KJ2900, BACHELOR THESIS IN CHEMISTRY

What is the best core scaffold for developing 3. generation EGFR inhibitors? This question is addressed by comparing the effect of two structurally different inhibitors, Osimertinib (AZD9291) and Avitinib (AC0010) on efficiency, selectivity, and toxicity.

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30.04.2021

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Abstract

Lung cancer is the leading cause of cancer-related deaths in Norway. Osimertinib and Avitinib are two structurally different inhibitors for the T790M mutation of epidermal growth factor receptor (EGFR) which is a major drug target in advanced non-small cell lung cancer. Both have selectivity over wild type EGFR. The balance between potency for T790M and selectivity over wild type EGFR has been achieved by tuning the properties of the chemical groups in the scaffold, based on data from the structure activity relationship for each inhibitor. In preclinical and clinical trials Osimertinib and Avitinib show adequate safety-profiles, and an effective shrinkage of non-small cell tumors in the lungs.

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1. Introduction

Cell division is the basis of life, the reason why organisms can grow and produce offspring. It is the mechanism enabling humans to develop from a single cell, a fertilized egg.[1] The process that gives life, when malfunctioning, is the same process that takes life. It is the reason why every single year around 35 000 people are diagnosed with cancer in Norway alone. In 2019, over 11 000 humans lost their lives because of this disease.[2]

One of the main concerns when developing new anti-cancer treatment is selectivity. To stop the cancerous cells from dividing and spreading, without affecting the healthy cells. This is a challenge, as the enzymes that bind ATP are numerous, and similar in structure around the binding site. However, by designing drugs that target a specific gene or protein that are mutated in the cancer cell, it is possible to develop selective drugs.

This approach is utilized in the development of new drugs against lung cancer, the third most common type of cancer in Norway and also the one that takes the most lives.[2] New drugs are being derived from existing structures that are known to have an effect, with the goal of improving them. In the treatment of a specific T790M mutation in the EGFR gene, a protein crucial for the development of non-small cell lung cancer, two core scaffolds are used. The goal of this report is to evaluate which of these is the best base for further optimalization. The comparison will be centered around the structure-activity relation of each drug, in addition to preclinical and clinical results for efficacy, selectivity, and toxicity. The metabolism of the drugs will not be in focus.

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2. Theory

2.1 Cancer

The cell cycle (Figure 1) of a normal cell is under strict regulation by internal and external molecules.[3] Depending on the demand for the cell, these signals control if the cell divides, or enters the non-dividing phase G_0. The control system also ensures that cells with damaged DNA undergo apoptosis, controlled cell death. Apoptosis can happen at all the checkpoints of the cell cycle.

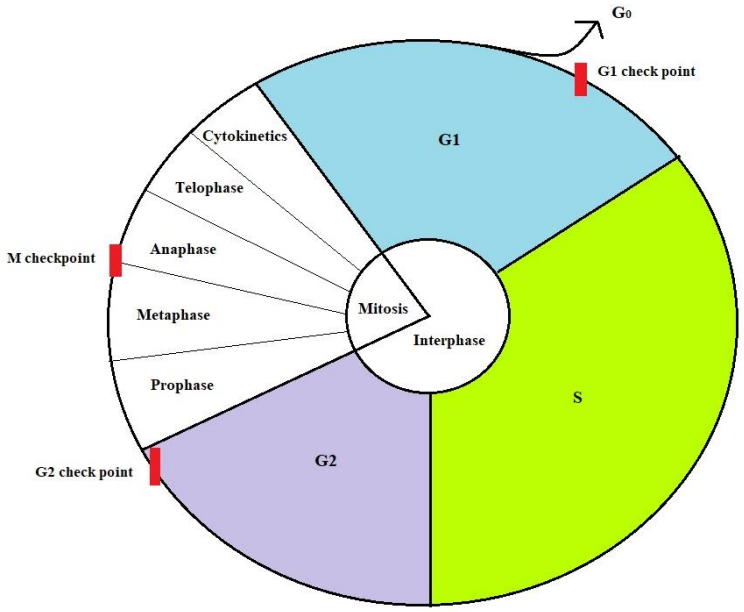

Figure 1. Overview of the phases in the cell cycle. Interphase is the phase where the cell does not divide, it consists of G1, S -and G2-phase. Mitosis is the phase where the cell divides. The red squares symbol the checkpoints of the cell cycle. Figure adapted from Campbell *et al.*[3]

Cancer is defined as uncontrolled growth and spread of cells.[3] The ability to override the control system is a result of mutations in the cell, affecting the genes coding for growth factors, their receptors or other molecules that are part of the signaling pathway for cell proliferation, migration, apoptosis etc.[3] When one abnormal cell divides unchecked, it can result in a tumor, a mass of abnormal cells within healthy tissue. There are two types of tumors; benign and malignant. Malignant tumors can spread from their origin and impair new tissues. The cancer-type is named after what tissue it has its origin in.

Lung cancer has its origin in the lungs. It can further be subdivided into small cell and non-small cell cancer.[2] The non-small cell lung cancer (NSCLC) is the most common of the two. A mutation in the epidermal growth factor receptor (EGFR) giving uncontrolled cell division is the main reason NSCLC is developed.[4] This receptor is therefore an important target for anti-cancer drugs.

