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EDITORIAL

Tyrosine kinase inhibitors: Multi-targeted or single-targeted?

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Abstract

Since in most tumors multiple signaling pathways are involved, many of the inhibitors in clinical development are designed to affect a wide range of targeted kinases. The most important tyrosine kinase families in the development of tyrosine kinase inhibitors are the ABL, SCR, platelet derived growth factor, vascular endothelial growth factor receptor and epidermal growth factor receptor families. Both multi-kinase inhibitors and single-kinase inhibitors have advantages and disadvantages, which are related to potential resistance mechanisms, pharmacokinetics, selectivity and tumor environment. In different malignancies various tyrosine kinases are mutated or overexpressed and several resistance mechanisms exist. Pharmacokinetics is influenced by interindividual differences and differs for two single targeted inhibitors or between patients treated by the same tyrosine kinase inhibitor. Different tyrosine kinase inhibitors have various mechanisms to achieve selectivity, while differences in gene expression exist between tumor and stromal cells. Considering these aspects, one type of inhibitor can generally not be preferred above the other, but will depend on the specific genetic constitution of the patient and the tumor, allowing personalized therapy. The most effective way of cancer treatment by using tyrosine kinase inhibitors is to consider each patient/tumor individually and to determine the strategy that specifically targets the consequences of altered (epi)genetics of the tumor. This strategy might result in treatment by a single multi kinase inhibitor for one patient, but in treatment by a couple of single kinase inhibitors for other patients.

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Key words: Tyrosine kinase inhibitors; Targeted therapy; Epidermal growth factor receptor; Vascular endothelial growth factor receptor; Platelet derived growth factor; Breakpoint cluster region-Abelson murine leukemia oncogene homolog 1; Janus kinase

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INTRODUCTION

One of the most common treatments for cancer includes the use of cytotoxic chemotherapeutics. However, this type of treatment, which is based on the difference in cell division rate between normal and cancer cells, is accompanied by several side effects due to the general vulnerability of cells to cytotoxic therapeutics. In order to reduce or change these side-effects, targeted therapies, which specifically attack signaling pathways driving the growth of tumors, are being developed. In addition to different side-effects, these therapeutics may result in a higher efficacy. In practice, they may result in activity against other tumors. An important mechanism in signal transduction pathways in cells is protein phosphorylation, which is carried out by protein kinases. These kinases regulate the fundamental processes of proliferation, differentiation, migration, me-

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tabolism and anti-apoptotic signalling of the cell.

The most important protein kinases are the serine/threonine and tyrosine kinases, which are characterized by their ability to catalyze the phosphorylation of serine/threonine or tyrosine amino acid residues in proteins, respectively. This paper will mostly focus on tyrosine kinases. Two classes of tyrosine kinases are distinguished: receptor tyrosine kinases and cellular tyrosine kinases. Receptor tyrosine kinases consist of an extracellular ligand binding domain, a transmembrane domain and an intracellular catalytic domain (Figure 1). Dimerization of two receptor tyrosine kinases upon ligand binding results in autophosphorylation of the tyrosine residues of the intracellular catalytic domains, which leads to an active conformation and subsequent activation of the signal transduction cascade within the cell. In this downstream signal transduction cascade, cellular tyrosine kinases play a primary role. The latter are located in the cytoplasm or in the nucleus[1]. In Figure 1, an example of signal transduction pathways by protein phosphorylation by epidermal growth factor receptor (EGFR) signalling is given.

Because of their important effects on cells, tyrosine kinases are highly regulated. When these kinases become constitutively activated and independent of ligands by mutations or over-expression, cancer develops by unregulated cell proliferation amongst other mechanisms. For this reason, tyrosine kinase inhibitors can serve as anticancer agents by interfering with this unregulated process[2]. Tyrosine kinase inhibitors are divided in monoclonal antibodies and small molecule tyrosine kinase inhibitors (TKIs). The latter are the subject of this paper. TKIs appear to stabilize tumor progression in many tumor types, have minimal or different side effects compared to cytotoxic chemotherapeutic agents and are often synergistic in combination with radiotherapy and/or chemotherapy[3]. A current trend in the development of tyrosine kinase inhibitors is the assumption that multi targeted therapy, which targets several signaling pathways simultaneously, is more effective than single targeted therapy. Single targeted therapies have shown activity for only a few indications and most solid tumors show deregulation of multiple signaling pathways. For example, the combination of a vascular endothelial growth factor receptor (VEGFR) inhibitor and platelet derived growth factor receptor (PDGFR) inhibitor results in a cumulative antitumor efficacy[4].

The hypothesis that altered signal transduction pathways are most effectively inhibited by multi-kinase inhibitors leads to the subsequent question: is it better to use several single inhibitors or single inhibitors with multiple effects? The first part of this paper deals with several tyrosine kinase inhibitors that are in clinical development or are recently approved. Subsequently, issues that might be important in addressing the question "what's better: multi single or a single multi?" will be discussed.

TYROSINE KINASE INHIBITORS IN CLINICAL DEVELOPMENT

In the human genome, at least 90 tyrosine kinases have been identified[5]. Fifty-six receptor tyrosine kinases are expressed, which can be subdivided in 19 families (AATYK, ALK, AXL, DDR, EGFR, EPH, FGFR, INSR, MET, MUSK, PDGFR, PTK7, RET, ROR, ROS, RYK, TIE, TRK and VEGFR family)[6]. In addition, 32 cellular tyrosine kinases are expressed, which can be subdivided in 11 families [ABL, ACK, CSK, focal adhesion kinase (FAK), FES, FRK, JAK, SRC-A, SRC-B, TEC and SYK family]. Among these, the ABL, SCR, EGFR, PDGFR and VEGFR families have been the primary targets for development of tyrosine kinase inhibitors.

Tyrosine kinase inhibitors are being developed to block abnormal signalling of signal transduction pathways that are involved in cellular growth and proliferation. While some tyrosine kinase inhibitors specifically inhibit one or two tyrosine kinases, most of the tyrosine kinase inhibitors are designed to inhibit more tyrosine kinases in multiple signalling pathways. Some tyrosine kinase inhibitors of the most important cellular and receptor tyrosine kinase families will be discussed, as well as several approved tyrosine kinase inhibitors and tyrosine kinase inhibitors in development.

Cellular tyrosine kinase inhibitors

Many tyrosine kinase inhibitors are designed to target the fusion protein Bcr-Abl and members of the SRC tyrosine kinase family. In addition, several tyrosine kinase inhibitors in preclinical development are designed to target the JAK tyrosine kinase family. A few novel tyrosine kinase inhibitors are in preclinical development for the less well-known cellular tyrosine kinases. An example is piceatannol that is targeted against ZAP70, a member of the SYK tyrosine kinase family[7]. In Table 1, an overview is given of approved tyrosine kinase inhibitors and tyrosine kinase inhibitors in development that target the cellular tyrosine kinase families ABL, SRC and JAK.

