The Potential Role Of The Real-Time Targeted Next Generation Sequencing Of Patients With Musculoskeletal (MSK) Metastases From An Unknown Origin.

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INTRODUCTION: A significant number of cancer patients present with disseminated metastatic bone disease. For a subgroup of this cohort traditional radiological and molecular investigations fail to identify a primary tumour. This group of 'Cancer of Unknown Primary' (CUP) patients make up 2-5% of all cancer patients, and the inability to employ disease-specific therapies results in their dismal median survival of just 6-10 months. We present a novel, prospective pilot study investigating the potential role of targeted Next Generation Sequencing (NGS) analysis of musculoskeletal (MSK) metastases in CUP patients, reporting on potential diagnostic, therapeutic and prognostic benefits.

METHODS: Analysed patients were referred to our tertiary referral centre for an orthopaedic oncology opinion. Each patient presented with a symptomatic, MSK lesion consistent with a metastases and no history of cancer. After a CT chest, abdomen and pelvis failed to identify a primary tumour, patients underwent an image guided tissue biopsy of their presumed metastases to rule out a primary MSK malignancy (sarcoma). After metastatic carcinoma was confirmed, fresh frozen biopsy tissue was subsequently sent for sequencing. Tumour DNA and RNA was analysed using the pan cancer TruSight Oncology 500 (TSO500) kit. Data analysis was performed using a custom bioinformatics pipeline. Any variants identified were classified based on their potential clinical significance in line with a joint consensus from the Association for Molecular Pathology, American Society of Clinical Oncology and the College of American Pathologists. Sequencing data was reviewed at a Genomic Tumour Advisory Board consisting of clinical scientists, oncologists and surgeons where patients' clinical, radiological and genomic investigations were all discussed. All recommendations of the board were subsequently relayed to the patients' clinical team.

RESULTS: 19 patients (8F:11M, median age 70 years, range 40-76) were analysed. 18 patients had one or more skeletal lesion(s). 1 patient presented with an isolated soft tissue swelling. A wide range of anatomical locations were involved including the pelvis (7), femur (3), humerus (3), tibia (1), radius (1), sternum (1), clavicle (1), sacrum (1) and extremity (arm) soft tissues (1). 18 (95%) patients had ≥1 variant with a 'strong' or 'potential' clinical significance. Only 1 patient's tumour contained no variants. A wide range of alterations were identified including point substitutions, deletions, copy number alterations and gene fusions. These were located in a variety of both tumour suppressor genes and oncogenes. Variant(s) were identified in 8 patients (42% cohort) which highlighted them as eligible for ≥1 open clinical trial(s) (median 1 trial /patient, range 1-7). Variant(s) were also identified in 8 of the remaining 11 patients that highlighted them as potential trial candidates, although no suitable studies were currently recruiting for these individuals. 3 (16%) patients had ≥1 variant with potential therapeutic ramifications, including indications for *FGFR* inhibitor or Tyrosine Kinase Inhibitor use. Variant(s) with potential prognostic significance were also identified in 3 patients (16%). One potential germline pathogenic *BRCA1* variant was identified in 1 patient and flagged as a potential risk for future familial malignancy. Overall median survival of the cohort was 15 months (range 0 – 41). The addition of our genomic analysis to the routine immunohistochemical characterisation of patients allowed 58% of the cohort to be referred to an appropriate site-specific Multi-Disciplinary Team (MDT) for consideration of disease specific treatment. 5 of these 11 (45%) patients died during follow up (median survival 11 months, 4-32). This compared to 7 of the 8 (88%) patients managed by a CUP MDT (median survival 9 months, 0-18).

DISCUSSION AND CONCLUSION: In an era of personalised medicine the ability to identify a patient's primary tumour and any active pathophysiological pathways is paramount. Our novel data suggests that the use of real-time genomic sequencing to characterise CUP patients' MSK metastases in the way presented has multiple diagnostic and therapeutic benefits. These include identification of suitable clinical trials, prognostic variants (particularly vital for CUP patients), germline pathogenic variants and (perhaps most importantly) the facilitation of referral to disease specific MDT. Moving forwards larger prospective trials are needed to characterise the genomics of the MSK metastases of this under-reported cohort, and to also investigate any potential survival benefits of our analysis strategy.