Supplemental Methods

LC-MS Assay for NAD Concentration

The concentration of NAD in cells was determined by a non-validated liquid chromatography–tandem mass spectrometry (LC-MS/MS) assay using $^{13}C_5$-NAD as an internal standard (IS). 150µL of 0.5 N perchloric acid was added to each cell pellet and the sample was vortexed at 1000 rpm for 5 minutes, and 25uL was pipetted to a clean well. Calibration standards were prepared in water. 10µL of the IS solution (concentration 10 µg/mL in 50:50, v:v, methanol:H2O) was added to the sample. Then the sample was crashed with 200 µL of 0.5 N perchloric acid, vortexed at 1000 rpm for 5 min and centrifuged at 13,000 rpm for 10 min at room temperature. 100µL of supernatant was diluted with 100µL of 5 mM ammonium formate, and 5 µL was injected onto the analytical column Atlantis dC18 column (100x2.1 mm, 3µm, Waters, Milford, MA).

Sample analysis was carried out with a Shimadzu Nexera (Columbia, MD) coupled to an API 5500 Q trap Mass Spectrometer (AB Sciex, Foster City, CA) equipped with a turbo-electrospray interface in positive ionization mode. The aqueous mobile phase was water with 0.1% formic acid (A) and the organic mobile phase was acetonitrile with 0.1% formic acid (B). The gradient was 0% B for the first 0.1 min, increased to 30% B from 0.1 to 1.0 min, and decreased to 0% B within 0.1 min and maintained at 0% B for another 0.4 min. The flow rate is 0.8 ml/min and the cycle time (injection to injection) was approximately 1.8 mins. Quantitation was carried out using the multiple reaction monitoring (MRM) transition *m/z* 664 to136 for NAD and *m/z* 669 to 136 for the IS. The lower and upper limits of quantitation of the assay were 0.0308 µM and 603 µM, respectively. The optimized instrument conditions were as follows: source temperature, 550°C; curtain gas, 35 psi; nebulizing (GS1), 55 psi; heating (GS2), 60 psi; collision energy (CE), 67V for NAD and 59V for the IS. LC-MS/MS data were acquired and processed using Analyst software (v1.5.2 Applied Biosystems/MDS Sciex, Canada). The calibration curve was

constructed through plotting the analyte/internal standard peak area ratios versus the nominal concentration of analyte with a weighted $1/x^2$ quadratic regression.

QMSP Assay

50 ng of bisulfite converted DNA were amplified in a 20 µl reaction using 20X Custom Taqman Gene Expression Assays (Applied Biosystems, Cat No. 4331348) using TaqMan® Universal PCR Master Mix, No AmpErase® UNG (Applied Biosystems, Cat No. 4324018) with cycling conditions of 95˚C 10 min, then 50 cycles of 95˚C for 15 sec and 60˚C for 1 min. For the DNA from FFPE sections, a 14 cycle pre-amplification step was added before the QMSP. 15 ng bisulfite converted DNA was amplified in a 20 µl reaction as described above using 0.1x QMSP assay concentrations. One ul of the pre-amplification reaction was carried over into the QMSP assay. DNA content was confirmed using a pre-amplification with a reference meRNaseP Taqman assay and only samples that were positive for meRNaseP were included in further analysis of QMSP reactions. All reactions were performed in duplicate. qPCR expression analysis was performed using the *NAPRT1*-specific Taqman assay Hs01567621_g1 (Applied Biosystems). *NAPRT1* Ct values were normalized to *GAPDH* Ct values and expression levels were determined using a standard ddCt method.

Supplemental Tables

	Ave GNE-617 CyQ IC50 (nM)	St Dev	NA Rescue	NAMPT (1555167_s_at)	NAPRT1 (226707_at)
NCI-H661	0.6	0.2	N	7.90	6.88
LXFL_529	0.9	0.0	N	7.61	7.59
NCI-H322T	1.4	0.1	N	8.07	6.75
NCI-H522	1.5	0.1	N	8.24	6.88
NCI-H1155	1.5	0.5	N	8.37	6.67
ABC-1	1.6	0.3	Y	8.26	8.21
ChaGo-K-1	2.4	0.1	Y	ND	ND

