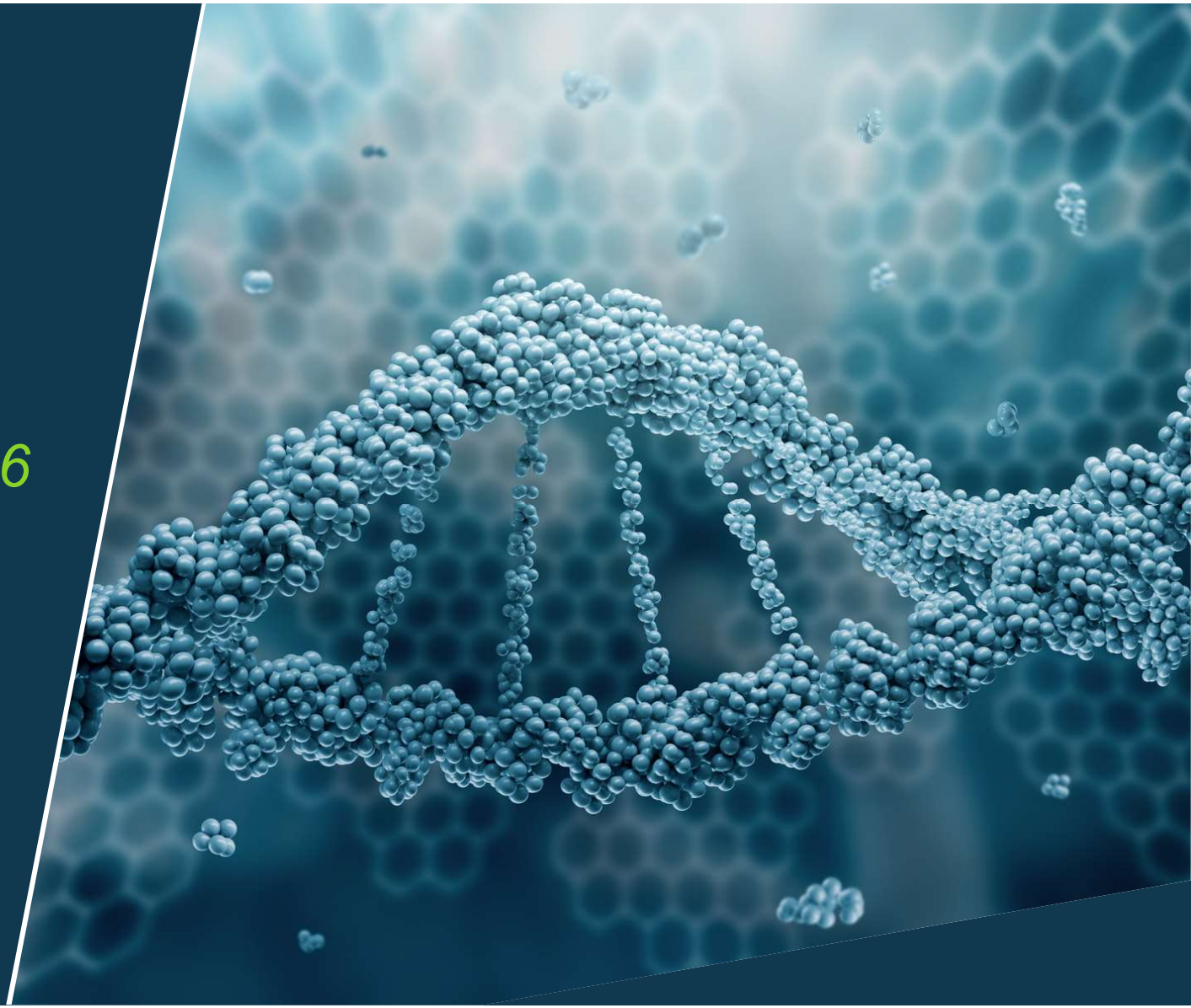
Donated Chemical Probe

# *AKT Inhibitor Probe BAY1125976*

**September 2021**

**Presenters:**
**Oliver Politz**
**(Lars Bärfacker)**



# AKT1/2 Probe BAY1125976

This is a new beginning of a terminated project……

Int J Cancer. 2017 Jan 15;140(2):449-459. doi: 10.1002/ijc.30457. Epub 2016 Oct 20.