2.2 Epidermal growth factor receptor

Receptors are specific, they are only activated by ligands with a complimentary chemical property to the binding site in the receptor.[3] Tyrosine kinase receptors (RTKs) is the most common of the receptor enzymes. EGFR is a RTK. Like all RTKs it has two regions, an extracellular ligand binding domain and an intracellular tyrosine kinase domain, which is connected by a single membrane spanning domain.

The extracellular region of EGFR consists of four domains: I, II, III, IV. Domain I and III are leucine rich ligand-binding domains, forming the interactions with epidermal growth factor (EGF).[5] The receptor exists in an equilibrium between two conformations: the extended (3.5% ± 1.5%) and the tethered (93.5% ± 1.5%),[6] shown in Figure 2. In the tethered structure the cysteine-rich domains II and IV interact, closing off the dimerization arm of domain II, so that it cannot dimerize. EGF will favor binding to the extended conformation, where it binds to both domain I and III simultaneously. This is not possible for the tethered structure, as the domains are too far apart.[6] A large concentration of EGF will drive the equilibrium towards the extended conformation, as it traps it in this conformation that can dimerize. The dimerized conformation of the extracellular region is the active state of EGFR.

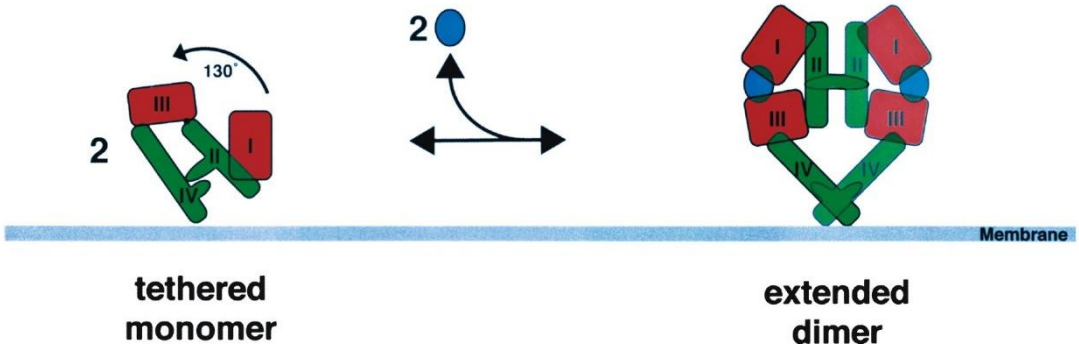

Figure 2. The two possible conformations of the EGFR extracellular domain. Figure reprinted with permission from Burgess *et al.* [6]

Unlike most RTKs, the intracellular tyrosine kinase region of EGFR is not autoinhibited.[6] It is not dependent on dimerization for stabilizing the active conformation of the activation loop, and as a result, the catalytic domain is always active.[7] When active, the catalytic domain can phosphorylate other proteins. The phosphorylation is done by transferring a phosphoryl group from ATP to the hydroxyl group of Tyrosine.[8] This starts a signaling cascade in the cytoplasm that has its target in the nucleus of the cell, where it stimulates the transcription and transduction of a set of genes needed for cell proliferation.[5]

The intracellular tyrosine kinase region of EGFR, also known as cytoplasmic region, consist of 12 subdomains as shown in Figure 3. ATP binding site is located in a deep cleft between the amino- and carboxy-terminal lobes.[8] On the N-lobe both the P-loop *(Gly695-700)*, a glycine-rich sequence, and the two subdomains forming the roof of the binding site, is located. At the point where N- and C-lobe connect, two gatekeeper subdomains are located, in addition to the hinge. The remaining six subdomains are located at the C-lobe: The activation loop *(Asp831-Val852)*, the catalytic loop *(Arg812-Asn818)*, the two subdomains of the DFG-motif *(Asp831-Gly883)* and the front pocket with one subdomain close to the ATP binding site, and one far from it.[7]

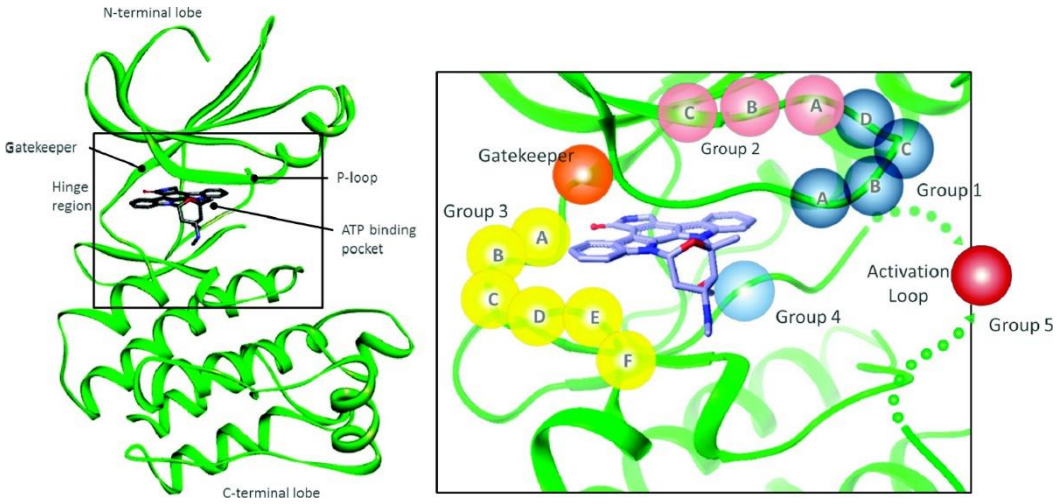

Figure 3. The subdivision of the catalytic domain of EGFR, surrounding the ATP binding site. Group 1 is the P-loop. Group 2 is the roof. The gatekeeper is located right above group 3. Group 3 A-E is the hinge, and F is the front pocket. Group 4 is the DGF-motif. Group 5 is the activation and catalytic loop. Reprinted with permission from Barf *et al.* [9]

