

Supplemental Material to:

Mari lida, Toni M. Brand, David A. Campbell, Megan M. Starr, Neha Luthar, Anne M. Traynor and Deric L. Wheeler

Targeting AKT with the allosteric AKT inhibitor MK-2206 in non-small cell lung cancer cells with acquired resistance to cetuximab

Cancer Biology & Therapy 2013; 14(6)

<http://dx.doi.org/10.4161/cbt.24342>

<http://www.landesbioscience.com/journals/cbt/article/24342/>

Supplemental data

Table 1: Phosphorylation levels of AKT substrate proteins in HC4 cells vs HP cells and HC4 cells treated with MK-2206 vs vehicle control.

Protein List	HC4 vs HP
4E-BP1(Ab-36)	0.90
4E-BP1(Ab-45)	0.89
4E-BP1(Phospho-Thr36)	1.14
4E-BP1(Phospho-Thr45)	1.26
AFX(Ab-197)	0.99
AFX(Phospho-Ser197)	1.02
Akt(Ab-308)	0.82
Akt(Ab-473)	0.89
Akt(Phospho-Ser473)	1.52
Akt(Phospho-Thr308)	1.55
Akt2(Ab-474)	0.89
Akt2(Phospho-Ser474)	0.82
ASK1(Ab-83)	0.91
ASK1(Ab-966)	0.65
ASK1(Phospho-Ser83)	1.13
ASK1(Phospho-Ser966)	1.19
BAD(Ab-112)	0.95
BAD(Ab-136)	1.00
BAD(Ab-155)	1.07
BAD(Phospho-Ser112)	1.02
BAD(Phospho-Ser136)	0.99
BAD(Phospho-Ser155)	1.39
BCL-2(Ab-56)	0.94
BCL-2(Ab-70)	1.01
BCL-2(Phospho-Ser70)	1.00
BCL-2(Phospho-Thr56)	0.97
BCL-XL(Ab-62)	1.06
BCL-XL(Phospho-Ser62)	0.91
Beta Actin	0.77
c-Jun (Phospho-Thr239)	0.85
c-Jun(Ab-170)	1.01
c-Jun(Ab-239)	0.89
c-Jun(Ab-243)	0.89
c-Jun(Ab-63)	0.98
c-Jun(Ab-73)	0.96
c-Jun(Ab-91)	1.02
c-Jun(Ab-93)	0.68
c-Jun(Phospho-Ser243)	0.90
c-Jun(Phospho-Ser63)	1.29
c-Jun(Phospho-Ser73)	1.10
c-Jun(Phospho-Thr91)	1.13
c-Jun(Phospho-Thr93)	1.14
c-Jun(Phospho-Tyr170)	1.45
c-Kit(Ab-721)	0.99
c-Kit(Phospho-Tyr721)	1.12
elF4E(Ab-209)	1.07
elF4E(Phospho-Ser209)	1.33
FAK(Ab-397)	0.99
FAK(Ab-861)	1.16
FAK(Ab-925)	0.93
FAK(Phospho-Tyr861)	1.33
FAK(Phospho-Tyr925)	1.34
FKHR(Ab-256)	0.77
FKHR(Ab-319)	0.95
FKHR(Phospho-Ser256)	1.05
FKHR(Phospho-Ser319)	1.12
FKHRL1(Ab-253)	0.84
FKHRL1(Phospho-Ser253)	1.16
Gab1(Ab-627)	0.87
Gab1(Phospho-Tyr627)	0.88
GAPDH	0.89
GSK3 alpha(Ab-21)	1.05
GSK3 alpha(Phospho-Ser21)	0.98
GSK3 beta(Ab-9)	0.96
GSK3 beta(Phospho-Ser9)	1.16
IKB-alpha(Ab-32/36)	0.98
IKB-alpha(Ab-42)	0.83
IKB-alpha(Phospho-Ser32/Phospho-Ser36)	0.98
IKB-alpha (Phospho-Tyr42)	1.19
IKK alpha(Ab-23)	0.94

