

METHOD

Field of the Invention

The present invention relates to the treatment of cancer.

5 Background of the Invention

Lymphoid (B-cell and T-cell) tumours account for a significant proportion of human malignancies. The spectrum of different, but related, B cell malignancies includes B-cell acute lymphocytic leukemia (B-ALL), B-cell chronic lymphocytic leukemia (B-CLL), B-cell chronic myelogenous leukemia (B-CML), B-cell prolymphocytic leukemia (B-PLL), hairy cell leukemia (HCL), various B-cell non-Hodgkin's lymphomas (B-NHLs) (including diffuse large B cell lymphoma (DLBCL), Follicular Lymphoma (FCL or FL), mantle cell lymphoma (MCL), marginal zone lymphoma (MZL), Primary effusion lymphoma (PEL)) and Multiple Myeloma (MM). The spectrum of T-cell malignancies includes T-cell leukaemia, peripheral T-cell lymphoma (PTCL), T-cell lymphoblastic lymphoma (T-CLL), cutaneous T-cell lymphoma (CTCL) and adult T-cell lymphoma (ATCL). Treatment of non-Hodgkin's lymphomas including both B-cell and T-cell tumours, chronic lymphocytic leukemias (CLL) and multiple myelomas (MM) is frequently unsatisfactory and attempts to link clinical or cellular characteristics of the disease to prognosis and treatment have met with difficulties.

Summary of the Invention

20 The present invention is based, in part, on methods that can be used to treat a patient having cancer with a TGF-beta activated kinase 1 (TAK1, MAP3K7) inhibitor. The invention further includes selecting patients having cancer who would be responsive to treatment with a TAK1 inhibitor. Moreover, the invention includes methods for determining for the presence of one or more deregulated TAK1 signal transduction molecules in a tumour cell. The presence of a
25 deregulated TAK1 signal transduction molecule indicates that a TAK1 inhibitor should be administered.

In one aspect, the invention includes inhibiting B cell tumour cell proliferation by contacting a B cell tumour cell with a TAK1 inhibitor. The B cell tumour can be a non-Hodgkin's lymphoma, a chronic lymphocytic leukaemia, or a multiple myeloma.

In another aspect, the invention includes inhibiting the growth of a solid tumour by contacting the tumour with a TAK1 inhibitor. The solid tumour can be a tumour of the head and neck, breast, ovary, lung, pancreas, colon, prostate, liver, kidney or skin.

In another aspect, the invention includes inhibiting proliferation of a T-cell leukemia and T-cell lymphoma by contacting the T-cell leukaemia and T-cell lymphoma with a TAK1 inhibitor. A T cell leukemia can include T-cell acute lymphoblastic leukemia (T-ALL), T-lymphoblastic lymphoma, T-CLL, CTCL or other T-NHLs. The TAK1 inhibitor can be administered either as a single agent or in combination with other anti-cancer agents or anti-cancer antibodies.

In another aspect, the invention includes a method of treating cancer. In one aspect, the invention includes a method of treating a patient having a B cell tumour by administering a TAK1 inhibitor. The B-cell tumour can be a non-Hodgkin's lymphoma, a chronic lymphocytic leukaemia or a multiple myeloma. In one example, the non-Hodgkin's lymphoma can be a follicular lymphoma, a diffuse large B cell lymphoma (DLBCL) of activated B cell (ABC) type, a diffuse large B cell lymphoma (DLBCL) of germinal center B cell (GCB) type, a mantle zone lymphoma (MZL), Mantle cell lymphoma (MCL), or MALT Lymphoma.

In another example, the non-Hodgkin's lymphoma has a t(14;18)(q32;q21) translocation, t(11;18)(q21;q21) translocation, t(1;14)(p22;q32), amplification of chromosome 18, addition of chromosome 18q21, amplification of chromosome 6, or amplification, as defined by comparative genomic hybridisation, of specific regions including BCL-10, CARD11, TRAF6 and TAK1.

In another aspect, the invention includes treating a patient having a solid tumour by administering a TAK1 inhibitor. The solid tumour can be a tumour of the head and neck, breast, ovary, lung, pancreas, colon, prostate, or skin.

In another aspect, the invention includes treating a patient having a T-cell leukemia by contacting the T-cell leukemia with a TAK1 inhibitor. A T-cell leukemia can include T-cell acute lymphoblastic leukemia (T-ALL), T-lymphoblastic lymphoma, T-CLL, CTCL or other T-NHLs.

In yet another aspect, the invention includes a method of treating a patient having a deregulated TAK1 signalling transduction molecule by administering a TAK1 inhibitor. The

TAK1 signalling transduction molecule can be MALT1, BCL-10, TAB1, TAB2, TRAF6, TRAF2, TAK1, CARD11, IRAK1, IRAK4, API1, API2, API3, API4 (survivin), BCL2 or NFkB target genes. The TAK1 signalling molecule can be a DNA molecule in either mutated or amplified or translocated form. The TAK1 signaling molecule can be a protein in its naïve form or modified, either by phosphorylation, ubiquitination, changed in sequence due to mutation, etc. The TAK1 signaling molecule can also be monitored by its subcellular localization. One example of such alterations in subcellular localization is shown by increased nuclear localization of BCL10 due to the gene amplification in a diffuse large B cell lymphoma with IGH-BCL2 fusion (Ye H et. al Haematologica. 2006;91 (6 Suppl)).

In another embodiment, a deregulated TAK1 signalling transduction molecule can be one or more of the molecules listed in Table 1 or Table 2 below.

Gene Symbol	Entrez	Pathway	U133A/B
RANK	8792	Rank-L	207037_at;238846_at;
RANKL	8600	Rank-L	210643_at;211153_s_at;241248_at;
TNFR1	7132	TNF	207643_s_at;241944_x_at;
TNFR2	7133	TNF	203508_at;
TRADD	8717	TNF	1729_at;205641_s_at;213443_at;
TANK	10010	TNF	207616_s_at;209451_at;210458_s_at;241191_at;243376_at;
GCK	2645	TNF	211167_s_at;
RIP	3267	TNF	213926_s_at;213928_s_at;
RAIDD	8738	TNF	209833_at;242638_at;
PROCASP2	835	TNF	208050_s_at;209811_at;209812_x_at;211140_s_at;34449_at;226032_at;226036_x_at;
UBC13	7334	TNF	201523_x_at;201524_x_at;212751_at;
UEV1A	7335	TNF	240129_at;
TLR2	7097	TLRs	204924_at;
TLR4	7099	TLRs	221060_s_at;224341_x_at;232068_s_at;240948_at;
TLR3	7098	TLRs	206271_at;
TLR7	51284	TLRs	220146_at;222952_s_at;
TLR9	54106	TLRs	223903_at;
TLR10	81793	TLRs	223750_s_at;223751_x_at;
MYD88	4615	TLRs	209124_at;
TOLLIP	54472	TLRs	217930_s_at;222469_s_at;233881_s_at;
IRAK	3654	TLRs	201587_s_at;
TIRAP	114609	TLRs	236687_at;239796_x_at;
PKR	5610	TLRs	204211_x_at;
TBK1	29110	TLRs	218520_at;
TGFB	7040	TGF-beta	203084_at;203085_s_at;
IL1R	3554	IL1	202948_at;215561_s_at;
TOLLIP	54472	IL1	217930_s_at;222469_s_at;233881_s_at;
MYD88	4615	IL1	209124_at;
CD19	930	B-Cell	206398_s_at;

CD22	933	B-Cell	
CD45	5788	B-Cell	207238_s_at;212587_s_at;212588_at;
CD79A	973	B-Cell	205049_s_at;
CD79B	974	B-Cell	205297_s_at;
LYN	4067	B-Cell	202625_at;202626_s_at;210754_s_at;239555_at;243633_at;
SHP1	8431	B-Cell	206410_at;
SYK	6850	B-Cell	207540_s_at;209269_s_at;226068_at;244023_at;
GAB1	2549	B-Cell	207112_s_at;225998_at;242572_at;
GAB2	9846	B-Cell	203853_s_at;238403_at;238405_at;241004_at;
SHP2	5781	B-Cell	205867_at;205868_s_at;209895_at;209896_s_at;212610_at;
CSK	1445	B-Cell	202329_at;
PAG	55824	B-Cell	225622_at;225626_at;
VAV	7409	B-Cell	206219_s_at;
VAV2	7410	B-Cell	205536_at;205537_s_at;226063_at;
GRB2	2885	B-Cell	215075_s_at;223049_at;228572_at;
BLNK	29760	B-Cell	207655_s_at;244172_at;
SOS	6654	B-Cell	212777_at;212780_at;229261_at;230337_at;232883_at;242018_at;242682_at;
PLCG2	5336	B-Cell	204613_at;
PKCB	5579	B-Cell	207957_s_at;209685_s_at;227817_at;227824_at;228795_at;230437_s_at;
PRKCQ	5588	B-Cell	210038_at;210039_s_at;
PRKCQ	5588	T-Cell	210038_at;210039_s_at;
PKCB	5579	T-Cell	207957_s_at;209685_s_at;227817_at;227824_at;228795_at;230437_s_at;
CD28	940	T-Cell	206545_at;211856_x_at;211861_x_at;
CD3	917	T-Cell	206804_at;
CD3E	916	T-Cell	205456_at;
CD3D	915	T-Cell	213539_at;
CD4	920	T-Cell	203547_at;216424_at;
UBC4	7322	T-Cell	201343_at;201344_at;201345_s_at;215604_x_at;240139_at;
UBC5	6923	T-Cell	200085_s_at;213877_x_at;200085_s_at;
CIAP1	329	T-Cell	202076_at;
CIAP2	330	T-Cell	210538_s_at;230499_at;
CD8	925	T-Cell	205758_at;
CD8B	926	T-Cell	207979_s_at;215332_s_at;230037_at;
CD45	5788	T-Cell	207238_s_at;212587_s_at;212588_at;
ZAP70	7535	T-Cell	214032_at;
FYN	2534	T-Cell	210105_s_at;212486_s_at;216033_s_at;217697_at;243006_at;
CLTA4	1493	T-Cell	221331_x_at;231794_at;234362_s_at;234895_at;236341_at;
PI3k	5286	T-Cell	213070_at;226094_at;235792_x_at;241905_at;
PIK3C2B	5287	T-Cell	204484_at;
PIK3C2G	5288	T-Cell	215129_at;
PIK3C3	5289	T-Cell	204297_at;215394_at;232086_at;239300_at;
PIK3CA	5290	T-Cell	204369_at;215212_at;
PIK3CB	5291	T-Cell	212688_at;217620_s_at;
PIM1	5292	T-Cell	209193_at;
PIK3CD	5293	T-Cell	203879_at;211230_s_at;
PIK3CG	5294	T-Cell	206369_s_at;206370_at;
VAV	7409	T-Cell	206219_s_at;
VAV2	7410	T-Cell	205536_at;205537_s_at;226063_at;
VAV3	10451	T-Cell	218806_s_at;218807_at;224221_s_at;

