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ALK inhibitors for clinical use in cancer therapy

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1. ABSTRACT

Anaplastic lymphoma kinase (ALK) is a receptor tyrosine kinase protein implicated in a variety of tumors, both solid and hematological. Few years ago crizotinib, an inhibitor of the receptor tyrosine kinases c-Met and ALK, demonstrated its activity in ALK positive non-small-cell lung cancer and other tumors with excellent toxicity profile. Subsequently several ALK inhibitors have been developed, offering new personalized treatment options. This review addresses some clinical considerations on the use of ALK inhibitors in ALK positive tumors and on the development of resistance to them.

2. INTRODUCTION

In 1994 Steve Morris identified ALK as a novel oncogene involved in a specific 2;5 chromosomal translocation and fused to the *nucleophosmin (NPM)* gene in Anaplastic Large Cell Lymphoma (ALCL) (1). Clinical development of ALK inhibitors took off however only in 2007, when aberrant activation of *anaplastic lymphoma kinase (ALK)* gene was found in non-small-cell lung cancer (NSCLC) and was identified as an *echinoderm microtubule-associated protein-like 4 (EML4)-ALK* rearrangement (2). From this point the interest in ALK has increased and a number of inhibitor entered in preclinical and clinical studies, offering new treatment options in tumours driven by abnormal ALK signalling.

ALK is a tyrosine kinase (TK) receptor (1,3), normally expressed only in the nervous system (4,5) with a role in neural development and differentiation (6-8). ALK is expressed in various type of cancers such as infiammatory myofibroblastic tumor (IMT (9), glioblastoma (6,10), infiammatory breast cancer (11), neuroblastoma (12), Ewing sarcoma (13), retinoblastoma (14), diffuse-large-B-cell lymphoma (DLBCL) (15) urothelial carcinoma (16), fetal lung interstitial tumor (17) and melanoma (18).

ALK pathological expression is due to chromosomal translocations that lead to the formation of ALK-containing oncogenic fusion proteins (19) that interrupt the chromosome at the level of *ALK* gene at 2p23. Currently 16 different ALK fusion proteins have been identified (17,20) with different predominance in each type of tumors. ALK fusions cause constitutive activation of its kinase domain (21) so, ALK fusion proteins are deregulated, ectopically expressed and constitutively activated in neoplastic cells altering the phosphorylation of intracellular substrates (4,22) and activating several transduction pathways (23-31).

For this reason, the relationship between the development ALK-containing fusions and malignant transformation makes ALK a potential therapeutic target. This evidence is less solid when a fusion or an activating point mutation is missing. During last years several ALK inhibitors have been developed (32-35): these molecules represent an excellent proof-of-principle for targeted therapy (36). As it has been observed with other TK inhibitors (TKIs), resistance has recently emerged in patients treated with ALK inhibitors too (37-41).

3. ALK INHIBITORS

3.1. The development

ALK is a good candidate for the development of targeted treatment not only because the lack of widely expression in normal adult tissues that should not give potentially important toxic effects (31,38,42) but also because it is causally linked to the transformation process. Potential strategies for targeting ALK include immunotherapy, gene silencing, inhibition of downstream signalling pathways and direct inhibition of its catalytic activity through small-molecules inhibitors in order to inhibit ALK dependent cancer cell growth (20). The aim of this target therapy is to obtain a maximum tumor specific effect with low toxicities, opposite to a conventional cytotoxic chemotherapy (43). Kinase inhibitors are directed against the ATP-binding site of the catalytic domain, which is highly conserved in ALK TK, with the goal of obtain a successful ALK inhibition.

However, the presence of different ALK fusions confers different sensitivity to ALK inhibitors (44) and also point mutations may be the cause of secondary drug resistance as found first in neuroblastoma (45,46).

Initial testing of ALK inhibitors were performed using inhibitors derived from natural products such as staurosporine derivatives which are not specific inhibitors of ALK (47). Synergies with heat shock protein 90 (HSP-90) inhibitors were also observed (48). Subsequently, almost 20 different classes of small molecule inhibitors of ALK have been developed (Table 1).

