# New Chemical Affinity Probe for Quantitative Proteomic Profiling of PI3Ks

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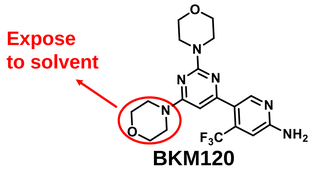
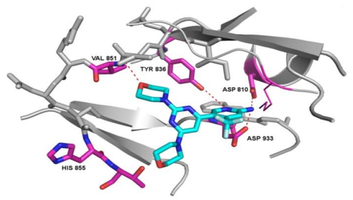
## ABSTRACT


- With MIB/MS techniques, kinome activation profiles of multiple tumors can be compared or global changes in kinase activation can be determined after drug treatment. But the coverage of kinome by immobilized inhibitor beads is still not complete because of the lack of suitable affinity probes for certain kinases such as PI3Ks.
- With the PI3K (PI3K-AKT-MTOR) pathway being probably the most commonly activated pathway in human cancers, and PIK3CA being one of the most frequently mutated genes in cancer, there is a strong rationale to therapeutically target the PI3K/AKT/mTOR pathway in human cancer.
- To include the important PI3Ks in the kinome mapping using MIB/MS techniques, a chemical affinity probe targeting PI3Ks, including the mutated PI3Ks has been developed in this study.
- The newly developed PI3K chemical affinity probe: 1) had similar activity towards PI3Ks and other kinases as BKM120; 2) could capture PI3Ks and their regulatory subunits from a variety of breast cancer cell lines and PDXs; 3) exhibited competitive capture of PI3Ks from SKBR3 cells with $IC_{50}$ values calculated from dose-dependent curves for BYL719 and BKM120 comparable to the published values; 4) demonstrated capacity to capture PIK3CA H1047R mutant form in bioengineered SKBR3 cells harboring H1047R mutant and from A375 mutated cell with H1047R mutant.


## INTRODUCTION



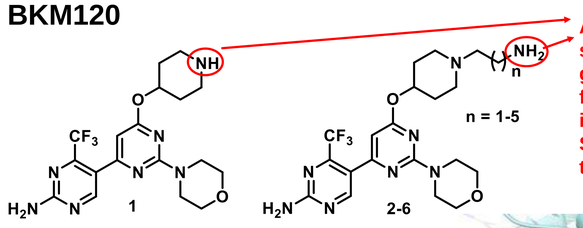




**Development of PI3K chemical affinity probe based on NVP-BKM120**

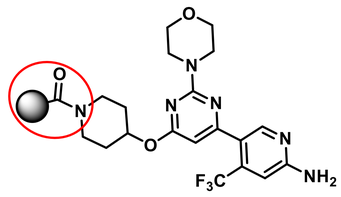
- BMK120 is selective pan-class II PI3K inhibitor with $IC_{50}$ values of 52/166/116/262 nM for p110α/β/δ/γ in cell-free assays respectively.
- BKM120 also has similar activity towards the most common P110α mutants E545K and H1047R with $IC_{50}$ values of 99 nM and 58 nM respectively.
- BKM120 exhibits lower potency against class III and class IV PI3Ks including mTOR.
- BKM120 structural template is amiable for multi-dimensional modification.
- Previous SAR findings and co-crystal structure of BKM120 with PIK3CA approved that BKM120 does tolerate chemical modification at C4 position of the pyrimidine core and retain activity against PI3Ks.

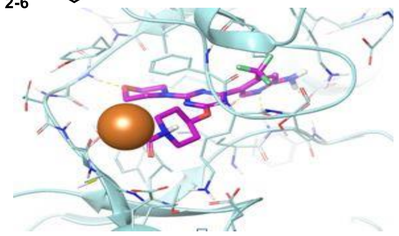
## METHODS & RESULTS

### 1. Design of PI3K chemical affinity probe based on NVP-BKM120



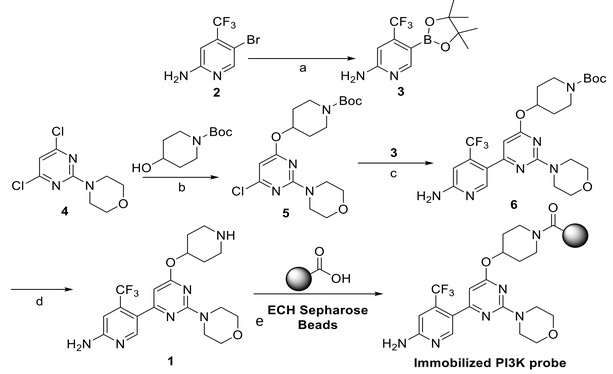

**A terminal primary or secondary amine group will be needed for the analog to be immobilized on ECH Sepharose beads through amide bonds**





We designed a series of qualified BKM120 analogs and evaluated them by computer docking to make sure the chemical probe, after linking to ECH Sepharose beads, maintains high binding affinity towards PI3K. The best compounds were synthesized and tested *in vitro* for its capability to capture and quantify PI3Ks.

