# Leveraging the active site steric hindrance for GTP hydrolysis in mutant K-Ras variants to achieve selectivity in kinase inhibitors for cervical cancer

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**Mutations in the GTP hydrolysis site of human K-Ras is known to cause constitutively active downstream signalling resulting in cellular proliferation. Especially in the context of cervical cancer, it has been shown previously that these mutations play a significant role as observed in the clinical samples. In this study, structural analysis of K-Ras mutants revealed that amino acid substitutions in the GTP hydrolysis site cause steric hindrance for successful GTP hydrolysis which in turn leads to a constitutively active K-Ras protein. We further propose that one can leverage this differential rate of GTP hydrolysis as the basis for selectivity of kinase inhibitors to achieve successful inhibition of the mutant K-Ras variants sparing the wild type untouched.**

**Keywords:** Cervical cancer, GTP-hydrolysis, inhibitor selectivity, steric hindrance, K-Ras.

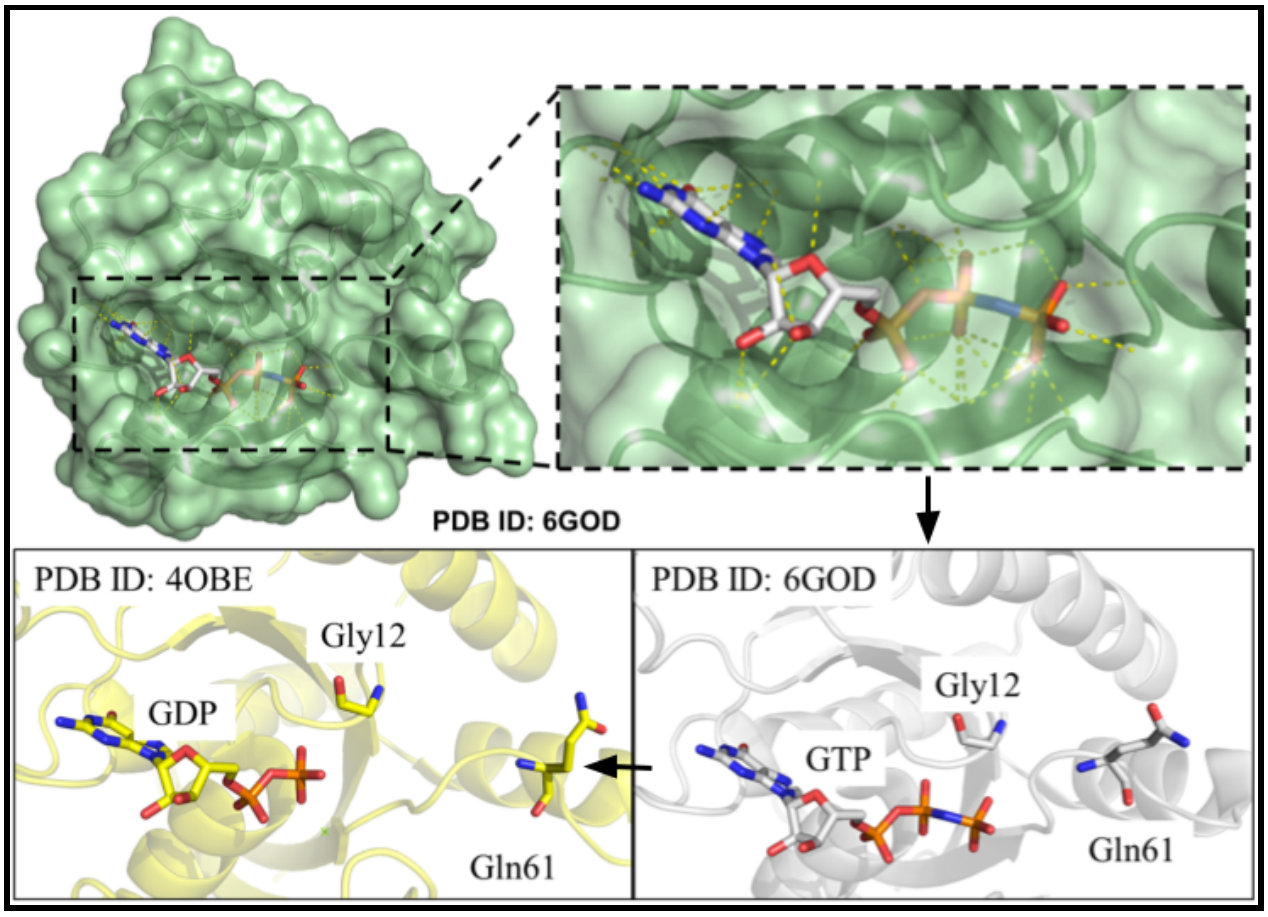


**Figure 1.** GTP hydrolysis site of wild type human K-Ras showing the binding orientation of GTP in the top panel with molecular details in the bottom panel. GDP binding is shown in the bottom left panel and GTP binding is shown in bottom right panel.

