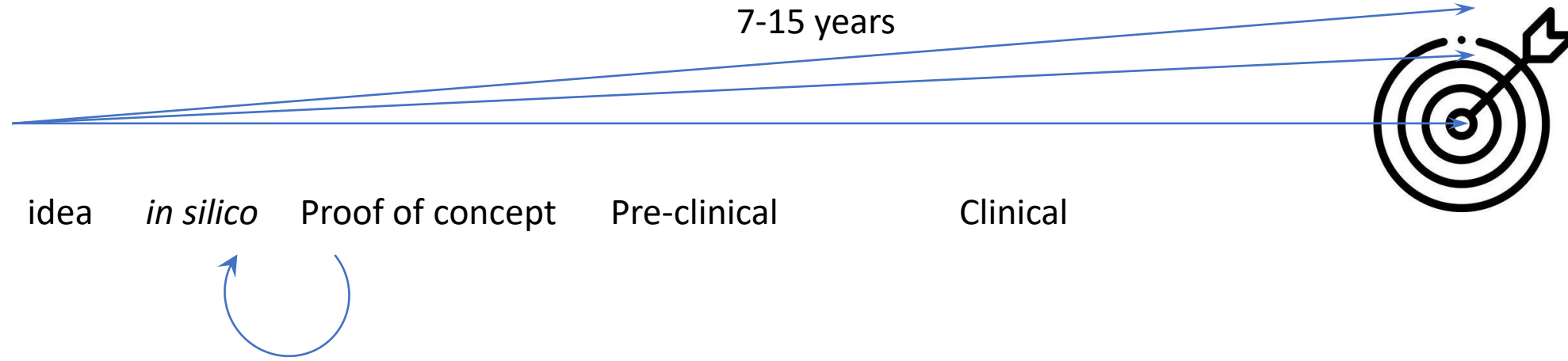


In silico small molecule drug discovery from the pharma company point of view

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Head of Global R&D
JSC, Valenta Pharmaceuticals

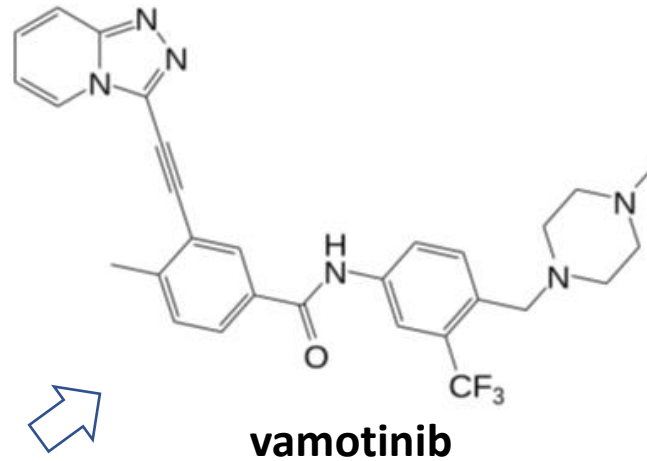
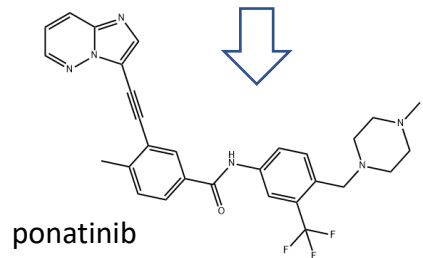
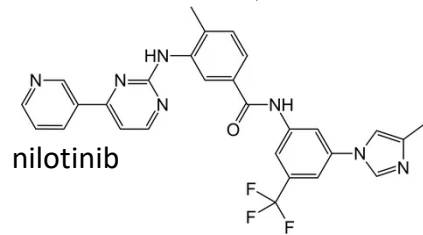
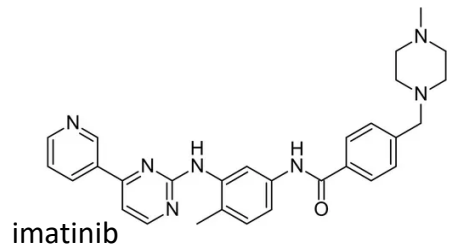
Why *in silico* stage matters a lot



