Breast cancer is the most frequently diagnosed cancer and the leading cause of mortality in females Worldwide. Breast cancer is classified into four subtypes: (i) Estrogen receptor α (ERα) positive or hormone receptor-positive (HR+) breast cancer (ii) Human epidermal growth factor receptor 2 (HER2) overexpressing or HER2 positive breast cancer, (iii) HER2 negative, (iv) Triple negative (Hormone Receptor-negative HR-/HER2-). Among all the breast cancer types Estrogen receptors-α (ERα) found to be expressed in more than 70% of breast cancer cases. Despite the success of screening programs and the development of adjuvant therapies, a significant percentage of breast cancer patients suffers from metastasis and develops resistance to therapies. Consequently, there is a growing need to identify new target implicated in this process. Over the past decade, many studies have shown a causal role of protein kinase dysregulations, hyper-activations or mutations in different human cancers including breast cancer, turning the protein kinases into a valid candidates for targeted therapies. These hyper-activated protein kinases with oncogenic activities have been the target of signal transduction-based therapies using small inhibitor molecules. Protein kinases are the enzymes which modify other proteins by chemically adding a phosphate group to tyrosine, threonine or serine amino acids and this process is called phosphorylation. Protein kinases share a conserved catalytic core that generally consists of 250-300 amino acids common in both serine/threonine and tyrosine protein kinase. N-terminal lobe of protein kinases favor ATP binding which is glycine rich loop (P-loop), and the C-terminal lobe regulates the phosphorylation activity.

In ERα positive breast cancer, interactions between kinases and the ERα are thought to be a critical signaling pathway in the majority of human breast cancer. Wherein ERα phosphorylation by different kinases appears to contribute to endocrine therapy resistance by regulating its transcriptional activity and altering stability. Giamas and group in the year 2011 identified a novel oncogenic kinase that is Lemur Tyrosine Kinase-3 (LMTK3) which belongs to a class of serine-threonine-tyrosine kinase and the functional kinase domain is conserved which ranges from 133–411 amino acids. Giamas and group reported LMTK3 to be a prognostic oncogenic ERα regulator with a central role in endocrine resistance and considered to be a new therapeutic target in breast cancer. LMTK3 regulates ERα activity through phosphorylation and protects ERα from proteasomal degradation

and triggers endocrine resistance which in turn induces the breast cancer cells to undergo proliferation and progression. The hindrance of LMTK3 function has been known to down regulate the ERα mRNA expression levels and thereby having control over the progression of ERα-positive breast cancer. So inhibiting the LMTK3 activity could control breast cancer progression. And the interaction between ERα and LMTK3 is also critical. Before designing drug against LMTK3, there is a need to understand the 3-D structure in more details. As the experimental 3-D structure is not available for LMTK3 domain, we have modelled the structure and then performed molecular dynamics (MD) simulation to characterize the salient structural and dynamic features. Our findings showed the 3-D modelled structure of LMTK3 domain to be stable and we noticed flexibility in the coils and loop regions, there are a significant number of intermolecular hydrogen bonds and hydrophobic contacts which are essential for the proper folding of the protein to stabilize the structure. And we also identified the ATP binding pocket in LMTK3 domain. The highest binding free energy (BFE) of ATP-LMTK3 complex reveals the favorable energy towards the formation of complex. From MD simulation study the complex was found to be stable in the dynamic system. Additionally we also refined the BFE of the complex in the dynamic system by performing molecular mechanics energy combined with the Poisson–Boltzmann or generalized Born and surface area continuum solvation (MM-PBSA/GBSA) calculations and the BFE is observed to be favorable and attain stability of the complex. The 3-D structure prediction of LMTK3 and its ATP binding mechanism study could be a first step towards the designing of ATP competitive potential inhibitors against LMTK3 to inhibit its activity, thereby controlling breast cancer progression.

It is also important to understand the protein-protein interaction between LMTK3 and ERα as LMTK3 phosphorylating ERα leads to the breast cancer progression. Molecular docking was performed for ERα and LMTK3 using PatchDock server and the molecular interactions between them was studied using PDBsum server. We identified the probable interface area, interacting residues across ERα-LMTK3 interface and identified probable phosphorylation site in ERα at N-terminal region. MD simulation study reveals the stability of ERα-LMTK3 complex. These findings might be helpful to obstruct the protein-protein

interactions between ERα and LMTK3 by designing drugs against interacting interface area and residues.

