Crizotinib
(Xalkori®)
A Small Molecule Tyrosine Kinase Inhibitor
By: Graham McLaren
Lung Cancer

- Small Cell Lung Cancer (15%)
- Non Small Cell Lung Cancer (NSCLC) (85%)
- Adenocarcinoma (~50%)
- Squamous-Cell Carcinoma
- Large-Cell Lung Carcinoma
- ALK+ EML4-ALK Fusion (~2-7% of NSCLC)
- Other Oncogene Cancers
Non Small Cell Lung Cancer

• Non Small Cell Lung Cancer (NSCLC) is the most common cause of cancer-related deaths
• EML4-ALK Fusion Protein present in ~2-7% of NSCLC cases
  – 10,000 new cases per year in the United States alone
  – Most common in younger, never smokers
Overview

• Fights ALK+ Cancers
  – EML4-ALK oncogene (NSCLC)
    • Translocation in short arm of chromosome 2
    • Encodes a protein that can undergo ligand independent dimerization
      – Is therefore constitutively active and unregulated
  – ALK-NPM (ALCL)
    • Present is anaplastic large cell lymphoma

• Other tyrosine kinases
  – c-MET

• Competitively binds to ATP binding site
  – Shuts down pro-survival second messenger signaling
Background on EML4-ALK

• A translocation on the short arm of chromosome 2

• Fusion between:
  – The N-terminal end of the echinoderm microtubule-associated protein-like 4 (EML4) gene
    • Several truncations observed
      – Always contains coiled-coil domain
  – Everything downstream of intron 20 of the anaplastic lymphoma kinase (ALK) gene
    • encodes the entire intracellular tyrosine kinase domain

• EML4-ALK fusion is an oncogene
EML4-ALK Translocation
What does ALK do?

• The function of ALK in healthy individuals is not fully understood.
  – May play an important role in embryonic neurogenesis.

• It is present in high levels in utero and shortly after birth but then declines to very low levels that are maintained throughout adulthood.
  – May be partially responsible for the regeneration of axons in damaged motor neurons.
The ALK Tyrosine Kinase Makes a Good Target

• Vulnerable
  – Attacking it has anti-tumor effects

• Not necessary (in mice at least)
  – ALK knockout mice (mice without any ALK proteins) were found to be viable and fertile.
    • If ALK proteins serve few functions in adults then inhibiting healthy ALK proteins should produce few side effects

• Unintended side benefits
  – ALK knockout mice tended to perform better than their wild type counterparts in experimental models of clinical depression.
    • Possible research into ALK inhibitors as an antidepressant.
What does EML4-ALK do?

• The EML4-ALK fusion gene codes for a protein called, cleverly, the EML4-ALK protein.
  – Oncoprotein
  – Is capable of using EML4’s coiled-coil domain to dimerize proteins and constitutively activate ALK’s tyrosine kinase domain.
    • Type of trans-autophosphorylation
    • No ligand is needed
Signaling Pathways

• 3 main, slightly disputed pathways
  – Ras/Raf/MEK/ERK pathway (agreed)
  – PI3K-AKT pathway (disputed)
  – STAT3 pathway (disputed)
Ras/Raf/MEK/ERK pathway

• Long cascade that ends with the phosphorylation on of extracellular signal-regulated kinase (ERK)

• Phosphorylation of ERK is responsible for:
  – Increased cell proliferation
  – Decreased apoptosis
PI3K-AKT pathway

• Phosphorylation and activation of Protein Kinase B (PKB or AKT)
  – Triggers other signaling cascades
    • Increase growth
    • Increase proliferation
    • Evade apoptosis
STAT3 pathway

• Abridged JAK/STAT pathway
• When active, the STAT3 pathway induces the production of a protein called ‘survivin.’
  – Survivin prevents apoptosis by inhibiting caspase proteins
  – Allows cells to live longer than healthy cells
Crizotinib

Crizotinib Facts

• Chemical Formula
  – $C_{21}H_{22}Cl_2FN_5O$

• IUPAC Name
  – 3-((R)-1-(2,6-dichloro-3-fluorophenyl)ethoxy)-5-(1-(piperidin-4-yl)-1H-pyrazol-4-yl)pyridin-2-amine

• Molecular Weight
  – 450.34 g/mol
Where did Crizotinib come from?