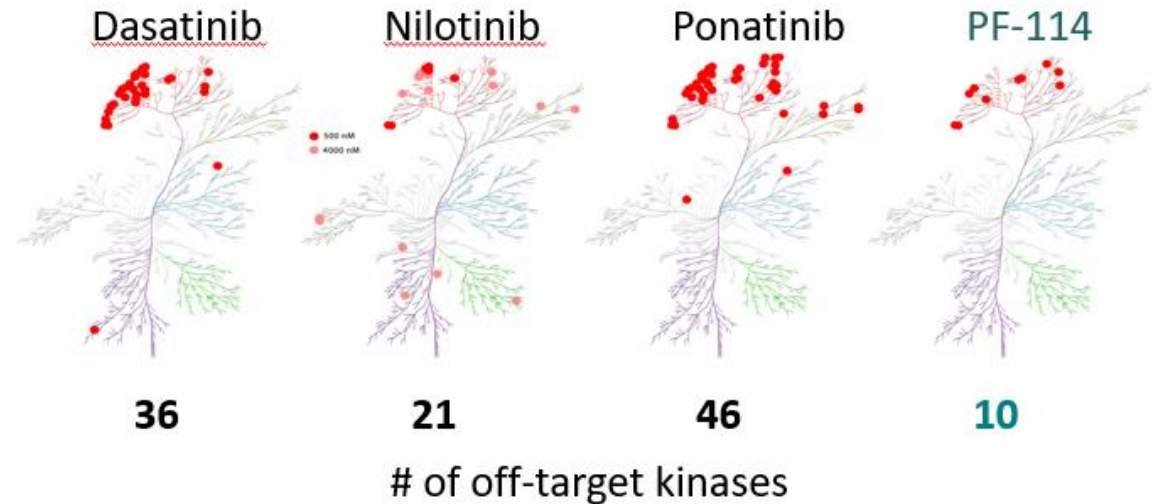
- The drug discovery and development process takes many years from the idea to drug approval, and it does not get easier because of regulatory landscape changes and competition from different drug products and drug modalities
- Slight deviations from the desired product profile at the beginning may result into big differences in the properties of the final product which may not fit market expectations
- Therefore, alignment of the desired product profile with *in silico* modeling stage and further translation into proof of concept stage is crucial

Case study: vamotinib - a 3rd generation Bcr-Abl inhibitor

- Vamotinib (PF-114): 3rd generation inhibitor of Bcr-Abl (following 1st generation inhibitor imatinib and 2nd generation – nilotinib)
- Rationally designed to avoid multiple off-target effects and improve safety compared to another 3rd generation inhibitor ponatinib
- Current status: filed for marketing authorization in Russia



Kinase inhibition profile of ATP-competitive drugs



in silico stage setup

- **Key indicators**

- Clearly defined therapeutic target (target discovery stage completed)
- Clearly defined mode of action
- translated into a hypothesis for the new molecular entity (which unique properties should a new molecule have)
- a feasible proof of concept

- **Implementation**

- ⇒ • Bcr-Abl fusion protein, kinase activity
- ⇒ • ATP-competitive binding site (alternatively – allosteric site)
- ⇒ • A molecule with affinity towards an Abl kinase mutant of interest and no affinity to off-target kinases
- ⇒ • Activity vs certain Abl mutants and a better kinase selectivity profile; no severe toxicity in animal models

in silico stage: therapeutic target and mode of binding

- **Capture the “physics” of a target**

- “Simple” ligand binding in the existing site (e.g. competitive enzyme inhibitors)
- Allosteric binding where the binding site emerges during the protein structure movement
- Functional influence on the protein (aiding CFTR channel function, blocking/activating other ion channels, etc)
- PDB structure may not be sufficient!