The three-dimensional structure around the ATP binding site has many similarities to the other 518 protein kinases in the human genome.[8] In addition, there are over 2000 other nucleotide-dependent enzymes that has potential binding sites.[10] As a result of this, selectivity is the biggest challenge when developing covalent inhibitors for EGFR. However, selectivity is not impossible when exploiting the grooves and fine differences in structure close to where the adenine base of ATP binds.[11] When covalently inhibiting this region, cysteines near the ATP binding site are targeted, as their positions are unique for EGFR. The availability of the cysteines varies, the easiest to access are located in the front-pocket, P-loop and DFG-motif. Cys797 in the front-pocket is one of the cysteines giving selective reactivity and inhibition.[12]

2.3 Irreversible covalent inhibition of EGFR

A covalent inhibitor is a molecule that forms a covalent bond with the receptor, decreasing its activity, or inactivating the receptor. The binding happens in two stages. First, the inhibitor associates with the enzyme, placing the different groups of the inhibitor in correct conformation and proximity to the enzyme. This stage is reversible and is directed by noncovalent interactions. In the resulting complex, the reactive group of the inhibitor forms a covalent bond with the enzyme through a Michael addition, making the inhibition irriversible.[8] The goal for any covalent inhibitor is to find the right balance of safety and efficacy, this is done by tuning the reactivity of the scaffold and warhead.

One of the biggest dangers with irreversible covalent inhibition, compared to reversible, is the potential for off-target reactivity, and the toxic effects related to this. In the treatment of life-threatening diseases, covalent inhibitors have become more common during the last ten years, as the benefits from the treatment outweigh the potential risks.[13] The advantages over reversible inhibitors are the strong target affinity and the longer effect in patients. The irreversible nature of the binding allows the design of a drug that is cleared rapidly, decreasing the chance for off-target toxicity. This does not affect the effect as the target-inhibitor complex will persist after the unbound drug is cleared, and until the receptor is re-synthesized.[9] Another advantage is that the covalent bond allows high potency even for small molecules, not depending on the big size for adequate Van der Waals interactions. A low molecular mass is optimal for the metabolic properties of the drug.[13]

Given the central role of EGFR in the development of cancerous cells, it is a natural target for inhibitory drugs.[5] The drugs are classified into first, second, third and fourth generation inhibitors.

2.3.1 First generation inhibitors

Ten percent of patients with NSCLC has one or several activating mutations.[4] The majority of the activating mutations are located on exon 19 or 21, coding for the EGFR receptor; small in-frame deletions and amino acid substitutions.[14, 15] The in-frame deletions happen on exon 19, and common for all is the deletion of the four amino acids Leu, Arg, Glu, and Ala on codons 747 to 750, termed as Del19. On exon 21 the most frequent amino acid substitution is the switch of Leu to Arg on codon 858, termed as L858R, in the activation loop clustered around the ATP-binding pocket.

Gefitinib and Erlotinib (Figure 4) are two reversible inhibitors that act by competing with ATP. They are anilinoquinazolines that inhibit the ATP binding site where they are stabilized by hydrogen-bonds to some critical amino acids that flank the binding site.[14] The activating mutations increase the affinity for these first-generation inhibitors while lowering the affinity for ATP.[16]

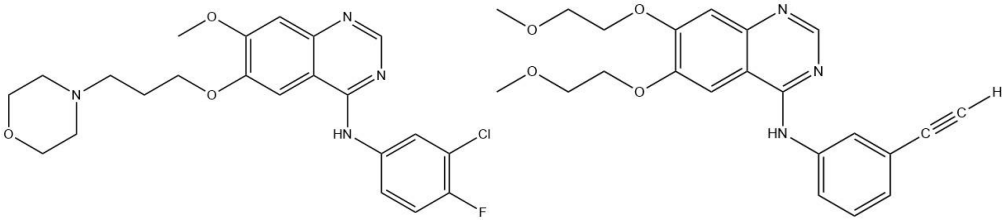

Figure 4. The chemical structure of Gefitinib (left) and Erlotinib (right), two reversible inhibitors for EGFR.

One of the struggles in treating NSCLC is that the cancer cells develop drug resistance by new mutations. The primary mechanism of resistance against first- and second-generation inhibitors is a mutation of the gatekeeper Thr 790, T790M. This threonine is located at the entrance of a hydrophobic pocket in the ATP binding site, and the mutation restores the affinity for ATP, rendering the reversible first generation inhibitors inactive.[17]

2.3.2 Second generation inhibitors

The second-generation inhibitors aim to treat the T790M mutation. Afatinib is the only second-generation inhibitor that is approved. It has the reversible anilinoquinazoline scaffold common for the first-generation inhibitors, in addition to an electrophile acrylamide making it irreversible, as shown in Figure 5. One of the biggest challenges with the second-generation inhibitors is that they are not selective for the T790M mutant, but also inhibit wild-type EGFR (WT-EGFR).[16] This gives a low dose-limit before toxicity arise, resulting in skin rash and diarrhea. Third generation inhibitors are being developed to overcome this problem and give high selectivity against T790M over the WT-EGFR.