NCI-H1568	2.8	0.8	Y	8.47	9.43
A427	3.0	0.2	N	8.69	6.63
NCI-H2030	3.1	0.9	Y	8.33	9.64
NCI-H23	3.3	0.2	Y	8.80	9.04
RERF_LC_MS	3.4	0.9	N	10.14	6.92
NCI-H2405	3.4	0.6	Y	9.04	8.68
NCI-H1650	3.5	1.8	N	8.10	7.06
NCI-H838	3.5	0.7	Y	8.81	9.68
NCI-H1355	3.6	0.0	N	ND	ND
RERF_LC_OK	4.0	0.2	N	8.76	6.96
NCI-H520	4.3	1.5	Y	8.86	8.71
VWRC-LCD	4.7	1.8	N	ND	ND
NCI-H1770	4.8	0.9	N	ND	ND
NCI-H2106	5.8	0.5	N	ND	ND
SK-MES-1	7.1	0.4	Y	7.97	9.09
SW1573	7.5	1.2	Y	8.86	7.65
NCI-H1703	8.5	0.8	N	9.40	6.86
EKVX	9.2	0.5	Y	8.66	10.07
Calu-6	9.4	1.8	Y	9.32	8.93
NCI-H1299	9.5	0.5	Y	8.54	8.60
HCC2279	11.1	0.9	Y	ND	ND
NCI-H1781	12.1	2.8	Y	9.53	8.67
NCI-H1435	13.4	1.8	Y	8.77	11.58
HOP18	14.3	5.4	Y	9.54	9.28
NCI-H292	14.8	5.5	Y	9.20	9.03
HOP62	17.4	8.8	Y	8.60	8.43
NCI-H1975	18.0	3.2	Y	10.57	7.89
NCI-H2126	18.7	7.6	N	9.84	6.67
A549	18.9	7.4	Y	10.29	7.71
KNS-62	26.3	1.2	Y	10.29	9.90
HCC827	27.7	0.7	N	10.57	7.29
NCI-H1563	31.9	5.4	Y	ND	ND
UMC-11	43.0	2.0	N	ND	ND
EBC-1	53.3	16.4	Y	11.16	9.86
HOP92	58.1	38.9	Y	11.07	7.63
NCI-H1838	59.1	61.7	Y	10.04	9.72
NCI-H460	59.6	20.5	N	ND	ND
NCI-H1793	85.2	18.4	Y	11.50	8.32
NCI-H650	128.2	124.2	Y	9.95	7.40
NCI-H2122	197.0	5.9	Y	12.10	10.45

NCI-H727	>1000.0	0.0	Y	ND	ND
NCI-H1651	1688.9	2867.5	Y	9.72	9.11
NCI-H226	1753.9	2811.5	Y	9.97	8.08
NCI-H647	2038.4	2569.6	Y	11.74	10.63
NCI-H441	3367.0	2828.4	Y	10.87	9.61
RERF_LC_KJ	>5000.0	0.0	Y	8.86	11.39

Table S1 GNE-617 IC50 and mRNA Levels of NAMPT and NAPRT1.
Average IC50 values of three independent assays. NAMPT and NAPRT1 expression were measured on an Affymetrix HGU-133P array, values are Log2 RMA normalized. NAMPT level was significantly correlated with GNE-617 IC50 ($p<0.0001$, Spearman $r=0.80$, $n=43$), NAPRT1 was also correlated with IC50 in the absence of NA ($p=0.003$, Spearman $r=0.45$, $n=43$).

Cell Lines Not Rescued with NA
105KC
143B
786-O
AN3 CA
C33 A
C3A
Caki-1
CHP-212
CoCM-1
COV434
DB
DOR13
EFM-192A
G361
G120
G124
G140
G59
G61
G84
GR-M
H322T
HCC1162
HCC1534
HCC38
HCC78
HCC827
HepG2
HEY
HGC-27
Hs 852.T
IMR-32
JJ012
KELLY
KLM-1
LCLC-103H
MDA−MB−157

MDST8
MG−63
MKN−74
NCI−H1355
NCI−H1703
NCI−H2170
NCI−H2810
NCI−H322T
NCI−H460
NCI−H661
NCI−PC3
PA−1
PC−3
PLC/PRF/5
QGP−1
REC−1
RERF−LC−MS
RERF−LC−OK
SC−1
SF268
SK−N−DZ
SK−N−FI
SK−N−SH
SNU−398
SNU−423
SNU−484
SR
T−47D
T98G
TK−10
U−2 OS
UM−UC−3
UMC−11
VMRC−LCD
WSU−FSCCL

Table S2 Cell Lines Not Rescued with 10uM NA. Cell lines that were not rescued from the cytotoxicity of 10uM GNE-617 with 10uM NA.

