Development of cell-free DNA based biomarkers to detect metastatic bladder cancer

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Systematic review:

I have performed a systematic review of blood-based nucleic acid and circulating tumour cells biomarkers for bladder cancer. This systematic review provides a comprehensive overview of the blood-based circulating tumour cell (CTC) and nucleic acid biomarkers that have been investigated. In total 47 studies were identified, with 21, 19 and 3 studies reporting DNA, RNA and CTC biomarkers respectively. An overlap in interest of targets between studies suggests that these could be promising biomarkers, but few biomarkers achieve high sensitivity and specificity, and fewer still have been validated in a separate cohort of patients. The systematic review is registered with the PROSPERO database (CRD42016051201), and has been published in Cancer Treatment Reviews (Impact Factor: 8.122)[1].

Clinical study

Recruitment

I recruited 95 patients referred for radical cystectomy (RC) prospectively at Urology clinics in UCLH, under the UCL/UCLH Biobank for Studying Health and Disease (Ethics approval (NC06.11) (HTA License: 12055). Of these 95 patients, 80 proceeded to RC after peri-operative assessment.

Work Package I: Of the 15 patients did not proceed to have RC, 5 had metastatic disease and the remainder opted for radical radiotherapy instead of surgery due to patient preference or anaesthetic concerns. The 5 patients with metastatic disease had blood drawn for cell-free DNA extraction as well as enumeration of circulating tumour cells. A follow-up blood sample was also taken following 2 cycles of chemotherapy.

Work package II: The 80 patients who proceeded have RC were consented to provide sequential blood samples at baseline and 1, 3, 6 and 12 months post-RC. Additionally, I maintained a database of their clinical findings, namely their CT scan results to monitor for a metastatic recurrence of their bladder cancer. For patients with known recurrence, samples from time of recurrence and before recurrence were analysed.

Work package III: I submitted the IRAS for the iROC trial (ClinicalTrials.gov Identifier: NCT03049410), which received HRA approval. The trial's primary outcome is to compare peri-operative recovery in open vs robotic cystectomy for bladder cancer, and there is an embedded translational sub-study for collection of blood samples on the same schedule as work package II. 140 patients have consented to this sub-study so far, and sample collection is ongoing. This biorepository will serve to validate the work of work package II. The results of work package III are not included in this report, as clinical follow-up is ongoing and analysis will only be performed after sample collection and follow-up have been completed.

Sample storage and processing

Plasma was separated from the blood samples taken from these patients, and stored at -80°C. Subsequently, cell-free DNA (cfDNA) was extracted from plasma using QIAamp Circulating Nucleic Acid Kit. Targeted

amplification was performed using pre-designed duel-indexed primers, and next generation sequencing (NGS) performed using 22 bladder-cancer specific mutations.

Results

Work package 1:

We initially sought to determine if the presence CTCs, Circulating Tumour Cells could be used as a marker to predict the presence of residual or metastatic disease. In a small POC cohort of 5 patients undergoing chemotherapy, only 3/5 patients had detectable CTCs, at baseline, in pre- chemotherapy samples despite all patients having radiologically confirmed metastatic disease. Analysis of samples collected post-chemotherapy during routine follow up CTCs were detectable in only a single patient. These data suggest that enumeration of CTCs, at least using the CELLSEARCH® Circulating Tumor Cell Kit, is not a reliable method to allow the monitoring of patients' response to therapy, at least for bladder cancer.

