RESEARCH HIGHLIGHT

How missense mutations in receptors tyrosine kinases impact constitutive activity and alternate drug sensitivity: insights from molecular dynamics simulations

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The fundamental oncology-related research is required for a deeper understanding of the molecular mechanisms associated with the normal and/or abnormal protein functions, which are closely related with structure and dynamics of the macromolecules involved in these process. The most common origin of oncogenic events is related to missense mutations. Mutation-induced structural effects promoted by oncogenic mutations in receptor tyrosine kinases (RTKs), are not yet fully characterized. Computational biology completes and enriches experimental data, producing a novel vision of molecular mechanisms governing RTKs activity. In series of our papers, we studied the structural and dynamical features of native and mutated RTKs from III family (KIT and CSF-1R), yielding a detailed description of their mechanisms of activation, ligand-depend for the native proteins and constitutive for the distinct mutants. The mechanisms of RTKs activation are described in terms of allosteric regulation between coupled regulating fragments of the protein, juxta-membrane region (JMR) and activation (A-) loop. As some mutations promote resistance to the clinically-used drugs, we analyzed the affinity of imatinib to these therapeutic targets. The computationally-obtained (*in silico*) data were correlated with *in vivo* and *in vitro* observations, thus validating our numerically-based accounts. Going forward, clinical validation of cancer-related models and simulations are cornerstones key of translation of *in silico* data into biomedical research, at clinical and pharmacological levels.

Keywords: RTKs; KIT; CSF-1R; oncogenic mutations; constitutive activation; imatinib; resistance; molecular dynamics simulation; allosteric regulation

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Introduction

Receptor tyrosine kinases (RTKs) are cell-surface transmembrane proteins that control signal transduction pathways in cells through tightly regulated allosteric mechanisms [1]. They act as sensors for extracellular ligands,

