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(54) Title: 14 GENE SIGNATURE DISTINGUISHES BETWEEN MULTIPLE MYELOMA SUBTYPES



FIG. 3

(57) Abstract: The present invention provides methods for diagnosing and treating multiple myeloma and its high risk subtypes and differentiating between an early failure and a sustained control high risk subtype by measuring expression levels of a subset of 14 genes. The genes *MEIS1*, *SPIB*, *RNF43*, *SHISA*, *SLC43A3*, *PLK1*, *RUNX2*, or *PMAIP1* in the subset are overexpressed in multiple myeloma where overexpression of *SHISA* is indicative of the early failure high risk subtype. The genes *C20orf142*, *TP53INP*, *ST6GAL1*, *235659_at*, *YIPF6*, or *MAN2A1* in the subset are underexpressed in multiple myeloma where underexpression of *TP53INP* is indicative of the sustained control high risk subtype.

5 **14 GENE SIGNATURE DISTINGUISHES BETWEEN MULTIPLE
MYELOMA SUBTYPES**

10 Cross-Reference to Related Applications

 This international application claims benefit of priority under 35 U.S.C. §120 to provisional application U.S. Serial No. 61/396,917, filed June 4, 2010, now abandoned, the entirety of which is incorporated by reference herein.

15

BACKGROUND OF THE INVENTION

Field of the Invention

20 The present invention generally relates to the field of cancer research. More specifically, the present invention relates to the use of gene expression profiling to identify genomic signatures specific for high and low risk myeloma useful for predicting and improving clinical outcome and survival.

25 Description of the Related Art

 Multiple myeloma (MM) is an invariantly fatal tumor of terminally differentiated plasma cells (PCs) that home to and expand in the bone marrow. Monoclonal gammopathy of undetermined significance (MGUS) and multiple myeloma are the most frequent forms of monoclonal gammopathies. Monoclonal
30 gammopathy of undetermined significance is the most common plasma cell dyscrasia with an incidence of up to 10% of population over age 75. The molecular basis of monoclonal gammopathy of undetermined significance and

multiple myeloma are not very well understood and it is not easy to differentiate these two disorders. Diagnosis of multiple myeloma or monoclonal gammopathy of undetermined significance is identical in 2/3 of cases using classification systems that are based on a combination of clinical criteria such as the amount of bone marrow plasmocytosis, the concentration of monoclonal immunoglobulin in urine or serum, and the presence of bone lesions. Especially in early phases of multiple myeloma, differential diagnosis is associated with a certain degree of uncertainty.

Furthermore, in the diagnosis of multiple myeloma, the clinician must exclude other disorders in which a plasma cell reaction may occur. These other disorders include rheumatoid arthritis, connective tissue disorders, and metastatic carcinoma where the patient may have osteolytic lesions associated with bone metastases. Therefore, given that multiple myeloma is thought to have an extended latency and clinical features are recognized many years after development of the malignancy, new molecular diagnostic techniques are needed for differential diagnosis of multiple myeloma, e.g., monoclonal gammopathy of undetermined significance versus multiple myeloma, or recognition of various subtypes of multiple myeloma.

Multiple myeloma initially resides in the bone marrow, but typically transform into an aggressive disease with increased proliferation (resulting in a higher frequency of abnormal metaphase karyotypes), elevated lactate dehydrogenase (LDH) and extramedullary manifestations (Barlogie B. et al., 2001). Although aneuploidy is observed in more than 90% of cases, cytogenetic abnormalities in this typically hypoproliferative tumor are informative in only about 30% of cases and are typically complex, involving on average 7 different chromosomes.

Given this genetic chaos, it has been difficult to establish correlations between genetic abnormalities and clinical outcomes. Only recently has chromosome 13 deletion been identified as a distinct clinical entity with a grave prognosis. However, even with the most comprehensive analysis of laboratory parameters, such as b2-microglobulin (b2M), C-reactive protein (CRP), plasma cell labeling index (PCLI), metaphase karyotyping, and fluorescence in situ hybridization (FISH), the clinical course of patients afflicted with multiple

myeloma can only be approximated, because no more than 20% of the clinical heterogeneity can be accounted for. Thus, there are distinct clinical subgroups of multiple myeloma and modern molecular tests may identify these entities. Overall, the progress in understanding the biology and genetics of multiple myeloma has been slow.

The advent of high-density DNA microarray has made possible a simultaneous analysis of messenger RNA (mRNA) expression patterns of thousands of genes. In the past the decade, microarray technology has been utilized in multiple myeloma patients to interrogate thousands of genes simultaneously. Combined with powerful tools such as hierarchical clustering analysis, genes which have high diagnostic and therapeutic value have been identified.

The prior art is deficient in correlating gene expression profiling with defined risk groups. More specifically, the prior art is deficient in a 14 gene model which discriminates between ultra-high early failure and sustained control risks. The present invention fulfills this long-standing need in the art.

SUMMARY OF THE INVENTION

The present invention is directed to a method for diagnosing multiple myeloma in a subject. The method comprises obtaining a biological sample from the subject and performing gene expression profiling on the sample. Expression levels of a subset of genes that are *C20orf142*, *TP53INP*, *ST6GAL1*, *235659_at*, *YIPF6*, *MAN2A1*, *MEIS1*, *SPIB*, *RNF43*, *SHISA*, *SLC43A3*, *PLK1*, *RUNX2*, and *PMAIP1* are compared to control levels where one or both of an overexpression or an underexpression of the genes compared to control is indicative of multiple myeloma in the subject.

The present invention is directed to a related method for diagnosing a subtype of high-risk multiple myeloma in a subject, comprising obtaining a bone marrow sample from the individual; measuring an expression levels of a subset of genes consisting of *C20orf142*, *TP53INP*, *ST6GAL1*, *235659_at*, *YIPF6*, *MAN2A1*, *MEIS1*, *SPIB*, *RNF43*, *SHISA*, *SLC43A3*, *PLK1*, *RUNX2*, and *PMAIP1*

comprising plasma cells in the sample; and comparing the measured expression levels to those in a control sample, wherein one or both of an overexpression or an underexpression of the genes in the subset compared to control is indicative of a subtype of high risk multiple myeloma, thereby diagnosing the multiple myeloma subtype in the individual.

The present invention also is directed to a method for diagnosing a subtype of high-risk multiple myeloma in a subject. The method comprises obtaining a bone marrow sample from the individual and measuring an expression levels of genes consisting of *C20orf142*, *TP53INP*, *ST6GAL1*, *235659_at*, *YIPF6*, *MAN2A1*, *MEIS1*, *SPIB*, *RNF43*, *SHISA*, *SLC43A3*, *PLK1*, *RUNX2*, and *PMAIP1* in plasma cells in the sample. The measured expression levels are compared to those in a control sample, where one or both of an overexpression or an underexpression of the genes in the subset compared to control is indicative of a subtype of high risk multiple myeloma, thereby diagnosing the multiple myeloma subtype in the individual.

The present invention is directed further to a method for differentiating between high-risk multiple myeloma subtypes in a subject having multiple myeloma. The method comprises obtaining a plasma cell sample from the subject; and measuring expression levels of a subset of genes consisting of *C20orf142*, *TP53INP*, *ST6GAL1*, *235659_at*, *YIPF6*, *MAN2A1*, *MEIS1*, *SPIB*, *RNF43*, *SHISA*, *SLC43A3*, *PLK1*, *RUNX2*, and *PMAIP1* comprising the sample. An overexpression of *SHISA* compared to control is indicative of an early failure high risk subtype and an underexpression *TP53INP* compared to control is indicative of a sustained control high risk subtype, thereby differentiating the high risk multiple myeloma subtypes in the subject.

The present invention is directed further still to a method for treating multiple myeloma in a subject. The method comprises inhibiting the expression of one or more genes *MEIS1*, *SPIB*, *RNF43*, *SHISA*, *SLC43A3*, *PLK1*, *RUNX2*, or *PMAIP1*; or amplifying the expression of one or more genes *C20orf142*, *TP53INP*, *ST6GAL1*, *235659_at*, *YIPF6*, *MAN2A1* or a combination thereof, thereby treating the multiple myeloma in the subject.

Other and further objects, features, and advantages will be apparent from the following description of the presently preferred embodiments of the invention, which are given for the purpose of disclosure.

