The promises of molecular targeted therapies and the challenges of the intrinsic and acquired resistance

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FONDAZIONE IRCCS
ISTITUTO NAZIONALE
DEI TUMORI

NEW SCENARIO

High-throughput tools

Molecular characterization of tumors

Molecular classification

Targeted therapy

MOLECULAR TARGETED THERAPY

- "Drugable" direct target (onco)gene products

- "Drugable" indirect target altered pathways (TSG)

MOLECULAR TARGETED THERAPY

"Drugable" direct target (onco)gene products

a) Cell addiction (viability)

c) Cell growth dependance

MOLECULAR TARGETED THERAPY

- **CELL ADDICTION** - **BCR/ABL** **in CML**

  (oncogene addiction) - **c-Kit** **in GIST**

b) **CELL GROWTH**

DEPENDANCE - **PDGFRB in DFSP**

(low oncogene addiction) - **PDGFRB in Chordomas**

MOLECULAR CLASSIFICATION ⟺ TARGETED THERAPY

Imatinib Paradigm

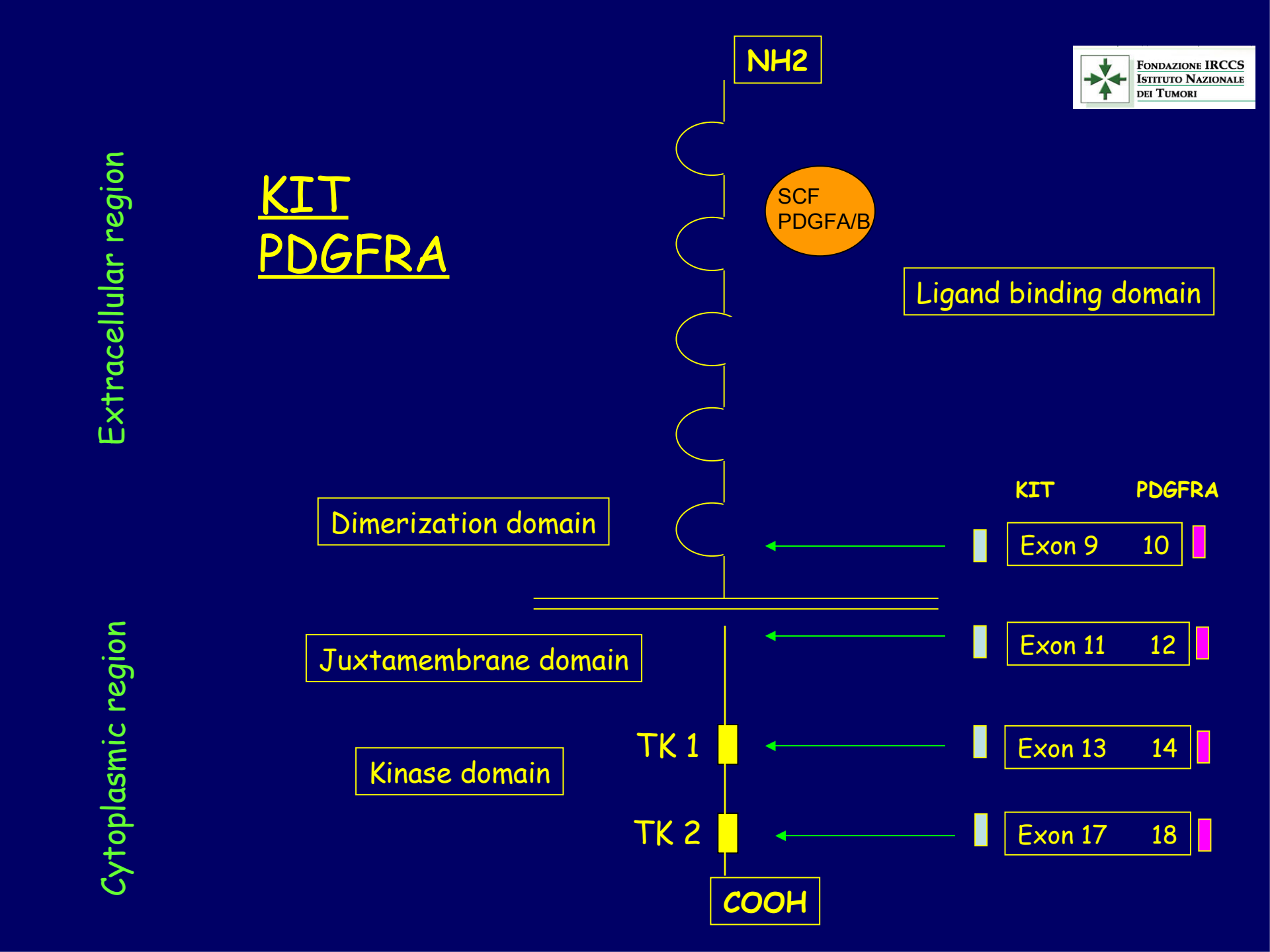
One drug for several histologically different tumors:

- **CML** (haematopoyetic)
- **GIST** (stromal-derivation)
- **DFSP** (sarcoma)
- **Chordomas** (notochorda derivation)

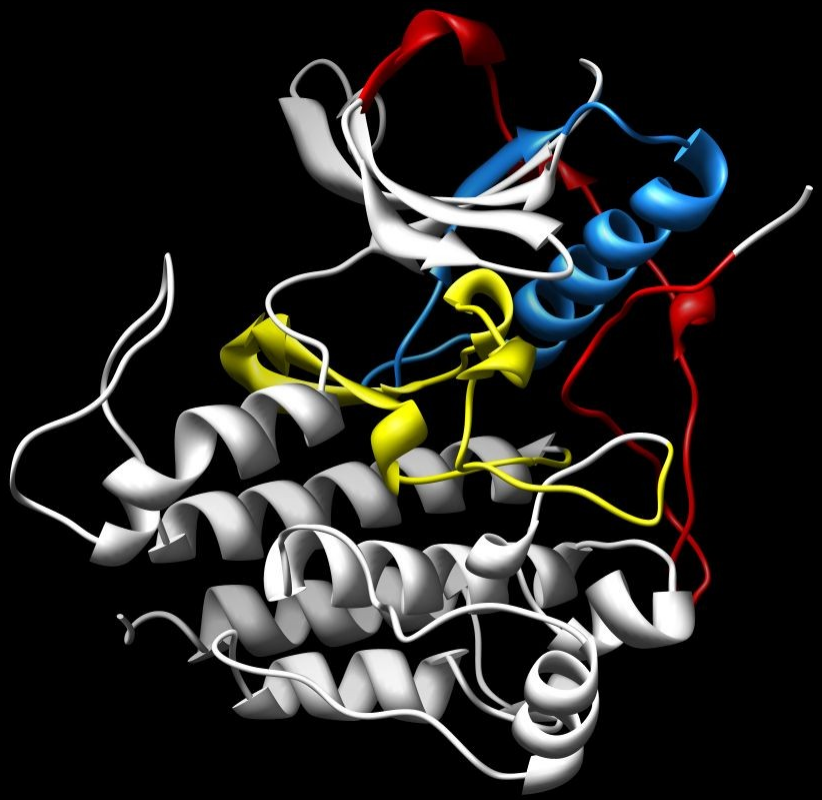
GIST: Intra-abdominal mesenchymal/stromal neoplasm, most probably from interstitial cells of Cajal origin, displaying KIT (CD117) immunopositivity

