# TYROSINE KINASE INHIBITORS (TKIs) – Challenges for anticancer therapy and regulatory perspectives

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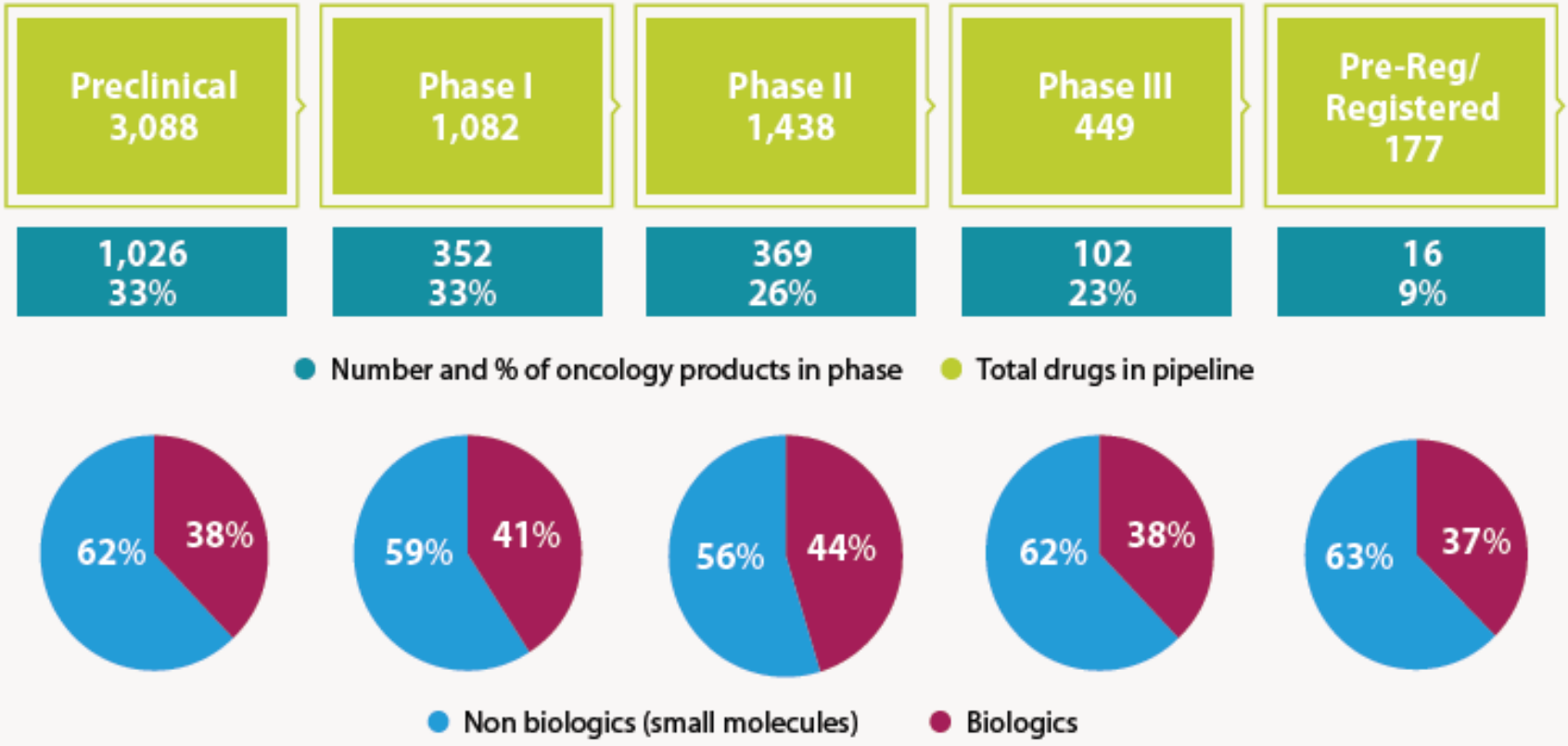
**Bioequivalence, Dissolution, Biosimilarity**
**From the "Chain Bridge" to other Bridges of the Pharmaceutical World**
**25 April, 2015, Antalya, Türkiye**

# Outline of the presentation

- Introduction
  - Protein Kinases (PKs)
  - Protein Tyrosine Kinases (PTKs)
  - Receptor Tyrosine Kinases (RTKs)
  - Targeted therapy
- Protein tyrosine kinase inhibitors (PTKIs)
- FDA *v.s.* EMA approvals of PTKIs
- Some research project results
- Conclusions

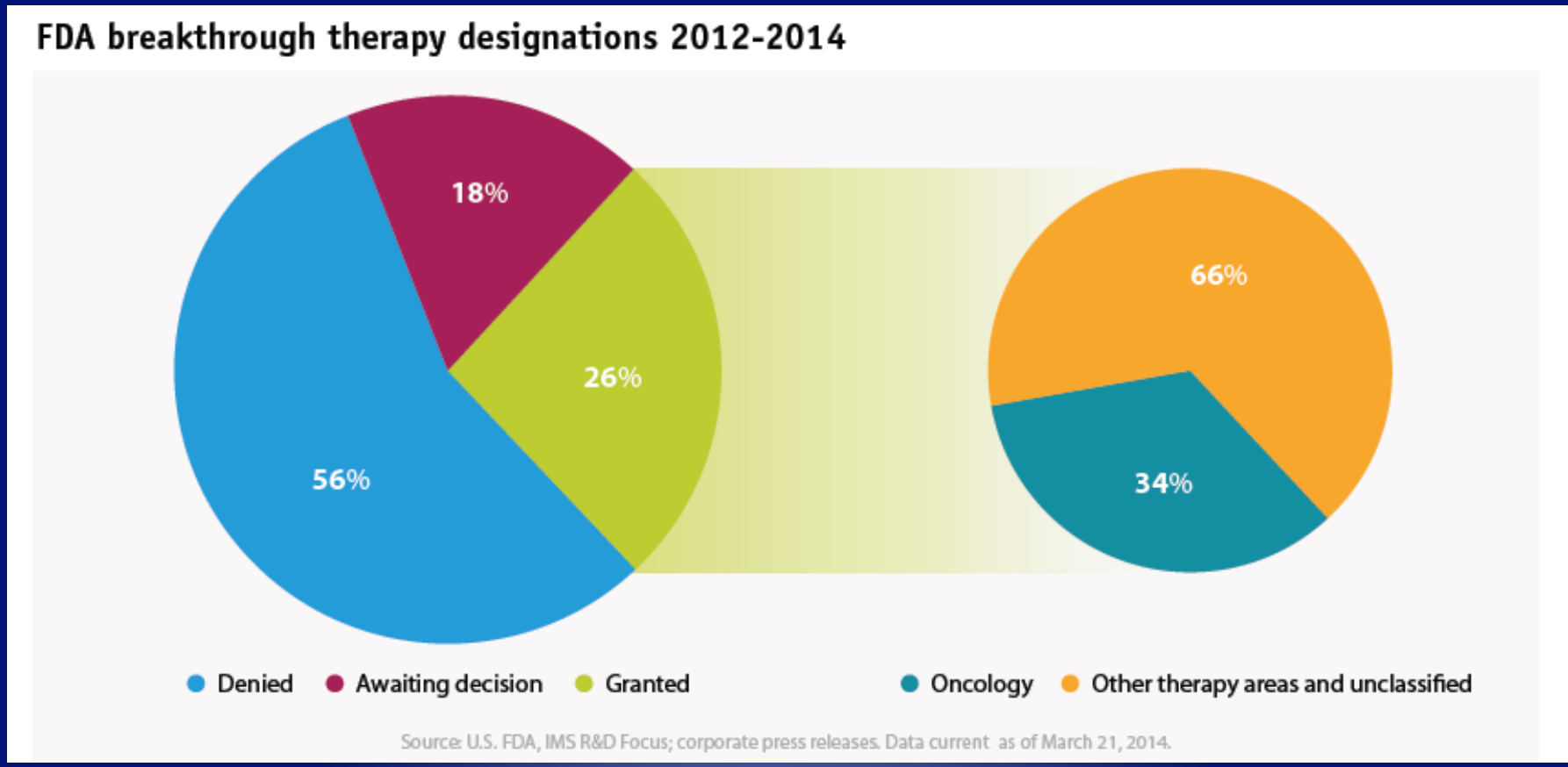
# Oncology is the largest area of focus in R&D with almost 2000 products in the pipeline

**Number of active products in the pipeline to date = 6,234**




Source: IMS Institute for Healthcare Informatics, Feb 2014

# Manufacturers seek accelerated approvals under regulatory provisions to reduce time-to-market

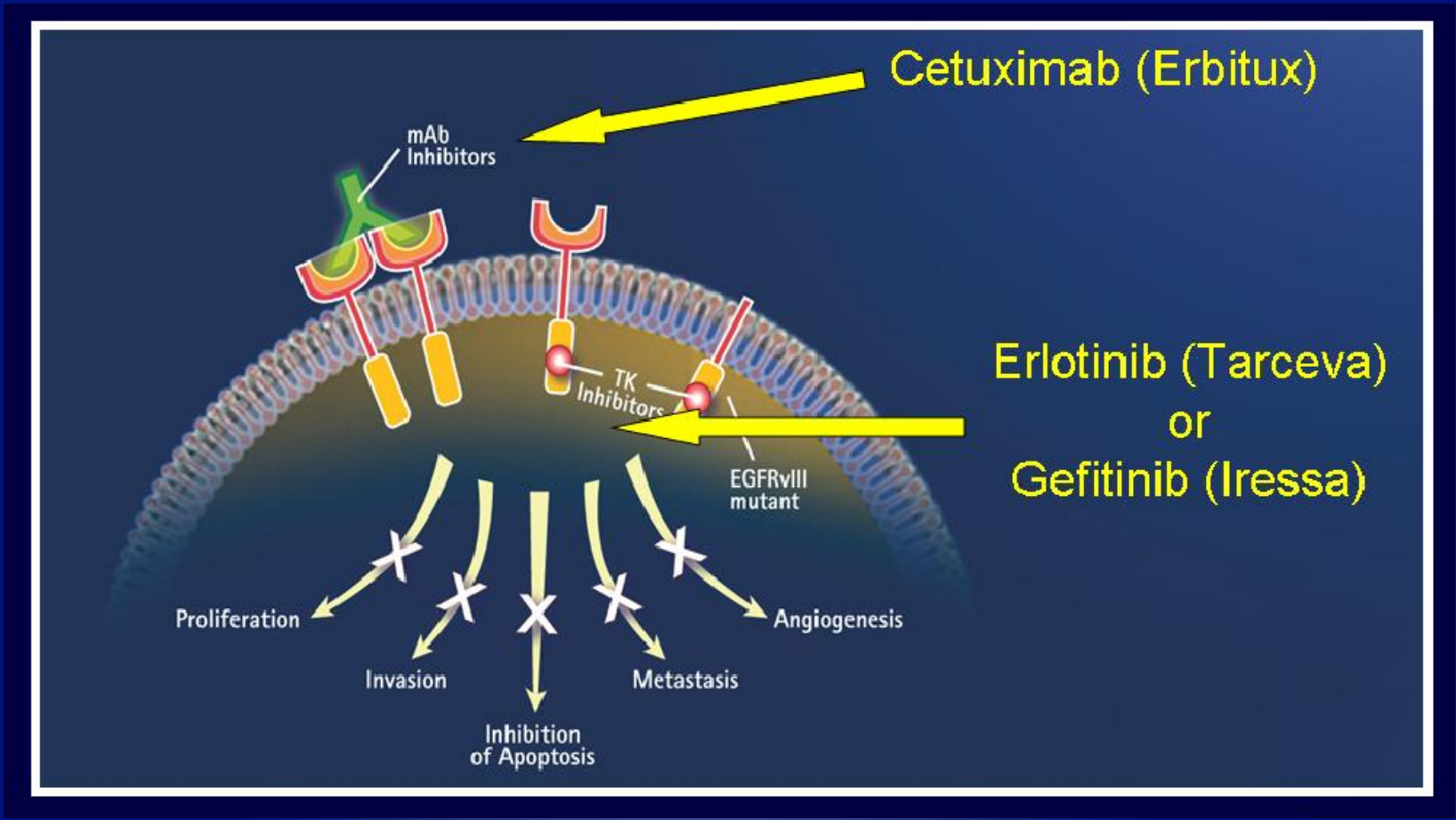



http://www.imshealth.com

# CURRENT DEVELOPMENT OF TARGETED THERAPY IN ONCOLOGY

- The development is particularly active and concerns principally in two types of agents which are monoclonal antibodies (MABs) and tyrosine kinase inhibitors (TKIs).
- Epidermal growth factor receptor (EGFR) signaling pathways play a key role in the regulation of cell proliferation, survival and differentiation.
- Consequently, EGFR is one of the most-studied ligand–receptor systems and specific EGFR inhibition approaches are currently among the most promising and the most advanced in the clinical setting.