Cell Line	NAPRT1 (226707_at)	Cancer Type
RT4	7.014	bladder
UMUC-11	7.000	bladder
EFM192A	6.976	breast
HCC1419	6.922	breast
MB468	6.960	breast
MDA175	6.484	breast
S03-21319 B1	6.819	breast
T47D	6.836	breast
COLO 320DM	6.012	colorectal
COLO 320HSR	6.210	colorectal
MDST8	6.692	colorectal
MKN-74	6.930	gastric
SNU-484	6.955	gastric
A172	7.004	glioma
G120	7.138	glioma
SW1783	7.172	glioma
Alexander	7.032	liver
C3A	6.318	liver
HepG2	6.438	liver
HLE	6.854	liver
HLF	6.640	liver
JHH-2	7.165	liver
PLC/PRF/5	6.773	liver
SK-HEP-1	6.690	liver
SNU-398	6.498	liver
SNU-423	6.828	liver
G-361	7.130	melanoma
HS852T	6.492	melanoma
AMO-1	6.796	MM
KMS-21BM	6.450	MM
NCI-H929	7.174	MM
OPM-2	6.463	MM
RPMI 8226	6.650	MM
U266	6.800	MM
A3/Kawakami	6.792	NHL
A4/Fukada	6.712	NHL
BJAB	6.929	NHL
CA-46	6.487	NHL

DB	7.028	NHL
DOHH2	6.724	NHL
EB-1	6.801	NHL
EB-3	7.004	NHL
GA-10	6.603	NHL
HS-SULTAN	7.188	NHL
JeKo-1	6.869	NHL
KARPAS-1106P	6.929	NHL
Karpas-422	6.784	NHL
MHH-PREB-1	7.198	NHL
NAMALWA	6.995	NHL
NU-DUL1	6.599	NHL
OCI-Ly3	6.798	NHL
OCI-LY7	7.117	NHL
RAJi	6.663	NHL
SCC-3	6.558	NHL
SU-DHL-4	6.991	NHL
SU-DHL-5	7.099	NHL
SU-DHL-8	7.004	NHL
WSU-FSCCL	7.124	NHL
A427	6.631	NSCLC
NCI-H1155	6.667	NSCLC
NCI-H1650	7.060	NSCLC
NCI-H1703	6.858	NSCLC
NCI-H2126	6.668	NSCLC
NCI-H322T	6.752	NSCLC
NCI-H522	6.876	NSCLC
NCI-H661	6.877	NSCLC
NCI-H1703	7.052	NSCLC
NCI-H460	6.998	NSCLC
RERF-LC-MS	6.918	NSCLC
RERF-LC-OK	6.960	NSCLC
RERF-LCA-D1	6.929	NSCLC
A2780ADR	6.836	ovarian
COV434	6.533	ovarian
COV644	7.081	ovarian
DOR 13	7.032	ovarian
HEY	6.704	ovarian
IGROV-1	6.932	ovarian
OVCAR420	6.094	ovarian

OVCAR429	6.695	ovarian
KLM-1	6.758	pancreatic
KP4	6.810	pancreatic
MIA PaCa-2	6.761	pancreatic
PK-8	6.647	pancreatic
PC3M	7.117	prostate
NCI-H378	7.042	SCLC
NCI-H889	7.140	SCLC

Table S3 Cell Lines with Low NAPRT1 Expression

NAPRT1 expression was measured on an Affymetrix HGU-133P array, values are presented as Log2, RMA normalized. MM=multiple myeloma, NSCLC=non-small cell lung cancer, SCLC=small cell lung cancer.

Supplemental Figure Legend

Figure S1 A) Chemical structure of GNE-617, B) cellular NAD and ATP levels in H522 cells after exposure to 4nM GNE-617, C) correlation of NAPRT1 mRNA and protein (n=32, spearman r= 0.88, p,0.0001).