The ABL family: The most important cellular target for tyrosine kinase inhibitor development has been the fusion protein Bcr-Abl, which is the responsible protein for the cancer types chronic myeloid leukemia (CML) and B-cell acute lymphoblastic leukemia (B-ALL)[8]. The approved tyrosine kinase inhibitor imatinib mesylate (STI571, Gleevec) induces complete response in 91% of chronic phase CML patients. However, in later stages of the disease resistance is often experienced[9]. Nilotinib (AMN107) and dasatinib (BMS354825) are designed to overcome imatinib resistance in CML. Nilotinib is a selective Bcr-Abl inhibitor, which is more potent (20 fold) than imatinib against wild-type Bcr-Abl and is also active against 32 of 33 (except for T315I) imatinib-resistant Bcr-Abl mutants. The inhibitor has been evaluated in phase I and Ⅱ studies in imatinib-resistant CML and phase I studies in ALL[10,11] and has recently been approved[12], along with dasatinib, which is a dual inhibitor of both Bcr-Abl and SRC, which reversed 14 of 15 imatinib-resistant mutants[13]. Another promising dual Bcr-Abl/SRC inhibitor is PD166326, which shows 100-fold more potency than imatinib and may also reverse acquired resistance to imatinib[14,15].

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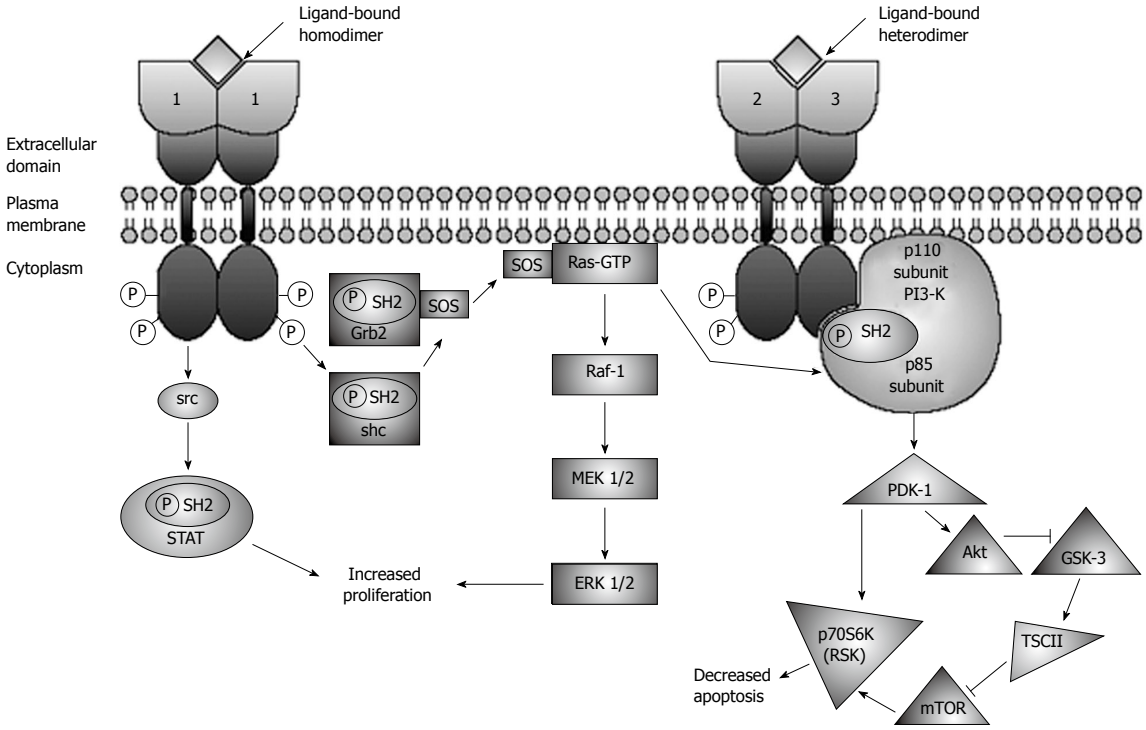

Figure 1 Homodimerization or heterodimerization of two epidermal growth factor receptors activates a cascade of tyrosine kinases phosphorylation resulting in increased proliferation and decreased apoptosis.

Receptor tyrosine kinase inhibitors

Most tyrosine kinase receptor inhibitors are designed to target the EGFR, PDGFR and VEGFR tyrosine kinase families. A few receptor tyrosine kinase inhibitors targeting tyrosine kinase receptors that are suggested to be relatively less important are also being developed. Examples are the MET inhibitor PHA-665752 and the IGF1R inhibitors AG1024 and picropodophyllin. These inhibitors, however, are still in the preclinical phase.

An overview of single-targeted and multi-targeted EGFR tyrosine kinase inhibitors is given in Table 2.

The EGFR family: Receptor tyrosine kinases play an important role in almost all types of cancer[16]. The EGFR family consists of the tyrosine kinase receptors EGFR (ErbB1, Her1), ErbB2 (Her2), ErbB3 (Her3) and ErbB4 (Her4). The activation of these kinases results in deregulation of cell growth, avoidance of apoptosis and angiogenesis in epithelial malignancies[17,18]. EGFR mutations play an important role in non-small cell lung cancer (NSCLC), while several mutations enhance the sensitivity to EGFR tyrosine kinase inhibitors gefitinib and erlotinib[19,20]. The tyrosine kinase inhibitor gefitinib inhibits mutated EGFR and is registered for treatment of NSCLC. Gefitinib is active in patients who have mutations in the EGFR kinase domain and to a lesser extent EGFR amplification, which corresponds with 10% of the patient group[20,21]. In the IPASS study patients were randomized to gefitinib alone or docetaxel and carboplatin. Molecular analysis for about a third of the patients suggested that the benefit of gefitinib was limited to patients with EGFR mutations with a progression free survival of 9.5 mo and those treated with docetaxel-carboplatin (6.3 mo) ($P < 0.001$). Gefitinib treatment was detrimental for patients without mutations (progression-free survival less than 3 mo)[22]. When patients were selected for gefitinib treatment based on EGFR mutation status (WJTOG study), patients had longer progression-free survival (9.2 mo) if they were treated with gefitinib than if they were treated with cisplatin plus docetaxel (6.3 mo) ($P < 0.0001$)[23].

Clinical trials are ongoing for other types of cancer. Another approved inhibitor of EGFR, erlotinib, which is used in a selected patient group with NSCLC, is usually preferred to gefitinib. However, although an initial response rate of 75% has been shown in patients with mutations in EGFR, these patients only rarely achieve complete response. In half of the patients this is due to a T790M mutation in EGFR[24]. Though lung tumors might be resistant to erlotinib, metastases of these tumors to the brain can be sensitive to the drug[25]. Another EGFR inhibitor is lapatinib, which in addition to EGFR, inhibits ErbB2[26]. This inhibitor gave good results in Phase Ⅲ ErbB2 positive breast cancer trials[27]. Canertinib is an inhibitor of all EGFR family members[28]. Phase Ⅱ studies are ongoing in metastatic NSCLC and breast cancer. In addition, many other single and multiple EGFR inhibitors are in clinical development.

