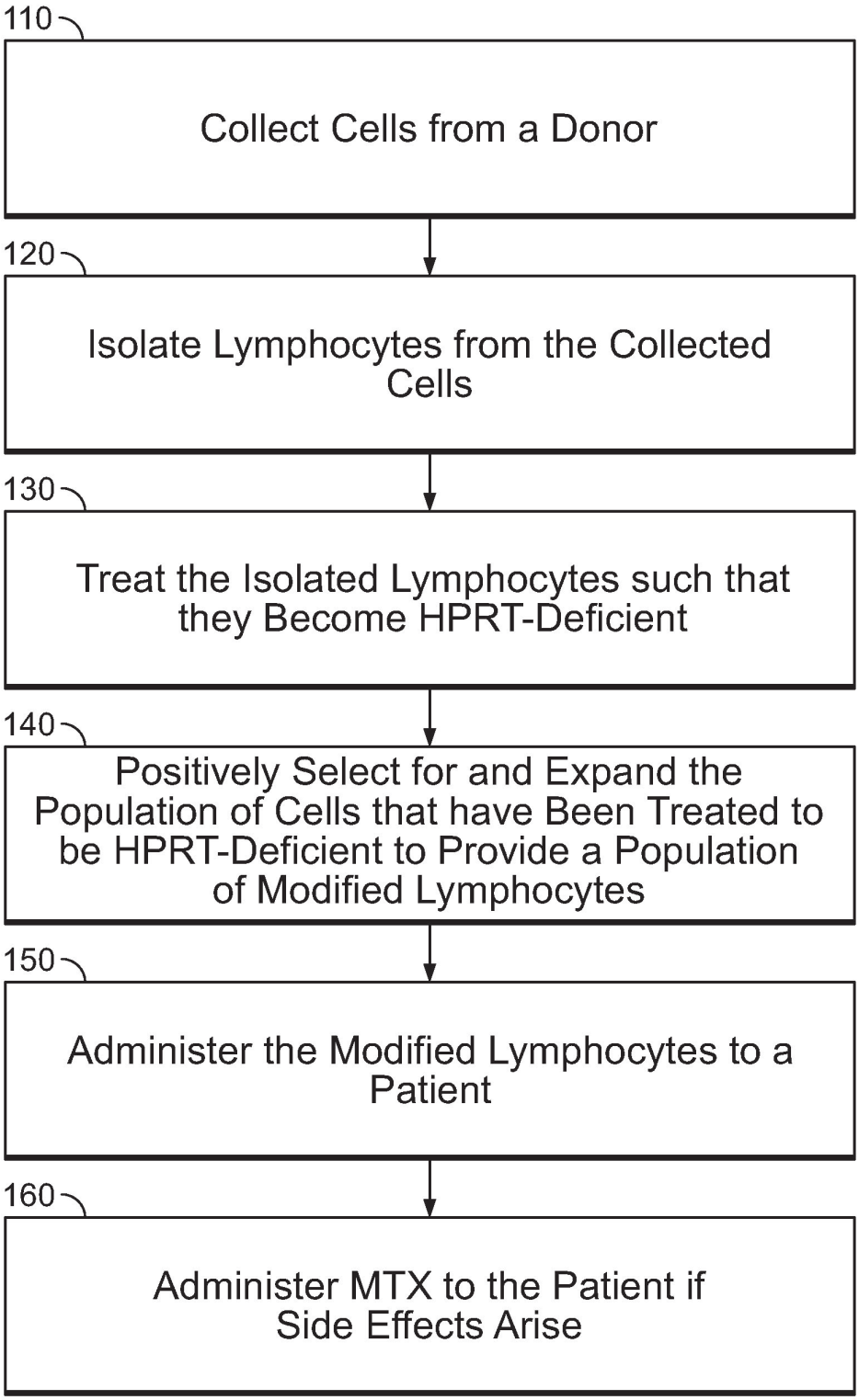
110
Collect Cells from a Donor
120
Isolate Lymphocytes from the Collected Cells
130
Treat the Isolated Lymphocytes such that they Become HPRT-Deficient
140
Positively Select for and Expand the Population of Cells that have Been Treated to be HPRT-Deficient to Provide a Population of Modified Lymphocytes
150
Administer the Modified Lymphocytes to a Patient
160
Administer MTX to the Patient if Side Effects Arise

FIG. 1

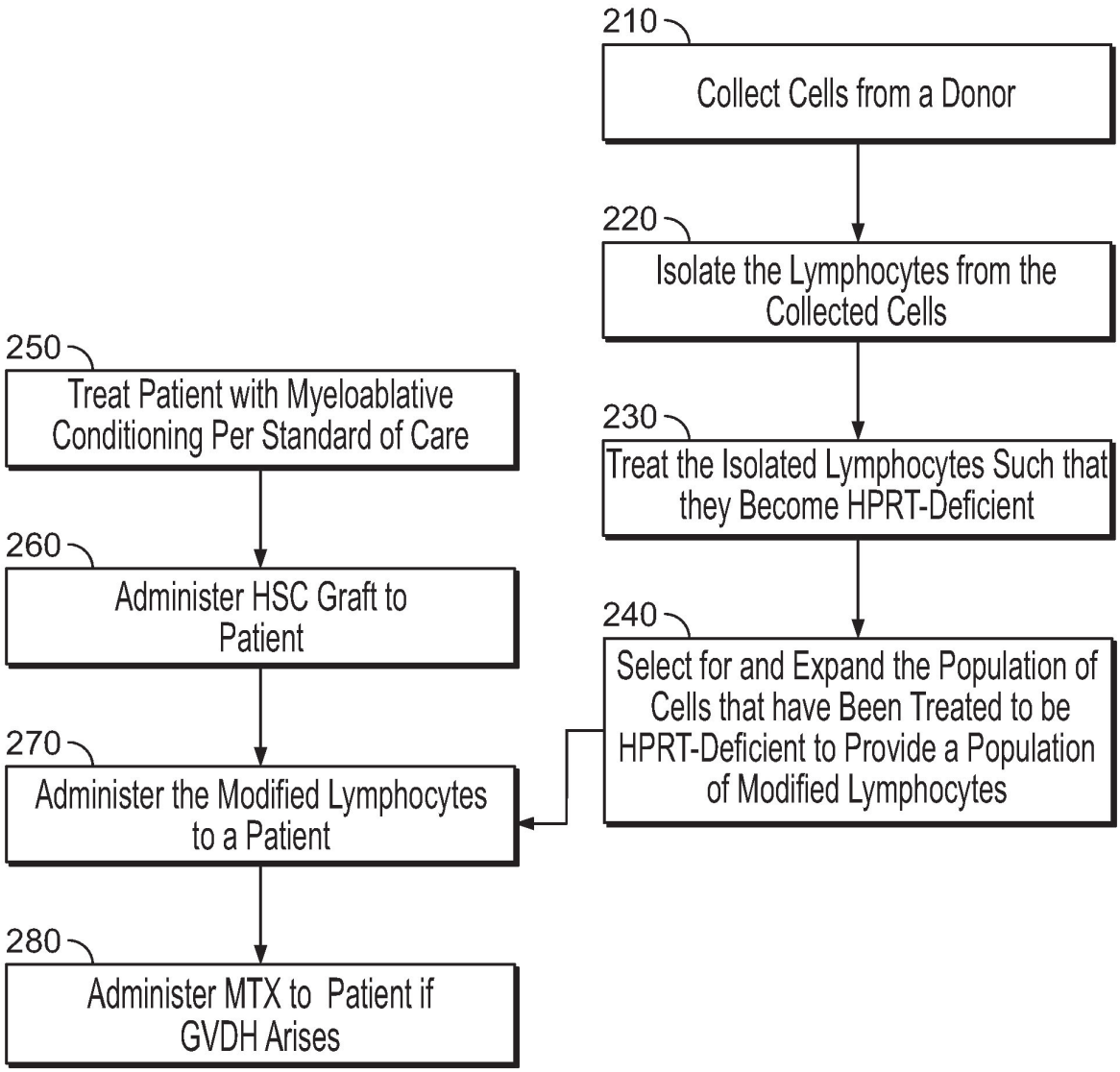
210
Collect Cells from a Donor
220
Isolate the Lymphocytes from the Collected Cells
230
Treat the Isolated Lymphocytes Such that they Become HPRT-Deficient
240
Select for and Expand the Population of Cells that have Been Treated to be HPRT-Deficient to Provide a Population of Modified Lymphocytes
250
Treat Patient with Myeloablative Conditioning Per Standard of Care
260
Administer HSC Graft to Patient
270
Administer the Modified Lymphocytes to a Patient
280
Administer MTX to Patient if GVDH Arises

