METHOD OF DETECTING RESISTANCE TO CANCER THERAPY

FIELD

The present invention relates to the fields of medicine, cell biology, molecular biology and genetics.

BACKGROUND

CML is a cancer of haematopoietic stem cells, and is caused by the presence of the oncogenic fusion gene, termed BCR-ABL, that is found in all patients with CML.

BCR-ABL encodes for a constitutively active tyrosine kinase that mediates the increased survival and proliferation of CML cells when compared to their normal counterparts. Effective treatment for CML exists in the form of a class of drugs that inhibits the kinase activity of BCR-ABL, which are commonly called tyrosine kinase inhibitors (TKI).

However, a small proportion of patients will exhibit primary resistance to TKIs and not respond, while others will have an initial response but over time, develop secondary resistance to these drugs, an event that is often associated with transformation to blast crisis (BC) CML, and shorter survival. In about 60% of patients with TKI-resistance (ref A1), BCR-ABL kinase activity is found to be reactivated, either through mutations in the *BCR-ABL* gene that render TKIs less able to bind to BCR-ABL, or via amplification and overexpression of the 'wild type' BCR-ABL gene or protein.

In the remaining 40% of cases, the cause of the TKI-resistance is unknown. The discovery of mechanisms which mediate TKI-resistance in the absence of reactivation of BCR-ABL, also termed BCR-ABL-independent TKI-resistance, are critically important to determine strategies to overcome TKI-resistance, and better manage patients with CML.

Thus, therapy with BCR-ABL inhibitors has resulted in high rates of disease control in the majority of patients with chronic phase chronic myelogenous leukemia (CML). However, a significant proportion of patients still fail to achieve optimal responses,[1] and almost all patients with late-stage disease succumb to their illness.[2]

Attempts utilizing clinically-driven risk scores to stratify patients for clinical outcome have had limited success,[3, 4] in part because such scores do not take into account the molecular features of the disease or patient-specific genetic factors that contribute to clinical outcomes.

Indeed, almost nothing is known of host genetic factors that are predictive of response in chronic phase CML.[5-7]

As a result, current clinical recommendations are to treat all patients with the same starting dose of imatinib, and to monitor for benchmark clinical responses at regular intervals. These include normalization of peripheral blood counts, and degree of cytogenetic and molecular responses at three- to six-monthly intervals.[5] Only upon failure to attain benchmarks is therapy altered. As such decision points, options include increasing the dose of imatinib, switching to more potent tyrosine kinase inhibitors, as well as preparation for high-dose chemotherapy and transplantation.

Ideally, it would be possible to tailor therapy according to both the leukemia and the patient so as to achieve the most rapid response as well as avoid the emergence of drug-resistance or disease progression. For these reasons, reliable markers for predicting response and guiding therapy are needed. Recent work has relied on array-based expression profiling to provide insights into genetic factors associated with drug resistance and disease progression in CML.[8] However, such approaches are limited by their inherent bias as well as their inability to define the underlying genetic events that contribute to these expression profiles.

SUMMARY

According to a 1st aspect of the present invention, we provide a polymorphic variant of a *BIM* (*BCL2L11*) gene which comprises, in 5' to 3' order, the nucleotide sequence set out in SEQ ID NO: 5 followed immediately by the nucleotide sequence set out in SEQ ID NO: 7.

There is provided, according to a 2nd aspect of the present invention, a polymorphic variant of a *BIM* (*BCL2L11*) gene characterised by lacking the nucleotide sequence set out in SEQ ID NO: 6.

The nucleotide sequence may be obtainable from a *BIM* polymorphic variant as set out above. The nucleotide sequence may be obtainable by nucleic acid amplification.

The polymorphic variant of a *BIM* (*BCL2L11*) gene or the nucleotide sequence may be associated with resistance to treatment with tyrosine kinase inhibitors for chronic myelogenous leukaemia in an individual comprising such a polymorphism. This may be in the absence of BCR-ABL reactivation (BCR-ABL-independent TKI-resistance).

The polymorphic variant of a *BIM* (*BCL2L11*) gene or the nucleotide sequence may be associated with resistance to treatment with tyrosine kinase inhibitors for gastrointestinal stromal tumours (GIST) in an individual comprising such a polymorphism. This may be in the absence of c-KIT/PDGFR reactivation (c-KIT/PDGFR-independent TKI-resistance).

The polymorphic variant of a *BIM* (*BCL2L11*) gene or the nucleotide sequence may be associated with resistance to treatment with tyrosine kinase inhibitors for non-small cell lung cancer (NSCLC) in an individual comprising such a polymorphism. This may be in the absence of EGFR reactivation (EGFR-independent TKI-resistance).

The polymorphic variant of a *BIM* (*BCL2L11*) gene or the nucleotide sequence may be associated with resistance to treatment with tyrosine kinase inhibitors for a myeloproliferative disorder in an individual comprising such a polymorphism. This may be in the absence of JAK2reactivation (JAK2-independent TKI-resistance).

We provide, according to a 3rd aspect of the present invention, a nucleotide sequence as set out in SEQ ID NO: 3. We provide ,a nucleotide sequence as set out in SEQ ID NO: 4. We provide for a combination of (a) and (b). The combination may comprise a primer set.

The method of detecting the presence of a *BIM* (*BCL2L11*) polymorphism in an individual may comprise detecting a nucleic acid amplification product comprising a sequence set out in SEQ ID NO: 1. The method may make use of a primer set as set out above.

As a 4th aspect of the present invention, there is provided a method of predicting whether an individual susceptible to or suffering from cancer or myeloproliferative disorder is likely to develop resistance to treatment with a tyrosine kinase inhibitor. The method may comprise determining whether the individual has a *BIM* (*BCL2L11*) polymorphism as described above.

The method may comprise detecting the presence of a nucleic acid amplification product comprising a sequence set out in SEQ ID NO: 1. The method may comprise nucleic acid amplification using a primer set as described above.

The method may be such that if the individual is determined to have a *BIM* (*BCL2L11*) polymorphism, then the individual is likely to develop resistance to treatment with a tyrosine kinase inhibitor. The determination that the individual has a *BIM* (*BCL2L11*) polymorphism

may be by detection of the presence of a nucleic acid amplification product comprising a sequence set out in SEQ ID NO: 1.

The method may be such that if the individual is determined not to have a *BIM* (*BCL2L11*) polymorphism, then the individual is less likely to develop resistance to treatment with a tyrosine kinase inhibitor. The determination that the individual does not have a *BIM* (*BCL2L11*) polymorphism may be by detection of the presence of a nucleic acid amplification product comprising a sequence set out in SEQ ID NO: 2

The amplification may comprise nucleic acid amplification by use of a primer set as set out above.

We provide, according to a 5th aspect of the present invention, a method of choosing a therapy for an individual with cancer or myeloproliferative disorder, the method comprising determining whether a patient is likely to develop resistance to treatment with a tyrosine kinase inhibitor by a method as set out above. The method may be such that where the individual is determined as being likely to develop such resistance, a therapy is chosen which involves more frequent monitoring of the patient. The therapy chosen may involve more frequent blood and bone marrow tests. The therapy chosen may involve bone marrow transplantation. The therapy chosen may involve administration of a more potent tyrosine kinase inhibitor (TKI). The TKI may comprise nilotinib or dasatinib. The therapy chosen may involve administration of a BH3-mimetic. The BH3-mimetic may comprise ABT-263. The BH3-mimetic may be administered in combination with a TKI. The therapy chosen may involve increasing the dose of the tyrosine kinase inhibitor, e.g., imatinib. In the case of imatinib, the dose of imatinib may be increased beyond the standard dose of 400mg/day to 600 or 800 mg/day. The therapy chosen may involve treatment with a drug that inhibits the pro-survival effect of the BCL2 group of proteins.

The present invention, in a 6th aspect, provides a method of determining the likelihood of success of a particular therapy on an individual with cancer or myeloproliferative disorder. The method may comprise comparing the therapy with the therapy determined by a method set out above.

The cancer or myeloproliferative disorder may comprise chronic myelogenous leukaemia (CML). The resistance to treatment with a tyrosine kinase inhibitor may comprise

for example BCR-ABL-independent TKI-resistance. The TKI resistance may comprise resistance to imatinib or other tyrosine kinase inhibitors.

The cancer or myeloproliferative disorder may comprise gastrointestinal stromal tumour (GIST). The resistance to treatment with a tyrosine kinase inhibitor comprises for example c-KIT/PDGFR-independent TKI-resistance The TKI resistance may comprise resistance to imatinib or other tyrosine kinase inhibitors.

The cancer or myeloproliferative disorder may comprise non-small cell lung cancer (NSCLC). The resistance to treatment with a tyrosine kinase inhibitor may comprise EGFR-independent TKI-resistance. The TKI resistance may comprise resistance to erlotinib or gefitinib, or other kinase inhibitors, e.g. sunitinib, nilotinib, and sorafenib.

The cancer or myeloproliferative disorder may comprise a myeloproliferative disorder such as selected from the group consisting of: polycythaemia vera, essential thrombocythaemia, and primary myelofibrosis. The resistance to treatment with a tyrosine kinase inhibitor may comprise JAK2-independent TKI-resistance. The TKI resistance may comprise resistance to imatinib or other tyrosine kinase inhibitors.

The cancer or myeloproliferative disorder may be selected from the group consisting of: haematologic malignancies, chronic lymphocytic leukaemia, acute lymphoblastic leukaemia, acute myeloid leukaemia, multiple myeloma, myeloproliferative disorders (including polycythaemia vera, essential thrombocythaemia, and primary myelofibrosis), solid tumours, small cell lung cancer, breast cancer, colorectal cancer, ovarian cancer, melanoma and neuroblastoma.

In a 7th aspect of the present invention, there is provided a method of treatment of a patient suffering from cancer or myeloproliferative disorder. The method may comprise determining if the cancer is a BCR-ABL-independent TKI-resistant CML cancer. The method may comprise determining if the cancer is a c-KIT/PDGFR-independent TKI-resistant GIST cancer. The method may comprise determining if the cancer is an EGFR-independent TKI-resistant NSCLC cancer.

The method may comprise determining if the myeloproliferative disorder is an JAK2-independent TKI-resistant myeloproliferative disorder.

The method may comprise a method as set out above. The method may further comprise treating the patient by performing a step selected as set out above in the 5th aspect of the invention.

The method may comprise detecting a nucleic acid amplification product comprising a sequence set out in SEQ ID NO: 1. This may be by use of a primer set described above.

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of chemistry, molecular biology, microbiology, recombinant DNA and immunology, which are within the capabilities of a person of ordinary skill in the art. Such techniques are explained in the literature. See, for example, J. Sambrook, E. F. Fritsch, and T. Maniatis, 1989, *Molecular Cloning: A Laboratory Manual*, Second Edition, Books 1-3, Cold Spring Harbor Laboratory Press; Ausubel, F. M. et al. (1995 and periodic supplements; *Current Protocols in Molecular Biology*, ch. 9, 13, and 16, John Wiley & Sons, New York, N.Y.); B. Roe, J. Crabtree, and A. Kahn, 1996, *DNA Isolation and Sequencing: Essential Techniques*, John Wiley & Sons; J. M. Polak and James O'D. McGee, 1990, *In Situ Hybridization: Principles and Practic*e; Oxford University Press; M. J. Gait (Editor), 1984, *Oligonucleotide Synthesis: A Practical Approach*, Irl Press; D. M. J. Lilley and J. E. Dahlberg, 1992, *Methods of Enzymology: DNA Structure Part A: Synthesis and Physical Analysis of DNA* Methods in Enzymology, Academic Press; Using Antibodies : A Laboratory Manual : Portable Protocol No. I by Edward Harlow, David Lane, Ed Harlow (1999, Cold Spring Harbor Laboratory Press, ISBN 0-87969-544-7); Antibodies : A Laboratory Manual by Ed Harlow (Editor), David Lane (Editor) (1988, Cold Spring Harbor Laboratory Press, ISBN 0-87969-314-2), 1855. Handbook of Drug Screening, edited by Ramakrishna Seethala, Prabhavathi B. Fernandes (2001, New York, NY, Marcel Dekker, ISBN 0-8247-0562-9); and Lab Ref: A Handbook of Recipes, Reagents, and Other Reference Tools for Use at the Bench, Edited Jane Roskams and Linda Rodgers, 2002, Cold Spring Harbor Laboratory, ISBN 0-87969-630-3. Each of these general texts is herein incorporated by reference.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows a view from the UCSC Genome Browser of Human genome (hg18) chr2:111,593,210-111,644,581 for UCSC Genes (top) and RefSeq Genes (GeneBank, bottom). The *BIM* gene and the deletion characterising the polymorphism is marked.

Figure 2 shows a DNA-PET analysis of six CML genomes.

Figure 2A summarizes the clinico-pathologic features of the patient samples and CML cell line used for DNA-PET analysis.