ITK	3702	T-Cell	211339_s_at;
SLP76	3937	T-Cell	205269_at;205270_s_at;244251_at;244556_at;244578_at;
LAT	27040	T-Cell	209881_s_at;211005_at;
PAG	55824	T-Cell	225622_at;225626_at;
CBL	867	T-Cell	206607_at;225231_at;225234_at;229010_at;243475_at;
LCK	3932	T-Cell	204890_s_at;204891_s_at;
SHB	6461	T-Cell	204656_at;204657_s_at;230459_s_at;234794_at;234795_at;243595_at;
PLCG1	5335	T-Cell	
LTBR	4055	LTBR	203005_at;232819_s_at;243400_x_at;
NIK	9020	LTBR	205192_at;
NIKBR	83696	LTBR	221672_s_at;221836_s_at;56829_at;229108_at;237001_at;237851_at;
GADD45A	1647	Suvival	203725_at;
GADD45B	4616	Suvival	207574_s_at;209304_x_at;209305_s_at;213560_at;
GADD45G	10912	Suvival	204121_at;
XIAP	331	Suvival	206536_s_at;225858_s_at;225859_at;228363_at;235222_x_at;243026_x_at;
BCL-XL	598	Suvival	206665_s_at;212312_at;215037_s_at;231228_at;
FLIP	8837	Suvival	208485_x_at;209508_x_at;209939_x_at;210563_x_at;210564_x_at;211316_x_at;211317_s_at;
IAP	330	Suvival	211862_x_at;214486_x_at;214618_at;217654_at;237367_x_at;239629_at;
SURVIVIN	332	Suvival	210538_s_at;230499_at;
TNFRSF10C			202094_at;202095_s_at;210334_x_at;
- DCR1	8794	Death	206222_at;211163_s_at;
TRAF1	7185	Death	205599_at;235116_at;
RELA	5970	Death	201783_s_at;209878_s_at;230202_at;
TNFRSF1A -			
TNFR1	7132	Death	207643_s_at;241944_x_at;
FADD	8772	Death	202535_at;
CASP8	841	Death	207686_s_at;213373_s_at;231218_at;
CASP3	836	Death	202763_at;236729_at;
BCL2	596	Death	203684_s_at;203685_at;207004_at;207005_s_at;232210_at;232614_at;237837_at;244035_at;
CARMA1	84433	Traf 2/6 Complex	223514_at;
BCL10	8915	Traf 2/6 Complex	205263_at;
MALT1	10892	Traf 2/6 Complex	208309_s_at;210017_at;210018_x_at;238157_at;
TRAF6	7186	Traf 2/6 Complex	204413_at;
TRAF2	7189	Traf 2/6 Complex	205558_at;
PAR1	2149	PAR	203989_x_at;
PAR4	5074	PAR	204004_at;204005_s_at;214090_at;214237_x_at;226223_at;226231_at;229515_at;
CRK	1398	SAPK/JNK	202224_at;202226_s_at;
CRKL	1399	SAPK/JNK	206184_at;212180_at;
SHC	6464	SAPK/JNK	201469_s_at;214853_s_at;
SHC2	53358	SAPK/JNK	206330_s_at;
SHC3	25759	SAPK/JNK	213464_at;
GRB2	2885	SAPK/JNK	215075_s_at;223049_at;228572_at;
SOS	6654	SAPK/JNK	212777_at;212780_at;229261_at;230337_at;232883_at;242018_at;242682_at;

HPK2	9448	SAPK/JNK	206571_s_at;218181_s_at;222547_at;222548_s_at;238769_at;244846_at;
TAB1	10454	TAK1 Complex	203901_at;233679_at;235480_at;235827_at;
TAB2	23118	TAK1 Complex	210284_s_at;212184_s_at;233957_at;243557_at;
TAB3	257397	TAK1 Complex	227357_at;
TAK1	6885	TAK1 Complex	206853_s_at;206854_s_at;211536_x_at;211537_x_at;
NLK	51701	NFkB	218318_s_at;222589_at;222590_s_at;
IKK1	1147	NFkB	209666_s_at;
IKK2	3551	NFkB	209341_s_at;209342_s_at;211027_s_at;
IKK3	8517	NFkB	209929_s_at;36004_at;
NFKB	4790	NFkB	209239_at;239876_at;
RELA	5970	NFkB	201783_s_at;209878_s_at;230202_at;
MKK4	6416	JNK	203265_s_at;203266_s_at;
MKK7	5609	JNK	209951_s_at;209952_s_at;216206_x_at;226023_at;226053_at;
JNK	5599	JNK	210477_x_at;210671_x_at;226046_at;226048_at;243280_at;
MAPK9	5601	JNK	203218_at;210570_x_at;225781_at;
MAPK10	5602	JNK	204813_at;
DUSP8	1850	JNK	206374_at;
IRS1	3667	JNK	204686_at;235392_at;
SMC	9918	JNK	201774_s_at;
HNRPK	3190	JNK	200097_s_at;200775_s_at;200097_s_at;
TP53	7157	JNK	201746_at;211300_s_at;224185_at;
ATF2	1386	JNK	205446_s_at;212984_at;
ELK1	2002	JNK	203617_x_at;210376_x_at;210850_s_at;
CJUN	3725	JNK	201464_x_at;201465_s_at;201466_s_at;213281_at;
NFAT4	4775	JNK	207416_s_at;210555_s_at;210556_at;229223_at;
NFATC1	4772	JNK	208196_x_at;209664_x_at;210162_s_at;211105_s_at;
NLK	51701	WNT	218318_s_at;222589_at;222590_s_at;
CTBP1	1487	WNT	203392_s_at;212863_x_at;213980_s_at;243180_at;
CBP	1387	WNT	202160_at;211808_s_at;235858_at;237239_at;
TCF	3172	WNT	208429_x_at;214832_at;214851_at;216889_s_at;230914_at;
GROUCHO	7091	WNT	204872_at;214688_at;216997_x_at;233575_s_at;235765_at;
HIPK2	28996	WNT	213763_at;219028_at;224016_at;224065_at;224066_s_at;225097_at;225115_at;225116_at;
cMYB	4602	WNT	225368_at;240294_at;
ATF2	1386	ALK	204798_at;215152_at;
CSX	1482	ALK	205446_s_at;212984_at;
GATA4	2626	ALK	206578_at;
MAP2K6	5608	p38	205517_at;230855_at;243692_at;
p38MAPK	5594	p38	205698_s_at;
MNK1	8569	p38	208351_s_at;212271_at;224620_at;224621_at;229847_at;242106_at;
PRAK	8550	p38	209467_s_at;243256_at;
HSP27	6294	p38	212871_at;
MAPKAP2	9261	p38	201747_s_at;201748_s_at;213635_s_at;
PLA2	5320	p38	201460_at;201461_s_at;215050_x_at;
STAT1	6772	p38	203649_s_at;
			200887_s_at;209969_s_at;AFFX-HUMISGF3A/M97935_3_at;AFFX-HUMISGF3A/M97935_5_at;AFFX-HUMISGF3A/M97935_MA_at;AFFX-HUMISGF3A/M97935_MB_at;232375_at;AFFX-HUMISGF3A/M97935_3_at;AFFX-HUMISGF3A/M97935_5_at;AFFX-HUMISGF3A/M97935_MA_at;AFFX-HUMISGF3A/M97935_MB_at;

MAX	4149	p38	208403_x_at;209331_s_at;209332_s_at;210734_x_at;214108_at;222174_at;
MYC	4609	p38	202431_s_at;239931_at;244089_at;
ELK1	2002	p38	203617_x_at;210376_x_at;210850_s_at;
CHOP	2521	p38	200959_at;215744_at;217370_x_at;231108_at;
MEF2	4205	p38	208328_s_at;212535_at;214684_at;239571_at;242176_at;
MEF3	4206	p38	
MEF4	4207	p38	205124_at;209926_at;
MEF5	4208	p38	207968_s_at;209199_s_at;209200_at;236395_at;239938_x_at;239966_at;244230_at;
MEF6	4209	p38	203003_at;203004_s_at;225641_at;
ATF2	1386	p38	205446_s_at;212984_at;
MSK1	9252	p38	204633_s_at;204635_at;
HMG14	3150	p38	200943_at;200944_s_at;
CREB	1385	p38	204312_x_at;204313_s_at;204314_s_at;214513_s_at;243625_at;

Table1

Table 2. TAK inhibitor sensitivity determining signature including the informative genes that differentiate 3 subclasses of DLBCL patients

Gene Symbol	Entrez	Pathway	U133A/B	Gene Group
TNFR1	7132	TNF	207643_s_at;	A
TNFR2	7133	TNF	203508_at;	A
TRADD	8717	TNF	1729_at;205641_s_at;	AA
TANK	10010	TNF	207616_s_at;209451_at;210458_s_at;	EEE
RAIDD	8738	TNF	209833_at;	B
			208050_s_at;209811_at;211140_s_at;34449_at	
			;	
PROCASP2	835	TNF	226032_at;	BBBBC
UBC13	7334	TNF	201523_x_at;201524_x_at;212751_at;	EEE
TLR2	7097	TLRs	204924_at;	E
TLR4	7099	TLRs	221060_s_at;224341_x_at;232068_s_at;	EEE
TLR7	51284	TLRs	220146_at;222952_s_at;	EE
TLR10	81793	TLRs	223750_s_at;223751_x_at;	FF
MYD88	4615	TLRs	209124_at;	C
TOLLIP	54472	TLRs	217930_s_at;233881_s_at;	ED
IRAK	3654	TLRs	201587_s_at;	E
PKR	5610	TLRs	204211_x_at;	E
TBK1	29110	TLRs	218520_at;	E
TGFB	7040	TGF-beta	203085_s_at;	C
IL1R	3554	IL1	202948_at;	E
TOLLIP	54472	IL1	217930_s_at;233881_s_at;	ED
MYD88	4615	IL1	209124_at;	C
CD19	930	B-Cell	206398_s_at;	F
CD45	5788	B-Cell	207238_s_at;212587_s_at;212588_at;	AAA
CD79A	973	B-Cell	205049_s_at;	F
CD79B	974	B-Cell	205297_s_at;	F
			202625_at;202626_s_at;210754_s_at;239555_a	
LYN	4067	B-Cell	t;	EEED
SHP1	8431	B-Cell	206410_at;	B
			207540_s_at;209269_s_at;226068_at;244023_a	
SYK	6850	B-Cell	t;	FFFF

