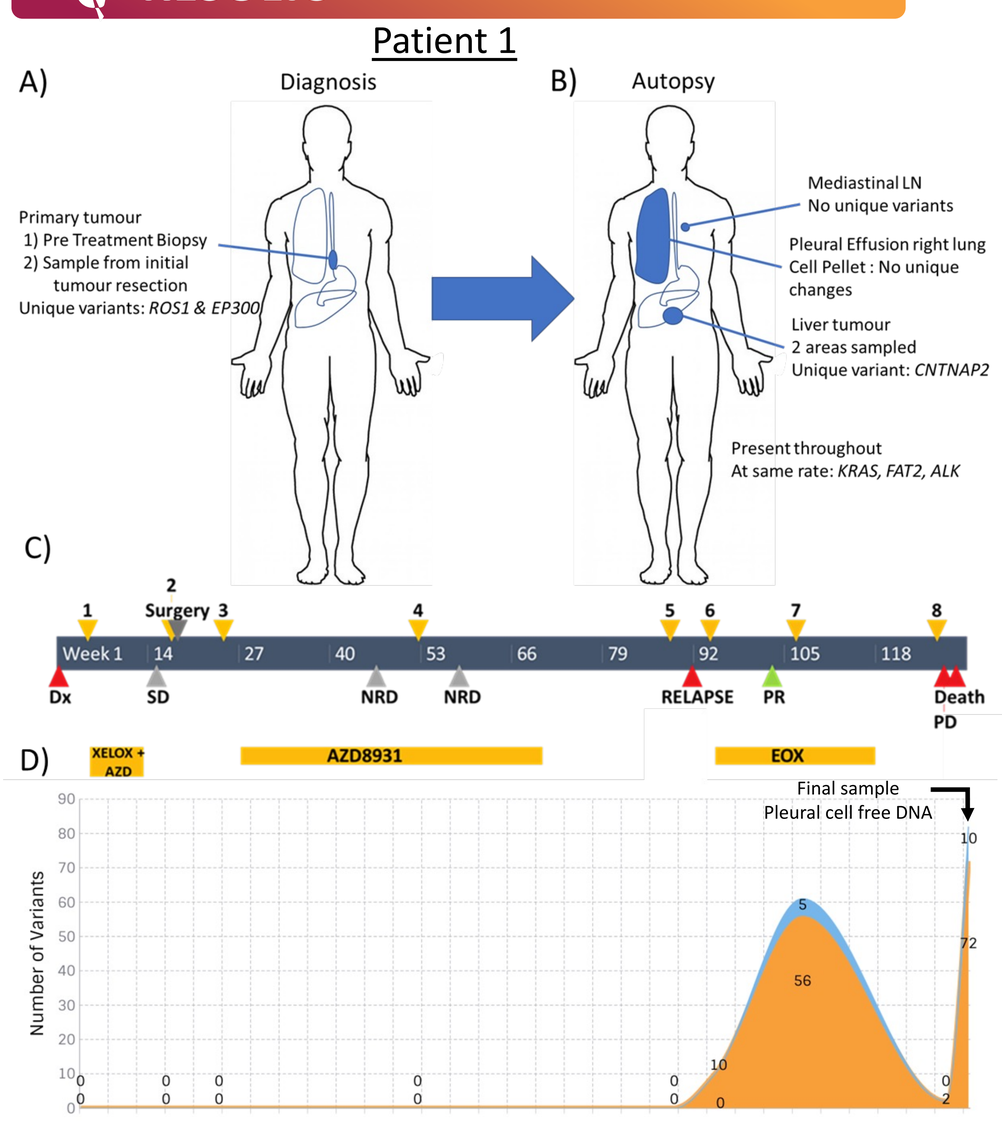
METHODS

Patients consented separately to sample storage at the University of Leicester Cancer Biobank, UHL11274 (REC: 13/EM/0196), and the PEACE study.