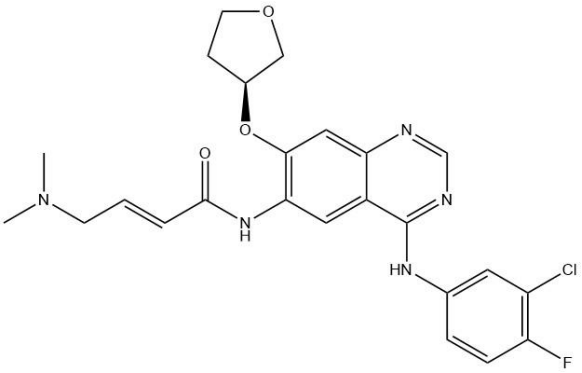

Figure 5. Chemical structure of the second-generation inhibitor Afatinib.

2.3.3 Third generation inhibitors

In 2009 Zhou *et al.*[16] identified another scaffold that could be used for inhibiting EGFR. It is based on an anilinopyrimidine scaffold, and the inhibitor was named WZ4002. Compared to inhibitors with an anilinoquinazoline scaffold it was 30- to 100-fold more potent against T790M, and 100-fold less potent against WT-EGFR, in vitro.[16] Based on this structure new inhibitors were developed. Among the third-generation inhibitors that have been through preclinical trials, there are two main scaffolds that are used, a 2-anilinopyrimidine and a 2-anilinopyrrolopyrimidine. Osimertinib (AZD9291) and Avitinib (Abivertinib, AC0010) are representative for these two scaffolds, respectively, as shown in Figure 6. Both inhibitors also have an acrylamide moiety attached to the scaffold that forms an irreversible covalent bond with the active thiol of Cys797. The crystal structure of the inhibitors in complex with EGFR are shown in Figure 7, indicating all interactions with the amino acids in the binding site.

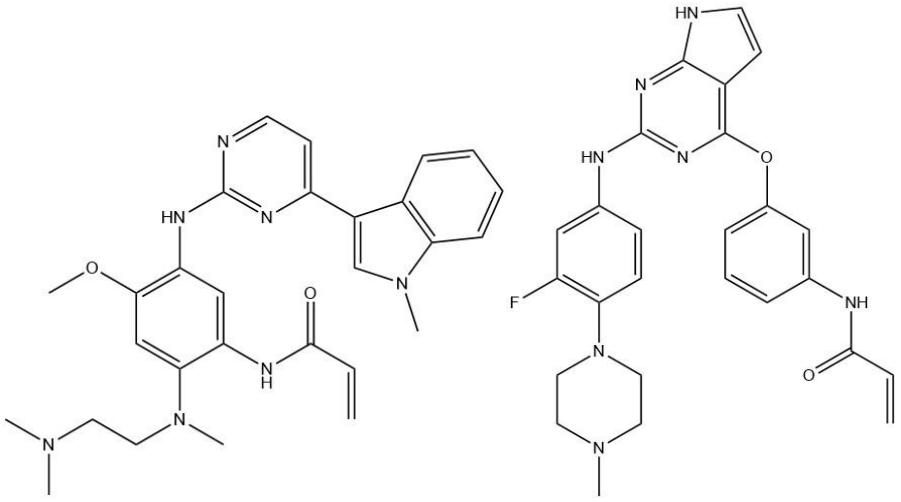

Figure 6. The chemical structure of the third-generation inhibitors Osimertinib (left) and Avitinib (right).

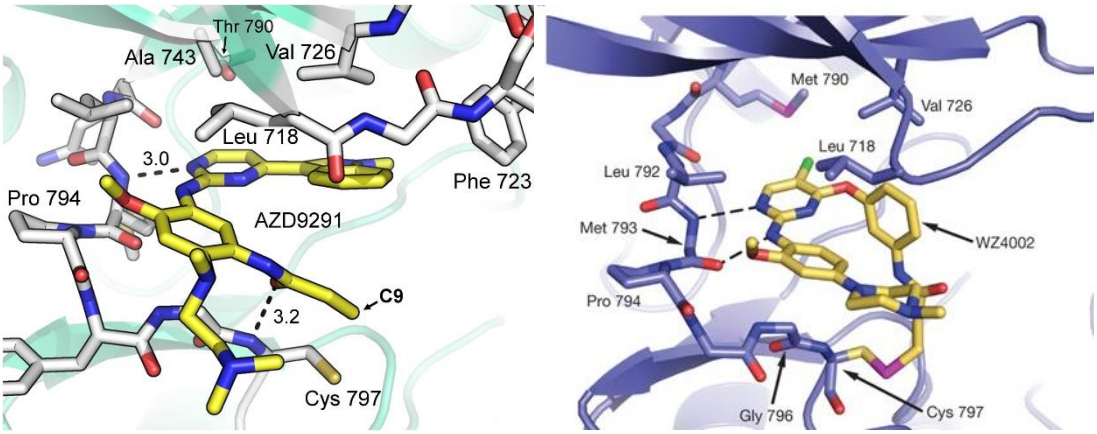

Figure 7. Crystal structure of EGFR in complex with Osimertinib (left), and WZ4002 (right). WZ4002 is used, as there is no available literature on the crystal structure of Avitinib in complex with EGFR. WZ4002 equals Avitinib in structure, with the exception that Avitinib has a pyrrole-group instead of a chlorine on the pyrimidine-core (stretching up towards Met790), and fluor instead of the methoxy on the aniline ring. Figures reprinted with permission from Squire *et al.* (left),[18] and Zhou *et al.* (right).[16]