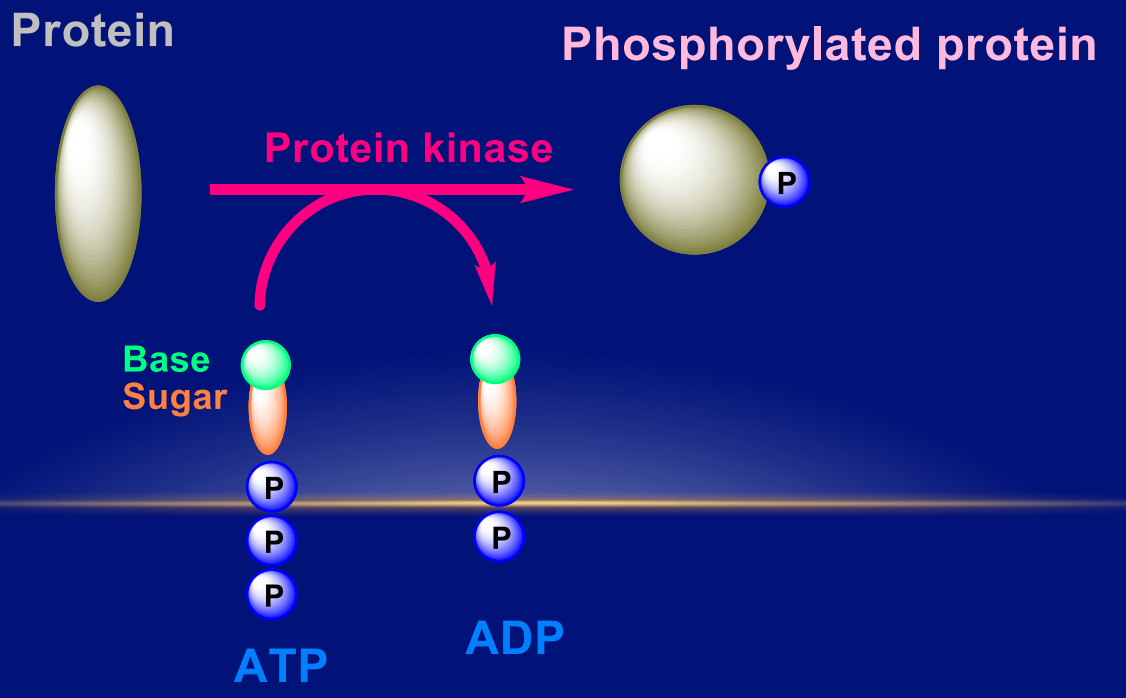
# Common Approaches for inhibiting the Epidermal Growth Factor (EGFR) Axis




Cetuximab, belonging to the MABs family, gefitinib and erlotinib, and other inhibitors belonging to the TKIs family, are among the most advanced anti-EGFR drugs at the clinical level.

# PROTEIN KINASES (PK)

- PKs are enzymes involved in phosphorylation and transfer of a phosphate group from adenosine-3-phosphates (ATP) to tyrosine, serine or threonine residues.
- Protein phosphorylation is one of the most important events in regulating cell activities.
- Some oncoproteins need phosphorylation for regulation and activation.

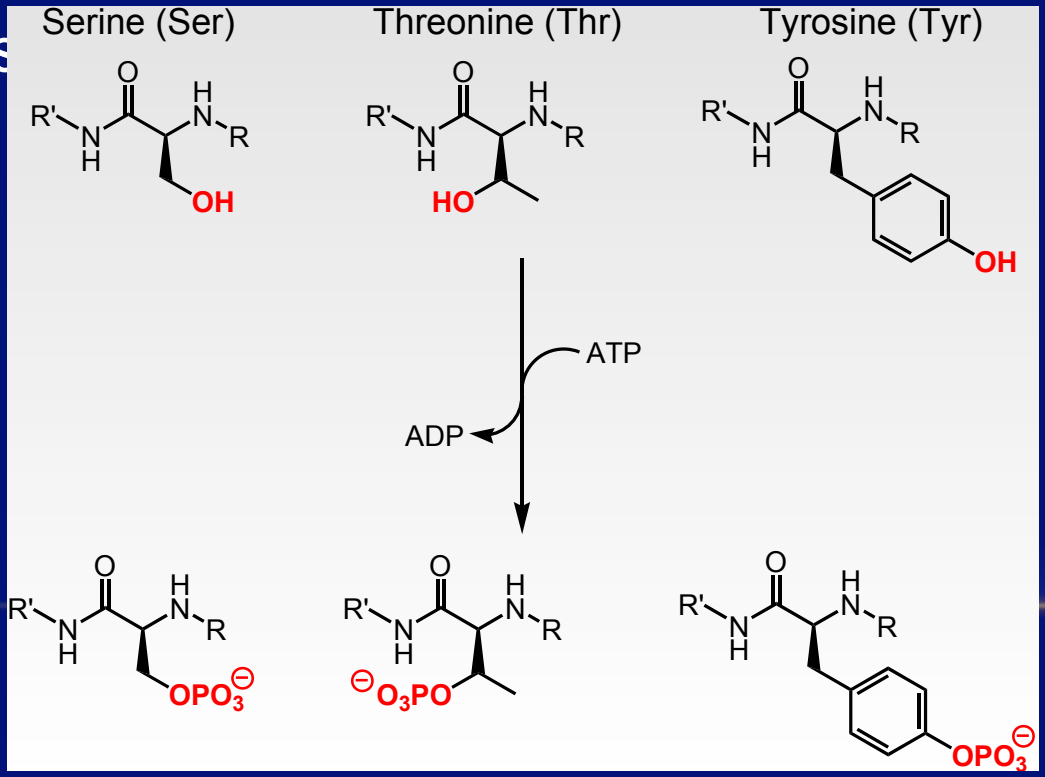


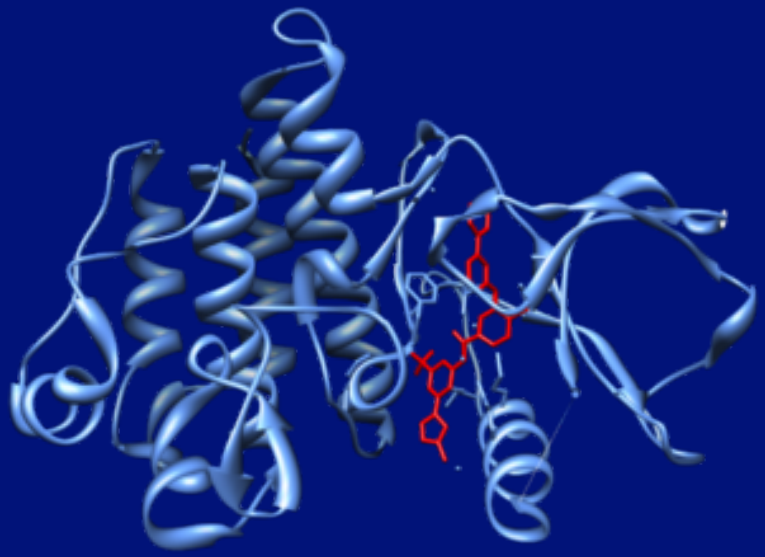
# CATEGORIES OF PROTEIN KINASES

① Kinases that specifically phosphorylate tyrosine residues

② Kinases that phosphorylate serine and threonine residues

③ Kinas

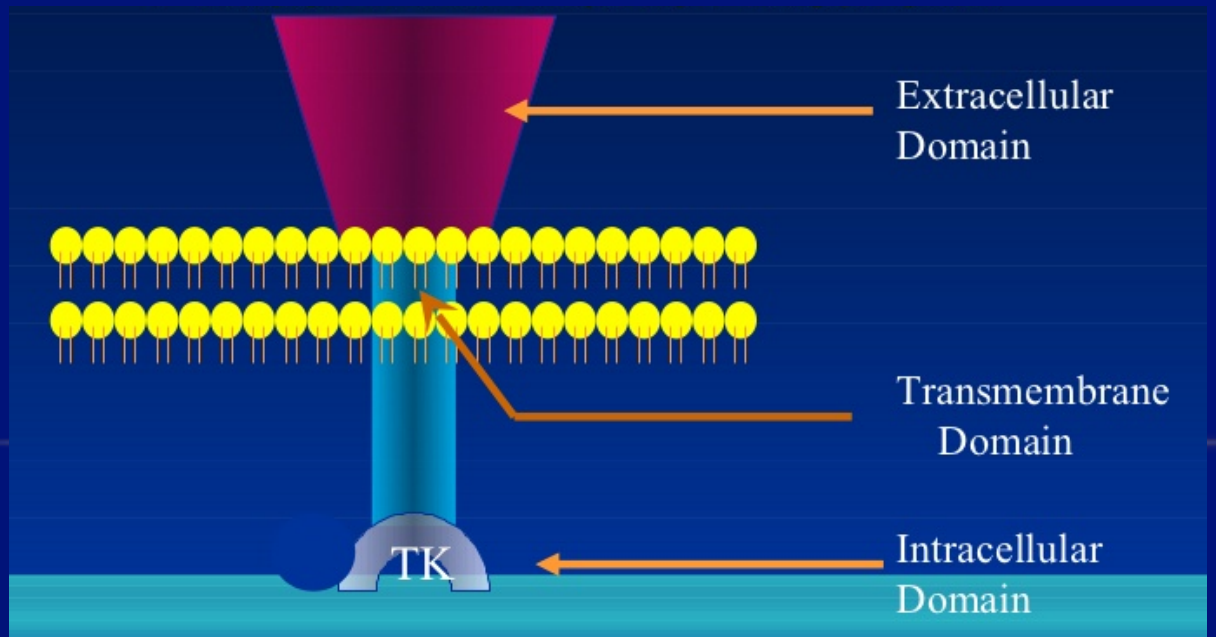




# PROTEIN TYROSINE KINASES (PTKIs)

# TYROSINE KINASE STRUCTURE

- RTKs' structure consists of three different parts: extracellular, transmembrane and intacellular or cytoplasmic regions (domains).
- The extracellular part is preceded by a cleavable signal sequence and holds the binding sites that interact with ligands.
- The extracellular domain is involved in the dimerization of RTKs, a process that is critical for the activation of intrinsic tyrosine kinase (TK) activity.