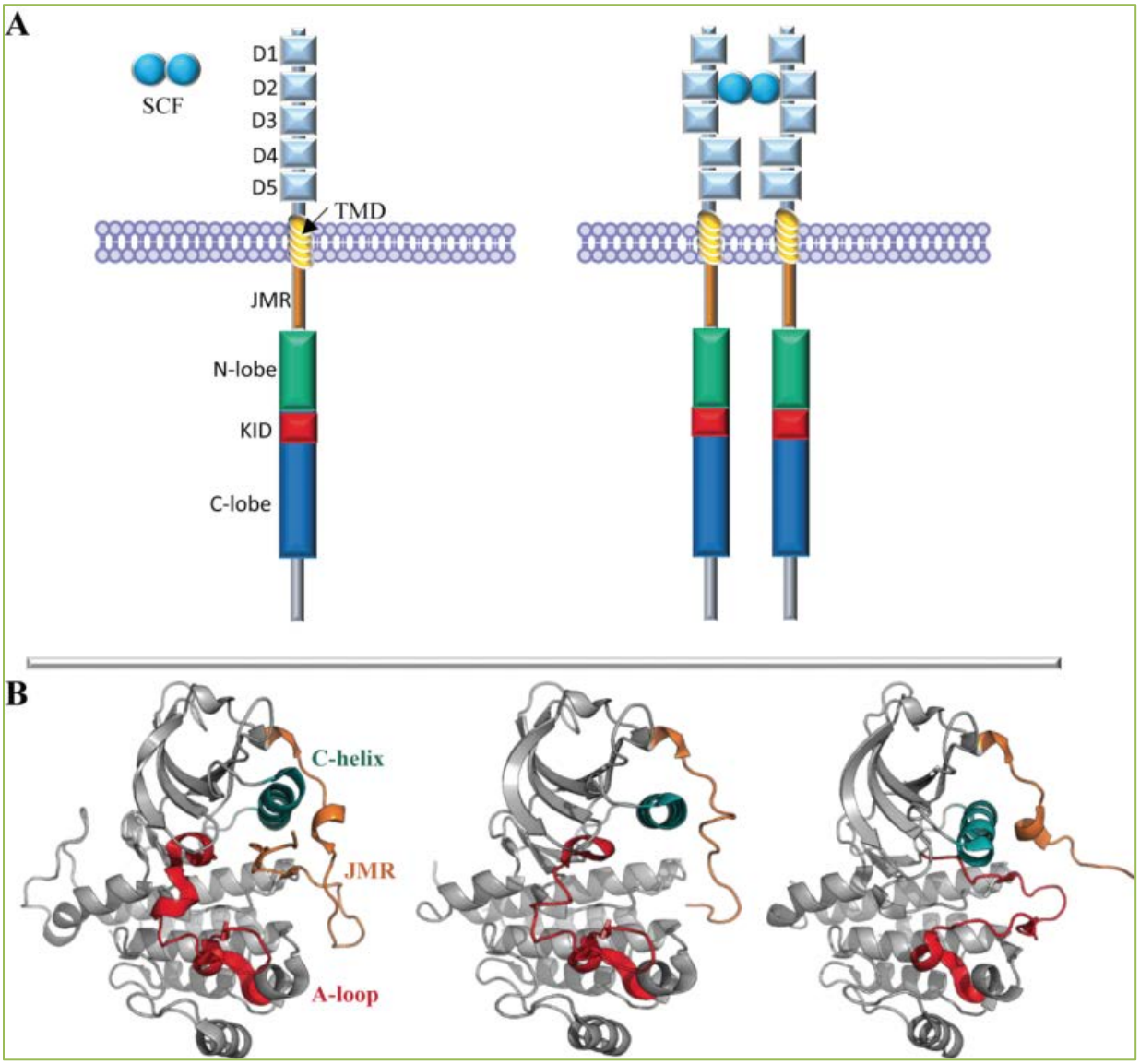

Figure 1. **Structural organisation of RTKs from III family illustrated with KIT**. KIT contains an extracellular domain with five Ig-like regions (D1-D5), a highly hydrophobic transmembrane domain (TMD, and an intracellular domain (kinase domain) composed of a juxta-membrane region (JMR), an ATP-binding region (N-terminal lobe) and the phosphotransferase domain (C-terminal lobe) spliced by a kinase insert domain (KID). (**A**) KIT is anchored at the cell membrane as a monomer maintained by electrostatic repulsion between other monomer units. Stem Cell Factor (SCF) binding on the extracellular domain of KIT induces a dimerization, promoting activation of the kinase domain. (**B**) The kinase domain conformational states of RTKs are demonstrated with the crystallographic structures of KIT, characterizing its inactive (1T45 [42]), active (1PKG [43]) and intermediate (1T46 [42]) states. In a cartoon representation of KIT, the A-loop, JMR and C-helix are in red, orange and cyan respectively.

the binding of which trigger receptor dimerization and activation of the kinase domain (KD) (**Figure 1A**). These intra-receptor processes lead to the recruitment, phosphorylation and activation of multiple downstream signaling proteins, which eventually govern the cell physiology [2]. In spite of remarkable conservation of the overall fold of RTKs, diversity in the sequences, generally of fragments regulating the kinase activity, is the ultimate factor specifying the response of each RTK to a unique set of signals to turn *on* or *off* their activity [3].

In solution, a RTK is at equilibrium between various conformations representing the receptor at different activation steps – from the inactive autoinhibited state to the active state, through multiple intermediate conformations. This equilibrium can be displaced by different effectors: ligand binding, phosphorylation event or point mutation. A mutation-induced shift of a RTK physiological balance may conduct to ligand-independent or constitutive RTK activation, leading to cell transformation.

Table 1. Pathologies associated with KIT point mutations of residue D816, as reported in literature

Pathology/Mutation	D816V	D816Y	D816H	D816N	D816F
mastocytosis	[43, 44]	[43, 44]	[44]	[43]	[45]
testicular germ cell tumor	[46, 47, 48]	[48]	[46, 47]	NA	NA
AML	[49]	[49]	[28]	[28]	NA
lymphoma	NA	NA	NA	[50]	NA

A vision on the molecular mechanisms of activation was elaborated from the structural studies of RTKs, which characterized each principle receptor state [4]. It was shown that, in normal cells, the RTK is activated upon ligand binding that initiates the departure of the auto-inhibiting juxtamembrane region (JMR) from the active site, prompting a switch of the kinase domain from the inactive autoinhibited state to the inactive non-autoinhibited state (**Figure 1 B**). This intermediary metastable receptor with the JMR removed from its autoinhibited position undergoes further extensive conformational changes in the kinase domain: (i) a relocation of the catalytic helix (C-helix) away from the active site and (ii) a deployment of the activation loop (A-loop) toward an extended conformation together with its displacement outside the active site. The catalytic site is then accessible to the ATP for binding, and the receptor appears in its active conformation. The activation process is followed by *trans* autophosphorylation of tyrosine residue located in the JMR [1, 5].

The deregulation of the type III RTKs, comprising the stem cell factor (SCF) receptor KIT, the macrophage colony-stimulating factor-1 (CSF-1) receptor CSF-1R (or FMS), the platelet-derived growth factor α and β (PDGFR-α and PDGFR-β) and the FMS-like tyrosine kinase 3 (FLT3) receptors, reveals their essential role in cancer development. Such deregulation is frequently associated with gain-of-function mutations leading to uncontrolled or constitutive activation of RTKs. Activating mutations of KIT are hallmarks in systemic mastocytosis [6] and gastro-intestinal stromal tumors (GISTs) [7], of FLT3 in acute myeloid leukemia (AML) [8, 9] and of PDGFRs in different tumors [4]. Activating mutations in CSF-1R have been rarely observed in human tumors, nevertheless, this receptor is a therapeutic target in oncology that either inhibits a paracrine loop promoting tumor growth [10] or re-educates tumor associated macrophages (TAMs) within tumor microenvironment [11].

