Inhibitors of the mutant form of KIT

The present invention relates to the treatment of KIT dependent diseases that are characterized by a mutant form of KIT whereby the mutant KIT is identified and an appropriate inhibitor of the mutant KIT is administered.

The c-kit gene encodes a receptor protein tyrosine kinase, which is herein referred to as KIT, but which is also known as mast/stem cell growth factor receptor. The amino acid sequence of KIT and the nucleotide sequence of the c-kit gene are known. See Swiss Prot.: P10721. Upon binding its ligand, stem cell factor, KIT forms a dimer that is autophosphorylated and activates signaling cascades that lead to cell growth. Mutations that lead to an activated form of KIT, especially forms that are activated independently of its ligand, are known and are believed to play a role in certain proliferative diseases, such as mast cell diseases, like mastocytosis, particularly systemic mastocytosis, acute myelogenous leukemia, gastrointestinal stromal tumors, sinonasal NK/T-cell lymphoma, seminomas and dysgerminomas.

Imatinib, which is marketed as its mesylate salt under the brandname GLIVEC or GLEEVEC, is known to inhibit wild type KIT and certain KIT mutations e.g. those in exons commonly found in gastrointestinal stromal tumors (GIST). However, it is also inactive or significantly less active against certain other mutant forms of KIT, for example the D816V mutation commonly found in systemic mastocytosis. The present invention is based upon research that correlates the treatment of a disease characterized by a mutant form of KIT with an appropriate alternative pharmaceutical therapy based on the alternative's ability to inhibit the mutant KIT.

Thus, the present invention relates to a method of treating a KIT dependent disease in a patient, which comprises

(a) identifying the mutant form of KIT associated with the KIT dependent disease; and

(b) administering to the patient an effective mutant KIT-inhibiting amount of an inhibitor selected from the group consisting of midostaurin, vatalanib and compound A.

KIT dependent diseases are generally proliferative diseases that are characterized by excessive KIT kinase activity due to an activating mutation in KIT. Such activating mutations are known in the art and are identified by techniques known in the art.

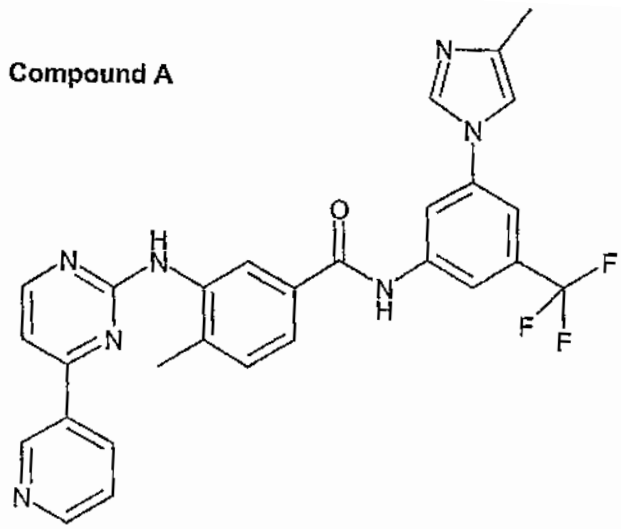
KIT dependent diseases include diseases characterized by the following known KIT mutations: D816F, D816H, D816N, D816Y, D816V, K642E, Y823D, Del 550-558, Del 557-561, N822K, V654A, N822H, Del 550-558 + V654A, Del 557-561 + V654A, Ins503AY, V560G, 558NP, Del 557-558, Del VV559-560, F522C, Del 579, R634W; K642E, T801I, C809G, D820Y, N822K, N822H, Y823D, Y823C and T670I.

In an important embodiment of the present invention, the KIT dependent disease is resistant to treatment with imatinib. A KIT dependent disease that is resistant to imatinib is generally a KIT dependent disease as described above wherein imatinib, administered at a dose of 400-1000 mg/day, does not provide sufficient inhibition of the mutant KIT to effect a significant therapeutic benefit. Generally, mutant KIT that is resistant to imatinib has an *in vitro* IC_{50} of the mutant KIT greater than about 3 micromolar. Imatinib resistant KIT mutations include D816F, D816H, D816N, D816Y, D816V, T670I and mutant forms that include V654A.

The selection of a compound that inhibits the mutant form of KIT is based on testing the compound or a number of compounds for their ability to inhibit the mutant KIT. Such testing is carried out by standard inhibition assays that are known in the art or within the skill of the artisan.