FIG. 2

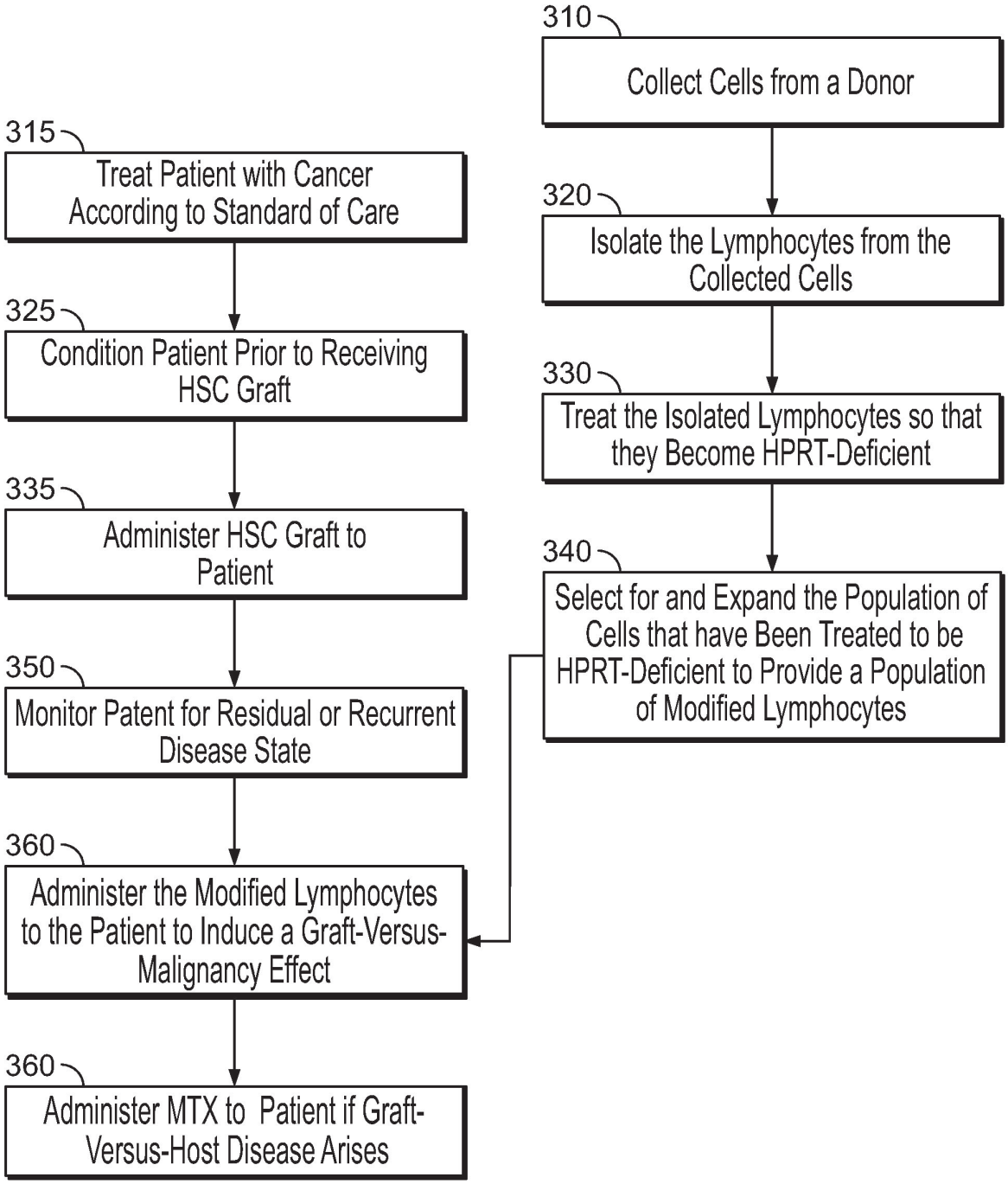
310
Collect Cells from a Donor
320
Isolate the Lymphocytes from the Collected Cells
330
Treat the Isolated Lymphocytes so that they Become HPRT-Deficient
340
Select for and Expand the Population of Cells that have Been Treated to be HPRT-Deficient to Provide a Population of Modified Lymphocytes
315
Treat Patient with Cancer According to Standard of Care
325
Condition Patient Prior to Receiving HSC Graft
335
Administer HSC Graft to Patient
350
Monitor Patent for Residual or Recurrent Disease State
360
Administer the Modified Lymphocytes to the Patient to Induce a Graft-Versus-Malignancy Effect
360
Administer MTX to Patient if Graft-Versus-Host Disease Arises

FIG. 3

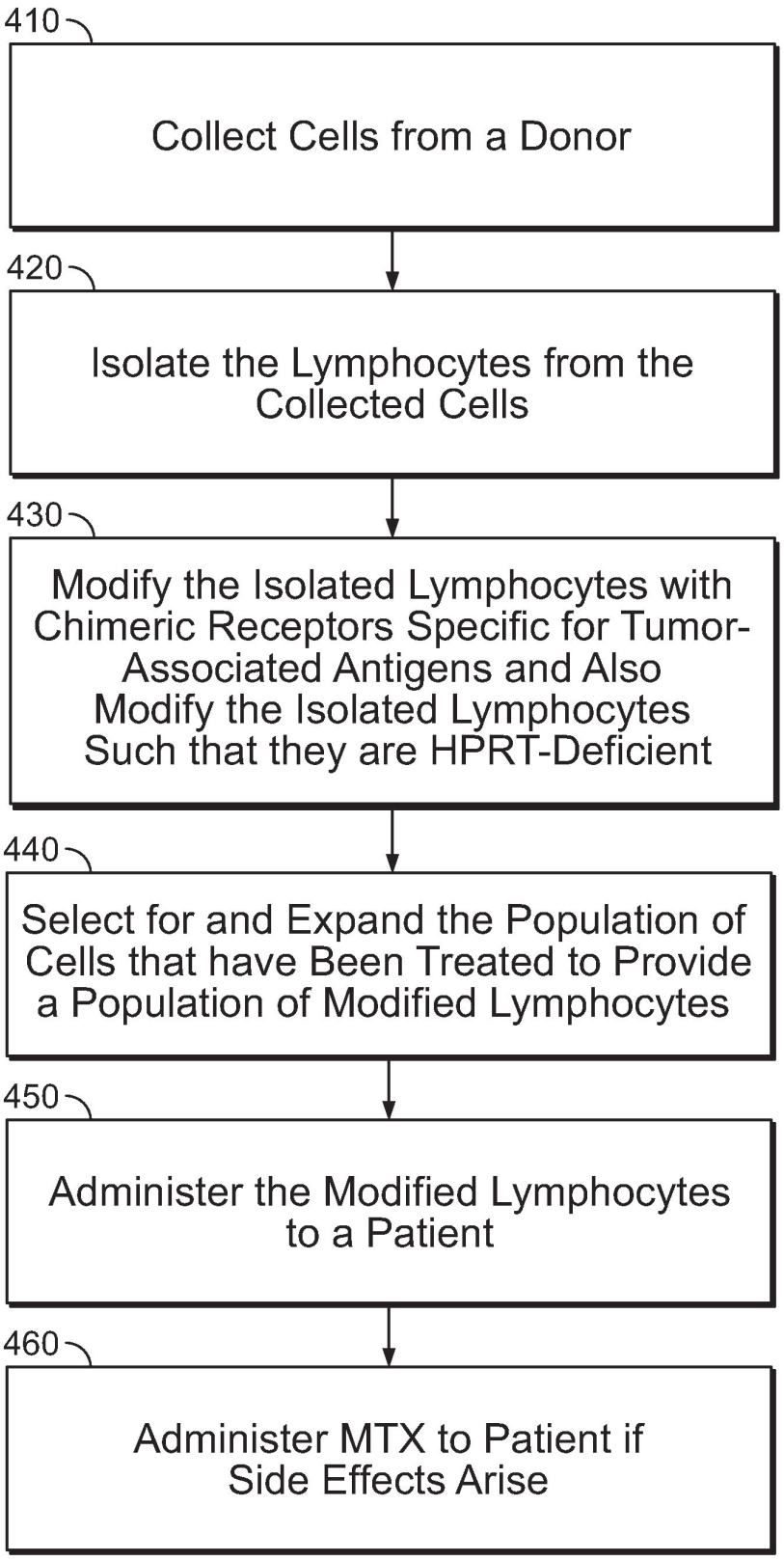
410
Collect Cells from a Donor
420
Isolate the Lymphocytes from the Collected Cells
430
Modify the Isolated Lymphocytes with Chimeric Receptors Specific for Tumor-Associated Antigens and Also Modify the Isolated Lymphocytes Such that they are HPRT-Deficient
440
Select for and Expand the Population of Cells that have Been Treated to Provide a Population of Modified Lymphocytes
450
Administer the Modified Lymphocytes to a Patient
460
Administer MTX to Patient if Side Effects Arise