5

BRIEF DESCRIPTION OF THE DRAWINGS

So that the matter in which the above-recited features, advantages and objects of the invention, as well as others which will become clear, are attained and can be understood in detail, more particular descriptions of the invention briefly summarized above may be had by reference to certain embodiments thereof which are illustrated in the appended drawings. These drawings form a part of the specification. It is to be noted, however, that the appended drawings illustrate preferred embodiments of the invention and therefore are not to be considered limiting in their scope.

Figure 1 shows that GEP-70 gene model defines high risk in ~20% of newly diagnosed multiple myeloma cases.

Figures 2A-29B show the clinical outcomes according to GEP-defined risk after the start of Total Therapy 2 for overall survival (Figure 2A; $P < 0.0001$) and event-free survival (Figure 2B; $P < 0.0001$).

Figure 3 shows the genomic signature based on 14 genes (GEP-14) used to distinguish EF and SC in GEP-70 defined high-risk multiple myeloma. $P < 0.0001$ (FDR: 15.8%)

Figure 4 shows the cure fraction (CF) in high-risk myeloma based on CR model based on Total Therapy 2 treatment.

Figure 5 shows the hazard rate over time in high vs low risk multiple myeloma based on Total Therapy 2 treatment.

Figure 6 shows the relative survival ratio in Total Therapy 2 treatment according to GEP-defined risk.

Figures 7A-7B show the shift to higher GEP risk score in EF (Figure 7A) vs SC (Figure 7B) high-risk myeloma. Figure 7A: p-value = 0.007; Figure 7B: p-value = 0.003.

Figure 8 shows the model which explains how over-expression of TP53INP1 on chromosome 8q22 is linked to SC with better outcome in high-risk myeloma.

Figure 9 shows the upregulation of TP53INP1 within 48 hours of administering bortezomib (VELCADE) and augmented by added thalidomide (VTDPACE).

Figure 10 shows that TP53INP1 can be up-regulated by test dose of melphalan 10mg/m² in 48 hours.

DETAILED DESCRIPTION OF THE INVENTION

As used herein, the following terms and phrases shall have the meanings set forth below. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art.

As used herein, the term, "a" or "an" may mean one or more. As used herein in the claim(s), when used in conjunction with the word "comprising", the words "a" or "an" may mean one or more than one. As used herein "another" or "other" may mean at least a second or more of the same or different claim element or components thereof. The terms "comprise" and "comprising" are used in the inclusive, open sense, meaning that additional elements may be included.

As used herein, the term "or" in the claims refers to "and/or" unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and "and/or".

As used herein, the term "about" refers to a numeric value, including, for example, whole numbers, fractions, and percentages, whether or not explicitly indicated. The term "about" generally refers to a range of numerical values (e.g., +/- 5-10% of the recited value) that one of ordinary skill in the art would consider equivalent to the recited value (e.g., having the same function or result). In some instances, the term "about" may include numerical values that are rounded to the nearest significant figure.

As used herein, the terms "subject", "individual" or "patient" refers to a mammal, preferably a human, who has, is suspected of having or at risk for having a pathophysiological condition, for example, but not limited to, multiple myeloma.

5 In one embodiment of the present invention there is provided a method for diagnosing multiple myeloma in a subject, comprising obtaining a biological sample from the subject; performing gene expression profiling on the sample; and comparing expression levels of a subset of genes that are *C20orf142*, *TP53INP*, *ST6GAL1*, *235659_at*, *YIPF6*, *MAN2A1*, *MEIS1*, *SPIB*,
10 *RNF43*, *SHISA*, *SLC43A3*, *PLK1*, *RUNX2*, and *PMAIP1* to control levels; where one or both of an overexpression or an underexpression of the genes compared to control is indicative of multiple myeloma in the subject.

In an aspect of this embodiment the overexpressed genes are *MEIS1*, *SPIB*, *RNF43*, *SHISA*, *SLC43A3*, *PLK1*, *RUNX2*, or *PMAIP1*. In this
15 aspect an overexpression of *SHISA* diagnoses a high risk early failure subtype of multiple myeloma. In another aspect of this embodiment the underexpressed genes are *C20orf142*, *TP53INP*, *ST6GAL1*, *235659_at*, *YIPF6*, or *MAN2A1*. In this other aspect an underexpression of *TP53INP* diagnoses a high risk sustained control subtype of multiple myeloma. In this embodiment and aspects
20 thereof one or both of overexpression or underexpression of the genes comprising the subset is indicative of ultra high risk multiple myeloma. Also the biological sample may be obtained from bone marrow.

In a related embodiment of the present invention there is provided a method for diagnosing a subtype of high-risk multiple myeloma in a subject,
25 comprising obtaining a bone marrow sample from the individual; measuring an expression levels of genes consisting of *C20orf142*, *TP53INP*, *ST6GAL1*, *235659_at*, *YIPF6*, *MAN2A1*, *MEIS1*, *SPIB*, *RNF43*, *SHISA*, *SLC43A3*, *PLK1*, *RUNX2*, and *PMAIP1* in plasma cells in the sample; and comparing the measured expression levels to those in a control sample, wherein one or both of
30 an overexpression or an underexpression of the genes in the subset compared to control is indicative of a subtype of high risk multiple myeloma, thereby diagnosing the multiple myeloma subtype in the individual. In this embodiment the overexpressed genes, the underexpressed genes and the diagnosis of high

risk early failure and sustained control subtypes are as described *supra*.

In yet another embodiment of the present invention there is provided method for differentiating between high-risk multiple myeloma subtypes in a subject having multiple myeloma, comprising obtaining a plasma cell sample
5 from the subject; and measuring expression levels of a subset of genes consisting of *C20orf142*, *TP53INP*, *ST6GAL1*, *235659_at*, *YIPF6*, *MAN2A1*, *MEIS1*, *SPIB*, *RNF43*, *SHISA*, *SLC43A3*, *PLK1*, *RUNX2*, and *PMAIP1* comprising the sample; wherein an overexpression of *SHISA* compared to control is indicative of an early failure high risk subtype and an underexpression
10 *TP53INP* compared to control is indicative of a sustained control high risk subtype, thereby differentiating the high risk multiple myeloma subtypes in the subject.

In yet another embodiment of the present invention there is provided method for treating multiple myeloma in a subject, comprising inhibiting
15 the expression of one or more genes *MEIS1*, *SPIB*, *RNF43*, *SHISA*, *SLC43A3*, *PLK1*, *RUNX2*, or *PMAIP1*; or amplifying the expression of one or more genes *C20orf142*, *TP53INP*, *ST6GAL1*, *235659_at*, *YIPF6*, *MAN2A1* or a combination thereof, thereby treating the multiple myeloma in the subject.

In this embodiment one or both of the inhibiting step or the
20 amplifying step may increase survivability outcome of high risk multiple myeloma in the subject. In an aspect of this embodiment the step of inhibiting *SHISA* treats an early failure subtype of high risk multiple myeloma. In another aspect the step of amplifying *TP53INP* may comprise administering one or more of bortezomib, thalidomide or mephalan to the subject. In this aspect amplifying *TP53INP* treats
25 a sustained control subtype of multiple myeloma. Also, amplifying expression of *TP53INP* induces apoptosis in multiple myeloma cells.

Provided herein are methods for utilizing a 14 gene signature for diagnosing multiple myeloma and its high risk subgroups, including distinguishing between subgroups. The 14 gene subset is identified from within a 70 gene
30 expression profiling model (GEP-70). GEP-70 was developed to identify high and low risk disease state in approximately 20% and 80% of newly diagnosed disease. GEP-70 has identified genes in chromosome 1 whose genetic abnormalities indicate certain disease states. Particularly, there are at least two

types of GEP-70 defined high risk groups. One group associated with ultra high risk is early failures (EF), while a second group has a more manageable form of the disease that can be controlled in a sustained control (SC) manner.

The genes in the 14 gene signature are *C20orf142*, *TP53INP*,
5 *ST6GAL1*, *235659_at*, *YIPF6*, *MAN2A1*, *MEIS1*, *SPIB*, *RNF43*, *SHISA*,
SLC43A3, *PLK1*, *RUNX2*, and *PMAIP1* (Table 1). In the signature an over
expression of one or more of the genes *MEIS1*, *SPIB*, *RNF43*, *SHISA*, *SLC43A3*,
PLK1, *RUNX2*, or *PMAIP1* and underexpression of one or more of the genes
10 *C20orf142*, *TP53INP*, *ST6GAL1*, *235659_at*, *YIPF6*, and *MAN2A1* are indicative
of multiple myeloma in a subject. Particularly, an overexpression of *SHISA* is
indicative of early failure high risk subset of multiple myeloma and an under
expression of *TP53INP* is indicative of the sustained control high risk subset of
multiple myeloma.