The KIT inhibitors utilized in accordance with the present method include midostaurin, vatalanib and compound A. Midostaurin (US5;093,330) and vatalanib (WO 98/35958) are known in the art. Compound A is a compound of the formula

Compound A



And may be produced according to WO 04/005281.

Appropriate dosages of midostaurin, vatanalib and compound A are determined by routine methods.

An appropriate dose of midostaurin is administered, e.g., once, twice or three times a day, for a total dose of 25 – 300 preferably 50-300 more preferably 50 -100 most preferably 100-300 mg daily, e.g., two or three times a day, for a total dose of 150-250 mg, preferably 225 mg daily.

An appropriate daily dose of vatanalib is an amount in the range from 300-4000 mg, e.g., in the range from 300-2000 mg/day or 300-1500 mg/day, in particular, 300, 500, 750, 1000, 1250, 1500 or 2000 mg/day, particularly 1250 mg/day.

The daily dose of compound A for a 70 kg/person is from approximately 0.05-5 g, preferably from approximately 0.25-1.5 g.

EXAMPLES

The human KIT gene encoding aa 544-976 was cloned into the baculovirus donor plasmid pFB-GST-01. This coding sequence was excised using restriction endonucleases Bam H1 and EcoR1 and ligated to a Bac-to-Bac donor vector pFB-GEX-P1 with compatible ends. Subsequently the desired mutations were brought into the KIT gene by methods know to a person skilled in the art. Due to a frame shift within the original plasmid that was used to generate the mutant coding sequences, the mutated plasmid inserts were excised and

inserted into the Bac-to-Bac donor vector pFB-GST-01 using the restriction enzymes BamH1-EcoR1 for each mutant shown in Figure 1. Automated sequencing confirmed the correct sequence to be present for each mutant plasmid.

Bacmid DNA was generated from 10 colonies each of DH10Bac cells transformed with pFB-GO1-KIT-mutant plasmid clones as described in materials and methods and these transfected into Sf9 cells. The transfected cells were pelleted and the resultant recombinant baculovirus present in the supernatant medium amplified. Western blotting was applied to the lysed cell pellets to confirm the expression of the GST-c-KIT fusion protein by the viral clones using anti-KIT and anti-GST antibodies for immonudetection.