The VEGFR/PDGFR family: The PDGFR family

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Table 1 Single-targeted and multi-targeted inhibitors of cellular tyrosine kinases ABL, SRC and janus kinase family

Inhibitor	Tyrosine kinase target	Neoplasm(s) targeted	Clinical status
Bcr-Abl inhibitors			
Imatinib mesylate (STI571)	Bcr-Abl, Kit, PDGFRβ	CML, ALL, GISTs	Approved
Nilotinib (AMN107)	BcrAbl, Kit, PDGFRβ	CML, Pcp ALL, HS, SM	Approved
Tyrphostin AG1024	Bcr-Abl, P-Akt	CML (refract to STI571)	Preclinical
ON012380	Bcr-Abl	CML	Phase Ⅰ
Tyrphostin AG957	Bcr-Abl	CML	Preclinical
Adaphostin	Bcr-Abl	CML	Preclinical
NS-187	Bcr-Abl, Lyn	CML, Pcp ALL	Preclinical
SRC family inhibitors			
Dasatinib (BMS354825)	Bcr-Abl, SRC	Adult CML, Pcp ALL	Approved
SKI-606	Bcr-Abl, SRC	Pcp leukemia, breast	Phase Ⅰ / Ⅱ
AZD0530	Bcr-Abl, SRC	Solid tumors	Phase Ⅰ
		Lung, liver, colorectal	Phase Ⅱ
TG100435	SRC, Lyn, abl, yes, lck, EphB4	Anti-cancer	Preclinical
PD166326	Bcr-Abl, SRC, lyn	CML	Preclinical
PD180970	Bcr-Abl, SRC, Kit	CML	Preclinical
AZM475271	SRC	Pancreatic	Preclinical
CGP76030	Bcr-Abl, SRC	CML, prost, bone met	Preclinical
PP1	FYN, Lck, Kit, Bcr-Abl	CML	Preclinical
PP2	SRC, Lck, Fyn, Hck	Colon	Preclinical
AP-23236	SRC	Bone metastases	Preclinical
PD173955 + 56	BA, Kit, csk, PDGFRβ, EGFR	CML	Preclinical
SU6656	Scr, Fyn, Yes, Lyn	Lymphoma	Preclinical
CGP77675	SRC	Bone	Preclinical
SU11333 + 36	SRC	Breast, colon	Preclinical
JAK inhibitors			
CP-690 550	JAK3	Immunogenic	Preclinical
AG-490	JAK2/3, EGFR	Leukemia, glioma	Preclinical
WHI-P154	JAK3, Hck, Syk	Neural precursor cells	Preclinical
WHI-P131	JAK3	Leukemia, GVHD	Preclinical
WP-1034	JAK, Stat	AML	Preclinical
LFM-A13	JAK2, BTK	Leukemia	Preclinical

BTK: Bruton's tyrosine kinase; CML: Chronic myeloid leukemia; Pcp: Philadelphia chromosome positive; ALL: Acute lymphoblastic leukemia; GIST: Gastrointestinal stromal tumors; HS: Hypereosinophilic syndrome; SM: Systemic mastocytosis; GVHD: Graft-versus-host disease; Cancer: Several cancer indications; PDGFR: Platelet derived growth factor receptor; EGFR: Epidermal growth factor receptor.

Table 2 Tyrosine kinase inhibitors of the epidermal growth factor receptor tyrosine kinase family

Inhibitor	Tyrosine kinase target	Neoplasm(s) targeted	Clinical status
Gefitinib (Iressa, ZD1839)	EGFR	Lung, breast	Approved
Erlotinib (Tarceva, OSI774)	EGFR	Lung	Approved
Lapatinib (Tykerb, GW-572016)	ErbB1, ErbB2	Breast, RCC, gastric, HN, NSCLC	Approved
Canertinib (CI-1033)	EGFR, ErbB2, ErbB3, ErbB4	Breast, NSCLC, HN	Phase Ⅰ/ Ⅱ
NVP-AEE788	EGFR, ErbB2, VEGFR	Solid tumors	Phase Ⅱ
XL647	EGFR, ErbB2, VEGFR, EphB4	Cancer	Phase Ⅰ
PKI-166	EGFR, ErbB2	Adv solid tumors	Phase Ⅰ
SU-11464	ErbB2	Cancer	Preclinical
EKB-569	EGFR, ErbB2	NSCLC, colorectal, solid	Phase Ⅱ
AG1478	EGFR	Nasopharyngeal	Preclinical
CP724,714	ErbB2	Breast, adv solid tumors	Phase Ⅰ/ Ⅱ
PD-158780	EGFR, ErbB2, ErbB4	Cancer	Preclinical
CP-654577	ErbB2	Breast	Preclinical
BIBW2992	EGFR, ErbB2	NSCLC, HN, breast	Phase Ⅱ
HKI-272	ErbB2	Breast, solid tumors	Phase Ⅰ/ Ⅱ
CL-387,785	EGFR, ErbB2	Cancer	Preclinical
CP358,774	EGFR	HN, ovarian, breast	Phase Ⅱ
MP 412	EGFR, ErbB2	NSCLC, breast, HN, pancreatic, prostate	Phase Ⅰ

Trk: Nerve growth factor receptor tyrosine kinase; PKC: Protein kinase C; RCC: Renal cell carcinoma; HN: Head and neck cancer; NSCLC: Non small cell lung cancer; EGFR: Epidermal growth factor receptor.

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Table 3 Tyrosine kinase inhibitors of the platelet derived growth factor receptor tyrosine kinase family

Inhibitor	Tyrosine kinase target	Neoplasm(s) targeted	Clinical status
Leflunomide (SU101)	PDGFRβ, EGFR, FGFR	Prostate cancer	Phase Ⅱ/Ⅲ
Lestaurtinib (CEP-701)	Flt3, Trk, VEGFR2	Leukemia, pancreatic, AML	Phase Ⅱ
Tandutinib (MLN518)	Flt3, PDGFRβ, Kit	AML	Phase Ⅰ/Ⅱ
SU14813	VEGFR2, PDGFRβ, Flt3	Adv malignancies	Phase Ⅰ, suspended
XL999	FGFR, VEGFR1/2/3, PDGFRβ, Flt3	Cancer	Phase Ⅱ, suspended
VX-322	Flt3, Kit	AML	Preclinical
SU5614	Flt3	AML, solid tumors	Preclinical, suspended
GTP-14564	Flt3	AML	Preclinical
KI23819	Flt3	AML	Preclinical
Gö6976	Flt3, JAK2	AML	Preclinical
AG1296	PDGFRβ, Kit, Flt3	AML	Preclinical
PKC412	Kit, PDGFRβ, PKC, Flt3, VEGFR2	Solid tumors	Phase Ⅱ
OSI 817	Kit, VEGFR	Solid tumors	Preclinical
CP-868596	PDGFRβ	Adv solid tumors	Phase Ⅰ
		NSCLC	Phase Ⅱ

Trk: Nerve growth factor receptor tyrosine kinase; PKC: Protein kinase C; RCC: Renal cell carcinoma; HN: Head and neck cancer; NSCLC: Non small cell lung cancer; PDGFR: Platelet derived growth factor receptor; EGFR: Epidermal growth factor receptor; AML: Acute myeloid leukaemia.

