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New therapeutic approaches to overcoming resistant *EGFR* exon 20 alterations

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Abstract

EGFR exon 20 alterations are rare events seen mainly in non-small cell lung cancer (NSCLC). They include *EGFR T790* and *C797S* mutations (associated with secondary resistance to classic EGFR tyrosine kinase inhibitors (TKIs)), and *EGFR* exon 20 in-frame insertions (associated with resistance to first- and second-generation EGFR TKIs). *In silico* modeling of structural changes in aberrant proteins has informed selection of compounds with potential clinical activity: poziotinib (whose smaller size permits access to the restricted kinase pocket created by *EGFR* and *ERBB2* exon 20 insertions); cetuximab (an antibody that attenuates dimerization caused by *EGFR* exon 20 insertions), and TAK-788 (another EGFR/ERBB2 TKI). Other alterations, such as *EGFR* T790M, are responsive to osimertinib, while the *EGFR C797S* alteration seen in osimertinib resistance demonstrates preclinical sensitivity to combined brigatinib and cetuximab. These observations indicate that clinical resistance can be overcome by utilizing advanced genomic interrogation coupled with computer modeling.

Keywords: NSCLC, EGFR, exon 20, TKIs, structural modeling

1.1 Introduction

Epidermal growth factor receptor (EGFR), an ErbB family member, is a tyrosine kinase enzyme involved in carcinogenesis. In non-small cell lung cancer (NSCLC), up to 90% of mutations are exon 19 deletions and point mutations in exons 18 and 21 (L858R), which sensitize tumors to EGFR tyrosine kinase inhibitors (TKIs) [1]. In contrast, *EGFR* exon 20 alterations make up a small subset of *EGFR* mutations, found mostly in NSCLC[1]. Per The Cancer Genome Atlas (TCGA) cohort (N = 7,099), alterations (of any type) in *EGFR* exon 20 represent ~11% of *EGFR* alterations across tumor types (N = 44/398 patients), are detected in ~1% of all patients with cancer (N = 44/7099 patients) (**Table 1**), and are present in ~3% of lung adenocarcinomas (N = 6/230 patients). *EGFR* exon 20 in-frame insertions of ≥3 base pairs are generally associated with primary resistance to 1st and 2nd generation TKI monotherapies (gefitinib, erlotinib, dacomitinib, neratinib, and afatinib) [1]. *EGFR* exon 20 T790M mutations correlate with secondary resistance, now treatable with osimertinib. The *EGFR* exon 20 *C797S* alteration is linked with osimertinib resistance. A single-center retrospective analysis found decreased survival in lung cancer patients carrying exon 20 alterations, compared to patients whose tumors harbor other molecular alterations [1]. Several studies proposing specific targeted therapies are currently ongoing (**Table 2**).

1.2 Therapeutic approaches aimed at EGFR exon 20 insertions in lung cancer

In-frame insertions of ≥3 base pairs in *EGF*R exon 20 were among the first EGFR mutations to be identified as oncogenic drivers in NSCLC. However, unlike the classical *EGFR* exon 19 deletions or *EGFR L858R* point mutations, which represent the majority of *EGFR* mutations in NSCLC, the uncommon *EGFR* exon 20 insertions correlate with *de novo* resistance to many targeted EGFR inhibitors and with a poor outcome.

A study investigating molecular structure found that certain *EGFR* exon 20 insertions, as well as corresponding *ErbB2*/*HER2* exon 20 insertions, caused structural changes restricting ATP-binding pocket's size [2]. Consequently, larger drugs such as lapatinib (ErbB2/HER2 inhibitor) and osimertinib (EGFR inhibitor) encountered difficulty binding to intended targets. One case report demonstrated tumor shrinkage following osimertinib treatment in a patient carrying an EGFR V769_D770InsASV variant, as well as *in vivo* effect in xenografts expressing common *EGFR* exon 20 insertions [3]; however, a larger study reported only a 6% response rate (RR) (1/17 patients) [4].

Conversely, poziotinib, a smaller TKI with a more flexible structure determined via computer modeling, demonstrated both *in vitro* and clinical activity [2]. Early results with poziotinib showed

response rates of ~60% in *EGFR* exon 20 insertion lung cancer and of ~50% in *ERBB2/Her2* exon 20 insertions [2], [5]. However, a recent press release regarding data in a phase 2 clinical trial investigating poziotinib in previously treated NSCLC patients with *EGFR* exon 20 insertions reported only a 14.8% objective response rate (17/115 patients), with a median duration of response of 7.4 months [6].