Figure 2B shows the karyo-genomic maps of six CML genomes which were generated from DNA-PET data using Circos,[29] and depicted as circular plots. SVs which matched those identified in at least one of 32 normal genomes were filtered out (these SVs correspond to those in Table E4). The different categories of structural variations (SV) are arranged in concentric layers as indicated in the key. The asterisks (*) indicate the presence of the *BCR-ABL1*/*ABL1-BCR* translocation.

Figure 3 shows somatic structural variations associated with blast transformation.

Figure 3A shows the Genome browser view of the novel rearrangement in the *EVI1* locus in P098. Genes known to be in this locus [University of California Santa Cruz (UCSC)] (Rhead et al. 2010) are shown in red and blue (top). Red tracks reflect the fragment coverage of concordant PETs. Dark red and pink horizontal arrowheads represent mapping regions (anchors) of discordant PETs indicating the presence of the insertion.

Figure 3B shows the expression levels of *EVI1* as measured by quantitative real-time PCR. *EVI1* levels are shown for two blast phase samples (P022 and P098) and four chronic phase samples (P308, P355, P490, and P500), normalized against expression of β-actin.

Figure 3C shows the florescence in situ hybridization analysis of the *EVI1* rearrangement using custom-made probes for chromosome 3 (RP11-137H17, green) and chromosome 8 (RP11-828L6, red; RP11-159N7, yellow) as depicted in Figure 3A. The *EVI1* rearrangement is absent in the normal control but can be seen as red-green-yellow fusion signal in the P098 sample.

Figure 4 shows a polymorphic 2903 bp deletion in intron 2 of *BIM* is present in all three resistant samples.

Figure 4A shows the detection of the *BIM* deletion by DNA-PET in three of three samples from patients with resistance to tyrosine kinase inhibitors (P308, P022, and P098), but not in samples from patients or cell lines sensitive to tyrosine kinase inhibitors (P145,

P440, and K562). Chr2:111580000..111650000 DNA-PET data of five clinical CML samples and K562 are shown in the Genome Browser. Dark red and pink horizontal arrow heads connected by green lines represent mapping regions (anchors) of discordant PETs indicating the presence of deletions. The vertical dashed lines depict the deleted region.

Figure 4B shows that the region is conserved in mammals as demonstrated by the UCSC Genome Browser '28-Way Cons' Track for the deletion (chr2:111,599,666..111,602,568) with the degree of conservation on the y-axis.

Figure 4C shows the validation of the deletion by PCR using genomic DNA samples from the five patients and K562 cells. PCR products with a size of 4.2 Kb correspond to the non-deletion allele, while PCR products with a size of 1.3 Kb correspond to the deletion allele. M, 1 Kb marker (Fermentas).

Figure 4D depicts the identical breakpoints found in all three deletion-containing samples by Sanger sequencing of PCR products. Deleted sequences are indicated in blue.

Figure 5 shows functional effects of the *BIM* polymorphism.

Figure 5A depicts the genomic organization of *BIM* showing exons for the major *BIM* isoforms including *BIMEL*, *BIML*, and *BIMS*, as well as for *BIMγ* (the only isoform known to contain exon 3).[30-32] The polymorphic deletion between exon 2 and 3 is highlighted by the red line. The exons containing the start codon (Start), dynein binding domain (DBD), BH3 domain (BH3), and stop codon/polyadenlyation signal sequence (Stop/PolyA) are also highlighted. The diagram is not drawn to scale.

Figure 5B shows the expression levels of exon-specific transcripts of *BIM*, as measured by quantitative real-time PCR in 23 CML patient samples [n=11 without the deletion (WT) and n=12 with the deletion (carriers)]. The levels of the various transcripts containing exons E2A, E3 or E4 are expressed as normalized ratios relative to exon 2A or β-actin. The mean and standard error of the mean are represented by the red lines and bars. Statistical significance (p) is calculated by Wilcoxon Ranks sums test.

Figure 5C shows the PCR reaction products from a collection of East-Asian and non-East-Asian CML cell lines performed to detect the deletion, using the method described in Example 2.

Figure 5D shows the ratio of exon 3- to exon 4-containing transcripts in CML cell lines with and without the deletion.

Figure 5E is a Western blot showing levels of BIMEL in cell lysates obtained from cell lines, with and without the deletion, following treatment with 1μM imatinib for 24 hours.

Figure 5F shows the growth curve of CML cells lines with and without the deletion cultured with 1μM imatinib.

Figure 5G shows the apoptotic activity of imatinib, dasatinib and ABT-737 against KCL22 and KYO-1 cells. Cells were treated with 2μM imatinib, 50nM dasatinib or 2.5μM ABT-737 for 48 hrs, and the percentage of dead cells was determined by flow cytometery.

Figure 6 shows association of the *BIM* polymorphism with clinical resistance to tyrosine kinase inhibitors. Patients seen at two South East-Asian referral centers with either chronic or accelerated phase CML were categorized according their sensitivity or resistance to tyrosine kinase inhibitors (TKI) by European LeukemiaNet criteria, as well as the presence or absence of mutations in *BCR-ABL* that are known to confer resistance. The incidence of the *BIM* polymorphism in each of these groups was then determined. P values were calculated using a two-tailed Fisher's exact test.

Figure 6A shows the frequency of the *BIM* deletion in patients with TKI-resistance with *BCR-ABL* mutations versus TKI-resistance in the absence of *BCR-ABL* mutations.

Figure 6B shows the frequency of the *BIM* deletion in patients with TKI-sensitive disease versus TKI-resistance in the absence of *BCR-ABL* mutations.

Figure 6C shows the frequency of the *BIM* deletion in patients with TKI-sensitive disease compared to all patients with TKI-resistance.

Figure 7 shows copy number information deduced from the cPET tag counts. Chromosomes are arranged on horizontal axis in alternating green and black as indicated on the bottom. Copy number values are represented on the Y-axis with smoothened window values indicated in red. Sample IDs are shown in the top left corners of plots. Note the different y-axis scale for K562.

Figure 8 shows genomic background of *BIM* deletion in East Asians.

Figure 8A. Seventy-four East Asian HapMap phase I individuals were genotyped for the intronic deletion in *BIM* and deletion genotypes were correlated with SNP genotypes using HaploView software (Barrett et al. 2005). LD based haplotype block is shown with SNPs in genomic order from left to right. Haplotype frequencies are show in gray on the right. Marker #49 represents the *BIM* deletion with A = no deletion and C = deletion. The deletion is on the background of the blue haplotype which is apart from the deletion identical with the most frequent red haplotype. Haplotype tagging SNPs are marked with an arrow head.

Figure 8B. IDs (Name) of tagging SNPs in A (#) are shown with their heterozygosity frequency (ObsHET), minor allele frequency (MAF) and alleles.

Figure 9. DNA paired-end tag (PET) sequencing method. Genomic DNA was hydrosheared, *EcoP15*I recognition sites were methylated and *EcoP15*I CAP adaptors were ligated to the ends of DNA fragments. The methylated DNA constructs were separated on agarose gel and 9 Kb sized fragments were selected for ligation resulting in circularized products where 5' (R3, dark red) and 3' (F3, pink) ends of 9 Kb fragments were connected by an internal biotinylated adaptor with two flanking non-methylated *EcoP15*I CAP adaptors. Constructs were digested by methylation sensitive *EcoP15*I to release 5' and 3' PET constructs. Sequencing adaptors were ligated to the PET constructs, which were then amplified by PCR and sequenced by the Applied Biosystems SOLiD system. Resulting PETs were mapped to the human reference sequence hg18.

Figure 10. Identification of structural variations (SVs) by dPET clusters. 'Interpretation' indicates the genomic structure of the sequenced genome deduced from the mapping pattern of the dPET clusters to the human reference sequence ('Mapping to reference'). Dark red arrows represent 5' anchor regions and pink arrows represent 3' anchor regions. Gray, blue, and red horizontal lines represent chromosomal segments. Red arrows indicate orientation of chromosomal segments.

Figure 11. Reconstruction of cytogenetically predicted isochromosome 17q. DNA-PET cluster of size 2 connects chromosome 17 position 17,595,066 minus strand with chromosome 17 position 28,282,853 plus strand. Top, Chromosome 17 ideogram is shown with break point locations indicated by red vertical lines. Dark red and pink arrows symbolise mapping positions and orientations of PETs. Middle, Genome browser view is shown for break point regions which correspond to red lines on top. Red track represents coverage by

concordant mapping PETs; genes are shown in green; dark red and pink arrows indicate mapping positions and orientations of PETs. Bottom, reconstruction of isochromosome 17q based on DNA-PET data. Arrows indicate direction of increasing genomic coordinates.

Figure 12. Somatic structural variations associated with blast transformation.

Figure 12A Shown are the genome structures of *BCR* and *ABL1* genes and location of translocation break points. Exons are indicated by boxes, introns are represented by feathered lines indicating direction of transcription (+ strand, left to right). Locations of break points are indicated by red vertical lines with respective sample IDs.

Figure 12B The ratio of the number of paired-end reads (cluster size normalized by coverage) connecting *BCR* with *ABL1* vs the reciprocal event correlates with disease progression. Genomic regions of *BCR* on chromosome 22 and *ABL1* on chromosome 9 are shown on horizontal axis with genes shown in green and blue. Copy number estimates are represented by purple tracks. Pink and dark red arrows indicate connectivity between *BCR* and *ABL1*. The number of PETs of the *BCR-ABL1* and *ABL1-BCR* translocations are shown in blue and grey, respectively. Dashed vertical lines indicate location of break points.

Figure 12C FISH validation of *BCR-ABL1* translocations identified by DNA-PET are shown.

Figure 13. Expression levels of exon-specific transcripts of *BIM*. Expression levels are measured by quantitative real-time PCR in 7 cell lines generated from normal individuals who are not affected by CML [n=3 without the deletion (WT), n=4 with the deletion (Carriers)]. The levels of the various transcripts containing exons E2A, E3 or E4 are expressed as normalized ratios relative to exon 2A or β-actin. Expression for the one homozygous carrier identified is highlighted in green. Statistical significance (p) is calculated by two-tailed Wilcoxon Ranks sums test (red) and two-tailed t-test (black).

SEQUENCE LISTING

SEQ ID NO: 1 is the sequence of PCR fragment amplified using BIM_del_F and BIM_del_R primers from a *BIM* gene with the deletion described (length 1,323 bp).

SEQ ID NO: 2 is the sequence of PCR fragment using BIM_del_F and BIM_del_R primers from a *BIM* wild type gene (length 4,226 bp).

SEQ ID NO: 3 is the sequence of the Bim_del_F forward primer (+chr2:111,599,051..111,599,070, hg18)

SEQ ID NO: 4 is the sequence of the Bim_del_R reverse primer (-chr2:111,603,257..111,603,276, hg18)

SEQ ID NO: 5 is the sequence of the 1000 bp flanking sequence upstream: chr2:111,598,666-111,599,665 of the Deletion in *BIM*: chr2:111,599,666-111,602,568 (hg18)

SEQ ID NO: 6 is the sequence of the deletion in *BIM*: chr2:111,599,666-111,602,568 (hg18)

SEQ ID NO:7 is the sequence of the 1000 bp flanking sequence downstream: chr2:111,602,569-111,603,568 of the deletion in *BIM*: chr2:111,599,666-111,602,568 (hg18)

DETAILED DESCRIPTION

This invention describes a novel polymorphism that we have found in East-Asian populations in the *BIM* gene. This polymorphism is associated with the development of drug-resistance in patients with chronic myelogenous leukaemia (CML).

Patients who have CML and who harbour this polymorphism are more likely to develop resistance to the standard therapy for CML than those who do not. Testing for this polymorphism is useful as a biomarker for the prediction of drug-resistance, and the early identification of patients who may benefit from alternative and/or more aggressive therapies. Furthermore, because the *BIM* gene is an important mediator of therapy-induced cell death in other cancer types, our invention may also be relevant to a wide range of human cancers in East-Asian populations.

In order to discover mechanisms of TKI-resistance, we applied a novel technology termed genomic paired-end ditag or DNA-PET (ref A2, ref A3), coupled with high-throughput sequencing to interrogate the genome of CML cells from patients with or without drug-resistance. In doing so, we uncovered a previously unknown deletion in the *BIM* gene that was associated with the development of BCR-ABL-independent TKI-resistance. Patients with this deletion are four to five times (17.4 vs 3.9%) more likely to have BCR-ABL-independent TKI-resistance than those without the deletion (p=0.04, two-tailed Fisher's exact test). In addition, we have also been able to correlate the presence or absence of the *BIM* deletion to

BCR-ABL-independent TKI-resistance in CML cell lines from patients, suggesting a direct mechanistic role for *BIM* deletions in TKI-resistance. Since it is known that upregulation of proapoptotic isoforms of the BIM protein is required for TKI-mediated CML cell death (refs A4-A7), we hypothesize that *BIM* deletions impair the expression of proapoptotic BIM.