FIG. 4

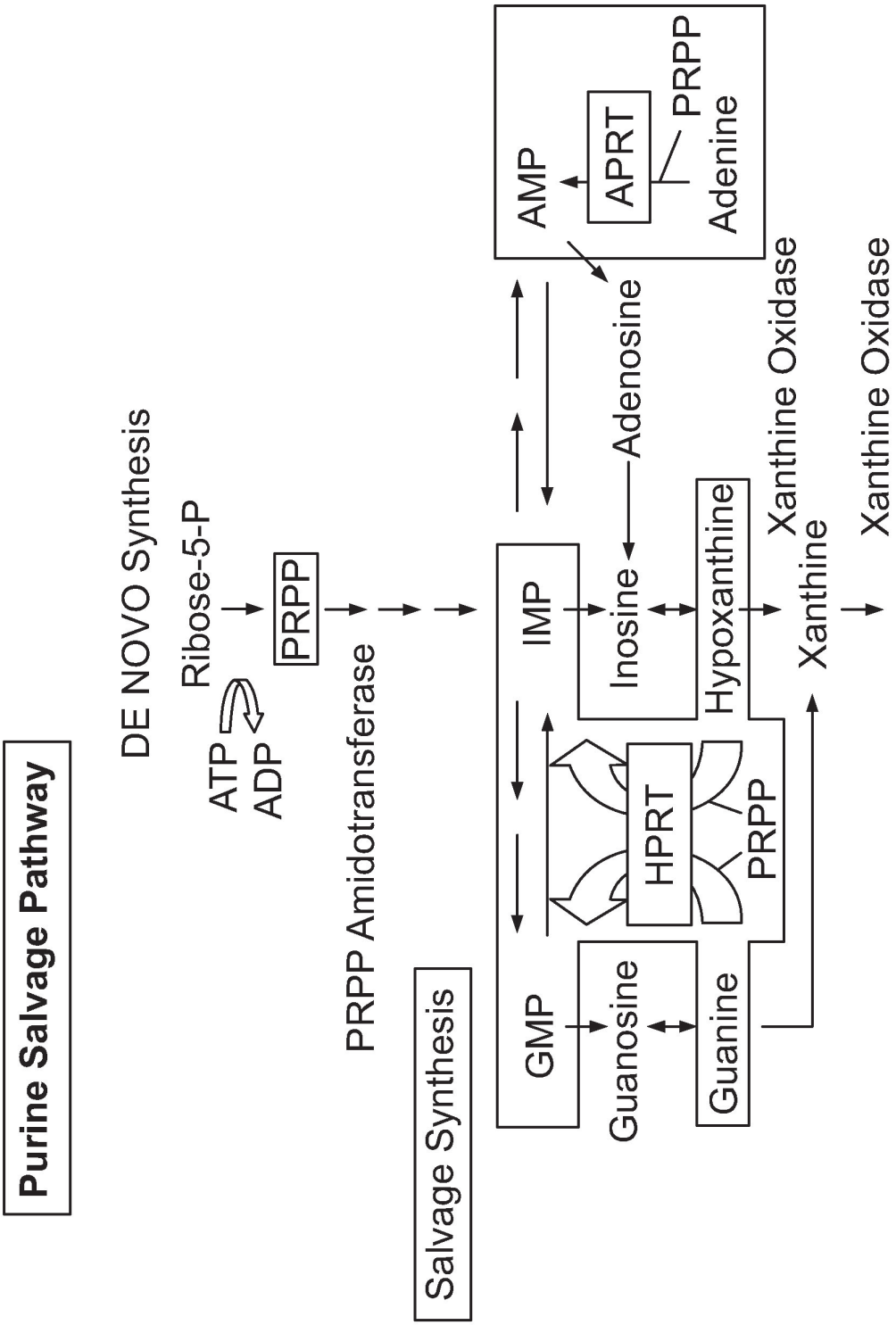
Purine Salvage Pathway
DE NOVO Synthesis
Ribose-5-P
ATP
ADP
PRPP
PRPP Amidotransferase
Salvage Synthesis
IMP
AMP
APRT
PRPP
Adenine
Adenosine
Inosine
Hypoxanthine
Xanthine Oxidase
Xanthine
Xanthine Oxidase
Uric Acid
GMP
Guanosine
Guanine
HPRT
PRPP

FIG. 5

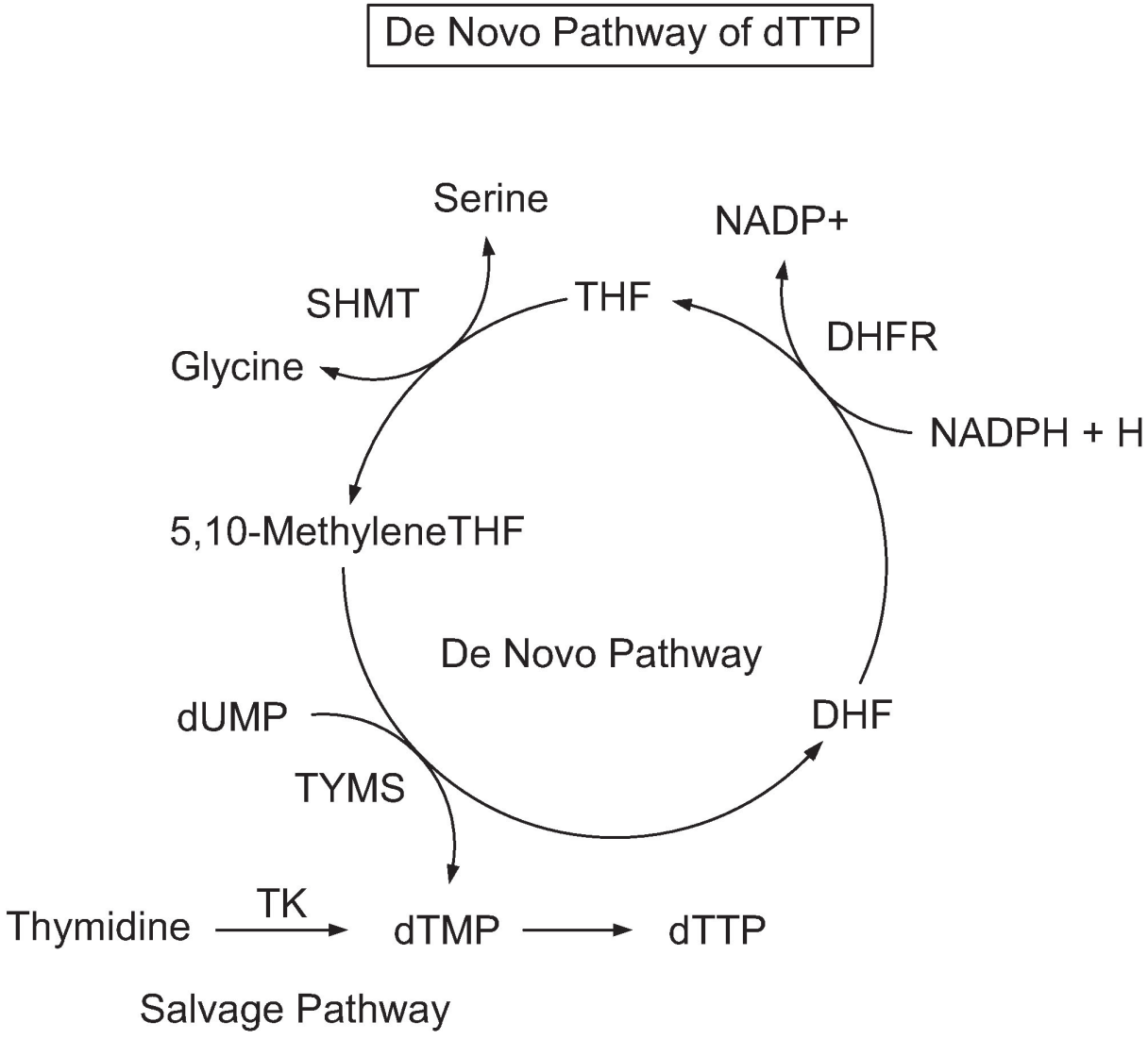
De Novo Pathway of dTTP
Serine
NADP+
SHMT
THF
DHFR
Glycine
NADPH + H
5,10-MethyleneTHF
De Novo Pathway
dUMP
DHF
TYMS
Thymidine
TK
dTMP
dTTP
Salvage Pathway