As such, the present invention also provides methods of treating
15 multiple myeloma. Treatment may be effected by an inhibition of one or more of
MEIS1, *SPIB*, *RNF43*, *SHISA*, *SLC43A3*, *PLK1*, *RUNX2*, or *PMAIP1*, an
amplification of one or more of *C20orf142*, *TP53INP*, *ST6GAL1*, *235659_at*,
YIPF6, or *MAN2A1* or a combination thereof. Preferably, amplifying expression
of *TP53INP* can induce apoptosis in cells associated with multiple myeloma. One
20 of ordinary skill in the art is well suited to determine a suitable therapeutic
regimen based on, *inter alia*, the subject's age, gender, health, previous
treatments, the type or subtype and the progression or regression of the multiple
myeloma.

The following examples are given for the purpose of illustrating
25 various embodiments of the invention and are not meant to limit the present
invention in any fashion. One skilled in the art will appreciate readily that the
present invention is well adapted to carry out the objects and obtain the ends and
advantages mentioned, as well as those objects, ends and advantages inherent
herein. Changes therein and other uses which are encompassed within the spirit
30 of the invention as defined by the scope of the claims will occur to those skilled in
the art.

EXAMPLE 1

Gene expression profiling

Plasma cell purifications and GEP, using the Affymetrix U133Plus2.0 microarray, were performed as previously described. The patient population includes 123 with high-risk myeloma in TT2 and TT3.

EXAMPLE 2

Statistical and microarray analyses

Affymetrix U133Plus2.0 micro-arrays were preprocessed using GCOS1.1 software and normalized using conventional GCOS1.1 scaling. Log rank tests for univariate association with disease-related survival were performed for each of the 54,675 'Signal' summaries. Specifically, log rank tests were performed for quartile 1 vs. quartiles 2-4 and quartile 4 vs. quartiles 1-3 in order to identify under- and over expressed prognostic genes, respectively.

A false discovery rate cut-off of 2.5% was applied to each list of log-rank P-values yielding 19 under- and 51 over expressed probe sets. Heat-map-column dendrograms were computed with hierarchical clustering using Pearson's correlation distances between patient pairs' log₂-scale expression. Column-dendrogram branches were sorted left-to-right based upon each patient's difference between average log₂-scale expression of the up-regulated and the down-regulated genes: this difference is interpreted as an up/down-regulated mean ratio (i.e. geometric mean) on the log₂ scale. This simple, univariate summary of the gene expression profile for each patient may enhance robustness to residual array effects (i.e. after MAS5.0 processing) that increase or decrease all genes multiplicatively, and is also independent of the MAS5.0 scale factor.

Weighting expression by hazard ratios, unstandardized or standardized (i.e. Wald statistics), does not improve this score, and the design was to use no supervision by OS or EFS beyond the gene-by-gene log rank tests. The log₂ up/down-regulated mean ratio was then clustered using K-means into 3 groups to separate out the small extreme right mode in the histogram: the two groups with lower up/down mean ratios were combined. The single extreme

mode in the up/down mean expression ratio is consistent with the extreme quartile log rank tests used in the differential expression analysis, though the histograms and the right-hand side of the heat maps suggest that the extreme patient group is smaller than 25%, closer to 13%.

5

EXAMPLE 3

14 gene subgroup of GEP70 model distinguishes between MM subtypes

10 The 14 identified genes suggest a strong relationship between sustained control with stress induced activation of TP53 and its target gene TP53INP1 while EF disease lacks this feature. EF disease is linked to the overexpression of SHISA3, which, like DKK1, can suppress Wnt signaling. The identification of these genes also brings forth, potential therapeutic targets which
15 can improve the clinical outcome of multiple myeloma.

 The survival variability of patients with multiple myeloma is not well accounted for with current laboratory parameters, such as beta-2-microglobulin and albumin levels employed in the ISS staging system (Table 1). *De novo* high-risk disease may be fundamentally different from myeloma acquiring drug
20 resistance and an aggressive clinical course after recurrent relapses.

Table 1

Higher frequency of unfavorable GEP features in EF vs SC myeloma.

SURVIVAL MODEL				CR DUR MODEL		
VARIABLE	EF (N=65)	SC (N=58)	P	EF (N=38)	SC (N=28)	P
B2M ≥5.5mg/L	45%	50%	0.55	45%	32%	0.30
Albumin <3.5g/dL	49%	45%	0.63	50%	29%	0.08
LDH ≥ 190 U/L	60%	43%	0.06	66%	50%	0.20
CA	78%	67%	0.18	78%	79%	0.99
GEP >median score	60%	38%	<0.02	68%	39%	0.02
GEP delTP53	22%	5%	<0.01	18%	7%	0.28

5

It is demonstrated that expression extremes of a subset of genes correlating with survival might be representative of the effects of DNA copy changes in myeloma disease progression. The present invention was thus able to identify a set of 70 genes, the expression levels of which permitted the identification of a small cohort 13% to 14% of patients at high risk for early disease-related death. High-risk disease defined by this model was an independent and highly significant prognostic variable to be validated in the context of other treatment approaches. Approximately 20% of all newly diagnosed patients fall into the high-risk group (Fig. 1).

The 14 gene signature is a refinement of the GEP-70 model which can distinguish between GEP-70 defined high-risk subgroups. It is contemplated

that ultra-high risk may correlate to molecular subgroups of multiple myeloma, for example, but not limited to, CD-1 subgroup which is primarily early failure subtype associated. Analysis of the high-risk subgroup in patients treated with Total Therapy 2 (TT2) and Total Therapy 3 (TT3) show that there is a breakpoint which typically occurs at year 3 (Figs. 2A-2B). Logistic regression analysis has been used to segregate the higher high-risk group, early failures (EF), from the lower high-risk group, sustained control (SC), based on GEP and standard variables (Fig. 3; Table 2).

Table 2

MV Logistic regression analysis of variables linked to EF vs SC

		% EF with				
		Factor				
Model	Variable	N	YES	NO	OR	P
Survival	GEP delTP53	120	80%	48%	4.60	0.025
	GEP very high risk*	120	64%	42%	2.41	0.022
CR Duration	GEP very high risk*	64	69%	41%	3.09	0.031

• > Median in high risk myeloma

Furthermore, analysis of the risk groups show curability (Fig. 4) is apparent earlier in high-risk than low-risk myeloma as evidenced by the steeper and more profound decline in hazard rate (Fig. 5). With the longer follow-up as in Total Therapy 2, the relative survival ratio in low-risk myeloma approaches the average survival expectation for the US population (Fig. 6). These results suggest a paradigm shift in multiple myeloma from no cure to cure and provide lessons for therapy of high-risk myeloma from SC subset (Figs. 7A-7B). These 14 genes may also provide new targets for multiple myeloma therapy.

EXAMPLE 3**Upregulation of TP53INP1 induces apoptosis**

Outcome differences in the 70 gene high risk myeloma in terms of EF and SC revealed that a shift in EF vs SC is indicated by higher 70 gene risk scores and lower delTP53 scores. On the other hand, SC is enabled by TP53INP1 (TP53-Dependent Damage-Inducible Nuclear Protein 1) overexpression which is linked to apoptosis events suggesting that SC high-risk group has cure potential (Fig. 8). This cell death is induced by DNA double-strand breaks (DSB's). Thus apoptosis is induced by the over-expression of TP53INP1. TP53INP1 may also regulate TP53-dependent apoptosis through phosphorylation of TP53 at Ser46, serving as a cofactor for the putative p53-Ser46 kinase.

Moreover, T53INP1 is upregulated within 48 hours after Total Therapy 3 treatment. Administration of bortezomib, augmented with thalidomide, demonstrates T53INP1 (Fig. 9). Also, during Total Therapy 4 and 5 treatment of 125 newly-diagnosed multiple myeloma patients, administration of 10 mg/m² of melphalan demonstrated upregulation of T53INP1 within 48 hours (Fig. 10). T53INP1 is rapidly inducible by bortezomib and melphalan in patients with low levels of TP53INP1 expression. Overexpression of TP53INP1 in SC subtypes is linked to apoptosis and better clinical outcome. Thus, TP53INP1 is not just a biomarker for multiple myeloma, but a therapeutic target by which cell sensitivity to anti-myeloma agents is restored.