# TYROSINE KINASE (TK)

- Enzyme that can transfer a phosphate group from ATP to a protein in a cell.
- It functions as an “on” or “off” switch in many cellular functions.
- The phosphate group is attached to the amino acid tyrosine on the protein.

TK types

- **Receptor tyrosine kinases**, eg. EGFR, PDGFR, FGFR
- **Non-receptor tyrosine kinases,** eg. SRC, ABL, FAK and Janus kinase

# RECEPTOR TYROSINE KINASES (RTKs)

- Among PKs, the RTKs comprise a well-known group and consist of a transmembrane receptor linked to the intracellular kinase domain.
- These proteins have emerged as key pharmacological targets in oncology.
- Phosphorylation of other RTKs, as well as intracellular intermediates by these kinases, is critical for signal transduction, regulation of cellular activity and function.
- Among 58 known RTKs, 30 of them have been shown to be necessary for oncogenesis in various tumors.

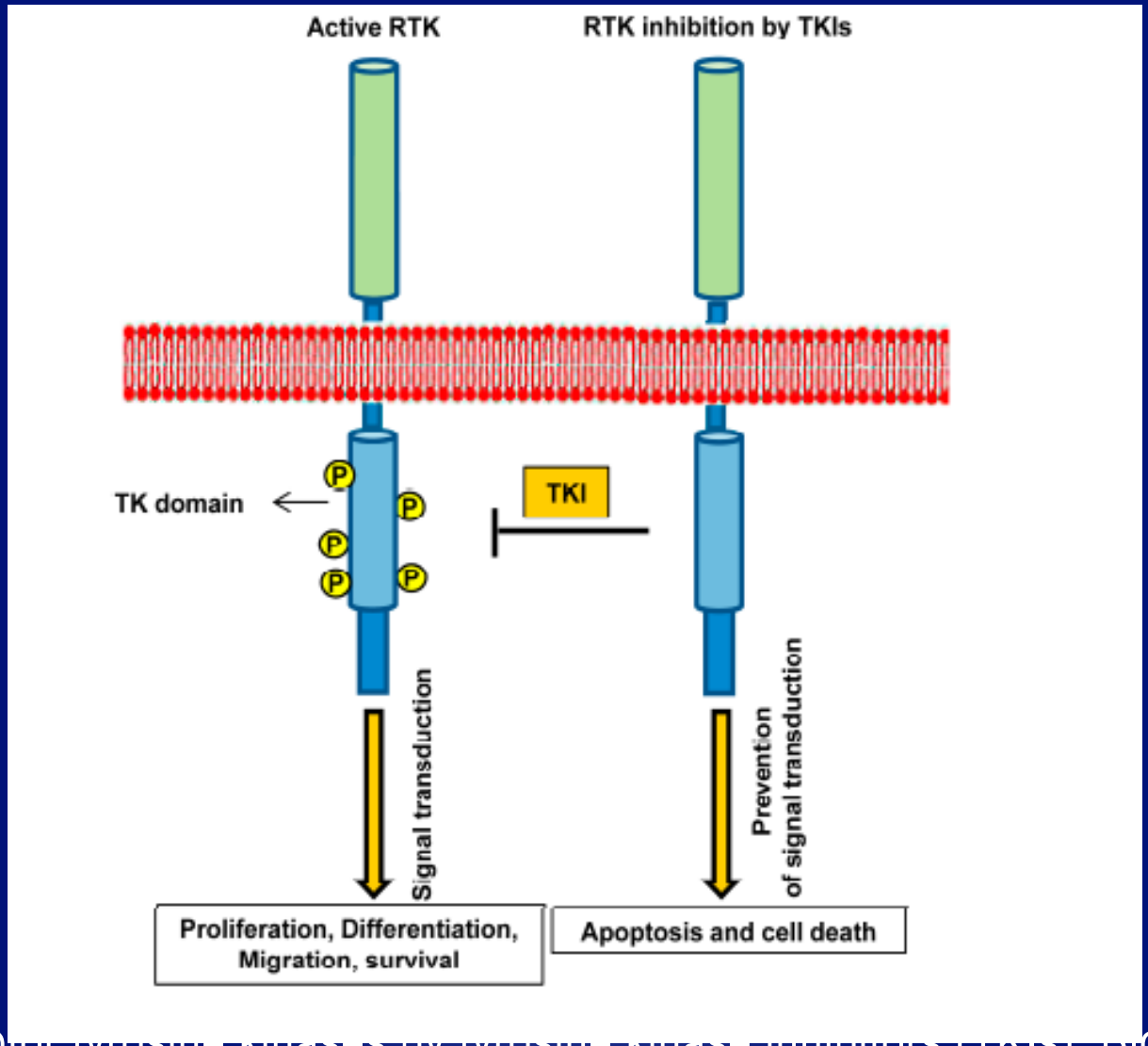
- The cytoplasmic region contains tyrosine residues that are phosphorylated upon ligand binding and activation, regulate catalytic function, and also serve as docking sites for SRC Homology 2 (SH2) domain-containing proteins.

- Deregulation of RTK activity is the major mechanism by which tumor cells escape from physiological constraints on survival and growth.

- Therefore, due to the interesting biological features, RTKs are of the main focus for developing new TKIs for therapeutic interventions in cancer patients.

# TYROSINE KINASE INHIBITORS (TKIs)

- TKIs, as well as other small inhibitors, are low molecular weight organic compounds.
- A cut off at 500 Daltons is recommended based on the observation that clinical attrition rates are significantly reduced when the molecular weight falls below 500 Daltons.
- The upper molecular weight is approximately 900 Daltons.




Targeting receptor tyrosine kinases by tyrosine kinase inhibitors (TKIs). Blocking small molecule inhibitors of kinase domain (TKIs) prevents the phosphorylation of the receptor at TK domain and interferes with cell proliferation, differentiation, migration, and survival and induces cell apoptosis. Phosphate groups are denoted as yellow circles. (*In J Mol Struc* 15, 2014, p.p. 13768 - 13801).

# TYROSINE KINASE INHIBITORS' (TKIs) CLASSIFICATION

- **BCR-ABL TKIs, eg. imatinib mesylate, dasatinib and nilotinib**
  - They bind to a segment of the kinase domain that fixes the enzyme in a closed or nonfunctional site in which the protein is unable to bind its supstrate/ phosphate donor, ATP.
- **Epidermal Growth Factor Receptor TKIs, e.g. gefitinob, lapatinib**
  - They inhibit the EGFR tyrosine kinase by virtue of competitive blockade of ATP binding (By blokade of downstream EGFR signal transduction pathways, cell cycle arest and inhibition of angiogenesis)
- **Vascualar Endothelial Growth Factor TKIs, eg. vatalanib, sunitinib, sorafenib**
  - Inhibition of multiple receptor tyrosine kinases, some of which are implicated in tumor growth, pathological angiogenesis and methasthatic progression of cancer
  - Competitive inhibit the binding of ATP to tyrosine kinase domain on the VEGF receptors.

# THREE MAIN GROUPS/TYPES OF PTKIs

- **Type I) ATP-competitive inhibitors** - Most of the current TKIs are classified as type I inhibitors.
  - Due to the highly conservative ATP-binding sites in TK domains and a high rate of competition with intracellular ATP, several difficulties obstruct the development of specific/selective TKIs of type I.

- **Types II) and TypeIII)** - non-ATP competitors and act through induction of structural changes in the RTKs.
  - The conformational shifts modify the TK domain in a way that the TK domain loses its kinase activity.
  - Moreover, these inhibitors can bind to residues within the TK domain and prevent tyrosine phosphorylation.

# SMALL MOLECULE INHIBITORS

## 'NIBs'

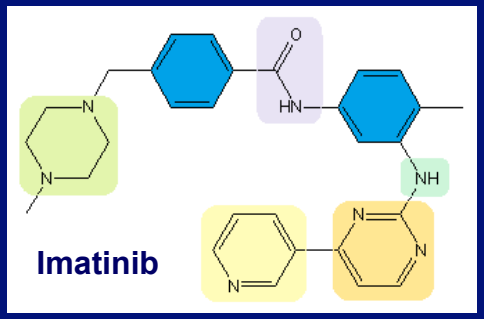
Different carboxamides:

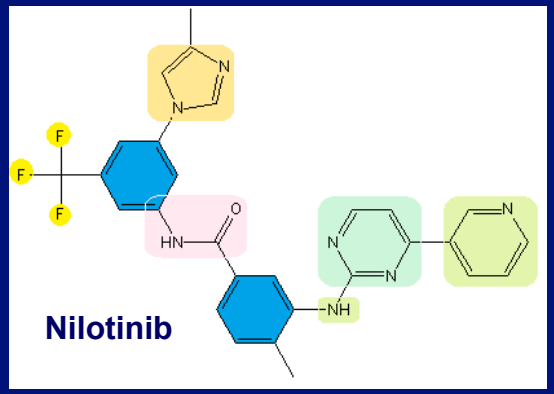
a) 2,4-dimethyl-1H-pyrrole-3-carboxamide