3.2. Crizotinib

The largest volume of available knowledge on ALK inhibitors relates to crizotinib. Crizotinib (PF-02341066, Pfizer) is the first in human ALK inhibitor developed. It is a derivate of aminopyridine and was originally developed as a potent, orally bioavailable, ATP-competitive small molecule inhibitor of mesenchymal epithelial transition growth factor/hepatocyte growth factor receptor (c-MET) (49). In 2011 it was approved by the Food and Drugs Administration in ALK positive NSCLC.

The phase I trial established 250 mg twice daily as the maximum tolerated dose (MTD) in patients with advanced cancer (excluding leukemias) (50). Preclinical and single-arm phase I studies have shown that patients with advanced stage ALK positive NSCLC can be successfully treated with crizotinib with overall response rate (ORR) of 61%. Progression free survival (PFS) was 10-11 months and there were very few grade 3 and 4 adverse events (AEs) (51,52). The most common AEs were: ocular flashes, nausea, emesis, fatigue, and diarrhoea; all manageable and reversible. Fatigue was the dose-limiting toxicity (DLT), occurring at grade 3 in 2/6 patients treated with crizotinib at 300 mg twice a day. Recently published post-marketing AEs described nephrotoxicity in a patient with previous idiopathic cronic kidney disease (53), esophagitis (54, 55), alveolar hemorhage (56) and interstitial lung disease (57). However, the combined administration of crizotinib with steroids has been shown to overcome this last AE (58). A recent phase III trial (NCT00932893) confirm the phase I-results with a PFS of 8 months with crizotinib versus 3 months with platinum-based chemotherapy and a ORR 65% versus 20% respectively (59). Others randomized phase III trials are testing crizotinib versus standard chemotherapy in first-line treatment in ALK positive non squamous lung cancer (NCT01154140) and adjuvant crizotinib versus placebo in NSCLC removed by surgery (NCT02201992).

Crizotinib has shown its efficacy also in rarer tumor like ALCL, DLBCL, IMT and other type of *ALK*-rearranged cancers. The first treatment of an ALK positive ALCL patient occurred in 2010. A report from *New England Journal of Medicine* described 2 adult patients with recurrent ALK positive ALCLs that achieved complete response (CR) shortly after receiving crizotinib as single agent, administered under a compassionate drug program (60). The final results of the study were recently published demonstrating clearly the activity of crizotinib in ALK positive ALCLs. ORR was 91% with 82% CR and 1/11 partial response (PR); 2-year PFS of 64% (61). All relapses developed within the initial 3 months of treatment with no parameter predicting for the development of a durable response, other than having failed an ABMT. These results were recently confirmed in a sponsored trial in which reported an ORR of 64% among 14 ALK positive lymphoma patients (62). A recent phase I study also reported this good safety profile with high response rates in children with relapsed ALK positive ALCL (63).

Crizotinib can be efficacious also in the treatment of ALK positive DLBCL: the compassionate study previously cited (61) reported 2 patients with ALK positive DLBCL treated with crizotinib with one rapid but transient response. Some experiences shows that crizotinib can be use as mantainence after allogenic bone marrow transplant, confirming its good safety profile (64).

Recently two cases of IMT and its epithelioid variant with systemic manifestations and ALK translocation were successfully treated with crizotinib (65,66). There were also reported some case reports on other ALK positive tumour successfully treated with crizotinib: an advanced pretreated sarcomatoid carcinoma of the upper aerodigestive tract has achieved with crizotinib clinical improvement and stable disease with minimal tumor shrinkage lasted for 4 months (67). A phase I/II trial of crizotinib in tumors other than NSCLC (NCT01121588) and in young patients (NCT00939770) is presently ongoing.