## BAY 1125976, a selective allosteric AKT1/2 inhibitor, exhibits high efficacy on AKT signaling-dependent tumor growth in mouse models

Oliver Politz [1], Franziska Siegel [1], Lars Bärfacker [2], Ulf Bömer [1], Andrea Hägebarth [1], William J Scott [3], Martin Michels [2], Stuart Ince [3], Roland Neuhaus [1], Kirstin Meyer [1], Amaury Ernesto Fernández-Montalván [1], Ningshu Liu [1], Franz von Nussbaum [4], Dominik Mumberg [1], Karl Ziegelbauer [1]

Affiliations + expand

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Free article

- BAY1125976 requested by the SGC
- Bayer project from 2017
- unique opportunity for a donated Chemical Probe

# AKT1/2 Probe BAY1125976

## Profile of BAY 1125976

- BAY 1125976 is a highly selective and potent allosteric inhibitor of AKT1 and AKT2 with similar potency (IC50 ~10 nM), while it displays weaker activity against AKT3 (IC50 ~500 nM) and is inactive against ~230 other protein/lipid kinases (>1 µM)
- Mechanistically, BAY 1125976 blocks AKT signaling by inhibiting the phosphorylation of AKT at both Thr308 and Ser473 (IC50 <1 nM), as well as downstream phosphorylation of 4E-BP1 (IC50<50 nM)
- The strong inhibition of cellular pAKT and downstream signaling by BAY 1125976 translates to a broad inhibition of tumor cell proliferation in vitro, with strongest effects in tumor cell lines that carry defects in the tumor suppressor PTEN, or oncogenic mutations in PIK3CA
- Daily oral dosing of BAY 1125976 in human xenograft tumor models induces strong pharmacodynamic inhibition of AKT phosphorylation that correlates with drug exposure
- In vivo, BAY 1125976 demonstrates dose-dependent antitumor efficacy in multiple xenograft tumor models of different histological types with AKT-mutations or PTEN deletions or PIK3CA mutations while being well tolerated
- When combined with anti-hormonal therapeutics in breast and prostate cancer cell lines, BAY 1125976 shows synergistic anti-proliferative effects, translating to enhanced antitumor efficacy with durable tumor regressions in vivo
- Combination of BAY 1125976 with the bone-targeting agent radium-223 dichloride in a breast cancer metastasis model results in a reduced total tumor and bone metastases burden
- Human PK for BAY 1125976 is favorable – once daily dosing as well as alternative schedules possible
- Excellent physicochemical profile