Of the two drugs, Osimertinib is the only one approved. The approval was made by the FDA in November 2015, based on the AURA clinical trials, the first in human phase I/II trial of the drug. In February 2016 it was also approved by the European Commission, after the additional phase II trials AURA extension and AURA2.[19] Avitinib is currently undergoing clinical trials at ACEA Biosciences, Hangzhou, and an application for approval in China has been made.[20]

In a study published in 2021 containing 34 patients being treated with Osimertinib, resistance to the drug occurred after a median of ten months.[21] Five patients had histological changes, and the remaining 29 kept their non-small cell histology. There are several different resistance mechanisms that were discovered for those who maintained the histology, the significance of each of them are still unknown. 24.1% kept the T790M mutation inn cis with C797 point mutation, MET amplification, or other downstream mutations. 75.9% lost the T790M mutation, giving rise to EGFR point mutations, activation of bypass RTK signaling, deviations in the signaling cascade or target loss.[21] Avitinib and Osimertinib show many of the same resistance mechanisms, but are affected by them to different degrees.

The C797S point mutation is common for all third-generation inhibitors. Ser is less nucleophile than Cys, so that the covalent bond cannot form.[22] Depending on the type of resistance, the progression free survival (PES) varies. Maintained T790M has a better prognosis than the loss of T790M, with a PES of respectively 13.81 to 5.36 months for treatment with Osimertinib. In both cases, MET amplification, worsened the outcome.[21]

As a result of the resistance to third generation inhibitors, new research on EGFR inhibitors for NSCLC has two main focuses: approving todays third generation inhibitors by making changes to the warhead and scaffold, and research into fourth-generation inhibitors.[23]

3. Discussion

All third-generation EGFR inhibitors have an acrylamide as a warhead. It is a relatively poor electrophile and is therefore dependent on other interactions from the scaffold binding non-covalently to the binding site of ATP, so that it is placed in a specific orientation and proximity to the target, enabling reaction.[13] The low reactivity of the warhead is an advantage, as it reduces the number of potential off-target reactions, and then gives less off-target toxicity. A well-designed scaffold is therefore the key to achieving selectivity and a good safety-profile.

Several studies have been published looking at the structure-activity relationship (SAR) for each of the inhibitors Osimertinib and Avitinib. Both Osimertinib and Avitinib center the structure of the scaffold around a 2- anilinopyrimidine. Pyrimidine occupies the adenine base binding site of ATP and forms hydrogen bonds with the hinge residue Met793.[24] The aniline ring gives hydrophobic interactions with the α-carbon on Gly796.[16] Both inhibitors also have a diamine attached to the 4-position of the aniline-ring. The effects of the different substitutes on this core are summarized in Figure 8, point 1-4.

Osimertinib does not have any 5-substituent on the pyrimidine, while Avitinib has a pyrrole group (**1**). The pyrrole gives selectivity for the double mutant L858R/T790M over WT-EGFR due to its favorable interaction with the gatekeeper Met790.[23] The double mutation results in a higher hydrophobicity, giving higher potency for this interaction. In addition it forms an additional hydrogen bond with the backbone carbonyl oxygen of Leu792, giving a higher potency.[25] On the other hand, it might be favorable to avoid a substituent in the 5-position of pyrimidine, as keeping the lipophilicity low is a goal.[26] A low lipophilicity will give better aqueous solubility and avoid binding to the plasma proteins. By not having a substituent in the 5-position, Osimertinib reduces its selectivity over WT, and potency for the double mutant. This can be compensated for by other groups on the scaffold. One of the reasons this is rational, is that a substituent in the 5-position increases the potency for the insulin-like growth factor 1 receptor (IGF1R) more than the increase in potency for the double mutant.[26] IGF1R is involved in the processing of insulin, and can lead to severe off-target toxicity in the form of hyperglycemia.[27]

Looking at the substituents of the aniline-ring, there are several differences between the two drugs. The diamine in the 4-position on Avitinib is a methyl substituted piperazine, while the one on Osimertinib is a linear ethylenediamine (**2**). In both cases the diamine serves as a basic

group, that Finlay *et al*. claims has a role of intramolecular base catalysis.[26] Analyzing the SAR, the piperazine give a higher potency for the double mutant, while ethylenediamine fragment gives the best selectivity over WT-EGFR.[26]

Osimertinib has a methoxy in 2-position, while Avitinib has a fluor in 3-position (**3**). The methoxy gives higher selectivity towards EGFR by extending towards Leu792 and Pro794 in the hinge region. In most other kinases, leucine is exchanged with a bulkier residue, something that would sterically interfere with the methoxy group. A similar potency and selectivity profile was shown for the fluor in 3-position.[25]