As observed in clinics, mutations (including missense point mutations and deletion/insertion) hotspot regions in type III RTKs are mainly located in the JMR and the A-loop, although mutations have been found in the extracellular and transmembrane regions [12]. Point residue substitutions are observed more frequently than deletion or insertion mutations. Many of the activating mutations in the same protein were observed in different pathologies. For example, the activating *KIT* mutation D816V in the A-loop described in melanomas, has also reported for gastrointestinal stromal tumors (GISTs) and mastocytosis [13-18], suggesting that a similar signaling pathway is shared between the two tumor types. On the other hand, it was reported that distinct substitutions of the same residue can promote different cancers. In KIT in particular, mutations of D816 residue are the principles cause of mastocytosis (D816V/Y/H/N/(F)), germ cell tumor (D816V/H/(Y)), lymphoma (D816N) and AML (D816V/Y/H/N) (**Table 1**). Similarly, the KIT point mutations of V599 located in the JMR (V559I, V559D) are the most common causes of GISTs and of acute nonlymphocytic leukemia (ANLL). Recently, KIT mutation S628N – in a loop close to the catalytic helix – has been revealed in patients with melanoma [19].

Structural features of the type III RTKs

Structural data, the principal basis for description of the molecular mechanisms at the atomic scale, are necessarily required in structure-based drug design or inhibitor optimization. Numerous X-ray studies contributed successfully to descriptions of the activation mechanism in many native RTKs and it was concluded that this mechanism is a common for different receptors. Characterization of structural effects promoted by mutations were performed mainly by computational methods which provide the data enable to describe the structure-dynamics-function relationships in the native protein, and further to characterize the alteration of protein structure and internal dynamics in the oncogenic or resistance mutants [20]. These theoretical methods also enable to describe intermediate conformational states of proteins, which can be used to guide the design of specific inhibitors acting as modulators of the protein functions by targeting putative allosteric sites [21, 22].

The structural, dynamics and thermodynamics features of the native and mutated KIT were characterized by molecular dynamic simulations, essential dynamics, normal modes analysis, binding free energy calculations and surfaces pockets analysis [19, 23, 24]. Application of different and