# AKT1/2 Probe BAY1125976

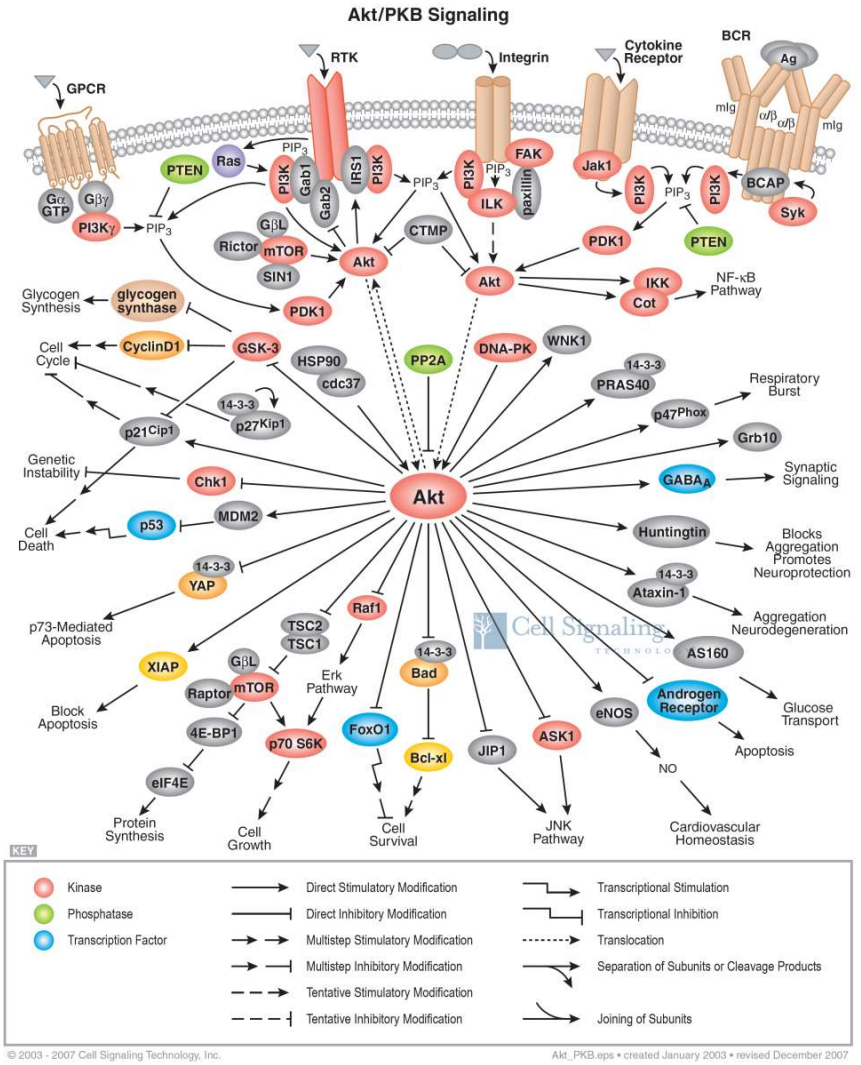
## Background & Scientific rationale

**Roles of AKT kinases in cell biology:**

- Akt is a central switch
  - Promotes cell growth, cell cycle progression, survival, DNA repair
  - Hormone receptor signaling
  - Role in immune regulation

- Akt pathway activation
  - Growth factor overexpression
  - Growth factor receptor activation
  - Mutations in **AKT** and PI3K
  - PTEN inactivation
  - Overexpression and amplification of Akt

- Downstream effectors
  - mTOR
  - **EIF4B, p70S6K,**
  - $p27^{KIP1}$
  - Mdm2
  - **DNA-PK**
  - BAD (Bcl-2 family), Caspase-9



# AKT1/2 Probe BAY1125976

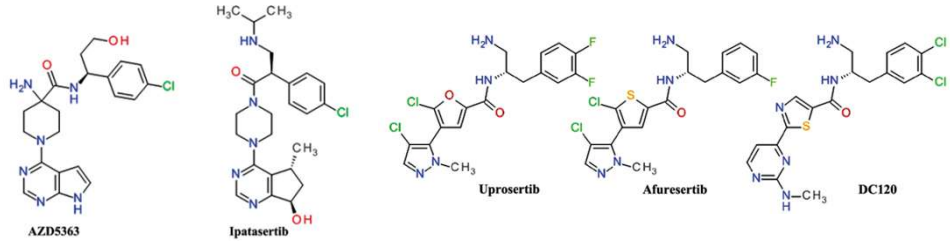
## Competitor compounds, a literature known, patents, commercially available

Table I. Akt-inhibiting drugs listed into major classes.