Protein List	HC4 2.5 uM MK2206 vs Vehicle control
4E-BP1(Ab-36)	1.03
4E-BP1(Ab-45)	0.86
4E-BP1(Phospho-Thr36)	0.90
4E-BP1(Phospho-Thr45)	0.85
AFX(Ab-197)	0.84
AFX(Phospho-Ser197)	0.92
Akt(Ab-308)	1.42
Akt(Ab-473)	1.35
Akt(Phospho-Ser473)	0.80
Akt(Phospho-Thr308)	0.88
Akt2(Ab-474)	1.05
Akt2(Phospho-Ser474)	0.79
ASK1(Ab-83)	1.05
ASK1(Ab-966)	0.91
ASK1(Phospho-Ser83)	1.01
ASK1(Phospho-Ser966)	1.05
BAD(Ab-112)	0.95
BAD(Ab-136)	0.89
BAD(Ab-155)	1.05
BAD(Phospho-Ser112)	1.09
BAD(Phospho-Ser136)	1.08
BAD(Phospho-Ser155)	1.25
BCL-2(Ab-56)	0.82
BCL-2(Ab-70)	0.79
BCL-2(Phospho-Ser70)	1.05
BCL-2(Phospho-Thr56)	1.23
BCL-XL(Ab-62)	0.78
BCL-XL(Phospho-Ser62)	1.13
Beta Actin	0.75
c-Jun (Phospho-Thr239)	0.82
c-Jun(Ab-170)	1.11
c-Jun(Ab-239)	1.13
c-Jun(Ab-243)	1.33
c-Jun(Ab-63)	1.02
c-Jun(Ab-73)	0.94
c-Jun(Ab-91)	1.00
c-Jun(Ab-93)	0.98
c-Jun(Phospho-Ser243)	0.86
c-Jun(Phospho-Ser63)	0.66
c-Jun(Phospho-Ser73)	0.88
c-Jun(Phospho-Thr91)	0.82
c-Jun(Phospho-Thr93)	0.90
c-Jun(Phospho-Tyr170)	0.88
c-Kit(Ab-721)	0.93
c-Kit(Phospho-Tyr721)	1.01
elF4E(Ab-209)	0.91
elF4E(Phospho-Ser209)	0.82
FAK(Ab-397)	0.96
FAK(Ab-861)	1.11
FAK(Ab-925)	1.21
FAK(Phospho-Tyr861)	0.89
FAK(Phospho-Tyr925)	0.79
FKHR(Ab-256)	0.93
FKHR(Ab-319)	0.82
FKHR(Phospho-Ser256)	0.87
FKHR(Phospho-Ser319)	1.01
FKHRL1(Ab-253)	0.79
FKHRL1(Phospho-Ser253)	0.86
Gab1(Ab-627)	0.88
Gab1(Phospho-Tyr627)	1.13
GAPDH	0.84
GSK3 alpha(Ab-21)	1.03
GSK3 alpha(Phospho-Ser21)	0.87
GSK3 beta(Ab-9)	0.95
GSK3 beta(Phospho-Ser9)	0.95
IKB-alpha(Ab-32/36)	1.06
IKB-alpha(Ab-42)	0.68
IKB-alpha(Phospho-Ser32/Phospho-Ser36)	1.09
IKB-alpha(Phospho-Tyr42)	1.07
IKK alpha(Ab-23)	0.93

IKK alpha(Phospho-Thr23)	1.10
IRS-1(Ab-307)	1.04
IRS-1(Ab-312)	0.80
IRS-1(Ab-636)	1.01
IRS-1(Ab-639)	0.81
IRS-1(Phospho-Ser307)	1.27
IRS-1(Phospho-Ser312)	0.97
IRS-1(Phospho-Ser636)	1.33
IRS-1(Phospho-Ser639)	1.13
JAK1(Ab-1022)	0.91
JAK1(Phospho-Tyr1022)	0.95
JAK2(Ab-1007)	0.98
JAK2(Ab-221)	1.02
JAK2(Phospho-Tyr1007)	1.11
JAK2(Phospho-Tyr221)	1.09
Met(Ab-1234)	0.83
Met(Ab-1349)	1.07
Met(Phospho-Tyr1234)	1.16
Met(Phospho-Tyr1349)	1.07
p21Cip1(Ab-145)	0.85
p21Cip1(Phospho-Thr145)	0.89
p27Kip1(Ab-10)	0.98
p27Kip1(Ab-187)	0.89
p27Kip1(Phospho-Ser10)	0.91
p27Kip1(Phospho-Thr187)	1.35
p53(Ab-15)	1.11
p53(Ab-18)	0.98
p53(Ab-315)	0.90
p53(Ab-33)	0.89
p53(Ab-37)	0.83
p53(Ab-46)	0.78
p53(Ab-6)	0.97
p53(Ab-9)	1.10
p53(Phospho-Ser15)	1.02
p53(Phospho-Ser315)	1.24
p53(Phospho-Ser33)	1.15
p53(Phospho-Ser37)	1.06
p53(Phospho-Ser46)	1.05
p53(Phospho-Ser6)	1.00
p53(Phospho-Ser9)	1.63
p53(Phospho-Thr18)	1.09
p70 S6 Kinase (Ab-424)	1.01
p70 S6 Kinase (Phospho-Ser424)	0.89
p70 S6 Kinase(Ab-411)	0.96
p70 S6 Kinase(Phospho-Ser411)	1.00
Paxillin(Ab-118)	0.85
Paxillin(Ab-31)	0.91
Paxillin(Phospho-Tyr118)	0.85
Paxillin(Phospho-Tyr31)	1.02
PDK1(Ab-241)	0.90
PDK1(Phospho-Ser241)	1.26
PTEN(Ab-370)	0.82
PTEN(Ab-380)	0.91
PTEN(Ab-380/382/383)	0.83
PTEN(Phospho-Ser370)	1.68
PTEN(Phospho-Ser380)	1.15
PTEN(Phospho-Ser380/Phospho-Thr382/383)	0.96
Pyk2(Ab-402)	0.99
Pyk2(Phospho-Tyr402)	1.00
Raf1(Ab-259)	1.29
Raf1(Ab-338)	0.95
Raf1(Phospho-Ser259)	1.22
Raf1(Phospho-Ser338)	0.95
S6 Ribosomal Protein(Ab-235)	1.11
S6 Ribosomal Protein(Phospho-Ser235)	2.34
Zap-70(Ab-319)	0.93
Zap-70(Ab-493)	1.07
Zap-70(Phospho-Tyr319)	1.14
Zap-70(Phospho-Tyr493)	1.03