An additional compound of interest is luminespib, an inhibitor of heat shock protein 90 (Hsp90), a chaperone protein that interacts with a variety of cellular proteins (including EGFR). One recent phase II clinical trial in lung cancer patients with exon 20 insertions demonstrated a 17% RR (5/29 patients), though median progression-free survival (PFS) was low (at 2.9 months) [7].

TAK-788, a novel EGFR2/HER2 inhibitor, is also being studied in the context of exon 20 insertions; a phase 1/2 study in *EGFR* exon 20 insertion lung cancer patients demonstrated responses in 14 of 26 lung cancer patients (54%); decreased target lesion size was also observed in 23 of 24 patients [8].

Elsewhere, another study utilized modeling to demonstrate that certain *EGFR* exon 20 alterations may promote receptor dimerization and could be sensitive to EGFR antibodies targeting this domain [9]. Two patients carrying *EGFR* D770_P772delinsKG and *EGFR* D770>GY, respectively, were treated with one such antibody—cetuximab--as part of their regimen, and achieved ongoing partial response at 6+ and 42+ months [9]. In another study, 3 of 4 patients receiving cetuximab combined with afatinib also achieved responses [10]. This strategy has been explored in *ErbB2*/*HER2* exon 20 mutation-positive patients: a trastuzumab-based regimen combined with lapatinib showed remarkable tumor regression in a case of metastatic lung adenocarcinoma [11]. Keeping in mind reporting bias, 6 of 7 reported patients with exon 20 insertions in *EGFR* or *ErbB2/HER2* achieved response with regimens that included a targeted antibody.

1.3 *EGFR* T790M and C797S mutations represent additional challenges

Aside from insertions, the *EGFR* T790M in exon 20 has also been identified as a significant driver of acquired resistance. Dual blockade with cetuximab and afatinib demonstrated a RR of 32% in T790M mutation-positive lung cancers, far exceeding the 7% response rate to afatinib monotherapy reported [12], [13]. However, the drug of choice is osimertinib. Osimertinib obtained Food and Drug Administration (FDA) and European Medicines Agency (EMA) approval as first-line treatment of metastatic NSCLC patients with common *EGFR* mutations (exon 19 deletions and exon 21 L285R mutations) on the strength of a clinical trial showing significantly longer PFS (18.9 months, compared to traditional EGFR TKIs at 10.2 months) [14]; objective response rates to osimertinib in patients with lung cancer and EGFR T790M alterations are 60-70%.

The *EGFR C797S* mutation represents another significant driver of acquired resistance that interferes with osimertinib binding. Structural analyses identified brigatinib as a compound able to fit in the altered ATP-binding pocket in *EGFR* "triple mutant" cells (containing C797S and T790M). Researchers demonstrated pre-clinical *in vitro* and *in vivo* activity, and noted that the efficacy of brigatinib appears enhanced when combined with cetuximab, presumably because of decreased surface and total EGFR expression [15]. This strategy of combining EGFR inhibitors and targeted antibodies may be successful as translational studies have suggested survival mechanisms for cancer cells independent of EGFR kinase activity [16].

Preclinical work has also identified additional molecules of potential importance for *EGFR* exon 20 insertion cancers. For instance, TAS6417 is a novel EGFR inhibitor targeting exon 20 insertions that shows *in vitro* activity in cell viability assays and *in vivo* activity in lung orthotopic implantation mouse models [17], [18]. Indeed, TAS6417 was a potent inhibitor against the most frequent *EGFR* mutations (exon 19 deletions and L858R) and against cells carrying *EGFR* T790M mutations; moreover, TAS6417 demonstrated activity in cells driven by less frequent alterations such as *EGFR* G719X, L861Q, and S768I mutations. For recalcitrant *EGFR* exon 20 insertion mutations, selectivity indices (wild-type *EGFR*/mutant *EGFR* ratio of inhibition) favored TAS6417 as compared to osimertinib and poziotinib, suggesting a wider therapeutic window [19]. Tarloxotinib, a hypoxia-activated EGFR TKI, has also shown activity in patient-derived lung cancer cell lines carrying *EGFR* exon 20 insertions [20].