- **Full atomic modeling of a protein target interaction**

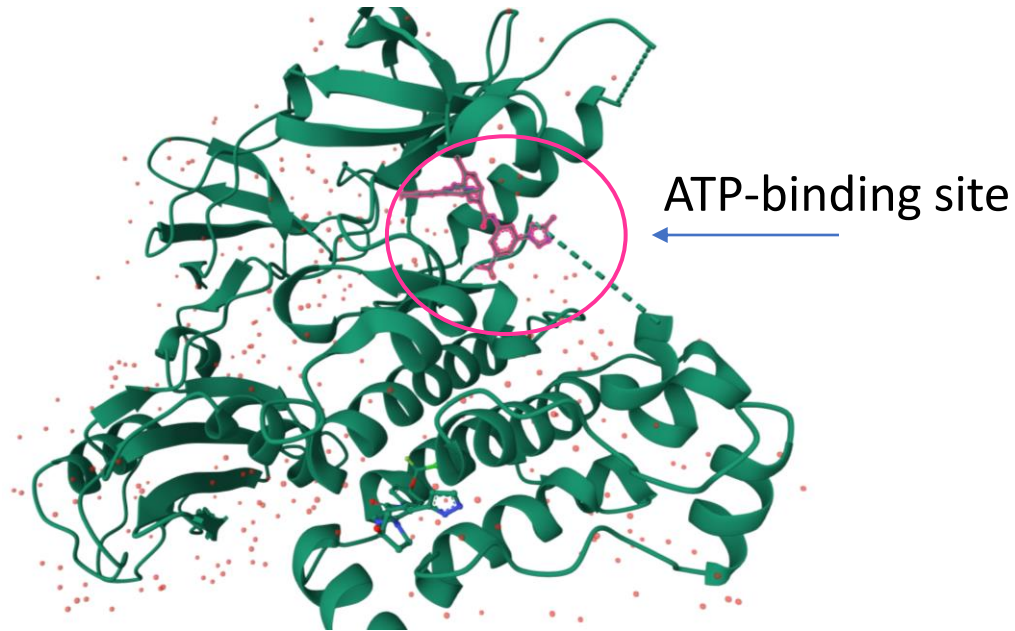
- Validation of a model using true positive and true negative examples
- Select proper method for model validation (docking, flexible docking, molecular dynamics, FEP)
- Gain understanding of ligand binding at structural biology level



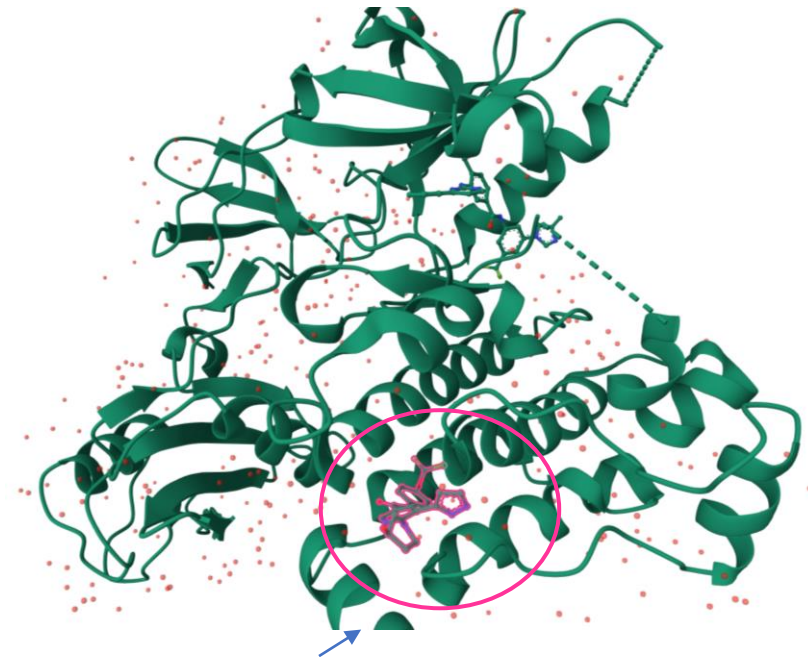
- Increase the success rate of designing a proper binder and shorten the time of drug candidate optimization vs large scale screening and empiric optimization of ligand structure

in silico stage: therapeutic target and mode of binding

Bcr-Abl inhibitors: Bcr-Abl – is a sole cause of disease, 2 inhibitor binding sites available