Table 1. ALK inhibitors in development

Name	Mechanism of action	Clinical trials in tumors	Responses	Adverse events	References
Crizotinib (PF-02341066 - Pfizer)	Selective ATP competitive oral inhibitor of ALK and MET tyrosine kinases and their variants	Phase I-II Phase III in second line Ongoing phase III in first line in NSCLC Studies with crizotinib as single agent or in combination	In NSCLC: 61-65% ORR, PFS 7.7. months In ALCL: ORR 64-91%, 2-years-PFS 64% Experiences in other solid tumors	Gastrointestinal adverse events (diarrhoea, emesis and nausea) Fatigue ALT elevation Ocular flashes	(51,52,59,61,62)
Alectinib (CH5424802 - Chugai Pharmaceutical, Roche)	Potent and selective orally available ALK and ROS1 inhibitor Activity against L1196M	Phase I/II trial in NSCLC patients in Japan Ongoing phase III in Japan versus crizotinib Ongoing phase I/II in US	In NSCLC: ORR 93.5.% with 60% ORR in crizotinib resistant NSCLC	Dysgeusia, ALT and creatinine elevation, rush, neutropenia	(94-96,117,118)
Ceritinib (LDK 378 - Novartis)	Selective ALK, IFG-1R and c-MET inhibitor Activity against L1196M	Phase I . in crizotinib naïve and resistant Phase II Phase III ongoing both as a first- and second-line therapy for ALK positive NSCLC	In NSCLC ORR 58% with 56% in crizotinib refractory, PFS 8.6. months	Nausea, diarrea, fatigue and ALT elevation	(84,90,91,119)
AP-26113 (Ariad)	Dual ALK/EGFR inhibitor and ROS1 inhibitor AP-26113 is 10 fold more potent compared to PF-02341066	Phase I/II in crizotinib naïve and resistant: ongoing	In NSCLC ORR 63% with ORR 73% in crizotinib resistance	Well tolerated, fatigue, nausea, diarrea, dyspnea, ALT elevation	(98,99,120)
ASP 3026 (Astellas Pharma)	ALK, ROS1 and EGFR inhibitor Activity against L1196M	Phase I: promising safety and tolerability	In crizotinib resistant NSCLC: 44% PR and 50% SD, PFS 5.9. months	Nausea, vomiting, fatigue, constipation, rush, AST/ALT elevation	(113,121)
PHA-E429 (nerviano medical science)	ATP competitive, crystal structure of the ALK kinase domain in a complex with PHA-E429	Phase I			(122)
CEP-14083 and CEP-14513 (Cephalon)	Selective ALK inhibitor	Phase I			
PF06463922 (Pfizer)	Selective inhibitor of anaplastic lymphoma kinase (ALK), EGFR and c-Ros oncogene 1 (ROS1)	Phase I/II			(109)
TSR-011 (Tesaro)	Inhibits ALK and tropomyosin-related kinase (TRK) A, B, and C receptor and oncogenic echinoderm microtubule associated protein like 4 (EML4)-ALK and tropomyosin (TPM)-TRKA dependent tumor growth in mice Activity against L1196M	Phase I/II	In NSCLC, 3/4 (all crizotinib-resistant) achieved PR Stable disease was observed in ALK- papillary thyroid, pancreatic and colorectal patients		(110)

(*Contd...*)

Table 1. (*Continued*...)

Name	Mechanism of action	Clinical trials in tumors	Responses	Adverse events	References
RXDX101 (Ignyta Inc.)	Inhibitor of TrkA, TrkB and TrkC, as well as ROS1 and ALK	Phase I in different type of advanced tumors	1/1 PR in ALK positive neuroblastoma	Well tolerated. Grade 1-2 paresthesias, nausea, dysgeusia, and diarrhoea	(114)
X396 (XCovery)	ALK inhibitor and synergic activity with mTOR inhibitor rapamycin Activity against L1196M	Phase I in NSCLC ongoing	Preliminary data: SD is 28% and PR 28%	Preliminary data: rash fatigue, nausea, vomiting and edema	(106, 107)
CEP-37440 (Cephalon)		Phase I in NSCLC			(123)
NMS-E628 (Ariad)	ALK inhibitor	Phase I			
X376 (XCovery)	ALK inhibitor Preclinical data avaible only				(107)
NVP-TAE684 (Novartis)	Selective ALK inhibitor	Phase I discontinued			(84)
WZ-5-126 (Ambit Biosciences)	ALK small molecules inhibitor Preclinical data avaible only				(124)
CRL151104A (chebridge st. jude)	ATP competitor Preclinical data avaible only				(125)
CEP-28122 (Cephalon)	Selective ALK inhibitor Preclinical data avaible only				(126)
F91873 and F91874 (Institute de rechercher Pierre Fabre)	ATP noncompetitive inhibitors, pyridoisoquinoline derivatives Preclinical data avaible only				
GSK1838705A (GlaxoSmithKlein)	Small molecule kinase inhibitor of insulin-like growth factor receptor. Preclinical data avaible only				(127)

