Arch. Pharm. Res. (2013) 36:1051–1054
DOI 10.1007/s12272-013-0157-8

Archives of Pharmacal Research
www.springer.com/12272

REPORT ON INVESTIGATIONAL DRUGS

Journey of the ALK-inhibitor CH5424802 to phase II clinical trial

Muhammad Latif · Aamer Saeed · Seong Hwan Kim

Received: 3 March 2013 / Accepted: 13 May 2013 / Published online: 23 May 2013

Abstract The anaplastic lymphoma kinase (ALK) receptor tyrosine kinase represents a potential therapeutic target. Specially, a variety of alterations in the ALK gene including mutations, overexpression, amplification, translocations and structural rearrangements, are involved in human cancer tumorigenesis. The second-generation ALK inhibitor CH5424802 (development code: AF802; Chugai Pharmaceutical, a subsidiary of Roche) achieves tumor regression with excellent tolerance and shows promising efficacy in patients with ALK-positive non-small cell lung cancer. CH5424802 shows good kinase selectivity, has a promising pharmacokinetics profile, and has strong anti-proliferative activity in several ALK-driven tumor models. CH5424802 has also shown anti-tumor activity in mouse xenograft studies. Here, we summarize recent advances and the evidence that CH5424802 acts as an ALK inhibitor. We also discuss its potential for further development as an anticancer drug in clinical trials.

Keywords Novel tetracyclic anaplastic lymphoma kinase inhibitor (CH5424802) · Clinical trials · Non-small cell lung cancer (NSCLC)

M. Latif
Cancer and Infectious Disease Research Center, Division of Drug Discovery Research, Korea Research Institute of Chemical Technology, Daejeon 305-600, Republic of Korea

M. Latif · S. H. Kim
Department of Medicinal and Pharmaceutical Chemistry, University of Science and Technology, Daejeon 305-350, Republic of Korea

A. Saeed
Natural Products and Heterocyclic Research Laboratory, Department of Chemistry, Quaid-i-Azam University, Islamabad 45320, Pakistan

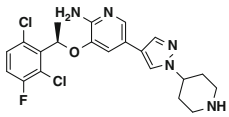
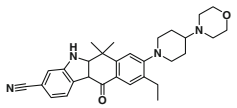
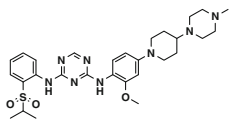
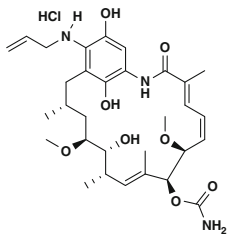
S. H. Kim (✉)
Laboratory of Translational Therapeutics, Pharmacology Research Center, Division of Drug Discovery Research, Korea Research Institute of Chemical Technology, P.O.Box 107, Yuseong-gu, Daejeon 305-600, Republic of Korea
e-mail: hwan@krict.re.kr

Lung cancer is often fatal and has a higher mortality rate than breast, colorectal and prostate cancer (Pirozynski 2006; Siegel et al. 2013). Clinically, lung cancers can be classified as small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC), with SCLC and NSCLC accounting for 80–85 % of all lung cancers. Despite committed efforts to improve lung cancer diagnosis and treatment, the 5-year survival rate for patients with NSCLC remains low at ~10–15 %. More than 50 % of patients are diagnosed at advanced stages of the disease; at that point, treatment has a palliative rather than a curative intent (Scagliotti 2007; McDermott et al. 2008).

A new era for cancer therapy has dawned in recent years with the development of novel methods for identifying and targeting tumors' molecular defects. Targeted therapies appear promising and offer new therapeutic models in the field of oncology. While numerous kinase inhibitors have been developed to treat a broad range of cancers, 208 NSCLC-targeting drugs are now in development: 17 in phase III, 130 in phase II, and 61 in phase I (White 2012). Targeted therapies dominate all stages of the NSCLC pipeline, and the major strategy of the late-phase drugs is inhibition of tyrosine kinases such as anaplastic lymphoma kinase (ALK).