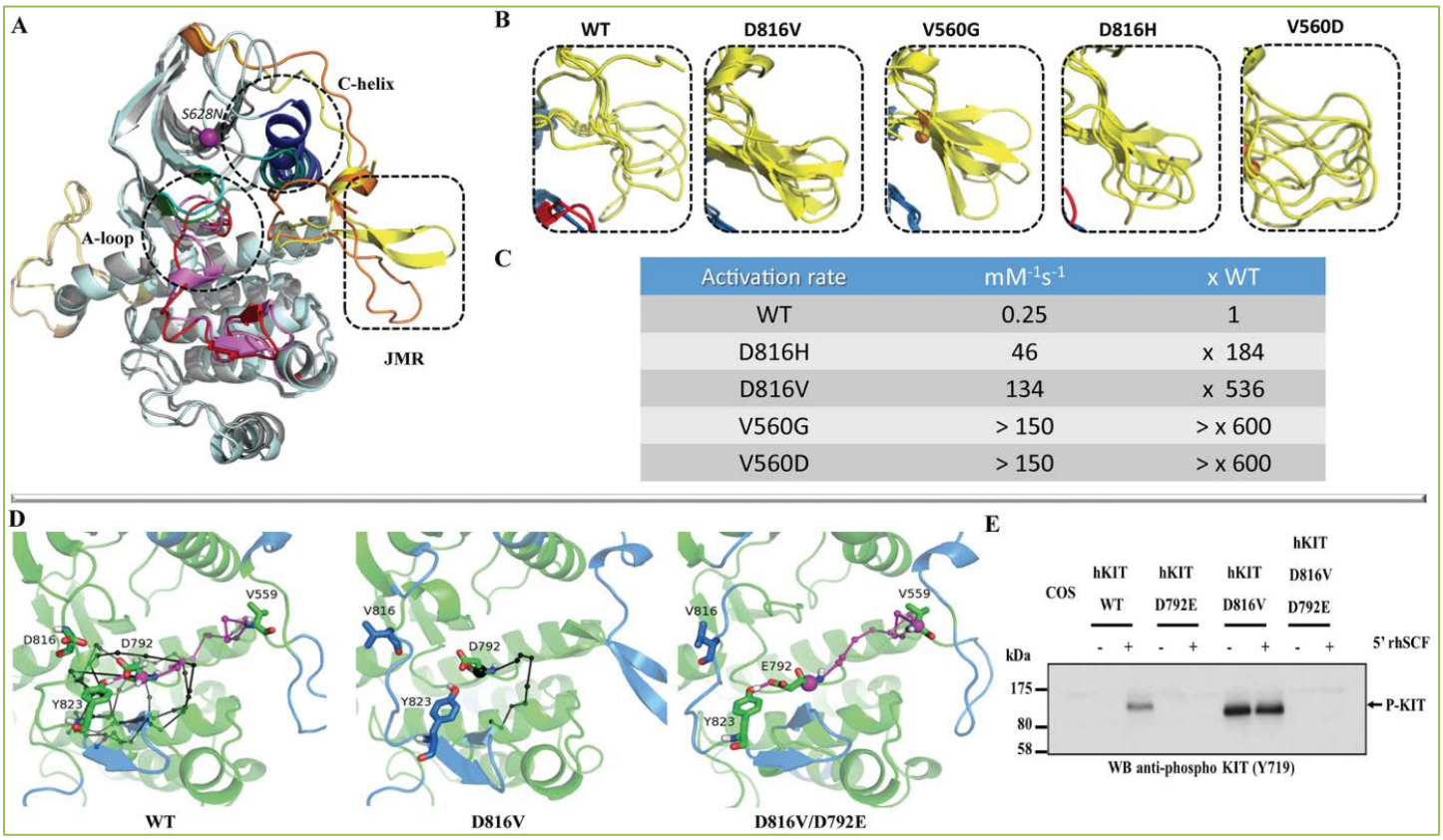

Figure 2. Mutation-induced structural effects in KIT. (**A**) Superimposed conformations of KIT cytoplasmic domain (inactive state) in the native protein (I) and its mutant S628N (II) are represented by ribbon diagrams, displaying the protein regions or fragments with different colors: (I) JMR in orange, Cα-helix in green, A-loop in magenta, rest in grey; (II) JMR in yellow, Cα-helix in blue, A-loop in red, rest in cyan. Point mutation in 628 position is labelled and denoted as a ball in magenta. Encountered fragments show the most significant structural difference between the native and mutated proteins. (**B**) Zoomed view on the JMR structure in the native KIT (WT) and in its mutants. (**C**) Activation rate of KIT WT and its mutants. (**D**) Intermolecular communication pathways between the JMR and A-loop in the native KIT (**left**), its oncogenic D816V mutant (**middle**) and the *in silico* predicted D816V/D792E double mutant (**right**). Communication pathway linking the JMR and A-loop through the catalytic loop is highlighted in magenta. Residues D/V816, Y828, D/E792 and V559 are represented as sticks. (**E**) Kinase activity of KIT WT and its mutants D816V, D792E and D816V/D792E expressed in Cos-7 cells. Cells were either stimulated with the KIT ligand SCF (+) or not stimulated (-). The Western blot shows the activation of KIT using an anti-KIT antibody (P-KIT).

independent computational methods to a comparative study of the native and mutated KIT evidenced the impact of KIT point mutations located on crucial regulating fragments – JMR (V560G/D), A-loop (D816V/H/N/Y) and loop preceding the Cα-helix (S628N) – on the structure and dynamics of the kinase domain.

Two types of structural effects were observed – local effects, detected at proximity of the mutation site, and long-range effects, manifested in regions distant from the mutation site (**Figure 2A**). The local effects consist in a loss of regular folding in the A-loop (in all studied KIT$^{D816V/H/N/Y}$ mutants) or in the loop preceding the Cα-helix (in KIT^{S628N} mutant). The long-range effects induced by the activating mutations are evidenced as a significant change in the protein structure (the JMR presents a coil in the native KIT and regular β-strands in KIT mutants), and conformational mobility (departure of the JMR from the kinase domain and bending of the C-helix). The spectacular JMR folding, and its departure from the kinase domain towards a solvent accessible position, together with the destabilization of the inactive conformation of the A-loop or of the loop preceding the Cα-helix, indicates that the KIT mutants are maintained in a state, different from the inactive autoinhibited KIT. This mutation-induced state may be described as the inactive non-autoinhibited form of the protein.

In all studied KIT mutants, the magnitude of the mutation-induced effects is not equivalent as it observed *in silico*, and depends on the mutation site location and on the type of substitution [23] (**Figure 2B**). The JMR mutations V560G/D induce only local structural change in KIT, evidenced by an alternation of JMR folding that shows either an increase or a decrease of regular structure, depending on the substitution. Together with the structural effect, the JMR moves from the kinase domain, as was observed in the