Resistant mutations: T315I, G250E, Y253H/F, E255K/V, H396P/R, F359V/C/I, L248V, M351T
~4% of patients develop mutations upon treatment with nilotinib front line

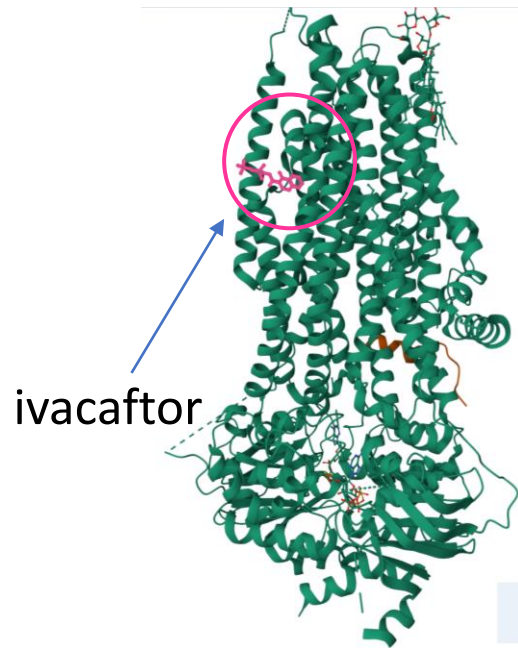


Allosteric binding site

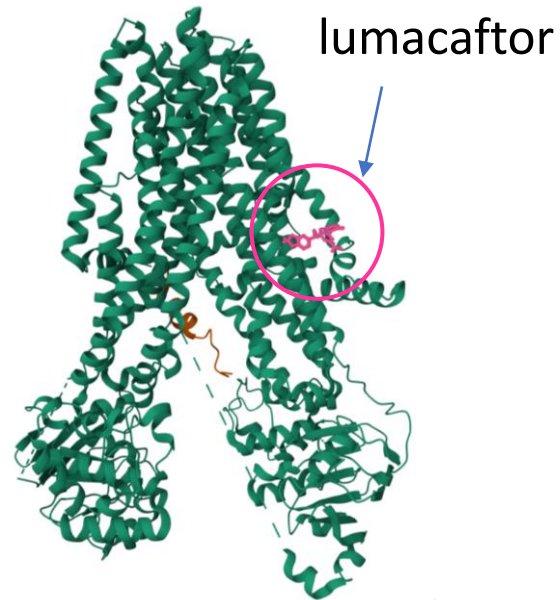
Resistant mutations: A337V/T, P465S, C464W, V468F, G463S, I502L, M244V, L248V, F317L, F359C/I/V
4% of patients develop mutations upon treatment with asciminib front line

in silico stage: therapeutic target and mode of binding

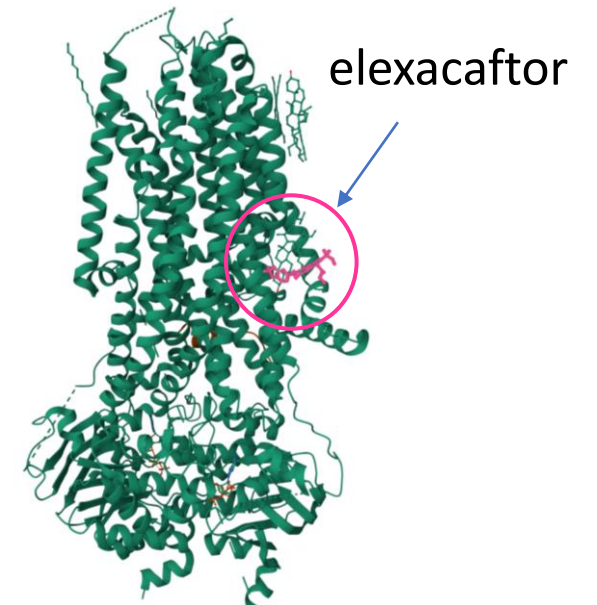
CFTR ion channel: different opportunities to assist functioning of inactivated mutant protein