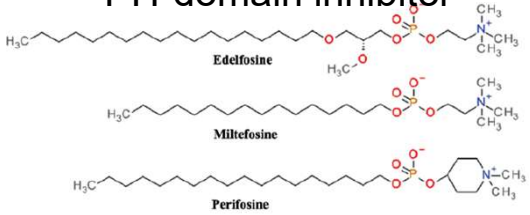
| Class | Description |
|---|---|
| ATP-competitive inhibitors | Orthosteric inhibitors targeting the ATP-binding pocket of the protein kinase B (Akt) |
| Isoquinoline-5-sulfonamides | H-8, H-89, NL-71-101 |
| Azepane derivatives | A series structures derived from (-)-balanol |
| Aminofurazans | GSK690693 |
| Heterocyclic rings | 7-azaindole, 6-phenylpurine derivatives, pyrrolo[2,3-d]pyrimidine derivatives, CCT128930, 3-aminopyrrolidine, anilinotriazole derivatives, spiroindoline derivatives, AZD5363, ipatasertib (GDC-0068, RG7440), A-674563, A-443654 |
| Phenylpyrazole derivatives | AT7867, AT13148 |
| Thiophenecarboxamide derivatives | Afuresertib (GSK2110183), 2-pyrimidyl-5-amidothiophene derivative (DC120), uprosertib (GSK2141795) |
| Allosteric inhibitors | Superior to orthosteric inhibitors providing greater specificity, reduced side-effects and less toxicity |
| 2,3-diphenylquinoxaline analogues | 2,3-diphenylquinoxaline derivatives, triazolo[3,4-*f*][1,6]naphthyridin-3(2*H*)-one derivative (MK-2206) |
| Alkylphospholipids | Edelfosine (1-O-octadecyl-2-O-methyl-rac-glycero-3-phosphocholine, ET-18-$OCH_3$) ilmofosine (BM 41.440), miltefosine (hexadecylphosphocholine, HePC), perifosine (D-21266), erucylphosphocholine (ErPC), erufosine (ErPC3, erucylphosphohomocholine |
| Indole-3-carbinol analogues | Indole-3-carbinol, 3-chloroacetylindole, diindolylmethane, diethyl 6-methoxy-5,7-dihydroindolo [2,3-*b*]carbazole-2,10-dicarboxylate (SR13668), OSU-A9 |
| Sulfonamide derivatives | PH-316, PHT-427 |
| Thiourea derivatives | PIT-1, PIT-2, DM-PIT-1, N-[(1-methyl-1*H*-pyrazol-4-yl)carbonyl]-N'-(3-bromophenyl)-thiourea |
| Purine derivatives | Triciribine (TCN, NSC 154020), triciribine mono-phosphate active analogue (TCN-P), 4-amino-pyrido[2,3-*d*]pyrimidine derivative API-1, 3-phenyl-3*H*-imidazo[4,5-*b*]pyridine derivatives, ARQ 092 |
| Other structures, derivatives | BAY 1125976, 3-methyl-xanthine, quinoline-4-carboxamide and 2-[4-(cyclohexa-1,3-dien-1-yl)-1*H*-pyrazol-3-yl]phenol, 3-oxo-tirucallic acid, 3$\alpha$- and 3$\beta$- acetoxy-tirucallic acids, acetoxy-tirucallic acid |
| Irreversible inhibitors | Natural products, antibiotics<br>Lactoquinomycin, Frenolicin B, kalafungin, medermycin, Boc-Phe-vinyl ketone, 4-hydroxynonenal (4-HNE), 1,6-naphthyridinone derivatives, imidazo-1,2-pyridine derivatives |