Anaplastic lymphoma kinase belongs to the family of tyrosine kinase receptors (TKRs). Recently, ALK-TKR has emerged as a potential biomarker and therapeutic target in

Table 1 ALK-targeted inhibitors

Inhibitor (company) structure	Some significant aspects[a]
Crizotinib (Pfizer) 	Crizotinib (PF-02341066, Xalkori) has been approved by FDA for treating NSCLC with ALK translocations. It is a dual c-MET and ALK inhibitor with IC_{50} of 11 and 24 nM, respectively in lymphoma cell lines showing the NPM-ALK oncogenic fusion protein.
CH5424802 (Chugai Pharmaceutical) 	CH5424802 is an orally active, potent, and highly selective ALK inhibitor with IC_{50} of 1.9 nM, and currently advancing in phase I/II clinical trials in NSCLC patients in Japan. The trials are planned to be finished in March 2014. This inhibitor has shown activity against the gatekeeper ALK mutant (Ardini and Galvani 2012).
AP-26113 (Ariad Pharmaceuticals) ND	AP-26113 is a potent dual inhibitor of ALK/EGFR with IC_{50} value of 0.62 nM. This inhibitor exhibits activity in crizotinib-resistant patients. Its approximately have tenfold higher potency and selectivity for ALK-positive cells compared to crizotinib (Rivera et al. 2010). Undergoing phase I/II clinical trials for this unique small inhibitor were initiated in September of 2011 (Zhang et al. 2010).
LDK378 (Novartis) ND	LDK378 is an orally available ALK inhibitor with IC_{50} of 0.15 nM and undergoing phase I trials in ALK rearranged tumors. LDK378 exhibits high efficacy in vivo and induces complete and strong tumor regression in an ALK-positive NSCLC dependent models. It is active in tumors bearing the C1156Y mutation that confers crizotinib-resistance (Li et al. 2011).
ASP3026 (Astellas Pharma) 	ASP3026 is an orally available ALK inhibitor with IC_{50} 3.2 nM and no preclinical data is available. The trial commenced in December 2010 and now in phase I trial in patients with advanced malignancies, B cell lymphoma, solid tumors, and ALK-positive tumors (La Madrid et al. 2012).
X-396 (Xcovery) ND	X-396 shows promising anti-tumor activity in vitro and in vivo on various ALK-dependent tumor models and appears to have potential to treat patients with resistance-conferring mutations. The distribution of X-396 in brain tissue suggests an interesting aspect and a clue towards activity against ALK-positive brain metastases. (Lovly et al. 2011). Its ALK enzyme inhibition IC_{50} value is <0.4 nM.
Retaspimycin hydrochloride (Infinity Pharmaceuticals) 	Retaspimycin hydrochloride (IPI-504) has been investigated as heat shock protein inhibitor with considerable activity in ALK-positive NSCLC patients. Now progressing in phase II trials in combination with docetaxel versus placebo/docetaxel. In phase IB/II, study is underway in combination with everolimus in KRAS-mutant NSCLC.
Preclinical agent	Several preclinical ALK inhibitors are under testing which include CEP-28122 (Teva), GSK-1838705A (GlaxoSmithKline) and 3-39 (Novartis). Clinical grade anti-ALK antibody is also under development. Combining an anti-ALK antibody with ALK inhibitors might be more effective than either agent alone and is evident from early preclinical data.

ND Chemical structure has not been disclosed yet

[a] www.prous.com

solid and hematologic tumors (Cabezón-Gutiérrez et al. 2012; La Madrid et al. 2012). In the ALK gene, a variety of genetic alterations such as mutations, overexpression, amplification, translocations, or other structural rearrangements, have been implicated in anaplastic large cell lymphoma (ALCL) and in a subset of NSCLC, suggesting *ALK* addiction in human cancer (Azarova et al. 2011; Soda et al. 2007; Shigematsu et al. 2005; Webb et al. 2009).

ALK tyrosine kinase activity is necessary for its transforming activity and oncogenicity (Sasaki et al. 2010; Koivunen et al. 2008). Crizotinib (PF-02341066, Xalkori) as the first-generation of ALK inhibitor was developed by

Pfizer and approved as a dual MET/ALK inhibitor for treating NSCLC with ALK translocations in August of 2011. Crozotinib-mediated inactivation of EML4–ALK kinase caused the disengagement of oncogenic signaling pathways and induced growth arrest and cell death, but made tumors acquire resistance. Therefore, numerous ALK kinase inhibitors (Table 1) have been in clinical evaluation as potential therapies as second-generation ALK inhibitors (Soda et al. 2008; McDermott et al. 2008). This report highlights the clinical, biological, and molecular features of CH5424802 and discusses the use of CH5424802 as a 'druggable' ALK inhibitor for cancer therapy.