is involved in the pathogenesis of several tumor types. PDGFR is important in cellular growth, proliferation, differentiation and angiogenesis[29]. FLT3 duplications and point mutations are implicated in the pathogenesis of acute myeloid leukaemia (AML)[30]. KIT mutations are involved in the pathogenesis of AML, gastrointestinal stromal tumors (GIST) and systemic mast cell disease (SMCD)[31]. Multiple PDGFR inhibitors are in clinical development for cancer therapy, most of which are directed against several tyrosine kinases. In addition to its activity against Bcr-Abl, imatinib also inhibits mutated c-KIT and PDGFR. For this reason, it is used for the treatment of GIST, where 90% of the tumors harbor a c-KIT mutation and of the 10% that do not, 30%-50% harbor a mutation in PDGFR[32]. Whereas imatinib is effective against the juxtamembrane mutated c-KIT in GIST, it has no activity against active site mutations that occur in AML and systemic mastocytosis (SM). Tandutinib (MLN518), which was initially developed as a FLT-3 inhibitor, also shows activity against wild-type and juxtamembrane mutated and active site mutated (D816V) c-KIT[33]. It is being evaluated in phase Ⅱ studies for relapsed or refractory AML[34]. Another PDGFR kinase inhibitor is leflunomide. In addition to PDGFR, it partially inhibits the kinase receptors EGFR and FGFR. The drug is being evaluated for prostate and glioblastoma multiforme cancer patients in phase Ⅱ and Ⅲ trials and for several types of cancer in preclinical phases[3]. Leflunomide is also being used for treatment of rheumatoid arthritis[35]. In Table 3, an overview is given of single-targeted and multi-targeted PDGFR tyrosine kinase inhibitors in different phases of development.

Several multi kinase inhibitors that are in development target the VEGFR family in addition to the PDGFR family. The rationale behind this is that tumor survival, growth and metastasis depend on tumor cell proliferation and angiogenesis, the last of which is mediated by endothelial cell proliferation. Targeting these processes simultaneously by blocking RTKs on tumor cells and VEGF activity on endothelial cells can be relevant in cancer therapy. Semaxinib inhibits the tyrosine kinase receptors VEGFR-2, c-KIT and FLT-3. The inhibitor showed good results in phase Ⅱ AML trials[36]. However, no objective response rates were obtained in other types of cancer. A more selective angiogenesis inhibitor is vatalanib, which inhibits VEGFR1, 2 and 3. At higher concentrations the receptors PDGFRβ and c-Kit are also inhibited. Clinical studies in several types of cancer have been concluded[37,38]. The inhibitor sunitinib targets multiple kinase receptors including VEGFR, PDGFR, c-KIT, and FLT-3, thereby resulting in both direct antitumor as well as antiangiogenic activity. It is approved for progressed GIST after imatinib therapy, for intolerated imatinib therapy for GIST and for advanced renal cell carcinoma[39]. Another approved tyrosine kinase inhibitor for advanced renal cell carcinoma is sorafenib, which targets both proliferation and angiogenesis by inhibiting c-KIT and Flt-3 on the one hand and VEGFR and PDGFR on the other. In addition, it inhibits the serine/threonine RAF/MEK/ERK pathway[40]. Phase Ⅲ trials were performed in NSCLC[41] in which the drug showed moderate activity with disease stabilization (median survival of 2.7 mo) and the drug is in development for several other types of cancer. Its activity is particularly promising in hepatocellular carcinoma. The combination of sorafenib and erlotinib showed a good response (28%) and disease stabilization (46%) in NSCLC even in patients with K-ras mutations, with a median time to progression of 10 mo[42], which is thought to be due to simultaneous inhibition of multiple signaling pathways, such as the Ras pathway. The combination was additive to synergistic in NSCLC cells, with the most pronounced effect found in cells with mutant K-ras[43]. This combination is also being evaluated in hepatocellular carcinoma for which sorafenib is also registered[44]. In Table 4, an overview is given of both approved tyrosine kinase inhibitors and tyrosine kinase inhibitors in development that are targeted against both the PDGFR and the VEGFR tyrosine kinase families.

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Table 4 Tyrosine kinase inhibitors of the vascular endothelial growth factor receptor tyrosine kinase families

Inhibitor	Tyrosine kinase target	Neoplasm(s) targeted	Clinical status
Sunitinib (Sutent, SU11248)	VEGFR1/2/3, PDGFRα/β, Kit, Flt3, CSF1R, RET	GIST (imatinib resist)	Approved
		Adv RCC	Approved
		NSCLC	Phase Ⅲ
Vandetanib (ZD6474)	VEGFR2, EGFR, RET	Solid tumors	Phase Ⅰ/Ⅱ
		Adv NSCLC	Approved
		Medullary thyroid	Phase Ⅱ
Vatalanib (PTK787/ZK222584)	VEGFR1/2/3, PDGFRβ, Kit	Colorectal, prostate, RCC, MDS, AML, MM	Phase Ⅲ
			Phase Ⅰ/Ⅱ
Sorafenib (BAY 43-9006)	B-Raf, VEGFR2/3, PDGFRβ, Flt3, Kit, RAF, RET	Adv RCC	Approved
		NSCLC	Phase Ⅲ
		Melanoma, prostate	Diff phases
Semaxinib (SU5416)	VEGFR2, Kit, Flt3, Stat5, AKT	AML	Phase Ⅱ
Cediranib (AZD2171)	VEGFR1/2/3, PDGFRβ, Kit	Solid tumors	Phase Ⅰ/Ⅱ/Ⅲ
CEP-7055	VEGFR1/2/3, Flt3	Solid tumors	Phase Ⅰ/Ⅱ
Dovitinib lactate (TKI258; CHIR258)	VEGFR1/2, FGFR1/3, PDGFRβ, Flt3, Kit, CSFR1	Multiple myeloma	Phase Ⅱ/Ⅲ
CP-547,632	VEGFR2, FGFR	Ovarian, lung	Phase Ⅰ/Ⅱ
Pazopanib (GW786034)	VEGFR1/2/3, PDGFRα/β, Kit	RCC,	Approved;
		Multiple myeloma;	Phase Ⅰ/Ⅱ
		solid tumors	Phase Ⅰ/Ⅱ
OSI-930	VEGFR, Kit	Cancer, anti-angiogenic	Phase Ⅰ/Ⅱ
Foretinib (XL-880; GSK 1363089)	VEGFR, c-MET	HCC, gastric	Phase Ⅰ/Ⅱ
KRN-951	VEGFR1/2/3, PDGFRβ, Kit	Adv cancer	Phase Ⅰ/Ⅱ
SU10944	VEGFR	Solid tumors	Preclinical
ZK-CDK	VEGFR1/2/3, PDGFRβ, Cdks	Breast, prostate	Phase Ⅰ/Ⅱ, suspended
Axitinib (AG-13736)	VEGFR1/2, PDGFRβ, Kit	Solid tumors	Phase Ⅱ/Ⅲ
Motesanib diphosphate (AMG706)	VEGFR1/2, PDGFRβ, Kit, RET	Anti-angiogenic	Phase Ⅱ
		GIST (Imatinib resist)	Phase Ⅱ
		NSCLC	Phase Ⅲ
Brivanib (BMS-582664)	VEGFR2, FGFR	Solid tumors	Phase Ⅰ/Ⅱ
PD173074	VEGFR, FGFR	Solid tumors, anti-angio	Preclinical
Linifanib (M10-963; ABT-869	PDGFRβ, VEGFR2, CSF1R	NSCLC, HCC, RCC	Phase Ⅱ/Ⅲ
BAY57-9352	VEGFR2/3, PDGFRβ, Kit	Anti-angiogenic	Phase Ⅰ
SU11657	TELPDGFRβ, VEGFR, Kit, Flt3	CMML	Preclinical
Regorafenib (BAY 73-4506)	VEGFR-2, Tie-2	RCC; HCC	Phase Ⅱ
XL-184	VEGFR-2, c-MET, c-Kit, Tie-2	NSCLC, Glioblastoma, lymphoma	Phase Ⅱ
BIBF1120	VEGFR2, PDGFR, FGFR	Ovarian	Phase Ⅱ
		Adv solid tumors	Phase Ⅰ
AZD6244	MEK	NSCLC, pancreas melanoma	Phase Ⅱ
TSU-68 (SU6668)	VEGFR2, PDGFRβ, FGFR, Kit	Adv solid tumors	Phase Ⅰ/Ⅱ/Ⅲ