Ivacaftor stabilizes open conformation of a channel



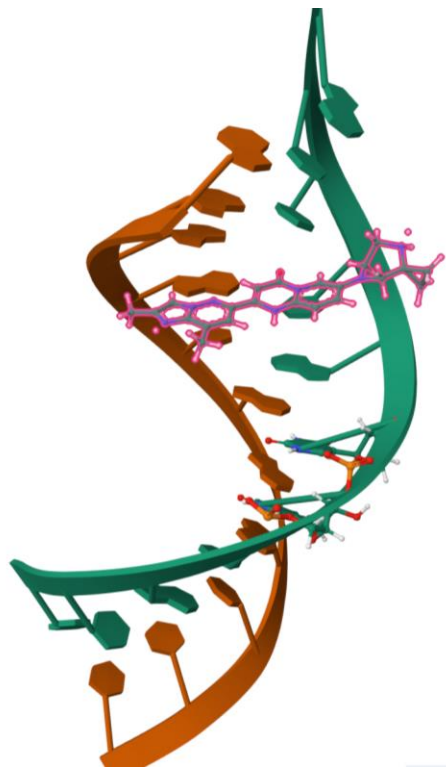
Lumacaftor stabilizes the correctly folded conformation of a channel



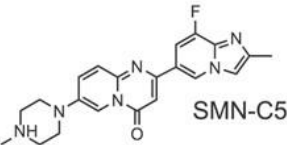
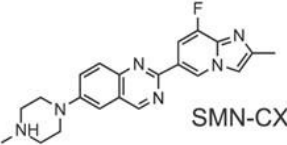
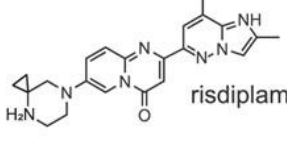
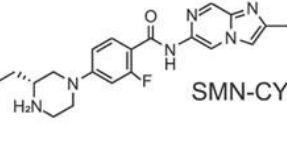
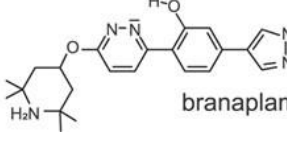
Elexacaftor stabilizes protein interface and dimerization

in silico stage: therapeutic target and mode of binding

Even more complicated modality: Risdiplam mechanism of action



Solution structure of Risdiplam bound to the RNA, PDB 8R62

A-1 splicing modifier	IC ₅₀ [nM]	K _D [μM]
 SMN-C5	30 nM ^a	28 ± 9 μM ^f
 SMN-CX	36 nM ^b	74 ± 17 μM ^f
 risdiplam	23 nM ^c	12 ± 1 μM ^g
 SMN-CY	29 nM ^d	60 ± 13 μM ^f
 branaplam	20 nM ^e	18 ± 1 μM ^g