4. MECHANISMS OF RESISTANCE TO ALK INHIBITORS

The development of drug resistance remains one of the major limitations of successful treatment of advanced cancers: the study mentioned above(61) have shown that resistance can emerge in some patients who have demonstrated initial response to ALK inhibition. Mechanism of acquired resistance can be divided in 3 groups, very similar to what we know on chronic myeloid leukaemia (CML) (68). First is ALK mutation, followed by resistance due to mechanisms that upregulates ALK (such as gene amplification or copy number gain), and by the activation of alternative signal transduction pathways (69-71). Mutations in ALK kinase domain confer a structural change in the ATP-binding site (72) and reduced drugs sensitivity both *in vitro* and *in vivo* with variable level of drug resistance like for BCR-ABL in EML4-ALK. Mutations can involve the the "gatekeeper" residue L1196, (for example L1196M (72)), and other "non-gatekeeper" residues distributed throughout the kinase domain: G1202R, S1206Y, G1269A, 1151Tins, F1174L, L1152R and C1156Y (69,73). The prevalence and the significance of these point mutations need yet to be clarified but, it is known that C1156Y, G1269A and L1196M, conferred clinical resistance to crizotinib in NSCLC patients and another one, F1174L, causes resistance to the same drug in an IMT patient (72,74). The acquired resistance to ALK-targeted therapy has been distinguished in "ALK-dominant" and "ALK-non-dominant" mechanisms.

In "ALK-dominant" mechanisms, the acquired resistance is due to the development of novel ALK kinase domain mutation alone or in combination with the increase of rearranged *ALK* gene copies in cancer cells, so the signalling remains dominant. Cells are still dependent on that pathway for survival and "second generation" inhibitors may be sufficient to bypass the resistance. Multiple ALK inhibitors have been tested against crizotinib-resistant mutants (75). These authors found that L1196Q substitution conferred resistance to crizotinib but not to AP26113 and NPV-TAE 694, while cells carrying I1171N mutation were resistant to all tested inhibitors (37). In the clinical report described above (61) deep sequencing of 2 blood samples of relapsed ALCL revealed the presence of different mutations: Q1064R at high prevalence (95%) in 1 patient, and I1171N

(33%) plus M1328I (14%) in the other patient. All these mutations were not present in samples obtained before crizotinib treatment. I1171N was already discovered in an *in vitro* screening (5): it commands an intermediate level of resistance to crizotinib which however is cross resistant with other ALK inhibitors such as AP26113 and NVP-TAE684. Mechanisms of acquired resistance are heterogeneous and may evolve dynamically in response to different ALK TKIs. Recently, consistent with preclinical data, clinical evidence was found that ALK mutations in two residues, G1202R and F1174V, can mediate resistance also to ceritinib (76) and alectinib (77).

In contrast, "ALK-non-dominant" mechanisms are cases in which different second oncogenic drivers were activated (for example, *KRAS, KIT, NTRK1 or EGFR*), coexisting in the same cell with the *ALK* rearrangement (78,79). These events should, in theory, bypass the dominance of ALK signalling and replace its oncogenic potential (37); in such a situation, "second generation" inhibitors would be ineffective.In order to overcome resistance, it will be important to differentiate patients that preserve ALK dominance versus those that have diminished ALK dominance.

It is reassuring however that "second generation" inhibitors work better than what could be expected on the basis of "non-dominant" models, with ORR from 55 to 60% (80). This complexity of mechanisms of acquired resistance suggests that other therapeutic options, including the use of radiation to treat isolated areas of progression and adding or switching to cytotoxic chemotherapy could be required (81), or the combination of some of these TKIs with other therapies such as for example, HSP-90 inhibitors or immunotherapy (82,83). None of these approaches have been subjected to controlled studies.