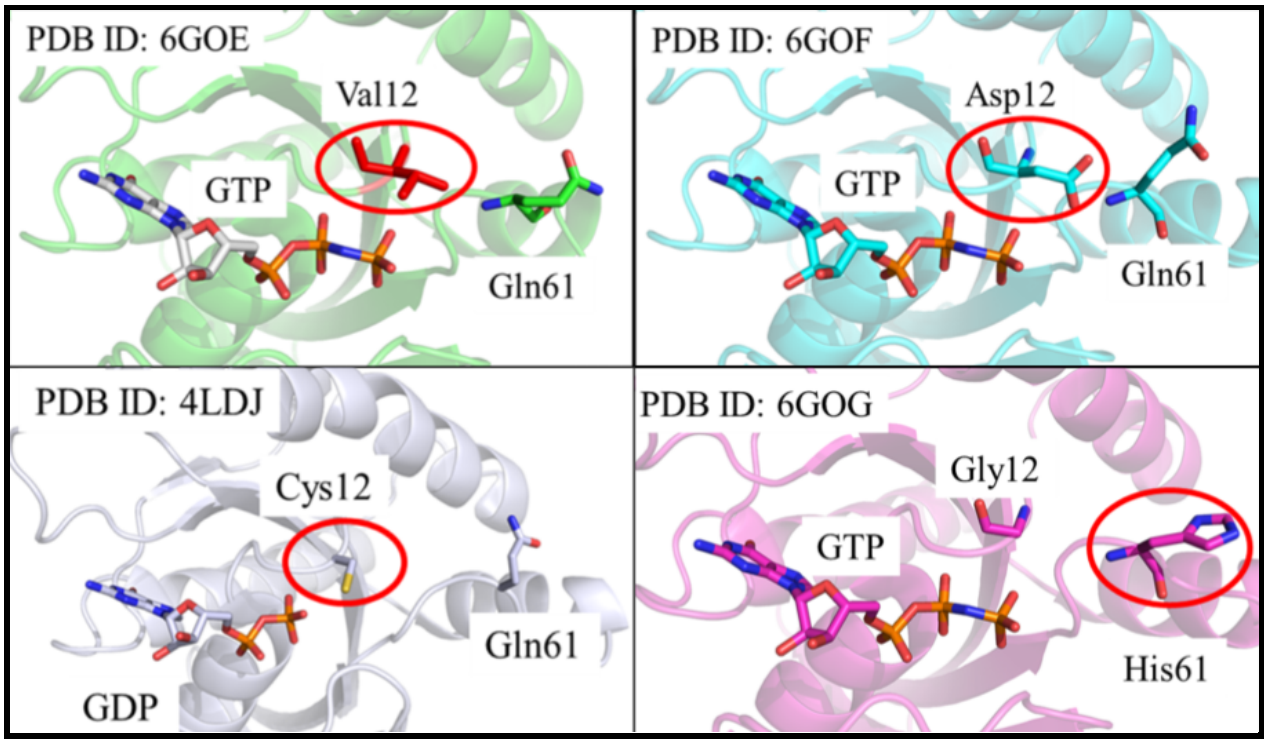


**Figure 2.** Molecular details of K-Ras mutants bound to GTP/GDP.

Cervical Cancer (CC) is one of the global health issues targeting young women. In combination with the human papilloma virus (HPV), CC can be life threatening. In most cases the HPV infection is the initial reason for the transformation of normal cervical epithelium. In such cases, one can prevent CC by administering the HPV vaccine. Sometimes CC may be asymptomatic while most of the times, symptoms such as bleeding in between the periods, white discharge, back pain, etc. are seen. It may take 10-20 years to develop CC from the precancerous stage. Majority of CC cases are squamous cells. Squamous cells are the epithelial layer of the cervix. Dysplasia of the cervical epithelial cells is a warning for CC which, in most cases, can be screened for HPV followed by HPV vaccination. Like in any other types of Cancer, early detection is always the best option. Usually Pap smear test is a simple test where the cervical epithelial cells are manually collected and analyzed for any pre-cancerous symptoms to make therapeutic recommendations accordingly. Besides HPV infections, mutations in the K-Ras (Kirsten RAt Sarcoma virus oncogene homolog) protein that affect the normal Ras pathway, have been reported clinically in patients diagnosed with CC thus making the K-Ras protein an attractive drug target in CC.

K-Ras is a GTPase which hydrolyzes a molecule of GTP to GDP (Figure 1). K-Ras, when bound to GTP, is activated and phosphorylates Raf protein which in turn initiates the downstream signalling events until transcription factors bind DNA towards gene expression promoting cell proliferation and inhibition of apoptosis. Following the GTP hydrolysis, the GDP bound K-Ras (Figure 1) is inactive thus inhibiting the downstream signalling events. Mutations at Gly12 and Gln61 of K-Ras have been reported to cause constitutively active forms of K-Ras due to defective GTP hydrolysis. Constitutively active K-Ras pathway promotes continuous cell proliferation and in combination with defective p53 protein, which promotes apoptosis, K-Ras causes Cancer/CC. In this study, we hypothesize that by using

small molecules one can selectively target the mutant K-Ras variants for degradation by the host ubiquitin-proteasome system. In order to understand the overall organization of the mutant vs. wild type K-Ras proteins, 3-dimensional analysis of their active sites was performed using Computational Biology tools. Structures were downloaded from the protein data bank (PDB IDs: 4OBE, 4LDJ, 6GOD, 6GOE, 6GOF and 6GOG). The analysis includes evaluation of the secondary structure (alpha-helices and beta-strands) using PyMOL molecular graphics software. Hydrogen bonding analysis was performed.