***BIM* (*BCL2L11*)**

BIM is also known as *BAM; BIM; BOD; BimL; BimEL; BIM-beta6; BIM-beta7; BIM-alpha6; BCL2L11*.

GenBank Accession numbers and UCSC Gene IDs of the *BIM* gene and polypeptide are as follow:

GeneBank accession numbers:

Gene (transcript)	Protein
NM_207002	NP_996885
NM_138621	NP_619527
NM_006538	NM_006538

UCSC Gene IDs:

Gene (transcript)	Protein
uc002tgw.1	No protein
uc002tgx.1	No protein
uc010fkd.1	No protein
uc002tgy.1	No protein
uc002tgz.1	O43521
uc010fke.1	No protein
uc002tha.1	O43521
uc002thb.1	No protein
uc002thc.1	No protein
uc002thd.1	O43521

The following transcript descriptions correspond to the Gene Bank accession numbers and UCSC Gene IDs listed above.

NCBI GeneBank (RefSeq)

NM_207002.2

Homo sapiens BCL2-like 11 (apoptosis facilitator) (*BCL2L11*), transcript variant 9, mRNA.

NM_138621.3

Homo sapiens BCL2-like 11 (apoptosis facilitator) (*BCL2L11*), transcript variant 1, mRNA.

NM_006538.3

Homo sapiens BCL2-like 11 (apoptosis facilitator) (*BCL2L11*), transcript variant 6, mRNA.

UCSC Genes

bim-alpha1 (uc002tgw.1)
Bim-alpha1, complete cds.
RefSeq (NM_006538):

bim-alpha1 (uc002tgx.1)
Bim-alpha1, complete cds.
RefSeq (NM_006538):

bim-alpha1 (uc010fkd.1)
Bim-alpha1, complete cds.
RefSeq (NM_006538):

bim-alpha1 (uc002tgy.1)
Bim-alpha1, complete cds.
RefSeq (NM_006538):

bim-alpha1 (uc002tgz.1)
BCL2-like 11 transcript variant 9.
RefSeq (NM_006538)

bim-alpha1 (uc010fke.1)
Bim-alpha1, complete cds.
RefSeq (NM_006538):

BCL2L11 (uc002tha.1)
BCL2-like 11 isoform 9
RefSeq (NM_138621):

bim-alpha1 (uc002thb.1)
Bim-alpha1, complete cds.
RefSeq (NM_006538):

bim-alpha1 (uc002thc.1)
Bim-alpha1, complete cds.
RefSeq (NM_006538):

BCL2L11 (uc002thd.1)
BCL2-like 11 isoform 9
RefSeq (NM_006538):

Literature for UCSC Genome Browser database/data

Rhead B, Karolchik D, Kuhn RM, Hinrichs AS, Zweig AS, Fujita P, Diekhans M, Smith KE, Rosenbloom KR, Raney BJ, Pohl A, Pheasant M, Meyer L, Hsu F, Hillman-

Jackson J, Harte RA, Giardine B, Dreszer T, Clawson H, Barber GP, Haussler D, Kent WJ. The UCSC Genome Browser database: update 2010. Nucleic Acids Res. 2010 Jan;38(Database issue):D613-9. Epub 2009 Nov 11.

The protein encoded by this gene belongs to the BCL-2 protein family. BCL-2 family members form hetero- or homodimers and act as anti- or pro-apoptotic regulators that are involved in a wide variety of cellular activities. The protein encoded by this gene contains a Bcl-2 homology domain 3 (BH3). It has been shown to interact with other members of the BCL-2 protein family, including BCL2, BCL2L1/BCL-X(L), and MCL1, and to act as an apoptotic activator. The expression of this gene can be induced by nerve growth factor (NGF), as well as by the forkhead transcription factor FKHR-L1, which suggests a role of this gene in neuronal and lymphocyte apoptosis. Transgenic studies of the mouse counterpart suggested that this gene functions as an essential initiator of apoptosis in thymocyte-negative selection. Several alternatively spliced transcript variants of this gene have been identified. [provided by RefSeq]

PREDICTION OF RESISTANCE TO TYROSINE KINASE INHIBITOR TREATMENT IN CHRONIC MYELOGENOUS LEUKAEMIA (CML)

Our discovery may have clinical utility in the following circumstances.

Patients with *BIM* deletions may benefit from more frequent monitoring of their condition. This may allow earlier use of alternative and/or more aggressive therapies, e.g. bone marrow transplantation, and can potentially lead to a better clinical outcome.

BIM deletions may serve as a therapeutic guide. For example, patients with the deletion may represent a subgroup of TKI-resistant patients who may be particularly sensitive to a class of drugs known as BH3-mimetics (refs A8-A12). Such drugs, like ABT-263 (currently in early phase clinical trials), selectively target pro-survival members of the BCL2 family of proteins, and which would normally be opposed by the pro-death family of BIM proteins.

CML is a cancer of haematopoietic stem cells, and is caused by the presence of the oncogenic fusion gene, termed *BCR-ABL*, that is thought to be causative for the disease. *BCR-ABL* encodes for a constitutively active tyrosine kinase that mediates the increased survival and proliferation of CML cells when compared to their normal counterparts.

Effective treatment for CML exists in the form of a class of drugs which inhibit the kinase activity of BCR-ABL, which are commonly called tyrosine kinase inhibitors (TKI). However, over time, a proportion of patients develop clinical resistance to these drugs, an event that is often associated with transformation to blast crisis (BC) CML, and shorter survival.

In about 60% of patients, TKI-resistance is associated with reactivation of BCR-ABL through mutations in the *BCR-ABL* gene that render TKIs less able to bind to BCR-ABL, or overexpression of the 'wild type' *BCR-ABL* gene or protein. In both cases, BCR-ABL kinase activity is restored in the presence of the TKI, hence the term BCR-ABL-dependent TKI-resistance. In the remaining 40% of cases, TKI-resistance occurs in the absence of BCR-ABL reactivation, and is termed BCR-ABL-independent TKI-resistance.

To date, the causes of BCR-ABL-independent TKI-resistance are unknown, and an increased understanding of the mechanisms that mediate this form of resistance will be important to determine strategies to overcome TKI-resistance. Because TKI-resistance might be mediated by either acquired or inherited genetic differences between patients who develop TKI-resistance compared to those who do not, we reasoned that identification of such differences would be a promising approach to uncover mechanisms of TKI-resistance.

Accordingly, we applied a novel technology termed genomic paired-end ditag or DNA-PET, coupled with high-throughput sequencing, to interrogate the genome of primary CML cells from patients with or without drug-resistance, and with and without BCR-ABL kinase domain mutations.

In this disclosure, we describe the following novel discoveries made by our team.

Through the use of DNA-PET, we have uncovered a previously unknown deletion in the *BIM* gene that is associated with the development of BCR-ABL-independent TKI-resistance. Using a clinically annotated set of CML patient samples, we find that patients with this deletion are four to five times more likely to have BCR-ABL-independent TKI-resistance than those without the deletion ($p=0.04$, two-sided Fisher's exact test).

Secondly, we have found that the presence of the *BIM* deletion in patient-derived CML cell lines is strongly associated with BCR-ABL-independent TKI-resistance. Using a panel of 7 patient-derived CML cell lines, we have found one cell line with the deletion. Importantly,

only the *BIM*-deletion containing cell line exhibited BCR-ABL-independent TKI-resistance. These results suggest a direct mechanistic role for *BIM* deletions in BCR-ABL-independent TKI-resistance. Since it is known that upregulation of BIM protein is required for TKI-mediated CML cell death, we hypothesize that *BIM* deletions impair the expression of proapoptotic isoforms of BIM.

Further analysis of the *BIM* deletion (using the PCR assay as described in Example 2, with the primers described by Sequences No. 3 and No. 4 as shown in Figure 4C, SNP analysis and HapMap data) has revealed that the deletion is actually a normal polymorphism that it has a frequency of approximately 10% in individuals of East-Asian descent. The deletion polymorphism is likely to be non-existent in Caucasians, based on the screening of 60 Caucasian HapMap samples and 446 German blood donors, or African (Yoruba) populations based on the screening of 60 Yoruban HapMap samples (Table E6). Consistent with the ethnic segregation of the *BIM* deletion, the cell line with the deletion described in b. was found to have been derived from a Japanese individual with CML (ref A31).

	Genotype			Allele frequency
	wt/wt	wt/del	del/del	del
European, HapMap (n=60)	60	0	0	0
East Asian, HapMap (n=74)	61	12	1	0.095
African, HapMap (n=60)	60	0	0	0
German (n=446)	446	0	0	0

Table E6 Genotype and allele frequency of the deletion polymorphismus in HapMap and German individuals.

This finding has several implications and potential clinical uses, as described in the following sections.

The invention may be used as follow:

It is currently not possible to predict at presentation which CML patients will develop TKI-resistance. By identifying a genetic factor that is associated with an increased risk of developing TKI-resistance, it may now be possible, in East-Asian populations, to identify such individuals. Patients with *BIM* deletions would then be followed more closely by their

physicians, and may be advised to have more frequent monitoring of their disease status than is currently recommended. These would include more frequent blood and bone marrow tests.

In addition, the presence of the *BIM* deletion may predict for sensitivity to specific alternative therapeutic strategies. These include increasing the dose of imatinib beyond the standard dose of 400mg/day to 600 or 800 mg/day. Alternatively, such patients may potentially be treated with a novel class of drugs that inhibit the pro-survival effect of the BCL2 group of proteins (which the BIM protein normally opposes). This latter possibility is currently being explored in our laboratory.

Importantly, because TKIs and other targeted therapies are very costly, physicians managing patients who are found to have *BIM* deletions, may use this finding as a rationale for justifying the increased cost associated with the use of higher drug doses, or changing therapies, to their patients and/or third party payors, or avoiding these strategies in the absence of a deletion.

OTHER DISEASES

The presence of BIM deletions in patients with other cancers may also be used as a predictor for the development of drug-resistance, as well as a guide for alternative therapies.

This would be analogous to the situation described for CML, and may encompass other haematologic malignancies (chronic lymphocytic leukaemia, acute lymphoblastic leukaemia, acute myeloid leukaemia, and multiple myeloma), myeloproliferative disorders (including polycythaemia vera, essential thrombocythaemia, and primary myelofibrosis), as well as the most common solid tumours (non-small cell and small cell lung cancer, breast cancer, colorectal cancer, ovarian cancer, melanoma, and neuroblastoma), and gastrointestinal stromal tumours (GIST).

Thus, the BIM polymorphism disclosed here may also be used to detect resistance to kinase inhibitor treatment in other diseases, such as EGFR-driven non-small cell lung cancers (NSCLC) and gastrointestinal stromal tumours (GIST, Gordon and Fisher, 2010).

Accordingly, patients with such cancers and tumours and who harbour the BIM polymorphism disclosed here are more likely to be resistant to therapies targeting their respective oncogenic kinases.

Furthermore, reference is made to Will et al. *Apoptosis induced by JAK2 inhibition is mediated by Bim and enhanced by the BH3 mimetic ABT-737 in JAK2 mutant human erythroid cells*. Blood 8 April 2010 Vol 115, number 14. This document describes myeloproliferative disorders, which are characterized by JAK2 activating mutations. This article provides evidence that JAK inhibitors also require BIM expression for sensitivity.

Accordingly, the BIM polymorphism disclosed here may further be used to detect resistance to kinase inhibitor treatment in myeloproliferative disorders, such as polycythaemia vera, essential thrombocythaemia, and primary myelofibrosis. Patients with myeloproliferative disorders such as those harbouring the BIM polymorphism disclosed here are more likely to be resistant to therapies targeting their respective oncogenic kinases.

We disclose a polymorphic variant of a *BIM* (*BCL2L11*) gene which is associated with resistance to treatment with a kinase inhibitor in an individual suffering from an EGFR-driven non-small cell lung cancer (NSCLC), a c-KIT/PDGFR-driven gastrointestinal stromal tumour (GIST) or a JAK2 driven myeloproliferative disorder, in an individual comprising such a polymorphism.

Examples of kinase inhibitors used to treat EGFR-driven non-small cell lung cancers (NSCLC) include gefitinib and erlotinib, while an example of a kinase inhibitor used to treat gastrointestinal stromal tumours (GIST) is imatinib. Myeloproliferative disorders may be treated with kinase inhibitors against their causative kinases, e.g. inhibitors against JAK2.

The BIM polymorphism may comprise a polymorphic variant of a *BIM* (*BCL2L11*) gene which comprises, in 5' to 3' order, the nucleotide sequence set out in SEQ ID NO: 5 followed immediately by the nucleotide sequence set out in SEQ ID NO: 7. The polymorphic variant *BIM* (*BCL2L11*) may be characterised by lacking the nucleotide sequence set out in SEQ ID NO: 6.