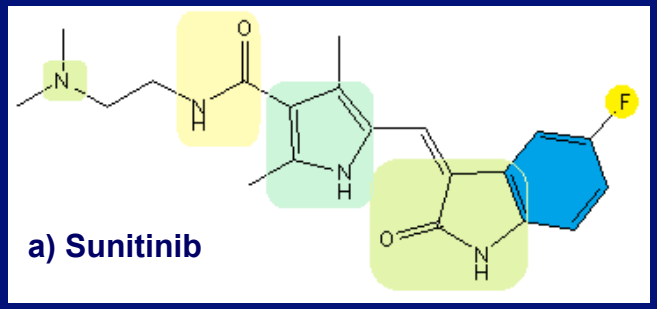
b) *N*-methyl-pyridine-2-carboxamide

c) 5-thiazole carboxamide

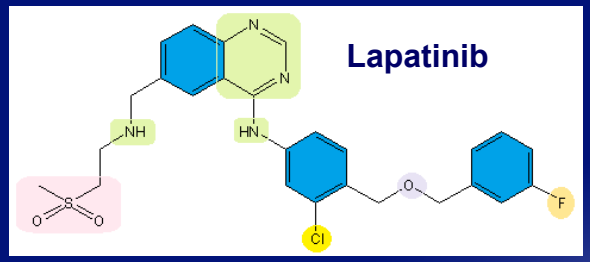
### Derivatives of 4-aminobenzamides

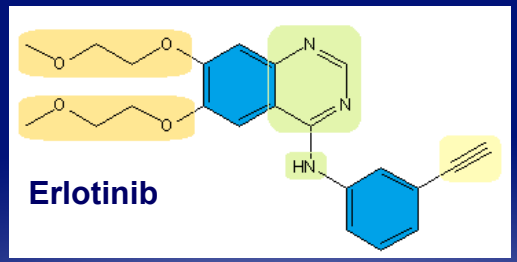


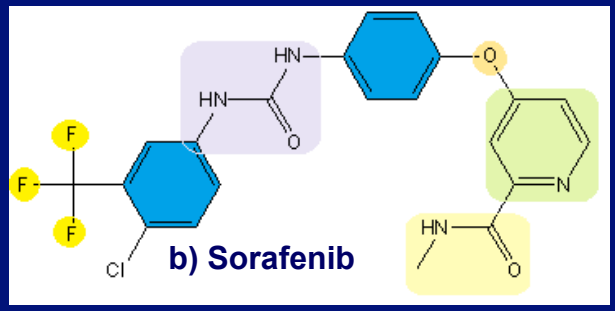



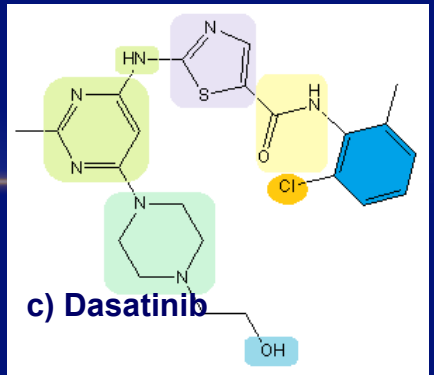




### Derivatives of quinazolin-4-amine



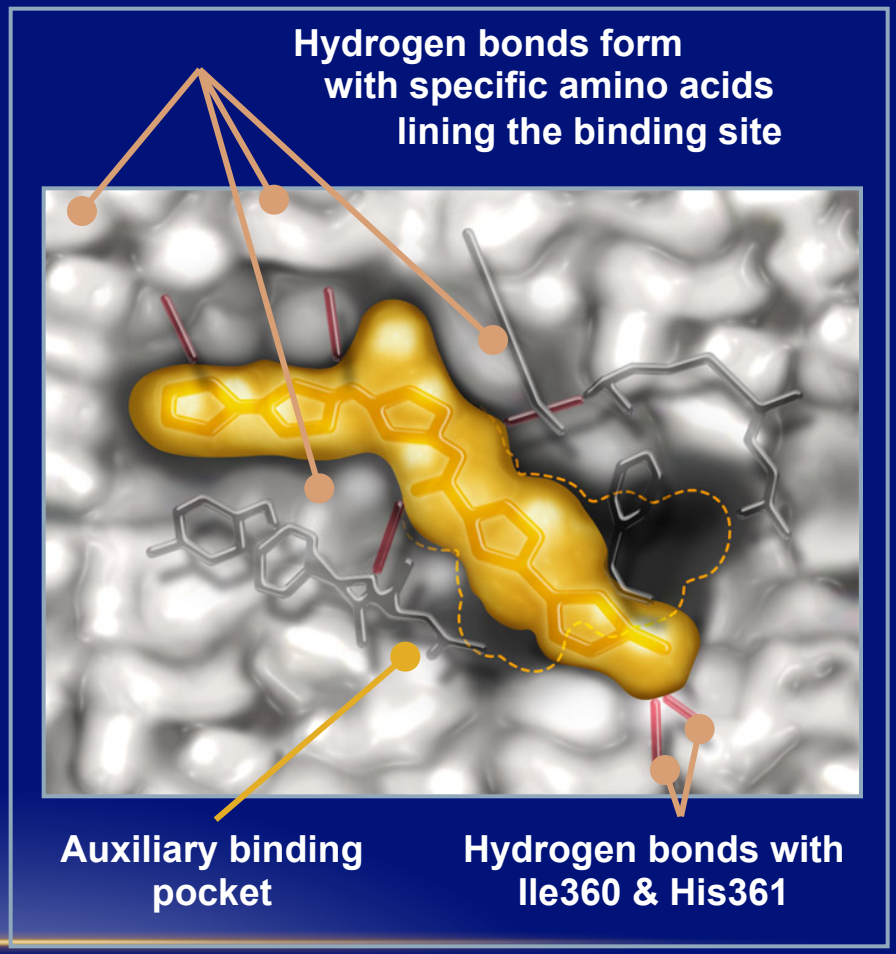



# Imatinib: First targeted therapy for cancer

- Imatinib mesylate is a first molecular targeted PTKI received the FDA approval (May 2001).
- It targets the BCR-ABL tyrosine kinase which underlines chronic myelogenous leukemia (CLM) and present in virtually all patients with CLM.
- It inhibits the binding of adenosine triphosphate (ATP) and thus blocks the downstream BCR-ABL signaling pathway.

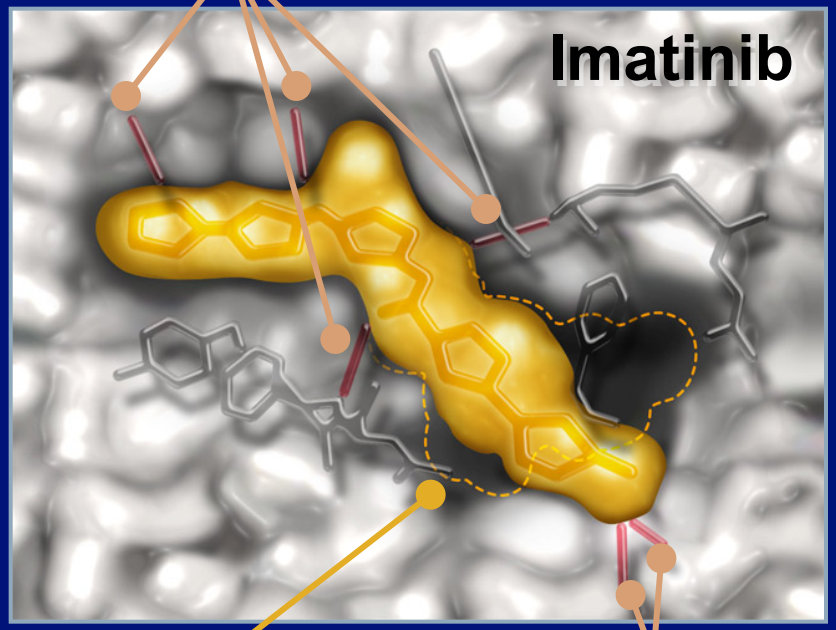

Weisberg E, et al. *Cancer Cell.* 2005;7:129-141.

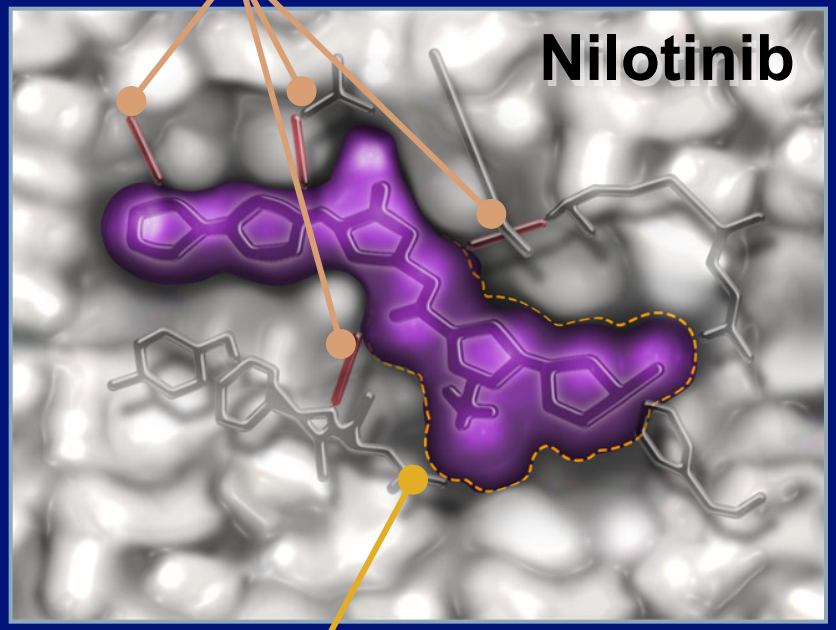
ABL

oncoprotein (a protein product of the fused BCR-ABLI oncogene produced by the Philadelphia chromosome, which plays a key role in initiating and maintaining leukaemia)

Hydrogen bonds form with specific amino acids lining the binding site

Only maintains 4 hydrogen bonds




Auxiliary binding pocket

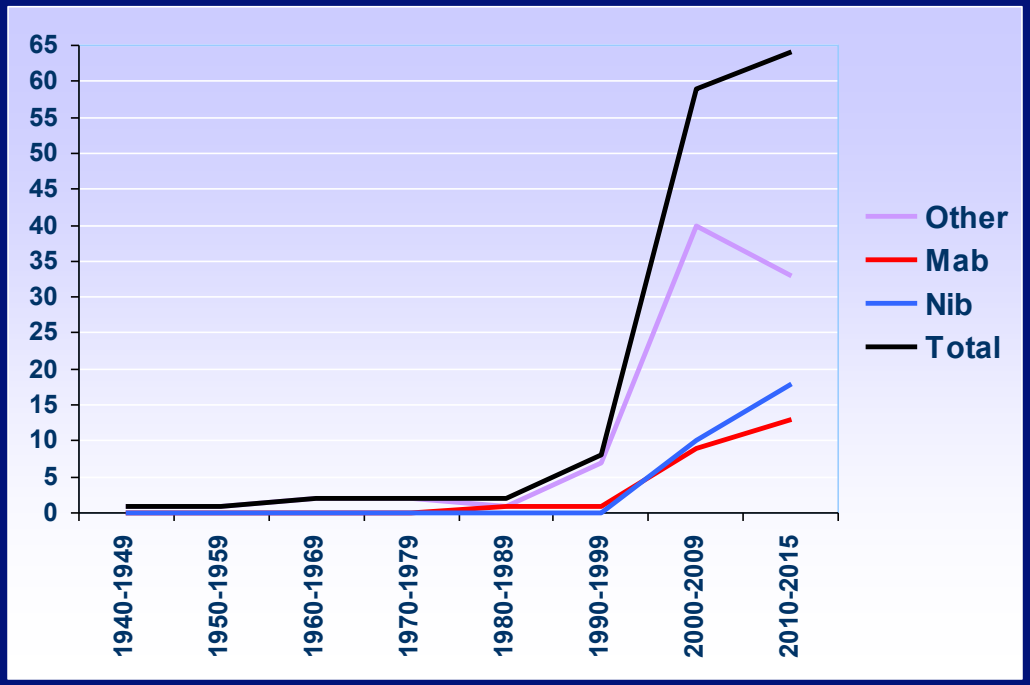
Hydrogen bonds with Ile360 & His361

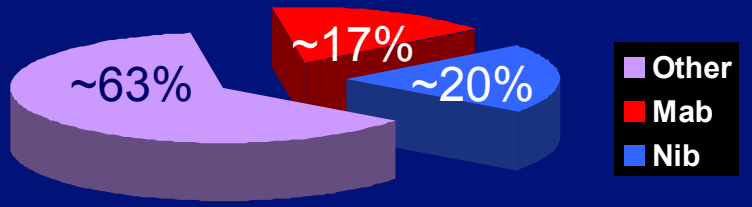
Improved fit to auxiliary pocket, via lipophilic interactions, making it less susceptible to point mutations

Weisberg E, et al. *Cancer Cell.* 2005;7:129-141.