CMML: Chronic myelomonocytic leukemia; MDS: Myelodysplastic syndromes; MM: Multiple myeloma; HCC: Hepatocellular carcinoma; RCC: Renal cell carcinoma; NSCLC: Non small cell lung cancer; PDGFR: Platelet derived growth factor receptor; EGFR: Epidermal growth factor receptor; AML: Acute myeloid leukaemia.

WHAT'S BETTER: MULTI SINGLE OR A SINGLE MULTI?

The considerations to determine whether multiple single kinase inhibitors or a single multi kinase inhibitor is preferable in cancer therapy are based on aspects concerning efficacy, resistance, pharmacokinetics, selectivity and tumor environment.

Efficacy

Large differences exist in the extent and number of expressed tyrosine kinases between different tumor types. Whereas in AML (only) 20 different receptors are expressed, brain tumors may express 50 different receptors[45]. The variability between tumor types is large, but tumors of the same histological type tend to have more similar receptor tyrosine kinase profiles, with disease specific expression, both in number and type of receptors.

Müller-Tidow *et al*[45] postulated that in cancer types where relatively few tyrosine kinases are (over)-expressed as in AML, the importance of each kinase might be relatively higher. For this reason, specific targeting of these single kinases, will offer a greater chance of an effective treatment compared with other tumors that have a higher number of alterations in receptor tyrosine kinase expression. However, this statement only holds when all kinases have an equal share in the contribution to carcinogenesis. But, also in AML some receptors are relatively highly overexpressed compared to others and these seem most suited as targets. For cancers with many overexpressed kinases, targeted inhibition of the important kinases is likely to be more effective than randomly inhibiting a couple of receptors using a multi inhibitor. The use of either single or multi targeted inhibitors must not depend on the num-

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Table 5 Resistance mechanism to tyrosine kinase inhibitors

	Mechanism	Target/drug/disease	Ref.
1	(Secondary) mutation of the tyrosine kinase	Bcr-Abl in CML, FLT3 in AML, EGFR in NSCLC, c-KIT in GIST	[12]
2	Gene amplification and subsequent overexpression of the protein kinase	Bcr-Abl in CML + c-KIT in GIST	[12]
3a	Activation of other signaling pathways	PDGFR mutation in c-KIT mutated GIST, MET overexpression in EGFR mutated NSCLC	[50]
3b	Overexpression of kinases downstream of the kinase	LYN in CML	
4	Lower intracellular drug concentrations because of:		
4a	Extracellular sequestration of the inhibitor by binding to α acid glycoprotein	PKC412, imatinib	[99]
4b	Decreased expression or activity of drug influx pumps	OCT1, imatinib	[52]
4c	Increased expression or activity of drug efflux pumps	BCRP, P-glycoprotein (imatinib)	[51,60]

NSCLC: Non small cell lung cancer; PDGFR: Platelet derived growth factor receptor; EGFR: Epidermal growth factor receptor; CML: Chronic myeloid leukemia; GIST: Gastrointestinal stromal tumors; AML: Acute myeloid leukaemia.

ber of expressed kinases but on the importance of particular kinases in a particular type of cancer. In the case where several kinases are overexpressed and several appear to contribute to the carcinogenesis, then a single multikinase inhibitor would be most effective. A high number of RTKs are overexpressed in NSCLC[46]. Although a multikinase inhibitor seems to be advantageous because of its ability to inhibit several of the receptors that are overexpressed, it has to be noted that in contrast to the data concerning several other cancer types[45], the variability between the expression of RTKs in NSCLC is high. This variability includes both the number of expressed kinases (at least 9 in 75%, 18 in more than 50% and more than 33 in 25%) and the type of kinases and also depends on the subtype of the lung tumor. Although in some cases EGFR mutations cause tumor pathogenesis, overexpression of EGFR and other receptor tyrosine kinases is the most important mechanism of lung carcinogenesis. Besides this variability, some receptor tyrosine kinases highly increase the risk of metastasis. In addition to the EGFR family, which is known to play an important role in metastasis, other receptors such as insulin receptor (INSR) and neurotrophic tyrosine receptor kinase 1 (NTRK1) are even more important and increase the risk for metastasis up to 7-fold[46]. The tyrosine kinase receptors DKFZ1 and EPHB6 reduce the risk of metastasis. For this reason, it is important to specifically inhibit the crucial receptor tyrosine kinases that increase the metastasis risk and not to touch the suppressive ones. This specific inhibition will be more difficult using a single multi kinase inhibitor.

In addition to the variability of expressed receptor tyrosine kinases between tumor types and subtypes, the possibility exists that some receptor kinases are tumor suppressor genes or that the role of the same receptor tyrosine kinases expressed in various types of cancer can differ. As a consequence, the possibility exists that non-selective multiple kinase inhibitors can promote cancer growth. For example, although its role in cancer is not completely elucidated, evidence exists that ErbB4 can function as a tumor suppressor gene in breast, prostate and kidney epithelia[47]. As a consequence, a tyrosine kinase inhibitor like canertinib - which at the moment is evaluated in clinical trials for the treatment of breast cancer -, might stimulate rather than suppress tumor growth because it non-selectively targets members of the EGFR family. In addition, whereas EPHB6 is highly overexpressed in AML and consequently can be assumed to play a role in its carcinogenesis, the same receptor reduces the risk of metastasis in NSCLC[45,46]. Besides, the expression of several receptor tyrosine kinases is downregulated in AML[45], which questions their role as oncogenes.