WHAT IS CLAIMED:

1. A method for diagnosing multiple myeloma in a subject,
5 comprising:
obtaining a biological sample from the subject;
performing gene expression profiling on the sample; and
comparing expression levels of a subset of genes that are
10 *C20orf142*, *TP53INP*, *ST6GAL1*, *235659_at*, *YIPF6*, *MAN2A1*, *MEIS1*, *SPIB*,
RNF43, *SHISA*, *SLC43A3*, *PLK1*, *RUNX2*, and *PMAIP1* to control levels;
wherein one or both of an overexpression or an underexpression of
the genes compared to control is indicative of multiple myeloma in the subject.

2. The method of claim 1, wherein the biological sample is obtained
15 from bone marrow.

3. The method of claim 1, wherein one or both of
overexpression or underexpression of the genes comprising the subset is
indicative of ultra high risk multiple myeloma.

4. The method of claim 1, wherein the overexpressed genes
20 are *MEIS1*, *SPIB*, *RNF43*, *SHISA*, *SLC43A3*, *PLK1*, *RUNX2*, or *PMAIP1*.

5. The method of claim 4, wherein an overexpression of *SHISA*
25 diagnoses a high risk early failure subtype of multiple myeloma.

6. The method of claim 1, wherein the underexpressed genes
are *C20orf142*, *TP53INP*, *ST6GAL1*, *235659_at*, *YIPF6*, or *MAN2A1*.

7. The method of claim 6, wherein an underexpression of
30 *TP53INP* diagnoses a high risk sustained control subtype of multiple myeloma.

8. A method for diagnosing a subtype of high-risk multiple myeloma in a subject, comprising:

obtaining a bone marrow sample from the individual;

measuring an expression levels of genes consisting of *C20orf142*,
5 *TP53INP*, *ST6GAL1*, *235659_at*, *YIPF6*, *MAN2A1*, *MEIS1*, *SPIB*, *RNF43*,
SHISA, *SLC43A3*, *PLK1*, *RUNX2*, and *PMAIP1* in plasma cells in the sample;
and

comparing the measured expression levels to those in a control sample, wherein one or both of an overexpression or an underexpression of the
10 genes in the subset compared to control is indicative of a subtype of high risk multiple myeloma, thereby diagnosing the multiple myeloma subtype in the individual.

9. The method of claim 8, wherein the overexpressed genes
15 are *MEIS1*, *SPIB*, *RNF43*, *SHISA*, *SLC43A3*, *PLK1*, *RUNX2*, or *PMAIP1*.

10. The method of claim 9, wherein an overexpression of *SHISA* diagnoses a high risk early failure subtype of multiple myeloma.

20 11. The method of claim 8, wherein the underexpressed genes are *C20orf142*, *TP53INP*, *ST6GAL1*, *235659_at*, *YIPF6*, or *MAN2A1*.

12. The method of claim 11, wherein an underexpression of *TP53INP* diagnoses a high risk sustained control subtype of multiple myeloma.

25 13. A method for differentiating between high-risk multiple myeloma subtypes in a subject having multiple myeloma, comprising:

obtaining a plasma cell sample from the subject; and

measuring expression levels of a subset of genes consisting of
30 *C20orf142*, *TP53INP*, *ST6GAL1*, *235659_at*, *YIPF6*, *MAN2A1*, *MEIS1*, *SPIB*,
RNF43, *SHISA*, *SLC43A3*, *PLK1*, *RUNX2*, and *PMAIP1* comprising the sample;

wherein an overexpression of *SHISA* compared to control is indicative of an early failure high risk subtype and an underexpression *TP53INP*

compared to control is indicative of a sustained control high risk subtype, thereby differentiating the high risk multiple myeloma subtypes in the subject.

14. A method for treating multiple myeloma in a subject,
5 comprising:

inhibiting the expression of one or more genes *MEIS1*, *SPIB*,
RNF43, *SHISA*, *SLC43A3*, *PLK1*, *RUNX2*, or *PMAIP1*; or

amplifying the expression of one or more genes *C20orf142*,
TP53INP, *ST6GAL1*, *235659_at*, *YIPF6*, *MAN2A1* or a combination thereof,

10 thereby treating the multiple myeloma in the subject.

15 15. The method of claim 14, wherein one or both of the inhibiting
step or the amplifying step increases survivability outcome of high risk multiple
myeloma in the subject.

16. The method of claim 14, wherein the step of inhibiting *SHISA*
treats an early failure subtype of high risk multiple myeloma.

17. The method of claim 16, wherein the step of amplifying
20 *TP53INP* comprises administering one or more of bortezomib, thalidomide or
mephalan to the subject.

18. The method of claim 17, wherein the step of amplifying
TP53INP treats a sustained control subtype of multiple myeloma.

25 19. The method of claim 14, wherein amplifying expression of
TP53INP induces apoptosis in multiple myeloma cells.