Of interest, pretreatment *EGFR* T790M mutations have been associated with genetic susceptibility to lung cancer [21]–[23] Because germline transmission of this mutation is a possibility, genetic counseling is recommended in these patients.

1.4 Conclusions

EGFR exon 20 alterations are heterogeneous and include both sensitive and resistant mutations [24], [25]. For instance, S768I is a mutation that does not restrict sensitivity to different TKIs [26]–[28] . It is often associated with other mutations, such as exon 18 G718X, with the latter being sensitive to several drugs including afatinib, neratinib and osimertinib [29], [30]. On the other hand, *EGFR* exon 20 insertion alterations as well as *T790M* and *C797S* mutations mediate both primary and secondary resistance to traditional EGFR TKIs and were previously considered undruggable [30], [31]. However, with structural protein analysis, careful preclinical studies, and clinical innovation, multiple new treatment

strategies now appear viable (**Table 2**). This family of alterations demonstrates how advanced genomics and computer modeling can be exploited in the clinic in order to overcome resistance.

Table 1: Frequency of non-silent *EGFR* mutations in The Cancer Genome Atlas (TCGA) (https://portal.gdc.cancer.gov/). (Total N = 7,099 patients)

	All patient s N	*EGFR*-mutated patient s N (% of patients)	*EGFR* exon 20 altered patients N (% of patients)
All tumor types	**7,099**	**398 (6%)**	**44 (1%)**
Colon adenocarcinoma	154	75 (49%)	22 (14%)*
Glioblastoma multiforme	290	74 (26%)	5 (2%)
Lung adenocarcinoma	230	72 (31%)	6 (3%)
Lower Grade Glioma	286	35 (12%)	1 (0%)
Cutaneous Melanoma	343	20 (6%)	0 (0%)
Head/Neck squamous cell carcinoma	279	20 (7%)	0 (0%)
Stomach adenocarcinoma	289	19 (7%)	0 (0%)
Rectum adenocarcinoma	69	9 (13%)	2 (3%)
Endometrial Carcinoma	248	8 (3%)	0 (0%)
Bladder Urothelial Carcinoma	130	7 (5%)	0 (0%)
Diffuse Large B-cell Lymphoma	48	7 (15%)	0 (0%)
Kidney renal clear cell carcinoma	417	6 (1%)	1 (0%)
Ovarian serous adenocarcinoma	316	6 (2%)	1 (0%)
Hepatocellular carcinoma	198	6 (3%)	3 (2%)
Lung squamous cell carcinoma	178	6 (3%)	0 (0%)
Breast invasive carcinoma	977	5 (1%)	0 (0%)
Cervical squamous cell & adenocarcinoma	194	5 (3%)	1 (1%)
Esophageal carcinoma	185	5 (3%)	0 (0%)
Prostate adenocarcinoma	332	3 (1%)	0 (0%)
Sarcoma	247	2 (1%)	0 (0%)
Acute Myeloid Leukemia	197	2 (1%)	0 (0%)
Adrenocortical carcinoma	90	2 (2%)	0 (0%)
Kidney renal papillary cell carcinoma	161	1 (1%)	0 (0%)
Pancreatic adenocarcinoma	150	1 (1%)	1 (1%)
Testicular Germ Cell Tumors	149	1 (1%)	0 (0%)
Cholangiocarcinoma	35	1 (3%)	1 (3%)
Thyroid carcinoma	402	0 (0%)	0 (0%)
Pheochromocytoma/ Paraganglioma	179	0 (0%)	0 (0%)
Thymoma	123	0 (0%)	0 (0%)
Uveal Melanoma	80	0 (0%)	0 (0%)
Kidney Chromophobe	66	0 (0%)	0 (0%)
Uterine Carcinosarcoma	57	0 (0%)	0 (0%)
Description of the EGFR alterations observed (N (%))			
All EGFR non-silent mutations		605 (100%)	
Non-exon 20 mutations		558 (92.2%)	
Exon 20 alterations		47 (7.8%)**	
Insertions	p.S768_V769insVDS	1 (0.2%)	
	p.V769_D770insASV	2 (0.3%)	
	p.D770_N771insGL	1 (0.2%)	
	p.H773_V774insH	1 (0.2%)	
	p.H773_V774insNPH	1 (0.2%)	
	p.H773_V774insVH	1 (0.2%)	
Point mutations	p.Y764H	1 (0.2%)	