Whole exome sequencing (WES) of primary and metastatic tumour DNA was performed. All non-synonymous predicted pathogenic variants in the primary biopsy were assigned to major and minor clones (denoted clone A & clone B variants). Variants not present in the initial primary tumour (VAF <5%) but present in ≥1 metastatic sample (VAF ≥20%) were termed Emergent Variants (EVs).

Cell3™Target MRD patient specific panels (NONACUS) were designed to capture clone A, B and Emergent Variants in plasma DNA. Patient specific NGS was performed (median depth 1500X), using 20ng of plasma DNA and Cell3™Target Library Preparation.

RESULTS

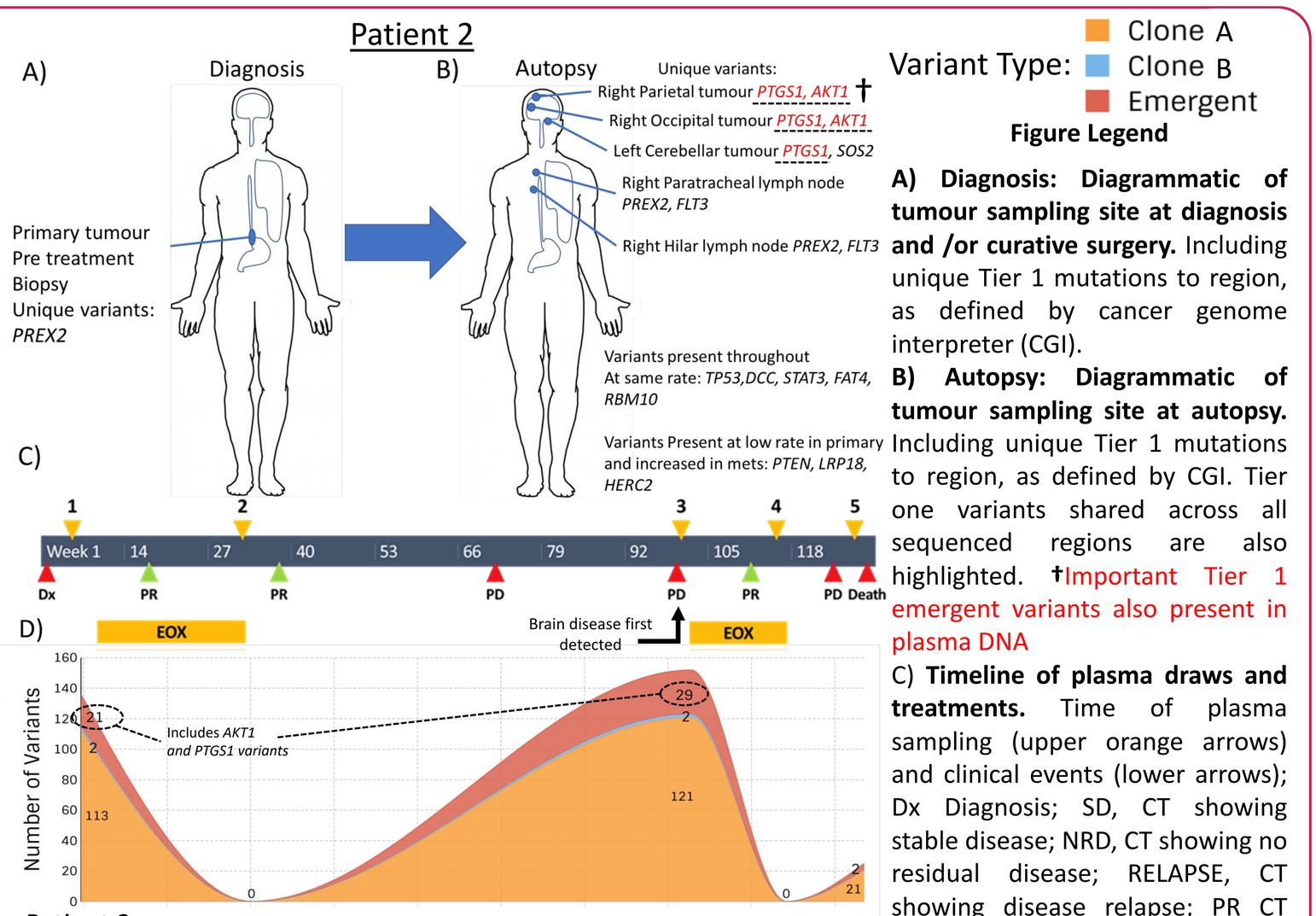


Patient 1

OA treated with curative intent. After relapse and death, metastases were sampled from liver, thoracic lymph nodes and pleural fluid. 132 non-synonymous tumour WES variants were detected. Five tier 1 variants were identified (clone A=3, EVs=2).