# FDA *v.s.* EMA Approvals in Oncology

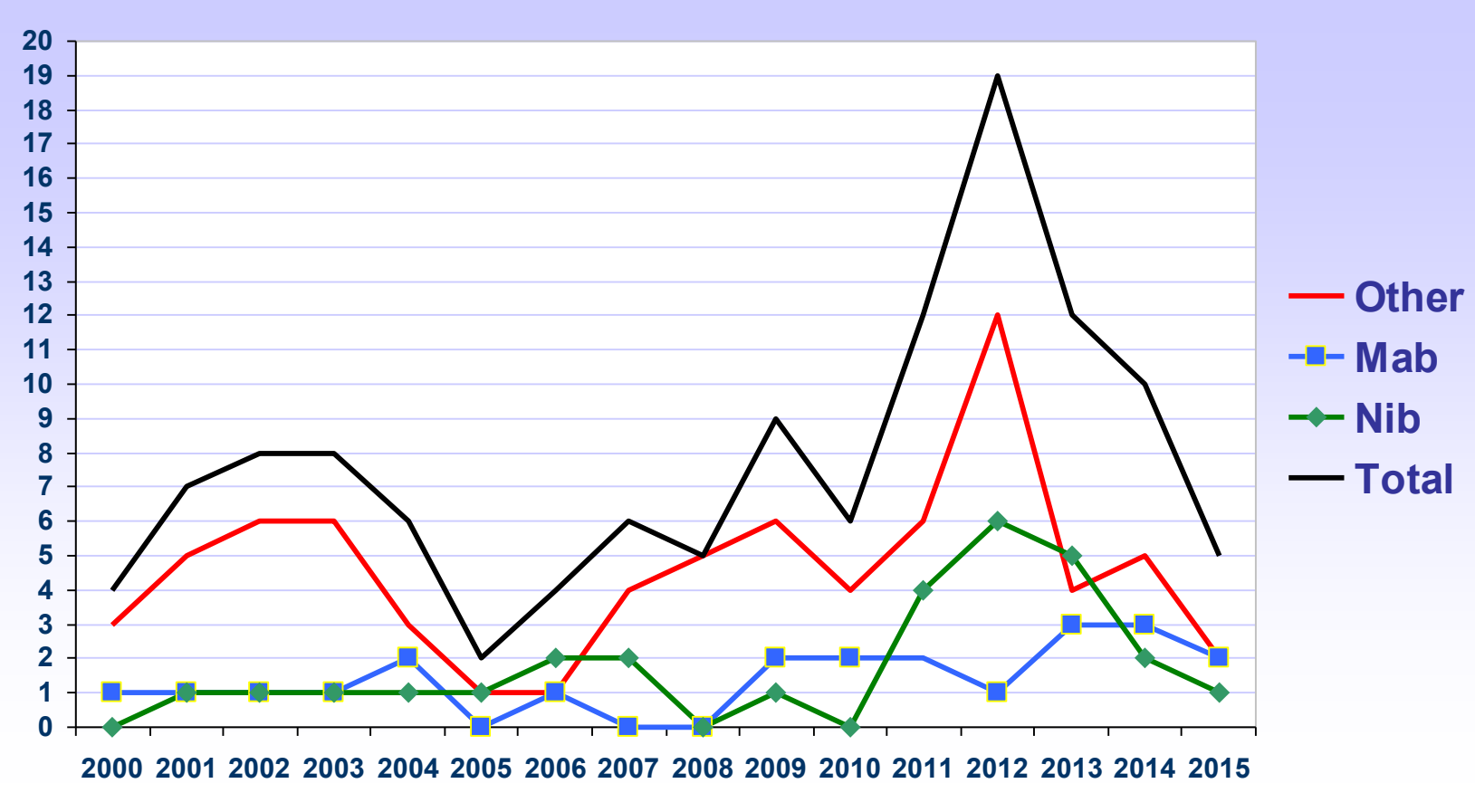
# Cronology of FDA approved antitumor drugs in humans per decades (1949 -2015)




http://www.centerwatch.com/drug-information/fda-approved-drugs/therapeutic-area/12/oncology

# FDA approvals of antitumor drugs in humans for the period 2000-2015



# FDA

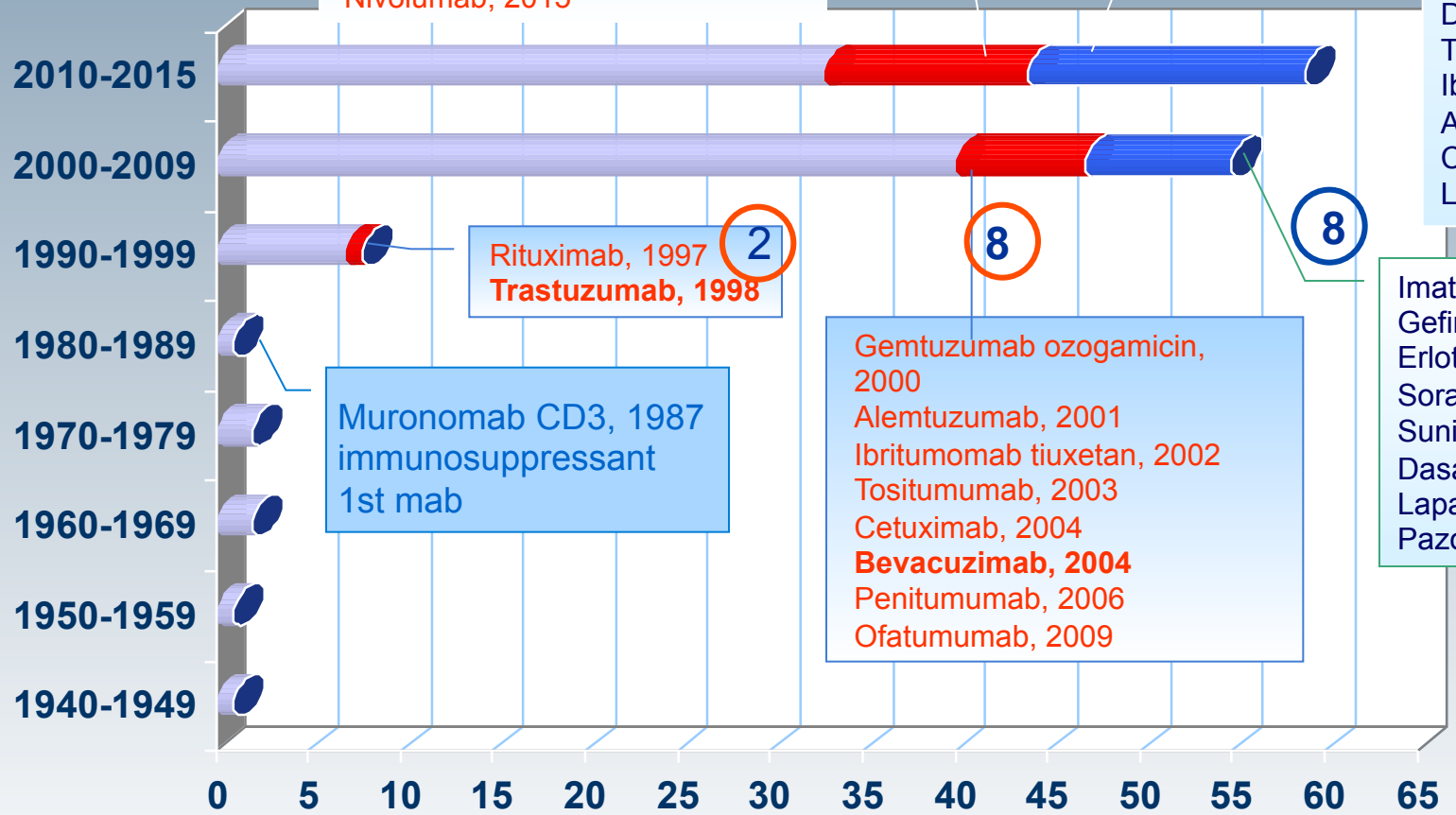
44 Targeted drugs in total: 21 MAB and 23 NIB

**Denosumab, 2010**
Ipilimumab, 2011
Brentuximab vedotin, 2011
Pertuzumab, 2012
Ado-trastuzumab emtansine, 2013
Obinutuzumab, 2013
Pembrolizumab, 2014
Blinatumomab, 2014
Ramucirumab, 2014
Dinutuximab, 2015
Nivolumab, 2015

11

15

Vemurafenib, 2011
Crizotinib, 2011
Vandertanib, 2011
Sunitinib maleate, 2011
Regorafenib,2012
Axotinib, 2012
Ponatinib, 2012
Cabozantinib, 2012
Bosutinib, 2012
Dabrafenib, 2013
Trametinib, 2013
Ibrutinib, 2013
Afatinib, 2013
Ceritinib, 2014
Lenvatinib, 2015