In addition to ERα regulation, Xu et al in 2014 highlighted the LMTK3-regulatory pathways and its involvement in invasion and metastasis in triple-negative breast cancer. They revealed that elevated cytoplasmic LMTK3 abundance in triple-negative breast cancer promotes tumor invasion and metastasis by interacting with GRB2 and subsequently activates proteins and promotes transcription of integrin β1 and results in breast cancer invasion and metastasis, provided an example of ERα-independent action of LMTK3. Therefore molecular interaction between GRB2 and LMTK3 is critical. We docked GRB2 with LMTK3 using ClusPro server and using PDBsum server we have identified the probable interface area, interacting residues across GRB2-LMTK3 interface. In addition we also identified hotspot residues at GRB2 and LMTK3 interacting interfaces. Hotspot residues contribute highly to stabilise energy of the protein-protein complex, provide specificity at their binding sites. Identifying these hotspot residues within the protein-protein interfaces can help us to better understand the protein-protein interactions and may also be helpful to modulate protein-protein binding. MD simulation study reveals the stability of the complex. With the knowledge of interacting residues, interface area and hotspot residues at GRB2 and LMTK3 interface, designing of inhibitors could be possible to obstruct the protein-protein interactions between GRB2 and LMTK3 domain which facilitate breast cancer invasion and metastasis.

Before a protein kinase to catalyze the regulatory phosphorylation reaction, they themselves should get activated and regulated through phosphorylation by other kinases. The regulatory phosphorylation event occurs at kinase activation loop. The activation loop phosphorylation is a major mechanism that induces the dynamic changes and stabilizes the active conformation of the phosphorylated kinase and catalyzes the phosphoryl transfer reaction of γ-phosphate of an ATP molecule to its substrate. Recently it has been reported that the activation of LMTK3 through phosphorylation by cycline dependent kinase 5 (CDK5) directed to a new cellular pathway that results in breast cancer tumour progression. Therefore, molecular interaction between CDK5 and LMTK3 is critical. In addition, identification of probable phosphorylation site at activation loop of

LMTK3 domain is also important. Using PatchDock server we docked CDK5 with LMTK3 and using PDBsum server we identified the probable intermolecular interactions between them and identified the probable phosphorylation sites in LMTK3 at activation loop. MD simulation study reveals the stability of the CDK5-LMTK3 complex. Additionally we studied the effect of activation loop phosphorylation in LMTK3 domain in the dynamic system, wherein we induced *in silico* phosphorylation at probable phosphorylation site in activation loop of LMTK3 domain. Overall, from these studies we can see that protein–protein interaction between LMTK3 with its binding partners is critical in signaling pathways which causes breast cancer progression, invasion and metastasis. So information regarding the protein-protein interaction could be helpful in designing appropriate inhibitors accordingly to obstruct undesirable protein-protein interaction.

Finally we have made an attempt to design potential inhibitors against LMTK3 using two different approaches: (i) ATP competitive approach (ii) Potential inhibitor using E-pharmacophore modelling approach. Since most of the kinase inhibitors are ATP competitive so we made an attempt to identify ATP competitive inhibitors using DOCK Blaster (a virtual screening server) and molecular docking. We narrowed down six potential inhibitors which bind to the ATP binding pocket with high binding affinity than ATP. Using (MM-PBSA/GBSA) calculations we refined the binding free energy in the dynamic system. The six lead compounds were found to have more binding affinity than ATP. Further, potential of mean force (PMF) study for ATP and lead compounds with LMTK3 had been performed to explore the unbinding processes and the free energy barrier. From the PMF results, it has been observed that the lead compounds showed higher dissociation energy barrier than the ATP. Our findings suggest that these lead compounds may compete with ATP, and could act as probable potential inhibitors for LMTK3.

In our second approach using Schrodinger Drug design suite, we narrowed down three best inhibitors (based on high Glide XP score) bound to the site away from the ATP pocket in a distinct allosteric manner. These three inhibitors contain some pharmacophoric features (Hydrogen bond donor and acceptor, two aromatic rings) that may inhibit LMTK3 activity. Since specific ATP competitive inhibitors

remain a major challenge due to the highly conserved nature of protein kinase domain, this non-ATP competitive approach may be helpful to inhibit LMTK3 activity in a specific manner. And the pharmacophoric features may be useful in the development of efficient LMTK3 inhibitors to control breast cancer progression, invasion and metastasis. Inhibiting this novel therapeutic target (LMTK3) may reduce the load of breast cancer amongst women.