GAB1	2549	B-Cell	207112_s_at;242572_at; 205868_s_at;209895_at;209896_s_at;212610_a	BE
SHP2	5781	B-Cell	t;	DDEE
CSK	1445	B-Cell	202329_at;	C
PAG	55824	B-Cell	225622_at;225626_at;	DD
VAV	7409	B-Cell	206219_s_at;	C
VAV2	7410	B-Cell	205536_at;226063_at;	FF
GRB2	2885	B-Cell	215075_s_at;223049_at;	EE
BLNK	29760	B-Cell	207655_s_at;244172_at; 212777_at;212780_at;229261_at;230337_at;	FF
SOS	6654	B-Cell	232883_at;242018_at;	CCDDDE
PLCG2	5336	B-Cell	204613_at; 207957_s_at;209685_s_at;227817_at;	C
PKCB	5579	B-Cell	227824_at;228795_at;	DDDDD
PRKCQ	5588	B-Cell	210038_at;210039_s_at;	AB
PRKCQ	5588	T-Cell	210038_at;210039_s_at; 207957_s_at;209685_s_at;227817_at;	AB
PKCB	5579	T-Cell	227824_at;228795_at;	DDDDD
CD28	940	T-Cell	206545_at;211856_x_at;211861_x_at;	AAA
CD3	917	T-Cell	206804_at;	A
CD3E	916	T-Cell	205456_at;	A
CD3D	915	T-Cell	213539_at;	A
CD4	920	T-Cell	203547_at; 201343_at;201345_s_at;215604_x_at;	A
UBC4	7322	T-Cell	240139_at;	FFDC
UBC5	6923	T-Cell	200085_s_at;200085_s_at;	EE
CIAP1	329	T-Cell	202076_at;	E
CIAP2	330	T-Cell	210538_s_at;230499_at;	EE
CD8	925	T-Cell	205758_at;	A
CD8B	926	T-Cell	207979_s_at;215332_s_at;	AE
CD45	5788	T-Cell	207238_s_at;212587_s_at;212588_at;	AAA
ZAP70	7535	T-Cell	214032_at; 210105_s_at;212486_s_at;216033_s_at;	A
FYN	2534	T-Cell	217697_at;243006_at; 221331_x_at;231794_at;234362_s_at;	AAABA
CLTA4	1493	T-Cell	236341_at;	AAAA
PI3k	5286	T-Cell	213070_at;235792_x_at;241905_at;	EDD
PIK3C2B	5287	T-Cell	204484_at;	D
PIK3C2G	5288	T-Cell	215129_at;	B
PIK3C3	5289	T-Cell	204297_at;215394_at;239300_at;	EBA
PIK3CA	5290	T-Cell	204369_at;215212_at;	EE
PIK3CB	5291	T-Cell	212688_at;217620_s_at;	DD
PIM1	5292	T-Cell	209193_at;	E
PIK3CD	5293	T-Cell	203879_at;211230_s_at;	CC
PIK3CG	5294	T-Cell	206369_s_at;206370_at;	DD
VAV	7409	T-Cell	206219_s_at;	C
VAV2	7410	T-Cell	205536_at;226063_at;	FF
VAV3	10451	T-Cell	218806_s_at;218807_at;	EE
ITK	3702	T-Cell	211339_s_at;	A
SLP76	3937	T-Cell	205269_at;205270_s_at;244251_at;244556_at;	AAAA
LAT	27040	T-Cell	209881_s_at;211005_at;	AA

PAG	55824	T-Cell	225622_at;225626_at; 206607_at;225231_at;225234_at;229010_at;	DD
CBL	867	T-Cell	243475_at;	BCCCD
LCK	3932	T-Cell	204890_s_at;204891_s_at;	AA
SHB	6461	T-Cell	204656_at;204657_s_at;	DB
LTBR	4055	LTBR	203005_at;	B
NIK	9020	LTBR	205192_at;	D
NIKBR	83696	LTBR	221672_s_at;221836_s_at;56829_at;	CCC
GADD45A	1647	Suvival	203725_at;	E
GADD45B	4616	Suvival	207574_s_at;209304_x_at;209305_s_at; 206536_s_at;225858_s_at;225859_at;	EEE
XIAP	331	Suvival	228363_at;235222_x_at;243026_x_at;	BEEEEEE
BCL-XL	598	Suvival	206665_s_at;212312_at;215037_s_at; 208485_x_at;209508_x_at;209939_x_at; 210563_x_at;210564_x_at;211316_x_at; 211317_s_at;211862_x_at;214486_x_at;	EEE AAAAAAAAA
FLIP	8837	Suvival	214618_at;217654_at;237367_x_at;239629_at;	BBAA
IAP	330	Suvival	210538_s_at;230499_at;	EE
SURVIVIN	332	Suvival	202094_at;202095_s_at;210334_x_at;	EEB
TNFRSF10C -				
DCR1	8794	Death	206222_at;	B
TRAF1	7185	Death	205599_at;235116_at;	DD
RELA	5970	Death	201783_s_at;209878_s_at;	CC
TNFRSF1A -				
TNFR1	7132	Death	207643_s_at;	A
FADD	8772	Death	202535_at;	E
CASP8	841	Death	207686_s_at;213373_s_at;	EA
CASP3	836	Death	202763_at;	E
BCL2	596	Death	203685_at;207005_s_at;232210_at;232614_at; 244035_at;	EEEEEE
CARMA1	84433	Traf 2/6 Complex	223514_at;	E
BCL10	8915	Traf 2/6 Complex	205263_at;	F
MALT1	10892	Traf 2/6 Complex	208309_s_at;210017_at;210018_x_at;	EEE
TRAF6	7186	Traf 2/6 Complex	204413_at;	E
TRAF2	7189	Traf 2/6 Complex	205558_at;	D
PAR1	2149	PAR	203989_x_at;	B
PAR4	5074	PAR	204004_at;204005_s_at;226223_at;226231_at;	FFFF
CRK	1398	SAPK/JNK	202224_at;202226_s_at;	EE
CRKL	1399	SAPK/JNK	206184_at;212180_at;	BC
SHC	6464	SAPK/JNK	201469_s_at;214853_s_at;	DA
SHC3	25759	SAPK/JNK	213464_at;	B
GRB2	2885	SAPK/JNK	215075_s_at;223049_at;	EE
SOS	6654	SAPK/JNK	212777_at;212780_at;229261_at;230337_at; 232883_at;242018_at;	CCDDDE
HPK2	9448	SAPK/JNK	206571_s_at;218181_s_at;222547_at; 222548_s_at;238769_at;244846_at;	DDDDDD

TAB1	10454	TAK1 Complex	203901_at;233679_at;	DE
TAB2	23118	TAK1 Complex	210284_s_at;212184_s_at;243557_at;	BAA
TAB3	257397	TAK1 Complex	227357_at;	E
TAK1	6885	TAK1 Complex	206853_s_at;206854_s_at;211536_x_at; 211537_x_at;	BBBB
NLK	51701	NFkB	218318_s_at;222589_at;222590_s_at;	DDD
IKK1	1147	NFkB	209666_s_at;	E
IKK2	3551	NFkB	209341_s_at;209342_s_at;211027_s_at;	CDD
IKK3	8517	NFkB	209929_s_at;36004_at;	EC
NFkB	4790	NFkB	209239_at;239876_at;	CD
RELA	5970	NFkB	201783_s_at;209878_s_at;	CC
MKK4	6416	JNK	203265_s_at;203266_s_at;	BE
MKK7	5609	JNK	216206_x_at;226053_at;	DD
JNK	5599	JNK	226046_at;226048_at;	EE
MAPK9	5601	JNK	203218_at;210570_x_at;225781_at;	EED
MAPK10	5602	JNK	204813_at;	B
DUSP8	1850	JNK	206374_at;	C
IRS1	3667	JNK	204686_at;235392_at;	DD
SMC	9918	JNK	201774_s_at;	C
HNRPK	3190	JNK	200097_s_at;200775_s_at;200097_s_at;	EEE
TP53	7157	JNK	201746_at;211300_s_at;	DD
ATF2	1386	JNK	205446_s_at;212984_at;	DE
ELK1	2002	JNK	203617_x_at;210376_x_at;210850_s_at; 201464_x_at;201465_s_at;201466_s_at; 213281_at;	CBB ABAB
cJUN	3725	JNK	207416_s_at;210555_s_at;210556_at;	DDD
NFAT4	4775	JNK	208196_x_at;210162_s_at;211105_s_at;	ECC
NFATC1	4772	JNK		
NLK	51701	WNT	218318_s_at;222589_at;222590_s_at;	DDD
CTBP1	1487	WNT	203392_s_at;212863_x_at;213980_s_at;	CCC
CBP	1387	WNT	202160_at;237239_at;	CD
TCF	3172	WNT	208429_x_at;214832_at;214851_at; 216889_s_at;	BBBE
GROUCHO	7091	WNT	204872_at;214688_at;216997_x_at; 233575_s_at;235765_at;	DDDDD
HIPK2	28996	WNT	213763_at;219028_at;225097_at;	
cMYB	4602	WNT	225116_at;225368_at;	BBBBB
ATF2	1386	ALK	204798_at;	E
MAP2K6	5608	p38	205446_s_at;212984_at;	DE
p38MAPK	5594	p38	205698_s_at;	E
MNK1	8569	p38	208351_s_at;212271_at;224621_at;	BBD
PRAK	8550	p38	209467_s_at;243256_at;	CB
HSP27	6294	p38	212871_at;	E
MAPKAP2	9261	p38	201747_s_at;201748_s_at;213635_s_at;	CCB
			201460_at;201461_s_at;215050_x_at;	BBB