We disclose a method of predicting whether an individual suffering from a non-small cell lung cancer (NSCLC) is likely to develop resistance to treatment with a kinase inhibitor,

the method comprising determining whether the individual has a *BIM* (*BCL2L11*) polymorphism as described. The resistance may be independent of EGFR reactivation. The non-small cell lung cancer (NSCLC) may comprise a non-small cell lung cancer (NSCLC) associated or caused by EGFR activation such as an EGFR driven non-small cell lung cancer (NSCLC).

We further disclose a method of predicting whether an individual suffering from a gastrointestinal stromal tumour (GIST) is likely to develop resistance to treatment with a kinase inhibitor, the method comprising determining whether the individual has a *BIM* (*BCL2L11*) polymorphism as described. The resistance may be independent of c-KIT/PDGFR reactivation. The gastrointestinal stromal tumour (GIST) may comprise a gastrointestinal stromal tumour (GIST) associated or caused by c-KIT/PDGFR activation such as a c-KIT/PDGFR driven gastrointestinal stromal tumour (GIST).

We also disclose a method of predicting whether an individual suffering from a or a myeloproliferative disorder is likely to develop resistance to treatment with a kinase inhibitor, the method comprising determining whether the individual has a *BIM* (*BCL2L11*) polymorphism as described. The resistance may be independent of JAK2 reactivation. The myeloproliferative disorder may comprise a myeloproliferative disorder associated or caused by JAK2 activation such as a JAK2 driven myeloproliferative disorder.

We disclose a method comprising detecting the presence of a nucleic acid amplification product comprising a sequence set out in SEQ ID NO: 1, for example by use of a primer set comprising a nucleotide sequence as set out in SEQ ID NO: 3 and a nucleotide sequence as set out in SEQ ID NO: 4, in which if the individual is determined to have the *BIM* (*BCL2L11*) polymorphism, then the individual is likely to develop resistance to treatment of a non-small cell lung cancer (NSCLC) with a kinase inhibitor.

We further disclose such a method, in which if the individual is determined to have the *BIM* (*BCL2L11*) polymorphism, then the individual is likely to develop resistance to treatment of a gastrointestinal stromal tumour (GIST) with a kinase inhibitor.

We also disclose such a method, in which if the individual is determined to have the *BIM* (*BCL2L11*) polymorphism, then the individual is likely to develop resistance to treatment of a myeloproliferative disease with a kinase inhibitor.

Conversely, if such an individual is determined not to have the *BIM* (*BCL2L11*) polymorphism (for example if the presence of a nucleic acid amplification product comprising a sequence set out in SEQ ID NO: 2 is detected), then the individual is less likely to develop resistance to treatment of a non-small cell lung cancer (NSCLC) with a kinase inhibitor.

Similarly, in such a method, in which if the individual is determined not to have the *BIM* (*BCL2L11*) polymorphism, then the individual is less likely to develop resistance to treatment of a gastrointestinal stromal tumour (GIST) with a kinase inhibitor.

Also, in such a method, in which if the individual is determined not to have the *BIM* (*BCL2L11*) polymorphism, then the individual is less likely to develop resistance to treatment of a meyloproliferative disease with a kinase inhibitor.

We describe a method of determining the likelihood of success of a particular therapy on an individual with non-small cell lung cancer (NSCLC) or gastrointestinal stromal tumour (GIST) or myeloproliferative disease, or any combination of the above, the method comprising comparing the therapy with the therapy determined by a method set out above.

We further describe a method of diagnosis of kinase inhibitor resistant non-small cell lung cancer (NSCLC) in an individual, the method comprising detecting the presence of a *BIM* (*BCL2L11*) polymorphism, as described above, in an individual. We also describe a method of diagnosis of kinase inhibitor resistant gastrointestinal stromal tumour (GIST) in an individual, the method comprising detecting the presence of a *BIM* (*BCL2L11*) polymorphism, as described above, in an individual. We describe a method of diagnosis of kinase inhibitor resistant myeloproliferative disease in an individual, the method comprising detecting the presence of a *BIM* (*BCL2L11*) polymorphism, as described above, in an individual.

We provide for method of treatment of a patient suffering from non-small cell lung cancer (NSCLC) and/or gastrointestinal stromal tumour (GIST) and/or a myeloproliferative disease, the method comprising determining whether the cancer is kinase-resistant cancer by a method described above and treating the patient.

EXAMPLES

Example 1. Assay for Screening for the Presence of the Deletion in Bim-alpha1

Standard Protocols/Kits for DNA extraction

Qiagen Blood & Cell Culture DNA Midi Kit (cat. No. 13343) for the DNA extraction from blood (see QIAGEN_Genomic_DNA_Handbook.pdf for details).

or

MasterAmp™ Buccal Swab DNA Extraction Kit for DNA extraction from buccal swabs.

It is also possible to use the Qiagen Allprep DNA/RNA mini kit (cat. No 80204). Qiagen Inc, Valencia, California, United States of America.

Example 2. PCR Assay Using Genomic DNA for Bim Deletion Detection

Primers (100uM)	0.2µl each	
Genomic DNA	1µl	(50ng)
dNTP (10mM)	2.5µl	
Jumpstart Taq	2.5µl	(Sigma; Cat. D1313)
10 x Buffer	5µl	
Add H_2O to	50µl	

PCR program

Step 1: 96^0C for 30sec

Step 2: 94^0C for 15sec

Step 3: 64^0C for 30sec

Step 4: 68^0C for 5 min

Step 5: Repeat steps 2-4 x 11

Step 6: 94^0C for 15sec

Step 7: 60^0C for 30sec

Step 8: 68^0C for 5 min

Step 9: Repeat steps 6-8 x 17

Step 10: 68^0C 20mins

16^0C forever

Primers

Bim_del_F AATACCACAGAGGCCCACAG (+chr2:111,599,051..111,599,070)

Bim_del_R GCCTGAAGGTGCTGAGAAAG (-chr2:111,603,257..111,603,276)

PCR products are run on a 1% agarose gel with ethidium bromide and products of the sizes

4,226 bp without deletion

1,323 bp with deletion

are visualised on a UV screen.

Example 3. Materials and Methods: Patients and Samples

Clinical samples were obtained from patients seen at the Singapore General Hospital and University of Malaya according to IRB-approved protocols.

Mononuclear cells were isolated by Ficoll centrifugation, and DNA/RNA extracted using AllPrep DNA/RNA Mini Kit (Qiagen) according to the manufacturer's instructions.

Example 4. Materials and Methods: DNA-PET Analysis and Validation

Genomic DNA was hydrosheared to 5, 7, and 9 Kb fragments, used for construction of sequencing libraries, and sequenced using a massively parallel SOLiD sequencer (see Table E1 and description below).

	Sample	Tags	Mappable Tags	PET	PET (NR)[1]	cPET[2] span range [bp]	cPET (NR)[1]	Coverage[3]	dPET[4] (NR)[1]	dPET[4] cluster[5]
DHH014IHH039	P440	1,100,736,719	654,761,718	237,888,286	60,483,853	6,430-10,460	54,566,588	156.7	5,917,265	1,255
IHH040	P145	406,158,202	187,588,800	57,282,295	30,574,510	7,339-10,440	27,920,763	86.5	2,653,747	782
IHH038	P308	983,240,880	486,558,654	137,899,606	39,548,846	5,930-8,090	31,886,502	77.8	7,662,344	1,211
IHH037	P098	856,013,096	477,870,346	132,002,088	53,906,020	7,655-10,272	45,363,132	143.3	8,542,888	1,444
IHH034	P022	1,536,669,332	854,022,398	256,381,192	50,259,818	4,410-6,429	38,068,014	69.7	12,191,804	1,555
IHK006007E	K562	376,077,182	223,850,946	133,675,193	44,065,447	6,846-10,248	40,261,305	118.4	3,804,142	1,153

[1] non redundant
[2] concordant PET
[3] physical coverage
[4] discordant PET
[5] filtered clusters based on in silico cluster size probability

Table E1. Statistics of massively parallel PET sequencing on the SOLiD platform

Paired-end (Applied Biosystems terminology: mate-paired) libraries were constructed as shown in Figure 9. In brief, 2x25bp ditag constructs, which corresponded to the ends of the 5-9 Kb DNA fragments, were generated using *EcoP15*I. High throughput sequencing of the 2x25 bp libraries was performed on SOLiD sequencers according to the manufacturer's recommendations (Applied Biosystems). Sequence tags were mapped to the human reference sequence (NCBI Build 36, hg18) and paired using SOLiD System Analysis Pipeline Tool, Corona Lite (Applied Biosystems), allowing 2 color code mismatches per tag. Paired-end tags (PETs) which were within the library insert distribution were categorized as concordant PETs (cPETs). The PETs which were rejected by cPET criteria were classified as discordant PETs (dPETs). These were further split into five distinct categories; (i) two tags mapped on different chromosome, (ii) two tags mapped on the same chromosome, but different strand, (iii) two tags mapped on the same chromosome, but wrong ordering (5' downstream of 3'), (iv) two

tags mapped on the same chromosome, same strand, correct ordering, but larger span distance than 1.1x the maximum library size, (v) two tags mapped on same chromosome, same strand, correct ordering, but smaller span distance than the minimum library size. Category (v) has been excluded from further analysis. dPETs of which the sequence tags mapped on both sides to the same genomic regions within 10 Kb were clustered together. The 10 Kb search windows to the left and right of the first tags were extended to 10 Kb according to the outermost tag coordinates. The regions to which the left (5') and right (3') tags mapped were described as anchor regions. Clusters of the size 3 and higher were kept for the identification of SVs. Single dPET clusters could identify SVs with one rearrangement point such as deletions if the 5' mapping anchor region was far apart from the 3' mapping anchor region, tandem duplications if the mapping order was 3' to 5' instead of the normal 5' to 3', unpaired inversion if the mapping orientation was reversed (on different strand), and isolated inter-chromosomal translocation if the 5' and 3' anchors mapped to different chromosomes. Two closely positioned dPET clusters could be used to deduce the SVs with two rearrangement points such as inversions, insertions, and balanced translocations as described in Figure 10.

Comparison of clusters across different genomes was performed based on an overlap of the 5' and 3' anchor regions extended by 10 Kb on both sides. If the 5' anchor region of a cluster of a second library was overlapping with the 5' extended anchor region of a cluster of the first library and the same was true for the 3' anchor regions, the two clusters were grouped together and the 10 Kb extension of the anchor regions were adjusted according to the outermost start and end anchor coordinates. Gene annotations were based on RefSeq Genes downloaded from UCSC (http://genome.ucsc.edu/; Rhead et al. 2010) on May 14 2009 using library specific breakpoints. Identified SVs were filtered based on SVs of 32 normal individuals and DNA-PET quality criteria as described elsewhere in this document.

Some loci showed an accumulation of dPET clusters. At these loci it might be misleading to assign a particular SV to a dPET cluster (e.g. if the breakpoints of a tandem duplication are surrounded by deletions and/or translocations, the rearrangement might not be interpreted as a tandem duplication). Therefore, a breakpoint based interconnection network was established to separate breakpoints in complex regions from isolated and less complex SVs. To determine the neighbourhood of a breakpoint, the start and end points of each dPET cluster anchor region were extended by the maximum insert size of the respective genomic library as search windows. If windows of neighbouring clusters overlapped with each other,

dPET clusters were grouped together into a supercluster. The procedure allowed an indirect connection of cluster A via B to C. The number of dPET clusters that could be joined together into a supercluster was represented by the supercluster size. In cases where >3 dPET clusters were interconnected, the breakpoint pairs were classified as 'complex' (intra- and inter-chromosomal).

Fluorescence *in situ* hybridization (FISH) was performed as follows. Nuclei were harvested by treating cells with 0.75M KCl for 20 min at 37°C. Then, after few fixations, nuclei were dropped on slides for FISH. *EVI1* probe (clone RP11-137H17) was labelled green and insertion of chr8: 127,950,637 to 130,664,919 (clones RP11-828L6 and RP11-159N7, respectively) was labelled in red and yellow, respectively. For fosmid probe preparation, DNA was labelled by nick translation in the presence of biotin-16-dUTP using Nick translation system (Invitrogen). In presence of 1μg / μl of Cot1 DNA, DNA cosmid clone was resuspended at a concentration of 5ng / μl in hybridization buffer (2SSC, 10% dextran sulfate, 1 X PBS, 50% formamide). Prior to hybridization, nuclei slides were treated with 0.01% pepsin at 37°C for 5 min followed by 1 X PBS rinse, 1% formaldehyde 10 min treatment, 1 X PBS rinse (5 min) and dehydratation through ethanol series (70%, 80%, and 100%). Denaturated probe was applied to these pretreated slides and codenaturated at 75°C for 5 min and hybridized at 37°C overnight. Two posthybridization washes were performed at 45°C in 2SSC / 50% formamide for 7 min each followed by 2 washes in 2SSC at 45°C for 7 min each. After blocking, the slides were revealed with avidin-conjugated fluorescein isothiocyanate (FITC) (Vector Laboratories). After washing, slides were mounted with vectashield and observed under epifluorescence microscope. Image analysis was done using Metasystem software.