Both Osimertinib and Avitinib also have a substituent in the 4-position of the pyrimidine. Osimertinib has a methyl substituted indole, while Avitinib has a phenol fragment (**4**). For Osimertinib there are Van der Waals interactions between the indole and the P-loop non-polar residues Val726 and Phe723.[24] The edge of the indole also interact with the backbone of Leu718 and Gly719.[18] All these interactions increase the potency and compensates for the lower affinity for the double mutant due to the lack of a 5-substituent on pyrimidine. The methyl on the indole increases the selectivity over WT.[26]

The biggest difference is that Osimertinib has the acrylamide attached to the 5-position of the aniline ring, while Avitinib has it on the other side of the pyrimidine, at the 2-position of the phenol fragment. This results in a different folding of the scaffold for the two drugs (Figure 7). In the case of Avitinib, the positioning of the acrylamide allows it to form a hydrogen bond with the backbone of Met793, helping with the positioning for the covalent interaction with Cys797, and increasing the potency.[25] For Osimertinib, the positioning of the warhead increase the selectivity over other kinases, as the covalent bonding in addition require the P-loop to be able to bend.[18] When binding, Osimertinib induce changes in the P-loop by attracting the Phe723 towards the binding site, and not out into the solvent region as it points originally. This loop-movement induced by the scaffold on Osimertinib, pushes the acrylamide towards Cys797, consequently a covalent binding can happen.

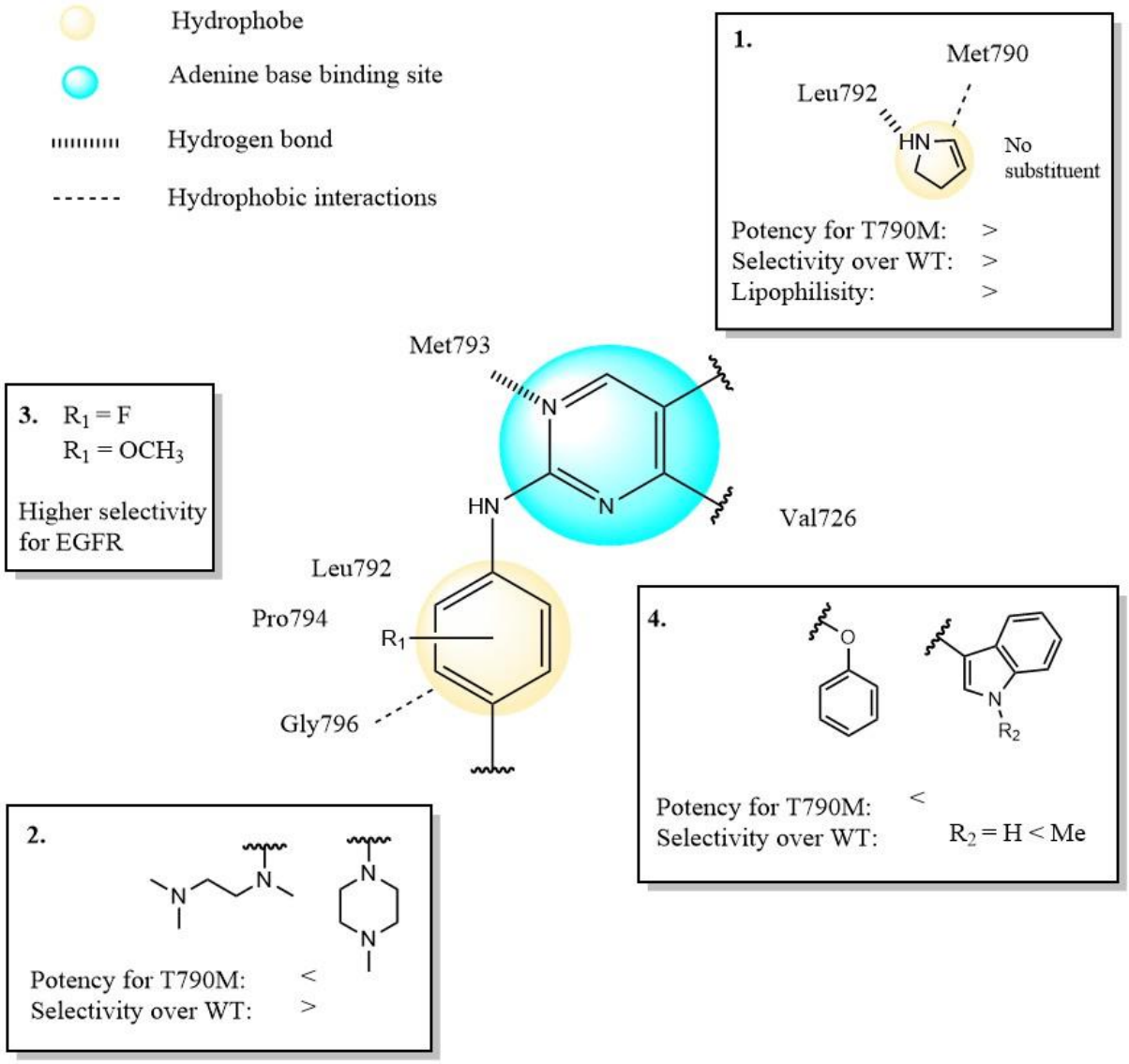

Figure 8. Overview of SAR for selected fragments of Osimertinib and Avitinib.