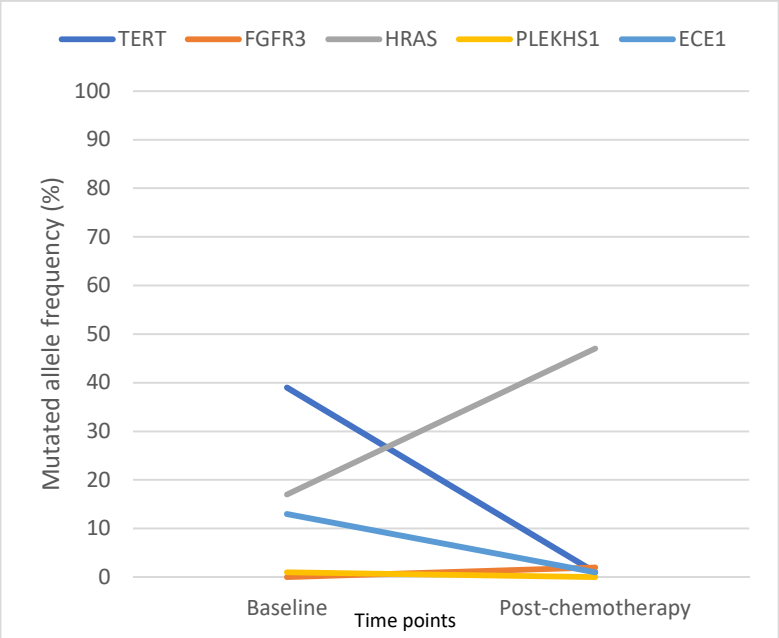
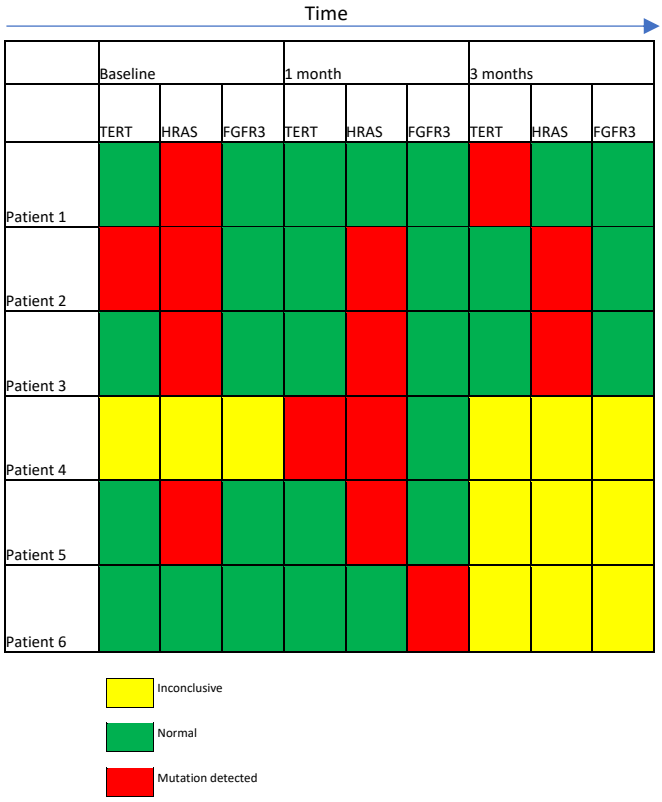


Figure 1: Allele frequency for patient 1103 before and after chemotherapy for metastatic bladder cancer.

In order to assess if alternative blood-based markers could you utilised to detect disease recurrence and metastasis, I also isolated cell free DNA (cfDNA) from patients undergoing treatment. cfDNA was detectable in all patients however, the level of cfDNA present did not appear to corelate with disease burden, although a larger sample cohort would be needed to confirm this.

We subsequently performed targeted sequencing of the cfDNA isolated from plasma, using a panel of 22 genes which have been previously shown to be mutated in bladder cancer. An example of the mutational burden from a single patient (1103) is shown in Figure 1, which shows the presence of *TERT*, *HRAS*, and *ECE1* mutations at baseline, nor or barely detectable *FGFR3* or *PLEKHS1*. The mutational burden post 2 cycles of chemotherapy, with a reduction in the level of *TERT* and *ECE1* mutations, but a significant increase in *HRAS* mutations (and the emergence of and *FGFR2* mutation). Similar results were observed for other patients. The ability to track the presence of cancer specific mutations in patients with a non-invasive blood test, could have clinical implications in monitoring patients for disease progression, where CT imaging findings can sometimes be inconclusive.

Work package 2: Monitoring of disease recurrence in patients undergoing cystectomy



WP2 focused on a similar work stream as WP1, in a cohort of patients undergoing radical cystectomy. For this patient group, CTC enumeration was not performed due to inconclusive findings even in high disease-burden metastatic patients in WP1, as well as being costly, approximately £400/sample. Five patients were diagnosed with metastatic disease after 6 months following radical cystectomy. Patient 6 had a detectable post-operative mutation signal for *FGFR3* at 1 month, but no clinical recurrence at 6 months. Cell-free DNA isolated from their blood samples was analysed using NGS, alongside a control set of patients with no known bladder cancer. Figure 2 summarises the results of sequential sampling in 6 patients at three different timepoints. Four samples had inconclusive results due to insufficient DNA or failed quality control (QC) check. Following the removal of their bladder cancer, all 6 patients had detectable mutation signal at either the one or three months post-operative timepoint. This means that patients had a detectable cell-free DNA mutation signal associated with bladder cancer before they had a clinically diagnosable recurrence. Patient 6 was the only patient without a detectable cancer signal at baseline. This could be attributed to the fact that the baseline sample was provided following neoadjuvant chemotherapy.

Clinical follow-up of these and the rest of the cohort is currently ongoing, and approximately 90% of metastatic recurrences happen in the first two years of RC[2].

Through ongoing work in the laboratories of Prof. Kelly and Dr Feber, we are looking to expand this POC data in the larger cohort collected through Work Package 3. This will involve changing the technology platform utilised in this application, this will allow potentially allow the development of a kit which could be used in routine NHS genetic laboratories to improve the monitoring of patients undergoing treatment for bladder cancer.

Conference abstracts and accolades:

American Urology Association Annual Meeting in May 2017[3] - Best Poster

British Association of Urological Surgeons Annual Meeting 2018[4] - Best Abstracts Session

European Association of Urologists Annual Meeting in Copenhagen 2018[5] – Extended presentation

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Published manuscripts during funding period

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