Diagnosis of Gastrointestinal Stromal Tumors: A consensus Approach.
Hum Pathol 2002 May;33:459-465

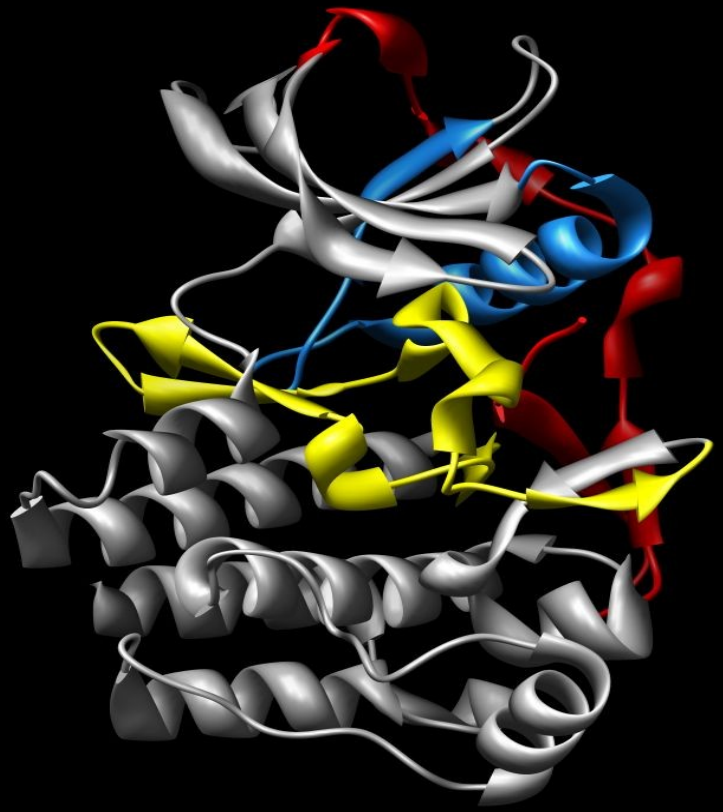
KIT and PDGFRA molecular modeling for GIST sensitivity and resistance to Imatinib


KIT
PDGFRA
NH2
SCF
PDGFA/B
Ligand binding domain
Extracellular region
Dimerization domain
KIT
PDGFRA
Exon 9
10
Exon 11
12
Juxtamembrane domain
Cytoplasmic region
TK 1
Exon 13
14
Kinase domain
TK 2
Exon 17
18
COOH

KIT Kinase domain



PDGFRA Kinase domain



Red: exon 11 wt

Blue: exon 13

Yellow : exon 17

juxtamembran domain

β-hairpin loop

TK1 C-Helix

TK2 Activation Loop

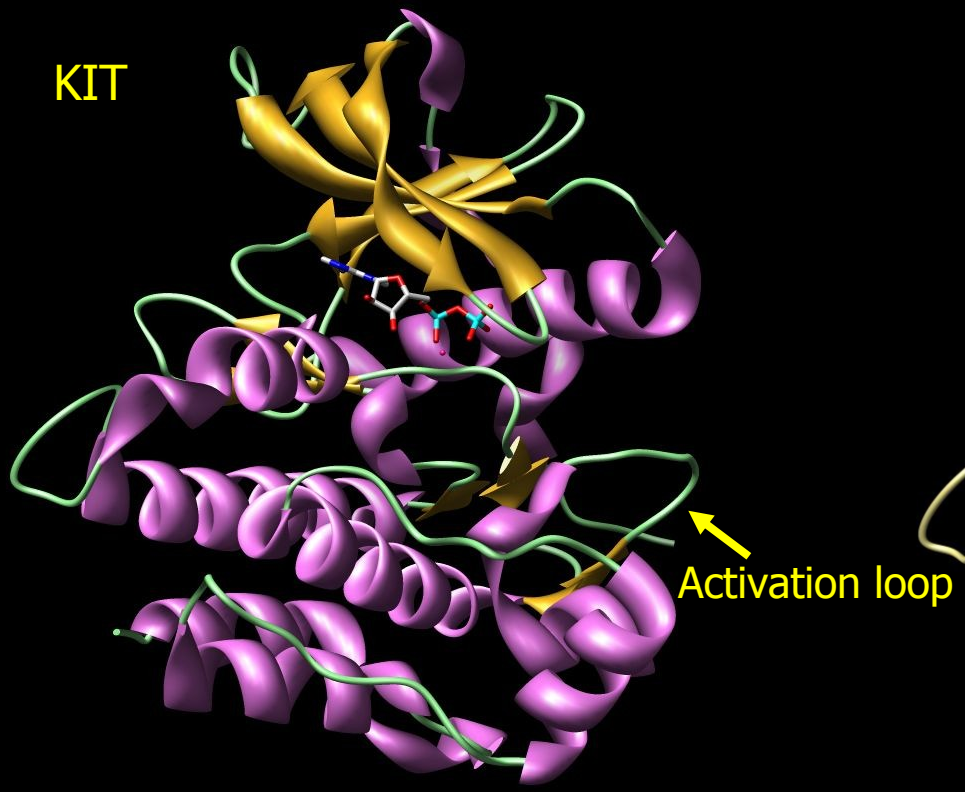
Red: exon 12 wt

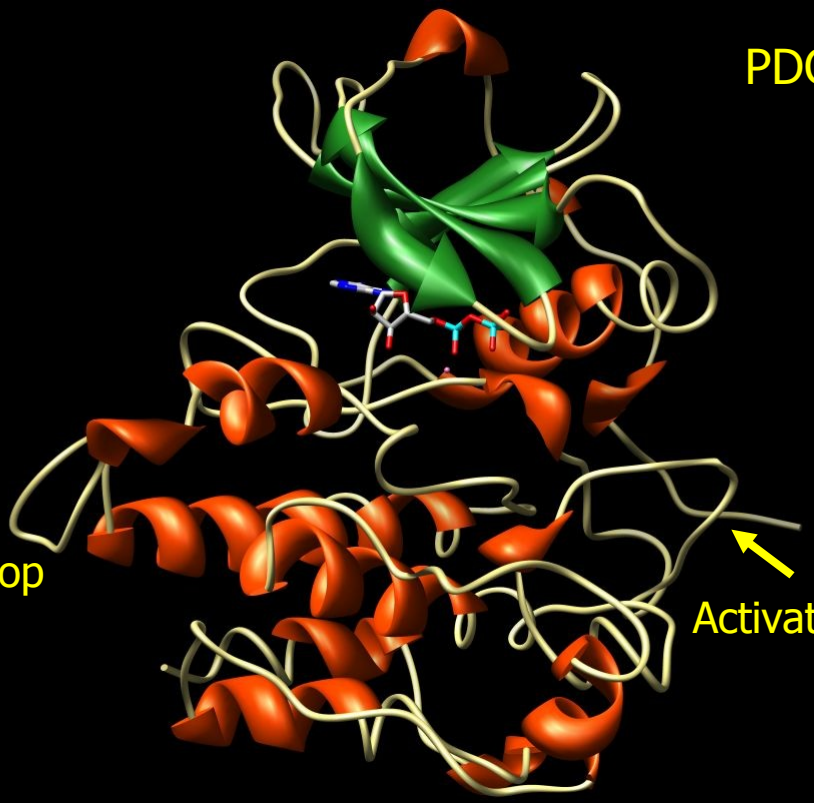
Blue: exon 14

Yellow : exon 18

S. Pricl, E Tamborini et al., Submitted

How ATP is located into the pocket:



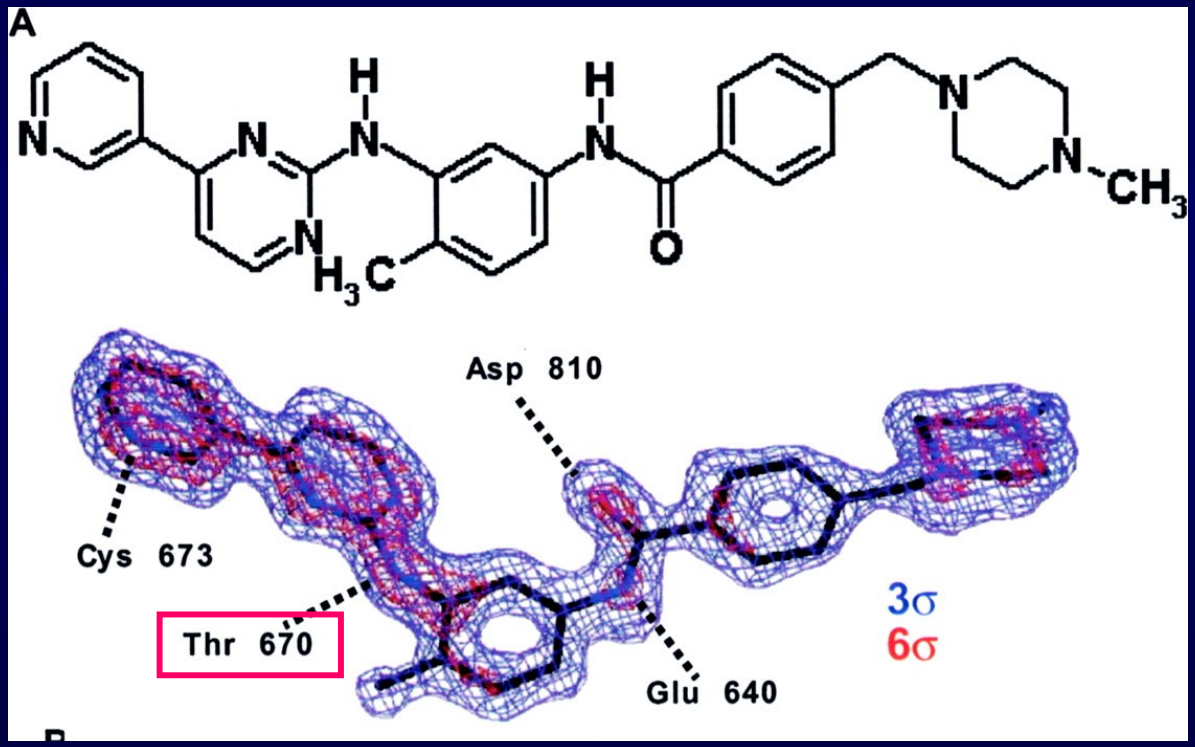


It binds the **ACTIVE/OPEN** conformation of the kinase

S. Pricl, E Tamborini et al., Submitted

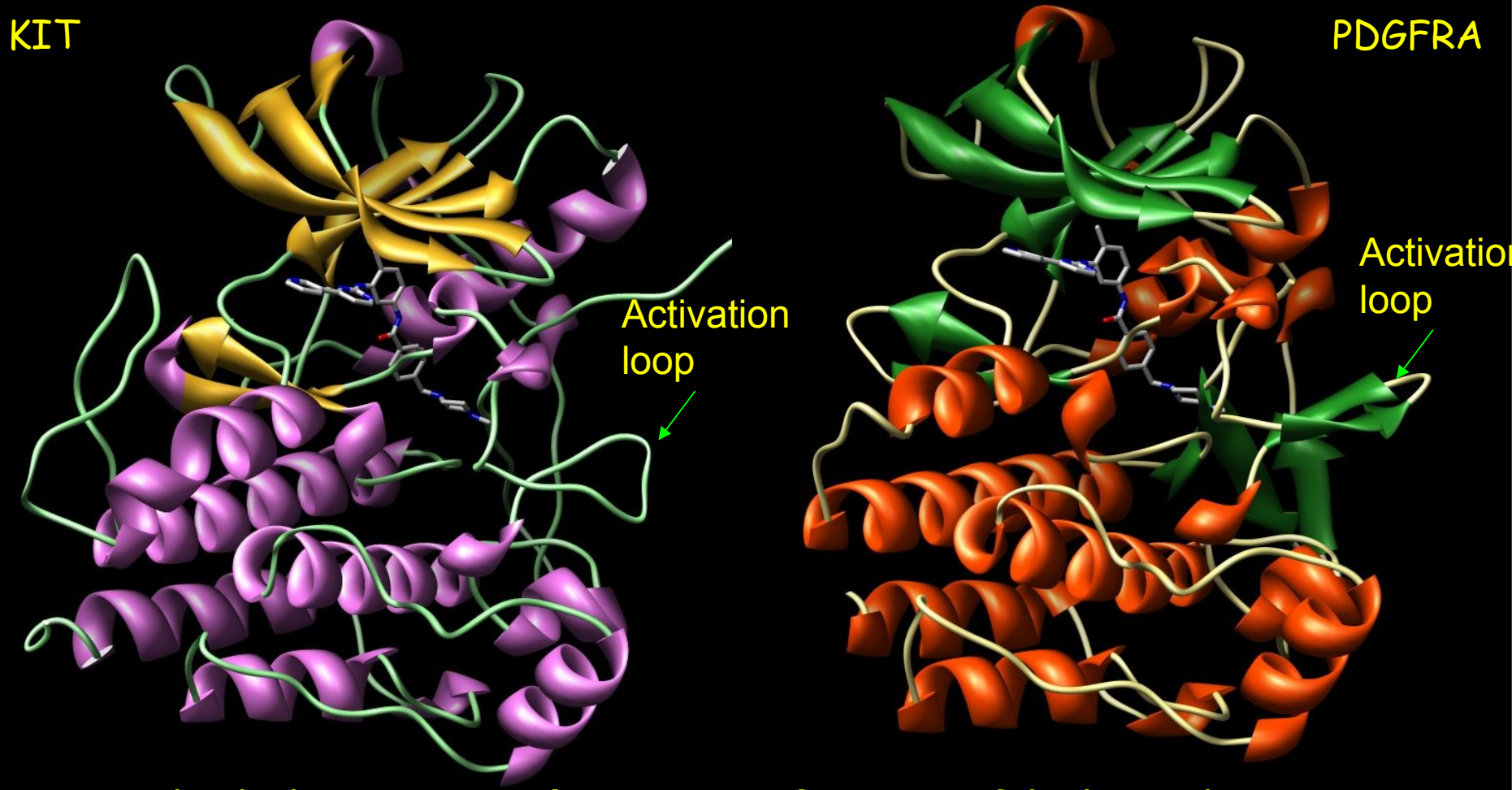
An intracellular inhibitor:

Gleevec/Imatinib/STI571



2-phenylaminopyrimidine derivative

How Imatinib is located into the pocket:



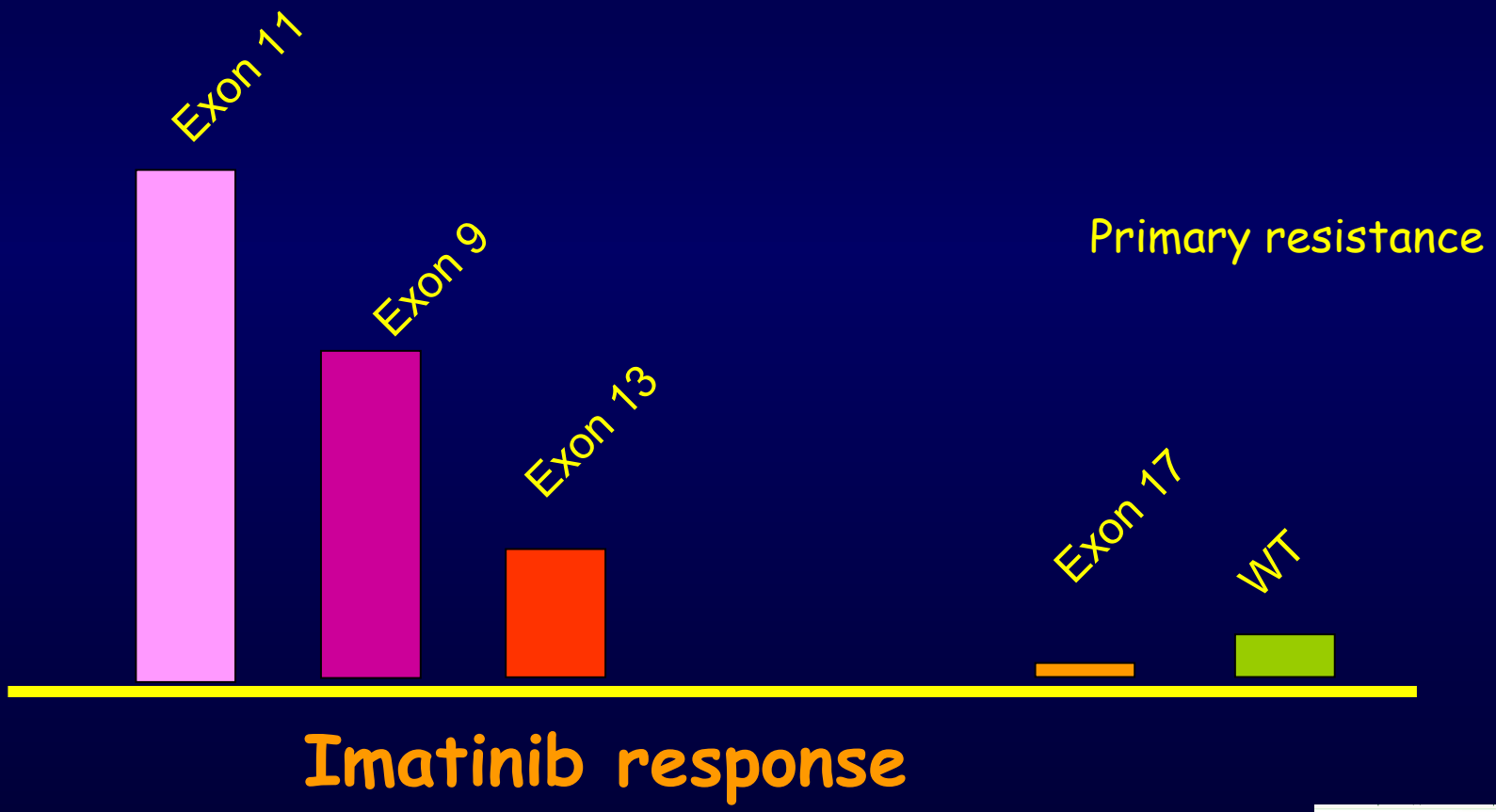
It binds the **INACTIVE/CLOSED** conformation of the kinase hampering the entrance of ATP

S. Pricl, E Tamborini et al., Submitted

Mutation HOT-SPOTs in GISTs

- Exon 11 KIT (juxtmembrane domain) exon 12 PDGFRA
- Exon 9 KIT (extracellular domain)
- Exon 13 KIT (I part of TK domain) exon 14 PDGFRA
- Exon 17 KIT (II part of TK domain) exon 18 PDGFRA