Osimertinib and Avitinib both aim to increase potency for the T790M mutation, while still maintaining a selectivity over the WT. However, they differ in what substituent that contributes the most for each parameter. Preclinical and clinical results give an indication to what extent the drugs succeed at achieving a balance between selectivity and potency.

Preclinical in vitro results for Osimertinib and Avitinib are given in Table 1. As expected, the reported activity on enzymes is higher than that in cellular assays. The selectivity ratio for T790M over WT-EGFR differs substantially from enzyme activity to cellular activity. Osimertinib exhibit a greater selectivity then Avitinib for enzyme activity, while Avitinib have the highest selectivity ratio for cellular activity. This can indicate a difference in cell permeability between the two inhibitors.

The literature value for cellular inhibition of EGFR by Osimertinib differs substantially from the experimental value that is given here, 15 against 214.[28] In order to compare Osimertinib and Avitinib, the experimental value will be used, as it was given from the same cellular assays and with the same method as the values for Avitinib.[27] The in vitro cellular activity show that Avitinib are more potent against the EGFR double mutant, and 4-fold more selective over WT-EGFR than Osimertinib. For cellular inhibition, Avitinib have a selectivity ratio almost 20-fold greater than that of Osimertinib. This can suggest that the increased potency for T790M Osimertinib gets from the indole (**4**), does not outweigh the increased potency Avitinib obtain from pyrrole (**1**) and piperazine (**2**). In addition, it can also imply that the pyrrole on Avitinib (**1**) has a bigger impact on selectivity over WT-EGFR, than the ethylenediamine (**2**) of Osimertinib.

The metabolites of Osimertinib can propose an additional explanation for its higher potency for WT-EGFR in cell proliferation. Osimertinib has two active metabolites: AZ7550 and AZ5104. AZ7550 has a similar profile as the parent drug, while AZ5104 exhibits a greater potency against the activating and double mutant, but also a lower selectivity against WT-EGFR, compared to Osimertinib.[28] In a cell line for EGFR proliferation and EGFR phosphorylation, none of the metabolites of Avitinib showed activity against mutated or wild type EGFR.[27] This gives a unique selectivity profile, and can possibly give an advantage over Osimertinib, that has a metabolite that is more potent against WT-EGFR than the parent drug.

Potency is here measured as the concentration of the drug that is needed to inhibit 50% of the target (IC_{50}-value). Since the inhibition is irreversible, the IC_{50}-value is time dependent.[9] The enzymatic values are recited from two different studies.[27, 28] However, their credibility is maintained as both studies compared the value to the first-generation inhibitor Gefitinib, and the ratio between Gefitinib-Osimertinib and Gefitinib-Avitinib corresponds with the Osimertinib-Avitinib ratio given.

Table 1. In vitro preclinical results for Osimertinib and Avitinib.[27]

		IC_{50} (nM)	
Study	Details	**Osimertinib**	**Avitinib**
Enzymatic inhibition of EGFR (phosphorylation)	EGFR (L858R/T790M) EGFR (WT)	1 184	0.18 7.68
Cellular inhibition of EGFR (phosphorylation - ELISA assay)	NCI-H1975 (L858R/T790M) A431 (WT)	214 1392	7.3 837
Cellular activity (cell proliferation - WTS assay)	NCI-H1975 (L858R/T790M) A431 (WT)	21.1 474	8.3 645

Table 2 gives the in vivo results for Osimertinib[28] and Avitinib.[27] The results contrast the in vitro efficacy. Here, Osimertinib shows inhibition at lower drug concentrations and has more durable tumor shrinkage than Avitinib, suggesting that it has a higher potency for the double mutant. Another explanation can be the metabolic stability of the drugs, and especially the amount that makes it way to the bloodstream. In an additional in vivo study of Osimertinib, it also showed moderate inhibition of tumor growth in A431 cells (WT).[28] Avitinib did not exhibit any antitumor activity in the A431 xenograft.[27] This supports the in vivo results of Avitinib having a higher selectivity over WT-EGFR than Osimertinib.

Table 2. In vivo preclinical results for Osimertinib and Avitinib, evaluated in mouse xenograft.[27]

			Osimertinib			**Avitinib**
Pharmacodynamic study – daily doses						
	Dose [mg/kg/day]		H1975 (L858R/T790M)	Dose [mg/kg/day]		H1975 (L858R/T790M)
Dose-dependency for inhibition of tumor growth. Measured at day 14.	1 5		<30% 75%	12 50 200		71% 79% 78%
	5[mg/kg/day]		H1975 (L858R/T790M)	200[mg/kg/day]		H1975 (L858R/T790M)
Durability of tumor shrinkage.[a] [Complete response]	Day 40 Day 200		10/12 10/12	Day 21 Day 143		14/14 7/14
Pharmacodynamic study – single dose						
Dose-dependency for inhibition of EGFR phosphorylation [inhibition]	Dose [mg/kg]	Time after dose [h]	H1975 (L858R/T790M)	Dose [mg/kg]	Time after dose [h]	H1975 (L858R/T790M)
	5	1 6 16 24 30	No Yes Yes Yes Yes	50 200	1 4 8 24 1 4 8 24	Yes Yes Yes No Partial Yes Yes Yes

Avitinib and Osimertinib show a dose-dependent inhibition in the preclinical studies. The efficacy of the drugs have further been evaluated in phase I/II clinical trials (Table 3) for patients who have previously been treated with a first-generation inhibitor, and developed resistance. Here they both show a similar overall response rate (ORR) at 50% and 51% for drug concentrations sufficient to give effect.