For validation of predicted SVs, PCR primers flanking the predicted break points were used to amplify rearranged regions for subsequent Sanger sequencing.

For real-time PCR assays, total cellular RNAs were extracted using the RNeasy Mini Kit (Qiagen) according to the manufacturer's instruction. RNA was reverse transcribed using Superscript III First-Strand Synthesis System (Invitrogen), and quantitatively assessed using the iQ5 Multicolor Real-Time Detection System (Bio-Rad) with a total reaction volume of 25ul. Primers were annealed at 59^0C for 20 seconds and the amplicon was extended at 72^0C for 30 seconds. The total number of cycles quantified was 40. Transcript levels of β-actin or

exon 2A of *BIM* were used to normalize between samples. Primers used are as the following: BIM exon 2A (Forward: ATGGCAAAGCAACCTTCTGATG; Reverse: GGCTCTGTCTGTAGGGAGGT), BIM exon 3 (Forward: CAATGGTAGTCATCC-TAGAGG; Reverse: GACAAAATGCTCAAGGAAGAGG), BIM exon 4 (Forward: TTCCATGAGGCAGGCTGAAC; Reverse: CCTCCTTGCATAGTAAGCGTT), β-actin (Forward: GGACTTCGAGCAAGAGATGG; Reverse: AGCACTGTGTTGGCGTAC-AG) and EVI1 (Forward: ACCCACTCCTTTCTTTATGGACC; Reverse: TGATC-AGGCAGTTGGAATTGTG).

Example 5. Materials and Methods: Cell Lines and Tissue Culture

CML lines were obtained from ATCC (MEG-01 and KU812), JCRB (NCO2) and DSMZ (KCL22, K562, KYO1, JK1, BV173 and NALM1). Cells were grown in RPMI 1640 medium supplemented with Penicillin, Streptomycin, Glutamine and 10% fetal bovine serum, incubated in humidified incubator at 37° with 5% CO_2.

Example 6. Results: CML Patient Samples

We selected four Philadelphia (Ph) chromosome-positive CML patient samples and one CML cell line for DNA-PET analysis (Figure 2A).

These samples represent patients exhibiting clinical sensitivity or resistance to tyrosine kinase inhibitors, and include two patients each in chronic and myeloid blast phase. One of the chronic phase patients and both blast phase patients were resistant to tyrosine kinase inhibitors, while one of the blast phase samples displayed additional karyotypic abnormalities besides the Ph chromosome.

We also included the K562 CML cell line and a remission sample from the treatment-sensitive patient as positive and negative controls respectively for the Ph chromosome.

Example 7. Results: DNA-PET Analysis of CML Genomes

We generated 72.1 Gb of mappable DNA sequence derived from >278 million non-redundant PETs and achieved, on average, 109-fold physical (fragment) coverage of each genome (Table E1 above). 85.4% of the PETs mapped concordantly to the reference genome, while 14.6% of the PETs did not. The latter were classified as discordant PETs (dPETs). The clustering of multiple dPETs connecting the same two genomic regions allowed us to identify

3,408 different structural variations (SV), as well as define the type of SV for each dPET (see Figure 10, and Tables E2 and E3 below).

	P440	P145	P308	P098	P022	K562
Deletion	434	413	510	497	537	448
Tandem duplication	58	45	63	61	61	173
Unpaired inversion	134	62	125	308	228	124
Inversion	92	72	74	94	60	68
Intra-chr. insertion	48	23	44	44	58	40
Inter-chr. insertion	10	8	10	12	14	15
Isolated translocation	45	25	49	43	59	50
Balanced translocation	0	2	8	0[1)]	4	0[2)]
Complex intra-chr.	209	56	147	150	197	152
Complex inter-chr.	225	76	181	235	337	83
Total	1,255	782	1,211	1,444	1,555	1,153

[1)] BCR-ABL1 translocation but not the reciprocal ABL1-BCR is present due to deletion of derivative chromosome 9
[2)] BCR-ABL1 translocation but not the reciprocal ABL1-BCR is present due loss of derivative chromosome 9 and/or complex rearrangements.

Table E2. SVs predicted by DNA-PET in 5 patient samples and K562

	SV[1)]	Non-redundant[2)] SV[1)]
Raw data	7,400	3,408
CML specific[3)]	1,301	1,220
After quality filter[4)]	495	459

1) SV statistics are reflected by numbers of dPET clusters (inversions, insertions, and balanced translocations are composed of two dPET clusters per event)
2) The same SV in different genomes is counted once.
3) After filtering the data by DNA-PET information of 23 normal libraries of 22 normal individuals and published paired-end sequencing data of normal samples (Kidd et al. 2008; Korbel et al. 2007).
4) dPET clusters were filtered which had a supercluster size > 100, or a Blast score >2000, or a Blast alignment type of EC, or a cluster size of 2 (see Methods)

Table E3. Filtering of predicted SVs by DNA-PET in 5 patient samples and K562

	P440	P145	P308	P098	P022	K562
Deletion	17	17	30	30	51	71
Tandem duplication	5	4	3	6	3	104
Unpaired inversion	5	3	3	16	8	26
Inversion	2	0	0	2	2	2
Intra-chr. insertion	0	0	1	1	0	0
Inter-chr. insertion	0	0	0	0	0	0
Isolated translocation	7	0	2	7	5	11
Balanced translocation	0	2	2	0[1]	2	0[2]
Complex intra-chr.	7	1	2	1	2	14
Complex inter-chr.	2	3	2	0	2	9
Total	45	30	45	63	75	237

[1)] BCR-ABL translocation but not the reciprocal ABL1-BCR is present due to deletion of derivative chromosome 9

[2)] BCR-ABL translocation but not the reciprocal ABL1-BCR is present due to loss of derivative chromosome 9 and/or complex rearrangements

Table E4. CML specific SVs predicted by DNA-PET in 5 patient samples and K562 after quality filtering

In order to exclude additional SVs that exist in the normal population, as well as decrease the proportion of false positives, the SVs were further filtered by additional DNA-PET data obtained from 32 normal and unrelated individuals, as well as by the following bioinformatics-defined quality criteria. Clusters were excluded if (i) they were interconnected with 100 or more other clusters indicated by a supercluster size >100, (ii) they had a high sequence similarity between the two joined breakpoint regions indicated by a Blast score >2000 between the two anchor regions including their extensions by 15 Kb towards the breakpoints, (iii) they showed a Blast score >300 between the two anchor/extension regions where the sequence similarity is within the anchor on one side and within the extension on the other side (EC type), (iv) they had a cluster size of 2. This resulted in 459 CML-specific SVs (Table E3 and Table E4) as well as copy number information (Figure 7), and allowed us to generate karyo-genomic maps for each genome (Figure 2B).

Importantly, using DNA-PET, we were able to identify the BCR-ABL translocation in all but the remission sample. This dataset was then used to identify SVs that tracked with either disease-stage or drug-resistance.

Example 8. Results: DNA-PET Analysis of Blast Transformation

The additional chromosomal abnormalities and molecular aberrations that are found in blast phase are thought to contribute to clinical behavior. However, a comprehensive assessment of either the number or structural nature of these events has been technically challenging, particularly for the detection of SVs which are copy number neutral. Accordingly, we turned our attention to the analysis of SVs that occurred upon development of blast phase. Here, as depicted in Table E4, we saw a progressive increase in the number of SVs in blast phase, which correlated well with karyotyping and FISH analysis (Table E7 and Figures 3D and 11). We then categorized the nature of the SVs, and found that deletions are the most prominent category of SVs in the two blast phase patients (P098 and P022, n=81 [58.7% of all SVs]), followed by unpaired inversions (n=24 [17.4%]), isolated translocations (n=12 [8.7%]) and tandem duplications (n=9 [6.5%]) and less than 5% for each of the other SV categories (Figure 2A). The blast phase cell line K562 showed, as expected, more rearrangements compared to the blast phase patient samples (237 vs. 63 and 75, respectively). Tandem duplications were the most prominent category in K562 (n=104 [43.9%), followed by deletions (n=71 [30%]), unpaired inversions (n=26 [11%]) and complex intra-chromosomal rearrangements (n=14 [5.9%]). Interestingly, no balanced translocations in addition to *BCR-ABL1* were observed in the two blast phase samples and the K562 cell line.

Cytogenetics	DNA-PET
P022	
46,XY,+8	3 copies of chr8 estimated on sequence based copy number (Figure 7)
t(9;22)(q34;q11.2)	*BCR-ABL1* translocation (Figure 2B)
i(17)(q10)[cp2]	a dPET cluster of size 2 indicates isochromosome 17 rearrangement between pos. 17,595,066 and 28,282,853 (Figure 11)
48,idem+der(22)t(9;22)[7]	copy number of 2.7 upstream of chr22:21,955,000 and downstream of chr9:132,605,000 (points of copy number change in Figure 7)
48idem+19[cp4]	no detection of higher copy number of chr 19
48idem,t(12;17)(p13;q11.2)+19[cp3]	not detected
49,idem,+8,+19[2]	3 copies of chr8 estimated on sequence based copy number (Figure 7)
46,XY[3]	
P098	
46,XY, t(9;22)(q34;q11.2)[cp20]	*BCR-ABL1* translocation (Figure 2B)
P145	
46,XY, t(9;22)(q34;q11.2)[cp20]	*BCR-ABL1* translocation (Figure 2B)
P440	
46,XY,	
P308	
46,XY, t(9;22)(q34;q11.2)[cp20]	*BCR-ABL1* translocation (Figure 2B)

Table E7 Validation of cytogenetic karyotypes by DNA-PET

We next explored which of the individual somatic SVs could contribute biologically to blast phase by intersecting the blast phase specific SVs with RefSeq genes downloaded from the UCSC Genome Bioinformatics homepage (http://genome.ucsc.edu/) (Rhead et al. 2010), and identified 205 candidate SVs predicted to affect gene function directly. Within this group, we observed the amplification of *BCR-ABL1* itself, which was appreciable as an increase in

the dPET cluster size, and is a recognized feature of transformation (Figure 12).[6, 10] We also observed an inverse correlation between the cluster size representing the reciprocal *ABL1-BCR* translocation and stage (Figure 12). Of note, DNA-PET detected the complete loss of *ABL1-BCR* in sample P098, consistent with a deletion in the der9 by FISH (Figure 12). Another candidate we identified was a 2.7 Mb insertion of chromosome 8 into intron 1 of MECOM (previously known as *EVI1* and *MDS1*) on chromosome 3 (Figure 3A) which we confirmed by FISH (Figure 3C). *EVI-1* is a zinc finger transcription factor and plays an essential role in the proliferation and maintenance of normal hematopoietic stem cells (HSC)[11-13], and when overexpressed in HSCs results in bone marrow hyperproliferation and myeloid differentiation block. To our knowledge, no comparable insertion within or upstream of *EVI1* has been reported, and analysis of the insertion site suggested that the insertion of chromosome 8 might alter the transcription level of the shorter transcripts, which we confirmed by RT-PCR (Figure 3B).

Taken together, our DNA-PET analysis has been able to identify both known and novel features of blast phase progression, and provides the first assessment of the number and nature of structural changes that occur in a human cancer model.

Example 9. Results: An East-Asian *BIM* Polymorphism in Imatinib-Resistant Samples

We next investigated resistance-associated SVs, and found two deletions that occurred in all three resistant samples and six SVs in at least two out of three. One deletion, 2.5 Kb in size, was located in intron 1 of *ZNF385D*, a gene that has not yet been connected to CML. Interestingly, the other deletion observed in all three resistant samples was in intron 2 of the pro-apoptotic gene *BCL2L11* (also known as *BOD*, *BIML*, *BIMEL*, *or BIM*) (Figure 4A). Importantly, others have reported that BIM upregulation by imatinib is required for the drug to induce apoptosis, since preventing *BIM* upregulation renders CML imatinib-resistant.[14-16] We confirmed the deletion by PCR and sequencing, and found an identical 2,903 bp deletion in all 3 samples (Figure 4C and Figure 4D), suggesting that the deletion is germline and thus constitutes a polymorphism. Accordingly, we screened 74 normal East-Asian samples from the International HapMap Project,[17] and determined the frequency of *BIM* deletion carriers to be 17.6% (12 heterozygous and 1 homozygous individual; allele frequency of 9.5%). Although the *BIM* deletion turned out to be a structural polymorphism rather than a recurrent

somatic event, we were intrigued by the fact that all three resistant patients were carriers of the *BIM* deletion, a gene that is required for imatinib-sensitivity.[14-16]

Example 10. Results: Functional Effects of the *BIM* Polymorphism

Analysis of *BIM* gene structure suggested that the deletion might result in alternative splicing of exon 3 and 4 in a mutually exclusive manner. This possibility was suggested by the deletion's proximity (107 bp) to the 5' end of the intron-exon boundary of exon 3, as well as the presence of a stop codon within exon 3 itself (Figure 5A). To test this hypothesis, we obtained primary CML samples with (n=12) and without (n=11) the deletion, and measured the expression levels of exon 3- and exon 4-containing transcripts, as well as exon 2-containing transcripts as a readout for general *BIM* transcription (since exon 2 is present in all *BIM* isoforms).[18] As shown in Figure 5B, we found that the deletion was associated with an increase in exon 3-containing transcripts together with a decrease in exon 4-containing transcripts, while general *BIM* transcription was unaffected. These results were mirrored in lymphoblastoid cell lines from normal individuals, indicating that the effect of the deletion is lineage-independent (Figure 13).