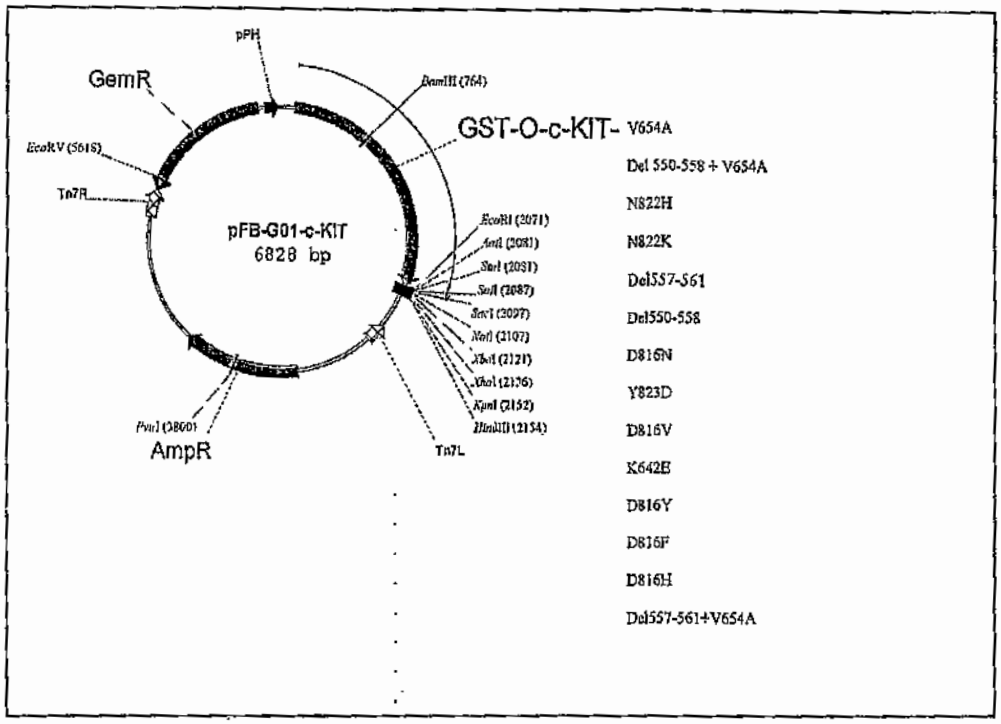


Figure 1

Kit Mutation	Vatalanib IC_{50} (μM) (avg)	Compound A IC_{50} (μM) (avg)
D816F	>10	>10
D816H	>10	>10
D816N	>10	<10
D816Y	>10	>10
D816V	>10	>10
K642E	<1	<10
Y823D	<1	<1
Del 550-558	<1	<2
Del 557-561	<1	<2
N822K	<2	<10
V654A	>10	>10
N822H	<2	<10
Del 550-558 + V654A	<10	<10
Del 557-561 + V654A	>10	>10

Midostaurin

HIS preparation	average IC50 µM	SEM	N° of values
HT-KIT-TA23 wt	1.7	0.15	2
HT-KIT TA23 -D820G	0.084	0.05	2
HT-KIT TA23 -T670I	0.89	0.21	2
GST preparation	average IC50 µM	SEM	N° of values
GST-KIT wt	1.8	0.26	10
GST-KIT Del 557-561	0.32	0.042	3
GST-KIT Del 550-558	0.53	0.057	3
GST-KIT Del 550-558+ V654A	0.27	0.079	5
GST-KIT Del 557-561 + V654A	0.34	0.11	5
GST-KIT V654A	0.46	0.16	5
GST-KIT K642E	0.64	0.036	4
GST-KIT R634W	0.33	0.13	2
GST-KIT T670I + Del 550-558	0.11	0.05	2
GST-KIT D816F	0.41	0.055	5
GST-KIT D816H	0.35	0.078	5
GST-KIT D816N	0.74	0.25	5
GST-KIT D816Y	0.29	0.11	9
GST-KIT D816V	0.25	0.039	3
GST-KIT D816H + R634W	0.08	0.04	2
GST-KIT N822H	0.37	0.12	5
GST-KIT N822K	0.15	0.058	5
GST-KIT Y823D	0.13	0.0075	3

Assay conditions: 1 µM ATP, 5 µg / ml Poly-EY, 10 min incubation at ambient temperature

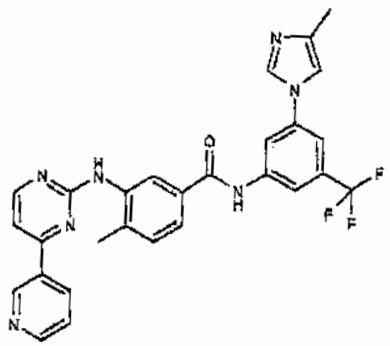
Virus containing media was collected from the transfected cell culture and used for infection to increase its titer. Virus containing media obtained after two rounds of infection was used for large-scale protein expression. For large-scale protein expression 100 cm^2 round tissue culture plates were seeded with 5 x 10^7 cells/plate and infected with 1 mL of virus-containing media (approximately 5 MOIs). After 3 days, the cells were scraped off the plate and centrifuged at 500 rpm for 5 minutes. Cell pellets from 10-20, 100 cm^2 plates, were re-suspended in 50 mL of ice-cold lysis buffer (25 mM Tris-HCl, pH 7.5, 2 mM EDTA, 1% NP-40, 1 mM DTT, 1 mM PMSF). The cells were stirred on ice for 15 minutes and then centrifuged at 5000 rpm for 20 minutes.

The centrifuged cell lysate was loaded onto a 2 mL glutathione-sepharose column (Pharmacia) and washed 3 x with 10 mL of 25 mM Tris-HCl, pH 7.5, 2 mM EDTA, 1 mM DTT, 200 mM NaCl. The GST-tagged proteins were then eluted by 10 applications (1 mL each) of 25 mM Tris-HCl, pH 7.5, 10 mM reduced-glutathione, 100 mM NaCl, 1 mM DTT, 10% glycerol and stored at -70°C.