Correlation between mutated KIT exons and response to Imatinib

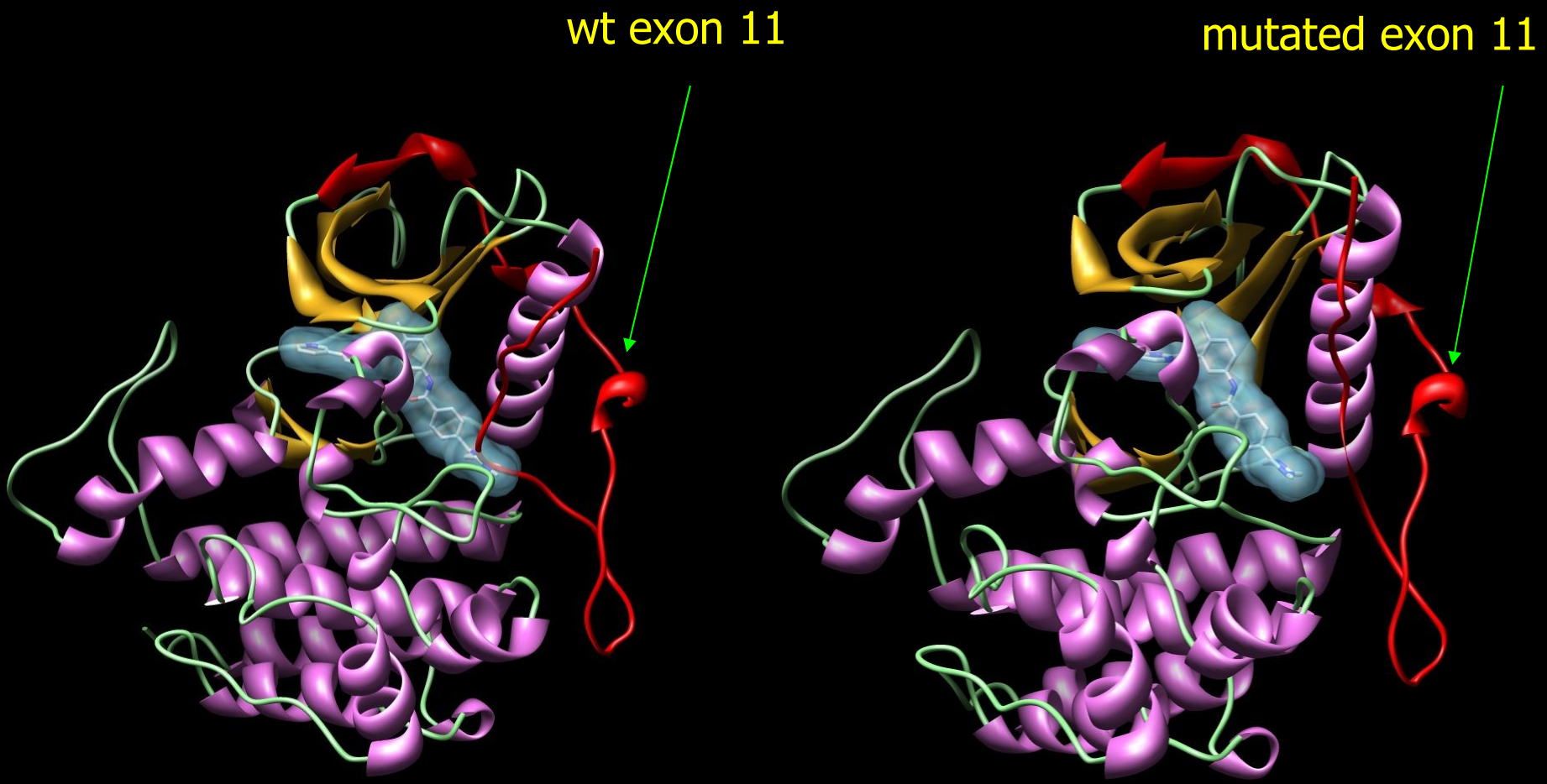


Primary or intrinsic resistance is due to a conformation of the ATP pocket which does not fit with the Imatinib entrance.

KIT molecular modeling for

Imatinib sensitivity of exon11 mutations:

KIT receptor



Sterical hindrance of β-hairpin
removed by exon 11 mutation

S. Pricl, E Tamborini et al., Submitted

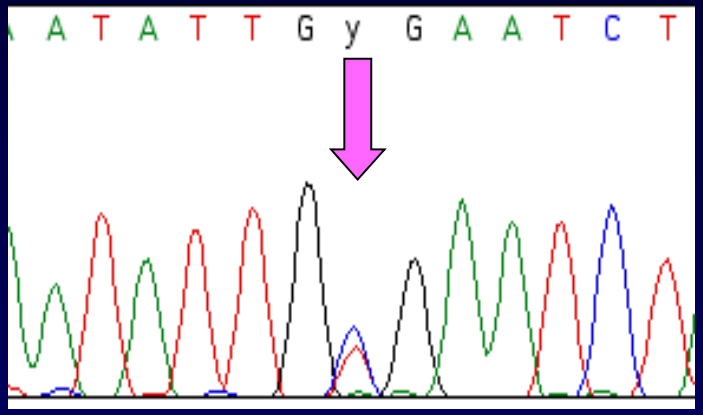
Secondary or acquired reisistance is due to secondary alterations affecting the 3D structure of the kinase domain

Many mechanisms have been reported to be responsible for secondary resistance to Imatinib, including gene amplification, loss of the target, functional resistance.

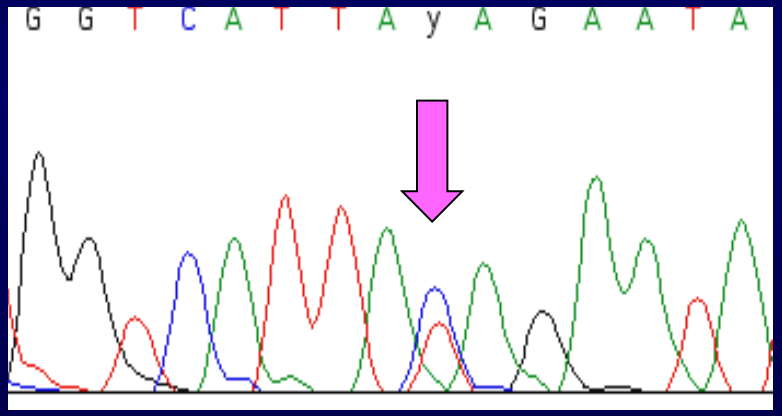
We will focus on secondary mutations affecting the ATP pocket of the receptors

To date secondary mutations are reported in KIT

Exon 13 **V654A**



Exon 14 **T670I**



Exon 17 **C809G D816E/G/H D820I/Y/N/A/G/E N822K/Y/H Y823D D716N**

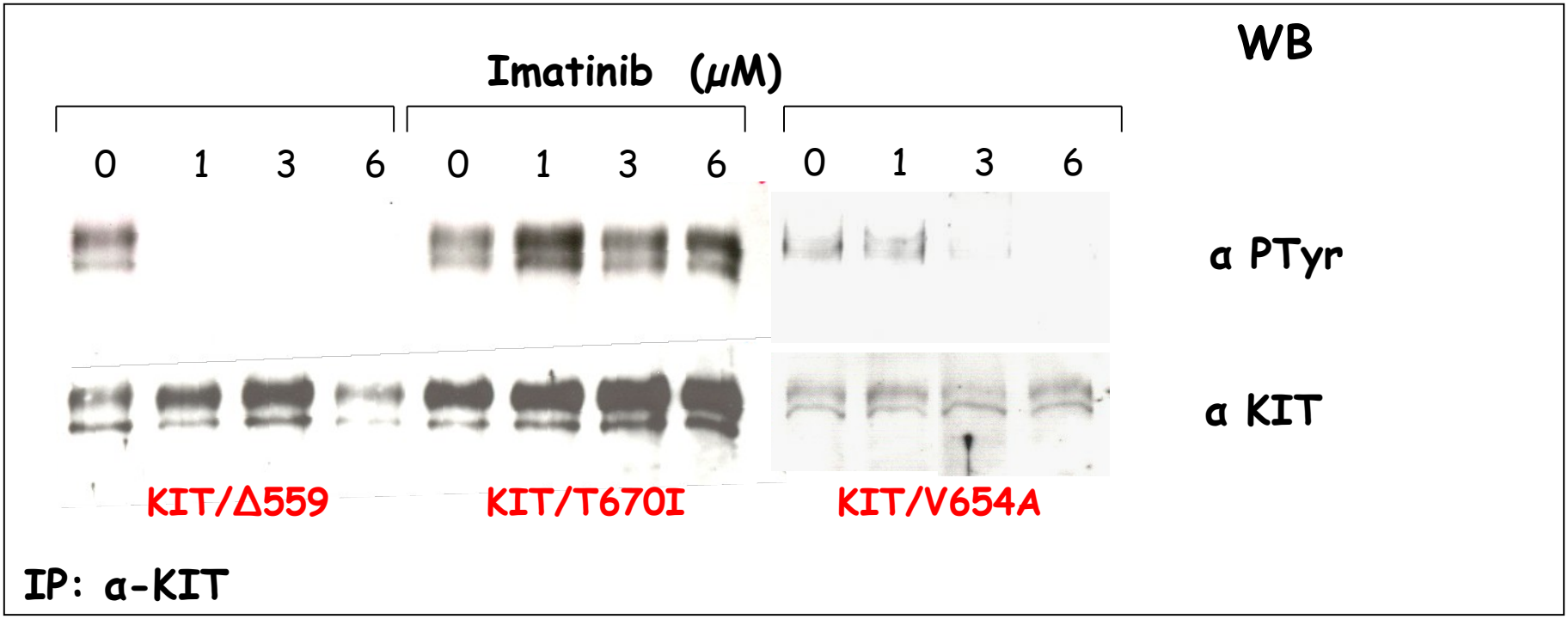
Functional effect of the identified secondary mutations