	p.M766V	1 (0.2%)
	p.S768G/I/T	5 (0.8%)
	p.V769L	1 (0.2%)
	p.N771S	1 (0.2%)
	p.P772R	1 (0.2%)
	p.V774A/M	3 (0.5%)
	p.L777P	1 (0.2%)
	p.S784F/P	2 (0.3%)
	p.T785I	1 (0.2%)
	p.V786M	1 (0.2%)
	p.I789M	1 (0.2%)
	p.T790M	2 (0.3%)
	p.G796S	1 (0.2%)
	p.L798P	1 (0.2%)
	p.D800G	2 (0.3%)
	p.Y801C	1 (0.2%)
	p.V802A	2 (0.3%)
	p.E804G	1 (0.2%)
	p.H805R	1 (0.2%)
	p.K806R	1 (0.2%)
	p.D807E/H	2 (0.3%)
	p.G810D	1 (0.2%)
	p.S811C	1 (0.2%)
	p.Y813C	1 (0.2%)
	p.L814M/P	2 (0.3%)
	p.C818F/R	2 (0.3%)

Abbreviation: % = percentage; EGFR = epidermal growth factor receptor; N = number of mutations or number of patients; TCGA = The Cancer Genome Atlas

* A variety of *EGFR* exon 20 alterations were present in colorectal cancer. Only one was an EGFR T790M and no EGFR insertions were seen. The functional impact of some of these alterations is unclear.

**47 EGFR exon 20 mutations were observed in 44 patients; three patients presented multiple *EGFR* exon 20 mutations.

Table 2: Examples of therapies targeting *EGFR* and *ERBB2* exon 20 alterations, mechanism of response, and response rate.

Drug/ therapy	Alteration(s) of interest	Mechanism of response	Response rate (all NSCLC)	Citation/ Year
***EGFR or ErbB2/HER2* exon 20 insertions**				
Lapatinib + trastuzumab + based regimen	*ErbB2/HER2* exon 20 insertion (*ErbB2* 774–775 AYVM)	Similar to EGFR, ErbB2/HER2 mAb (trastuzumab) may interfere with dimerization	Case report, **objective response in 1 of 1 patient**	[11] 2013
Cetuximab-based regimen	*EGFR* exon 20 insertion (EGFR D770_P772del_insKG and D770>GY)	EGFR mAb (cetuximab) interferes with dimerization of receptors (modeling showed *EGFR* exon 20 insertions brought dimerization domains closer together)	**Objective response in 2 of 2 patients**, previously resistant to EGFR tyrosine kinase inhibitors	[9] 2015
Osimertinib	*EGFR* exon 20 insertion (V769_D770InsASV)	Small molecular TKI	Case report, single patient with clinical improvement and tumor shrinkage	[3] 2017
Poziotinib	*EGFR* and *ERBB2* exon 20 insertion	Small molecule TKI Smaller size of poziotinib versus other EGFR TKIs allows binding despite restricted drug-binding pocket caused by exon 20 insertion	**Objective response in 7 of 11** patients with *EGFR* exon 20 mutations **(64%)**	[2] 2018
			Objective response in 23 of 40 patients with *EGFR* exon 20 mutations **(58%)** **Objective response in 6 of 12** patients with *HER2* exon 20 mutations **(50%)**	[5] 2018
			Objective response in **17 of 115** patients **(15%)**	[6] 2019
Cetuximab + afatinib combination	*EGFR* exon 20 insertion	Dual *EGFR* inhibition via irreversible TKI (afatinib) and antibody binding to extracellular domain (cetuximab)	**Objective response in 3 out of 4** patients	[10] 2018
Osimertinib	*EGFR* exon 20 insertion	Small molecular TKI	**Objective response in 1 of 17** patients **(6%)**	[4] 2018
Luminespib	*EGFR* exon 20 insertion	Heat shock protein 90 inhibition	**Overall response in 5 of 29** patients **(17%);** median **progression-free survival of 2.9 mos**	[7] 2017
TAK-788	*EGFR* exon 20 insertion	EGFR/HER2 TKI	**Objective response in 14 of 26** patients (54%)	[8]