CH5424802 as an orally active, potent, and highly selective ALK inhibitor, is currently advancing in human clinical trials (Kinoshita et al. 2011a, 2011b, 2012). As an ATP-competitive inhibitor, CH5424802 exhibits strong antiproliferative activity in NSCLC and ALCL. Molecular modeling analysis revealed its binding to the ATP site of ALK in the DFG-in mode (Sakamoto et al. 2011; Kinoshita et al. 2011b). In an enzymatic activity assay, CH5424802 inhibits ALK activity at nanomolar concentrations (IC_{50}, 1.9 nM; K_d, 2.4 nM; K_i, 0.83 nM) and exhibits good kinase selectivity; among 402 kinases, only three kinases, ALK, cyclin G-associated kinase (GAK), and leukocyte tyrosine kinase (LTK) showed greater than 50 % inhibition at 10 nM CH5424802 (Sakamoto et al. 2011). The inhibitory action of CH5424802 can be explained by the high sequence similarity of LTK to ALK (Iwahara et al. 1997). In NSCLC expressing echinoderm microtubule-associated protein-like 4 (EML4)-ALK fusion oncogene (Soda et al. 2007), CH5424802 exhibited preferential antitumor activity. CH5424802 also exhibited antitumor activity in ALCL expressing the nucleophosmin (NPM)-ALK fusion. About 50–60 % of ALCLs possesses a reciprocal chromosomal translocation that fuses NPM to ALK, thus forming a soluble chimeric and oncogenic NPM-ALK (Bischof et al. 1997).

The good pharmacokinetic profile of CH5424802 has been linked with its strong antiproliferative activity in ALK-driven tumor models (Sakamoto et al. 2011). The half-life and the oral bioavailability of CH5424802 in mice were 8.6 h and 70.8 %, respectively. No differences in body weight or gross signs of toxicity were observed in CH5424802-treated mice at any dose level. In the mouse xenograft model inoculating NCI-H2228 cells (a NSCLC-expressing EML4-ALK), the oral administration of CH5424802 dose-dependently inhibited tumor growth with an ED_{50} of 0.46 mg/kg, but not in the xenograft model inoculating A549 cells that is an NSCLC cell line that does not express ALK fusions.

In the mode of action study, CH5424802 prevents ALK autophosphorylation in NCI-H2228 cells (Sakamoto et al. 2011). CH5424802 also suppresses the phosphorylation of STAT3 and AKT in NCI-H2228. The inhibitory effect of CH5424802 on the phosphorylation of ALK and STAT3 was confirmed in in vivo models. In the ALK-positive ALCL cell line KARPAS-299, CH5424802 completely inhibited the phosphorylation of STAT3 (Sakamoto et al. 2011). STAT3 is required for ALK-mediated lymphomagenesis in ALCL (Chiarle et al. 2005).

Furthermore, CH5424802 exhibited anticancer potency against the L1196 M gatekeeper mutation of ALK accompanied with the inhibition of both ALK and STAT3 phosphorylation (Sakamoto et al. 2011). L1196 M, in which methionine is substituted for leucine at position 1,196 in ALK, exhibited increased kinase activity compared with wild-type ALK (Lu et al. 2009).

The results for the first-in-human phase I/II study of CH5424802 in patients with ALK-positive NSCLC has been reported recently (Kiura 2012). The highest dose level defined in the protocol, 300 mg b.i.d., did not reach the maximum tolerated dose, and toxicities were mild to moderate. Importantly, all patients achieved tumor regression at all dose levels, suggesting that it could be well tolerated. The promising efficacy of CH5424802 in patients with ALK-positive NSCLC hints at further success in the ongoing phase II and advanced trials. Hopefully, the effort to develop potent ALK inhibitors such as CH5424802 will result in clinical process in cancer treatment for patients with ALK-driven tumors.

Acknowledgments This work was supported by KRICT's project, SI-1304, funded by the Ministry of Knowledge Economy, Republic of Korea.

References

Ardini, E., and A. Galvani. 2012. ALK inhibitors, a pharmaceutical perspective. *Frontiers in Oncology* 2: 1–8. article 17.

Azarova, A.M., G. Gautam, and R.E. George. 2011. Emerging importance of ALK in neuroblastoma. *Seminars in Cancer Biology* 21: 267–275.

Bischof, D., K. Pulford, D.Y. Mason, and S.W. Morris. 1997. Role of the nucleophosmin (NPM) portion of the non-Hodgkin's lymphoma-associated NPM-anaplastic lymphoma kinase fusion protein in oncogenesis. *Molecular and Cellular Biology* 17: 2312–2325.

Cabezón-Gutiérrez, L., P. Khosravi-Shahi, V.M. Diaz-Muñoz-de-la-Espada, J.R. Carrión-Galindo, I. Eraña-Tomás, and M. Castro-Otero. 2012. ALK-mutated non-small-cell lung cancer: A new strategy for cancer treatment. *Lung* 190: 381–388.