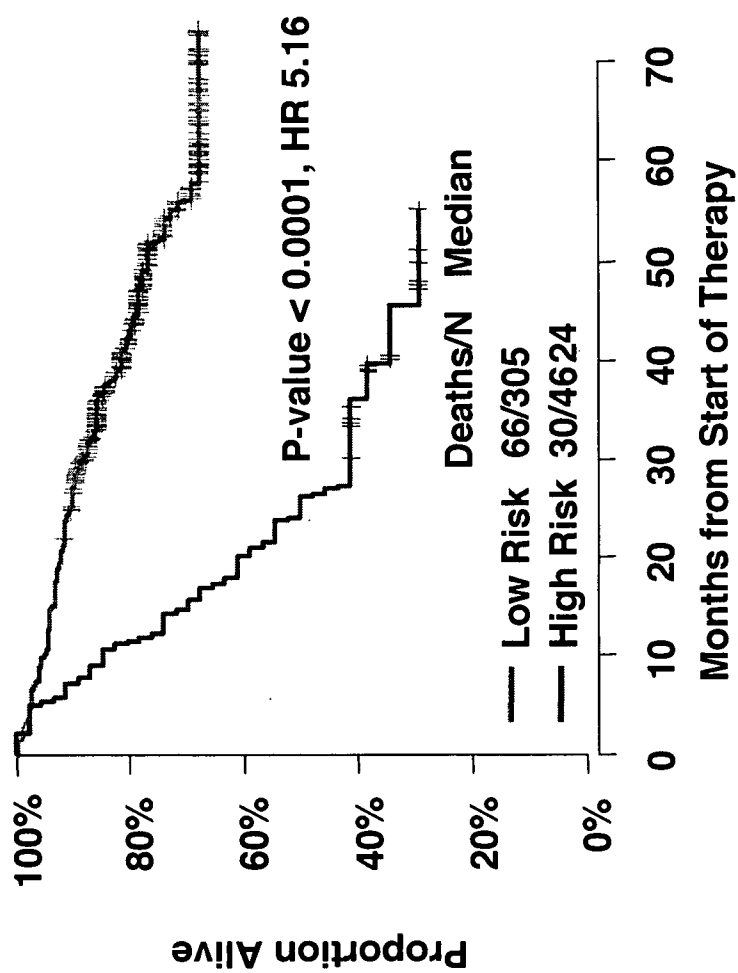


FIG. 1

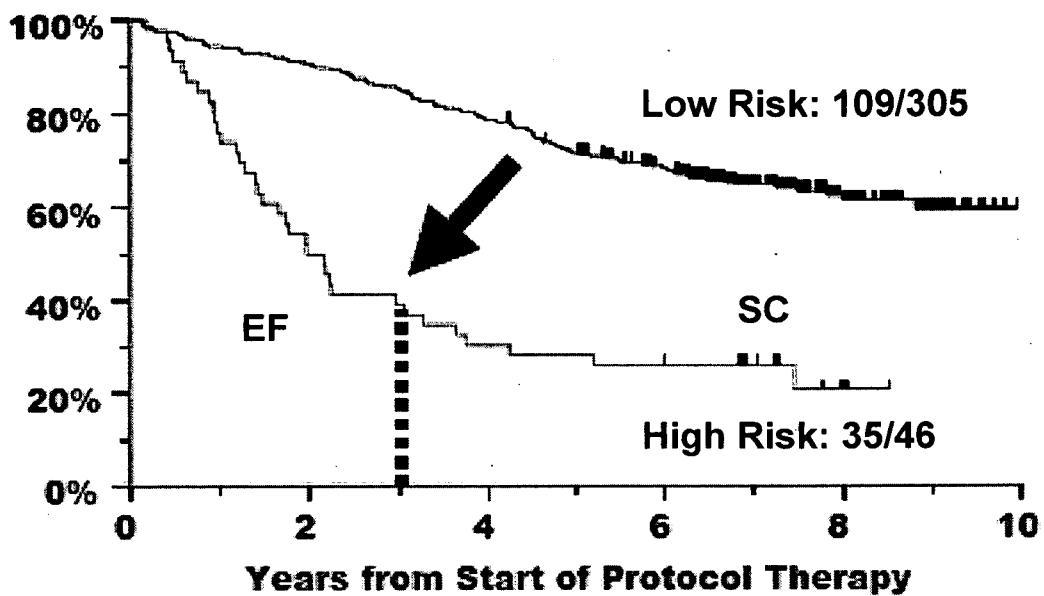


FIG. 2A

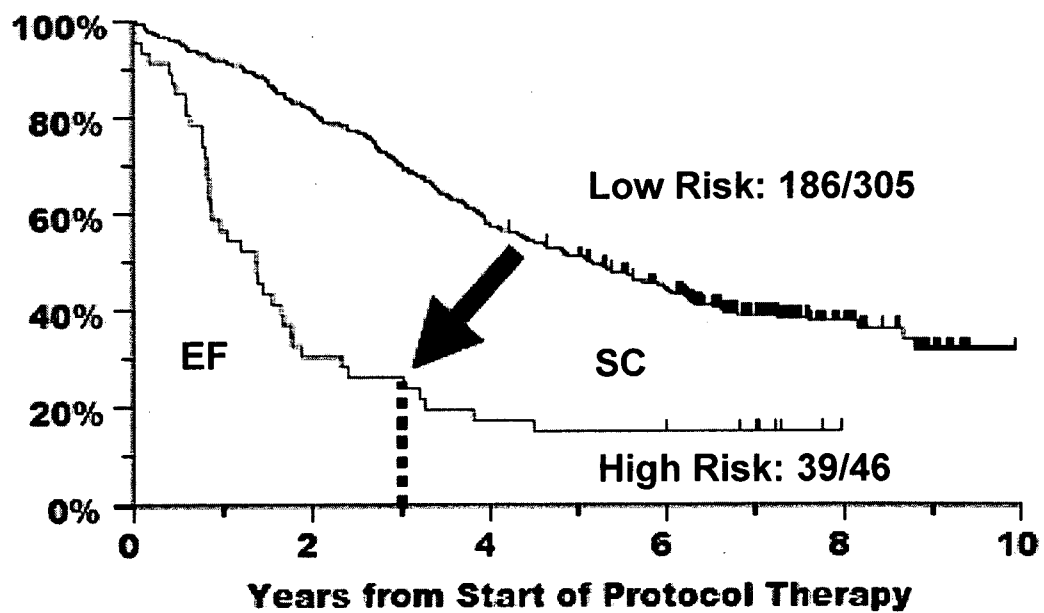


FIG. 2B

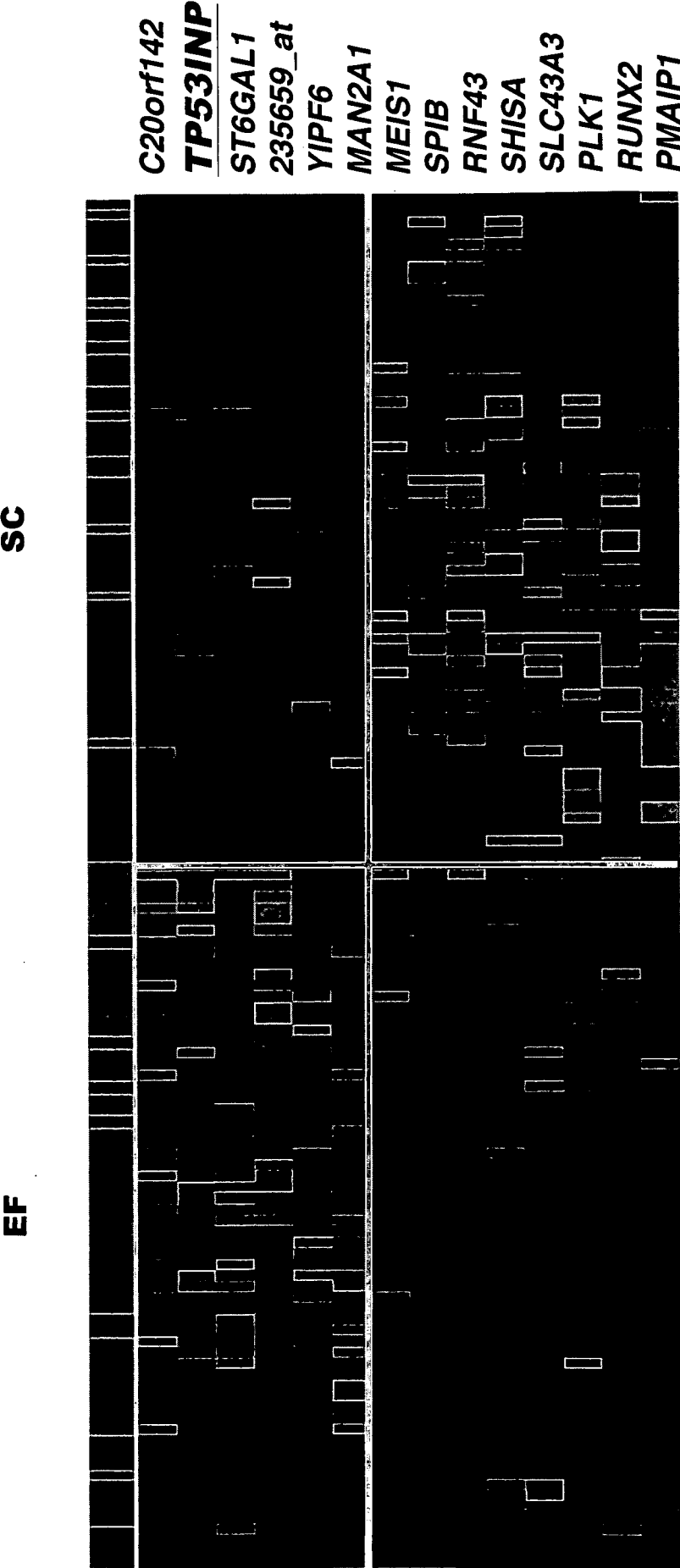


