Assessing the clinical applications of ctDNA in patients with advanced stage metastatic cancer using our enhanced TAm-Seq™ platform

1. Introduction

- ctDNA has numerous potential clinical applications (Figure 2) including:
  - Minimally invasive detection of cancer mutations
  - Molecular stratification of patients for treatment
  - Monitoring response to treatment
  - Identifying the emergence of resistance to therapy

2. Clinical study design

- Blood samples were taken at multiple time-points, before and during treatment for each patient.
- Plasma was separated from 10 mL of blood collected in a Vacutainer containing EDTA.
- ctDNA was extracted from the plasma, and targeted amplicon deep sequencing (TAm-Seq) was carried out.

3. What is TAm-Seq?

- TAm-Seq is an amplicon sequencing-based method that can be used to sample multiple genes and to detect and quantify rare mutations that are present in ctDNA in patient plasma (Figure 3A).
- We have developed enhanced TAm-Seq that allows for reduced background levels and increased sensitivity for identification of mutations in DNA from plasma samples.
- We use a TAm-Seq amplicon panel covering hotspots and entire coding regions in 35 genes (Figure 3B).

4. Case study A

- An initial sample of ctDNA was analysed by TAm-Seq which detected an EGFR T790M mutation, qualifying the patient for trial treatment with AZD9291.
- The patient has shown positive clinical response (Figure 4), and the frequency of most mutations (as monitored by TAm-Seq) also reduced to undetectable levels (Figure 5).

5. Case study B

- The patient was diagnosed with a xanthoastrocytoma, and treated with surgery followed by radiotherapy.
- A BRAF V600E mutation was detected using routine hospital assays. This was then confirmed with TAm-Seq.
- Monitoring the mutation showed that during treatment with off-label vemurafenib, the mutation reduced in frequency (Figure 6).

6. Case study C

- Patient C presented with stage IV NSCLC which did not respond to gefitinib (Figure 7).
- The second treatment was composed of cisplatin at 75 mg/m² and paclitaxel at 50 mg/m².
- Both detectable mutations were shown by TAm-Seq to reduce in frequency during the second round of treatment.

7. Conclusions

- ctDNA analysis can be used to identify the emergence of resistance mutations for patients where it was not possible to obtain sufficient material via conventional biopsies (see Case Study A).
- ctDNA analysis can be used to monitor responses to different therapeutic doses (see Case Study B) or to different therapies (see Case Study C).
- Monitoring multiple mutations by ctDNA analysis may indicate the differential response of tumor subclones to treatment (see Case Study A).
- ctDNA analysis shows good concordance with RECIST response in PET scan images (see Case Study A).
- ctDNA analysis may be used to indicate that a patient is unlikely to respond to further treatment with a targeted therapy (see Case Study B).
- These data demonstrate that TAm-Seq is a minimally invasive and sensitive technique that can be especially useful when conventional biopsies are unavailable, or do not provide enough material for analysis.