IKK alpha(Phospho-Thr23)	0.95
IRS-1(Ab-307)	0.92
IRS-1(Ab-312)	0.94
IRS-1(Ab-636)	0.90
IRS-1(Ab-639)	0.68
IRS-1(Phospho-Ser307)	0.86
IRS-1(Phospho-Ser312)	0.88
IRS-1(Phospho-Ser636)	0.70
IRS-1(Phospho-Ser639)	0.86
JAK1(Ab-1022)	1.01
JAK1(Phospho-Tyr1022)	1.13
JAK2(Ab-1007)	1.02
JAK2(Ab-221)	1.12
JAK2(Phospho-Tyr1007)	0.97
JAK2(Phospho-Tyr221)	0.90
Met(Ab-1234)	0.97
Met(Ab-1349)	1.02
Met(Phospho-Tyr1234)	0.78
Met(Phospho-Tyr1349)	1.10
p21Cip1(Ab-145)	1.34
p21Cip1(Phospho-Thr145)	1.32
p27Kip1(Ab-10)	0.95
p27Kip1(Ab-187)	1.22
p27Kip1(Phospho-Ser10)	1.34
p27Kip1(Phospho-Thr187)	0.91
p53(Ab-15)	0.94
p53(Ab-18)	0.97
p53(Ab-315)	1.29
p53(Ab-33)	1.17
p53(Ab-37)	1.15
p53(Ab-46)	1.06
p53(Ab-6)	0.89
p53(Ab-9)	0.94
p53(Phospho-Ser15)	0.98
p53(Phospho-Ser315)	0.90
p53(Phospho-Ser33)	0.80
p53(Phospho-Ser37)	0.86
p53(Phospho-Ser46)	0.92
p53(Phospho-Ser6)	1.22
p53(Phospho-Ser9)	1.17
p53(Phospho-Thr18)	0.99
p70 S6 Kinase (Ab-424)	0.94
p70 S6 Kinase (Phospho-Ser424)	1.01
p70 S6 Kinase(Ab-411)	0.99
p70 S6 Kinase(Phospho-Ser411)	1.05
Paxillin(Ab-118)	1.26
Paxillin(Ab-31)	1.03
Paxillin(Phospho-Tyr118)	1.52
Paxillin(Phospho-Tyr31)	1.04
PDK1(Ab-241)	0.79
PDK1(Phospho-Ser241)	0.76
PTEN(Ab-370)	1.32
PTEN(Ab-380)	0.98
PTEN(Ab-380/382/383)	1.56
PTEN(Phospho-Ser370)	0.88
PTEN(Phospho-Ser380)	0.96
PTEN(Phospho-Ser380/Phospho-Thr382/383)	0.96
Pyk2(Ab-402)	0.97
Pyk2(Phospho-Tyr402)	1.26
Raf1(Ab-259)	0.84
Raf1(Ab-338)	0.89
Raf1(Phospho-Ser259)	0.89
Raf1(Phospho-Ser338)	0.99
S6 Ribosomal Protein(Ab-235)	0.82
S6 Ribosomal Protein(Phospho-Ser235)	0.70
Zap-70(Ab-319)	1.00
Zap-70(Ab-493)	0.98
Zap-70(Phospho-Tyr319)	0.86
Zap-70(Phospho-Tyr493)	1.41