Chiarle, R., W.J. Simmons, H. Cai, G. Dhall, A. Zamo, R. Raz, J.G. Karras, D.E. Levy, and G. Inghirami. 2005. Stat3 is required for ALK-mediated lymphomagenesis and provides a possible therapeutic target. *Nature Medicine* 11: 623–629.

Iwahara, T., J. Fujimoto, D. Wen, R. Cupples, N. Bucay, T. Arakawa, S. Mori, B. Ratzkin, and T. Yamamoto. 1997. Molecular characterization of ALK, a receptor tyrosine kinase expressed specifically in the nervous system. *Oncogene* 14: 439–449.

Kinoshita, K., Y. Ono, T. Emura, K. Asoh, N.I.T. Furuichi, H. Kawada, S. Tanaka, K. Morikami, T. Tsukaguchi, H. Sakamoto,

T. Tsukuda, and N. Oikawa. 2011a. Discovery of novel tetracyclic compounds as anaplastic lymphoma kinase inhibitors. *Bioorganic and Medicinal Chemistry Letters* 21: 3788–3793.

Kinoshita, K., T. Kobayashi, K. Asoh, N. Furuichi, T. Ito, H. Kawada, S. Hara, J. Ohwada, K. Hattori, T. Miyagi, W.S. Hong, M.J. Park, K. Takanashi, T. Tsukaguchi, H. Sakamoto, T. Tsukuda, and N. Oikawa. 2011b. 9-Substituted 6,6-dimethyl-11-oxo-6,11-dihydro-5H-benzo[b]carbazoles as highly selective and potent anaplastic lymphoma kinase inhibitors. *Journal of Medicinal Chemistry* 54: 6286–6294.

Kinoshita, K., K. Asoh, N. Furuichi, T. Ito, H. Kawada, S. Hara, J. Ohwada, T. Miyagi, T. Obayashi, K. Takanashi, T. Tsukaguchi, H. Sakamoto, T. Tsukuda, and N. Oikawa. 2012. Design and synthesis of a highly selective, orally active and potent anaplastic lymphoma kinase inhibitor (CH5424802). *Bioorganic and Medicinal Chemistry* 20: 1271–1280.

Kiura, K., T. Seto, N. Yamamoto, M. Nishio, K. Nakagawa, and T. Tamura. 2012. A first-in-human phase I/II study of ALK inhibitor CH5424802 in patients with ALK-positive NSCLC. *Journal of Clinical Oncology* 30, suppl; abstr 7602.

Koivunen, J.P., C. Mermel, K. Zejnullahu, C. Murphy, E. Lifshits, A.J. Holmes, H.G. Choi, J. Kim, D. Chiang, R. Thomas, J. Lee, W.G. Richards, D.J. Sugarbaker, C. Ducko, N. Lindeman, J.P. Marcoux, J.A. Engelman, N.S. Gray, C. Lee, M. Meyerson, and P.A. Jänne. 2008. EML4-ALK fusion gene and efficacy of an ALK kinase inhibitor in lung cancer. *Clinical Cancer Research* 14: 427542–427583.

La Madrid, A.M., N. Campbell, S. Smith, S.L. Cohn, and R. Salgia. 2012. Targeting ALK: A promising strategy for the treatment of non-small cell lung cancer, non-Hodgkin's lymphoma, and neuroblastoma. *Target Oncology* 7: 199–210.

Li, N., P.-Y. Michellys, K. Sungjon, A. Culazzo Pferdekamper, J. Li, S. Kasibhatla, C.S. Tompkins, A. Steffy, A. Li, F. Sun, X. Sun, S. Hua, R. Tiedt, Y. Sarkisova, T.H. Marsilje, P. McNamara, and J. Harris. 2011. Activity of a potent and selective phase I ALK inhibitor LDK378 in native and crizotinib-resistant preclinical tumor models. *AACR-NCI-EORTC International Conference: Molecular Targets and Cancer Therapeutics, SanFrancisco, CA*, abstr. No. B232.

Lovly, C.M., J.M. Heuckmann, E. de Stanchina, H. Chen, R.K. Thomas, C. Liang, and W. Pao. 2011. Insights into ALK-driven cancers revealed through development of novel ALK tyrosine kinase inhibitors. *Cancer Research* 71: 4920–4931.

Lu, L., A.K. Ghose, M.R. Quail, M.S. Albom, J.T. Durkin, B.P. Holskin, T.S. Angeles, S.L. Meyer, B.A. Ruggeri, and M. Cheng. 2009. ALK mutants in the kinase domain exhibit altered kinase activity and differential sensitivity to small molecule ALK inhibitors. *Biochemistry* 48: 3600–3609.