### 2. Synthesis of PI3K Chemical Probe




Reagents and conditions: a) Bis(pinacolato) diboron, $K_2CO_3$, dioxane, $Pd(dppf)_2Cl_2$, 115°C, 6h, 46% yield; b) NaH, 0°C-r.t., overnight, 77% yield; c) $Pd(dppf)_2Cl_2$, $K_2CO_3$, DME/$H_2O$ = 4:1, microwave, 120°C, 30 min., 72% yield; d) DCM, TFA, r.t., 1h., 92% yield; e) EDC, DMF/EtOH = 1:1, overnight.

### 3. New PI3K beads can capture PI3Ks from various cancer cell lines

**From HCC1806 cells**

| Kinase | Peptide | Unique Peptide | LFQ intensity |
|---|---|---|---|
| PI4KA | 18 | 18 | 1.66E+08 |
| PK3CA | 15 | 15 | 8.41E+08 |
| PK3CB | 12 | 12 | 3.42E+08 |
| PIK3C3 | 6 | 6 | 22315000 |
| PIK3R1 | 18 | 13 | 5.27E+08 |
| PIK3R2 | 22 | 20 | 8.39E+08 |
| PIK3R3 | 13 | 9 | 2.72E+08 |
| PIK3R4 | 5 | 5 | 2256400 |

**From BT474 cells**

| Kinase | Peptide | Unique Peptide | LFQ intensity |
|---|---|---|---|
| PI4KA | 29 | 29 | 8.32E+08 |
| PK3CA | 32 | 32 | 5.22E+09 |
| PK3CB | 22 | 21 | 1.95E+09 |
| PIK3C3 | 1 | 1 | 67523000 |
| PIK3R1 | 17 | 13 | 1.18E+09 |
| PIK3R2 | 19 | 27 | 4.86E+09 |
| PIK3R3 | 20 | 25 | 2.16E+09 |
| PIK3R4 | 2 | 2 | 18756000 |

**From SKBR3 cells**

| Kinase | Peptide | Unique Peptide | LFQ intensity |
|---|---|---|---|
| PI4KA | 25 | 25 | 2.03E+08 |
| PK3CA | 36 | 36 | 3.6E+09 |
| PK3CB | 16 | 15 | 4.79E+08 |
| PK3CD | 15 | 14 | 4.67E+08 |
| PIK3R1 | 32 | 23 | 3.01E+09 |
| PIK3R2 | 32 | 29 | 1.02E+09 |
| PIK3R3 | 23 | 18 | 1.1E+09 |

From all three breast cancer cell lines, the new PI3K beads can selectively and reliably capture PIK3CA, PIK3CB together with the regulatory subunits.

### 4. New PI3K beads can capture PI3Ks from various PDXs

**From WHIM2**

| Kinase | Peptide | Unique Peptide | LFQ intensity |
|---|---|---|---|
| PI4KA | 14 | 14 | 63687000 |
| PK3CA | 21 | 3 | 76957000 |
| PK3CB | 18 | 8 | 2.38E+08 |
| PK3CD | 17 | 4 | 39064000 |
| PIK3R1 | 28 | 14 | 2.75E+08 |
| PIK3R2 | 32 | 6 | 1.27E+08 |
| PIK3R3 | 10 | 6 | 68908000 |
| PIK3R4 | 16 | 4 | 23149000 |

**From WHIM12**

| Kinase | Peptide | Unique Peptide | LFQ intensity |
|---|---|---|---|
| PI4KA | 17 | 17 | 95110000 |
| PK3CA | 21 | 3 | 22630000 |
| PK3CB | 19 | 8 | 1.54E+08 |
| PK3CD | 21 | 8 | 2.74E+08 |
| PK3CG | 2 | 2 | 4484900 |
| PIK3R1 | 29 | 15 | 1.63E+08 |
| PIK3R2 | 33 | 7 | 5.19E+08 |
| PIK3R3 | 8 | 4 | 15545000 |

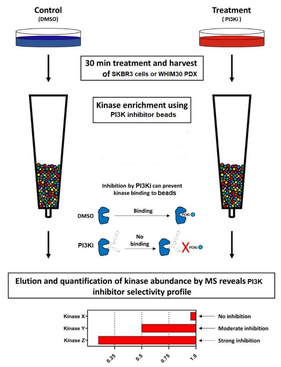
**From WHIM30**

| Kinase | Peptide | Unique Peptide | LFQ intensity |
|---|---|---|---|
| PI4KA | 13 | 13 | 56660000 |
| PK3CA | 27 | 4 | 2.29E+08 |
| PK3CB | 18 | 7 | 1.97E+08 |
| PK3CD | 19 | 5 | 76097000 |
| PIK3R1 | 30 | 16 | 3.48E+08 |
| PIK3R2 | 33 | 6 | 4.11E+08 |
| PIK3R3 | 11 | 7 | 1.85E+08 |
| PIK3R4 | 11 | 2 | 4895400 |

From all three breast cancer PDX tumors, the new PI3K inhibitor beads can selectively and reliably capture PIK3CA, PIK3CB, PIK3CD and the regulatory subunits.