TAS6417	*EGFR* exon 20 insertion	EGFR/HER2 inhibitor	Preclinical *in vitro* and *in vivo* activity	[17]–[19]
Tarloxotinib	*EGFR* exon 20 insertion	EGFR/HER2 TKI in hypoxia	Preclinical *in vivo* activity	[20]
***EGFR* exon 20 T790M or C797S**				
Cetuximab + afatinib combination	T790M	Dual *EGFR* inhibition via irreversible TKI (afatinib) and antibody binding to extracellular domain (cetuximab)	**Objective response** in **32%** of T790M-positive patients for afatinib plus cetuximab; (**Objective response in ~7% for afatinib alone**)	[12], [13] 2012, 2014
Osimertinib	T790M	Osimertinib is a third generation TKI	**Objective response rates of ~60-70%**	[14]
Brigatinib + cetuximab	C797S	Dual *EGFR* inhibition via TKI (brigatinib) and antibody binding to extracellular domain (cetuximab)	Preclinical *in vitro* and *in vivo* activity	[15] 2017

Abbreviations: EGFR = epidermal growth factor receptor; ErbB2/HER2 = human epidermal growth factor receptor 2; NSCLC = non-small cell lung cancer; PFS = progression-free survival; TKI = tyrosine kinase inhibitor

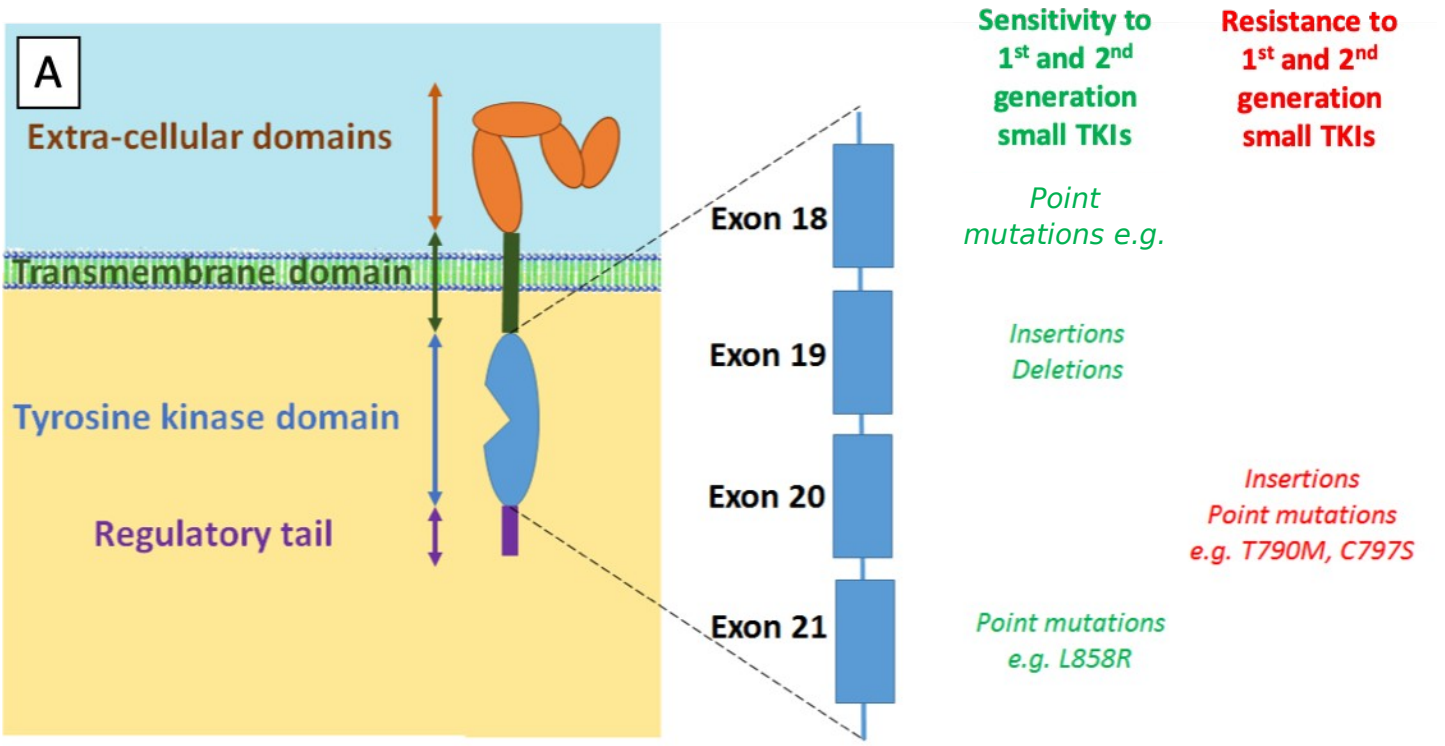
Figure 1: EGFR receptor structure, tyrosine kinase domain variants and sensitivity to small tyrosine kinase inhibitors.