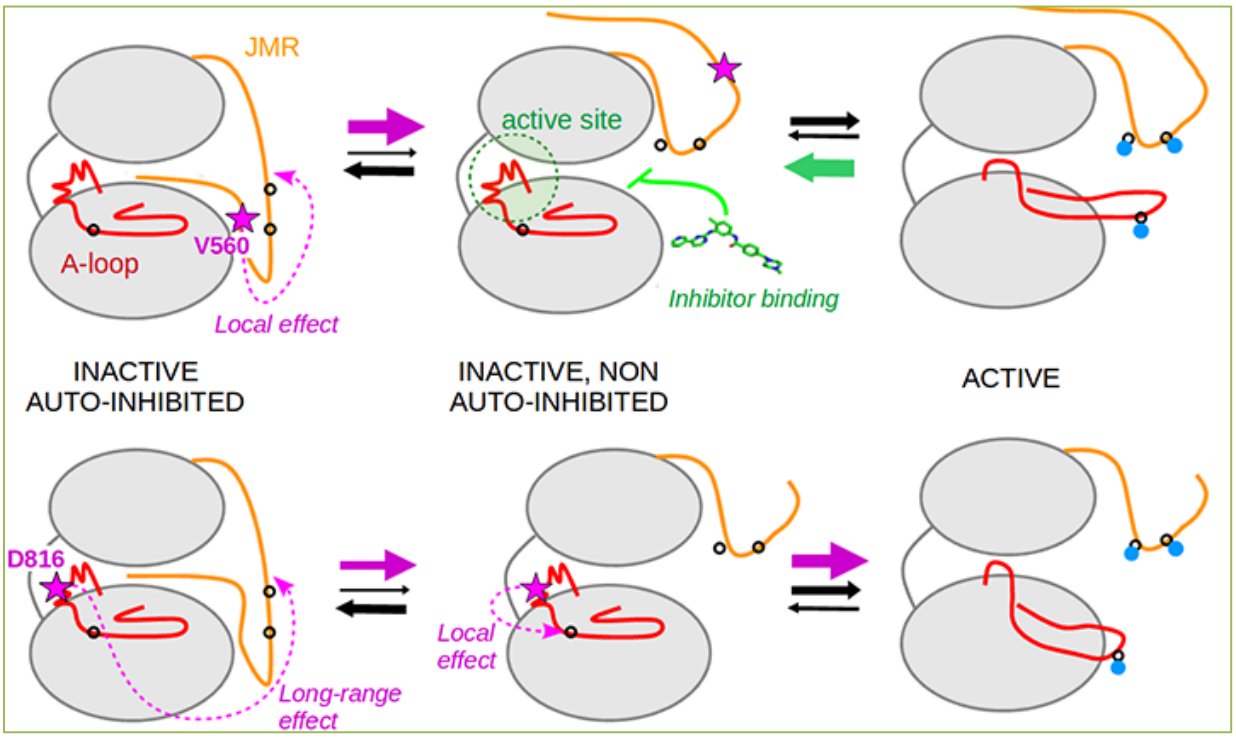

Figure 3. The inactive-to-active state switch of KIT tyrosine kinase domain. The inactive auto-inhibited state of the native KIT with A-loop and JMR adjacent to the active site (left) in the absence of SCF is in equilibrium with the active state in which A-loop and JMR are displaced from the kinase domain (right). The conformational equilibrium is changed by binding of the inhibitor to the active site in an intermediate non-autoinhibited state (middle). The point mutation in the JMR promote this inactive non-inhibited state **(top panel**). The mutation-induced effects in KIT^{D816V} promotes the appearance of the active form of the receptor (**bottom panel**).

KIT$^{D816V/H/Y/N}$ and KIT^{S628N} mutants. In KIT^{S628N} mutant, the three principal regulating fragments – JMR, A-loop and Cα-helix – are influenced significantly by the point mutation. This mutation promotes the effects observed in KIT having the point mutation in position 816 (A-loop) or 560 (JMR).

Insights on the mutation-induced activation mechanisms

Comparison of the mutation-induced effects evidenced by numerical approaches (*in silico*) with the auto-activation rate of mutants (*in vitro* and *in vivo* data), established a pertinent correlation [23]. The most pronounced structural effects were detected in KIT^{D816V}, the most clinically observed mutant in patients with mastocytosis and GISTs, and also found in AML and in germ cell tumors [25, 26]. This mutation prompts a constitutive kinase activity characterized by a considerably increased auto-activation rate (by a factor ~ 500) respectively to the native receptor, whereas the D816H/N mutation induces a more moderate increase (by a factor ~ 200) [27, 28]. The structural and dynamical manifestation of the mutation-induced effects in KIT^{D816V} and in KIT$^{D816H/N}$ in respect to the native protein fits well to their auto-activation rates. Moreover, the *in silico* evidenced impact of V560G mutation, the most frequent KIT mutations in GISTs and melanoma, on the JMR, is more marked than of D816V/H/Y/N mutations [23], consistent with a higher activating potency of this substitution [27, 29] (**Figure 2B, C**). Such relationships between the mutation-induced structural effects evidenced *in silico* and the *in vitro* (*in vivo*) activation rate (pathogenicity) measurements deliver novel elements to the activation mechanisms of these mutants. The KIT activating D816V mutation allosterically promotes a spontaneous detachment of JMR from kinase domain, achieving the first step of the inactive-to-active state transition in KIT (**Figure 3**). The JMR mutations V560D/G also disturb its auto-inhibited conformation locally, promoting KIT constitutive activation, similarly the long-range effects of A-loop mutations D618V/H/Y/N [23]. The KIT mutation S628N induces structural effects in all fragments regulating the kinase activity and induces a conformation promoting KIT activation in the absence of ligand stimulation [19].

Since the different RTKs show systematically equivalent (position and missense) mutations related to distinct pathologies, we were interested in further comparison of effects induced by homologous mutations in two RTKs, D816V in KIT and D802V in CSF-1R. Our comparative analysis of the two RTKs, showed considerable sequence identity and great structural similarities of their

auto-inhibited states, demonstrates that the homologous mutations do not have the same impact on the receptors conformation and dynamics, providing a plausible interpretation for the differential incidence of these mutations in oncology. Mutation D816V in KIT induces high oncogenic activation, while mutation D802V in CSF-1R is lowly oncogenic and does not direct influence on the auto-activation rate of this receptor [30]. This founding correlates with the *in silico* observations showed that D802V has either very little or no effect on the JMR [31].

Key-points to the allosterically-regulated mechanisms of RTKs activation

The RTKs activation is a tightly regulated process, initiated by binding of their ligands on extracellular domain that prompts intra-molecular processes in this region (conformational modification of the Ig-like fragments, dimerisation) [32, 33]. These consequent processes initiate a response of the principal regulatory fragments in cytoplasmic region – JMR, A-loop and Cα-helix – resulting in the activation of the kinase reaction and post-transduction events. Such intramolecular propagation of the ligand-controlled signal, may be changed by the disease-related (gain-of-function) mutations positioned on these functional fragments. Consequently, a mutation can be considered as a transforming perturbation of the functional intra-molecular signaling network. The mutation-induced long-distance structural effects and their correlation with the mutation-induced local effects suggest the allosteric nature of intra-molecular regulation controlling the structure/dynamics relationships between the A-loop, Cα-helix and JMR in RTKs. A notion of communication and its pathway as a physically significant means for establishing such control or perturbing was introduced [34-36].