FIG. 6

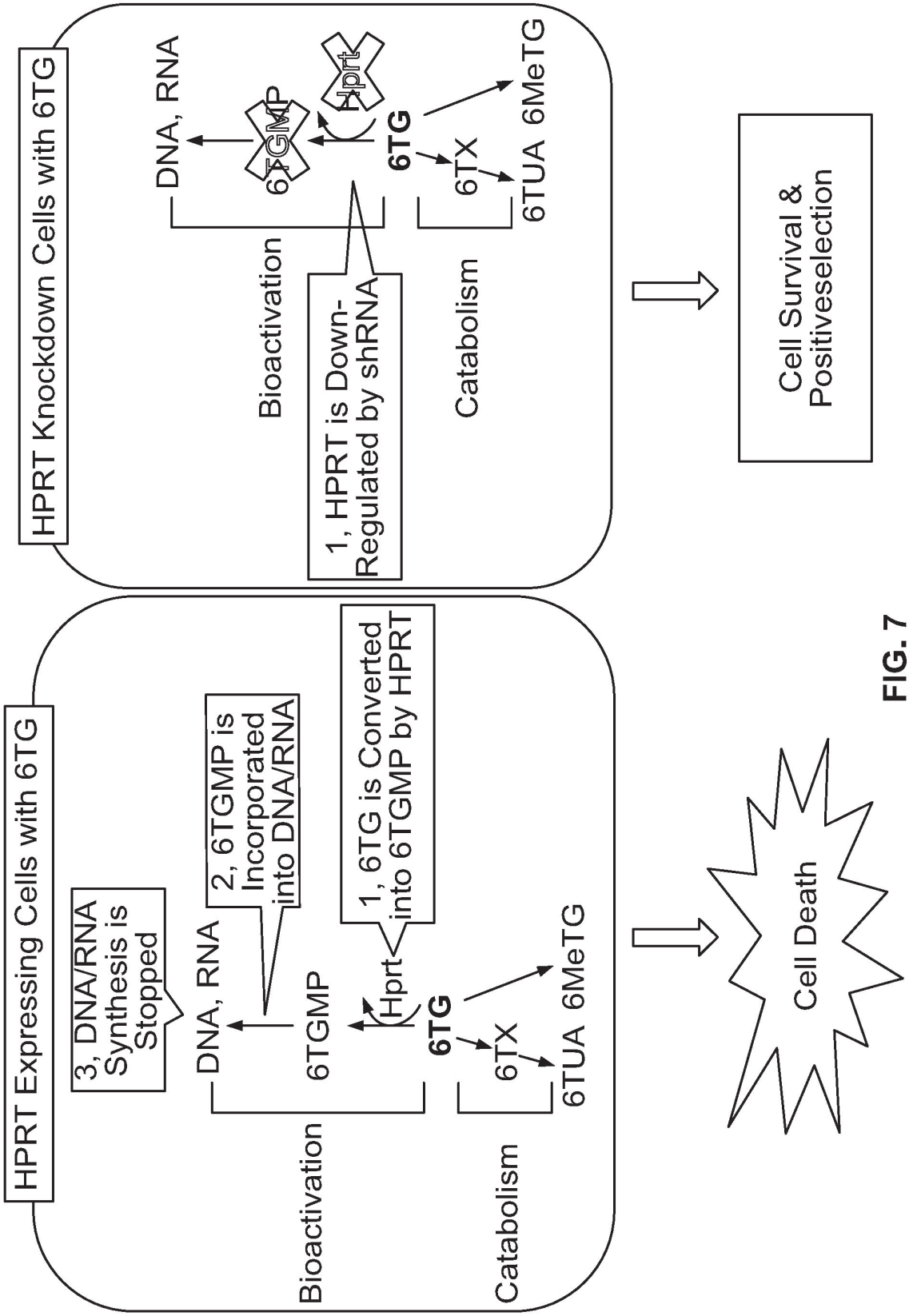
HPRT Expressing Cells with 6TG
3, DNA/RNA Synthesis is Stopped
DNA, RNA
2, 6TGMP is Incorporated into DNA/RNA
6TGMP
1, 6TG is Converted into 6TGMP by HPRT
Hprt
6TG
6TX
6TUA 6MeTG
Bioactivation
Catabolism
Cell Death
HPRT Knockdown Cells with 6TG
DNA, RNA
6TGMP
Hprt
6TG
6TX
6TUA 6MeTG
Bioactivation
1, HPRT is Down-Regulated by shRNA
Catabolism
Cell Survival & Positiveselection

FIG. 7

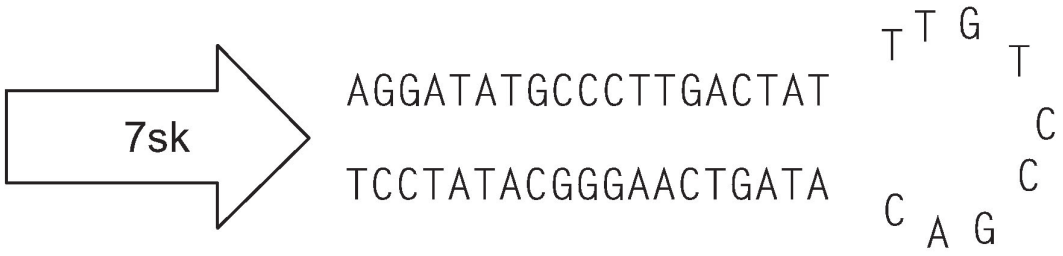
7sk
AGGATATGCCCTTGACTAT
TCCTATACGGGAACTGATA
T T G
T
C
C
C A G

FIG. 8

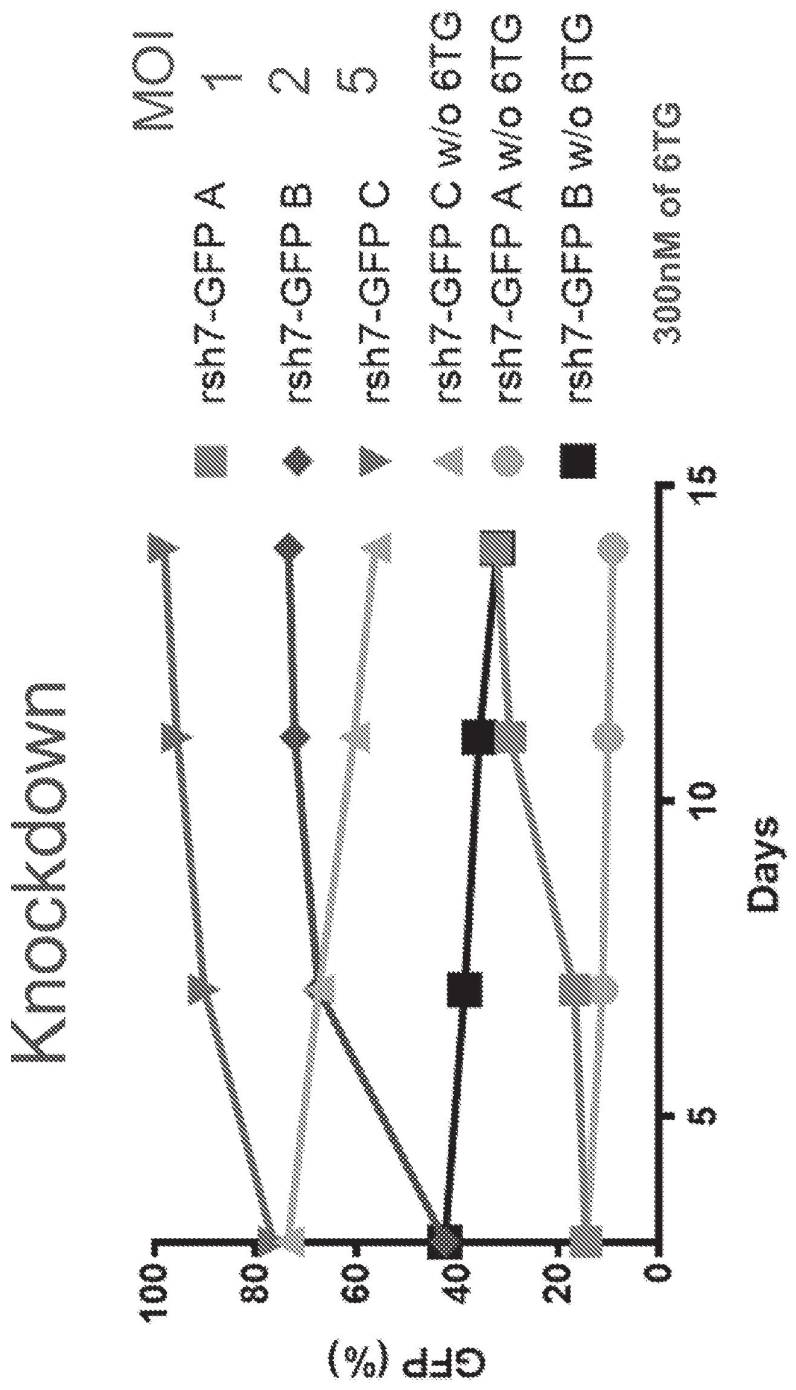
Knockdown
GFP (%)
100
80
60
40
20
0
5
10
15
Days
MOI
1
2
5
rsh7-GFP A
rsh7-GFP B
rsh7-GFP C
rsh7-GFP C w/o 6TG
rsh7-GFP A w/o 6TG
rsh7-GFP B w/o 6TG
300nM of 6TG