In silico driven hypothesis for the drug candidate

Hypothesis: a more selective kinase inhibitor is required for improved safety

Ponatinib



Kinase inhibition profile

WARNING: ARTERIAL OCCLUSION, VENOUS THROMBOEMBOLISM, HEART FAILURE, and HEPATOTOXICITY

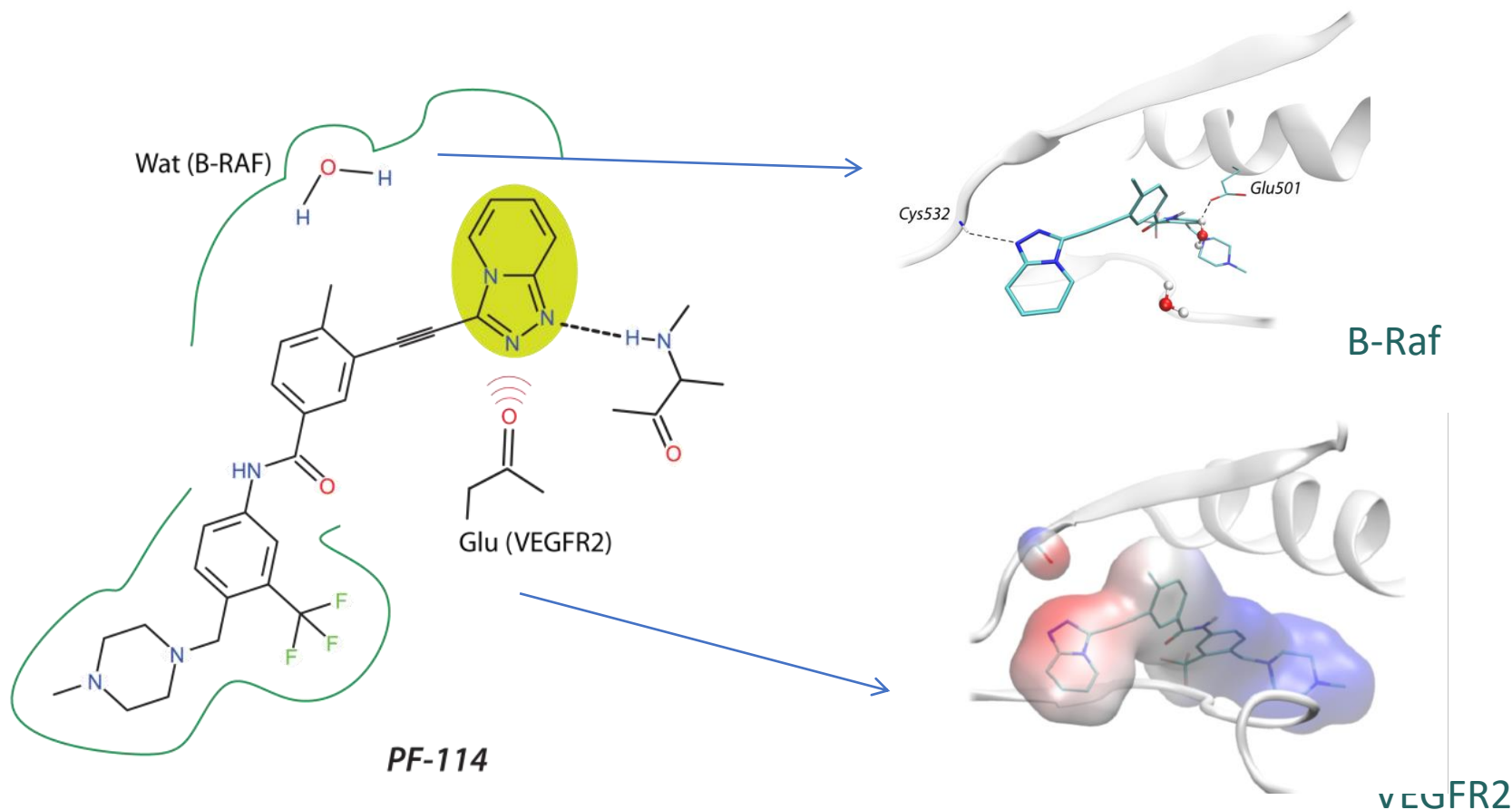
See full prescribing information for complete boxed warning.

- Arterial occlusion has occurred in at least 35% of Iclusig-treated patients including fatal myocardial infarction, stroke, stenosis of large arterial vessels of the brain, severe peripheral vascular disease, and the need for urgent revascularization procedures. Patients with and without cardiovascular risk factors, including patients less than 50 years old, experienced these events. Interrupt or stop Iclusig immediately for arterial occlusion. A benefit-risk consideration should guide a decision to restart Iclusig (5.1).
- Venous thromboembolism has occurred in 6% of Iclusig-treated patients. Monitor for evidence of thromboembolism. Consider dose modification or discontinuation of Iclusig in patients who develop serious venous thromboembolism (5.2).
- Heart failure, including fatalities, occurred in 9% of Iclusig-treated patients. Monitor cardiac function. Interrupt or stop Iclusig for new or worsening heart failure (5.3).
- Hepatotoxicity, liver failure and death have occurred in Iclusig-treated patients. Monitor hepatic function. Interrupt Iclusig if hepatotoxicity is suspected (2.3, 5.4).