5. "SECOND GENERATION" ALK INHIBITORS

Ceritinib (LDK378, Novartis Inc.), alectinib (CH5434802/RO5424802, Chugai Pharmaceutical/ Roche), AP26113 (ARIAD Pharmaceuticals) are some examples of "new generation" inhibitors (Table 1). Ceritinib specifically inhibits the ALK fusion protein and is not active against C-Met. It is 20-times more potent than crizotinib (84,85): (39). In particular, ceritinib effectively inhibits ALK harboring L1196M, G1269A (the two most common crizotinib-resistant mutations), I1171T, and S1206Y mutations. However, we observed that ceritinib did not overcome 2 crizotinib-resistant ALK mutations, G1202R and F1174C (86). The first-phase I trial in human, limited to relapsed/refractory NSCLC patients demonstrated objective responses (87,88), confirming preclinical datas (89). ORR was 58% at the dose of 400 mg once daily, in particular ORR of 56% was reported in crizotinib resistant patients (90,91): this response rate is similar for patients not previously treated with ALK inhibitor or treated with crizotinib. Also PFS can support a front-line treatment scenario: PFS was 8.6. months, and was 3 months longer in patients who had not received crizotinib previously. Major adverse events were gastrointestinal AEs (primarily grade 1 or 2 nausea and diarrhea), fatigue and elevation of ALT very similar to crizotinib; however only half the patients required dose modification when compared with crizotinib(90). However ceritinib showed a higher drug-related grade 3 or 4 diarrhea and nausea (respectively 7% and 5%) compared with crizotinib (0% and 1%) (52,59,90). Ceritinib has been approved in the US under 'Breakthrough Therapy' designation for the second-line treatment of ALK-positive NSCLC; EMEA response is still pending (92). Currently phase II and III trials (NCT01964157, NCT01947608, NCT01828112 and NCT01828099) are ongoing both in crizotinib naïve or not patients. There is also a phase I study in paediatric patient investigated ceritinib (NCT01742286).

Alectinib is a potent and selective ALK inhibitor (93). Preclinical data have demonstrated that alectinib specifically inhibits EML4-ALK with higher potency than crizotinib, and is also active in cell lines with the most common crizotinib resistance mutations, including L1196M, C1156Y, and F1174L (94). A phase I/II trial in crizotinib-naïve patients with ALK positive NSCLC demonstrated objective responses in 93.5.% patients enrolled at the MTD (300 mg twice day without DLT), with 2 CR and 41 PR and not yet determined PFS (95). The activity was associated with mild adverse events (no grade 4 were reported): mostly dysgeusia, liver dysfunction, neutropenia, rush and creatinine elevation. Visual effects and gastrointestinal disorders (diarrhea, vomiting, and nausea) were rare. During a phase I study there were an ORR of 60% and good safety profile in patients who are refractory to crizotinib (96): for this reason, a single-arm phase I/II study in crizotinib resistant patients is ongoing (NCT01801111). There is also an ongoing phase III trial comparing alectinib versus crizotinib in advanced NSCLC (NCT02075840).

AP26113 (Ariad) is a dual ALK and EGFR inhibitor: in preclinical setting it has shown efficacy also in crizotinib resistant L1196M (97). It has clinical activity in crizotinib-naïve and crizotinib-resistant NSCLC, with an ORR of 63% or 73% in crizotinib resistant patients (98). Increased ALT and dyspnea have been noted as dose-limiting toxicities, and the most common grade 3 and 4 adverse events were pulmonary symptoms like hypoxia and dyspnea (at 180 mg/day), with also grade 1 or 2 fatigue and gastrointestinal AEs (99). The phase II study (NCT01449461 and NCT02094573) is ongoing at the dose of 180 mg daily both in crizotinib-resistant and crizotinib-naive patients and in patients with ALK wild-type NSCLC who have EGFR mutations resistant to available TKIs.

6. OTHER APPROACHES: HSP90 INHIBITORS

HSP-90 inhibitors and other natural-derived compounds inhibit ALK by increasing the proteosome-mediated degradation of ALK protein through binding to HSP-90 (100,101). This strategy could be useful to manage acquired resistance to second-generation ALK inhibitors (102). Various inhibitors such as ganetespib (STA-9090), AUY922, IPI504 and retispamycin are under investigation. They have shown ORR between 66% and 40% for IPI504 (103) and ganetespib (104) respectively, with PFS of 7-8 months. AEs are constituted mainly by fatigue, diarrhea and nausea. AUY922 (105) and ganetespib (101) has demonstrated activity also in crizotinib-resistant patients. There are several phase I/II trials examining all HSP-90 inhibitors alone or in combination with crizotinib or ceritinib in patients with advanced ALK positive tumors: IPI-504 (Infinity pharmaceuticals, NCT01228435), ganetespib (Syntax pharmaceuticals, NCT01579994 and NCT01562015), AT13387 (Astex pharmaceutical, NCT01712217), AUY922 (Novartis, NCT01772797, NCT01124864 and NCT01752400) and DS 2248 (Daiichi Sankyo, NCT01288430).