FIG. 3

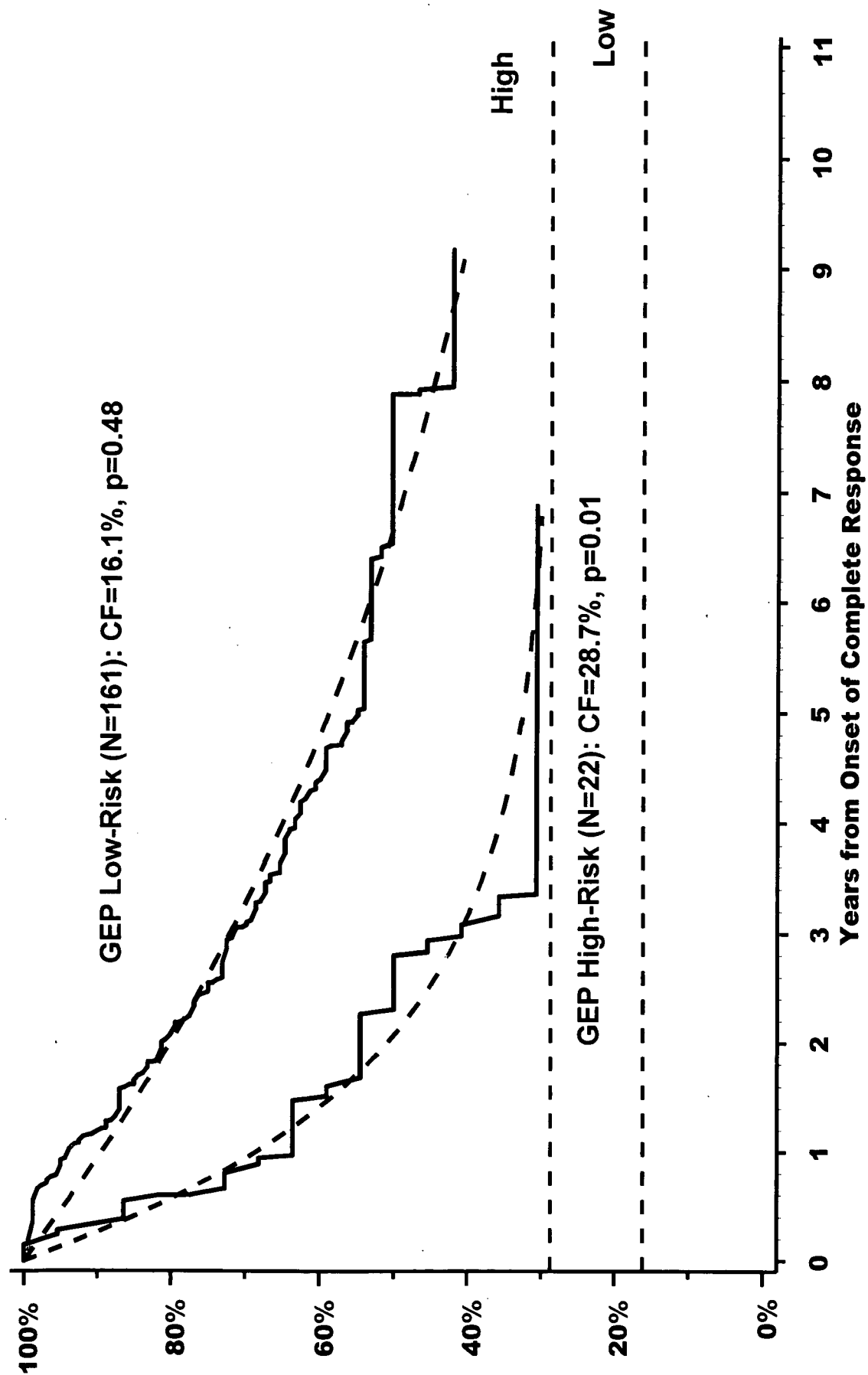


FIG. 4

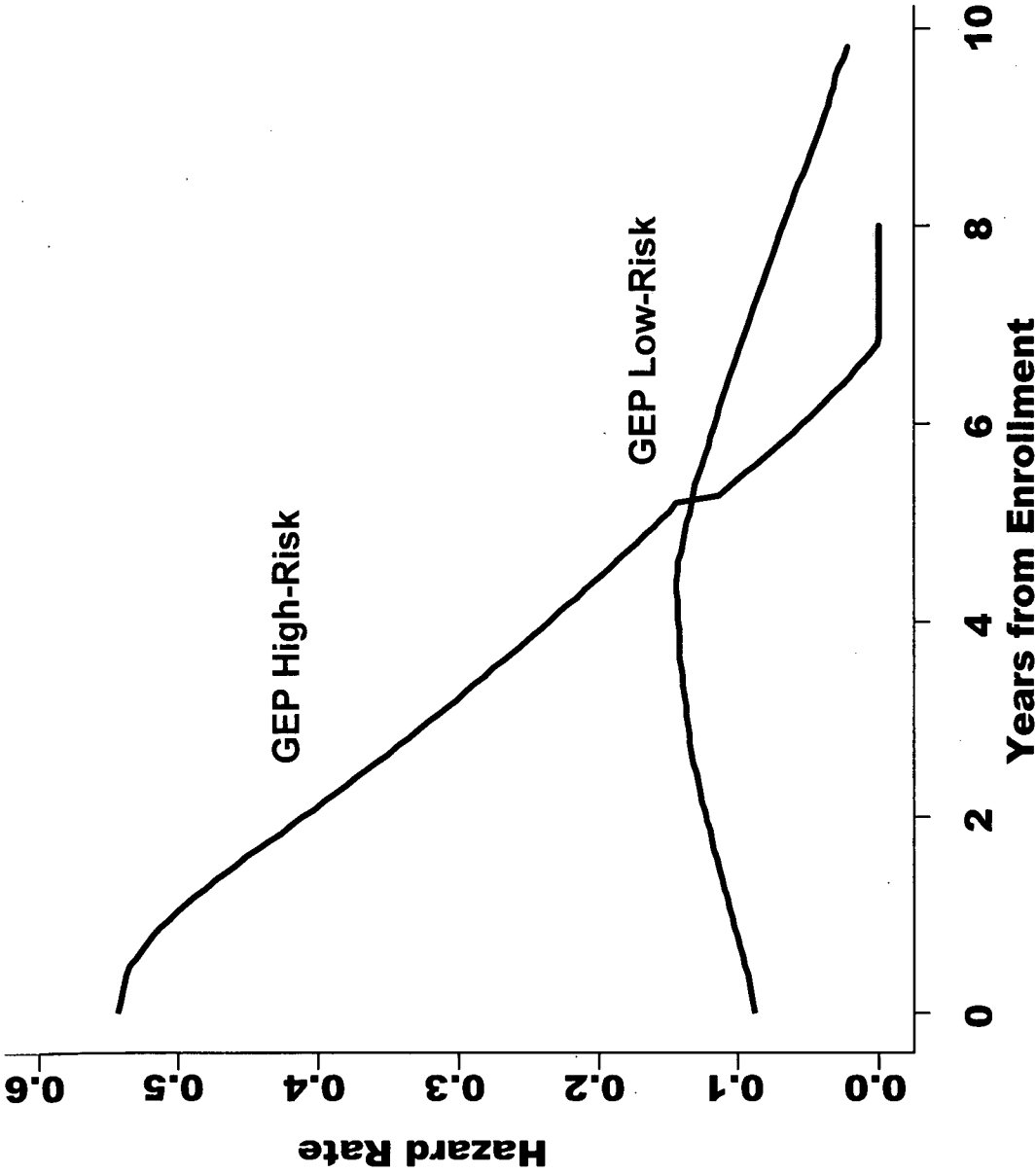


FIG. 5

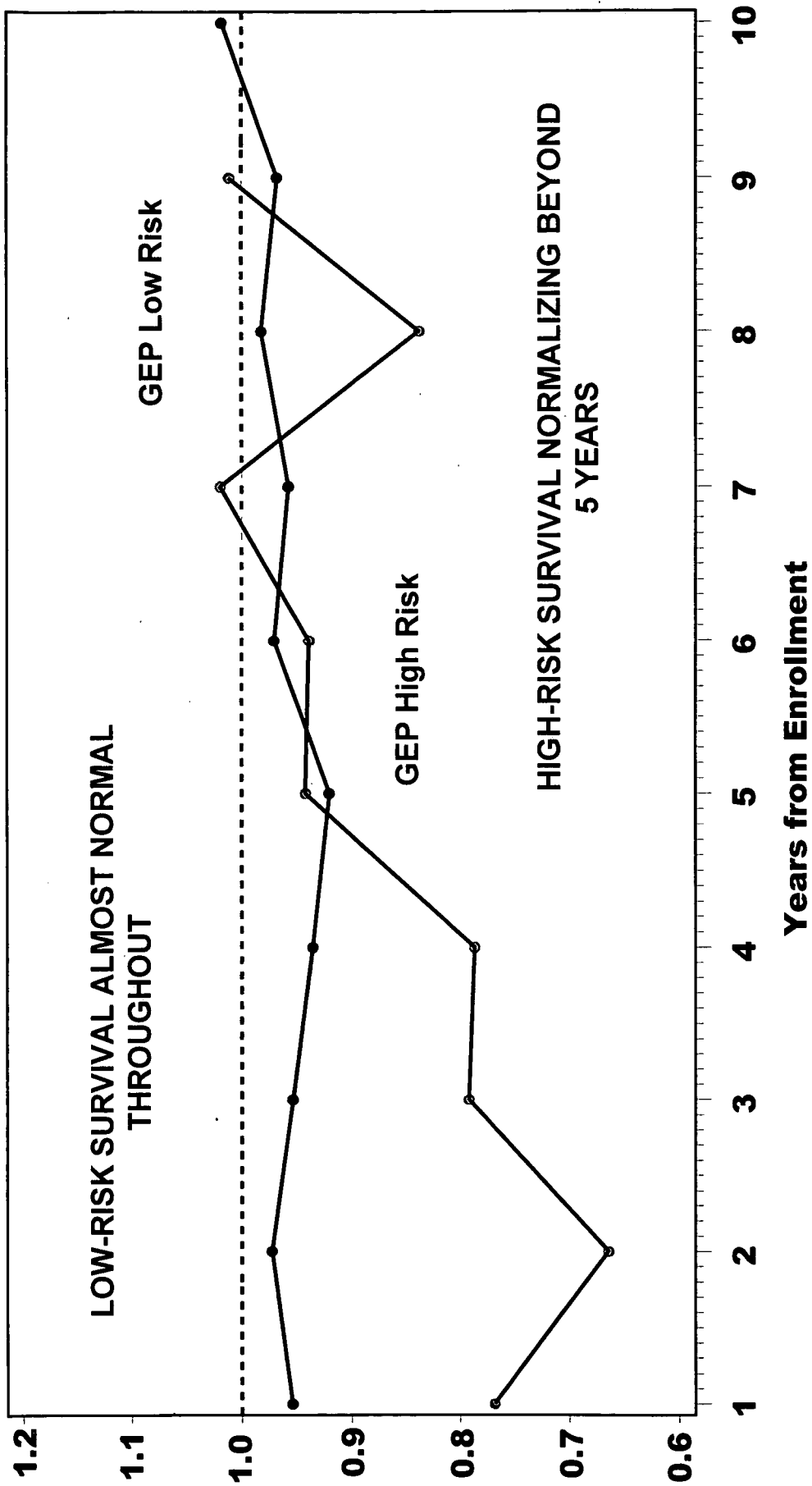


FIG. 6

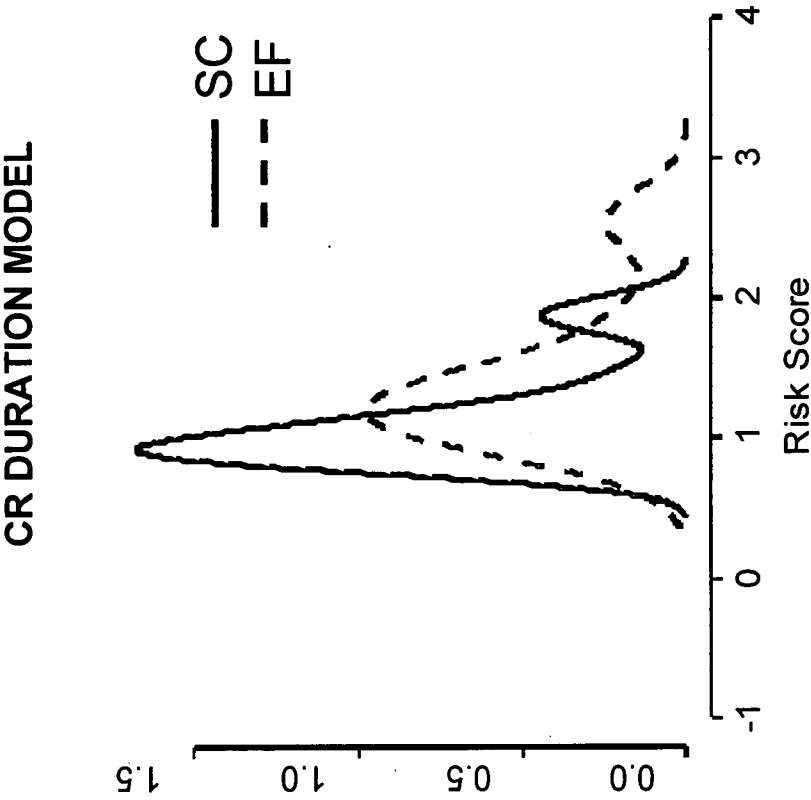