• Discovered by accident... kind of
  – Pfizer was working on a c-MET inhibitor
    • c-MET is another tyrosine kinase receptor
  – Found a drug with great pharmacodynamics but poor pharmacokinetics
  – Pfizer continued to tweak the drug until they came up with crizotinib
  – Crizotinib binds to the lipophilic ATP binding pocket of RTK
  – Marketed as Xalkori®
Crizotinib Timeline

- Crizotinib was developed in 2005
- Target was discovered in 2007
- Target validated in 2009
- FDA approved in 2011
Cost

- Costs $9,600 per month without insurance
- With insurance, co pay can be as low as $30
Why Crizotinib works

- Binds efficiently ($IC_{50} = 20\text{nM}$)
- The 2-aminopyridine NH group forms a Hydrogen bond with the hinge residue Glu 1197 while the ring nitrogen hydrogen bonds with the hinge residue Met 1199.
- The alpha-methyl group aids the structure of the benzyl group and makes favorable hydrophobic interactions in the lipophilic pocket.
  - Only the $R$ configuration will fit.
What that all means.

ATP CANNOT BIND
EML4-ALK positive cells

ALK inhibitions

Inhibition of ERK  Inhibition of STAT3

BIM induction  Down-regulation of survivin

Apoptosis
No ATP, No Autophosphorylation

• With the EML4-ALK protein no longer active, the aforementioned signaling cascades are all inhibited.
• This leads to decreased cell proliferation and increased apoptosis.
• Clinically, an objective response rate (ORR) of 55% was seen with 3 complete responses. (n=255)
Shortcomings

• Crizotinib works very well, very quickly...
  – 80% with objective response saw improvement within the first 8 weeks

• But, it also looses its effectiveness quickly.
  – The average length of response was about 42 weeks.
  – Resistance formed through secondary mutation
    • Usually consists of a larger amino acid residue being substituted into the pocket, preventing crizotinib from binding
    • Author’s speculation that the cleft in which the 3-benzyloxy group sits would be a fitting place for the substitution.
Pharmacokinetics

• Administration
  – 250mg taken orally, twice daily

• Absorption
  – Peak plasma levels are reached in 4-6 hours after dosing
    • ~131ng/mL after a single dose
  – Steady state is reached in about 15 days
    • ~256ng/mL minimum
  – The bioavailability ranged from 32-66%
  – Absorbs best at low pH
Pharmacokinetics

• Distribution
  – The $V_D$ is 1772 L
    • Indicative of extensive uptake by tissues
    • Of the drug left in circulation, 91% was bound to plasma proteins
  – Half life $\sim$42 hours

• Metabolism
  – Primarily metabolized by chytochrome P450 (CYP3A4/5)
    • Oxidized the piperidine ring,
    • Dealkylation
      – Conjugation
Pharmacokinetics

• Elimination
  – 63% through feces
    • 53% unchanged
  – 22% through urine
    • 2.3% unchanged
Side Effects

• Most are manageable
• Over half (62%) report low grade visual disturbances
  – Light trails, vitreous floaters and blurred vision
• GI disturbances were generally low grade
  – Nausea, diarrhea, vomiting and constipation
• 1.6% of patients reported life-threatening interstitial lung disease
• Cases of hepatotoxicity (7%)
Contraindications

• Avoid taking crizotinib with any CYP3A substrates, inducers or inhibitors
• Avoid taking crizotinib with anything that can raise the stomach pH
  – Antacids
  – Crizotinib is best absorbed at low pHs
• Because crizotinib is such a new drug, many drug interactions are untested
Why am I interested?

• My mom has been taking crizotinib since New Years
• First diagnosed in 2006
  – stage 3 adenocarcinoma
  – Surgery, chemo and radiation put it into remission
• Scan shows masses in brain Fall 2010
  – Erlotinib (Tarceva) and whole brain radiation shrunk both
• Stage 4 diagnosed December 2012 after finding her sacrum full of disease
  – Palliative radiation to the area relived pain and crizotinib was started early January 2013
  – Currently doing well
  – Bought a dog on Tuesday (23 April 2013)
# Acronym Table

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<td>ALK</td>
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<tr>
<td>EML4</td>
<td>Echinoderm Microtubule-Associated Protein-Like 4</td>
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<tr>
<td>TKR</td>
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References


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