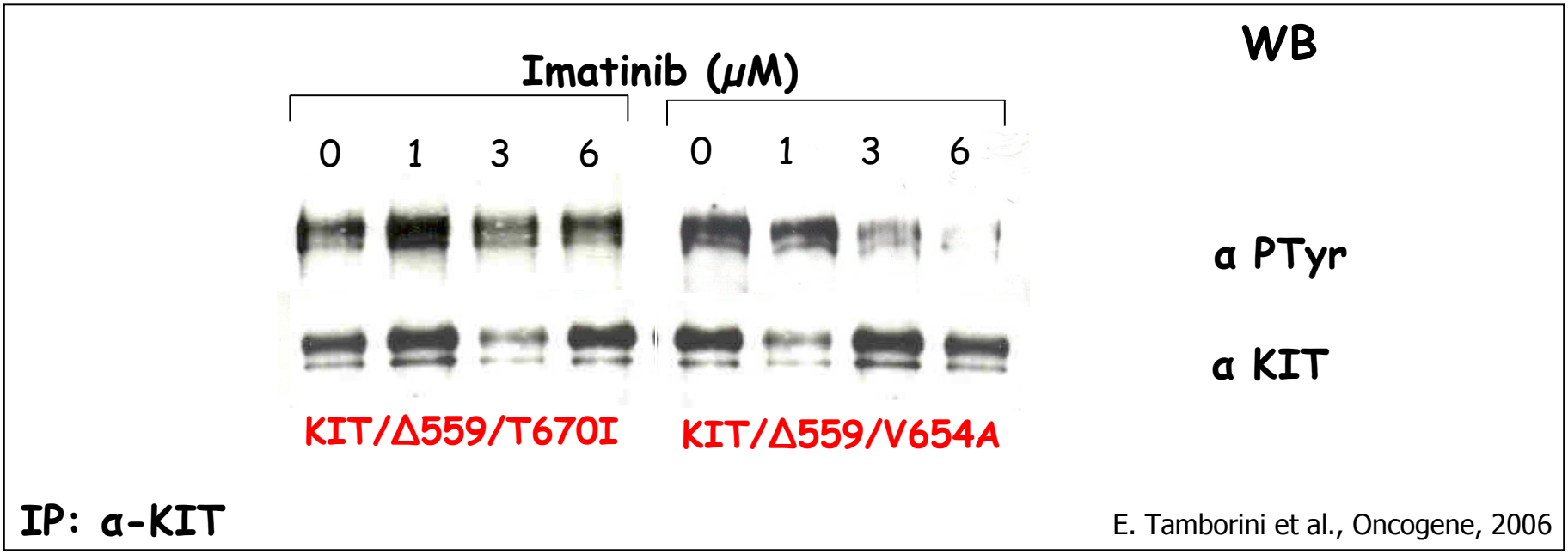
Experimental design:

1. Transient transfection of COS1 cells with different forms of c-KIT cDNA

2. Imatinib treatment of transfected cells

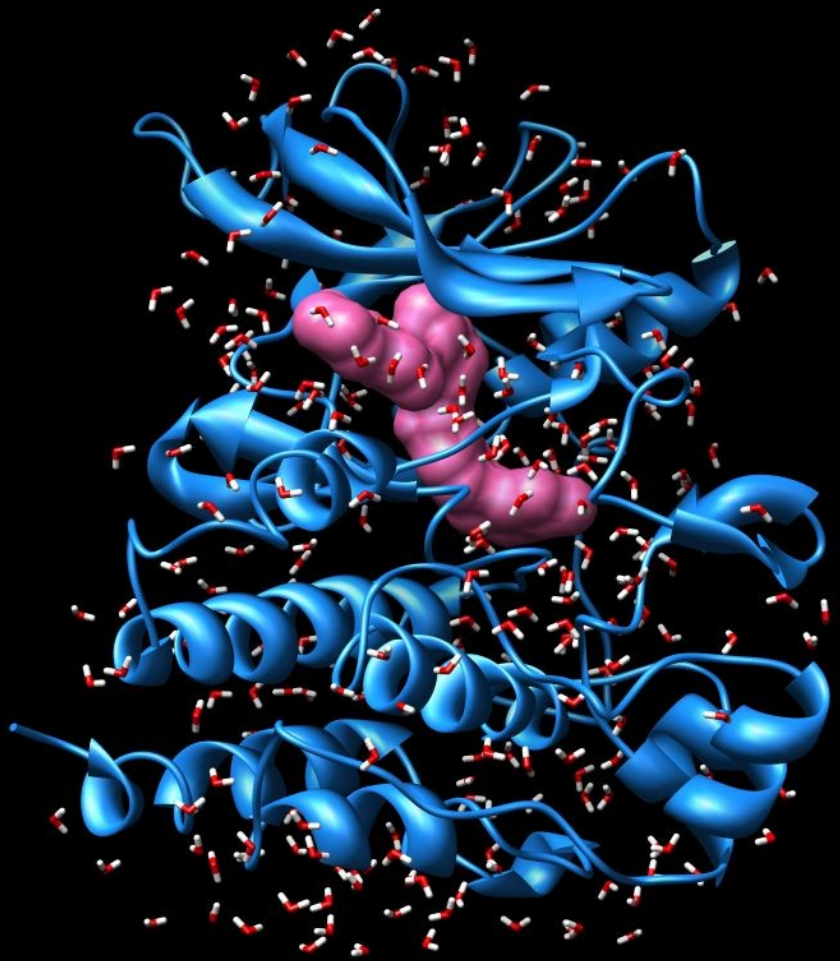
3. Biochemical analyses of phopshorylation (activation) status of KIT receptor.





