Crizotinib: A Tyrosine Kinase Inhibitor

Graham McLaren  
*Western Michigan University, mclareng@me.com*

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A Small Molecule Tyrosine Kinase Inhibitor

Graham McLaren

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Background on Lung Cancer and the EML4-ALK Oncogene

Lung cancer is the most prevalent of cancers and non-small cell lung cancer (NSCLC) is the most fatal. Lung cancer accounts for about 32% of cancers in men and 25% in women. Of those cases of lung cancer, about 85% are NSCLC and it is usually detected at an advanced stage (9, 11). Of all the NSCLC, 2-7% are ALK-positive, meaning they have a genetic mutation that causes the overproduction of an unregulated, activated oncoprotein responsible for intracellular communication. The constitutively active signaling that is unchecked leads to ‘oncogene addiction.’ ‘Oncogene addiction’ means that the cancer’s sole driving force is this specific mutated gene and its products (4,1). The ALK-positive mutation is most commonly found in younger, never (or former, light) smokers that have an adenocarcinoma subtype (1). While the stated percentage of possible patients is relatively low, the 2-7% of NSCLC that is ALK-positive translates into more than 10,000 new cases annually in the United States alone (7).

The specific cancerous target of crizotinib that will be focused on is the EML4-ALK protein. This protein appears in cancers with a genetic rearrangement that occurs in the short arm of chromosome 2 (1). This genetic mutation involves the fusion of the N-terminal end of the echinoderm microtubule-associated protein-like 4 (EML4) gene and the portion of the anaplastic lymphoma kinase (ALK) gene that encodes the entire intracellular tyrosine kinase domain. There have been many different truncations of the EML4 gene observed in these mutations but they always contain the coiled-coil domain and the ALK gene has always been observed to break at intron 20 (8). The result of the mutation is the EML4-ALK oncogene that encodes an enzyme that is capable of activating pro-survival signaling pathways that prevent cell apoptosis, increase cell
growth and proliferation and aid in cell survival (1,6). The lack of death in these cells, and their ability to live and thrive for longer periods of time then their un-mutated counterparts, allows them to multiply and spread rapidly, making the EML4-ALK fusion protein the prominent driver of tumor growth. Cancers expressing the EML4-ALK oncoprotein are termed ALK-positive.

The un-mutated ALK enzyme is believed to play an important role in embryonic neurogenesis but its expression decreases significantly shortly after birth and the low levels are maintained throughout adulthood (8). In adults, it is believed that ALK may be partially responsible for the regeneration of axons in damaged motor neurons but mice with ALK knocked out are viable and fertile (1). In fact, they tended to perform better than their wild type counterparts in experimental models of clinical depression. This could lead to studies that look at the effectiveness of ALK inhibitors when used to treat mood and cognitive disorders (1).

The EML4-ALK protein is dangerous because the tyrosine kinase domain from the ALK gene can undergo ligand independent dimerization. This is possible because of a coiled-coil domain from the EML4 gene. This interaction keeps the enzyme in a constitutively active state, without the need for a ligand to regulate the process (6). As long as ATP is present and able to bind, the EML4-ALK protein will keep pro-survival signaling pathways active (8,6).

The general consensus is that the two main signaling pathways that are activated by the active EML4-ALK fusion protein are the Ras/Raf/MEK/ERK pathway and an abridged version of the JAK/STAT pathway (8,6). These two pathways are
illustrated in Figure 1. The activation of these pathways results in the phosphorylation of the extracellular signal-regulated kinase (ERK) and the signal transducer and activator of transcription 3 (STAT3) proteins, respectively (6). The Ras/Raf/MEK/ERK pathway is responsible for increasing the rate of cell proliferation and decreasing apoptosis while the phosphorylation of STAT3 decreases cell apoptosis by up-regulating a pro-survival protein called survivin (4,6). Ardini et. al. (1) claims that the STAT3 pathway is not a contributing factor in NSCLC, but instead the PI3K-AKT pathway is more significant (1). The PI3K-AKT pathway leads to the phosphorylation and activation of Protein Kinase B (PKB or AKT). This triggers other signaling cascades, which can increase cell growth and proliferation, aid in survival, and even help evade apoptosis (1).