Because the proapoptotic properties of BIM reside in the BH3 domain, which is found only in exon 4 (Figure 5A),[18, 19] we asked if the decrease in exon 4-containing transcripts might be associated with imatinib-resistance. Fortunately, we were able to identify one CML cell line, KCL22, which harbored the deletion and used these cells to address this question (Figure 5C). Notably, this line was originally obtained from a Japanese patient, and in contrast to most other CML lines, exhibited imatinib-resistance *ab initio*.[20-22] We confirmed that the cells expressed an increased exon 3/exon 4 transcript ratio (Figure 5D) and that this was associated with decreased protein expression of the major exon 4-containing *BIM* isoform, *BIMEL*, in KCL22 cell lines both before and after imatinib exposure, when compared to lines without the deletion (Figure 5E). We also confirmed that KCL22 cells were resistant to imatinib, as well as the more potent second-generation tyrosine kinase inhibitors (Figure 5F).

These results led us to ask if BH3 mimetics, which are in early phase clinical trials,[23] might sensitize KCL22 cells to imatinib. As shown in Figure 5G, we found that this was indeed the case, and that the combination of imatinib or dasatinib and ABT-737 acted synergistically to induce apoptosis in KCL22 cells but not KYO-1 cells.

Example 11. Results: Association of the *BIM* Polymorphism with BCR-ABL Independent Clinical Resistance to Tyrosine Kinase Inhibitors in Chronic Myelogenous Leukaemia

We next determined the frequency of the polymorphism in a larger cohort of East-Asian CML patients, and found it to be present in 12.0% (19 out of 158). We also tested the hypothesis that the polymorphism could predict for clinical resistance. In this analysis, we reasoned that the presence of the polymorphism, as exemplified by KCL22 cells, might be sufficient to confer resistance to tyrosine kinase inhibitors, whereas in its absence, resistance would require the emergence of clones bearing resistance-conferring *BCR-ABL* mutations.[24]

Accordingly, we divided the samples from all patients with resistance into those with or without *BCR-ABL* mutations, and found a significant difference in the frequency of the polymorphism between the two groups: 2 out of 42 (4.8%) with *BCR-ABL* mutations vs 13 out of 55 (23.6%) without (p=0.01) (Figure 6A).

When we determined the frequency of the polymorphism in patients with sensitive vs resistant disease in the absence of a *BCR-ABL* mutation, the difference was also significant: 4 out of 61 in imatinib-sensitive patients versus 13 out of 55 for imatinib-resistant patients (p=0.02) (Figure 6B). However, there was no significant difference in the frequency of the polymorphism between imatinib-sensitive patients (4 out of 61), and all imatinib-resistant patients (15 out of 97) (p=0.13) (Figure 6C).

This result might be expected, given that statistical significance here would depend on the frequency of the *BIM* polymorphism in the general CML population.

Example 12. Discussion

We report a novel polymorphism in intron 2 of the *BIM* gene that is associated with both *in vitro* and *in vivo* clinical resistance to tyrosine kinase inhibitors. The frequency of the deletion carriers in the normal East-Asian population is 17.6%, and in the CML patients seen at two South-East Asian referral centers, 12.0%. In this cohort, the polymorphism accounted for a quarter (13/55 or 23.6%) of the cases with resistance in the absence of a *BCR-ABL* mutation. We believe an etiologic role is unlikely given the lack of geographical variations in the worldwide incidence of CML.[25]

We also show that the presence of the polymorphism results in the increased expression of transcripts containing exon 3 vs exon 4 of the *BIM* gene, and found a significant association between the development of clinical resistance and the presence of the polymorphism, such that individuals with the deletion had a 1.34-fold relative risk (heterozygous genotype relative risk; 95% confidence interval 0.94 – 1.59) of developing resistance than those without. In addition, by determining that resistance is independent of BCR-ABL inhibition, we were able to predict that patients with resistance and the deletion would be less likely to harbor CML clones with resistance-conferring mutations in BCR-ABL than those without the deletion. This finding is consistent with the idea that such clones only emerge under the selective pressure of therapy, and in the absence of other resistance-conferring mechanisms.[24]

Current clinical guidelines advocate either increasing the dose of tyrosine kinase inhibitor or changing to more potent tyrosine kinase inhibitors upon development of resistance.[5] Hence it is of concern that among 4 patients with *BIM* polymorphism-associated resistance, and in whom these guidelines were followed and subsequent response data available, none responded. While anecdotal, this observation is consistent with our finding that *BIM* deletion-associated resistance is BCR-ABL-independent. However, our *in vitro* observations also suggest that resistance in this setting can be overcome by combining tyrosine kinase inhibitors with BH3 mimetics. Thus, the *BIM* polymorphism may be used as a predictor of both resistance to tyrosine kinase inhibitors and of response to BH3 mimetics. Screening for this polymorphism may therefore be useful in the management of East-Asian CML patients. The applicability of our findings to other cancers where drug-sensitivity is dependent on BIM-mediated apoptosis also warrants investigation.[26-28]

Examples 13 and 14. Association of the *BIM* Polymorphism with EGFR Independent Clinical Resistance to Tyrosine Kinase Inhibitors in Non-Small Cell Lung Cancer (NSCLC)

It has previously been shown that mutant EGFR-driven NSCLCs require BIM expression for TKIs to be able to kill NSCLC cell lines (Cragg et al. PLOS Medicine, 2007; Costa et al. PLOS Medicine, 2007).

Accordingly, we predict that cell lines, and by extension patients, bearing the BIM polymorphism will be resistant to drugs which target the mutant EGFR.

In addition, we predict that resistant cell lines will be sensitive to the combination of BH3 mimetic drugs (e.g. ABT-737 and ABT-263) and the anti-EGFR drug.

Example 13. Association of the *BIM* Polymorphism with EGFR Independent Clinical Resistance to Tyrosine Kinase Inhibitors in Non-Small Cell Lung Cancer (NSCLC) - *in vitro* Correlations

NSCLC cell lines with activating mutations in EGFR will be identified.

Cell lines will then be analyzed for the presence or absence of the BIM polymorphism by PCR analysis as previously described.

The sensitivity and/or resistance of the various cell lines to anti-EGFR drugs will be determined using standard cellular assays for cell growth, proliferation, and apoptosis.

Cell lines will also be tested for their sensitivity to BH3 mimetics, either as single agents or in combination with drugs targeting EGFR.

The presence or absence of the polymorphism will then be correlated with the degree of sensitivity/resistance of the NSCLC cell line to the drugs being tested.

We expect that there will be a positive correlation between the presence of the BIM polymorphism and drug-resistance.

Specifically, we predict that NSCLC lines with activating mutations in EGFR bearing the BIM polymorphism will be more resistant to anti-EGFR drugs than those not bearing the BIM polymorphism.

Furthermore, we expect that cell lines with the BIM polymorphism and which are resistant to drugs targeting EGFR will be sensitive to the combination of BH3 mimetic drugs (e.g. ABT-737 and ABT-263) and the anti-EGFR drug.

Example 14. Association of the *BIM* Polymorphism with EGFR Independent Clinical Resistance to Tyrosine Kinase Inhibitors in Non-Small Cell Lung Cancer (NSCLC) - Patient Correlations

Patients with NSCLC with activating mutations in EGFR will be identified.

DNA from patients and/or their tumours will be analyzed for the presence or absence of the BIM polymorphism by PCR analysis as previously described.

The patient's response to the anti-EGFR therapy will then be determined using clinical criteria. These data will include clinical parameters such as tumour size, time to progression, progression free survival, overall survival, and performance status.

The presence or absence of the polymorphism will then be correlated with the clinical parameters mentioned above.

We expect that there will be a positive correlation between the presence of the BIM polymorphism and an inferior response to anti-EGFR therapy compared to patients without the polymorphism. The may be seen as a failure of the tumour to decrease in size.

In addition, such patients may have a shorter time to progression, progression free survival, and/or overall survival.

Examples 15 and 16. Association of the *BIM* Polymorphism with c-KIT/PDGFR Independent Clinical Resistance to Tyrosine Kinase Inhibitors in Gastrointestinal Stromal Tumours (GIST)

It has previously been shown that c-KIT/PDGFR-driven GISTs require BIM expression for TKIs to be able to kill GIST cell lines (Gordon et al. JBC, 2010).

Accordingly, we predict that cell lines, and by extension patients, bearing the BIM polymorphism will be resistant to drugs which target the mutant c-KIT.

In addition, we predict that resistant cell lines will be sensitive to the combination of BH3 mimetic drugs (e.g. ABT-737 and ABT-263) and the anti-c-KIT drug.

Example 15. Association of the *BIM* Polymorphism with c-KIT/PDGFR Independent Clinical Resistance to Tyrosine Kinase Inhibitors in Gastrointestinal Stromal Tumours (GIST) - *in vitro* Correlations

c-KIT-driven GIST cell lines with activating mutations in c-KIT will be identified.

Cell lines will then be analyzed for the presence or absence of the BIM polymorphism by PCR analysis as previously described.

The sensitivity and/or resistance of the various cell lines to anti-c-KIT drugs will be determined using standard cellular assays for cell growth, proliferation, and apoptosis.

Cell lines will also be tested for their sensitivity to BH3 mimetics, either as single agents or in combination with drugs targeting c-KIT.

The presence or absence of the polymorphism will then be correlated with the degree of sensitivity/resistance of the GIST cell line to the drugs being tested.

We expect that there will be a positive correlation between the presence of the BIM polymorphism and drug-resistance.

Specifically, we predict that GIST lines with activating mutations in c-KIT bearing the BIM polymorphism will be more resistant to anti-c-KIT drugs than those not bearing the BIM polymorphism.

Furthermore, we expect that cell lines with the BIM polymorphism and which are resistant to drugs targeting c-KIT will be sensitive to the combination of BH3 mimetic drugs (e.g. ABT-737 and ABT-263) and the anti-c-KIT drug.

Example 16. Association of the *BIM* Polymorphism with c-KIT/PDGFR Independent Clinical Resistance to Tyrosine Kinase Inhibitors in Gastrointestinal Stromal Tumours (GIST) - Patient Correlations

Patients with GIST with activating mutations in c-KIT will be identified.

DNA from patients and/or their tumours will be analyzed for the presence or absence of the BIM polymorphism by PCR analysis as previously described.

The patient's response to the anti-c-KIT therapy will then be determined using standard clinical criteria. These data will include clinical parameters such as tumour size, time to progression, progression free survival, overall survival, and performance status.

The presence or absence of the polymorphism will then be correlated with the clinical parameters mentioned above.

We expect that there will be a positive correlation between the presence of the BIM polymorphism and an inferior response to anti-c-KIT therapy compared to patients without the

polymorphism. The may be seen as a failure of the tumour to decrease in size. In addition, such patients may have a shorter time to progression, progression free survival, and/or overall survival.

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Each of the applications and patents mentioned in this document, and each document cited or referenced in each of the above applications and patents, including during the prosecution of each of the applications and patents ("application cited documents") and any manufacturer's instructions or catalogues for any products cited or mentioned in each of the applications and patents and in any of the application cited documents, are hereby incorporated herein by reference. Furthermore, all documents cited in this text, and all documents cited or referenced in documents cited in this text, and any manufacturer's

instructions or catalogues for any products cited or mentioned in this text, are hereby incorporated herein by reference.

Various modifications and variations of the described methods and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in molecular biology or related fields are intended to be within the scope of the claims.