Summarized, in addition to (receptor) tyrosine kinases' known function of acting as an oncogene in one setting, there is a possibility of a tumor suppressive role of these proteins in another. This underscores the necessity of caution in inhibiting them while there is a risk of (widely) testing these inhibitors for several types of cancer.

Resistance

The use of tyrosine kinase inhibitors is often accompanied by resistance. This resistance to (receptor) tyrosine kinase inhibitors can develop in several ways. Different mechanisms are summarized in Table 5.

In those types of cancer where resistance is frequently caused by a mutation in a tyrosine kinase receptor that plays an important role in the carcinogenesis, two single inhibitors with a high potency for this kinase might be more effective than one inhibitor against this kinase and several other kinases. The reason for this is that the mutation decreases the affinity of the kinase to the inhibitor. An example is given by CML, where insensitivity to imatinib most often results from point mutations in the kinase domain of Bcr-Abl[48]. A case of resistance to imatinib due to a mutation in PDGFR in addition to mutation in KIT was reported for GIST. However, resistance caused by the activation of another kinase seems unlikely in imatinib resistant leukemic patients, unless there is the possibility of resistance induction by activation of LYN[49-51]. In addition, amplification of Bcr-Abl gene is associated with resistance.

Resistance can also be caused by differential expression of the drug transporters hOCT1 and MDR1 (ABCB1, P-glycoprotein), which mediate the active cellular influx and efflux transport of imatinib, respectively[52]. Also BCRP (ABCG2) has been reported to be implicated in re-

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sistance to imatinib[53]. Binding of imatinib to a1-acid glycoprotein can cause resistance as well as the overexpression of Bcl-2 or loss of Bim and Bad. The latter can be overcome by combination therapy with the BH3 mimetic ABT-737[54]. The first way to bypass imatinib resistance is to develop inhibitors that bind Bcr-Abl with a higher affinity and therefore are able to avoid the development of resistant leukemic clones. However, it is expected that the effect of these new inhibitors will only be temporary since resistance will develop again. Since most resistance mechanisms are developed by mutations, it has been hypothesized that a combination of Bcr-Abl inhibitors, which both have different mutation profiles, might be effective to prevent the development of imatinib resistant clones[55]. Synergistic effects to imatinib-resistant Bcr-Abl cells have been observed *in vivo* when both nilotinib and imatinib were administered[56]. Since mutants arise from the way of binding with the inhibitor, a combination of imatinib or nilotinib with dasatinib should give an even more effective result. Imatinib and nilotinib bind only to the inactive conformation of Abl while dasatinib binding is independent of the conformation of Bcr-Abl[10]. It would be even better to use a combination of an ATP competitor (imatinib, nilotinib or dasatinib) and a substrate competitor such as ON012380 to inhibit each other's resistance inducing mutants by attacking different parts of the kinase[57].

In several types of cancer, resistance is caused by means of overexpression of the target kinase. In these cases inhibiting a kinase downstream of the tyrosine kinase receptor in addition to the target receptor itself will be effective because these downstream kinases are not amplified. A single multi kinase will be preferred because the sensitivity to the inhibitor is not decreased by amplification. In addition, inhibition of the kinases is not specific for cancer cells and will result in toxicity to normal cells. To minimize these side-effects it will be better to use a single inhibitor instead of two.

Fifty percent of the resistance to gefitinib and erlotinib is caused by a secondary mutation (T790M, D751Y or exon 20 insertion) in the EGFR gene[58] and in some cases by the multidrug transporter ABCG2[59,60]. K-ras mutation and p-Akt overexpression are considered as resistance mechanisms for erlotinib and gefitinib too[61,62]. Loss of PTEN has not yet been found to be associated with p-Akt overexpression or with resistance to gefitinib[62,63]. In addition to the resistance mechanisms of a secondary mutation and amplification of the target kinase, another mechanism was found to play a role in the resistance of NSCLC to gefitinib and erlotinib. Amplification of MET appeared to be responsible for another 22% of these lung tumors. The shift to this alternative tyrosine kinase receptor resulted in the activation of PI3K/Akt signaling by ErbB3 phosphorylation without the involvement of EGFR and ErbB2[64]. Based on the involvement of a second gene in the development of resistance, the use of combination therapies of MET inhibitors and EGFR inhibitors can offer effective treatment for patients that are resistant to gefitinib or erlotinib. In this case, a second single tyrosine kinase inhibitor against MET should be administered in addition to gefitinib or erlotinib. The same approach is used for RAS mutations that are responsible for resistance to erlotinib and gefitinib.

It appears that resistance in one cancer type can be caused by several resistance mechanisms. In case of a secondary mutation in EGFR, a second inhibitor of EGFR with a different matching profile would be preferable. However, in case of resistance mediated by overexpression of MET, this approach wouldn't induce any effect.

Regarding the reduced intracellular drug concentration as a mechanism for resistance, it depends on the type of cancer and the tumor cell characteristics which type of treatment will be most effective. A single multi kinase inhibitor can be very effective, but if it becomes the target of extracellular sequestration or substrate of an efflux transport pump, it will be lost. The same holds for the single inhibitors, however, in this case, more flexibility exists thanks to the possibility of switching to other single kinase inhibitors when resistance by this mechanism is detected.

It is believed that several mutations and amplifications of genes that cause primary resistance are already present before treatment is started. These mutant receptor kinases remain sensitive for particular tyrosine kinase inhibitors. For example imatinib resistant c-KIT remains sensitive to PKC412 while AP23464 is very potent in CML resistant to imatinib[65,66]. In addition, PD166326 shows increased activity against the SRC family member LYN[14]. For this reason, it is very important to identify the mutation by which the kinase is activated in order to be able to decide the most effective type of therapy that is insensitive to resistance development.

Pharmacokinetics

The pharmacokinetics of a drug is described by its absorption, distribution, metabolism and excretion (ADME), which determines the bioavailability of the drug. The pharmacokinetic properties of tyrosine kinase inhibitors are also associated with their molecular weight, hydrophobicity/hydrophilicity, hydrogen bonding and active transport; CYP enzymes and various transporters also play a major role[67-68]. Regarding these properties, a single multi inhibitor will be preferred to two single inhibitors if one of the last is a substrate for a drug transport pump or is metabolised. Such a drug will become second choice when its pharmacokinetic properties are worse. A possible problem associated with the administration of two single inhibitors is that the drug metabolism of the one single drug can interfere with the metabolism of the other. In that case, a single multi-kinase inhibitor is preferred; otherwise there is no preference. Although much is known about the pharmacokinetics of tyrosine kinase inhibitors, a complete overview is beyond the scope of this paper and only some examples will be given.

Li *et al*[69] compared the pharmacokinetics of the metabolism of two approved EGFR inhibitors gefitinib and erlotinib. It was shown that the metabolic clearance of gefitinib was higher than the metabolic clearance of erlotinib. In addition, the importance of various cytochrome P450 enzymes involved in the metabolism was different

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between both inhibitors[69]. However, to determine and compare the pharmacokinetic properties of tyrosine kinase inhibitors in a predictive way in order to show a preference for one of the types is very difficult. First, tyrosine kinase inhibitors that reached the market during the last five years have a satisfactory to good bioavailability. Second, regardless of the type of inhibitor, pharmacokinetics of a particular drug can differ between patients because of differences in metabolism and can change over time in the same patient. In this context, additional factors like the influence of smoking on the metabolism of the inhibitors play a role too. By comparing the pharmacokinetics of erlotinib in smokers and non-smokers, it has been shown that in current smokers the metabolic clearance of the inhibitor is increased[70].