			200887_s_at;209969_s_at;AFFX-HUMISGF3A/M97935_3_at; AFFX-HUMISGF3A/M97935_5_at; AFFX-HUMISGF3A/M97935_MA_at; AFFX-HUMISGF3A/M97935_MB_at; 232375_at; AFFX-HUMISGF3A/M97935_3_at; AFFX-HUMISGF3A/M97935_5_at; AFFX-HUMISGF3A/M97935_MA_at; AFFX-HUMISGF3A/M97935_MB_at; 208403_x_at;209331_s_at;209332_s_at; 210734_x_at;214108_at;222174_at; 202431_s_at; 203617_x_at;210376_x_at;210850_s_at; 200959_at;217370_x_at;231108_at; 208328_s_at;212535_at;214684_at;239571_at; 242176_at; 205124_at; 207968_s_at;209199_s_at;209200_at; 236395_at;239938_x_at;239966_at;244230_at; 205446_s_at;212984_at; 204633_s_at;204635_at; 200943_at;200944_s_at; 204312_x_at;204313_s_at;204314_s_at; 214513_s_at;	AAAAAAAAA AA DDCDBB E CBB CCC DDDDD F DDDDDDD DE EE FF EEEC
STAT1	6772	p38		
MAX	4149	p38		
MYC	4609	p38		
ELK1	2002	p38		
CHOP	2521	p38		
MEF2	4205	p38		
MEF4	4207	p38		
MEF5	4208	p38		
ATF2	1386	p38		
MSK1	9252	p38		
HMG14	3150	p38		
CREB	1385	p38		

In still another aspect, the invention includes a method of selecting a patient having a tumour that is susceptible to treatment with a TAK1 inhibitor. The method can include determining if the patient has a genetic mutation of a t(14;18)(q32;q21) translocation, a t(11;18)(q21;q21) translocation, a t(1;14)(p22;q32) translocation, or amplification of chromosome 18, whereby the presence of a mutation indicates the tumour is susceptible to treatment.

Alternatively, the method can include determining if the patient has a deregulated TAK1 signalling transduction molecule, wherein the presence of the deregulated TAK1 signalling transduction molecule is an indication that the patient is susceptible to treatment with a TAK1 inhibitor.

In any of the methods described herein, the TAK1 inhibitor can be administered either as a single agent or in combination with other anti-cancer agents or anti-cancer antibodies.

In another aspect, the invention includes a kit for predicting a patient's response to a TAK1 inhibitor, said kit comprising (a) one or more phospho-specific antibodies against a TAK1

signal transduction molecule, and (b) a reagent suitable for detecting binding of said antibodies to the TAK1 signal transduction molecule.

In yet another aspect, the invention includes methods to classify a tumor as a TAK1 inhibitor sensitive tumor. By measuring the relative levels of particular deregulated TAK1 signalling transduction genes or proteins in tumour tissue it is possible to determine if the tumor is responsive to a TAK1 inhibitor. The present invention can be used to predict the suitability of administering a TAK1 inhibitor to a cancer patient.

According to one aspect of the present invention there is provided a method of selecting a mammal having or suspected of having a tumour for treatment with a TAK1 inhibitor drug. The method includes providing a biological sample from a subject having a B cell tumour, a solid tumor, or a T cell leukemia and testing the biological sample for expression of any one of the genes listed in Table 1 or Table 2, or their gene products, thereby to predict an increased likelihood of response to the TAK1 inhibitor drug. In one embodiment, the method includes testing the biological sample for at least 5, 10, 20, 30, 40, 50 or 100 of the genes listed in Table 1 or Table 2.

Brief description of the drawings

Fig. 1 shows a schematic of the TAK1 signaling pathway in B cell lymphocytes (BCR) and T cell lymphocytes (TCR).

Fig. 2 shows a bar chart showing the effect of TAK1 knock down by shRNA on the viability of B-cell lymphoma cells with a t(14;18)(q32;q21) translocation.

Fig. 3 shows a bar chart showing the effect of a TAK1 kinase inhibitor on the viability of B-cell lymphoma cells with a t(14;18)(q32;q21) translocation.

Detailed Description

The present invention is based, in part, on the finding that certain lymphomas, in particular B cell tumors, solid tumors or T cell leukemia's, are selectively responsive to a TAK1 inhibitor.

Moreover, the invention includes identifying tumours carrying particular mutations including a t(14;18)(q32;q21) translocation, a t(11;18)(q21;q21) translocation, a t(1;14)(p22;q32) translocation, an amplification of chromosome 18, an addition of chromosome 18q21, an

amplification of specific regions (detected by comparative genomic hybridization), changes in the subcellular localization, over- or under-expression of a deregulated TAK1 signalling transduction molecule, or posttranscriptional modifications in the proteins containing TAK1 pathway signaling molecules including MALT1, BCL-10, TAK1, TRAF6, CARD11, IRAK1, TAB1, TAB2, TRAF2, IRAK4, API1, API2, API3, API4 (survivin), BCL2 or NF-kB target genes or deletion of specific regions containing API2. NF-kB target genes include any gene that is regulated by the NF-kB transcription factor, for example a set of NF-kB target genes is provided in the reference Dave SS et. al. N. Engl. J. Med. 2006; 354(23): 2431-42. These tumours have been identified to be particularly susceptible to treatment using a TAK1 inhibitor.

The identification of the above particular mutations of the present invention can be used to determine if a patient is a responder or non-responder to a TAK1 inhibitor. By responders and non responders it is meant objective tumour responses according to the Union International Contre le Cancer/World Health Organization (U ICC/WHO) criteria are categorised as follows: complete response (CR): no residual tumour in all evaluable lesions; partial response (PR): residual tumour with evidence of chemotherapy-induced 50% or greater decrease under baseline in the sum of all measurable lesions and no new lesions; stable disease (SD): residual tumour not qualified for CR; and progressive disease (PD): residual tumour with evidence of 25% or greater increase under baseline in the sum of all measurable lesions or appearance of new lesions. As defined herein non-responders are PD.

Deregulated TAK1 signal transduction molecules

The invention further includes identifying a tumour for a deregulated TAK1 signaling transduction molecule. A deregulated TAK1 signaling transduction molecule is any molecule that is directly or indirectly modified, for example activated or deactivated, in the MALT pathway compared to a normal cell. See Fig. 1. A deregulated TAK1 signaling molecule also includes tumor cells that have a deregulated TAK1 signaling molecule, for example, molecules that are over or under-expressed in signalling pathways involving TAK1. Such signalling pathways include antigen receptor signalling on T and B cells, IL-1 and TLR family signalling, TNF signalling, etc.

Tumours having deregulated TAK1 signaling molecules have been identified to be particularly susceptible to treatment using a TAK1 inhibitor. The present invention includes a number of different biomarkers that can be used to predict a patient's responsiveness to a TAK1 inhibitor. The biomarkers of the invention include genetic mutations whereby the presence of a mutation indicates that the tumour is susceptible to treatment. Examples of genetic mutations that lead to deregulated TAK1 signaling are the t(14;18)(q32;q21) translocation that causes MALT1 (and BCL2) to be overexpressed, the t(11;18)(q21;q21) translocation that results in a fusion protein of MALT1 with API2 and the t(1;14)(p22;q32) translocation that results in overexpression of BCL-10. Further examples of genetic mutations that lead to deregulated TAK1 signaling include amplification of chromosome 18 resulting in overexpression of MALT1 or BCL2, and amplifications or deletions of specific regions identified by comparative genomic hybridization containing key components of the TAK1 signalling pathway including MALT1, BCL-10, TAK1, TRAF6, TRAF2, TAB1, TAB2, CARD11, IRAK1, IRAK4, API1, API2, API3, API4 and NF-kB target genes.

The biomarkers of the invention also include deregulated TAK1 signalling transduction molecules, wherein the presence of the deregulated TAK1 signalling transduction molecule is an indication that the patient is susceptible to treatment with a TAK1 inhibitor. Examples of deregulated TAK1 signal transduction molecules include molecules modified by posttranslational alterations such as phosphorylation including phosphorylated TAK1, phosphorylated IKKbeta, phosphorylated p65, phosphorylated MKK4, phosphorylated MKK6, phosphorylated p38 and phosphorylated JNK. Other molecules that serve as deregulated TAK1 signal transduction molecules include molecules modified by ubiquitinylation including ubiquitinylated TRAF6, ubiquitinylated IKKgamma and ubiquitinylated IkappaBalpha. Other markers that serve as deregulated TAK1 signal transduction molecules include alterations in subcellular localization such as translocation of molecules such as BCL-10, API4, p65 and RelA from the cytoplasm to the nucleus.

A deregulated TAK1 signaling molecule also includes any molecule that is over- or under-expressed in the TAK1 signalling pathway. By measuring the relative levels of one or more deregulated TAK1 signaling molecules as shown in Table 1 or Table 2, i.e., measuring gene expression or protein expression or activity in a tumour tissue it is possible to determine if the

tumor is responsive to a TAK1 inhibitor. The present invention can thus be used to predict the suitability of administering a TAK1 inhibitor to a cancer patient.

Assays

- 5 The present invention provides a number of biomarkers that can be used to predict a patient's responsiveness to a TAK1 inhibitor. One exemplary method for detecting the presence of a biomarker includes obtaining a tumour sample from a test subject and determining for the presence of the biomarker.
- 10 Any appropriate sample can be used to determine for the presence of a biomarker of the invention. In one example the sample is a suspected B cell tumour and determination of whether that tumour has a genetic mutation is performed. Examples of genetic mutations include a t(14;18)(q32;q21) translocation, a t(11;18)(q21;q21) translocation, a t(1;14)(p22;q32), an amplification of chromosome 18, or addition of chromosome 18q21.
- 15 These markers can be characterized by fluorescent in situ hybridization, comparative genomic hybridization (CGH) and cDNA microarrays for gene expression profiling and copy number changes.