McDermott, U., A.J. Iafrate, N.S. Gray, T. Shioda, M. Classon, S. Maheswaran, W. Zhou, H.G. Choi, S.L. Smith, L. Dowell, L.E. Ulkus, G. Kuhlmann, P. Greninger, J.G. Christensen, D.A. Haber, and J. Settleman. 2008. Genomic alterations of anaplastic lymphoma kinase may sensitize tumors to anaplastic lymphoma kinase inhibitors. *Cancer Research* 68: 3389–3395.

Pirozynski, M. 2006. 100 years of lung cancer. *Respiratory Medicine* 100: 2073–2084.

Rivera, V.M., R. Anjum, F. Wang, S. Zhang, J. Keats, Y. Ning, S.D. Wardwell, L. Moran, E. Ye, D.Y. Chun, Q.K. Mohemmad, S. Liu, W.-S Huang, Y. Wang, M. Thomas, F. Li, J. Qi, J. Miret, J.D. Iuliucci, D. Dalgarno, N.I. Narasimhan, T. Clackson, and W.C. Shakespeare. 2010. Efficacy and pharmacodynamic analysis of AP26113, a potent and selective orally active inhibitor of anaplastic lymphoma kinase (ALK). *Proceedings of the 101th annual meeting of the American association for cancer research (AACR), Washington, DC*, 17–21 April 2010, poster 3623.

Sakamoto, H., T. Tsukaguchi, S. Hiroshima, T. Kodama, T. Kobayashi, T.A. Fukami, N. Oikawa, T. Tsukuda, N. Ishii, and Y. Aoki. 2011. CH5424802, a selective ALK inhibitor capable of blocking the resistant gatekeeper mutant. *Cancer Cell* 19: 679–690.

Sasaki, T., S.J. Rodig, L.R. Chirieac, and P.A. Jänne. 2010. The biology and treatment of EML4-ALK non-small cell lung cancer. *European Journal of Cancer* 46: 1773–1780.

Scagliotti, G.V. 2007. Potential role of multi-targeted tyrosine kinase inhibitors in non-small-cell lung cancer. *Annals of Oncology*, Suppl 10: x32–x41.

Shigematsu, H., L. Lin, T. Takahashi, M. Nomura, M. Suzuki, I.I. Wistuba, K.M. Fong, H. Lee, S. Toyooka, N. Shimizu, T. Fujisawa, Z. Feng, J.A. Roth, J. Herz, J.D. Minna, and A.F. Gazdar. 2005. Clinical and biological features associated with epidermal growth factor receptor gene mutations in lung cancers. *Journal of the National Cancer Institute* 97: 339–346.

Siegel, R., D. Naishadham, and A. Jemal. 2013. Cancer statistics, 2013. *CA: A Cancer Journal for Clinicians* 63: 11–30.

Soda, M., Y.L. Choi, M. Enomoto, S. Takada, Y. Yamashita, S. Ishikawa, S. Fujiwara, H. Watanabe, K. Kurashina, H. Hatanaka, M. Bando, S. Ohno, Y. Ishikawa, H. Aburatani, T. Niki, Y. Sohara, Y. Sugiyama, and H. Mano. 2007. Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. *Nature* 448: 561–566.

Soda, M., S. Takada, K. Takeuchi, Y.L. Choi, M. Enomoto, T. Ueno, H. Haruta, T. Hamada, Y. Yamashita, Y. Ishikawa, Y. Sugiyama, and H. Mano. 2008. A mouse model for EML4-ALK-positive lung cancer. *Proceedings of the National Academy of Sciences of the United States of America* 105: 19893–19897.

Webb, T.R., J. Slavish, R.E. George, A.T. Look, L. Xue, Q. Jiang, X. Cui, W.B. Rentrop, and S.W. Morris. 2009. Anaplastic lymphoma kinase: Role in cancer pathogenesis and small-molecule inhibitor development for therapy. *Expert Review of Anticancer Therapy* 9: 331–356.

White, C. 2012. Non-small cell lung cancer pipeline. *Datamonitor.*

Zhang, S., F. Wang, J. Keats, Y. Ning, S.D. Wardwell, L. Moran, Q.K. Mohemmad, E. Ye, R. Anjum, Y. Wang, X. Zhu, J.J. Miret, D. Dalgarno, N.I. Narasimhan, T. Clackson, W.C. Shakespeare, and V.M. Rivera. 2010. AP26113, a potent ALK inhibitor, overcomes mutations in EML4-ALK that confers resistance to PF-02341066. *101st AACR Annual Meeting, Washington, DC.*