

## Project on Drug Development

Drug chosen: Nilotinib

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### Introduction

Nilotinib is a tyrosine kinase inhibitor used targeted therapy for cancer, Chronic myelocytic leukemia (CML), which is caused by the constitutively active tyrosine kinase Bcr-Abl. Nilotinib binds to the inactive Bcr-Abl protein, blocking Bcr-Abl mediated proliferation of leukemic cells. Nilotinib blocks the signals from these proteins and helps stop cancer cells from growing.

Nilotinib is used in treatment of leukemia and it is considered a "second-generation" Bcr-Abl tyrosine kinase inhibitor, building on the success of imatinib. Studies show it is 20 to 50 times stronger than imatinib and it is also intended to be specific for BCR-ABL. Therefore, this new medicine is widely use nowadays.

### **(A)Lead Compound Discovery**

Imatinib is the first-generation drug to cure blood cancer. It can inhibit the Bcr-Abl protein to avoid excess white blood cells formation. However, in the past 5 years, IRIS Medical Foundation found that there were 15% of CML patients had already resistant on imatinib and 4.2% were unresponsive to it. As a result, chemists created nilotinib with the idea of improving the target specificity of an earlier kinase inhibitor, imatinib. Nilotinib possesses an in vitro Bcr-Abl binding potency 30 times greater than imatinib in imatinib-resistant cells, and 5-7 times greater than imatinib in imatinib-susceptible leukemic cells.

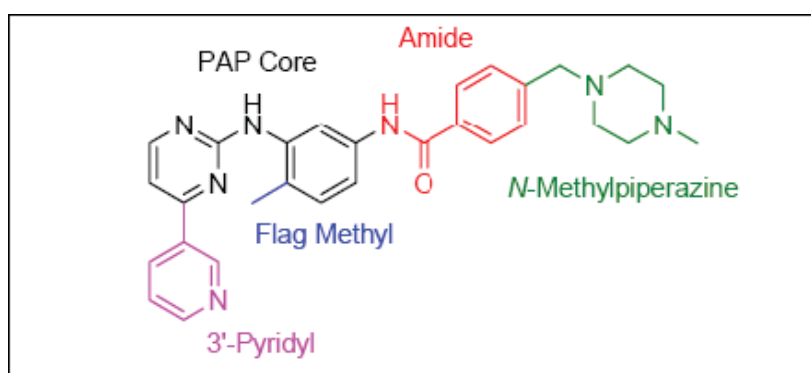
### **(B) Molecular Modification**

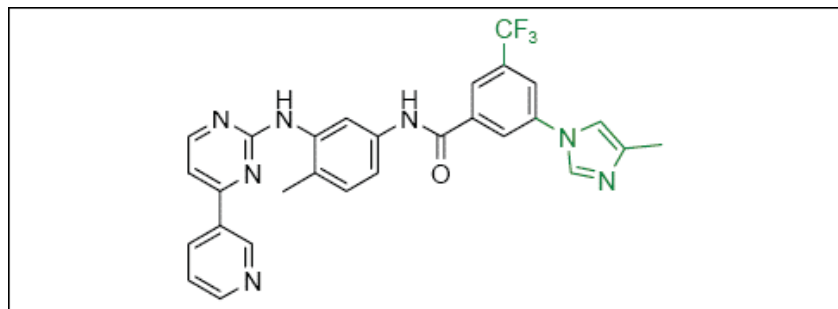
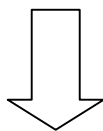
Nilotinib (also known as AMN107) was developed by modifying the chemical structure of imatinib by using a rational drug design strategy based on the replacement of the methylpiperazinyl group of imatinib and optimization to optimize potency against the ABL kinases. It retains the kinase specificity profile of imatinib, with inhibition confined to ABL kinases, ARG, Kit, and PDGFR kinases. Nilotinib, in contrast to dasatinib, binds the kinase domain of ABL kinases in the inactive conformation.

### **Imatinib (First-generation drug)**

IUPAC name:

4-[(4-methylpiperazin-1-yl)methyl]-N-[4-methyl-3-[(4-pyridin-3-yl)pyrimidin-2-yl)amino]phenyl]benzamide





### **Nilotinib (Second-generation drug)**

IUPAC name:

4-methyl-*N*-[3-(4-methyl-1*H*-imidazol-1-yl)- 5-(trifluoromethyl)phenyl]-3-[(4-pyridin-3-yl)pyrimidin-2-yl] amino]benzamide

### Working Principle:

Like imatinib, nilotinib does not inhibit Src kinase and binds only to the inactive conformation of Bcr-Abl, with P-loop folding over the ATP-binding site, and the activation-loop blocking the substrate binding site, to disrupt the ATP-phosphate-binding site and inhibit the catalytic activity of the enzyme. Nilotinib makes 4 hydrogen-bond interactions with the Abl kinase domain, involving the pyridyl-N and the backbone-NH of Met318, the aniline-NH and the side chain hydroxyl of Thr315, the amido-NH and side chain carboxylate of Glu286, as well as the amido-C=O of Asp381 and a fluorine atom in the trifluoromethyl group of nilotinib. This close interaction made changes in the core of imatinib prohibitive. The improvement in binding affinity for Bcr-Abl maintains the ability to inhibit also Kit and PDGFR, but with less affinity of imatinib (Bcr-Abl PDGFR Kit).

### **(C) Formulation Development**

Nilotinib drug substance, a monohydrate mono-hydrochloride, is a white to slightly yellowish to slightly greenish yellow powder.