All timepoints (n=5) prior to relapse were ctDNA negative. Relapse and on treatment timepoints (n=3) were ctDNA positive. Only clone A variants (n=10) were identified at relapse. 61 variants (56 clone A, 5 clone B) were identified on treatment. No EVs were detected. *KRAS* p.G12D was the only tier 1 variant detected in ctDNA.

83 variants were detected in the cell free pleural fluid sample including 72 clone A and 10 clone B variants. Of the tier 1 variants KRAS (VAF 17.8%) and ALK (VAF 9.0%) were both detected.



Patient 2

Irresectable OA at diagnosis with locally extensive LN disease. Metastases were sampled from brain and abdominal lymph nodes after death (See Figure above).

287 non-synonymous tumour WES variants were detected. 13 tier 1 mutations were identified (clone A = 8, Clone B=1, EVs=4)

ctDNA was only detectable at diagnosis and disease progression, no ctDNA was detected during tumour response to chemotherapy. 132-157 tumour variants were detected at timepoints 1 (diagnosis, including 21 EVs), and 3 respectively (including 26 EVs).

Two tier 1 EVs (in *AKT1* and *PTGS1*) specific to the metastatic brain tumours were detectable in ctDNA at diagnosis, 90 weeks prior to clinical diagnosis.

ctDNA was undetectable in samples taken prior to death in both cases, although plasma DNA levels were high.

Variant Type: Clone A, Clone B, Emergent

Figure Legend

A) Diagnosis: Diagrammatic of tumour sampling site at diagnosis and /or curative surgery. Including unique Tier 1 mutations to region, as defined by cancer genome interpreter (CGI).

B) Autopsy: Diagrammatic of tumour sampling site at autopsy. Including unique Tier 1 mutations to region, as defined by CGI. Tier one variants shared across all sequenced regions are also highlighted. †Important Tier 1 emergent variants also present in plasma DNA

C) **Timeline of plasma draws and treatments.** Time of plasma sampling (upper orange arrows) and clinical events (lower arrows); Dx Diagnosis; SD, CT showing stable disease; NRD, CT showing no residual disease; RELAPSE, CT showing disease relapse; PR CT showing Partial response; PD, CT showing progressive disease. Treatment: Chemotherapy orange bars, XELOX = Capecitabine and oxaliplatin, AZD8931 a novel dual-EGFR/HER2/HER3 inhibitor, EOX = Epirubicin, oxaliplatin and capecitabine.

D) **All Variants detected in plasma over time.** Number of variants, Clone A variants orange, Clone B variants Blue, Emergent Variants Red. *Note* final sample / data point for patient 1 is the plasma DNA data from the pleural fluid and not plasma DNA.

CONCLUSIONS

As expected ctDNA was detectable at disease progression or relapse but not in prior time points or at times of disease response to therapy.

Despite high plasma DNA levels, ctDNA analysis was not useful close to death, potentially due to widespread necrosis of non-tumour cells.

Interestingly, brain metastases specific ctDNA was detectable up to 90 weeks prior to clinical appearance. This unexpected finding may be explained by ctDNA detecting;

1. Variants missed by the initial diagnostic biopsy due to intra / intertumoral heterogeneity that subsequently led to metastases
2. Micro-metastatic disease that only became clinically apparent later.

This preliminary study provides evidence for the utility of ctDNA monitoring in OA, to detect both primary tumour and emergent variants. This ability to confidently track metastatic specific alterations during disease progression promises new insights into OA evolution.

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