Table1 from: Nitulescu, G. M., et al. (2016). Int J Oncol **48(3): 869-885.**

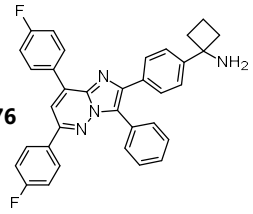
### ATP competitive Inhibitor




### PH-domain inhibitor




### Allosteric Inhibitor

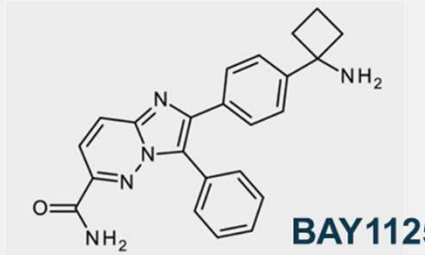
# AKT1/2 Probe BAY1125976

## Pharmacology of AKT inhibitors

| Data | BAY 1125976 | MK2206 | Ipatasertib (GDC-0068) | Capivasertib (AZD5363) |
|---|---|---|---|---|
| MoA | allosteric | allosteric | ATP comp. | ATP comp. |
| **Biochemical activity**: | | | | |
| **AKT1**/**2**/3$^{wt}$ [nM] | 5/18/428 | 19/16/103 | 5/18/8 | 3/3/7 |
| **AKT1**/**2**$^{E17K}$ [nM] | 1.6µM/>2µM | >2µM/>2µM | 3/11 | 2.7/4.8 |
| **Cellular MoA** (model) | | | | |
| pAKT-S473 AKT1$^{wt}$ [nM] | 1.5 (KPL-4) | 6.6 (KPL-4) | Strong increase >200% | Strong increase >200% |
| pAKT-S473 AKT1$^{E17K}$ [nM] | 0.8 (LAPC-4)<br>83 (KU-19-19) | 180 (KU-19-19) | Strong increase >200% | Strong increase >200% |
| p4EBP1-T70 | 35 (LAPC-4)<br>100 (KU-19-19) | 460 (KU-19-19) | | |
| pPRAS40-T246 AKT1$^{wt}$ [nM] | 228 (LNCaP) | *No data* | 157 (LNCaP) | *No data* |
| pPRAS40-T246 AKT1$^{E17K}$ [nM] | 140 (LAPC-4) | *No data* | 100 (LAPC-4) | *No data* |
| **Proliferation** | | | | |
| AKT1$^{wt}$ | 60nM (MCF-7) | 380nM (MCF-7) | | *No data* |
| AKT1$^{E17K}$ | 40nM (LAPC4) | 150nM (LAPC4) | 533 (LAPC4) | *No data* |
| **In vivo MoA** (% inh over time [h]) | | | | |
| KPL4 AKT1$^{wt}$ | pAKT-S473 >80% / 24h | pAKT-S473 >80% / 24h | pPRAS40 >80% / ~8h (PC3) | |
| KU-19-19 AKT1$^{E17K}$ | pAKT-S473 >80% / 24h | pAKT-S473 >80% / 24h | | |
| **In vivo tumor growth inhibition** (T/C) | | | | |
| Model, dose mg/kg, response | AXF984 AKT1$^{E17K}$, 50, SD<br>PC3, 50, SD | AXF984 AKT1$^{E17K}$, 240, SD | PC3, 100, SD | |

# AKT1/2 Probe BAY1125976

## Technical In vitro profile




BAY1125976

| POTENCY ($IC_{50}$ [nM]) | |
|---|---|
| AKT1 (at 10 µM ATP) | 5.2 |
| AKT1 (at 2 mM ATP) | 44 |
| AKT2 (at 10 µM ATP) | 18 |
| AKT2 (at 2 mM ATP) | 36 |
| AKT3 (at 10 µM ATP) | 427 |

| Properties & Physchem | |
|---|---|
| LogD @ pH 7.5 | 2.3 |
| LLE | 6.0 |
| Sw @ pH 6.5 [mg/L] | 308 |
| MW / TPSA [g*mol / Å²] | 383 / 99 |
| Stability (r/h/d plasma, 37°C, 2h) [%] | 100 |

| *in vitro* DMPK Properties | | | | | | |
|---|---|---|---|---|---|---|
| Caco2 Permeability | $P_{app}$(A-B) [nm/s] | | $P_{app}$(B-A) [nm/s] | | efflux ratio | |
| | 135 | | 412 | | 3.1 | |
| metabolic stability | | | CL [L/h/kg] | | $F_{max}$ [%] | |
| | rat hepatocytes | | 1.3 | | 70 | |
| | dog hepatocytes | | 1.2 | | 43 | |
| | human hepatocytes | | 0.64 | | 52 | |
| CYP inhibition $IC_{50}$ [µM] | 1A2 | 2C8 | 2C9 | 2D6 | 3A4 | 3A4 preinc. |
| | > 20 | > 20 | > 20 | > 20 | > 20 | > 20 |
| CYP Induction | 3A4: NOEL 5000 µg/L | | | | | |

| Selectivity | |
|---|---|
| In-house kinase panel | High selectivity see next slide |
| Eurofins @ 1 µM (kinase panel) | See next slide |

