

## Primary versus Metastasis using IonTorrent Next Generation Sequencing (PRIMINGS)

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Cancer patients who have undergone tumour resection with curative intent may present subsequently with a tumour nodule at a distant anatomical site. For planning treatment and prognosis, it is vital to know whether this represents a metastasis from the original tumour, or a new primary cancer. Typically a biopsy of the second tumour will provide sufficient information on conventional histology and immunohistochemical profiling to determine whether this represents a new primary or metastasis. There are tumour types and tissue types in which the sensitivity of immunohistochemistry is limited however and not infrequently a diagnosis is given on the 'balance of probability'.

Targeted next generation sequencing (NGS) has been shown to be useful in case reports of difficult cases but has not been tested on a series of clinical cases with a definitive diagnosis of secondary primary or metastatic tumour.

A series of patients were selected from the recent diagnostic archive representing those with unequivocal dual primary tumours and confirmed primary and distant metastatic tumours. DNA was extracted from both tumours and uninvolved lymph node tissue and tested using the Ion AmpliSeq™ Cancer Hotspot Panel v2. Sequencing data was used to predict whether the second tumour represented a second primary or metastasis, blinded to histopathological and clinical information.

Pairs of primary and metastatic tumours showed common mutations, with sufficient overlap to predict confidently their shared clonal origin. The pairs of separate primary tumours showed differing mutational profiles with no overlap to suggest metastasis. Testing germline DNA from uninvolved lymph node tissue allowed the exclusion of single nucleotide variants from the analysis and therefore improved the specificity of the test.

In patients presenting with a second distant tumour following cancer resection, targeted NGS has the potential to be a useful clinical test to identify this as a metastasis or primary.