Black box warning from the FDA label

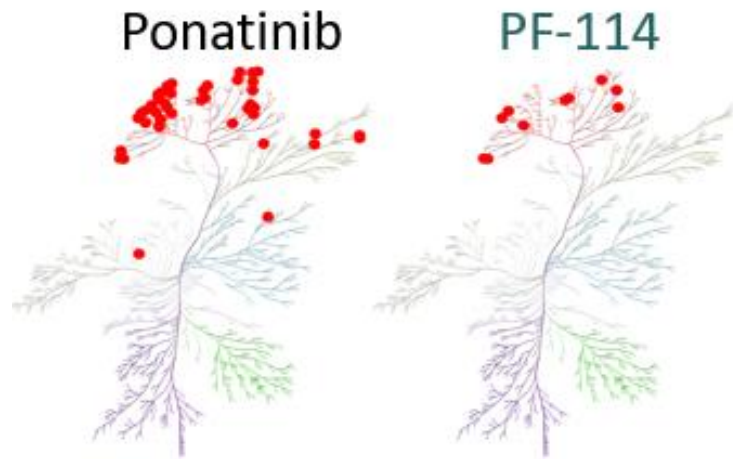
In silico driven hypothesis for the drug candidate

Selectivity of vamotinib by design: disrupting typical off-target interactions e.g. with VEGFR2 and B-Raf

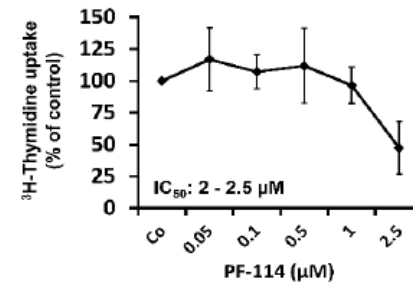
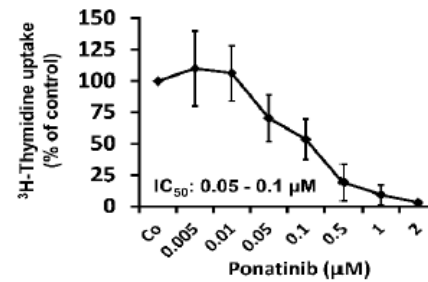


In silico driven hypothesis for the drug candidate

Kinase selectivity profile of vamotinib (PF-114) may explain its reduced cardio toxicity



- PF-114 as opposed to ponatinib is not toxic to human umbilical vein endothelial cells (Medical University of Vienna, Prof. Peter Valent lab)

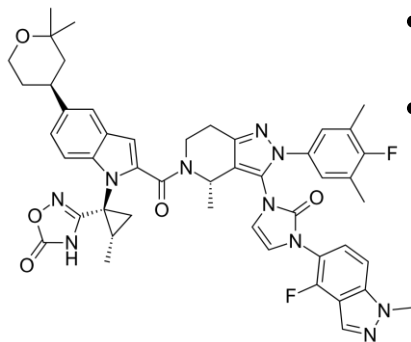


- PF-114 as opposed to ponatinib is not toxic to cardiomyocytes, does not cause ventricular fractional shortening in zebrafish, does not induce cardiac dysfunction in ApoE null mice as compared to ponatinib (University of Vanderbilt, Prof. Hind Lal lab)

Basing on the comparison of strongly inhibited kinases by ponatinib vs vamotinib and other inhibitors the following kinases may stand for cardiovascular toxicity: EPHA6, EPHA7, TAK1, TIE2, VEGFR2, ZAK

Other molecular properties of interest: cost of manufacturing

- For a company working in the other-the-counter (OTC) segment the cost of drug is crucial.
- In Russia, the cost of API per monthly course of an OTC drug should not exceed ~5 USD, which translates to 2-4k USD/kg of API
- Considering obesity as an indication for OTC drug therapy:



- Monthly cost of semaglutide in Russia is ~70 USD
- How can small molecule chemicals like orforgliprone reach that price level?

- In silico tools to be evaluated: Rondaxe CoGS, ChemPrice, CoPriNet, MolPrice, RetroPriceNet

Other molecular properties of interest: intellectual property

- Novelty
 - Your molecule does not appear somewhere else explicitly
- Inventive step
 - Your molecule differs from existing ones in a non-trivial for an expert way
- Freedom to operate
 - Your molecule and all related to it (manufacturing, use, etc) does not violate the IP rights of others
- Patenting salt forms of a drug molecule, crystal forms
 - Worth for prolongation of patent protection