FIG. 7B

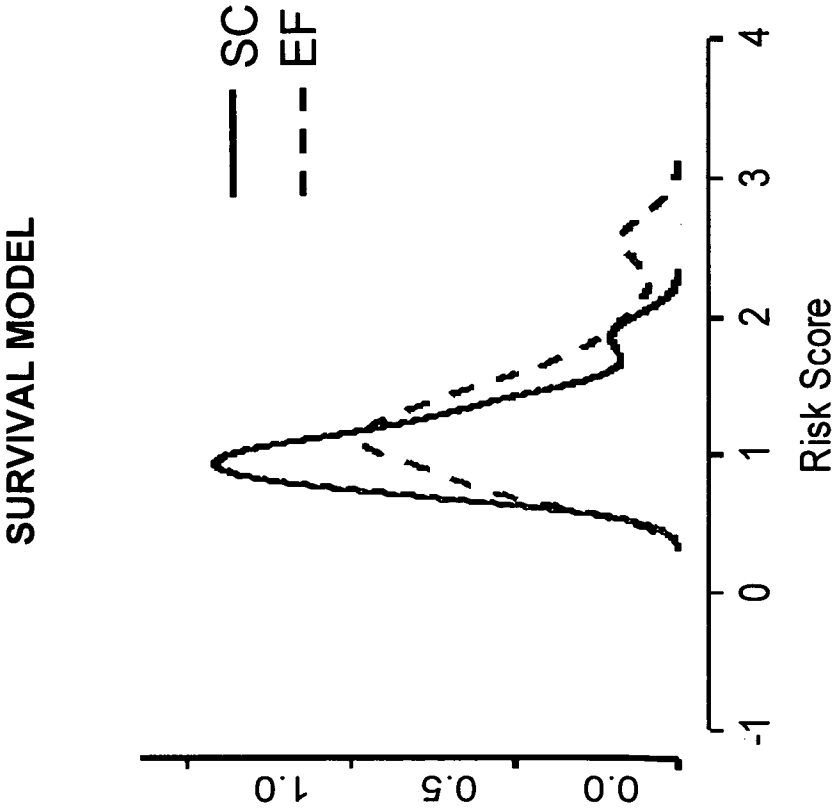


FIG. 7A

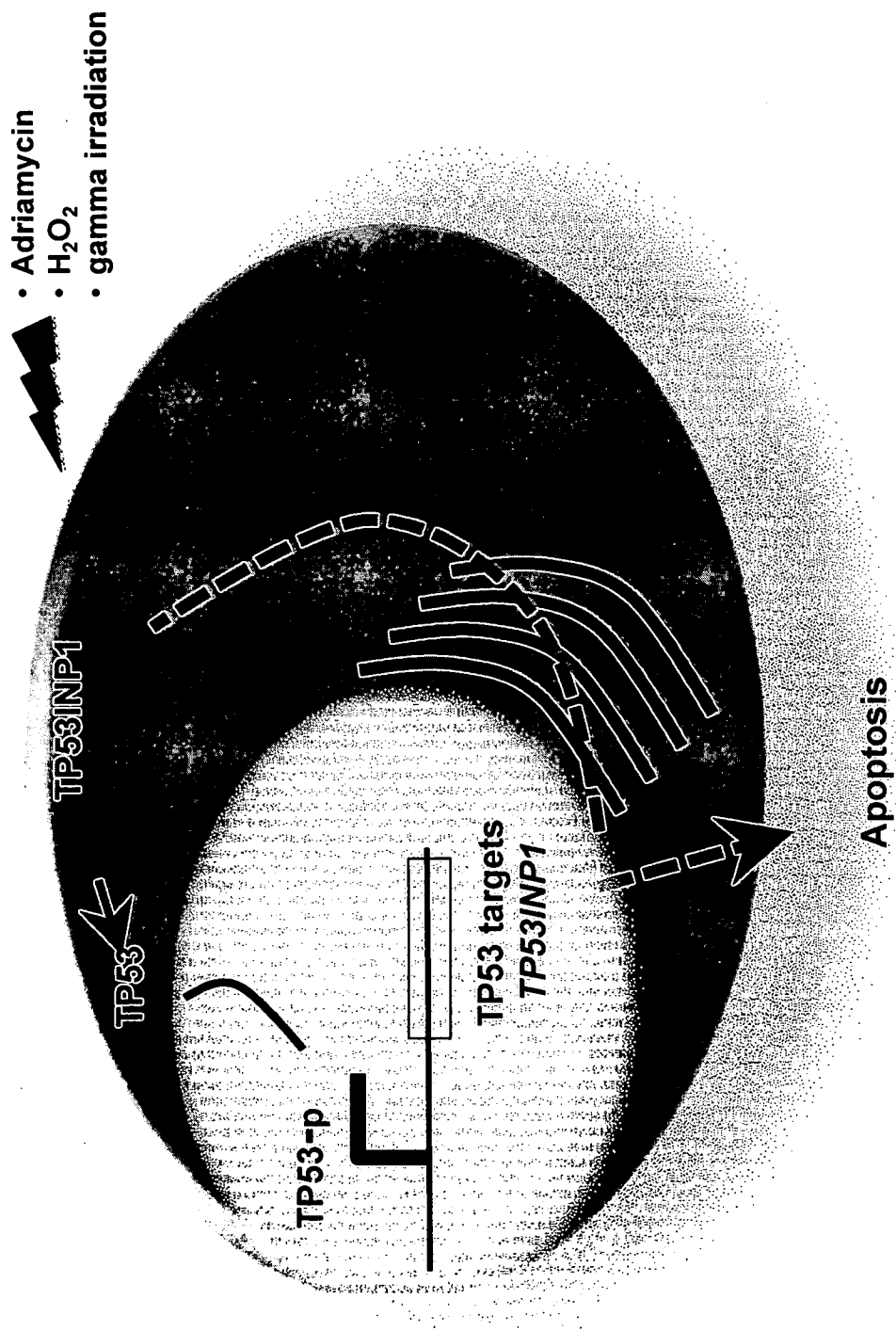
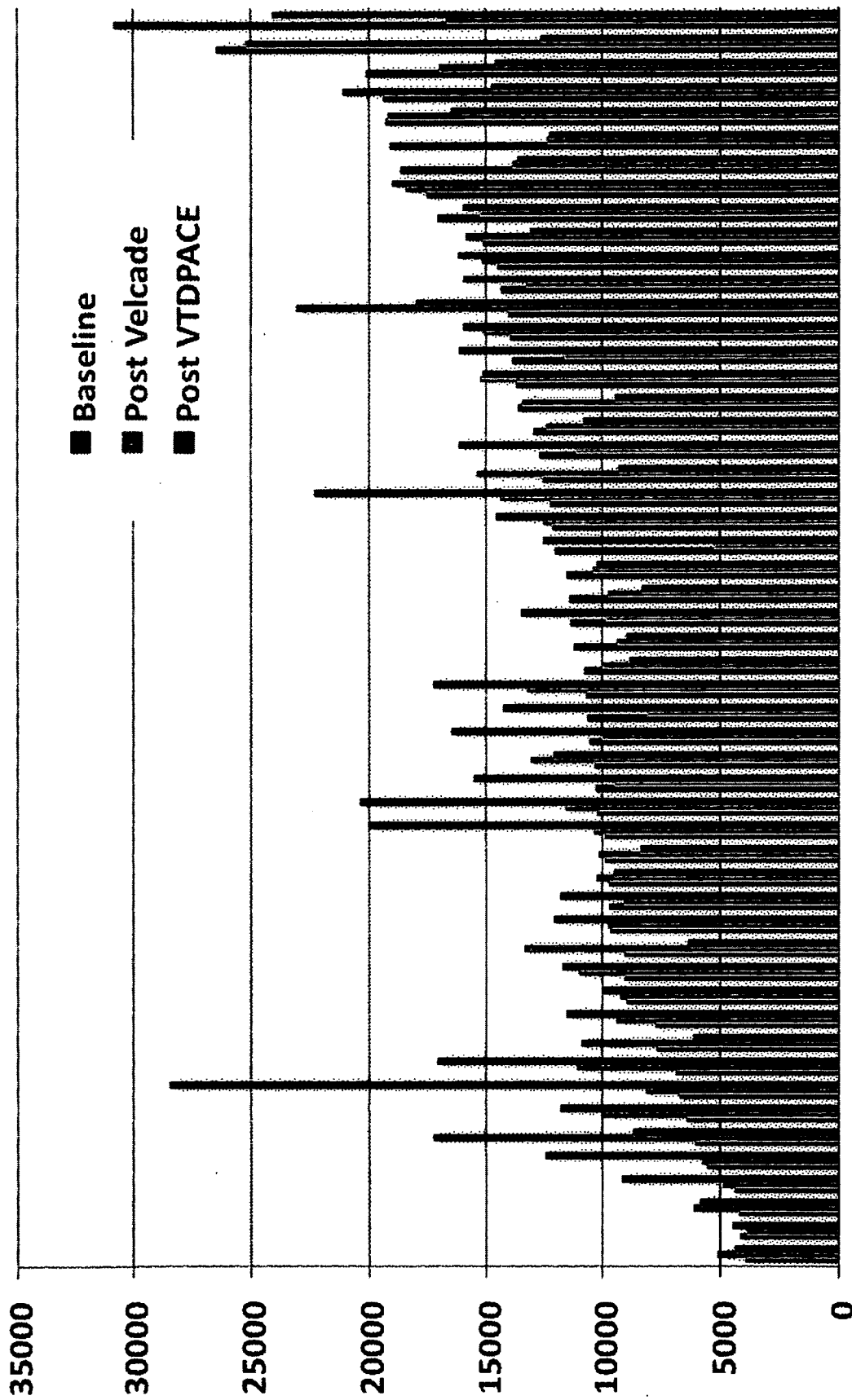
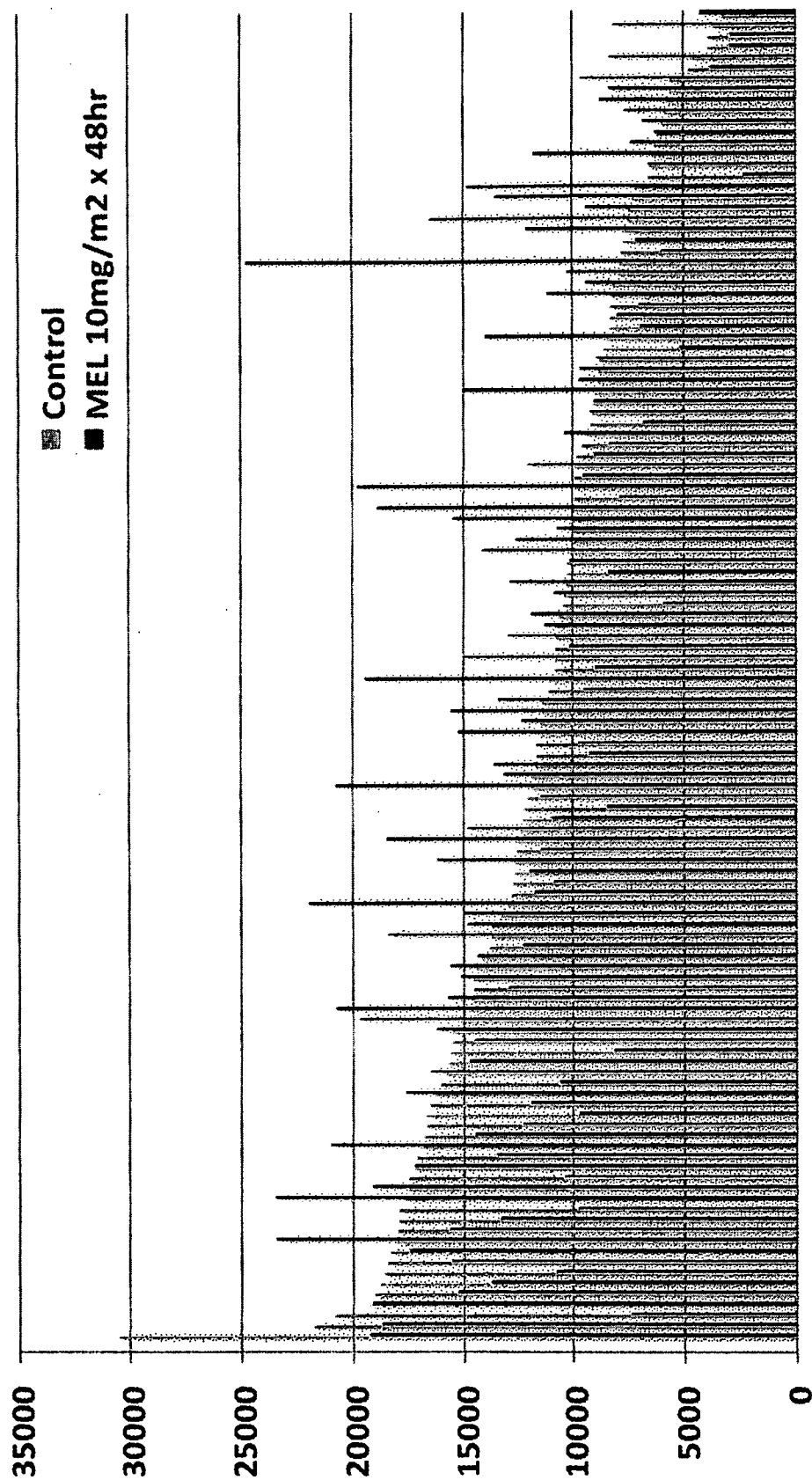


FIG. 8



**RANKING TP53INP1 BASELINE VALUES FROM LOW TO HIGH,
48HR F/U POST-VEL & VTDPACE AS PART OF TOTAL THERAPY 3**

FIG. 9



125 Newly Diagnosed Cases Enrolled in Total Therapy 4 and 5

FIG. 10