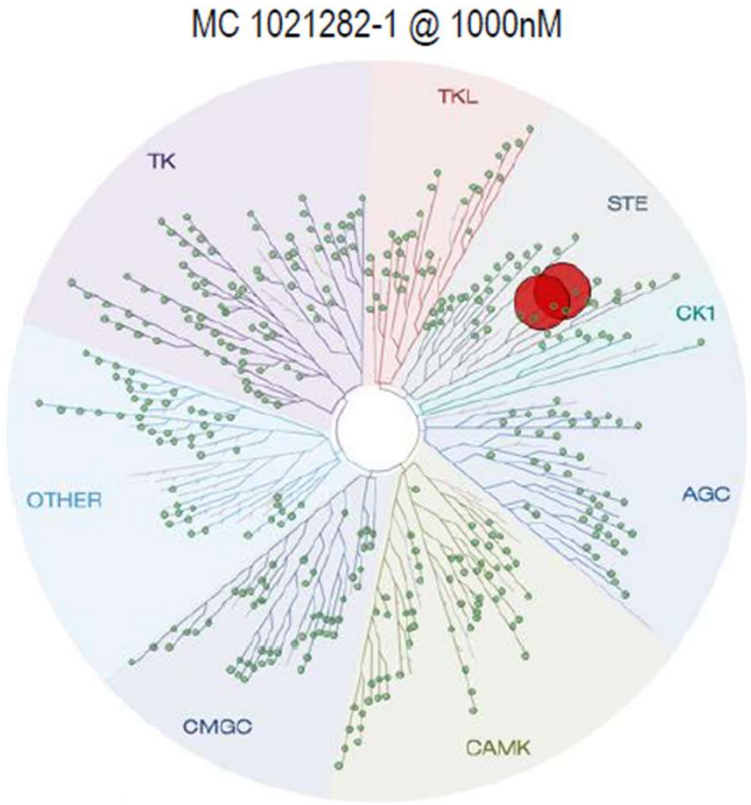
| SAFETY | |
|---|---|
| Ames | negative |
| hERG $IC_{50}$ [µM] | 8.7 |

- BAY-1125976 has high in vitro potency and selectivity
- BAY-1125976 has high solubility and high permeability

# AKT1/2 Probe BAY1125976

Selectivity Profile in more detail

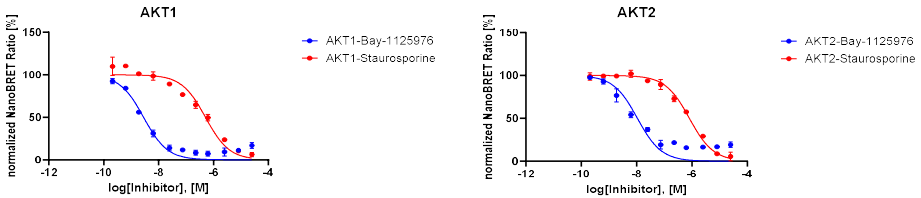
- AKT1, AKT2, AKT3 currently measured @ Benedict-Tilman Berger lab
- Additional compounds sent for comparison
- Data on BAY1125976 will provide more insight into selectivity of  Chemical Probe
- Data will identify most suitable negative control in direct comparison / same selectivity assay

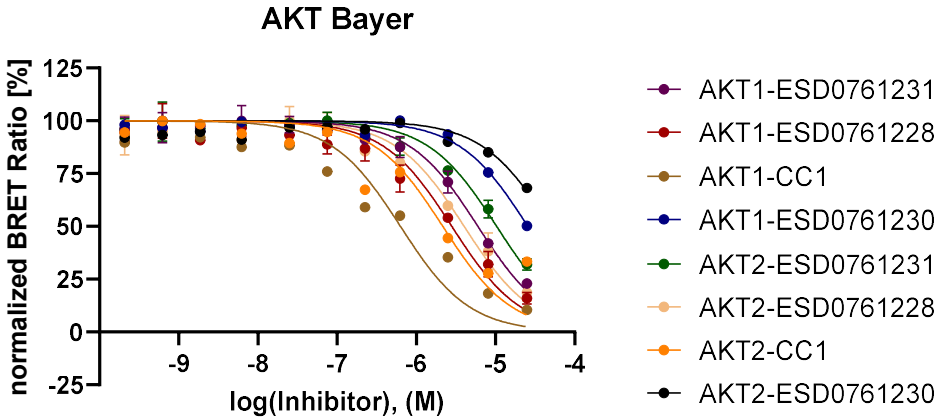



Kinome scan of BAY 1125976 for selectivity profiling at 1µM

# AKT1/2 Probe BAY1125976

## NanoBRET profiling data from Berger lab




| IC50, [M] | ESD0761231 | ESD0761230 | ESD0761228 | CC1 (Ctrl) | Bay-1125976 | Staurosporine |
|---|---|---|---|---|---|---|
| AKT1 | 6.00E-06 | 2.56E-05 | 2.81E-06 | 6.32E-07 | 2.84E-09 | 5.10E-07 |
| AKT2 | 1.04E-05 | 4.97E-05 | 4.03E-06 | 2.16E-06 | 1.07E-08 | 8.09E-07 |