Regarding imatinib a large interindividual variation was found which is related to efficacy of the drug[71]. This variation was related to alpha-acid glycoprotein binding, while a 22% reduction in clearance was observed in heterozygous compared with wild-type patients corresponding to BCRP (ABCG2 c.421C>A ($P < 0.05$). This large variation was earlier related to the expression of BCRP and P-glycoprotein[72,73]; e.g. in mice with a BCRP knock out the clearance was increased while the accumulation in brain was also increased[73]. SNPs in the BCRP gene were also associated with toxicity of sunitinib and gefitinib, possibly due to aberrant pharmacokinetics[74,75].

Regarding pharmacokinetics, it is difficult to predict which type of inhibitor will show the most optimal results in cancer patients. This is due to similarities in physicochemical properties between tyrosine kinase inhibitors and interindividual differences in drug metabolism. In addition, small differences in inhibitors that are independent of the inhibitor type can make them, for example, substrate for a drug transport pump. So, in order to determine the most optimal TKI type, comparative pharmacokinetic studies for particular TKIs in a specific setting have to be performed and will result in different conclusions depending on the investigated inhibitors and the setting.

Selectivity

The tyrosine kinase domain of protein tyrosine kinases consists of an N-terminal lobe and a C-terminal lobe, the last of which is the binding site for downstream proteins. The ATP binding site is in the cleft between the two lobes[76]. It turned out to be difficult to design small molecule inhibitors that specifically attack the substrate binding site[77]. One of the few examples is the Bcr-Abl inhibitor ON012380, which because of its possibility to bind the substrate binding site of Bcr-Abl, is very effective in inhibiting the kinase domain of mutated Bcr-Abl (10 fold more potent than imatinib)[57]. In order to obtain selectivity, tyrosine kinase inhibitors are designed to specifically attack the ATP binding site of tyrosine kinases. Although the ATP binding site is highly conserved, selectivity of tyrosine kinase inhibitors is realized by exploiting the proximal regions of the ATP binding site. Hydrogen bonds of the adenine region are used by inhibitors and increase their potency. The sugar region is used for selectivity in EGFR inhibitors because of a different amino acid residue compared to the other receptors[78]. The hydrophobic pocket (also called the selectivity pocket) and channel, which is not used by ATP-binding, play an important role in inhibitor selectivity and binding affinity, respectively. The phosphate binding region itself can be used to improve selectivity[79].

In addition to the substrate binding site and the ATP binding site of the tyrosine kinase domain, a new target useful in drug development might be the juxtamembrane region.

This region is located between the transmembrane helix and the kinase domain and autoinhibits the catalytic activity of receptor tyrosine kinases. Because of lack of sequence similarity amongst juxtamembrane regions between different receptor tyrosine kinase families, this region has been suggested to be a good target to obtain specificity and increased affinity[80].

Because it is difficult to design inhibitors that specifically inhibit single kinases, it is inevitable that other kinases, at least to a lower degree, are inhibited as well, resulting in side effects. In this way, multi kinase inhibitors are less specific and might consequently lead to more side effects.

In addition to the extent of selectivity between tyrosine kinases, regarding the side effects, it is also important to focus on selectivity for tumor cells compared to normal cells. An inhibitor like gefitinib is directed against a specific mutation, which only occurs in tumor cells. Non-small-cell lung cancer patients are selected for gefitinib and erlotinib based on their mutational status. Deletion mutations in EGFR exon 19 and EGFR L858R point mutation, which are associated with a never smoking history, female sex and Asian ethnicity, are predictive of response to these tyrosine kinase inhibitors[81]. However, if the resistance caused by MET overexpression is attacked by a MET inhibitor, this approach might lead to many side effects because this inhibitor will inhibit MET in both normal and tumor cells. So, the extent of side effects depends on the degree of targeted therapy.

In order to avoid cross reactivity of tyrosine kinase inhibitors, Fernández *et al*[82] obtained selectivity of kinase inhibitors for tyrosine kinases by comparing kinase dehydron patterns since dehydrons - the underdehydrated backbone hydrogen bonds of a kinase - are not conserved between kinases[82].

It has been suggested that the most important condition for an inhibitor to achieve specificity for a particular kinase, is the ability to adapt to multiple conformational states of the enzyme. This ability seems to be more important than differences in sequence of the kinase domain or differences in interactions with binding site residues[83]. This mechanism of specificity is shown by erlotinib, which is suggested to be dependent on the recognition and high affinity binding of many conformations of EGFR. Another specificity mechanism dependent on conformation is shown by imatinib, which shows highly specific inhibition of PDGFR, c-KIT and Abl. Whereas SRC is phylogenetically less divergent from Abl than PDGFR and c-KIT are, imatinib shows no inhibition of SRC. This is explained by specific inhibition of the inactive protein conformation

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that is unique for PDGFR, c-KIT and Abl. Another example of a specificity mechanism is that of dasatinib, which is suggested to inhibit both Abl, c-KIT, SRC and LCK because of its ability to recognize multiple conformational states of many different targets[83]. The systematic analysis of crystal structures of tyrosine kinases is suggested to be useful in the design of more potent and selective tyrosine kinase inhibitors[67].

To increase potency and to minimize side-effects, it is essential to design tyrosine kinase inhibitors that selectively block the tyrosine kinase or kinases that are involved in the aberrant signaling. For the purpose of finding selectivity of the tyrosine kinase inhibitors several mechanisms, like differences in sequences, kinase dehydron patterns and conformation states of the kinases are investigated.

Tumor microenvironment

Tumors are not built up solely of tumor cells. An important part consists of connective tissue or stroma, made up of stromal cells and extracellular matrix, which is produced by these cells. Examples of stromal cells are fibroblasts, endothelial cells and macrophages. Stromal cells also play an important role in the carcinogenesis, where they are characterized by upregulation or induction of growth factors and their receptors, adhesion molecules, cytokines, chemokines and proteolytic enzymes[84,85].

In order to discuss a possible choice between single multi kinase inhibitors and multiple single kinase inhibitors, one has to compare cancer cells and stromal cells and to look at different types of tumor stroma. The receptor associated tyrosine kinases VEGFR-1 and VEGFR-2 on endothelial and tumor cells play a central role in the promotion of cancer by their involvement in angiogenesis. Inhibition of these receptors by, for example, the tyrosine kinase inhibitors sunitinib, vatalanib and sorafenib has led to good results in cancer therapy. Another important tyrosine kinase is FAK, which is required for the activity of integrin and growth factor receptors in endothelial cells and fibroblasts. FAK regulates cellular adhesion, migration and survival. Inhibition of FAK could be a good way to prevent survival of these tumor stromal cells. In addition, the growth factors TGF-β, PDGF and FGF2 secreted by cancer cells transform normal fibroblasts into tumor associated fibroblasts, which make their receptors a suitable target for inhibition by tyrosine kinase inhibitors[85]. Finally, the EPH tyrosine kinase receptors have been shown to be important in tumor cells and tumor stroma by mediating cell-cell interactions[86]. Although VEGFR, PDGFR and EPHR are important targets on both tumor cells and tumor stroma cells, kinases like FAK only function in stromal cells and other oncogenes often only function in tumor cells. Regarding this difference in gene expression between tumor cells and tumor stromal cells, a multi kinase inhibitor directed against a receptor tyrosine kinase in cancer cells, might not efficiently target this tyrosine kinase in tumor stromal cells, but it might target another one.