E. Tamborini et al., Oncogene, 2006

Molecular modeling of KIT carrying T670I



KIT Exon 11 mutation (delta 559)

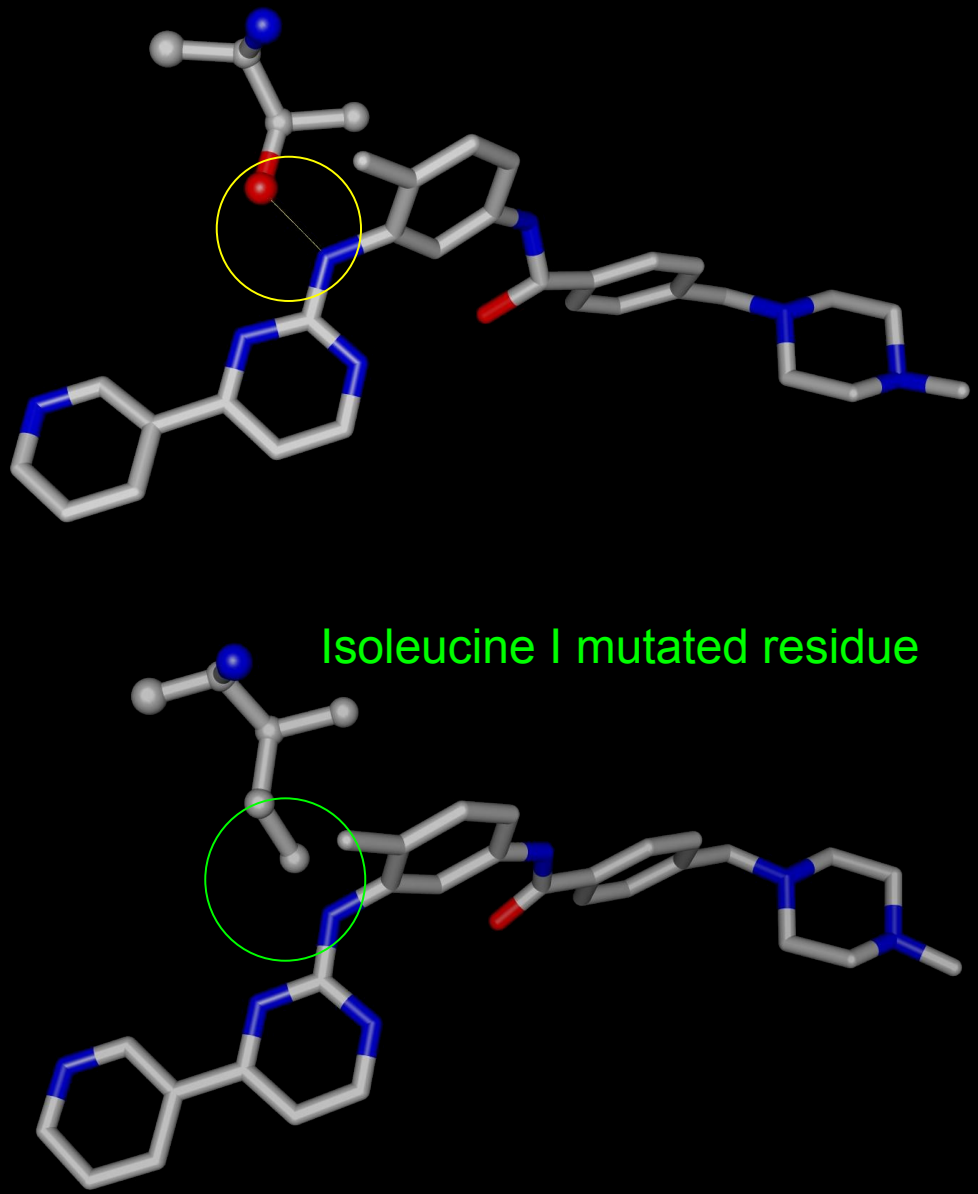
KIT Exon 11 mutation (delta 559) + T670I

E. Tamborini et al., Oncogene, 2006

T670I

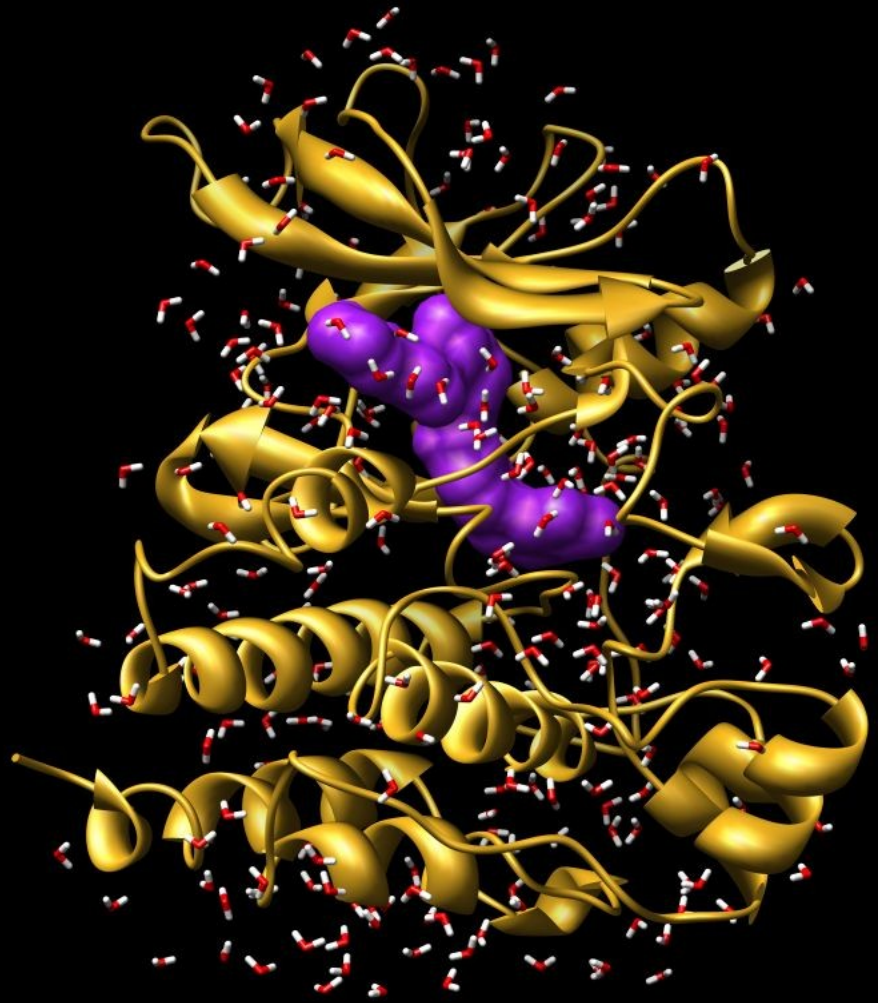
In a sort of a domino effect:

the topical stabilizing H-bond between aminopyridine nitrogen of Imatinib and the side chain Og1 atom of the gatekeeper residue T no longer exists