Rituximab, 1997
**Trastuzumab, 1998**

2

8

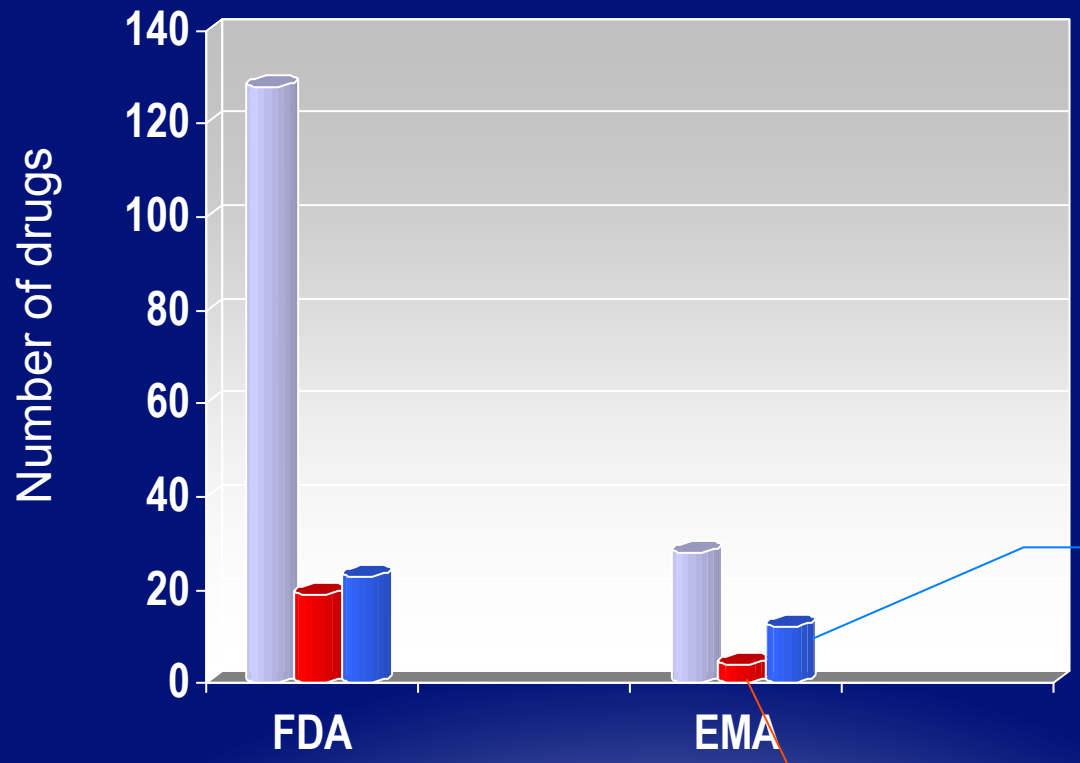
8

Muronomab CD3, 1987
immunosuppressant
1st mab

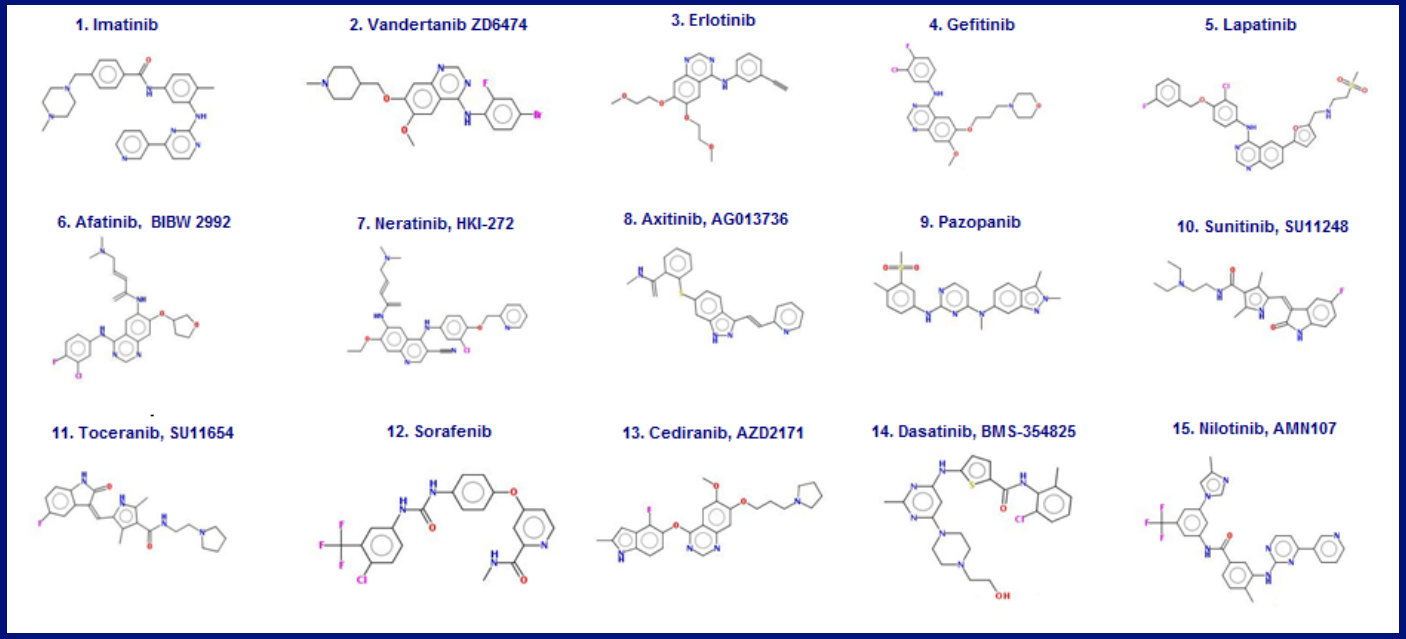
Gemtuzumab ozogamicin, 2000
Alemtuzumab, 2001
Ibritumomab tiuxetan, 2002
Tositumumab, 2003
Cetuximab, 2004
**Bevacuzimab, 2004**
Penitumumab, 2006
Ofatumumab, 2009

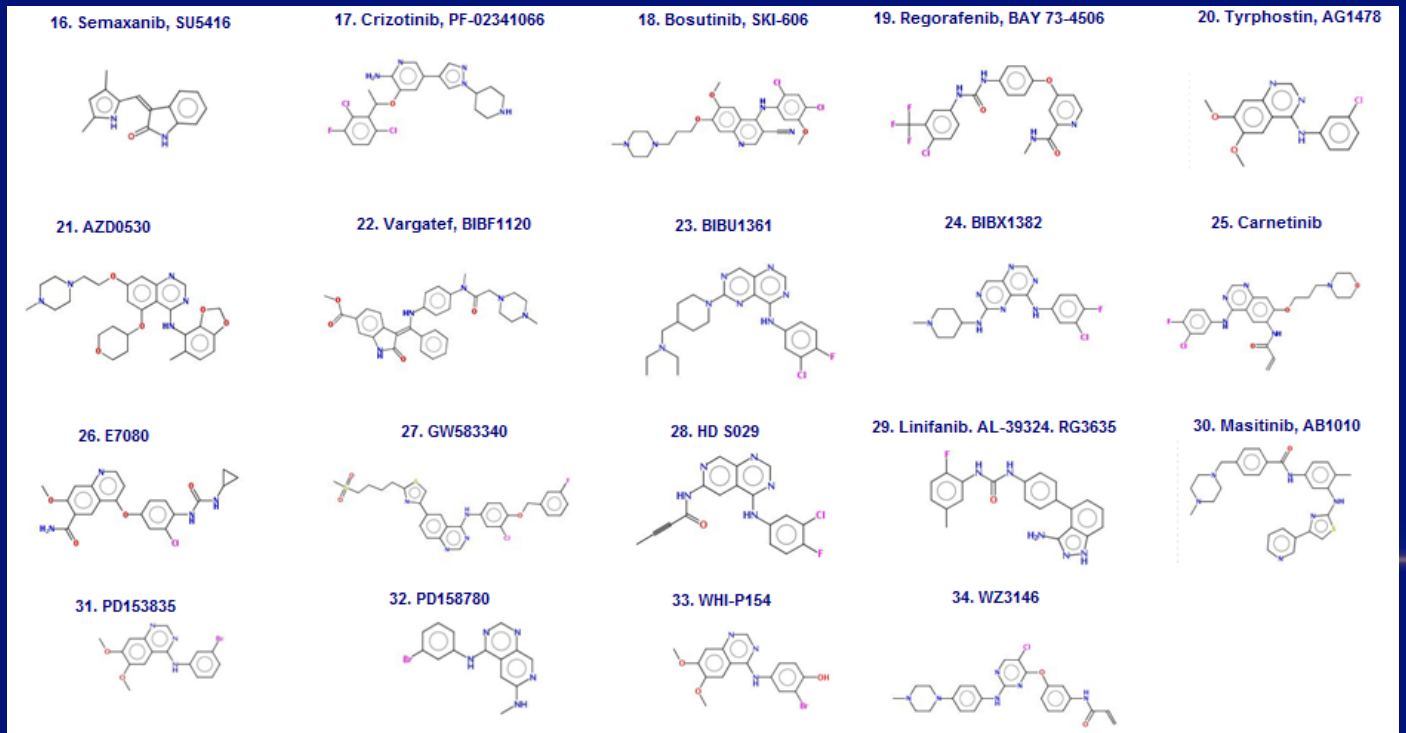
Imatinib mesilate, 2001
Gefinitib, 2003
Erlotinib (OSI774), 2004
Sorafenib, 2005
Sunitinib, 2006
Dasatinib, 2006
Lapatinib, 2007
Pazopanib, 2009

# FDA and EMA profile of approved drugs for oncology (1995-2015)



# RESEARCH PROJECT RESULTS




**Quinazolin-4-amine** (2, 3, 4, 5, 6, 13, 20, 21, 25,, 27, 31, 33)

**Carboxamide** (10, 11, 12, 14, 19)

**Benzamide** (1, 8, 15, 30)

**Quinoline** (7, 18)

**Indazole** (9, 29)

**Indol-2-one** (16, 22)

**Pyrimido[5.4-d]pyrimidin 4-amine** (23, 24)

**Pyrido [3.4-d]pyrimidin-4,6 diamine** (28, 32)

**Pyridin-2-amine** (17)

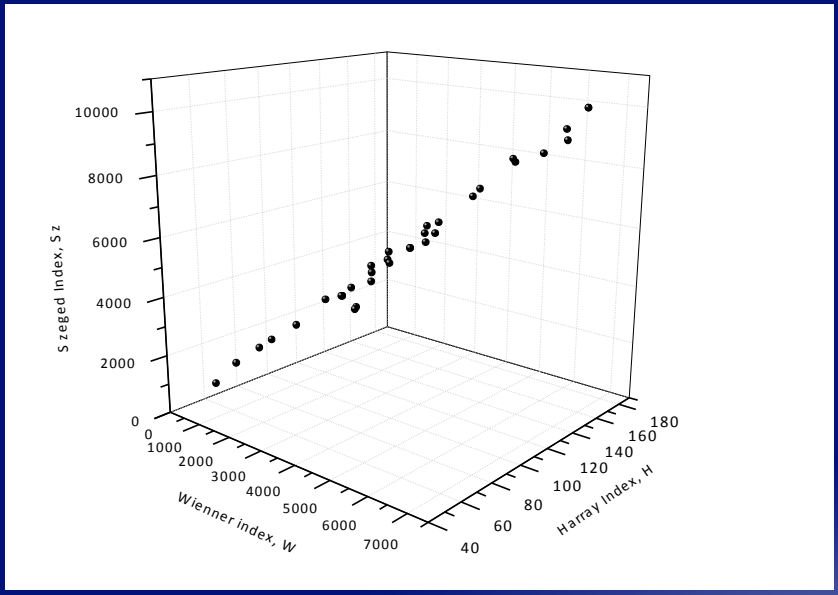
**Quinolin-4-carboxamide** (26)

**Acrylamide** (34)

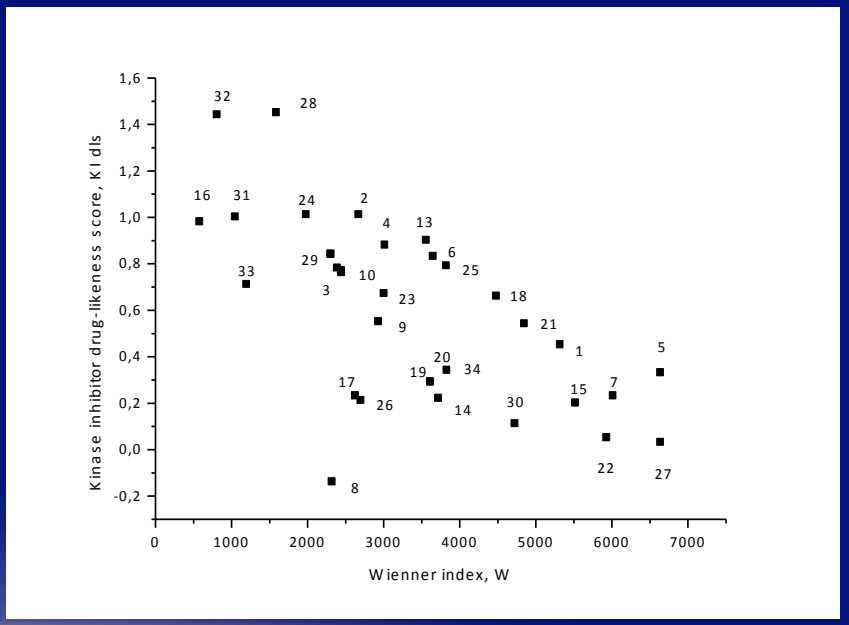
The relationship between calculated molecular descriptors (MDs), drug-likeness parameters and predicted ADMET properties were explored.

Study results showed significant collinearity between MDs: relative molecular mass, ($M_r$), volume ( $V$), $n_{atoms}$ and topological polar surface area (TPSA) and topological indices (TIs), *i.e.* Wiener index (W), Haray index (H), Randić connectivity index ($\chi 1$) and Szeged index (Sz) (r = 0.88691 - 0.98726).

The decrease of kinase-likeness scores (KI dls) was observed with increase of TIs values.