**Drug Facts**

Crizotinib is a small molecule tyrosine kinase inhibitor sold by Pfizer under the brand name Xalkori®. In some material it is referred to by its pre-market product name, PF-2341066. It is approved for use to treat advanced stage or metastasized non-small cell lung cancer that is ALK-positive (9). It is normally taken orally, twice daily in a 250 mg tablet. If needed, the dose can be reduced to 200 mg twice daily and further reduced to 250 mg once daily. Crizotinib can be taken with or without food (4).
Chemically, crizotinib is 3-((R)-1-(2,6-dichloro-3-fluorophenyl)ethoxy)-5-(1-(piperidin-4-yl)-1H-pyrazol-4-yl)pyridin-2-amine and is shown in Figure 2. This gives it a molecular formula of C_{21}H_{22}Cl_{2}FN_{5}O and a molecular weight of 450.34 Da (3). Crizotinib is synthetically manufactured and was originally designed as a c-MET (a different tyrosine kinase) inhibitor. It was found that the tyrosine kinase domains in both the c-MET and ALK proteins are similar enough that the specific structure of crizotinib effectively binds with both (3). The conjugated ring structure provides structural resonance and accurate positioning of binding sites (3). The strategically placed 2-aminopyridine group and the ring nitrogen both form hydrogen bonds to amino acid residues in the tyrosine kinase receptor, Glu 1197 and Met 1199 respectively. These bonds induce a conformational change that allows the other substituents of the drug to block the binding of ATP. The chiral C-21 carbon has a methyl group that must be in the R configuration in order for the drug to properly fit and the methyl group’s hydrophobic properties also make the drug more attractive to the lipophilic ATP binding pocket (8).

Crizotinib has moved with remarkable speed through the development and testing phases. The specific abnormality that is the intended target (the ALK-positive mutation) was not discovered until 2007 (9). It took until 2010 to validate ALK-positive proteins as an effective drug target and by 2011 the FDA had approved the drug for clinical use (6). At this time no generic alternative is available so patients are forced to spend as much as
$9600 a month on this new drug. Private insurance can reduce the cost considerably (to as low as $30 a month) but because this is an oral medication and not intravenous like typical chemotherapies, Medicare and Medicaid coverage is significantly lacking, but that’s another paper.

**Pharmacodynamics**

As mentioned before, crizotinib enters the cell and binds to the lipophilic ATP binding pocket. While bound, it acts as a competitive antagonist to ATP, forming hydrogen bonds between the drug and amino acid residues in the tyrosine kinase receptor. These bonds hold the drug in place while the rest of the molecule blocks the binding of ATP through both placement and inducing a conformational change in the EML4-ALK protein. Without ATP, the protein no longer has access to the phosphate groups that it used to activate downstream cascades. This prevents the protein from ever reaching its active state, essentially turning it off (8). The binding affinity of crizotinib is ten times greater than that of ATP, so relatively low amounts of crizotinib are needed to produce the desired effect (3).

The EML4-ALK protein is prevented from entering its active state because crizotinib acts as a competitive antagonist to ATP. When crizotinib binds to the tyrosine kinase domain, it induces a conformational change in the activation loop, preventing it from accepting ATP, and thus, from becoming active (8). By preventing the activation of the enzyme, crizotinib also indirectly inhibits the phosphorylation of ERK and STAT3 (and arguably, ATK). This leads to an increase in the rate of apoptosis in ALK-positive cells (6).
Okamoto and Nakagawa found that the inhibited ERK pathway increased BIM expression via induction by TAE684 (6). To put more simply, when crizotinib is present, the ERK pathway becomes inhibited, indirectly. The inhibition of the ERK pathway leads to an increase in concentration of the molecule TAE684. TAE684 then induces the expression of pro-apoptotic BIM proteins. These proteins are pro-apoptotic because they bind to and antagonize pro-survival members of its Bcl-2 family of proteins. The antagonization of the pro-survival proteins negates their effects leads to apoptosis of the cell (6).

STAT3 inhibition (via ALK inhibitors) also plays a role in the destruction of ALK-positive cells. Survivin, a protein that prevents apoptosis by inhibiting caspase proteins, is down-regulated to normal levels when the STAT3 pathway is no longer in a constitutively active state. The decreased survivin levels lead to an increase in ALK-inhibitor induced apoptosis (6).

As an alternative form of treatment, targeting each of these pathways individually could, theoretically, work, but it would be, at best, impractical, if not clinically impossible. Targeting each affected signaling pathway directly would likely be less effective than ALK inhibition because of how much is unknown about the specific pathways that are being utilized by the EML4-ALK protein. Even if all the pathways were discovered and could be effectively inhibited, doing so would create much more and more server side effects than a tyrosine kinase inhibitor because these pathways are used for constructive means in healthy cells. They are necessary for survival in cells that are disease-free unlike the ALK receptor (8).
Crizotinib binds to the ALK tyrosine kinases so easily that concentrations as low as 20 nM produce half of the inhibitory effect (IC\textsubscript{50}) (3). This poses a potential issue for side effects. Normally, when an enzyme is over-expressing itself, the goal is to reduce the expression to normal levels. Crizotinib binds so well that it essentially turns the EML4-ALK protein off. The reason this is not too much of a concern is because ALK expression is not required for healthy living, as demonstrated by the ALK knockout mice (1). Crizotinib also has the potential to bind to tyrosine kinase inhibitors other than those in ALK. The molecular design process has tailored crizotinib to work best in ALK’s binding pocket (3) and the therapeutic dose is low enough that most side effects are completely manageable.