FIG. 9A

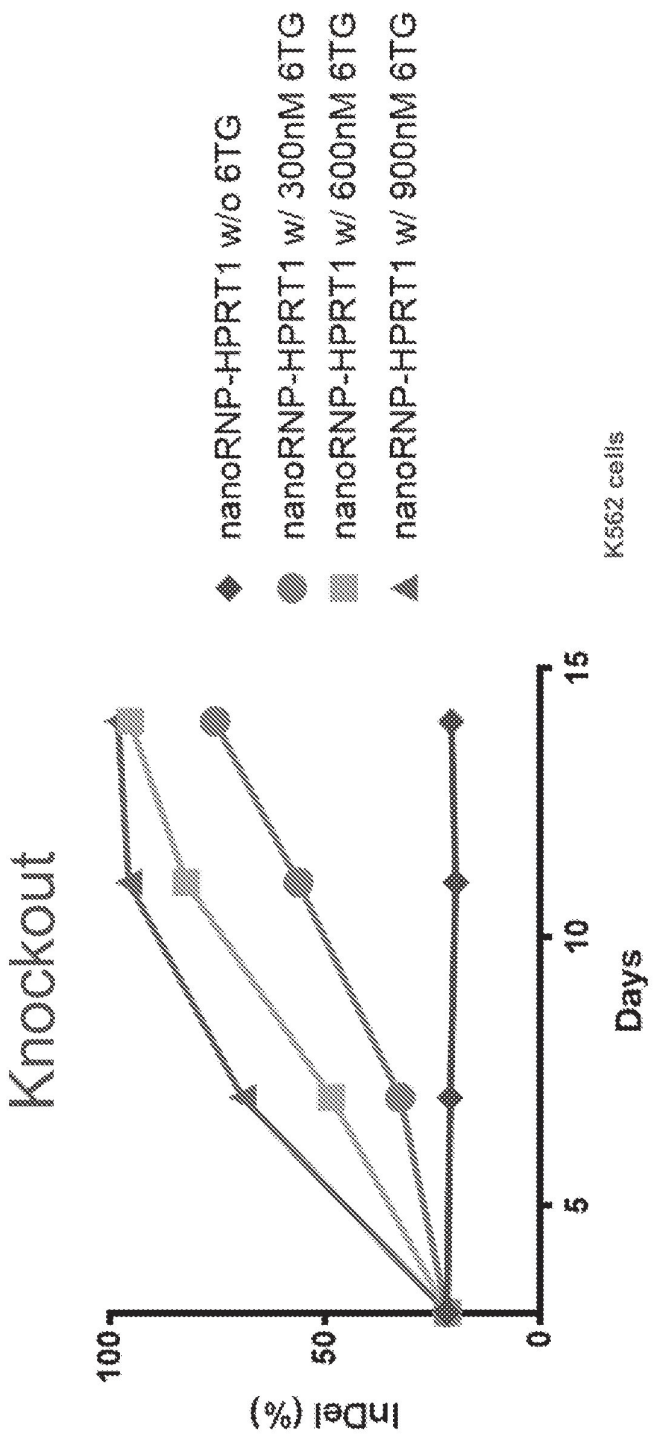
Knockout
InDel (%)
100
50
0
5
10
15
Days
nanoRNP-HPRT1 w/o 6TG
nanoRNP-HPRT1 w/ 300nM 6TG
nanoRNP-HPRT1 w/ 600nM 6TG
nanoRNP-HPRT1 w/ 900nM 6TG
K562 cells

FIG. 9B

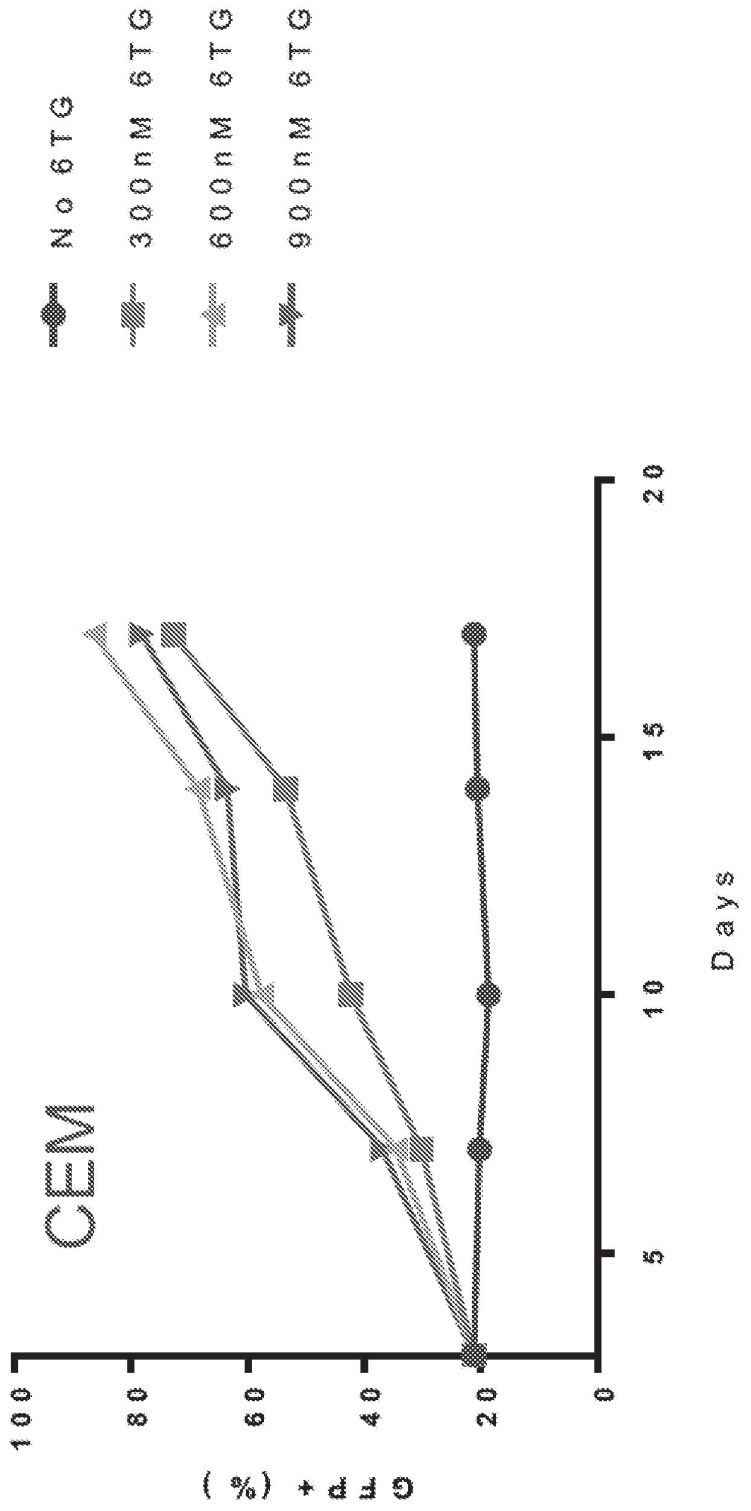
No 6TG
300nM 6TG
600nM 6TG
900nM 6TG
CEM
GFP+ (%)
100
80
60
40
20
0
5
10
15
20
Days