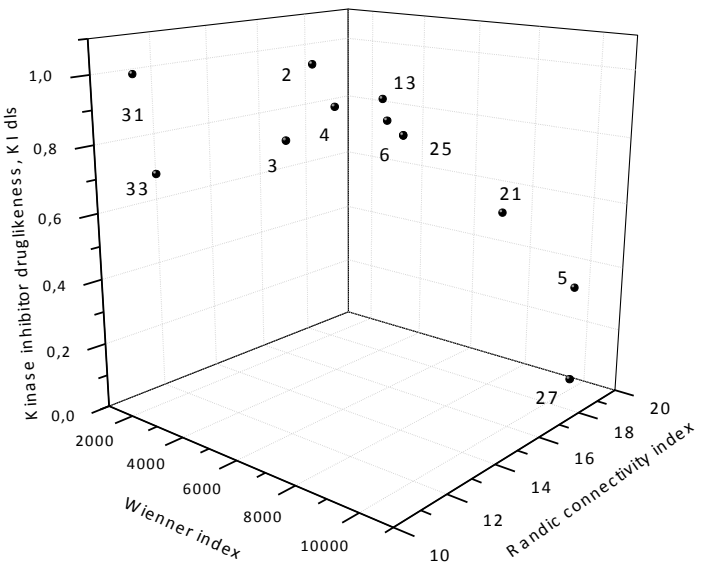


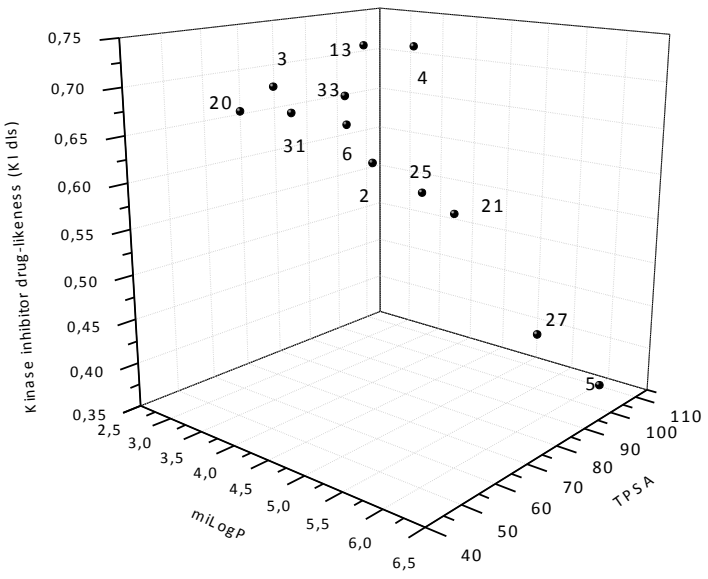

Correlations of Winner (W), Harray (H) and Szeged (Sz) indices of investigated inhibitors (n=34)




Correlations of kinase inhibitor drug-likeness scores (KI dls) and Wienner index (W) of investigated inhibitors (n=4)

The results in a subgroup of quinazoline-4-amino derivatives study revealed the optimal Log P between 3.5 - 4.5, TPSA < 60, *Mr* < 400, and topoligical indices W and X1 up to 2000 and 15, respectively.

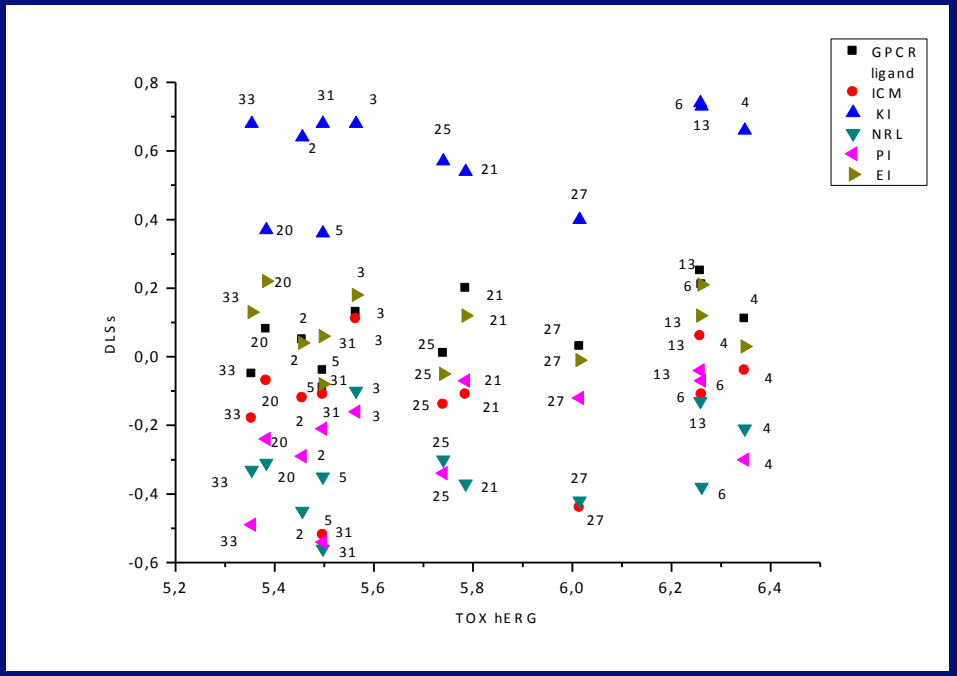


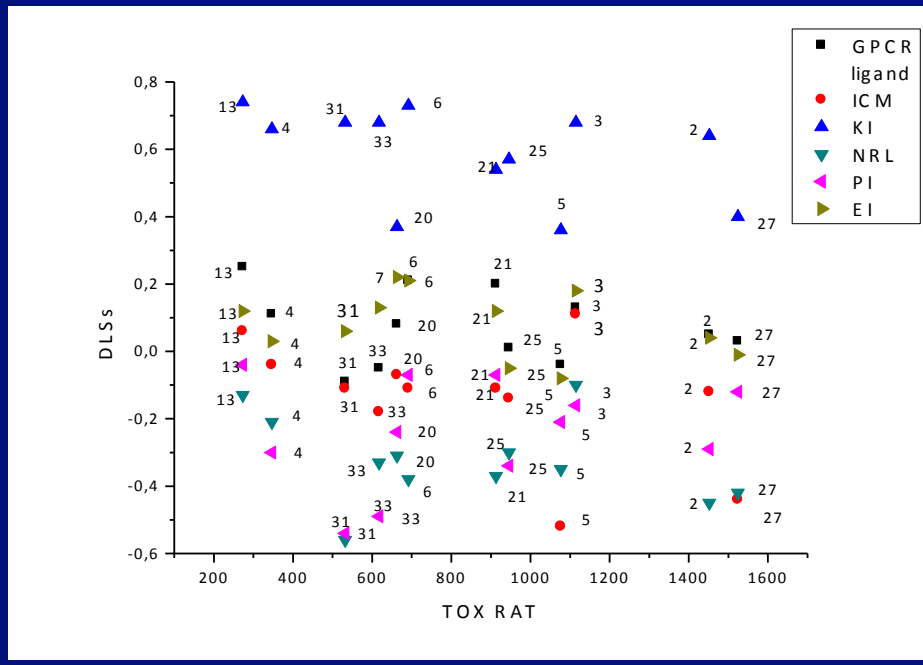

The relationship of kinase inhibitor likeness scores (KI dls) and topological indices, *i.e.,* Wienner index (W) and Randić index (X1) of quinazoline inhibitors.

The relationship between kinase inhibitor likeness scores (KI dls), miLogP and TPSA of quinazoline inhibitors.

The highest KI dls (0.90 - 1.27) were computed for pyrimido[5.4-d]pyrimidin-4-amine and pyrido [3.4-d]pyrimidin-4,6-diamine derivatives, while for quinazoline derivatives KI-dls with lower values (0.36 - 0.74) were obtained.




Relationships of drug-likeness scores (DLSs) and TOX hERG parameters (ADMET Predict 6.5) of quinazoline derivatives.




Relationships of drug-likeness scores (DLSs) and TOX RAT parameters (ADMET Predict 6.5) of quinazoline derivatives.

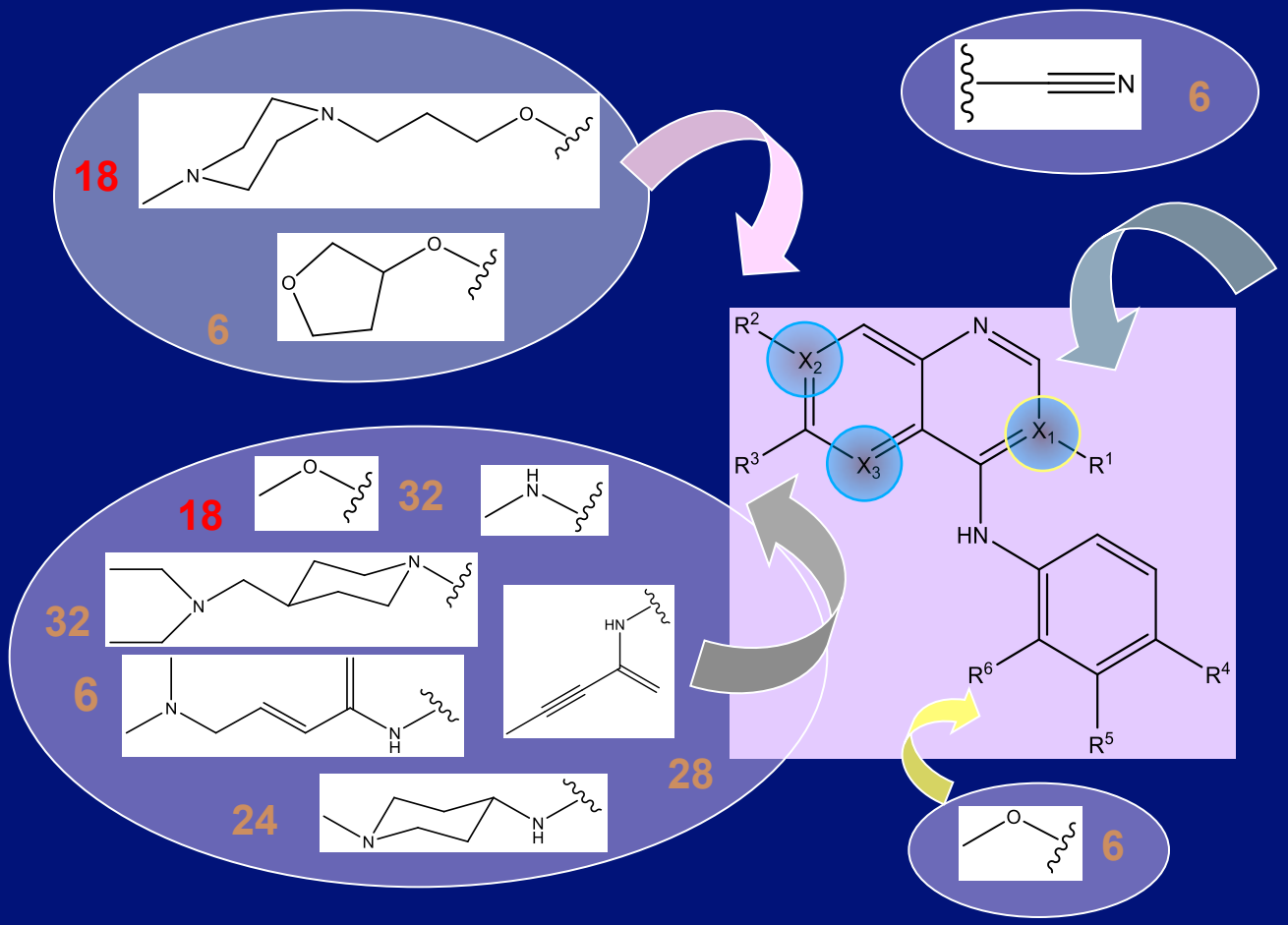
- With more *N* atoms in central bicyclic ring system – bigger kinase inhibitor scores (KI dls) were computed

- $n_{ON}$ = **5** – **8** (28 & 32); **7** (23 & 24); **8** (6 & 18), N atoms dominant
- $n_{OHNH}$ = **1** (18, 23) or **2** (6, 24, 28, 32)

- For inhibitors with obtained highest kinase inhibitor scores (KI dls = 0.9 – 1.27) the multiple DLSs were also computed.