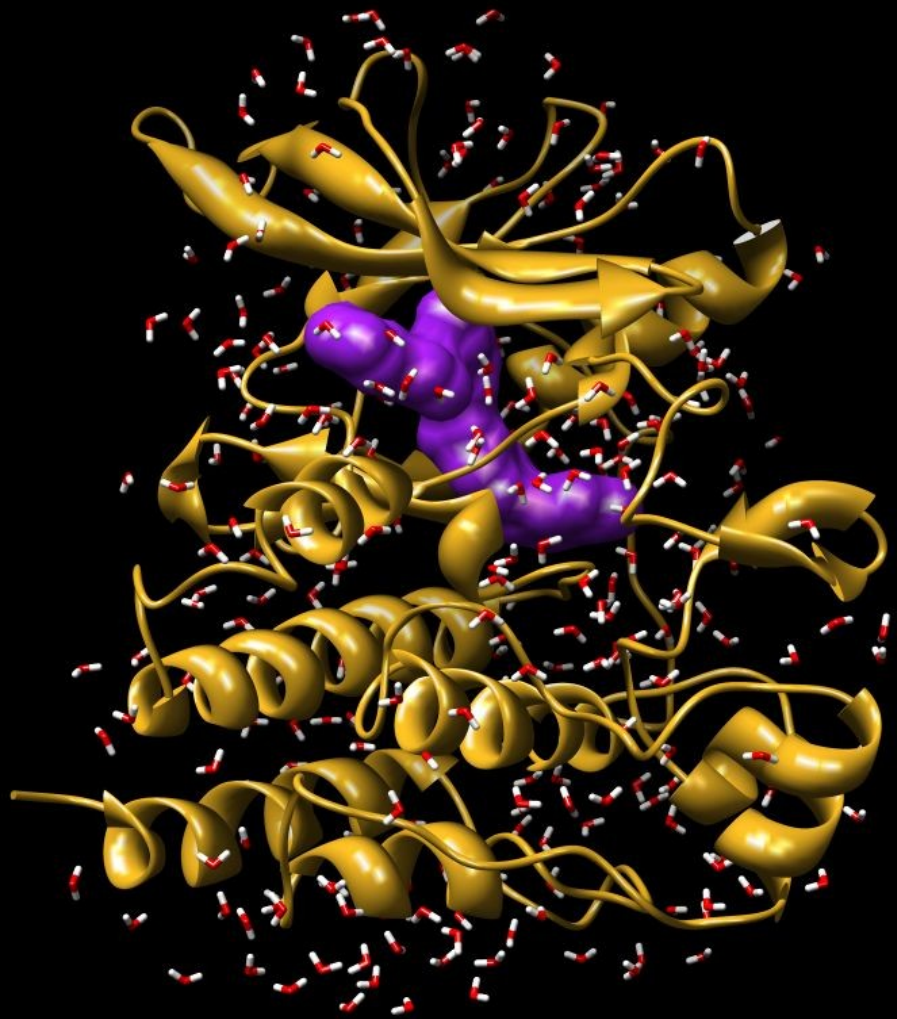


E. Tamborini et al., Oncogene, 2006

Molecular modeling of KIT carrying V654A



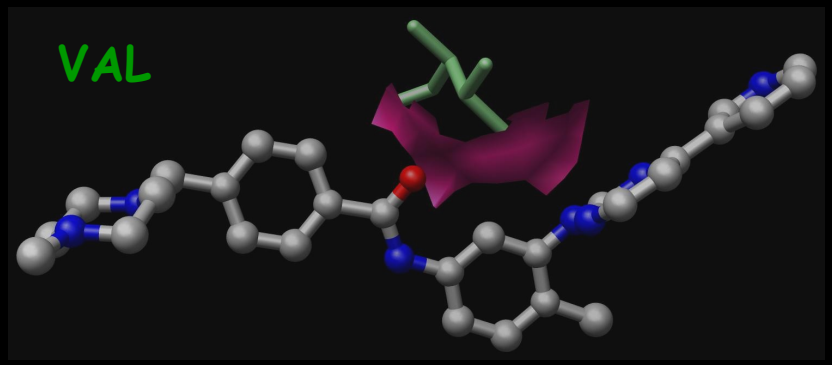
KIT Exon 11 mutation (delta 559)

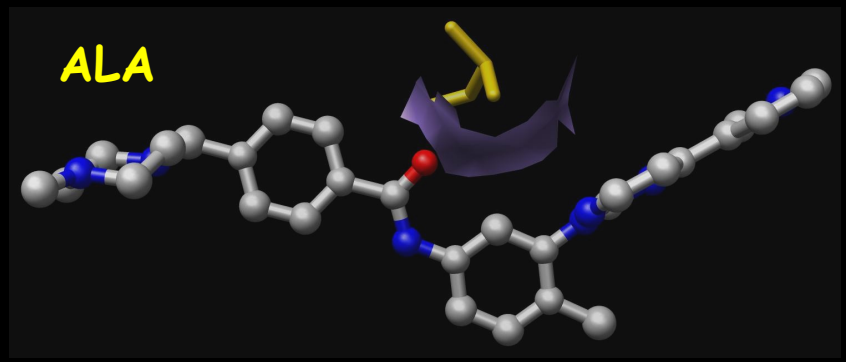


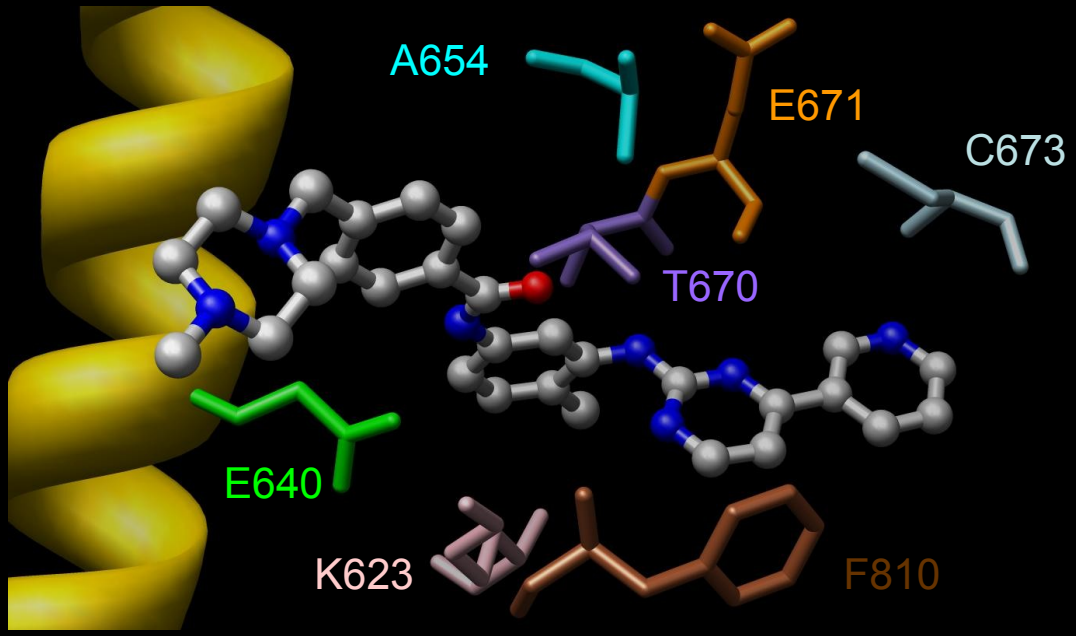
KIT Exon 11 mutation (delta 559) + V654A

E. Tamborini et al., Oncogene, 2006

Molecular modeling of KIT carrying V654A







E. Tamborini et al., Oncogene, 2006

In conclusion:

Two types of KIT mutations have been defined associated with Imatinib Secondary/Acquired resistance:

Type I (T670I)
which profoundly affects ATP pocket structure rendering fully ineffective Imatinib.

Type II (V654A)
Which decreases Imatinib affinity for ATP binding pocket. A responsiveness is detectable increasing the dose.

Molecular modeling has provided the structural bases of the biological results and has suggested different therapeutic modalities.

Experimental Molelcular Pathology

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