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An NRAS mutation in a case of Erdheim-Chester disease

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Sir,

Erdheim Chester disease (ECD) is a rare, non-Langerhans' cell histiocytic neoplastic disorder that usually presents in patients in their fifties. ECD often presents as a multi-system macrophagic infiltration and common sites of involvement are the central nervous system, cardiovascular system, respiratory system, retroperitoneum, and skin. Extra-skeletal involvement is usually responsible for death due to secondary complications and ECD has had until recently an extremely poor prognosis (*vide infra*), with less than half of patients surviving three years (1).

The exact aetiology of ECD is unknown, but recent molecular studies have shown that that over half of the cases contain a *BRAF* V600 mutation (2). However, Diamond *et al.* recently reported a single case of ECD with an *NRAS* codon 61 substitution in the absence of a *BRAF* mutation (3). Here we report the second case of ECD with an *NRAS* mutation identified through review of the pathology archives: we identified a case of a 77 year-old retired man with multiple co-morbidities who presented with progressive bone pain around the right knee. Radiographs showed areas of increased density in both tibiae and the right femur, but no widespread bony lesions. In addition, T1-weighted magnetic resonance imaging showed confluent areas of abnormal marrow signal in the femur and tibia, with heterogeneous fat adjacent to the femoral lesion (Figure 1). Due to the patient's frailty, serial imaging was not performed.

Microscopic examination of a needle biopsy specimen taken from the presenting bony lesion in the right tibia showed diffuse chronic macrophagic infiltration of bone with associated bone remodelling (Figure 1). In spite of presentation with only localised disease, ECD was suggested. The patient did not receive active treatment for ECD due to co-morbidities, including bladder cancer.

To characterise further the abnormal infiltrate in bone, a next generation sequencing approach was used to examine mutations in common oncogenes. 10ng of DNA was extracted from three 10µm formalin-fixed paraffin-embedded sections from the needle biopsy, and examination of the haematoxylin and eosin-stained section showed that the lesion had a neoplastic cell content of approximately 50%. The Fluidigm access array was used to analyse 50 amplicons covering the hotspots reported in COSMIC (<http://cancer.sanger.ac.uk>) in 11 genes (*EGFR*, *KRAS*, *BRAF*, *TP53*, *PTEN*, *NRAS*, *c-KIT*, *IDH1*, *IDH2*, *PDGFRA* and *PIK3CA*), and sequencing was performed using the Ion Torrent Personal Genome Machine (PGM) (Life Technologies). An *NRAS* mutation was identified using the Integrative Genomics Viewer (IGV); a mutation was deemed present on the basis of a 35% base substitution with a ≥500 read depth. A substitution mutation was identified in *NRAS* codon 61, c.182A>G, causing an amino acid change from glutamine to arginine, p.Q61R (NCBI Reference Sequence: NG_007572.1; COSMIC mutation ID COSM584). No mutations were detected in *BRAF* (Figure 2) or any of the other genes.

A molecular diagnosis can be valuable for providing a robust objective diagnosis and is particularly useful in this rare disease where the morphology can mimic a spectrum of reactive conditions. Genetic alterations in ECD are also important for guiding patient management because the identification of a *BRAF* V600E substitution in such patients, even with multisystemic disease which is refractory to first-line interferon-α treatment has been shown to respond to the *BRAF* inhibitor vemurafenib (4). In contrast, ECD harbouring an *NRAS* mutation would not be expected to respond to such targeted therapy. Although direct inhibition of *NRAS* has proven difficult, the downstream mitogen-activated protein kinase (MAPK) and phosphatidylinositide 3-kinases (PI3K) pathways are potential targets and MEK inhibitors, which act on the

MAPK pathway, have been used successfully in *NRAS*-mutated melanoma and may therefore be useful in other *NRAS*-dependent neoplastic diseases.

This case highlights the value of being able to provide a robust tissue diagnosis in addition to identifying mutations that could be exploited for targeted therapy or in this case deciding not to provide a targeted therapy from which most cases of ECD would benefit. This report of the second *NRAS* mutation in ECD argues for the need for more extensive multi-gene mutation profiling in addition to *BRAF* in patients with ECD. More research is warranted to reveal if the current findings have therapeutic consequences, but this time- and cost-efficient DNA sequencing technology may enable new therapeutic options in otherwise rare and devastating diseases with limited treatment options.

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Figure 1

(A-C) Photomicrographs of sclerotic intramedullary lesions showing diffuse chronic macrophagic infiltration of bone with bone remodelling. (A) H&E, scale bar equals 500µm (B) H&E, scale bar equals 200µm; (C) H&E, scale bar equals 100µm. (D)

Anteroposterior radiograph of the right knee. Areas of increased density are seen in the right femur and tibia (arrows), in addition to osteoarthritis. (E) Sagittal T1-weighted image of the right knee showing confluent areas of abnormal marrow signal in the femur and tibia (arrows), with heterogeneous fat adjacent to the femoral lesion (asterisk).

Figure 2

IGV showing sequence of DNA extracted from the needle biopsy specimen taken from the presenting bony lesion in the right tibia. (A) 35% substitution mutation in *NRAS* c.182A>G, p.Q61R ; (B) no mutation detected in *BRAF*.

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