The most common reported side effects were gastrointestinal issues (nausea, vomiting and diarrhea) and vision disorders (visual trailing and blurriness) (4). Most of these side effects were either manageable or diminished over the course of treatment. In less common, more severe instances, there were reports of compromised immune systems, pneumonitis, hepatotoxicity and more intense, prolonged GI disturbances (4).

On a clinical scale, crizotinib has shown to be effective at reducing tumor size in ALK-positive patients. Over the course of two trials, encompassing 255 patients, there were 3 complete responses and 136 partial responses. This translates to an objective response rate of about 55% (9,4). Of those with an objective response, about 80% of them occurred within the first 8 weeks of treatment (4). While these results look promising, there is a significant downside. The median duration of the response was limited to about 42 weeks (4). That means that, on average, the cancer will develop a resistance to the drug in under a year. The most common mechanism for developing a
resistance is a secondary mutation within the tyrosine kinase domain of ALK that minimizes crizotinib’s binding affinity. Normally, the secondary mutation would involve substituting a larger amino acid into the area that forms the ATP binding pocket. The larger amino acid, if placed correctly, could prevent crizotinib from binding but still allow ATP to bind (9). This mutation could potentially open up new vulnerabilities in the cancer that could be exploited by another drug.

**Pharmacokinetics**

Crizotinib is normally administered orally, twice daily in 250 mg pills. Peak plasma concentrations ($C_{\text{max}}$) after a single dose were typically reached within 6 hours at a level of 131 ng/mL (4). With a half-life ($t_{1/2}$) of 42 hours, it takes 15 days of administration to reach the steady state plasma concentration (4). The median minimum (trough) plasma concentration was 256 ng/mL, which is high enough to provide full efficacy (4). It was noted that both peak plasma concentration and area under the curve were about 1.5 times greater in Asian patients compared to their non-Asian counterparts (4). No explanation has been offered for this observation.

The volume of distribution averaged out to be 1772 L with 91% of the blood borne drug bound to plasma proteins (4). Elimination from healthy volunteers was primarily through the feces (63%) with 53% being unchanged. The urine accounted for the elimination of 22% of the drug (2.3% unchanged) (4). Primary metabolization was done by cytochrome P450 (CYP) 3A4/5. It was done by first, oxidizing the piperidine ring, followed by dealkylation. The dealkylated products were then conjugated (made water soluble) before being excreted (4).
Other

It was noted that, because crizotinib was metabolized by CYP 3A4/5, coadministration with either CYP3A inducers or inhibitors altered both the AUC and $C_{\text{max}}$. When taken in conjunction with strong CYP3A inducers, crizotinib’s AUC and $C_{\text{max}}$ dropped by 82 and 69% respectively (4). When taken with strong CYP3A inhibitors, those levels jumped 3.2 and 1.4 times, respectively (4). These findings make sense because with more CYP3A that is available to metabolize crizotinib (in the case with the inducer), the faster crizotinib could be broken down. In the case of the inhibitor, the drug can remain in the system longer because the enzyme responsible for its metabolization is otherwise occupied. Crizotinib itself is a moderate CYP3A inhibitor so care must be taken when crizotinib is administered in conjunction with something that is metabolized by CYP3A (4).

Crizotinib was chosen for the topic of this paper because the author’s mother is currently being treated with it. She was diagnosed with stage 3 adenocarcinoma in the spring of 2006. After surgery, chemo and radiation she had no evidence of disease until the fall of 2010. One of her routine scans turned up a spot on her lung and a subsequent MRI turned up two small lesions in her brain. Fortunately, a combination of whole brain radiation and erlotinib (Tarceva®) shrunk both abnormalities significantly. That course of treatment seemed to be holding the disease at bay for a time but a recent scraping from a bronchoscopy turned up live cancer cells and the following bone scan showed a large mass in her sacrum. Palliative radiation has reduced her sacral pain considerably and results regarding tumor shrinkage or bone regeneration are still pending. She started taking Xalkori® (crizotinib) in January of 2013 and she is experiencing minimal side
effects. She has reported small amounts of manageable nausea and some of the visual disorders described above. She does not find the visual disturbances too distracting and has claimed to enjoy and embrace the alternate perception of reality that they offer.

In clinical trials, crizotinib has seen great initial success. Through two studies, its objective response rate averaged about 55% with minimal side effects. Unfortunately, resistance developed in 100% of cases with an objective response in an average of 42 weeks. This was done mainly through secondary mutations in the tyrosine kinase domain of the ALK portion of the EML4-ALK gene. This drug has shown that medicine designed for a very specific disease has the potential to be effective.
References


6 - Okamoto, Isamu, and Kazuhiko Nakagawa. "Echinoderm Microtubule-Associated Protein-like 4-Anaplastic Lymphoma Kinase-Targeted Therapy for Advanced Non-


