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
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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

- Preclinical: 3,088 (33%)
- Phase I: 1,082 (33%)
- Phase II: 1,438 (26%)
- Phase III: 449 (23%)
- Pre-Reg/Registered: 177 (9%)

Source: IMS Institute for Healthcare Informatics, Feb 2014

http://www.imshealth.com
Manufacturers seek accelerated approvals under regulatory provisions to reduce time-to-market.

**FDA breakthrough therapy designations 2012-2014**

- **56%** Denied
- **26%** Awaiting decision
- **18%** Granted
- **34%** Oncology
- **66%** Other therapy areas and unclassified


[http://www.imshealth.com](http://www.imshealth.com)
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. Kinases with activity towards all three residues
PROTEIN TYROSINE KINASES (PTKIs)
RTKs’ structure consists of three different parts: extracellular, transmembrane and intracellular 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.
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
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.
TYROSYNE 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 substrate/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 blockade of downstream EGFR signal transduction pathways, cell cycle arrest 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 metastatic 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.
Derivatives of 4-aminobenzamides

- 2,4-dimethyl-1H-pyrrole-3-carboxamide
- N-methyl-pyridine-2-carboxamide
- 5-thiazole carboxamide

Derivatives of quinazolin-4-amine

- Lapatinib
- Erlotinib
- Nilotinib
- Sunitinib
- Sorafenib
- Dasatinib

SMALL MOLECULE INHIBITORS ‘NIBs’

1. Lapatinib
2. Erlotinib
3. Imatinib
4. Nilotinib
5. Sunitinib
6. Sorafenib
7. Dasatinib
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.

Nilotinib was rationally designed for more effective binding to BCR-ABL oncoprotein (a protein product of the fused BCR-ABL oncogene produced by the Philadelphia chromosome, which plays a key role in initiating and maintaining leukaemia).

- Improved fit to auxiliary pocket, via lipophilic interactions, making it less susceptible to point mutations
- Only maintains 4 hydrogen bonds

Hydrogen bonds form with specific amino acids lining the binding site

- Hydrogen bonds with Ile360 & His361

FDA v.s. EMA Approvals in Oncology
Chronology 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

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

1940-1949
- Muronomab CD3, 1987
- Immunosuppressant
- 1st mab

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- Afatinib, 2013
- Ceritinib, 2014
- Lenvatinib, 2015

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

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- Sorafenib, 2005
- Sunitinib, 2006
- Dasatinib, 2006
- Lapatinib, 2007
- Pazopanib, 2009

Other
Mab
Nib
FDA and EMA profile of approved drugs for oncology (1995-2015)

Number of drugs

FDA

EMA

Other

MAB

NIB

Imatinib

Erlotinib

Sunitinib

Sorafenib

Gefitinib

Pazopanib

Vandetanib

Crizotinib

Axitinib

Afatinib

Trastuzumab

Cetuximab

Bevacuzimab

Rmuicromumab
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 (\( V \)), \( n_{\text{atoms}} \) 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.
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 topological 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.
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.

**Chemical Structures:**

18: $R^4 = R^5 = Cl; X^1 = X_2 = X_3 = C$
6: $R^4 = H; R^5 = F; R^6 = Cl; X_1 = N; X_2 = X_3 = C$
28: $R^4 = H; R^5 = F; R^6 = Cl; X_1 = X_2 = N; X_3 = C$
32: $R^4 = R^5 = H; R^6 = Br; X_1 = X_2 = N; X_3 = C$
23: $R^4 = H; R^5 = F; R^6 = Cl; X_1 = X_2 = X_3 = N$
24: $R^4 = H; R^5 = F; R^6 = Cl; X_1 = X_2 = X_3 = N$

(stop KI dls)

+ GPCR ligand dls, EI dls
+ GPCR ligand dls, ICM dls, EI dls
+ GPCR ligand dls, ICM dls, EI dls
+ GPCR ligand dls, ICM dls, EI dls
+ GPCR ligand dls, EI dls
+ GPCR ligand dls, ICM dls, EI dls

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

- Multi-Targeted Kinase Inhibitors (Imatinib, Sunitinib, Sorafenib…)
- Target Specific Monoclonal Antibodies (Trastuzumab, Cetuximab…)
- Traditional Chemotherapy (Taxol, Anthracyclines…)
- Hormonal therapy
- Radiotherapy

Combinatorial Treatment
References


Thank you for your attention!
Boldog születésnapot, Imre!

Sretan rođendan!

Mutlu yıllar!

Feliĉan naskiĝtagon!

La mulți ani!

Happy Birthday!

Feliz aniversario!

χαρούμενα γενέθλια!

Alles Gute zum Geburtstag!

生日快樂，伊姆雷

幸せな誕生日，イムレ