Detection of a deregulated TAK1 signal transduction pathway molecule

- 20 Means of determining if a sample has a genetic mutation are known in the art. These methods include those described or claimed in the following publications, the entire disclosures of which are incorporated by reference herein. Methods to determine if the tumor has an amplification of chromosome 18 are described in Haematologica. 2006; 91(2):184-91. A t(14;18) translocation can be determined interphase fluorescence *in situ* hybridization (FISH)
- 25 as described by Godon A et.al, Leukemia. 2003;17(1):255-9 or by Farter JL et. al, 1: Diagn Mol Pathol. 2001; 10(4):214-22. A t(14;18)(q32;q21) translocation can be determined as described in Davies et al., Chromosome Res. 2005;13(3):237-48. The expression of AP12-MALT1 mRNA can be studied using reverse transcriptase (RT)-polymerase chain reaction (PCR) and nested PCR as described in Sanchez-Izquierdo D *et.al* Blood 2003 101: 11 4539-
- 30 4546 and Ye H *et.al* Journal of pathology 2005; 205: 293-301. A t(11;18)(q21;q21) translocation can be detected by RT-PCR of the AP12-MALT1 fusion transcripts and a t(14;18)(q21;q21) translocation can be detected by interphase fluorescence *in situ* hybridisation (FISH; Vysis Abott Labs).

The procedures and reagents needed to determine for a deregulated TAK1 signal transduction molecule are known in the art. In one example, an agent of interest that can be used to detect a deregulated TAK1 signal transduction molecule includes any molecule such as a peptidomimetic, protein, peptide, nucleic acid, small molecule, an antibody or other drug candidate, that can bind the protein. In one example, antibodies that are commercially available can be used to detect a deregulated TAK1 signal transduction molecule. For example, phosphorylated (phos) TAK1, Phos I κ B, Phos IKK, Phos P38 can be measured by using Phospho specific antibodies from Cell Signaling USA. Alternatively, a deregulated TAK1 signal transduction molecule such as MALT and BCL-10 can be performed using immunostaining with mouse monoclonal Antibodies.

Typically, the method includes determining from a tumour sample of a test patient for the presence of a deregulated TAK1 signal transduction pathway molecule. For example, phosphorylated-TAK1 can be visualized by reacting the proteins with antibodies such as monoclonal antibodies directed against the phosphorylated serine, threonine or tyrosine amino acids that are present in the proteins. For example, monoclonal antibodies useful for isolating and identifying phosphotyrosine-containing proteins are described in U.S. Pat. No. 4,543,439.

Typically, antibodies used for visualizing a deregulated TAK1 signal transduction molecule can be labeled by any procedure known in the art, for example, using a reporter molecule. A reporter molecule, as used herein, is a molecule which provides an analytically identifiable signal allowing one of skill in the art to identify when an antibody has bound to a protein that it is directed against. Detection may be either qualitative or quantitative. Commonly used reporter molecules include fluorophores, enzymes, biotin, chemiluminescent molecules, bioluminescent molecules, digoxigenin, avidin, streptavidin or radioisotopes. Commonly used enzymes include horseradish peroxidase, alkaline phosphatase, glucose oxidase and beta-galactosidase, among others. The substrates to be used with these enzymes are generally chosen for the production, upon hydrolysis by the corresponding enzyme, of a detectable color change. For example, p-nitrophenyl phosphate is suitable for use with alkaline phosphatase reporter molecules; for horseradish peroxidase, 1,2-phenylenediamine, 5-aminosalicylic acid or toluidine are commonly used. Incorporation of a reporter molecule onto an antibody can be by any method known to the skilled artisan.

After separation and visualizing the proteins, the amount of each protein species may be assessed by readily available procedures. For example, by using Western blot analysis which includes electrophoretically separating proteins on a polyacrylamide gel, and after detecting the separated proteins, the relative amount of each protein can be quantified by assessing its optical density. Alternatively, other methods such as FACS, immunohistochemistry, immunocytochemistry, fluorescence microscopy, ELISA, etc., can be used either for altered expression of naïve, posttranslationally modified proteins or for monitoring the alterations in the subcellular localization of the proteins.

- 10 In the methods of the invention one or more deregulated TAK1 signal transduction pathway molecules can be detected. For example, an assay system can be set up which can detect for the presence of multiple deregulated TAK1 signal transduction pathway molecules.

Expression profile

- 15 The invention also includes a method for determining an expression profile of an appropriate tumour sample to determine if that tumour is likely to be responsive to TAK1 inhibitor treatment. In one example, the present invention includes determining for the level of expression of the genes in Table 1 or Table 2 in the test tumour sample. The gene profile obtained is compared against controls, i.e., expression patterns, which is indicative that a tumour is responsive to TAK1 treatment. The gene sequences of each of the biomarkers listed in Table 1 or Table 2 can be detected using agents that can be used to specifically detect the gene or other biological molecules relating to it, for example, RNA transcribed from the gene or polypeptides encoded by the gene. Exemplary detection agents are nucleic acid probes, which hybridize to nucleic acids corresponding to the gene, and antibodies.

- 25 The biomarkers listed in Table 1 or Table 2 are intended to also include naturally occurring sequences including allelic variants and other family members. The biomarkers of the invention also include sequences that are complementary to those listed sequences resulting from the degeneracy of the code and also sequences that are sufficiently homologous and sequences which hybridize under stringent conditions to the genes listed in Table 1 or Table 2. Conditions for hybridization are known to those skilled in the art and can be found in Current Protocols in Molecular Biology, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. A preferred, non-limiting example of highly stringent hybridization conditions are hybridization

in 6 X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2 X SSC, 0.1% SDS at 50-65°C.

By "sufficiently homologous" it is meant a amino acid or nucleotide sequence of a biomarker which contains a sufficient or minimum number of identical or equivalent (e.g., an amino acid residue which has a similar side chain) amino acid residues or nucleotides to a second amino acid or nucleotide sequence such that the first and second amino acid or nucleotide sequences share common structural domains or motifs and/or a common functional activity. For example, amino acid or nucleotide sequences which share common structural domains have at least about 50% homology, preferably 60% homology, more preferably 70%-80%, and even more preferably 90-95% homology across the amino acid sequences of the domains and contain at least one and preferably two structural domains or motifs, are defined herein as sufficiently homologous. Furthermore, amino acid or nucleotide sequences which share at least 50%, preferably 60%, more preferably 70-80% or 90-95% homology and share a common functional activity are defined herein as sufficiently homologous.

The comparison of sequences and determination of percent homology between two sequences can be accomplished using a mathematical algorithm. A preferred, non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Karlin and Altschul (1990) Proc. Natl. Acad. Sci. USA 87:2264-68, modified as in Karlin and Altschul (1993) Proc. Natl. Acad. Sci. USA 90:5873-77. Such an algorithm is incorporated into the NBLAST and XBLAST programs (version 2.0) of Altschul, et al. (1990) J. Mol. Biol. 215:403-10. BLAST nucleotide searches can be performed with the NBLAST program, score=100, wordlength=12 to obtain nucleotide sequences homologous to TRL nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score=50, wordlength=3 to obtain amino acid sequences homologous to the protein sequences encoded by the genes listed in Table 1 or Table 2 . To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al., (1997) Nucleic Acids Research 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used. See <http://www.ncbi.nlm.nih.gov>. Another preferred, non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the ALIGN algorithm of Myers and Miller, CABIOS (1989). When utilizing the ALIGN program for

comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used.

Methods for determining nucleic acid sequences that are differentially expressed in a subject with cancer

The invention provides a list of genes or gene products that can be used to produce an expression profile signature which characteristically predicts TAK1 inhibitor sensitivity of a tumour cell. Any method known in the art can be used to determine whether a tumour cell is responsive to treatment with an TAK1 inhibitor.

In one embodiment, the method comprises determining mRNA and/or protein level of the biomarkers of a mammal, such as by Northern blot analysis, reverse transcription-polymerase chain reaction (RT-PCR), in situ hybridization, immunoprecipitation, Western blot hybridization, or immunohistochemistry. According to the method, cells may be obtained from a subject and the levels of the biomarker's protein or mRNA level are determined and compared to a control.

In one embodiment, the method comprises using a nucleic acid probe to determine whether a mammal is responsive to TAK1 inhibition. The method includes:

providing a nucleic acid probe comprising a nucleotide sequence, for example, at least 10, 15, 25 or 40 nucleotides, and up to all or nearly all of the coding sequence which is complementary to a portion of the coding sequence of a nucleic acid sequence listed in Table 1 or Table 2 ;

obtaining a tissue sample from a mammal having a cancerous cells;

contacting the nucleic acid probe under stringent conditions with RNA obtained from the sample (e.g., in a Northern blot or in situ hybridization assay); and

comparing the amount of hybridization of the probe with RNA derived from ; wherein the amount of hybridization is indicative of the presence of cancerous cells in the first tissue sample.

In another example, the methods of the invention include determining expression profiles with microarrays involves the following steps: (a) obtaining a mRNA sample from a subject and preparing labeled nucleic acids therefrom (the "target nucleic acids" or "targets"); (b)

contacting the target nucleic acids with an array under conditions sufficient for the target nucleic acids to bind to the corresponding probes on the array, for example, by hybridization or specific binding; (c) optional removal of unbound targets from the array; (d) detecting the bound targets, and (e) analyzing the results, for example, using computer based analysis methods, to indicate whether the mammal is responsive to TAK1 inhibition treatment

In the method detailed above, the method includes obtaining mRNA from the mammal's tumour sample. RNA may be extracted from tissue or cell samples by a variety of methods, for example, guanidium thiocyanate lysis followed by CsCl centrifugation (Chirgwin, et al., Biochemistry 18:5294-5299, 1979). RNA from single cells may be obtained as described in methods for preparing cDNA libraries from single cells (see, e.g., Dulac, Curr. Top. Dev. Biol. 36:245, 1998; Jena, et al., J. Immunol. Methods 190:199, 1996).

The RNA sample can be further enriched for a particular species. In one embodiment, for example, poly(A)+ RNA may be isolated from an RNA sample. In particular, poly-T oligonucleotides may be immobilized on a solid support to serve as affinity ligands for mRNA. Kits for this purpose are commercially available, for example, the MessageMaker kit (Life Technologies, Grand Island, N.Y.).