One interesting parameter is the half-life of the drugs. The half-life of Avitinib is very short, compared to that of Osimertinib (8 against 55 hours).[29, 30] One advantage with the rapid clearance of Avitinib is that it, due to the irreversible nature of the inhibition, gives a lower chance for off-target toxicity without going on compromise with the efficacy.[9]

Table 3. Results from phase I/II clinical trials for Osimertinib,[30] and Avitinib.[29]

		Osimertinib		**Avitinib**
Pharmacokinetic, PK				
Half-life of drug ($t_{1/2}$)	Dosing range 20-240mg	55h (29.6-145)	Dosing range 50-600mg	7.76h (7.15-8.12)
Steady-state concentration of drug		After 22 days		After 7 days
Time to reach maximum plasma concentration (T_{max})		6h (3-24)		3h (1.0-6.0)
Tumor activity				
Overall response rate (ORR)	Total (n=239)	51%	Daily dose ≥ 350mg (n=36) Daily dose < 350mg (n=16)	50% 6.25%
Disease control rate (DCR)	Total (n=239)	84%	Total (n=52)	65.4%

[a]ORR = proportion of patients who displayed a partial response (PR) or complete response (CR).

[b]DCR = proportion of patients who achieved PR, CR, or stable disease.

Regardless of how efficient a drug is, it will not be approved for use unless it also shows a good safety-profile. The safety-profile is a result of selectivity and toxicity, and can be evaluated both preclinically and clinically. The most common adverse effects from the clinical trial are given in Table 4.

The selectivity of the inhibitors Osimertinib [28] and Avitinib [27] [1µmol/L] have been assessed against a panel of 280 and 349 kinases respectively, in addition to selected molecular targets like ion channels, receptors and transporters. Avitinib showed no significant inhibition in cellular assays for pharmacologically relevant concentrations. Eight of the 280 kinases that Osimertinib were screened against showed more than 60% inhibition at cellular level, but none of these contained cysteine similarly situated as Cys797 in EGFR. As a result, all potential inhibition would be reversible and short lived, and therefore not account for significant toxicity. It did however show some inhibition against ERBB2/4 and BLK, in which both have an Cys797 analogue.

Included in the screening were insulin-like growth factor receptor and the insulin receptor.[27] The inhibitors did not exhibit significant activity in the cellular assay, nor did they affect the insulin levels or affect the blood glucose in rats dosed with the drug. Another important anti-target is hERG, where off-target inhibition can cause irregular heartbeat leading to seizures and death. None of the inhibitors showed significant inhibition of hERG.

The increase in transaminase as an adverse effect of Avitinib gave more severe toxicity for patients with preexisting liver impairment. It is not a common toxicity for Osimertinib, suggesting that for these patients, Osimertinib would be preferred over Avitinib.

In addition to the potential off-target toxicity, on-target toxicity is also an important factor. This is caused by inhibition of WT-EGFR in the skin and gastrointestinal organs, and results in skin rash and diarrhea.[28] None of the inhibitors gave a high percentage of diarrhea or skin rash of degree three or higher, suggesting that they have a sufficient selectivity over WT-EGFR for clinically relevant doses.

Table 4. The most common adverse events registered during clinical trials of Osimertinib,[30] and Avitinib.[29]

		Osimertinib, n=253	Avitinib, n=52
Treatment-emerged adverse effects [%]	Diarrhea (grade ≥ 3)	47 (4)	75 (0)
	Skin rash (grade ≥ 3)	40 (2)	48 (4)
	Increased alanine transaminase level	-	44
	Increased aspartate transaminase level	-	38
	Nausea	22	-
	Decreased appetite	21	-
Drug-related adverse effect of grade 3 or higher		32	19
Drug-related serious adverse effect		6	8
Dose giving increase in severity and adverse events		160mg/kg	350mg/kg

4. Conclusion

When developing new third generation inhibitors of EGFR, the scaffold is an important part. Its reversible interactions are the key to increasing the efficiency, and still maintaining a good safety profile. When comparing Osimertinib and Avitinib, it is not possible to conclude that one of the scaffolds are better than the other, for further optimalisation. They are a product of different substituents that as a sum provide a desired balance between potency for the T790M mutation and selectivity over WT, two properties that do not normally coincide. As a result, there is not one chemical group that would be good in all cases, but rather the knowledge that a balance is needed.

In the case of Osimertinib, the potent indole is balanced by the more selective ethylenediamine and the lack of a 5-substituent on the pyrimidine, giving a lower potency. This balance in the effects of the chemical groups on the scaffold, results in an inhibitor giving an overall response rate of 51%. The result is quite similar for Avitinib, with an overall response rate of 50%. On the other hand, the prioritizing of the chemical groups has been different. Avitinib has a pyrrole group in the 5-position of the pyrimidine, giving a higher potency for T790M and a higher selectivity over WT. The piperazine increases the potency for both T790M and WT, and as a result this needs to be balanced out with a less potent phenol-fragment in the 4-position of the pyrimidine.

Another argument for continuing to develop inhibitors with different scaffolds, is that the different scaffolds give rise to different toxicity. This is a factor that could be utilized in future development of personalized treatment.

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