The structure of wild type K-Ras contains multiple alpha-helices and beta-strands. As shown in Figure 1, the GTP molecule in the active site makes more than 20 hydrogen bonds and that the binding orientations of GDP and GTP look similar suggesting that these binding orientations are conserved. Towards the gamma phosphate group of GTP, two critical amino acids, Gly12 and Gln61 (Figure 1) which were reported to be clinically prone to mutations in CC. Secondary structural analysis revealed that both Gly12 and Gln61 are located on flexible loops rather than helices or beta-strands. Gly12 may not play a critical role in the overall architecture of the active site due to lack of any side chain. However, when Gly12 mutates, causing the amino acid substitutions such as G12V, G12D and G12C (Figure 2) then the side chains of the new amino acids may cause steric hindrance to the release of gamma phosphate upon GTP hydrolysis. In other words, the G12V, G12D and G12C mutant variants of K-Ras may slow down the rate of GTP hydrolysis in the active site due to which, these mutant variants of K-Ras are constitutively active boosting the downstream signal cascade towards cell proliferation. Interestingly, Gln61 with a neutral side chain may not make any significant contacts in the active site during the GTP hydrolysis. As shown in Figure 2, a commonly reported amino acid substitution, Q61H may however change the architecture of the active site because of the positive side chain of His61. Thus it may slow the GTP hydrolysis. The amino acid substitutions at positions 12 and 61 are on flexible loops instead of helices or beta-strands re-emphasized on possible structural changes during the GTP binding, GTP hydrolysis and the gamma-phosphate group release. These subtle changes were leveraged in this study to achieve the selectivity of an inhibitor series that were designed for selectivity. We are currently in the process of synthesis and evaluation of the inhibitor series. All data obtained from the inhibitor evaluation will be published in the future issues of TCABSE-J.

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**Conflict of interest:** The applications report presented here is a currently ongoing project at TCABS-E, Rajahmundry, India. The authors invite collaborations without any conflict of interest.