Characterization of the inter-fragments communication in KIT and CSF-1R were performed by Modular Network Analyses (MONETA) [37, 38]. This method based on a dual formalism – geometrical (topological) descriptors and dynamical correlations – detects cohesive groups of amino acids (clusters, or *Independent Dynamics Segments*) communicating with other groups via chains of residues (linkers, or *Communication Pathway*) [37, 38]. In both native receptors, communication between JMR and A-loop was evidenced through extended networks of H-bonds – the intramolecular *Communication Pathways* (*CPs*) – connecting these two remote regions (**Figure 2D**). In KIT/CSF-1R, these regulating fragments are linked non-covalently through residue D792/788 (the catalytic loop) forming with Y823/809 (A-loop) a strong and dynamically stable H-bond. In KIT and CSF-1R mutants, this JMR–A-loop communication pathway was interrupted, allowing a self-governed unconstrained JMR fold, controlled by the proper JMR polypeptide sequence [38].

This difference of the intramolecular communication pathways in the native and mutated proteins derived from distinct local dynamical properties of the proteins, the *Independent Dynamics Segments* (*IDSs*) that changed considerably in the mutants in respect to the native receptors [37-38]. The established *in silico* correlations for the residues movements in KIT and CSF-1R evidences relationships between the structural and dynamical properties of each protein and the physical factors underlying the signal propagation between the protein residues. Such dual characterization (structure-dynamics) of proteins, based only on computing, delivers *per se* identification of residues crucial for the protein functions. In particular, in KIT/CSF-1R, residues D792/788 and Y823/809 positioned in the catalytic and the A-loop respectively were identified as key residues in the communication pathway, regulating RTKs activation.

The H-bond between D792/788 and Y823/809 was disrupted in receptors possessing equivalent D816/802V mutations, the two residues being slightly torn apart by the local effect of the mutation. We suggested that introducing the second mutation in KIT^{D816V} mutant can restore the H-bond. Replacement of an aspartate to a glutamate (D792E) having longer side-chain that the aspartate and thus enable to accommodate a longer inter-residue distance, may stimulate a re-establishing of communication between the JMR and the kinase domain of KIT. The structure and dynamical properties of the $KIT^{D792E/D816V}$, the double mutant conceived and procured *in silico*, were found very similar to those of the native KIT [38]. As proposed, D792E mutation induces a compensate impact to the destructive effect of D816V mutation on the two regulatory fragments communication. This *in silico* mutagenesis-type prediction has been validated to a certain extend by an *in vitro* measurement of the autophosphorylation level [39] in the double mutant D792E/D816V respective to the native KIT and its oncogenic mutant (D618V) (**Figure 2 E**). In this experiment, the high trans-autophosphorylation signal in KIT^{D816V}, both in the presence and absence of the stem cell factor SCF, was vanished in the double mutant $KIT^{D816V/D792E}$. On the other side, mutation of highly conserved residue D792 may to damage considerably the kinase activity of KIT, an issue requiring the further investigations.

Structure-based insight on resistance/sensitivity of KIT/CSF-1R to imatinib

As discussed above, gain-of-function mutations in KIT and CSF-1R associated with human diseases promote the