7. "THIRD GENERATION" INHIBITORS

Other new ALK TKIs, such as ASP-3026 (Astellas Pharma), NMS-E628 (Nerviano Medical Sciences), X-396 (Xcovery), CEP-37440 (Teva Pharmaceutical Industries), TSR-011 (Tesaro), PF-06463922 (Pfizer) and RXDX101 (Ignyta Inc.) are currently starting clinical trials.

X-396 is a novel, potent ALK small molecule TKI with significant anti-tumor activity. Preliminary data on phase I (NCT01625234) study shows that it is generally well tolerated at doses up to 250 mg daily and induces responses in both crizotinib naïve and crizotinib resistant ALK positive NSCLC patients (106). Moreover, it can inhibit ALK kinase also when it is associated with its resistance point mutation L1196M and C1156Y (107).

PF06463922 is a new ALK inhibitor and a potent ROS1 kinase inhibitor and preclinical data show its great potential to overcome the resistance associated with ROS1 mutation (108,109). A phase I/II trial is ongoing (NCT01970865).

TSR-011 is currently used in a phase I/IIa trial (NCT02048488): its preclinical data show a high affinity for the ALK domain and preliminary data show promising results (110).

Recently ASP 3026 has shown activity in ALCL mice models (111,112). Preliminary data on "fast following" design trial (NCT01401504) demonstrated good activity in crizotinib resistant NSCLC with low toxicity (113). The phase II trial is recruiting patients with advanced malignancies (NCT01284192).

RXDX-101 is an oral small molecule inhibitor of TrkA, TrkB and TrkC, as well as ROS1 and ALK, with high potency and selectivity. It has demonstrated potent pharmacological activity in preclinical studies and it is well tolerated in patients with advanced solid tumors (114): The phase I/IIa is ongoing (NCT02097810).

8. CONCLUSIONS

Personalised treatment of cancer patients has become a reality in the last few years, with many drugs having been developed that target specific altered pathways. The initial identification of the genetic lesion at the basis of malignant transformation in ALK positive ALCLs, originally obtained in 1994 (1), was successfully exploited and brought to patient bedside in 2010 (60). This compares favorably with the time which elapsed between the discovery of the Philadelphia (Ph) chromosome in CML and the clinical development of imatinib (115). The success in identifying the ALK translocations and rapidly developing targeted drugs to exploit it paves the way for a better understanding of NSCLC and other tumors biology. The entry of crizotinib in the treatment of ALK positive tumors marked a new era in the therapy of these malignancies. The demonstration of high response rates, even in the setting of advanced and resistant disease, should prompt the development of clinical studies in less advanced conditions and in combinations with already active drugs. A particular emphasis should be placed on the need to decrease as much as possible the use of cytotoxic drugs. However, the best way in which ALK inhibitors should be administered in the setting of ALK-rearranged tumors remain to be fully elucidated (116). Indeed the future availability of several ALK TKIs will require studies to investigate the best use (sequential vs. combination) of these powerful tools. The possibility of obtaining responses in a substantial fraction of advanced, heavily pretreated ALK positive tumors illustrates our present inability to forecast the level of heterogeneity present inside the disease, such as a metastatic NSCLC, relapsed blast crisis CML or relapsed Ph positive acute lymphoblastic leukemia, in which monotherapy with TKIs seldom obtain durable responses. Further studies, such as exome sequencing of pre and post treatment samples could hopefully shed light and provide an useful indicator of the level of heterogeneity present inside a tumor at any given time. The next couple of years will hopefully see the fading of regimens based only on unspecific cytotoxic drugs in favor of more specific and hopefully less toxic approaches. The use of crizotinib and other ALK TKIs represent an useful example of how personalized medicine has improved patient care through the use of molecular-targeted therapy.

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