# Letter to the Editor - Molecular diagnoses of century old childhood tumours

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## Letter

Dear Sir

A major limitation of cancer genomics studies has been the lack of fresh frozen tumour material. As massively parallel sequencing has been evolving over recent years, it has become feasible to sequence archival tumour material preserved as formalin-fixed paraffin embedded (FFPE) tissue. The oldest specimen sequenced to date is a 32 year old tumour sample[1]. Here, we set out to study the oldest retrievable specimens from the pathology archive of Great Ormond Street Hospital for Children (GOSH).

We searched the pathology archives of GOSH, London, UK, for the oldest intact tumour specimens retrievable. GOSH is the oldest children's hospital in the English speaking world. It was founded by Charles West in 1852 in Bloomsbury, London, from charitable donations raised predominantly by Charles Dickens. From paper archives (**Figure 1a**) we identified and reviewed well documented potential tumour cases from the 1920s, the earliest period for which both reliable reports and tissue blocks were available. We chose three different examples and extracted DNA using standard methods. We performed massively parallel sequencing on an Illumina platform targeting coding exons of 366 cancer genes by a custom target enrichment bait design (Agilent, USA).

The quality of sequences we obtained was adequate. Compared to 96 FFPE tumour specimens of the last decade processed by the same pipeline, the evenness of coverage was unchanged. There was, however, a drop in capture efficiency in the older specimens, which is a known effect of age on FFPE derived DNA[1]. Curation of mutations revealed relevant driver events in each tumour. Case 1, an embryonal rhabdomyosarcoma, harboured a canonical *NRAS* Q61R mutation, which is a known driver of embryonal rhabdomyosarcoma (**Figure 1b**)[2]. Case 2, a lymphocytic neoplasm, was driven by a canonical mutation in *NOTCH1*, an established driver gene of haematological malignancies (**Figure 1c**)[3]. Case 3, a cellular capillary haemangioma, harboured an *ASXL1* nonsense mutation (**Figure 1d**). *ASXL1* in an established cancer gene in haematological malignancies that is not known to be

recurrently mutated in solid tumours. Interestingly, we previously identified a case with a truncating *ASXL1* mutation in a small sequencing studies of angiosarcoma[4]. Thus, it may be possible that *ASXL1* is a recurrent driver of solid vascular tumours.

It is most remarkable and fortunate that FFPE preservation of tumour tissue maintains DNA for almost 100 years in a condition that is suitable for massively parallel sequencing. This paves the way for studying ultra rare tumours. Furthermore, it may enable an archaeological investigation into environmental mutagens of the past, the signatures of which are encoded in cancer genomes[5].

## Figure legend

**Figure 1. Sequencing tumours of the past.**

**a.** Historical case register of the pathology archive of Great Ormond Street Hospital.

**b-d.** For each case a histological section is shown. For case 'a' this is stained for Desmin, the hallmark of embryonal rhabdomyosarcoma. For cases 'b' and 'c' these are H&E stained slides. Underneath each section, the raw sequencing data is depicted. Reads are represented by blue and salmon coloured bars. The highlighted base represents the mutant base.

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## References

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