Tasigna (nilotinib) capsules, for oral use, contain 150 mg or 200 mg nilotinib base, anhydrous (as hydrochloride, monohydrate) with the following inactive ingredients: colloidal silicon dioxide, crospovidone, lactose monohydrate, magnesium stearate and polyoxamer 188. The capsules contain gelatin, iron oxide (red), iron oxide (yellow), iron oxide (black) and titanium dioxide.

### **(D) Safety Tests and Human Trials**

#### **Animal Test (in mice)**

Ph-positive leukemias are caused by the aberrant fusion of the BCR and ABL genes. Nilotinib is a selective Bcr/Abl tyrosine kinase inhibitor related to imatinib, which is widely used to treat chronic myelogenous leukemia. Because Ph-positive acute lymphoblastic leukemia only responds transiently to imatinib therapy, we have used mouse models to test the efficacy of nilotinib against lymphoblastic leukemia caused by the P190 form of Bcr-Abl.

## Result:

After transplant of 10,000 highly malignant leukemic cells into compatible recipients, untreated mice succumbed to leukemia within 21 days, whereas mice treated with 75 mg/kg nilotinib survived significantly longer. We examined cells from mice that developed leukemia while under treatment for Bcr/Abl kinase domain point mutations but these were not detected. In addition, culture of such cells *ex vivo* showed that they were as sensitive as the parental cell line to nilotinib but that the presence of stromal support allowed resistant cells to grow out. Nilotinib also exhibited impressive anti-leukemia activity in P190 Bcr/Abl transgenic mice that had developed overt leukemia/lymphoma masses and that otherwise would have been expected to die within 7 days. Visible lymphoma masses disappeared within six days of treatment and leukemic cell numbers in peripheral blood were significantly reduced. Treated mice survived more than 30 days.

## Conclusion:

These results show that nilotinib has very impressive anti-leukemia activity but that lymphoblastic leukemia cells can become unresponsive to it both *in vitro* and *in vivo* through mechanisms that appear to be Bcr-Abl independent.

## **Human Trials**

There are many safety tests carried out in different country, for example, Japan, America and Hong Kong. The Department of Leukemia of University of Texas M.D. Anderson Cancer Center, which located in Houston, had designed a study to evaluate the safety and tolerability of nilotinib.

## **Phase I**

At the center, from May 25, 2004, to May 4, 2005, 119 patients whose disease was resistant to imatinib were enrolled. The patients were assigned to receive daily doses of nilotinib according to the following dosing schedule: 50 mg (7 patients), 100 mg (7), 200 mg (10), 400 mg (10), 600 mg (6), 800 mg (19), and 1200 mg (10) or to receive 400 mg twice daily (32) or 600 mg twice daily (18). No dose-limiting toxic effects were seen at doses of up to 600 mg daily. Dose-limiting toxic effects occurred among 18 patients at doses higher than 600 mg.

## Result:

The posterior probability of dose-limiting toxicity was 0.30. Among 119 patients, 17 were in chronic phase disease, 56 in accelerated phase (10 with clonal evolution only), 24 in myeloid blast phase, 9 in lymphoid blast phase, and 13 had Ph-positive ALL. Overall, of 33 patients with the blastic phase, 13 had a hematologic response to nilotinib (39%) and 9 patients (27%) had a cytogenetic response, 6 of whom had a major cytogenetic response (Ph-positive cells in metaphase, 35%). Of 46 patients with accelerated-phase CML (excluding those with clonally evolution only), 33 had a hematologic response and 22 had a cytogenetic response; 9 of these responses were major. Among the 10 patients who had clonally evolution as the only feature of the accelerated phase of CML, 5 had active disease and 5 were in complete hematologic remission. All 5 patients with clonally evolution and hematologic disease had a complete hematologic response; 6 of 10 had a major cytogenetic response.

### Conclusion:

This study of nilotinib defined the dose-limiting toxic effects and the maximum tolerated dose of the drug. Imatinib therapy has side effects that can be dose-limiting in 10 percent of patients. 23 in this study, nilotinib was not associated with the common toxic effects seen with imatinib (e.g. fluid retention, edema, and weight gain) or with pleural effusions. Frequently noted side effects of nilotinib were mild-to-moderate rashes; transient, clinically insignificant elevations of indirect bilirubin levels; and myelosuppression, which was dose-related and dose-limiting. In summary, nilotinib has a relatively favorable safety profile.

Afterward, another study was designated to evaluate the safety and efficacy.

### **Phase II**

The 316 chronic-phase patients in the study were heavily pre-treated for Ph+ CML, with a significant majority (72%) having received at least 600 mg of imatinib as well as having been treated earlier with interferon (65%) and hydroxyurea (83%).

### Result:

Among 279 assessable patients (i.e., those patients with at least six months of follow up) with chronic-phase disease, major cytogenetic response was observed in 145 (52%) of which 96 (34%) were complete. Complete hematologic response was reported in 137 (74%) of 185 assessable patients. Among 64 patients with accelerated-phase disease, major cytogenetic response was observed after at least 8 months follow-up in 23 patients (36%), of which 14 (22%) were complete. Confirmed hematologic response occurred in 38 (59%), of which 15 (23%) were complete.

### Conclusion:

The phase II study showed an acceptable tolerability profile with a low incidence of events related to fluid retention such as edema, a side effect common with other tyrosine kinase inhibitors. The study also showed virtually no non-hematologic cross-intolerance between imatinib and nilotinib.

### **(E) Approval for Marketing**

Based on the above safety test results, the Food and Drug Administration (FDA) approved Nilotinib for sale. FDA recommends that 400 mg orally and twice daily, approximately 12 hours apart and should not be taken with food. No food should be consumed for at least 2 hours before the dose is taken and no food should be consumed for at least one hour after.

Nilotinib was widely used in USA in June 2010; however, Hospital Authority is still considering whether this new medicine can be widely used in Hong Kong.