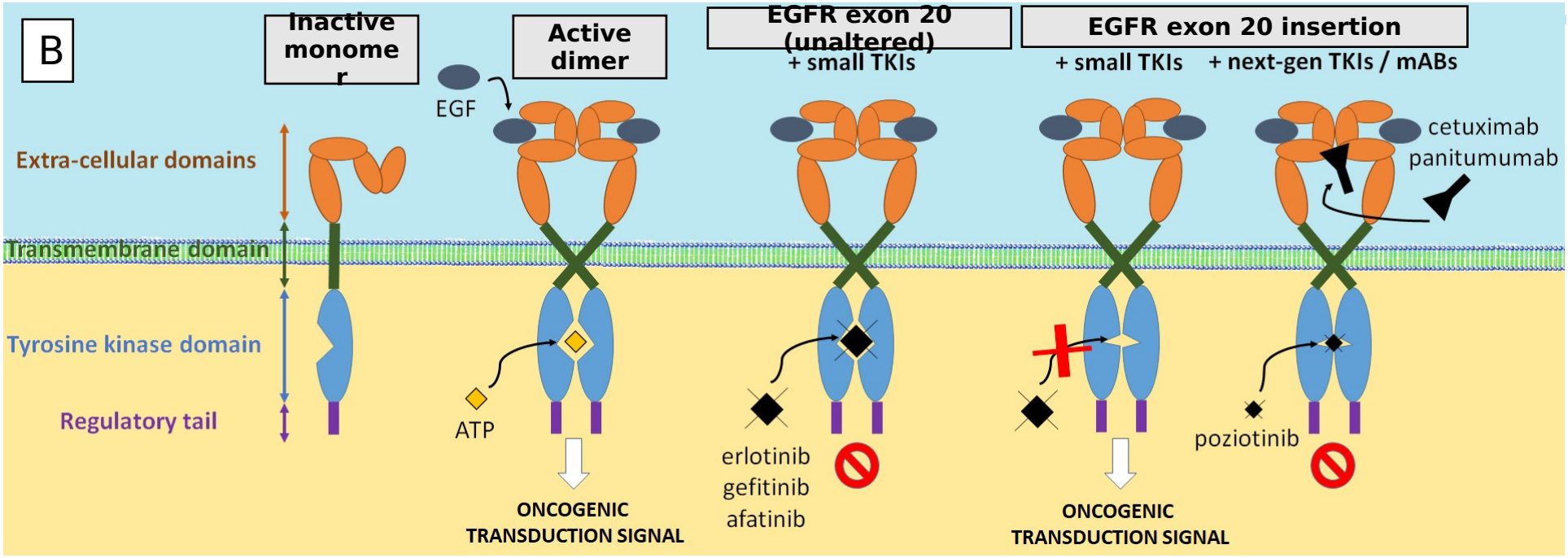
Panel A: The tyrosine kinase domain of EGFR is encoded by exons 18 to 21. Point mutations and small insertions/deletions located within exons 18, 19 and 21 generally confer sensitivity to first- and second-generation tyrosine kinase inhibitors. Point mutations and small insertions/deletions located within exon 20 confer resistance to first- and second-generation tyrosine kinase inhibitors.

Panel B: Upon ligand binding, the EGFR moieties dimerize, the tyrosine kinase domains come closer to each other (1 donor, 1 receiver). The ATP molecule binds to the tyrosine kinase, leading to the consecutive phosphorylation of the regulatory tail and later activation of the intracellular oncogenic signal.

In non-exon 20 mutated EGFR tumors, classical tyrosine kinase inhibitors (TKIs) compete with ATP for binding to the ATP-binding pocket and decrease/inhibit the intracellular transduction cascade.

In exon 20 mutated EGFR tumors, the structure of the ATP-binding pocket is smaller, and TKIs can no longer bind. The receptor remains active and the oncogenic signal persists. The use of monoclonal antibodies (mAb) or smaller next-generation TKIs may circumvent the resistance conferred by exon 20 alterations.





Abbreviations: ATP = adenosine triphosphate; EGFR = epidermal growth factor receptor; mABs = monoclonal antibodies; TKI = tyrosine kinase inhibitors

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