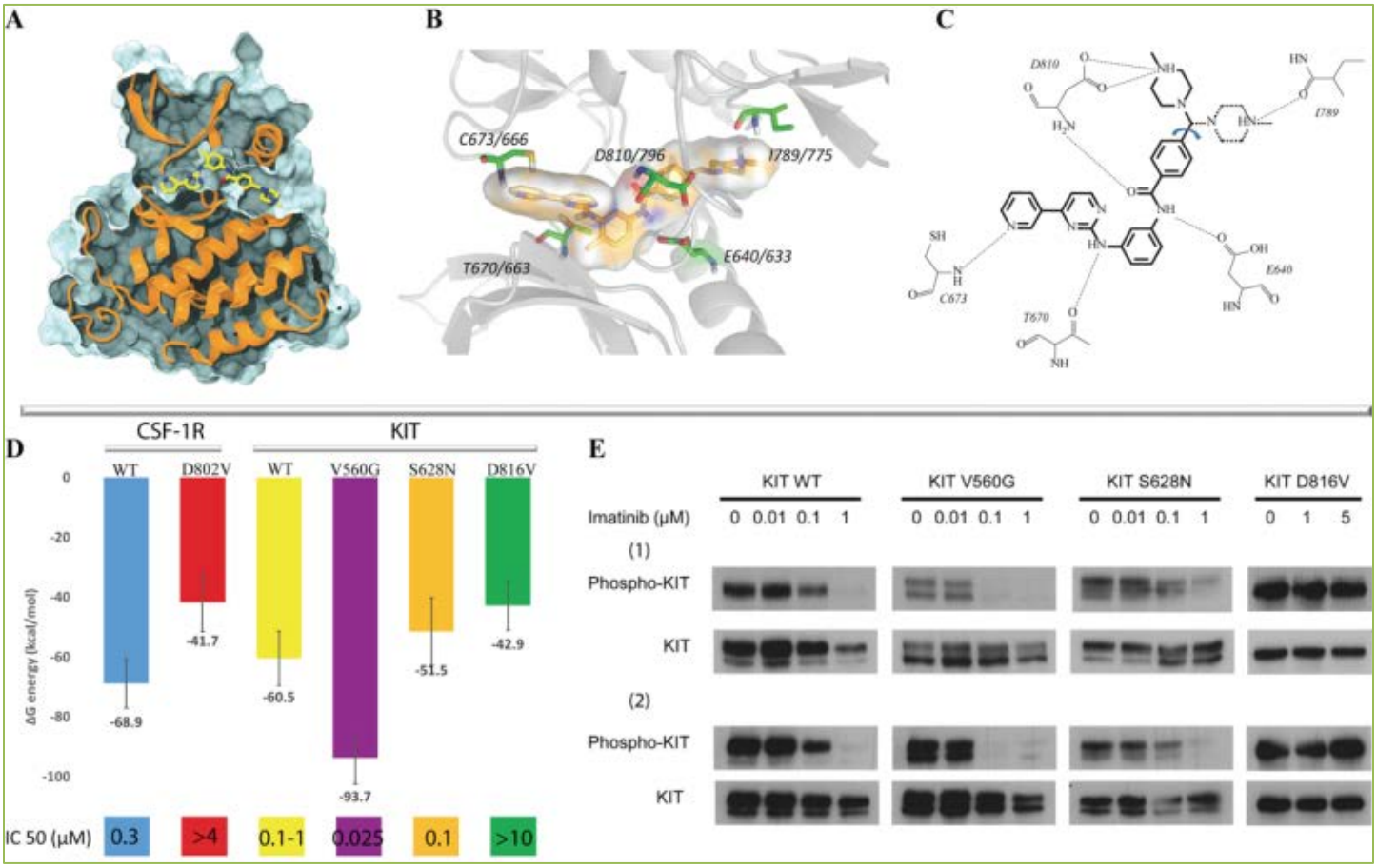

Figure 4. Imatinib binding to receptors tyrosine kinases (RTKs) CSF-1R and KIT. (**A**) General view of the studied imatinib-target complexes. Imatinib is presented as sticks. The surface of a target showed as a ribbon diagram and its binding site are countered. (**B**) Zoomed view of imatinib and interacting residues of KIT/CSF-1R showed by sticks. (**C**) The H-bonds (dashed lines) pattern stabilizing imatinib in complexes formed by the native KIT and its mutants. Alternative conformations of the methyl-piperazinyl moiety in imatinib is shown by solid (in KIT^{S628N}) and dashed ($CSF\text{-}1R^{WT}$, $CSF\text{-}1R^{D802V}$, KIT^{WT}, KIT^{D816V} and KIT^{V560G}) lines. (**D**) Graphical representation of the binding free energy (ΔG) of RTKs complexes formed by imatinib and target. The computed total ΔG value and the experimentally measured affinity is shown for each complex. (**E**) Inhibition test of KIT phosphorylation by imatinib. Cells expressing KIT^{WT}, KIT^{D816V}, KIT^{V560G} or KIT^{S628N} were treated with the indicated concentrations of imatinib. Protein lysates were analysed by western-blotting to reveal KIT protein expression (KIT) and KIT phosphorylation (Phospho-KIT) as readout of imatinib inhibition.

alteration of the kinase domain structure. This can lead to differences in the sensitivities of the mutants towards imatinib – a first-line RTKs inhibitor that produces remarkable results in the treatment of several cancers [26, 32]. Close similarity of the binding pockets of the two native proteins in the inactive state explains their comparable sensitivity to imatinib that is not effective for the mutants (KIT [27] or CSF-1R [30]). The mutation-induced structural effects observed *in silico* in KIT and CSF-1R were compared with the sensitivity of mutants to the drugs (*in vitro* and *in vivo* data) [23, 40]. Remarkably, *in silico* observations revealed that the local impact of the equivalent (A-loop) mutations, D816V in KIT and D802 in CSF-1R, inherent to the imatinib-binding site, is very similar and resemble to those detected in the other mutants, KIT^{D816H} and KIT^{S628N}. Such destabilisation of the A-loop conformation in mutants would be a major factor contributing to the loss of drug sensibility. The mutation-induced local unfolding of the A-loop facilitates the protein transition toward an extended conformation, which constitute the determinative step of receptor activation (**Figure 3**). This effect promotes destabilization of the inactive autoinhibited conformation of kinase domain, adapted for binding of inhibitors, leading to the RTKs resistance. In $KIT^{V560G/D}$ mutants, the structure of A-loop shows the better stability than in the native protein. The mutation promotes only a departure of the JMR from the kinase domain, thus inducing the inactive non-autoinhibited state and providing a greater drug sensitivity of these mutants compared to the native target [23].