In one embodiment, the RNA population may be enriched for sequences of interest, as detailed on Table 1 or Table 2. Enrichment may be accomplished, for example, by primer-specific cDNA synthesis, or multiple rounds of linear amplification based on cDNA synthesis and template-directed in vitro transcription (see, e.g., Wang, et al., Proc. Natl. Acad. Sci. USA 86:9717, 1989; Dulac, et al., supra; Jena, et al., supra).

The target molecules may be labeled to permit detection of hybridization of the target molecules to a microarray. That is, the probe may comprise a member of a signal producing system and thus, is detectable, either directly or through combined action with one or more additional members of a signal producing system. Examples of directly detectable labels include isotopic and fluorescent moieties incorporated, usually by a covalent bond, into a moiety of the probe, such as a nucleotide monomeric unit (e.g., dNMP of the primer), or a photoactive or chemically active derivative of a detectable label which can be bound to a functional moiety of the probe molecule.

In other embodiments, the target nucleic acid may not be labeled. In this case, hybridization may be determined, for example, by plasmon resonance (see, e.g., Thiel, et al., Anal. Chem. 69:4948, 1997).

- 5 Microarrays for use according to the invention include one or more probes of genes listed in Table 1 or Table 2 .

The method described above results in the production of hybridization patterns of labeled target nucleic acids on the array surface. The resultant hybridization patterns of labeled
10 nucleic acids may be visualized or detected in a variety of ways, with the particular manner of detection selected based on the particular label of the target nucleic acid. Representative detection means include scintillation counting, autoradiography, fluorescence measurement, calorimetric measurement, light emission measurement, light scattering, and the like.

- 15 One such method of detection utilizes an array scanner that is commercially available (Affymetrix, Santa Clara, Calif.), for example, the 417.TM. Arrayer, the 418.TM. Array Scanner, or the Agilent GeneArray.TM. Scanner. This scanner is controlled from a system computer with an interface and easy-to-use software tools. The output may be directly imported into or directly read by a variety of software applications. Scanning devices are
20 described in, for example, U.S. Pat. Nos. 5,143,854 and 5,424,186.

Proteins

- Detecting for the presence of a protein product encoded by one or more of the biomarker genes listed in Table 1 or Table 2 can be done by using any appropriate method known in the
25 art. For example, an agent of interest that can be used to detect a particular protein of interest, for example using an antibody. The method for producing polyclonal and/or monoclonal antibodies that specifically bind to polypeptides useful in the present invention is known to those of skill in the art and may be found in, for example, Dymecki, et al., (J. Biol. Chem. 267:4815, 1992); Boersma & Van Leeuwen, (J. Neurosci. Methods 51:317, 1994); Green, et
30 al., (Cell 28:477, 1982); and Arnheiter, et al., (Nature 294:278, 1981).

In one embodiment, an immunoassay can be used to quantitate the levels of proteins in cell samples. The invention is not limited to a particular assay procedure, and therefore, is

intended to include both homogeneous and heterogeneous procedures. Exemplary immunoassays that may be conducted according to the invention include fluorescence polarization immunoassay (FPIA), fluorescence immunoassay (FIA), enzyme immunoassay (EIA), nephelometric inhibition immunoassay (NIA), enzyme-linked immunosorbent assay (ELISA), and radioimmunoassay (RIA).

In another example, the presence of the marker protein in a tissue sample can be determined using immunohistochemical staining. For such staining, a multiblock of tissue may be taken from the biopsy or other tissue sample and subjected to proteolytic hydrolysis, employing such agents as protease K or pepsin. In certain embodiments, it may be desirable to isolate a nuclear fraction from the sample cells and detect the level of the marker polypeptide in the nuclear fraction.

In yet another embodiment, the invention contemplates using a panel of antibodies that are generated against the marker polypeptides of this invention. Such a panel of antibodies may be used as a reliable diagnostic probe for determining if a tumour is responsive to treatment with an TAK1 inhibitor.

Data analysis

To facilitate the sample analysis operation, the data obtained by the reader from the device may be analyzed using a digital computer. Typically, the computer will be appropriately programmed for receipt and storage of the data from the device, as well as for analysis and reporting of the data gathered, for example, subtraction of the background, deconvolution of multi-color images, flagging or removing artifacts, verifying that controls have performed properly, normalizing the signals, interpreting fluorescence data to determine the amount of hybridized target, normalization of background and single base mismatch hybridizations, and the like.

In one embodiment, a system comprises a search function that allows one to search for specific patterns, for example, patterns relating to differential gene expression, for example, between the expression profile of the test tumour cell and the expression profile of a tumour cell that is responsive to treatment with an TAK1 inhibitor. A system may also allow one to search for patterns of gene expression between more than two samples. Comparison of the

expression levels of one or more genes characteristic of responsiveness to an TAK1 inhibitor with reference expression levels, for example, expression levels that are characteristic of susceptibility to an TAK1 inhibitor may be conducted using computer systems.

5 *Subtyping diffuse large B-cell lymphoma (DLBL / DLBCL) Patients to determine if the patient is sensitive to a TAK1 inhibitor*

The present invention can be used to subtype DLBCL patients in order to determine if the patients are sensitive or likely insensitive to a TAK1 inhibitor. Specifically, patients can be categorized to determine if the patients fall within 3 distinct subclasses based on their
10 expression pattern of TAK1 genes. A patient sample that falls within Groups 1 and 3 as described below are believed to be TAK1 sensitive, while a patient sample that falls within Group 2 is likely to be TAK1 insensitive. A method for subtyping DLBCL patients is described below. Thus, the invention includes providing a test DLBCL sample and determining whether the sample falls within Groups 1, 2 or 3.

15 The method includes:

 mapping genes of Table 1 to Affymetrix probesets based on the annotations available from Affymetrix (<http://www.affymetrix.com/analysis/index.affx>);

 providing an Affymetrix U133A/B gene chip having gene expression data of 176 newly diagnosed diffuse large B cell lymphoma (DLBCL) patients;

20 verifying data quality such that 113 samples (Table 3) are kept for further analysis; and

 performing sample clustering such that three groups generated, wherein samples that fall within Groups 1 and 3 are TAK1 sensitive, while a sample falling within Group 2 is TAK1 insensitive.

25

 To test whether a DLBCL patient sample falls within Group 1, 2 or 3 the method includes:

 providing a test DBLCL patient sample;

 providing a data verified U133A/B gene chip;

30 normalizing the test sample with the 113 samples of Table 3;

 clustering the test sample and the 113 samples including the sensitivity determining signature gene set as in Table 2, wherein if the test sample falls within Groups 1 and 3 the

sample is TAK1 sensitive, whereas if the test sample falls within Group 2 the sample is TAK1 insensitive.

Details of how to perform the above method are provided below:

1. TAK1 pathways genes

Genes in TAK1 pathways can be assembled based on the public information. The genes that are involved in the signaling of ALK, FAS, MAP kinase, IL-1 receptor, TGF-beta, TNF receptor, thrombin and protease-activated receptor, Toll-like receptor, WNT, and antigen receptor are included. These genes can be mapped to Affymetrix probesets based on the annotations available from Affymetrix (<http://www.affymetrix.com/analysis/index.affx>) (Table1)

2. Gene expression data

The gene expression data of 176 newly diagnosed diffuse large B cell lymphoma (DLBCL) patients generated with Affymetrix U133A/B gene chip are publicly available by Margaret Shipp's group at Dana Faber Cancer Institute (Molecular profiling of diffuse large B-cell lymphoma identifies robust subtypes including one characterized by host inflammatory response Blood 105(5) 1851-1861). The raw data can be downloaded from <http://www.broad.mit.edu/cgi-bin/cancer/datasets.cgi>, and further processed and analyzed as described below.

3. Data preprocessing and analysis

3.1 QC:

In order to verify data quality, and generate gene expression results, the raw data (.CEL files) of the DLBCL samples can be loaded into Affymetrix Expression Console 1.0 (Affymetrix Inc.) and analyzed using MAS5 algorithm. The following criteria are used to filter out samples with low quality data: 1) scaling factor < 4; 2) rawQ < 5; 3) 3'/5' ratio for both actin and GAPDH < 5; 4) percentage of present call > 20 for chip A or > 10 for chip B. As a result of the QC procedure, 113 samples (Table 3) are kept for further analysis.

3.2 Normalization:

Array normalization: The parameters for MAS5 algorithm are set to normalize each array using all probesets on the array, and the trimmed mean value for each array is preset to 100.

Probeset normalization: The expression matrix generated by MAS5 can then be further normalized so that the mean of each probeset is centered to zero.

3.3 Sample clustering:

- 5 For unsupervised clustering analysis, the normalized expression matrix is loaded into GeneSpring GX 7.3.1 (Agilent Inc.). A 2-way hierarchical clustering is performed using only probeset identifications from table 2. Spearman correlation was used as the similarity measure in the clustering. The result from clustering reveals three subtypes, Group 1, 2 and 3.

10

4. Test DLBCL samples

- New test patient samples can be profiled using affymetrix U133A/B chips. After the data has been inspected following the same quality control (QC) procedure as described above 3.1, they can be added into the affymetrix U133A/B chip data with 113 samples (Table 3). The new test sample set (113 plus test sample) will be analyzed following the same process as outlined above 3.2-3.3. The new test samples will be clustered into one of the Groups 1-3. If the test sample falls within Group 1 and 3, the patient is likely to be TAK1 sensitive. If the test sample falls within Group 2, the patient is likely to be TAK1 insensitive.

