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Mutational analysis of c-KIT and PDGFRA receptors in gastrointestinal stromal tumours

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Introduction: The pathogenesis of gastrointestinal stromal tumors (GISTs) is generally associated with activating mutations of the proto-oncogene tyrosine-protein kinase Kit (c-KIT). However, about 15% of GISTs do not harbour c-KIT mutations. It is estimated that 5% of these GISTs have mutations in the platelet-derived growth factor receptor α (PDGFRA). Accurate diagnosis of GIST has become very important since the availability of targeted therapy with tyrosine kinase inhibitors, such as imatinib mesylate. The routine work-up for GIST diagnosis includes immunohistochemistry for CD117 (c-KIT polyclonal antibody), as it is estimated that 95% of GIST cases show positive immunoreactivity. However, it can be observed that the routinely used immunohistochemical analysis does not provide complete sensitivity for GIST diagnosis, as there are nearly 5% of GISTs that are negative for c-KIT immunohistochemistry. Mutational analysis for c-KIT and PDGFRA can confirm the diagnosis of GIST, particularly in CD117-negative suspect GIST. Moreover, specific mutations have a prognostic and/or a predictive value for response to therapy.

Aim: To establish a fast and cost-effective method of testing to identify mutational profiles of c-KIT and PDGFRA in GIST cases diagnosed in Malta.

Methodology: GIST cases diagnosed in the last 12 years were retrieved from the archives of the Histology Section at the Pathology Department (Mater Dei Hospital). Haematoxylin and eosin staining and immunohistochemical staining of CD117 were performed on serial sections of formalin-fixed, paraffin-embedded sections to identify tumoral areas. CD117-positive and negative tumoral tissue was sectioned and DNA was later isolated following standard protocols. Polymerase chain reaction (PCR) was used to amplify exons 9, 11, 13, and 17 of the c-KIT gene. Primers were designed to enable fusion of the amplified fragments, ultimately allowing sequencing of the concatemer in a single run. Future studies will utilize laser microdissection.

Results: Histologically examined GISTs were evaluated following CD117 immunohistochemical staining. Positive c-KIT immunostaining was present in 72% ($n=36$) out of a total of 47 GISTs. Currently, only one of the c-KIT positive GISTs was characterised by sequencing. A mutation in exon 11, which encodes the juxtamembrane domain, a known region harbouring numerous deletions, was identified.

Conclusion: This deletion is in a notable region of the c-KIT receptor known to activate its kinase activity. Moreover, exon 11 mutations are sensitive to imatinib mesylate. Hence, mutation analysis provides molecular classification of GISTs while predicting therapy outcome. Tumors with a wild-type c-KIT gene will be further screened for PDGFRA mutations.