- KI dls: **32** (1.27) > **24** (1.16) > **28** (0.96) > **23** (0.90) > **6** (0.73) > **18** (0.70)
- GPCR ligand dls: **24** (0.45) > **23** (0.34) > **32** (0.32) > **28** (0.26) > **6** (0.21) >**18** (0.11)
- ICM dls: **32** (0.33) > **28** (0.27) > **24** (0.26) > **23** (0.19) > **6** (-0.11) > **18** (-0.07)
- EI dls: **32** (0.39) > **24** (0.38) > **23** (0.25) > **28** (0.23) > **6** (0.21) > **18** (0.10)
- TOX Risk: **23** = **32** = **6** = 3; **24** = **28** = **18** = 4
- CYP Risk: **23** = **24** = **28** = **32** = **6** = 1; 18 = 2
- CYP 2D6 & CYP 3A4 substrates (**28** additionally as CYP 2C9).

The results of study revealed the structural features and physicochemical properties relevant to activity of investigated compounds as protein tyrosine kinase inhibitors, and the possible use of this methodology in exploration of target and potential anti-target features of these inhibitors.




**18**: $R^4 = R^5 = Cl$; $X^1 = X_2 = X_3 = C$ **(only KI dls)**
**6**: $R^4 = H$; $R^5 = F$; $R^6 = Cl$; $X_1 = N$; $X_2 = X_3 = C$; (+ GPCR ligand dls, EI dls)
**28**: $R^4 = H$; $R^5 = F$; $R^6 = Cl$; $X_1 = X_2 = N$; $X_3 = C$; (+ GPCR ligand dls, ICM dls. EI dls)
**32**: $R^4 = R^5 = H$; $R^6 = Br$; $X_1 = X_2 = N$; $X_3 = C$; (+ GPCR ligand dls, ICM dls, EI dls)
**23**: $R^4 = H$; $R^5 = F$; $R^6 = Cl$; $X_1 = X_2 = X_3 = N$; (+ GPCR dls, EI dls)
**24**: $R^4 = H$; $R^5 = F$; $R^6 = Cl$; $X_1 = X_2 = X_3 = N$; (+ GPCR dls, ICM dls, EI dls)

The likeness with GPCR ligand (GPCR dls, 0.21 – 0.45), ion channel modulator (ICM dls, 0.22 -0.33) and enzyme inhibitor (EI dls, 0.21 – 0.36) were also revealed with nine molecules, out of total 34.

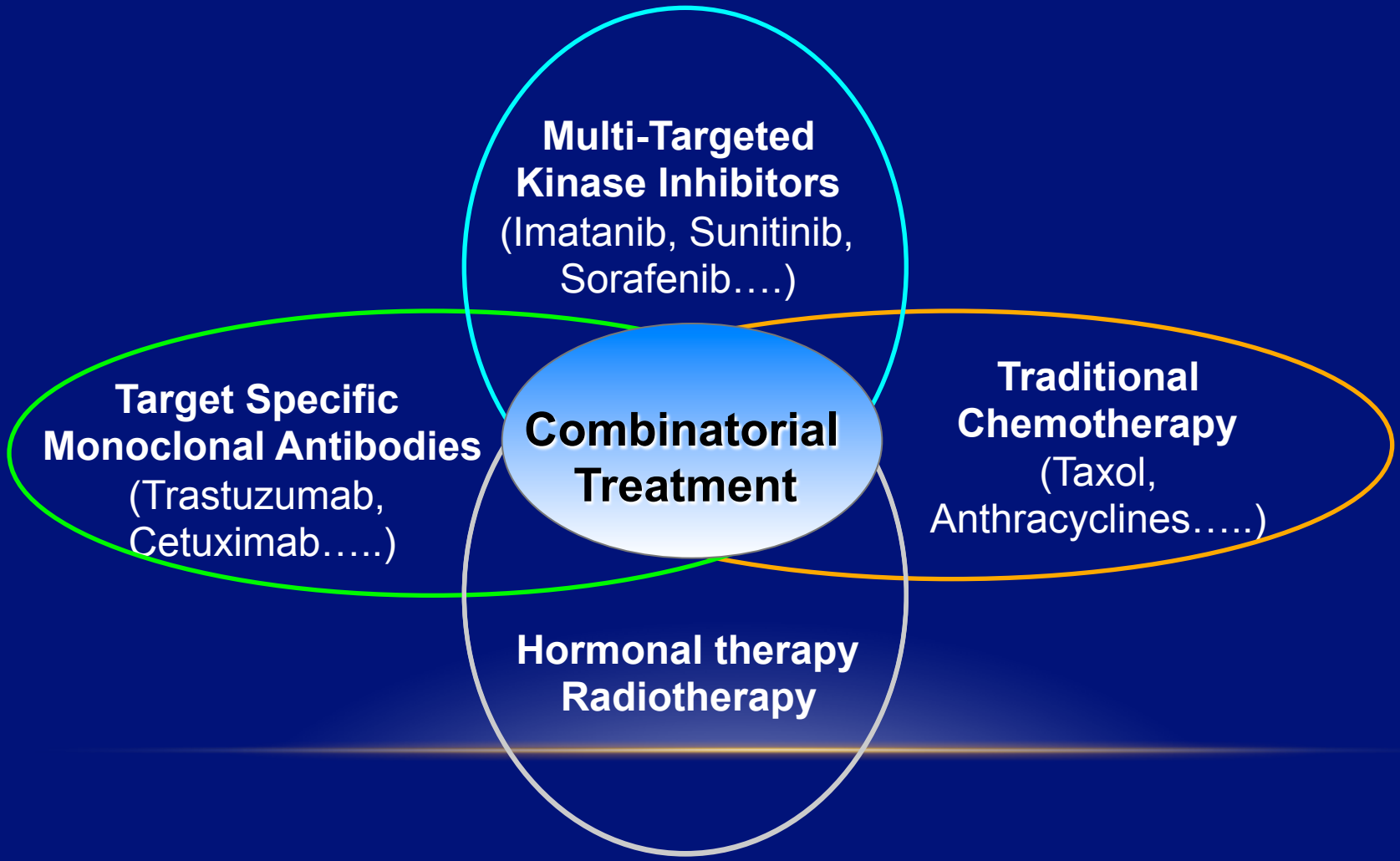
Additional drug-likeness properties are the most pronounced in a group of inhibitors (**23, 24, 28 and 32**) with the highest KI dls (0.9 – 1.27).

According to ADMET Predictor analyses, inhibitors with multiple drug-likeness scores were characterized as CYP 2D6 and CYP 3A4 substrates (28 additionally as CYP 2C9) with CYP Risk 1 or 2, CYP Code D6, and TOX Risk 3 or 4.

# Conclusions

- Most of the available targeted cancer therapy agents have significantly improved patients' progression-free survival, but none of them has yet proven to cure the disease.
- Numerous of RTK–TKIs and other inhibitors have been developed.
- Despite the considerable efforts from screening to clinical trials, which are expensive and time-consuming, the number of TKIs that have entered into clinical trials or have been approved by authorities for cancer treatment still remains low.
- Moreover, most TKIs as research tools or in the clinic are multi-targeted drugs.
- Multi-targeted property has several disadvantages, including side effects, a complication of the interpretation of results, and inducement of early resistance.

# Future of Individualized Cancer Therapy



# References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- Hojjat-Farsangi M (2014) Small-Molecule Inhibitors of the Receptor Tyrosine Kinases: Promising Tools for Targeted Cancer Therapies, *Int. J. Mol. Sci. 15*, p.p. 13768-13801.

Thank you for your attention!

# Boldog születésnapot, Imre!

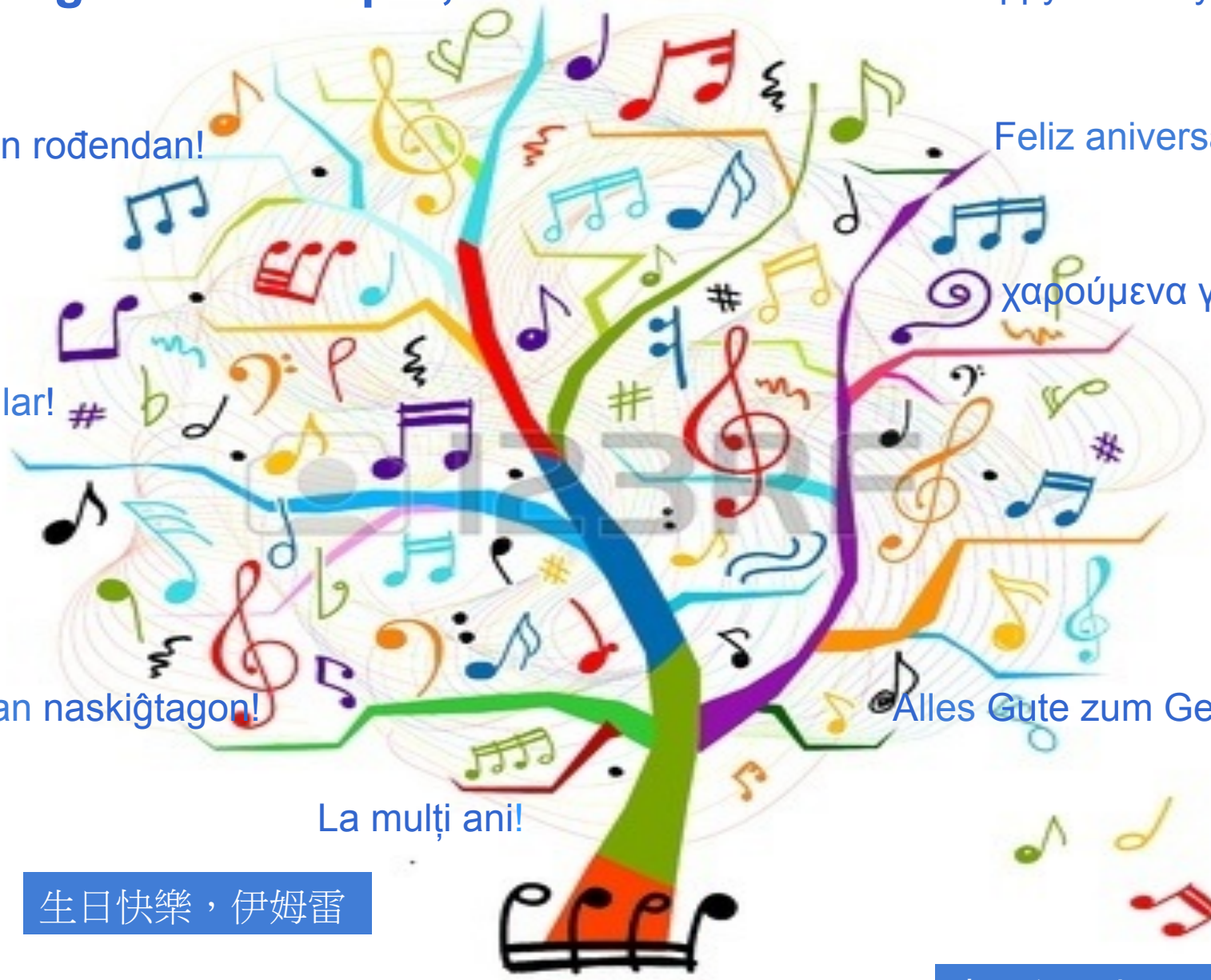
Happy Birthday!

Sretan rođendan!

Feliz aniversario!

χαρούμενα γενέθλια!

Mutlu yıllar!



Feliĉan naskiĝtagon!

Alles Gute zum Geburtstag!

La mulți ani!

生日快樂，伊姆雷

幸せな誕生日、イムレ