The mutation-induced effects on imatinib affinity of RTKs were analyzed through the comparative study of the structural, dynamical and thermodynamical properties of molecular complexes formed by imatinib bound to the clinically relevant

mutants of two RTKs, KIT and CSF-1R [40]. A strong correlation between the *in silico* calculated and *in vitro* measured binding affinities of imatinib to it targets was established. The free energy of binding is considerably different in the studied complexes (**Figure 4A, D)**, showing the lowest (-94 kcal/mol) and the biggest (-42/-43 kcal/mol) values in complexes formed by the most sensitive (KIT^{V560G}) and the most resistant ($CSF\text{-}1R^{D802V}$ and KIT^{D816V}) targets to imatinib, respectively. Based on the values of the binding free energy, the sensitivity of the studied targets to imatinib may be described by the following scale: $KIT^{V560G} > CSF\text{-}1R^{WT}/KIT^{WT} \geq KIT^{S628N} \geq CSF\text{-}1R^{D802V}/KIT^{D816V}$ which is qualitatively very consistent with our *in vitro* data.

By computing the number of conformations in our simulations ('occurrence') that displayed a given non-covalent interaction of imatinib to the target as a measure characterising the stability of this interaction, we evidenced that imatinib binding to the resistant KIT^{D816V} and $CSF\text{-}1R^{D802V}$ is diminished considerably [41]. The changes in the computed imatinib-target binding energy together with the mutation-induced perturbation of the imatinib-selective inactive conformation of the targets [23, 24, 38], correlate evidently with the resistant profile of the $KIT^{D816V/H}$ and $CSF\text{-}1R^{D802V}$ mutants, comforting our numerically-based observations. Conversely, KIT^{V560G}, showing a higher sensitivity to imatinib than the KIT^{WT}, binds imatinib through H-bonds demonstrating significantly greater occurrences than in KIT^{WT}. In the imatinib•KIT^{S628N} complex, formed by a mutant sensitive to imatinib, the pattern and occurrences of imatinib-target interactions is alterated. Specifically, the imatinib H-bonding with D810 residue occurs due to a flip of the methyl-piperazin fragment of imatinib and a change on the orientation of D810 side chain (**Figure 4B, C**). Such conformational adaptation the both interacting partners, target and inhibitor, induces a relevant decrease (or loss) of the inhibitor interaction with I789 and stabilises the double H-bond with D810 side chain, the two compensative interactions ensuring a good binding affinity of imatinib. The relative sensitivity of KIT^{WT} and KIT^{S628N}, experimentally determined through the treatment with different imatinib concentrations of COS7 cells transfected with the corresponding expression vectors, indicates for KIT^{S628N} the same sensitiveness to drug (**Figure 4E**) [40].

Conclusions

I*n silico* study provides the detailed description of molecular effects which are not yet empirically analyzed or are non-accessible for the direct experimental measurements. Our theoretical analysis established the impact of gain-of-function mutations on structural, dynamical and the imatinib-recognition properties of KIT and CSF-1R, two crucial clinical targets. The obtained data reveal novel and fine elements on molecular mechanisms leading to the RTKs activation in the normal and cancerous cells. We showed that the native KIT in the absence of stimulation by its physiological ligand (SCF), is maintained in the inactive state through an intra-molecular communication between the JMR and A-loop, the principle fragments regulating the RTKs activity. In KIT mutants, the role of SCF factor stimulating the functional KIT activation is replaced by a mutation-driven activation that interrupt this communication. Discontinuity in communication between the two regulatory fragments, JMR and A-loop, promotes stabilization of the cytoplasmic region in a conformation favoring a transition toward the fully active state of KIT mutants. The established cross-correlation between the local (on the fragments containing the mutation) and long-range (on the fragments distant from point mutation) structural and dynamical effects of KIT mutations prove the allosteric character of the gain-of-function mutations action.

Unlike the reversible activation/deactivation process initiated by SCF in the native KIT, the mutation-induced activation is irreversible. The gain-of-function mutation acts as a perpetual effector inducing a particular state leading to KIT constitutive activation. Distinct to the mutation-induced shift towards an active conformation in KIT^{D816V}, the equivalent mutation D802V in CSF-1R does not impacts the JMR structure. The divergent role of the equivalent mutations on the protein conformational dynamics may be related to the primary sequence between the two native receptors, particularly in the JMR. As a result, the CSF-1R D802V mutant does not confer a competitive advantage to the cell, thus is not a driver oncogenic event.

Resistance of receptors KIT and CSF-1R having the equivalent mutations (D816V and D802V respectively) to inhibitors targeting the inactive autoinhibited state is similarly promoted by the mutation-induced local effects, destabilising the binding-competent inactive non-autoinhibited conformation of the active site in kinase domain.

Description of the mutations-induced structural effects and of the physical support for allosteric coupling/decoupling provides a novel scope for the description of the mechanisms of KIT constitutive/oncogenic activation and contributes to the understanding of allosteric regulation in this protein. The described conformations of mutated KIT represent novel targets for *in silico* drug design. The conformational states specific to oncogenic and/or resistant mutated forms of RTKs will open a way for innovative rational strategies for the development of novel efficient anti-cancer targeted treatments delivered from both experimental evidence and

theoretical modeling and simulations.

Conflicting interests

The authors have declared that no competing interests exist.

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Author contributions

I.C.B. and L.T. wrote the paper.

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