20

Table 3

DLBCL.NEW.206	DLBCL.NEW.347
DLBCL.NEW.210	DLBCL.NEW.348
DLBCL.NEW.211	DLBCL.NEW.349
DLBCL.NEW.215	DLBCL.NEW.350
DLBCL.NEW.219	DLBCL.NEW.353
DLBCL.NEW.230	DLBCL.NEW.357
DLBCL.NEW.232	DLBCL.NEW.359
DLBCL.NEW.239	DLBCL.NEW.361
DLBCL.NEW.240	DLBCL.NEW.404
DLBCL.NEW.242	DLBCL.NEW.405
DLBCL.NEW.244	DLBCL.NEW.408
DLBCL.NEW.246	DLBCL.NEW.411
DLBCL.NEW.250	DLBCL.NEW.412
DLBCL.NEW.251	DLBCL.NEW.416
DLBCL.NEW.254	DLBCL.NEW.417
DLBCL.NEW.259	DLBCL.NEW.418
DLBCL.NEW.261	DLBCL.NEW.421
DLBCL.NEW.262	DLBCL.NEW.422
DLBCL.NEW.267	DLBCL.NEW.423
DLBCL.NEW.268	DLBCL.NEW.426

DLBCL.NEW.269	DLBCL.NEW.427
DLBCL.NEW.270	DLBCL.NEW.430
DLBCL.NEW.271	DLBCL.NEW.432
DLBCL.NEW.272	DLBCL.NEW.433
DLBCL.NEW.277	DLBCL.NEW.435
DLBCL.NEW.279	DLBCL.NEW.436
DLBCL.NEW.280	DLBCL.NEW.441
DLBCL.NEW.282	DLBCL.NEW.443
DLBCL.NEW.283	DLBCL.NEW.445
DLBCL.NEW.284	DLBCL.NEW.448
DLBCL.NEW.285	DLBCL.NEW.449
DLBCL.NEW.286	DLBCL.NEW.451
DLBCL.NEW.290	DLBCL.NEW.452
DLBCL.NEW.291	DLBCL.NEW.454
DLBCL.NEW.295	DLBCL.NEW.455
DLBCL.NEW.300	DLBCL.NEW.458
DLBCL.NEW.301	DLBCL.NEW.460
DLBCL.NEW.303	DLBCL.NEW.461
DLBCL.NEW.304	DLBCL.NEW.462
DLBCL.NEW.307	DLBCL.NEW.463
DLBCL.NEW.309	DLBCL.NEW.467
DLBCL.NEW.311	DLBCL.NEW.470
DLBCL.NEW.312	DLBCL.NEW.474
DLBCL.NEW.313	DLBCL.NEW.475
DLBCL.NEW.332	DLBCL.NEW.476
DLBCL.NEW.333	DLBCL.NEW.477
DLBCL.NEW.336	DLBCL.NEW.479
DLBCL.NEW.338	DLBCL.NEW.481
DLBCL.NEW.339	DLBCL.NEW.482
DLBCL.NEW.340	DLBCL.NEW.485
DLBCL.NEW.344	DLBCL.NEW.496
DLBCL.NEW.345	DLBCL.NEW.497
	DLBCL.NEW.498
	DLBCL.NEW.502
	DLBCL.NEW.503
	DLBCL.NEW.504
	DLBCL.NEW.507
	DLBCL.NEW.509
	DLBCL.NEW.514
	DLBCL.NEW.609
	DLBCL.NEW.617

TAK1 inhibitors

TAK1 inhibitors are known in the art, for example, the TAK1 inhibitor can include, for example, a peptide, an antibody, an antisense molecule or a small molecule. TAK1 inhibitors useful in the present invention include but are not limited to, those described or claimed in the

following publications the entire disclosures of which are incorporated by reference herein. Examples of small molecule TAK1 inhibitors include zearalenones those disclosed in WO 2002048135, TAK1 short interfering RNA (siRNA) are described in Takaesu et. al J Mol Biol. 2003;326(1):105-15 and an inactive mutant of TAK1 is described in Thiefes et. al., J Biol Chem. 2005; 280(30):27728-41.

The TAK1 inhibitor can be administered either as a single agent or in combination with other anti-cancer agents or anti-cancer antibodies including CHOP or rituximab.

Examples

Example 1: The following example was performed to determine inhibition of cell growth by a TAK1 inhibitor comprising of shRNA against TAK1.

TAK1 shRNAs and scrambled shRNAs were designed using the Ref Seq #: NM_003188 and constructed in to pSIREN RetroQ retroviral vector (Clontech). Initial validation of the shRNAs was done in a HeLa cell line by co-transfection of TAK1 shRNA with NF-kB Luc vector (Clontech's Mercury profiling systems). Takaesu et. al J Mol Biol. 2003;326(1):105-15. have demonstrated that TAK1 is critical for the NF-kB activation in HeLa cells. The shRNA construct that showed about 70% inhibition of NF-kB Luc assay and inhibited TAK1 protein levels by 70% was selected for further evaluation of the role of TAK in maintaining the survival of lymphoma cells. This construct along with the scrambled construct was transfected along with gag/pol plasmid and pVSV-G in to the 293T cells. The viral supernatant was harvested and used to infect the lymphoma cells in culture dishes. The four cell lines (OCI-LY19, DOHH2, Karpas231 and WSU-NHL carry the t(14;18) translocation) were plated at 25,000 cells/well in flat-bottomed 24 well plates and treated with 1ml of viral supernatant from TAK1 shRNA and scrambled shRNA in triplicate and incubated for a total of 72 hours. Following the incubation period, the extent of cell survival was measured by adding 1/10 (vol/vol) AlamarBlue reagent to every well and incubating the plates for a further 4 hours. The reaction was stopped by the addition of SDS to a final concentration of 0.1%. Fluorescence was measured at 545nm (excitation) and 600nm (emission). The cell survival data is represented in Fig. 2 as percent of live cells as compared to the scrambled shRNA treated groups.

Example 2: The following example was performed to determine inhibition of cell growth by a TAK1 inhibitor comprising a small molecule.

In order to demonstrate that the kinase function of TAK1 is critical for the Lymphoma cell survival, a small molecule inhibitor of TAK 1 was tested in the same set of Lymphoma cell lines as above carrying the t(14;18) chromosomal translocation. The chemical name of the compound is 3-[(aminocarbonyl)amino]-5-(4-{[4-(2-methoxyethyl)piperazin-1-yl]methyl}phenyl)thiophene-2-carboxamide.

More specifically, cell lines (OCI-LY19, DOHH2, Karpas231, WSU-NHL and SUDHL4 carry the t(14;18) translocation) were plated at 10,000 cells/well in flat-bottomed 96 well plates and dosed with test compounds in triplicate over a 10 point dosing range from 0 to 30 μmolesL^{-1} . All cell lines were incubated with test compounds for a total of 72 hours. Background levels were determined for a control (undosed) plate within 2 hours of dosing test compounds. Following the dosing period, the extent of proliferation was measured by adding 1/10 (vol/vol) AlamarBlue reagent to every well and incubating the plates for a further 4 hours. The reaction was stopped by the addition of SDS to a final concentration of 0.1%. Fluorescence was measured at 545nm (excitation) and 600nm (emission). GI50 values were determined for each test compound across the panel.

The four cell lines were found to be sensitive to a TAK1 inhibitor. See Fig. 3. The correlation between the sensitivity to TAK1 shRNA and small molecule kinase inhibitor is striking, emphasizing the role of TAK1 kinase activity in the survival of Lymphoma cells carrying the t(14;18).

Example 3: The following example was performed to determine inhibition of cell growth by a TAK1 inhibitor comprising a small molecule.

In order to demonstrate that the kinase function of TAK1 is critical for lymphoma cell survival, four small molecule inhibitors, i.e., compound 1 is 2-[(aminocarbonyl)amino]-5-[4-(morpholin-4-ylmethyl)phenyl]thiophene-3-carboxamide, compound 2 is 2-[(aminocarbonyl)amino]-5-[4-(1-piperidin-1-ylethyl)phenyl]thiophene-3-carboxamide, compound 3 is 3-[(aminocarbonyl)amino]-5-[4-(morpholin-4-ylmethyl)phenyl]thiophene-2-

carboxamide and compound 4 is 3-[(aminocarbonyl)amino]-5-(4-{{(2-methoxy-2-methylpropyl)amino)methyl}phenyl}thiophene-2-carboxamide, of TAK kinase were tested in a panel of leukaemia and lymphoma cell lines, five of which (OCI-LY19, DOHH2, Karpas231, WSU-NHL and SUDHL4) carry the t(14;18) translocation. The TAK1 inhibitors are known in the art (see for example, WO 2003010158, WO 2003010163 and WO2004063186 the disclosures of which are incorporated by reference herein). All cell lines were plated at 10,000 cells/well in flat-bottomed 96 well plates and dosed with test compounds in triplicate over a 10 point dosing range from 0 to 30 μmolesL^{-1} . All cell lines were incubated with test compounds for a total of 72 hours. Background levels were determined for a control (undosed) plate within 2 hours of dosing test compounds. Following the dosing period, the extent of proliferation was measured by adding 1/10 (vol/vol) AlamarBlue reagent to every well and incubating the plates for a further 4 hours. The reaction was stopped by the addition of SDS to a final concentration of 0.1%. Fluorescence was measured at 545nm (excitation) and 600nm (emission). Growth inhibition 50 (GI50) values were determined for each test compound across the panel. See Table 4.

Compound Number	TAK1 pIC50 (enzyme)					
	OCI-LY19	DOHH2	Karpas231	WSU-NHL	SUDHL4	
	DLBCL	FL	B-ALL	B-NHL	DLBCL	
1	6.9	0.124	0.127	0.30	0.37	6.39
2	7.3	0.005	0.014	0.14	0.25	2.42
3	7	0.085	0.175	0.16	0.42	2.72
4	7.2	0.054	0.101	0.06	0.27	3.08

Compound Number						
	MEC1	HEL92.1.7	KG1a	Jurkat	Raji	ARH77
	B-CLL	Erythro- leukemia	AML	T-ALL	Burkitts Lymphoma	Plasma cell leukemia
1	1.34	0.73	0.45	15.86	15.22	1.17
2	0.16	0.17	0.40	4.39	3.5	0.25
3	0.63	0.50	0.43	>30	>30	0.48
4	2.99	1.47	1.09	10.09	>30	3.72

Table 4 shows GI50 values (μM) for 4 test compounds against a panel of human haematological tumor cell lines.

The TAK1 inhibitor compounds were significantly more potent compared to the mean in four out of five cell lines that carried the t(14;18) chromosomal translocation. This profile was differentiated from other compounds that inhibit other pathways (data not shown).

Table 5 shows the GI50 values (μM) for a TAK1 kinase inhibitor against a panel of multiple myeloma tumour cell lines. The results indicate that a distinct set of myeloma cells are responsive to TAK1 inhibitors.