The protein kinase activities of the various Kit mutants 200-500 ng were assayed in the presence or absence of inhibitors, 20 mM Tris-HCl, pH 7.6, 3 mM $MnCl_2$, 3 mM $MgCl_2$, 1 mM DTT, 10 µM Na_3VO_4, 3 µg/mL poly(Glu,Tyr) 4:1, 1% DMSO, 1.5 µM ATP (γ-[^{33}P]-ATP 0.1 µCi). The assay (30 µL) was carried out in 96-well plates at ambient temperature for 30 minutes and the reaction terminated by the addition of 20 µL of 125 mM EDTA. Subsequently, 30 µl of the reaction mixture were transferred onto Immobilon-PVDF membrane (Millipore, Bedford, MA, USA) previously soaked for 5 minutes with methanol, rinsed with water, then soaked for 5 minutes with 0.5% H_3PO_4 and mounted on vacuum manifold with disconnected vacuum source. After spotting all samples, vacuum was connected and each well rinsed with 200 µL 0.5% H_3PO_4. Membranes were removed and washed 4 x on a shaker with 1.0% H_3PO_4, once with ethanol. Membranes were counted after drying at ambient temperature, mounting in Packard TopCount 96-well frame, and addition of 10 µL/well of Microscint (Packard). IC_{50} values were calculated by linear regression analysis of the percentage inhibition of each compound in duplicate, at 4 concentrations (usually 0.01, 0.1, 1 and 10 µM). One unit of protein kinase activity is defined as 1 nmole of ^{33}P transferred from [γ^{33}P]ATP to the substrate protein/minute/mg of protein at RT.

We claim:

1. Use of the compound of formula A



A

for the manufacture of a medicament for the treatment of a KIT dependent disease in a patient, which comprises:

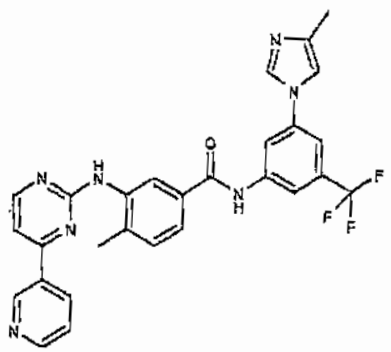
(a) identifying a mutant form of KIT associated with the KIT dependent disease; and

(b) administering to said patient an effective mutant KIT inhibiting amount of compound A,

wherein the mutant form of KIT is selected from D816F, D816H, D816N, D816Y, D816V, K642E, Y823D, Del 550-558, Del 557-561, N822K, V654A, N822H, Del 550-558 + V654A, Del 557-561 + V654A, Ins503AY, V560G, 558NP, Del 557-558, Del VV559-560, F522C, Del 579, R634W, K642E, T801I, C809G, D820Y, N822K, N822H, Y823D, Y823C and T670I.

2. Use according to Claim 1, wherein the mutant form of KIT is selected from D816F, D816H, D816N, D816Y, D816V, K642E, Y823D, Del 550-558, Del 557-561, N822K, V654A, N822H, Del 550-558 + V654A, Del 557-561 + V654A.

3. Use of the compound of formula A



A

for the manufacture of a medicament for the treatment of a KIT dependent disease in a patient, which comprises:

(a) identifying a mutant form of KIT associated with the KIT dependent disease; and

(b) administering to said patient an effective mutant KIT inhibiting amount of compound A,

wherein the KIT dependent disease is resistant to treatment with imatinib.

4. Use according to Claim 1, wherein the mutant form of KIT is selected from the group consisting of D816H, D816N, K642E, Y823D, Del 550-558, Del 557-561, N822K and N822H.

5. Use according to Claim 2, wherein the mutant form of KIT is D816F.

6. Use according to Claim 2, wherein the mutant form of KIT is D816H.

7. Use according to Claim 2, wherein the mutant form of KIT is D816N.

8. Use according to Claim 2, wherein the mutant form of KIT is D816V.

9. Use according to Claim 2, wherein the mutant form of KIT is K642E.

10. Use according to Claim 2, wherein the mutant form of KIT is Y823D.

11. Use according to Claim 2, wherein the mutant form of KIT is Del 550-558.

12. Use according to Claim 2, wherein the mutant form of KIT is Del 557-561.

13. Use according to Claim 2, wherein the mutant form of KIT is N822K.

14. Use according to Claim 2, wherein the mutant form of KIT is V654A.

15. Use according to Claim 2, wherein the mutant form of KIT is N822H.

16. Use according to Claim 2, wherein the mutant form of KIT is Del 550-558 + V654A.

17. Use according to any of Claims 1-16, wherein the KIT dependent disease is selected from mast cell diseases, acute myelogenous leukemia, gastrointestinal stromal tumors, seminomas and dysgerminomas.