FIG. 10A

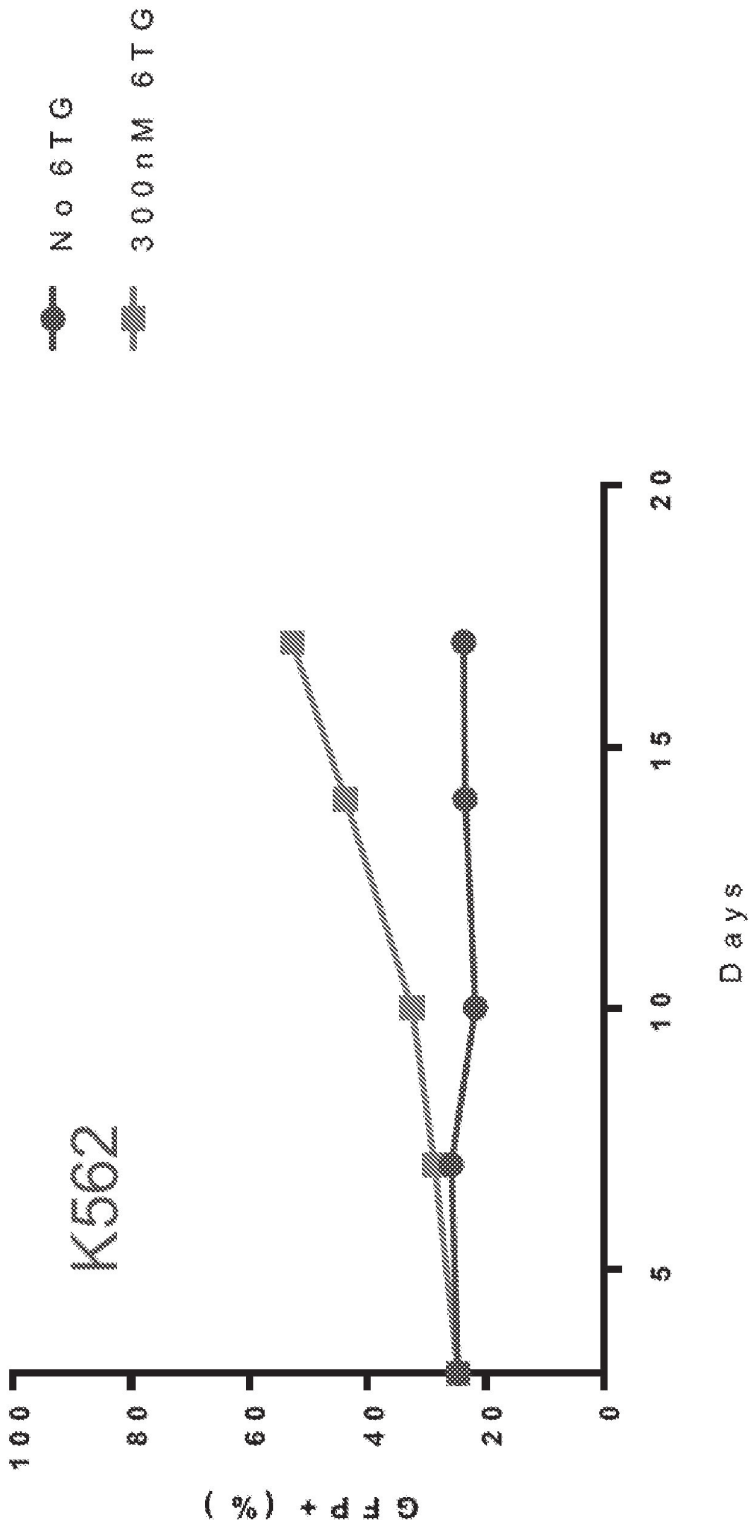

FIG. 10B

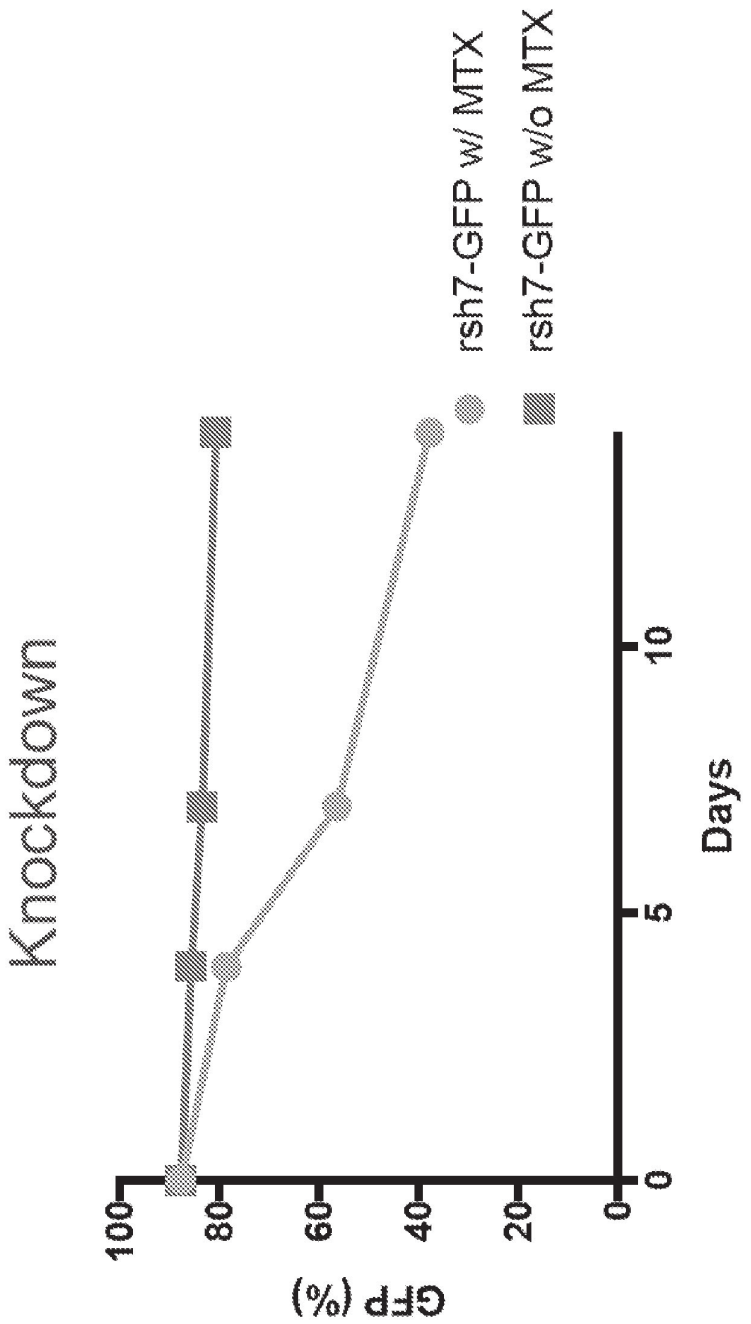
Knockdown
rsh7-GFP w/ MTX
rsh7-GFP w/o MTX
GFP (%)
100
80
60
40
20
0
0
5
10
Days