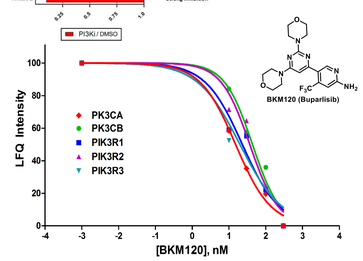
- The new PI3K inhibitor beads have been approved to be able to selectively and reliably capture PI3Ks and their regulatory subunits from breast cancer cell lines and PDX tumors.
- About 30 other kinases were captured by the new PI3K beads including ATR, PRKDC, DCK, MTOR, EBB2, ERBB3, INSR, JAK1 etc.

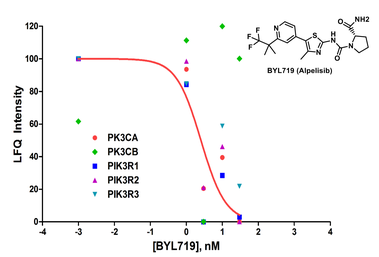
### 5. New PI3K beads compete with BKM120 and BYL719 for interaction with PI3Ks




Dose dependent curves for BKM120 and BYL719 determined by PI3Ks captured through new PI3K beads from SBRK3 cells treated with BKM120 (pan-class II PI3K inhibitor) or BYL719 (Alpelisib, p110α selective PI3K inhibitor).

- Cell lysates were treated with PI3K inhibitors, BYL719 or BKM120.
- BYL719: Alpelisib, FDA approval 05/2019 for certain types of breast cancer. PI3Kα–selective inhibitor.
- BKM120: Buparlisib, small molecule orally-available pan-class I PI3Ks inhibitor.
- The new PI3K inhibitor beads should compete with BYL719 and BKM120 for binding to PI3Ks in the test samples, therefore, should be able to generate dose dependent curves for both PI3K inhibitors.

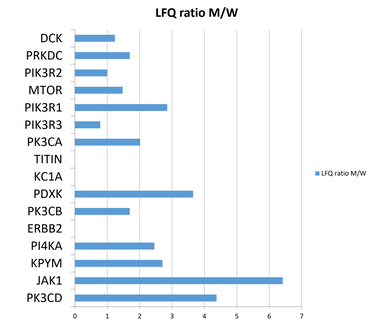



| IC50 (nM) | PK3CA | PK3CB | PIK3R1 | PIK3R2 | PIK3R3 |
|---|---|---|---|---|---|
| BKM120 | 15.15 | 43.49 | 23.10 | 36.73 | 19.89 |
| BYL719 | 2.54 | NA | NA | NA | NA |

### 6. New PI3K beads can capture PIK3CA H1047R from mutated SKBR3 cells

| Kinase | LFQ intensity_WT | LFQ intensity_MU | LFQ ratio M/W |
|---|---|---|---|
| PK3CD | 1.93E+08 | 8.44E+08 | 4.373711 |
| JAK1 | 1.47E+08 | 9.42E+08 | 6.418728 |
| KPYM | 12588000 | 34047000 | 2.704719 |
| PI4KA | 55297000 | 1.36E+08 | 2.45384 |
| ERBB2 | 24535000 | 0 | 0 |
| PK3CB | 1.57E+08 | 2.66E+08 | 1.692894 |
| PDXK | 50493000 | 1.84E+08 | 3.649615 |
| KC1A | 8422300 | 0 | 0 |
| TITIN | 56649000 | 0 | 0 |
| PK3CA | 6.01E+08 | 1.21E+09 | 2.012517 |
| PIK3R3 | 1.1E+09 | 8.61E+08 | 0.783624 |
| PIK3R1 | 1.02E+09 | 2.91E+09 | 2.85301 |
| MTOR | 1.89E+09 | 2.78E+09 | 1.475017 |
| PIK3R2 | 3.01E+09 | 3E+09 | 0.997112 |
| PRKDC | 4.5E+08 | 7.63E+08 | 1.696123 |
| DCK | 5.14E+09 | 6.35E+09 | 1.237193 |




Captured 20 kinases plus 3 PIK3 regulatory subunits in mutated SKBR3, 27 kinases plus 3 PIK3 Regulatory subunits in WT SKBR3.

## CONCLUSION

- **We have developed a chemical affinity probe for quantitative proteomic profiling of wide type and mutant PI3Ks.**
- **The new PI3K inhibitor beads not only can expand the kinome coverage of MIB-MS, but also can help analyze the dynamic changes of PI3Ks in various cancers and identify possible binding partners for desired/undesired biological effects.**
- **The results obtained may also provide guidance in formulating precision medicine for cancer patients.**

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