

Studies on *KIT* mutations and toceranib susceptibility
in canine mast cell tumor

Abstract

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In the treatment of canine mast cell tumor (MCT), a kinase inhibitor toceranib is frequently used and has been demonstrated anti-tumor activity in certain dogs. Toceranib is a multi-kinase inhibitor that targets receptor tyrosine kinase such as KIT, PDGFR, and VEGFR. One of the mechanisms underlying the action of toceranib is inactivation of KIT that is constitutively activated by a mutation in MCT cells. However, the therapeutic activity of toceranib does not necessarily correlate with the presence of the *KIT* mutation and thus individualized therapy with toceranib have not been established in canine MCTs. To make a foundation of individualized therapy with toceranib in canine MCT, following studies were performed: comprehensive mutation analysis of *KIT* was performed on genomic DNA samples of 164 dog MCTs using next-generation sequencing (NGS) approach. Moreover, recombinant KIT proteins containing mutations identified by NGS and reported but not characterized mutations were prepared and characterized. As a result, there are various types/characteristics of mutations in *KIT* in canine MCTs. Therefore, it is necessary to consider the difference of characteristics among each mutation type for development of individualized therapy with toceranib in canine MCTs. Moreover, low frequency but some mutations conferred toceranib resistance to *KIT*. Thus, it was considered that some MCT cases have minor clones that have a predisposition of toceranib resistance in tumor tissue before starting the toceranib treatment. Therefore, to clarify the

developmental process of toceranib-resistant *KIT* mutation, toceranib-resistant cell lines were generated from cloned MCT cell lines and performed NGS analysis of their *KIT* genes. In this analysis, it was considered that the secondary mutation of *KIT* plays an important role on resistance to toceranib in canine MCTs. Therefore, to develop a strategy to overcome toceranib-resistance in MCT, effects of SHP2 inhibition on the growth of toceranib-resistant MCT cell lines was examined. Although SHP099 alone did not show obvious growth inhibitory effect to toceranib-resistant MCT cell lines, it showed potent growth inhibitory effect when combined with toceranib. In conclusion, it is necessary to consider the difference of characteristics among each mutation type for development of individualized therapy with toceranib in canine MCTs. Particularly, it should be noted that there are tumor cells carrying toceranib-resistant *KIT* mutations that may pre-exist in toceranib-naïve tumor tissue or may occur *de novo* in tumor tissue during toceranib treatment. For canine MCTs carrying toceranib-resistant clones, a combination therapy of toceranib with SHP099 might be a potential therapeutic approach.