FIG. 11A

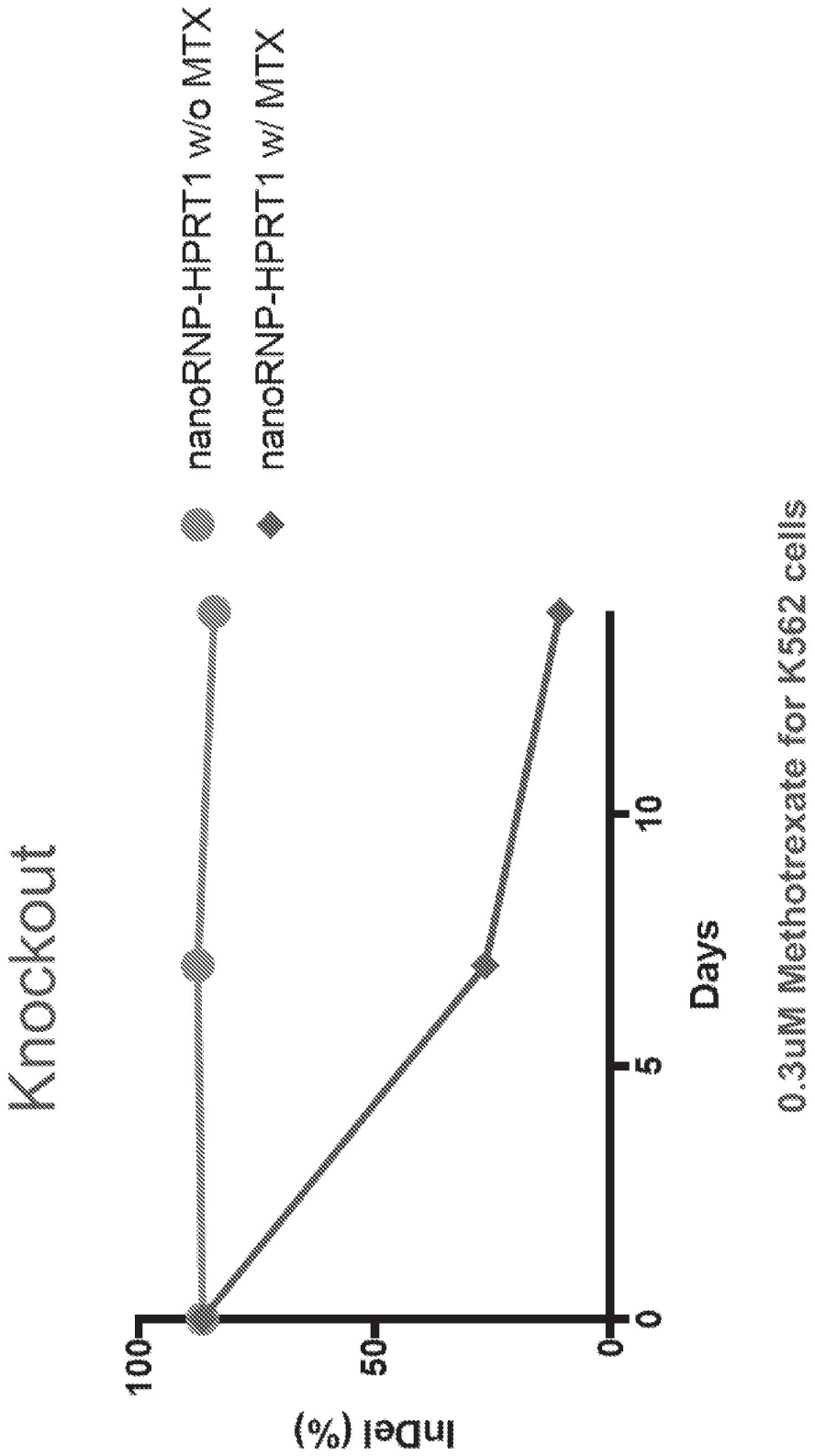
Knockout
nanoRNP-HPRT1 w/o MTX
nanoRNP-HPRT1 w/ MTX
InDel (%)
100
50
0
0
5
10
Days
0.3uM Methotrexate for K562 cells

FIG. 11B

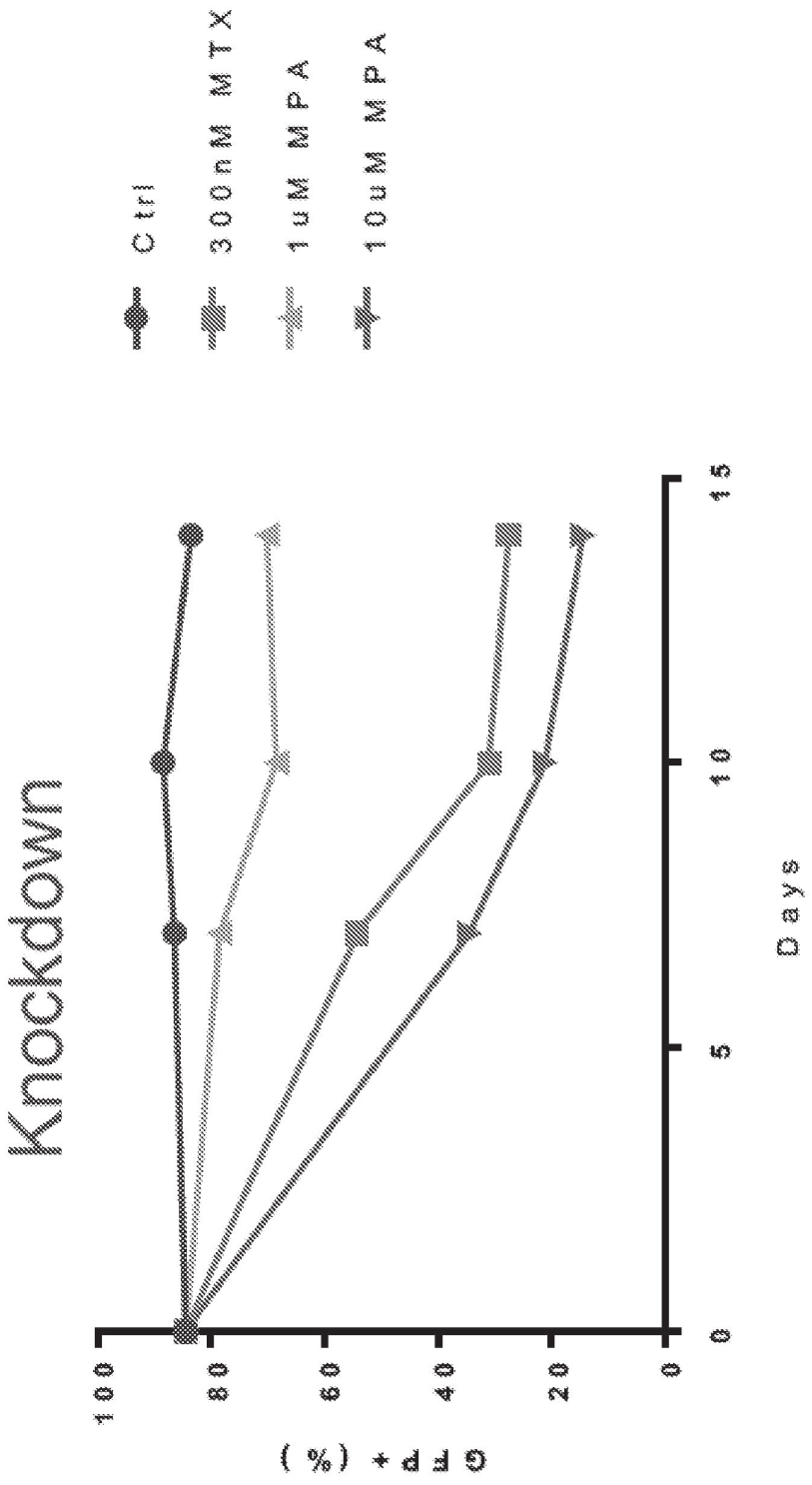
Knockdown
Ctrl
300nM MTX
1uM MPA
10uM MPA
GFP+ (%)
100
80
60
40
20
0
0
5
10
15
Days