SEQUENCE LISTING FREE TEXT

SEQ ID NO: 1

Sequence of PCR fragment amplified using BIM_del_F and BIM_del_R primers from the BIM (BCL2L11) gene with the deletion described (1,323 bp)

>hg18_dna range=chr2:111599051..111599665 strand=+ followed by hg18_dna range=111602569..111603276 strand=+

AATACCACAGAGGCCCACAGCAAAATCATGGAAGGAACTGACCTGGTGGAGACTGGTAAATTG
GAGAGTATTTGCCCCTTCTATGTTTGGGCCACACCTAATTGTGGCTGTGAGGGCATGTGGTCT
CAGGGTGGGTTTTCCTATATTGCAAGATAACCTAGGAATGCAAAATTGATGCCAGATACCCTG
TTTCAACATTGACAGCTGATTCAGATTTTGAAAACATTGTACAAGCTGAAAAGAAACATCTGC
AGACTTGATTTGGCCCTTGAACCTACAGCATGTGACTTTGGGTACATACTTTGGGTAACCTTG
GTGAGGGGCTGAGTCTGTGTTGATCGACTTGCTGTTCCCCACCAATGGAAAAGGTTCATGTCT
TGATCAGTAGTCAATCACATTGACCATTTGTCCAGATTAGCTTGCCATACATGAACAAGATAG
AAGTAAGTTGGTAGAGTTATCAATTAGGAAACCCAGTACAGAGTCTATTATAATTTAGATTGT
ACCTCATGATGAAGGCTAACTCAACAAACCCATCAGAACAGACACTGGAACAAAATGACATTT
CTAAATACCATCCAGCTCTGTCTTCATAGGCTTCAGTGAGGTAAATCACTGTTCTCCATAGAG
GCTGTGCCATTTTACATTCCCACCAACAGGGCACAAGGGTTCCAGTTTCTCCACATACTTACC
AACACTTTTTTTTTTTTTTTTTTAACAGTAGTCATCCTAGAGGATATAGGTGATCTTTCACTG
TGCTTTGGATTTATATTTACTGGCTTAGATTTGTATGGCCACCACCATAGTCAAGATACAGAA
CAACTCAACCACAAGGATTTCTCATGATACCTTTTTATAGCCACAGCCACCTCTCTCCCTCTT
CCTTGAGCATTTTGTCATATGGTCATTGGTGATTAAATAAAATGTATTTTAATATTGACTTTC
TCTGTTTCTTTCTACCTTTTTAAACATGGCTACTAGAAAAATGCACAATTAGATTTGTGGCTG
GTGTTCTGTTTCATCTAAACAGGCTGGCCTCACAGAGGAGCTGGAGTGTGCAGTGCTGCTCTA
GCAAGCCAGGCTTGACTCTTCCCACTCAGGGCACATCACTTCCATGAAGCTTACTCCTTGGGT
TGTTTGGTTGACTTAGGAGAATGGAAGTGATTAGCAGAATCTTGTAAGCATTTTAAACATTAA
ATGAGCATTGTAAACAGCGGCATTCTTCAGGCAAATACAGTTTTGTTTTACCTCTTTAAATTC
CATGGTATATTCGGACTTCAAAAAGTAGATGGTAGAGCACATGCTTTCTCAGCACCTTCAGGC

SEQ ID NO: 2

Sequence of PCR fragment using BIM_del_F and BIM_del_R primers from the BIM (BCL2L11) wild type gene (4,226 bp)

>hg18_dna range=chr2:111599051 111603276 strand=+

AATACCACAGAGGCCCACAGCAAAATCATGGAAGGAACTGACCTGGTGGAGACTGGTAAATTG
GAGAGTATTTGCCCCTTCTATGTTTGGGCCACACCTAATTGTGGCTGTGAGGGCATGTGGTCT
CAGGGTGGGTTTTCCTATATTGCAAGATAACCTAGGAATGCAAAATTGATGCCAGATACCCTG
TTTCAACATTGACAGCTGATTCAGATTTTGAAAACATTGTACAAGCTGAAAAGAAACATCTGC
AGACTTGATTTGGCCCTTGAACCTACAGCATGTGACTTTGGGTACATACTTTGGGTAACCTTG
GTGAGGGGCTGAGTCTGTGTTGATCGACTTGCTGTTCCCCACCAATGGAAAAGGTTCATGTCT
TGATCAGTAGTCAATCACATTGACCATTTGTCCAGATTAGCTTGCCATACATGAACAAGATAG

AAGTAAGTTGGTAGAGTTATCAATTAGGAAACCCAGTACAGAGTCTATTATAATTTAGATTGT
ACCTCATGATGAAGGCTAACTCAACAAACCCATCAGAACAGACACTGGAACAAAATGACATTT
CTAAATACCATCCAGCTCTGTCTTCATAGGCTTCAGTGAGGTAAATCAGGCAGGCCTTTGCCC
ATGTTATAGAATTGGAAAGAACCTCAGAGTGGTGGTCACTTGTCAGAGGTTGGGCACACCTGT
GAGGTGGTGGGGAGAAATGACAGACATCCCAGCAGCTACACATGCTGGCTGCACGTCTCTTGC
CAAATGCCAGGAGGTAATTTTTTAGGGTCCCTCCTTAGGGAAAGGGGCTGGAAGTTTTATTAT
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GTACTCATGAAAATGCTCCCCCATATATATGATCATTCTCGCTTACTATAACATCCTTGCTTA
CTAAATGAGTTAACAGGGCTTTATGGTGTGTATCGTGAAACACACGTGCATTAAAGACCCTCT
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TTCTCTAACCAGTTCCCTTTCAAGCAAAATGCATACATTGGTCTCTGTAGGTGATGGGTTAAT
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TGTAAAGACAGGCACAAATGCTTTTTTTGTTGATAAATACTATTTTACAAGCTAATTATAAGT
TAAGCACTGTTACTTGAGATGAAATATACAGGGCTTCAAAGATCATAATCTAAATAATTATGC
ACAGCTAATGGTTATACCTGTGAAGTAAAGTAGTGGATCCTGAGGTGTAATTTTATAGTATTA
GCTGCATTTCAGTAGATGGTGTGATGAAGAGTTTAATGCATAGGATTAAATGAGAAGTTACGA
GGAGTTTGTTTAAAGTTAATGTACCGAGGTAAGTTTTCAGTGTTAAGTTTTTGGGAGATTTGT
TTTGGGAGAGGATGAGTTGGGGTTGGGGGAGGAAAGGACTTAGCCAGATGTGAGTTTCTTAAA
TTGAAGCATAAAATTTACAATTTATGTAGTCCATAATTTTCTCTGGACATTCTACAGTCTTAG
TTCATGCCTGAAGACCACTGAAATAATGCTGAGTTGATAAGTGGTTCTCTTGACTTTGTTTAG
TATTCTTTACTCAACCCTATCCATGAAGTTCTTCAATGAAGCTTTTGATAATTTATTGCAAAA
TACATTTTCCACAAAGAAGTCATTATGATTGGTTTGAACTAGTGGAACACAAATGTGAGGTTA
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GGGGTAGAGATCAGTGTGTCTTGAAGTTTTGTTTTGCAAAACTTTGTTCTAATGAGAAAGTCA
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CCTTTTTGGATGAGTGTTTTATGCCATATGTGTTTTTAATGGAAATTAAAGTGTAGTCAGTAC
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TCCCTAGTGTCATGTTAGTAAATGTCTTAAATTTTATACTTGTTCCCTGGCACATTGGAATTC
ACAGATGGGAGTTAATGGCTTTCTTTTTTTTTTTTTTTTTTCCTCAGCGTCTTGTGGGTACT
TCTCTTATAGCTGGTACTTGTCTGACCCCTCCTTTAGTTTGTGAGCTCCCTGGGCGGGGAATA
ATGGCCTGCAGATGCTAGCGAGTGCCTGACAAAGAGGAGAAGCCCAGGAGATGTTGAGAGTCA
GTCCAGCTCTGCCTGTTAGCCTTTCAGACAAATAAAGTTGAAGAAGGCAGGTAGCAAGAAAAA
GATCCTGACCTCTGCTCTGCCAAAGTGTTTTTAATTACCTGGATCTAGCTGTAAGGTTTGCCA
CGTAGTGGTGACAGCTGAGGTCTAGCTCAGCACTACTCAGCAGGGAAGCCACACATGCATTAA
GCACTTGACATAGGACTAGTCTGAACTGAGTTGTGCTGTCATTATTGATACACACTGGATTTT
GAGGAGACAAAAAAGAATGCAAAATAGTTTAATTGTTTTCATATGGGTTACATGTTGAAATGG
TGTTTTAAATATATAGGTTAAATAAAATATAAACTTGTATTGCAGTTAAACACAAAGCGTAAA
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TTGACAACTTCAGATACCTTATATAAGTGAAATTTATATAGTATTTGCTCTTCCATGACAGGC
TTATTTCACTTAGCGTAATGTCGTCAGGGTTCATTTATCTTGCAACATGTCAGAATTTCCTTC
CTTTTTAAGGCTGAAGGTTGTTCCAGTGTGTGTATATCACATACTTCATTTATCCATTCATCC
ATCAGGAGATACTTGGGTTGCTTCCACTTTTTGGCTATTGTGAGTAGTGCTGCTATGAACATG
GGTATGCAAATATCTTTTGGGGGATTCTGCTTTGAATTTTTTTGGATATATACTTGGAAGTGG
AATTGCTGGATCATATGGTAATTCTATTTTTAATTTTTTGGGGAACCATCATGCTGTTCTCCA

TAGAGGCTGTGCCATTTTACATTCCCACCAACAGGGCACAAGGGTTCCAGTTTCTCCACATAC
TTACCAACACTTTTTTTTTTTTTTTTTTAACAGTAGTCATCCTAGAGGATATAGGTGATCTTT
CACTGTGCTTTGGATTTATATTTACTGGCTTAGATTTGTATGGCCACCACCATAGTCAAGATA
CAGAACAACTCAACCACAAGGATTTCTCATGATACCTTTTTATAGCCACAGCCACCTCTCTCC
CTCTTCCTTGAGCATTTTGTCATATGGTCATTGGTGATTAAATAAAATGTATTTTAATATTGA
CTTTCTCTGTTTCTTTCTACCTTTTTAAACATGGCTACTAGAAAAATGCACAATTAGATTTGT
GGCTGGTGTTCTGTTTCATCTAAACAGGCTGGCCTCACAGAGGAGCTGGAGTGTGCAGTGCTG
CTCTAGCAAGCCAGGCTTGACTCTTCCCACTCAGGGCACATCACTTCCATGAAGCTTACTCCT
TGGGTTGTTTGGTTGACTTAGGAGAATGGAAGTGATTAGCAGAATCTTGTAAGCATTTTAAAC
ATTAAATGAGCATTGTAAACAGCGGCATTCTTCAGGCAAATACAGTTTTGTTTTACCTCTTTA
AATTCCATGGTATATTCGGACTTCAAAAAGTAGATGGTAGAGCACATGCTTTCTCAGCACCTT
CAGGC

SEQ ID NO: 3

Bim_del_F forward primer (hg18_dna range=chr2:111,599,051..111,599,070 strand =+)

AATACCACAGAGGCCCACAG

SEQ ID NO: 4

Bim_del_R reverse primer (hg18_dna range=chr2:111,603,257..111,603,276 strand =-)

GCCTGAAGGTGCTGAGAAAG

SEQ ID NO: 5

1000 bp flanking sequence upstream of the deletion in the BIM (BCL2L11) gene: chr2:111,598,666-111,599,665

>hg18_dna range=chr2:111598666-111599665 strand=+

CTTTTGTGGCAGTGATGAGTTGAGGTCCAAACATTAGCTTTCAGGTCTGTCTTCATTAAGCTA
AAGTGTGTTTTAACCACCAGGCTTTACATAGTAATGACATTTTGCTTGAAAGGGAACTGATCA
TTTACAGAAAATAGCTTAATAATCAAAAGTGTAAAGAAAGATGACAATCATTTTTGAAAATAA
CACTTTTAAAAAAATGAACTAGTTCATGAAAGCAGTACCAACATAGAACCATGAAAATGGTTT
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TAACATAAATACCACAGAGGCCCACAGCAAAATCATGGAAGGAACTGACCTGGTGGAGACTGG
TAAATTGGAGAGTATTTGCCCCTTCTATGTTTGGGCCACACCTAATTGTGGCTGTGAGGGCAT
GTGGTCTCAGGGTGGGTTTTCCTATATTGCAAGATAACCTAGGAATGCAAAATTGATGCCAGA
TACCCTGTTTCAACATTGACAGCTGATTCAGATTTTGAAAACATTGTACAAGCTGAAAAGAAA
CATCTGCAGACTTGATTTGGCCCTTGAACCTACAGCATGTGACTTTGGGTACATACTTTGGGT
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CATGTCTTGATCAGTAGTCAATCACATTGACCATTTGTCCAGATTAGCTTGCCATACATGAAC
AAGATAGAAGTAAGTTGGTAGAGTTATCAATTAGGAAACCCAGTACAGAGTCTATTATAATTT
AGATTGTACCTCATGATGAAGGCTAACTCAACAAACCCATCAGAACAGACACTGGAACAAAAT
GACATTTCTAAATACCATCCAGCTCTGTCTTCATAGGCTTCAGTGAGGTAAATCA