A complication might be the different composition of stroma between tumors. Whereas the tumor cells in glioblastoma are kept together primarily by the blood vessels surrounding them, the tumor stroma in other tissues often consists of "fibroblastic connective tissue". In the first case, the stroma is made up almost entirely of cellular components, the most important of which are the endothelial cells. In the second case, the stroma consists of a few myofibroblasts, smooth muscle cells or pericytes and a lot of extracellular matrix specific for the type of cell by which it is produced. The type of cell is dependent on the structure of the host tissue[87]. The differences in both tumor cell types and the composition of the extracellular matrix may require different strategies to inhibit tumor stroma. Furthermore, tumor-associated fibroblasts of different tissues have significant differences in their gene expression. Differences between stroma cells even exist in a single region[85,88].

In addition to fibroblasts and endothelial cells, tumor stroma consists of immune cells. The infiltration of macrophages and T cells to the tumor may mean both pro and anti-tumor survival, which depends on the expression of particular chemokines[85,89,90]. The role of dendritic cells is still ambiguous[85]. Neutrophils are suggested to reduce tumorigenicity and natural killer cells inhibit the progression to metastasis[91,92]. So, inhibition of immune cells can also cause harm depending on the type of cell being inhibited and on the moment of immunologic escape. Many different settings and tumor characteristics make it difficult to prefer one kind of inhibitor above the other. It becomes even more complicated when metastasised disease has to be treated since metastases can contain either stromal cells and tumor cells with the same character or stromal cells of the new host tissue[93,94]. For some cancers it can be effective to use a multi kinase inhibitor, which both attacks tumor cells and tumor stromal cells efficiently, whereas another type of cancer needs separate inhibitors for the tumor and stromal cells because of different tyrosine kinase expression. Furthermore, it might turn out that for at least some types of cancer the role of tyrosine kinase(s) (receptors) is relatively less prominent in stromal tumor cells than their role in cancer cells. In this regard, the important role fulfilled by chemokines and their receptors, including responsibility for leukocyte infiltration and angiogenesis has to be considered[85]. Other crucial targets for therapy are CD105, TEM8, αvβ3 integrins, PMSA, Tenascin C, FAPα, MMPs, uPA and CAIX[84]. At the moment, drugs in development are designed to target this large diversity of molecules and receptors.

From practice, VEGFR has turned out to be a very important target and can well be inhibited by using multi targeted single inhibitors. EPHR is another important target to develop inhibitors against, possibly in combination with VEGFR. The importance of other tyrosine kinases of stromal cells in different tumor settings and the best way to inhibit them needs further investigation.

Toxicity

Another consideration in the choice between multiple single kinase inhibitors or a single multi-kinase inhibitor is the toxicity of these compounds alone and in com-

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bination. In general the same considerations as for any drug have to be taken into account: is toxicity acceptable compared to the observed antitumor efficacy? However, kinase inhibitors have specific toxicities, either related to the primary target kinase(s), an off-target effect or caused by a specific metabolite of the kinase inhibitor. Therefore toxicity profiles of each new drug have to be determined before using them in combination.

EGFR tyrosine kinase inhibitors, such as gefitinib and erlotinib cause a typical skin toxicity described as "acneiform" rash, which is dose dependent. In a recent study erlotinib induced rash was characterized more in detail and it appeared to be different from acne vulgaris and was characterized as inflammation of hair follicles, with infiltration of TRAIL-positive dendritic cells[95]. For several other specific tyrosine kinase inhibitors, such as imatinib, cardiac toxicity has also been found in a substantial number of patients[96]. This toxicity became apparent in the post-approval phase since this type of kinase inhibitors were usually not screened for cardiotoxicity.

In contrast to the general belief during early development of kinase inhibitors, these compounds also exhibit classical toxicities such as diarrhea and myelotoxicity, although to a different extent and with different determinants e.g. EGFR tyrosine kinase inhibitors also display diarrhea which for gefitinib could be related to polymorphisms in *EGFR* (191 C/A, -216 G/T and R497K)[97]. The toxicity of erlotinib is also related to its metabolism by cytochrome P450 3A4 which is induced in smokers compared to non-smokers[70]. Therefore care has to be taken that in case of e.g. erlotinib, combination drugs, either another tyrosine kinase inhibitor or a cytotoxic drug, do not inhibit erlotinib metabolism, or if so, the doses have been optimized. In case of combining two selective kinase inhibitors, toxicity of each compound is usually well characterized and this will help to predict toxicity of the combination.

Special care has to be taken when drugs targeting neoangiogenesis are being used either alone or in combination. Since tumors are usually driven by mutations in more than one signaling pathway, this has led to the design of multi-kinase inhibitors, which are by definition not selective and hence may also affect signaling pathways in normal tissues. In the early development of VEGR tyrosine kinase inhibitors, it became clear they can exhibit serious vascular problems, which was first shown for SU5416[98]. The next generation, including sunitinib and sorafenib, showed a much more acceptable toxicity profile, although cardiac toxicity can be a problem. Usually this is an off-target toxicity. Sunitinib, an inhibitor of VEGFR, PDGFR and c-kit, was also shown to inhibit AMP-activated protein kinase (AMPK), which led to a disturbance of the metabolic homeostasis in the heart[96], possibly accounting, at least in part, for sunitinib induced cardiotoxicity. Hence from this perspective multi-kinase inhibitors may be more likely to account for cardiovascular toxicity. Therefore combinations of two specific kinase inhibitors may be preferable. Next, it will be advisable to screen novel multi-kinase inhibitors for off-target toxicity such as cardiac toxicity, during preclinical development and include careful monitoring in early clinical development.

CONCLUSION

The essence of any therapy is to specifically attack a tumor based on the difference in genetic background between tumor and normal cells. Targeted therapy focuses on differences in signaling pathways driving the growth of tumors. However, these pathways are disturbed to a different extent as a reflection of the diversity in genetics among individuals, hence hitting one target is unlike to cure cancer, and therefore attacking more pathways increases the chance of success and bypassing resistance. However, the most effective way of cancer treatment by using tyrosine kinase inhibitors is to consider each patient/tumor individually and to determine the strategy that specifically targets the consequences of altered (epi)genetics of the tumor, with an acceptable toxicity. This strategy might result in treatment by a single multi-kinase inhibitor for one patient, whereas for other patients this means treatment by a combination of single kinase inhibitors.

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