FIG. 12A

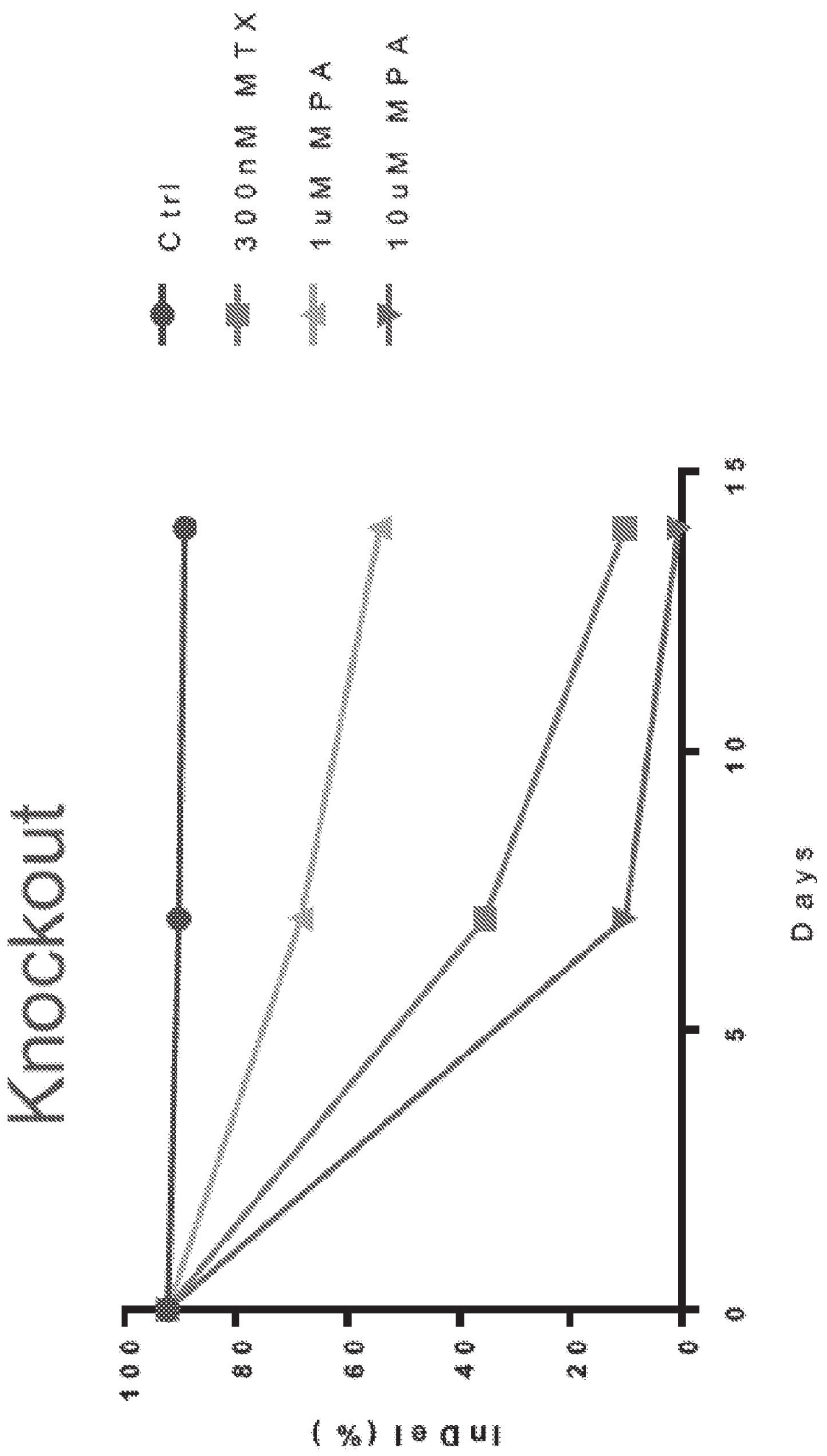
Knockout
Ctrl
300nM MTX
1uM MPA
10uM MPA
Indel (%)
100
80
60
40
20
0
0
5
10
15
Days

FIG. 12B

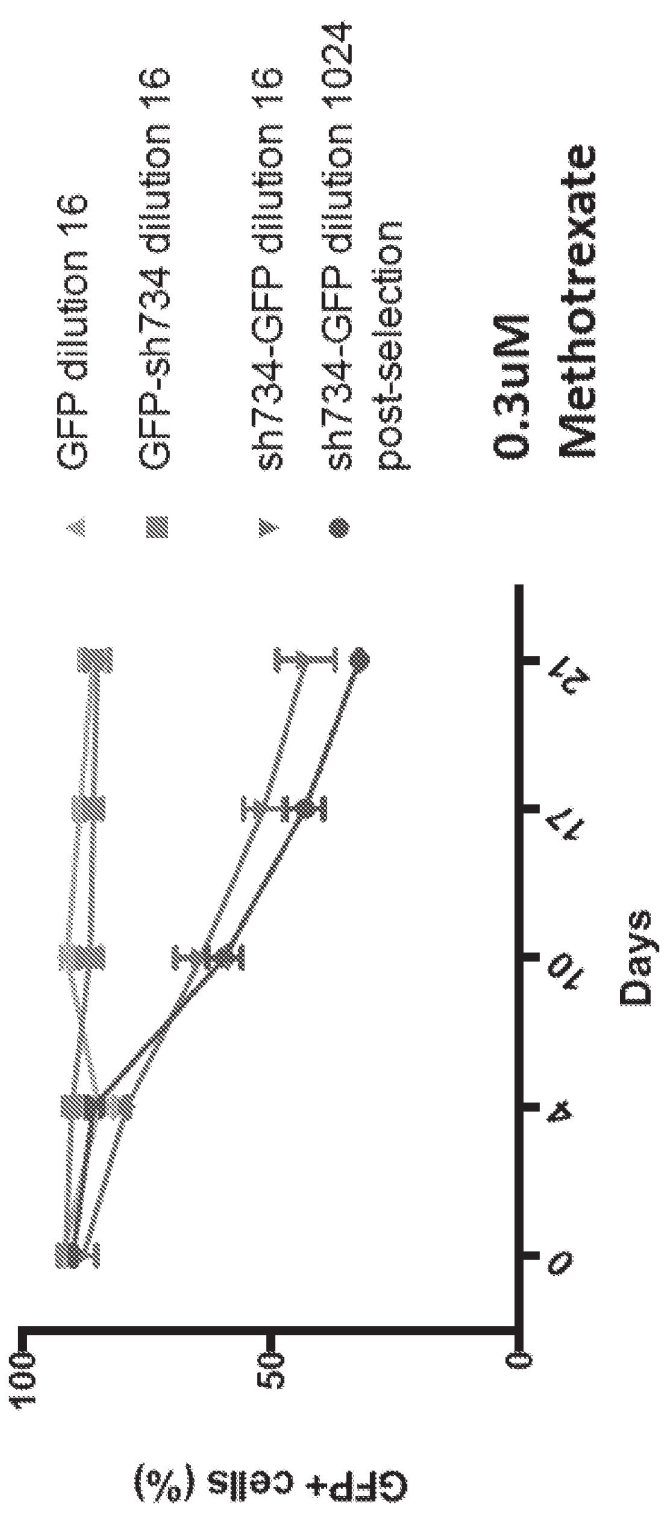
GFP dilution 16
GFP-sh734 dilution 16
sh734-GFP dilution 16
sh734-GFP dilution 1024
post-selection
0.3uM
Methotrexate
GFP+ cells (%)
100
50
0
0
4
10
17
24
Days

FIG. 13

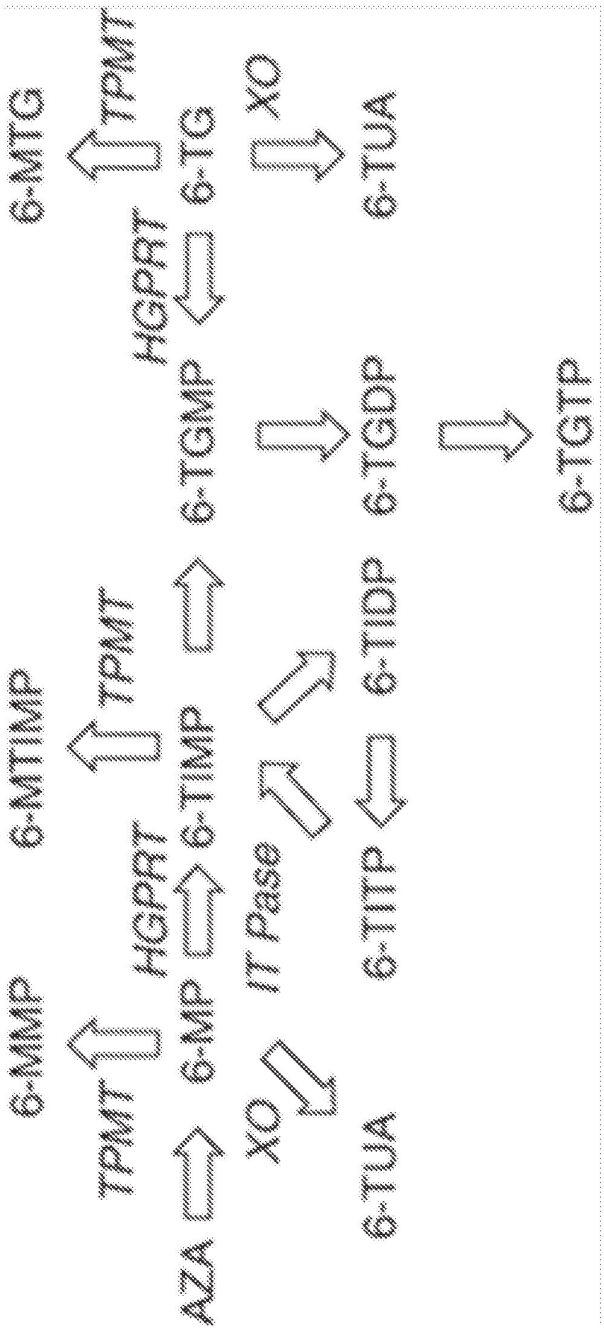
6-MTG
TPMT
HGPRT
6-TG
XO
6-TUA
6-MMP
6-MTIMP
TPMT
6-TGMP
6-TGDP
6-TGTP
TPMT
HGPRT
AZA
6-MP
6-TIMP
IT Pase
XO
6-TUA
6-TITP
6-TIDP

FIG. 14