SEQ ID NO: 6

Deletion in the BIM (BCL2L11) gene: chr2:111,599,666-111,602,568

>hg18_dna range=chr2:111599666-111602568 strand=+

GGCAGGCCTTTGCCCATGTTATAGAATTGGAAAGAACCTCAGAGTGGTGGTCACTTGTCAGAG
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CTTGTAGTTTTTGTTGTACTCATGAAAATGCTCCCCCATATATATGATCATTCTCGCTTACTA
TAACATCCTTGCTTACTAAATGAGTTAACAGGGCTTTATGGTGTGTATCGTGAAACACACGTG
CATTAAAGACCCTCTGGAAGGTATTAGCTTTTCACACTTTCACAACAAAAGCTTCACACTTGT
GGTTATTAAGCTATTTTCTCTAACCAGTTCCCTTTCAAGCAAAATGCATACATTGGTCTCTGT
AGGTGATGGGTTAATGCATGGAAATAGTTTCTCCTTCCCTGGAACTGGGAATAGTGGGTGAGA
TAGTGTATTTTTTAATGTAAAGACAGGCACAAATGCTTTTTTTGTTGATAAATACTATTTTAC
AAGCTAATTATAAGTTAAGCACTGTTACTTGAGATGAAATATACAGGGCTTCAAAGATCATAA
TCTAAATAATTATGCACAGCTAATGGTTATACCTGTGAAGTAAAGTAGTGGATCCTGAGGTGT
AATTTTATAGTATTAGCTGCATTTCAGTAGATGGTGTGATGAAGAGTTTAATGCATAGGATTA
AATGAGAAGTTACGAGGAGTTTGTTTAAAGTTAATGTACCGAGGTAAGTTTTCAGTGTTAAGT
TTTTGGGAGATTTGTTTTGGGAGAGGATGAGTTGGGGTTGGGGGAGGAAAGGACTTAGCCAGA
TGTGAGTTTCTTAAATTGAAGCATAAAATTTACAATTTATGTAGTCCATAATTTTCTCTGGAC
ATTCTACAGTCTTAGTTCATGCCTGAAGACCACTGAAATAATGCTGAGTTGATAAGTGGTTCT
CTTGACTTTGTTTAGTATTCTTTACTCAACCCTATCCATGAAGTTCTTCAATGAAGCTTTTGA
TAATTTATTGCAAAATACATTTTCCACAAAGAAGTCATTATGATTGGTTTGAACTAGTGGAAC
ACAAATGTGAGGTTATAAAGAGGTTCGCCTTAGCCAGGGGCTCCTTTAGCTGCAAAGCAGTTT
TTTGCTCAGCAACTTGGGGTAGAGATCAGTGTGTCTTGAAGTTTTGTTTTGCAAAACTTTGTT
CTAATGAGAAAGTCAAGTCTTAGGAGGAATGTATAGTAGTTGAGTGTTTGTATTAACACTGTT
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AAGTGTAGTCAGTACACTAAAGTGTAGTCAGTACAATTGGAAATAAGAGTTGAGAAAAGTCAG
GATATGGAGGAATGCTCCCTAGTGTCATGTTAGTAAATGTCTTAAATTTTATACTTGTTCCCT
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CCTGGGCGGGGAATAATGGCCTGCAGATGCTAGCGAGTGCCTGACAAAGAGGAGAAGCCCAGG
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TACACACTGGATTTTGAGGAGACAAAAAAGAATGCAAAATAGTTTAATTGTTTTCATATGGGT
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GTCAGAATTTCCTTCCTTTTTAAGGCTGAAGGTTGTTCCAGTGTGTGTATATCACATACTTCA
TTTATCCATTCATCCATCAGGAGATACTTGGGTTGCTTCCACTTTTTGGCTATTGTGAGTAGT

GCTGCTATGAACATGGGTATGCAAATATCTTTTGGGGGATTCTGCTTTGAATTTTTTTGGATA
TATACTTGGAAGTGGAATTGCTGGATCATATGGTAATTCTATTTTTAATTTTTTGGGGAACCA
TCATG

SEQ ID NO: 7

1000 bp flanking sequence downstream of the deletion in the BIM (BCL2L11) gene: chr2:111,602,569-111,603,568

>hg18_dna range=chr2:111602569-111603568 strand=+

CTGTTCTCCATAGAGGCTGTGCCATTTTACATTCCCACCAACAGGGCACAAGGGTTCCAGTTT
CTCCACATACTTACCAACACTTTTTTTTTTTTTTTTTTAACAGTAGTCATCCTAGAGGATATA
GGTGATCTTTCACTGTGCTTTGGATTTATATTTACTGGCTTAGATTTGTATGGCCACCACCAT
AGTCAAGATACAGAACAACTCAACCACAAGGATTTCTCATGATACCTTTTTATAGCCACAGCC
ACCTCTCTCCCTCTTCCTTGAGCATTTTGTCATATGGTCATTGGTGATTAAATAAAATGTATT
TTAATATTGACTTTCTCTGTTTCTTTCTACCTTTTTAAACATGGCTACTAGAAAAATGCACAA
TTAGATTTGTGGCTGGTGTTCTGTTTCATCTAAACAGGCTGGCCTCACAGAGGAGCTGGAGTG
TGCAGTGCTGCTCTAGCAAGCCAGGCTTGACTCTTCCCACTCAGGGCACATCACTTCCATGAA
GCTTACTCCTTGGGTTGTTTGGTTGACTTAGGAGAATGGAAGTGATTAGCAGAATCTTGTAAG
CATTTTAAACATTAAATGAGCATTGTAAACAGCGGCATTCTTCAGGCAAATACAGTTTTGTTT
TACCTCTTTAAATTCCATGGTATATTCGGACTTCAAAAAGTAGATGGTAGAGCACATGCTTTC
TCAGCACCTTCAGGCTGCCTGGAGCCTCCCAATAGAGGTGTCTTCGAGGGAGTCCCAGCTCTG
TCTCTGAAACCCCAAAGTTACTTGTTTGACACCAAGAGAAATAAGGAAACTTTTTAGGTCCTA
AGTGGGGAGAGAAAGTGCTAGAAGAGAAAGATATTTTTCTTTACTAGTTCCAAACACATTTAT
TAATTGTTAGTTACCCAATTTTAAATTTACATCTTAAAAAAATTTTTTTTCAGATAATTACAG
ATTCACATGCATTTATAGGAAATAATACAAAGAAATTGTATATGCCATTCACCCA

CLAIMS

1. A polymorphic variant of a *BIM* (*BCL2L11*) gene which comprises, in 5' to 3' order, the nucleotide sequence set out in SEQ ID NO: 5 followed immediately by the nucleotide sequence set out in SEQ ID NO: 7.

2. A polymorphic variant of a *BIM* (*BCL2L11*) gene characterised by lacking the nucleotide sequence set out in SEQ ID NO: 6.

3. A nucleotide sequence as set out in SEQ ID NO: 1, which is obtainable from a *BIM* polymorphic variant according to Claim 1 or 2, for example by nucleic acid amplification.

4. A polymorphic variant of a *BIM* (*BCL2L11*) gene according to Claim 1 or 2 or a nucleotide sequence according to Claim 3, which is associated with:

(i) resistance to treatment with tyrosine kinase inhibitors for chronic myelogenous leukaemia in the absence of BCR-ABL reactivation (BCR-ABL-independent TKI-resistance);

(ii) resistance to treatment with tyrosine kinase inhibitors for gastrointestinal stromal tumours (GIST) in the absence of c-KIT/PDGFR reactivation (c-KIT/PDGFR-independent TKI-resistance);

(iii) resistance to treatment with tyrosine kinase inhibitors for non-small cell lung cancer (NSCLC) in the absence of EGFR reactivation (EGFR-independent TKI-resistance); or

(iv) resistance to treatment with tyrosine kinase inhibitors for a myeloproliferative disorder in the absence of JAK2 reactivation (JAK2-independent TKI-resistance);

in an individual comprising such a polymorphism.

5. A nucleotide sequence (a) as set out in SEQ ID NO: 3, or a (b) as set out in SEQ ID NO: 4, or a combination of (a) and (b), for example, a primer set.

6. A method of detecting the presence of a *BIM* (*BCL2L11*) polymorphism according to Claim 1 or 2 in an individual, the method comprising detecting a nucleic acid amplification product comprising a sequence set out in SEQ ID NO: 1, for example by use of a primer set according to Claim 5.

7. A method of predicting whether an individual susceptible to or suffering from cancer or a myeloproliferative disorder is likely to develop resistance to treatment with a tyrosine kinase inhibitor, the method comprising determining whether the individual has a *BIM* (*BCL2L11*) polymorphism according to Claim 1 or 2.

8. A method according to Claim 7, which comprises detecting the presence of a nucleic acid amplification product comprising a sequence set out in SEQ ID NO: 1, for example by use of a primer set according to Claim 5.

9. A method according to any of Claims 7 or 8, in which (a) if the individual is determined to have a *BIM* (*BCL2L11*) polymorphism, for example if the presence of a nucleic acid amplification product comprising a sequence set out in SEQ ID NO: 1 is detected, such as by use of a primer set according to Claim 5, then the individual is likely to develop resistance to treatment with a tyrosine kinase inhibitor, or in which (b) if the individual is determined not to have a *BIM* (*BCL2L11*) polymorphism, for example if the presence of a nucleic acid amplification product comprising a sequence set out in SEQ ID NO: 2 is detected, such as by use of a primer set according to Claim 5, then the individual is less likely to develop resistance to treatment with a tyrosine kinase inhibitor.

10. A method of choosing a therapy for an individual with cancer or a myeloproliferative disorder, the method comprising determining whether a patient is likely to develop resistance to treatment with a tyrosine kinase inhibitor by a method according to any of Claims 7, 8 or 9, and where the individual is determined as being likely to develop such resistance, choosing a therapy comprising any one or more of the following:

(a) more frequent monitoring of the patient;

(b) more frequent blood and bone marrow tests;

(c) bone marrow transplantation;

(d) administration of a more potent tyrosine kinase inhibitor (TKI), such as nilotinib or dasatinib;

(e) administration of a BH3-mimetic, e.g., ABT-263, for example in combination with a TKI;

(f) increasing the dose of a tyrosine kinase inhibitor, e.g. imatinib, for example beyond the standard dose of 400mg/day to 600 or 800 mg/day; or

(g) treatment with a drug that inhibits the pro-survival effect of the BCL2 group of proteins.

11. A method of determining the likelihood of success of a particular therapy on an individual with cancer or a myeloproliferative disorder, the method comprising comparing the therapy with the therapy determined by a method according to Claim 10.

12. A method according to any of Claims 7 to 11, in which the cancer or myeloproliferative disorder comprises chronic myelogenous leukaemia (CML) and in which the resistance to treatment with a tyrosine kinase inhibitor comprises for example BCR-ABL-independent TKI-resistance, such as resistance to a tyrosine kinase inhibitor such as imatinib.

13. A method according to any of Claims 7 to 11, in which the cancer or myeloproliferative disorder comprises gastrointestinal stromal tumour (GIST), and in which the resistance to treatment with a tyrosine kinase inhibitor comprises for example c-KIT/PDGFR-independent TKI-resistance, such as resistance to a tyrosine kinase inhibitor such as imatinib.

14. A method according to any of Claims 7 to 11, in which the cancer or myeloproliferative disorder comprises non-small cell lung cancer (NSCLC), and in which the resistance to treatment with a tyrosine kinase inhibitor comprises for example EGFR-independent TKI-resistance, such as resistance to a tyrosine kinase inhibitor such as erlotinib or gefitinib, or other kinase inhibitors, e.g. sunitinib, nilotinib, and sorafenib.

15. A method according to any of Claims 7 to 11, in which the cancer or myeloproliferative disorder comprises a myeloproliferative disorder such as selected from the group consisting of: polycythaemia vera, essential thrombocythaemia, and primary myelofibrosis, and in which the resistance to treatment with a tyrosine kinase inhibitor comprises for example JAK2-independent TKI-resistance, such as resistance to JAK inhibitors.

16. A method according to any of Claims 7 to 15, in which the cancer or myeloproliferative disorder is selected from the group consisting of: haematologic

malignancies, chronic lymphocytic leukaemia, acute lymphoblastic leukaemia, acute myeloid leukaemia, multiple myeloma, myeloproliferative disorders (including polycythaemia vera, essential thrombocythaemia, and primary myelofibrosis), solid tumours, small cell lung cancer, breast cancer, colorectal cancer, ovarian cancer, melanoma and neuroblastoma.

17. A method of treatment of a patient suffering from cancer or a myeloproliferative disorder, the method comprising determining whether the cancer or myeloproliferative disorder is a BCR-ABL-independent TKI-resistant CML cancer, a c-KIT/PDGFR-independent TKI-resistant GIST cancer, an EGFR-independent TKI-resistant NSCLC cancer or a JAK2-independent TKI-resistant myeloproliferative disorder by a method according to any of Claims 7 to 15, and treating the patient by performing a step selected from (a) to (g) as set out in Claim 10.

17. A method according to Claim 15 or 16, which comprises detecting a nucleic acid amplification product comprising a sequence set out in SEQ ID NO: 1, for example by use of a primer set according to Claim 5.

18. A polymorphic variant, nucleotide sequence or method substantially as hereinbefore described with reference to and as shown in Figures 1 to 13 of the accompanying drawings.