Compd Number	TAK1	JJN-3	L-363	AM0-1	RPMI-8226	MOLP-8	IM-9	ARH-77	KARPAS-620
	pIC50	plasma cell leukemia	plasma cell leukemia	plasmacytoma	Multiple myeloma	Multiple myeloma	B lymph	plasma cell leukemia	plasma cell leukemia
4	7.2	0.52	0.35	0.91	1.60	0.09	0.21	3.72	0.14

Table 5 shows GI50 values (μM) for compound 4 against a panel of human multiple myeloma cell lines

Table 6 shows the GI50 values (μM) for a TAK1 kinase inhibitor against a panel of human B-cell lymphoma cell lines. The experiments to generate the results for both Tables 5 and 6 were performed as described above. The results indicate that a distinct set of human B-cell tumor cells are responsive to TAK1 inhibitors.

Compd Number	TAK1	SC-1	JEKO-1	NAMALWA	MEC-1
	pIC50	FL	MCL	Burkitts Lymphoma	B-CLL
4	7.2	2.88	0.29	2.96	2.99

Compd Number	JVM-3	DERL 2	WSU DLCL2	U937	Ramos	Raji
	T cell B-PLL	lymphoma	B cell lymphoma	Histiocytic lymphoma	Burkitts Lymphoma	Burkitts Lymphoma
4	0.14	0.31	1.95	0.12	0.21	>30

Table 6 shows GI50 values (μM) for compound 4 against a panel of human B cell lymphoma cell lines

Some of the TAK inhibitors used in the study belong to a large class of thiophene carboxamide ureas that are known to inhibit other enzymes with similar potency against TAK1, such as FLT3, CHK1, ARK5 and Aurora B kinase. Hence in order to rule out any off

target effects of TAK1 inhibitors in lymphoma and myeloma cell lines, we further utilized a commercially available TAK1 specific inhibitor, LL-Z-1640-2, which is a (3*S*,5*Z*,8*S*,9*S*,11*E*)-8,9,16-trihydroxy-14-methoxy-3-methyl-3,4,9,10-tetrahydro-1*H*-2-benzoxacyclotetradecine-1,7(8*H*)-dione (Iris Biotech, GmbH; see WO-00248135). Table 7 shows the GI50 values (μM) for the TAK1 kinase inhibitor, LL-Z-1640-2 against a panel of B-cell lymphoma cell lines.

Compd Number	TAK1 pIC50	SUDHL4 DLBCL	KARPAS231 B-ALL	OCI- LY19 DLBCL	WSU- DLCL B cell lymphoma	DOHH2 FL	MEC1 B- CLL	JVM- 3 B- PLL	JEKO- 1 MCL
LL-Z-1640-2	6.8	1.64	0.95	0.23	0.02	0.39	0.73	0.71	0.07

Table 7 shows GI50 values (μM) for another TAK1 kinase inhibitor against a panel of human B cell lymphoma cell lines

Table 8 shows the GI50 values (μM) for the TAK1 kinase inhibitor, LL-Z-1640-2 against a panel of multiple myeloma tumour cell lines. The experiments were performed as described above. The results indicate that, similar to the thiophene carboxamide ureas a distinct set of B-cell lymphoma and myeloma cells are responsive to TAK1 inhibitors.

Compd	TAK1 pIC50	JJN-3 plasma cell leukemia	L-363 plasma cell leukemia	AM0-1 plasmacytoma
LL-Z-1640-2	6.8	32.43	32.43	0.57

Compd	RPMI-8226 M.M	MOLP-8 Multiple myeloma	IM-9 B lymphoblastoid	ARH-77 plasma cell leukemia	KARPAS-620 plasma cell leukemia
LL-Z-1640-2	1.41	10.44	0.02	1.24	ND

Table 8 shows GI50 values (μM) for another TAK1 kinase inhibitor against a panel of human multiple myeloma cell lines

Example 4: Genomic analysis of TAK1 pathways in DLBCL

1. Tak1 pathways genes

Genes in Tak1 pathways were assembled based on the public information. The genes that are involved in the signaling of ALK, FAS, MAP kinase, IL1 receptor, TGF-beta, TNF receptor,

thrombin and protease-activated receptor, Toll-like receptor, WNT, and antigen receptor were included. The genes were mapped to Affymetrix probesets based on the annotations available from Affymetrix (<http://www.affymetrix.com/analysis/index.affx>) (Table1)

5 *2. Gene expression data*

The gene expression data of 176 newly diagnosed diffuse large B cell lymphoma (DLBCL) patients were generated with Affymetrix U133A/B gene chip and were made publicly available by Margaret Shipp's group at Dana Faber Cancer Institute (). The raw data were downloaded from <http://www.broad.mit.edu/cgi-bin/cancer/datasets.cgi>, and further processed
10 and analyzed as described below.

3. Data preprocessing and analysis

3.1 QC:

In order to verify data quality, and generate gene expression results, the raw data (.CEL files)
15 of the DLBCL samples were loaded into Affymetrix Expression Console 1.0 (Affymetrix Inc.) and analyzed using MAS5 algorithm. The following criteria were used to filter out samples with low quality data: 1) scaling factor < 4; 2) rawQ < 5; 3) 3'/5' ratio for both actin and GAPDH < 5; 4) percentage of present call > 20 for chip A or > 10 for chip B. As a result of the QC procedure, 113 samples (Table 3) were kept for further analysis.

20 *3.2 Normalization:*

Array normalization: The parameters for MAS5 algorithm were set to normalize each array using all probesets on the array, and the trimmed mean value for each array was preset to 100. Probeset normalization: The expression matrix generated by MAS5 were further normalized
25 so that the mean of each probeset was centered to zero.

3.3 Sample clustering:

For unsupervised clustering analysis, the normalized expression matrix was loaded into GeneSpring GX 7.3.1 (Agilent Inc.). A 2-way hierarchical clustering was performed using
30 only probeset IDs from table2. Spearman correlation was used as the similarity measure in the clustering.

Results:

The 113 newly diagnosed DLBCL samples were separated into 3 distinct subclasses based on their expression pattern of Tak1 genes. The informative genes (the genes that are

5 differentially expressed among the 3 patient subclass) were further divided into 7 groups (A-F) based on their distinct expression patterns. Most of the informative genes in Group 2 are down-regulated compared to the other 2 groups, suggesting the samples in this group represent a patient population that is insensitive to Tak1-targeted therapy.

What is claimed is:

1. A method of inhibiting B cell tumour cell proliferation by contacting a B cell tumour cell with a TAK1 inhibitor.

5

2. The method of claim 1 wherein the B cell tumour is a non-Hodgkin's lymphoma, a Hodgkin's lymphoma, a chronic lymphocytic leukaemia, or a multiple myeloma.

3. A method of treating a patient having a B cell tumour by administering a TAK1
10 inhibitor.

4. The method of claim 3 wherein the B-cell tumour is a non-Hodgkin's lymphoma, a Hodgkin's lymphoma, a chronic lymphocytic leukaemia (CLL) or a multiple myeloma.

15 5. The method of claims 2 or 4 wherein the non-Hodgkin's lymphoma is a follicular lymphoma, a diffuse large B cell lymphoma (DLBCL) of activated B cell (ABC) type, a diffuse large B cell lymphoma (DLBCL) of germinal center B cell (GCB) type, a mantle zone lymphoma (MZL), Mantle cell lymphoma (MCL), Primary mediastinal B-cell lymphoma (PMBCL) or MALT Lymphoma.

20

6. The method of claim 5 wherein the non-Hodgkin's lymphoma has a t(14;18)(q32;q21) translocation, a t(11;18)(q21;q21) translocation, a t(1;14)(p22;q32), an amplification of chromosome 18, an amplification of chromosome 6, or an amplification, as defined by comparative genomic hybridization, of specific regions of BCL-10, CARD11, TRAF6 or
25 TAK1.

7. The method of claims 2 or 4 wherein the B-cell tumour is CLL.

8. A method of treating a patient having a deregulated TAK1 signalling transduction
30 molecule by administering a TAK1 inhibitor.

9. The method of claim 8 wherein the TAK1 signalling transduction molecule is Malt1, BCL-10, BCL2, TAB1, TAB2, TAK1, TRAF2, TRAF6, TAK1, CARD11, IRAK1, IRAK4, API1, API2, API3, API4 or NFkappaB target genes.

10. A method of inhibiting the growth of a solid tumour by contacting the tumour with a TAK1 inhibitor.

5 11. The method of claim 10, wherein the solid tumour is selected from the group consisting of a tumour of the head and neck, breast, ovary, lung, pancreas, colon, prostate, or skin.

10 12. A method of treating a patient having a solid tumour by administering a TAK1 inhibitor.

13. The method of claims 10 or 12, wherein the solid tumour can be a tumour of the head and neck, breast, ovary, lung, pancreas, colon, prostate, liver, or skin.

15 14. A method of selecting a patient having a tumour that is susceptible to treatment with a TAK1 inhibitor, comprising determining if the patient has a genetic mutation of a t(14;18)(q32;q21) translocation, a t(11;18)(q21;q21) translocation, a t(1;14)(p22;q32) translocation, or an amplification of chromosome 18, whereby the presence of a mutation indicates the tumour is susceptible to treatment.

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15. A method of selecting a patient having a tumour that is susceptible to treatment with a TAK1 inhibitor, comprising determining if the patient has a deregulated TAK1 signalling transduction molecule, wherein the presence of the deregulated TAK1 signalling transduction molecule is an indication that the patient is susceptible to treatment with a TAK1 inhibitor.

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16. A method of inhibiting proliferation of a T cell leukemia and T-cell lymphomas by contacting a T cell leukaemia and T-cell lymphoma with a TAK1 inhibitor.

17. The method of claim 16, wherein the T cell leukemia is a T-cell acute lymphoblastic leukemia (T-ALL), or T-cell lymphomas, for example, peripheral T-cell lymphoma (PTCL), T-cell lymphoblastic lymphoma (T-CLL), cutaneous T-cell lymphoma (CTCL) and adult T-cell lymphoma (ATCL).

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18. A method of selecting a mammal having or suspected of having a tumour for treatment with a TAK1 inhibitor drug, the method comprising providing a biological sample from a subject having cancer and testing the biological sample for expression of any one of the genes listed in Table 1, or their gene products, thereby to predict an increased likelihood
5 of response to the TAK1 inhibitor drug.