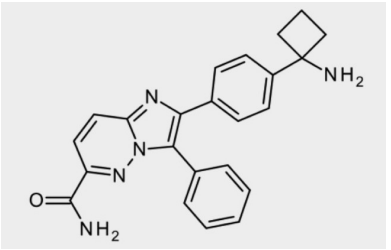
Negative control

Chemical probe

# AKT1/2 Probe BAY1125976

## In vitro proliferation in broad breast cancer cell line panel

| Cell line | subtype | ER | PR | ERBB2/ HER2 | IC50 (BAYER) | IC50 (Sanger) | AKT1 | BRAF | CDKN2A | KRAS | PIK3CA | PTEN | RB1 | TP53 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ZR-75-1 | L | + | - | - | 7,02E-08 | 7,02E-08 | wt | wt | wt | wt | wt | p.L108R | wt | wt |
| EVSA-T | L | | | | 1,20E-07 | 9,22E-08 | wt | wt | wt | wt | wt | p.T319fs*1 | wt | p.S241C |
| KPL-4 | LH | | | | 1,74E-07 | 1,74E-07 | wt | wt | wt | wt | p.H1047R | wt | wt | wt |
| MDA-MB-361 | L | + | + | + | | 4,61E-07 | p.D3N | wt | wt | wt | p.E545K; p.K567R | wt | wt | p.E56* |
| MDA-MB-415 | L | + | | + | | 4,65E-07 | wt | p.P74A | wt | wt | wt | p.C136Y | wt | p.Y236C |
| MFM-223 | L | | | | | 5,97E-07 | wt | wt | wt | wt | p.H1047R | wt | wt | p.K132R |
| HCC202 | L | - | - | + | | 8,01E-07 | wt | wt | wt | wt | p.E545K; p.L866F | wt | wt | p.T284fs*21 |
| HCC70 | B | - | - | - | 8,44E-06 | 1,11E-06 | wt | wt | wt | wt | wt | p.F90fs*9 | wt | p.R248Q |
| T47D | L | + | + | - | 6,03E-08 | 1,19E-06 | wt | wt | wt | wt | p.H1047R | wt | wt | p.L194F |
| MDA-MB-453 | LH | - | - | + | 1,22E-07 | 1,33E-06 | wt | wt | wt | wt | p.H1047R | wt | wt | wt |
| HCC1954 | B | - | - | + | | 1,39E-06 | wt | wt | wt | wt | p.H1047R | wt | wt | p.Y163C |
| EFM-19 | L | + | + | - | | 1,68E-06 | wt | wt | wt | wt | p.H1047L | wt | wt | p.H193R |
| HCC1187 | B | - | - | - | | 2,79E-06 | wt | wt | wt | wt | wt | wt | wt | p.G108delG |
| MCF7 | L | + | + | - | 6,33E-08 | 3,41E-06 | wt | wt | p.?fs | wt | p.E545K | wt | wt | wt |
| BT-474 | LH | + | + | + | 1,67E-08 | 4,20E-06 | wt | wt | wt | wt | p.K111N | wt | wt | p.E285K |
| CAMA-1 | L | + | | + | | 4,88E-06 | wt | wt | wt | wt | wt | p.F278fs*12; p.D92H | wt | p.R280T |
| HCC1569 | B | - | - | + | | 5,83E-06 | wt | wt | wt | wt | wt | p.K267fs*9 | wt | p.E294*; p.D228fs*19 |
| BT-20 | B | - | - | - | 3,85E-07 | 6,20E-06 | wt | wt | p.?del | wt | p.P539R | wt | wt | p.K132Q |
| EFM-192A | L | + | + | + | | 1,03E-05 | wt | wt | wt | wt | p.C420R | wt | wt | p.E271fs*1 |
| MDA-MB-175-VII | L | + | | | | 1,04E-05 | wt | wt | wt | wt | wt | wt | wt | wt |
| COLO-824 | B | - | | + | | 1,04E-05 | wt | wt | wt | wt | wt | wt | p.S350I | p.? |
| CAL-51 | B | - | NA | - | | 1,25E-05 | wt | wt | wt | wt | p.E542K | p.E288fs: p.T321fs | wt | wt |
| HCC2218 | L | - | - | + | | 1,41E-05 | wt | p.E296K | wt | wt | wt | wt | wt | wt |
| Hs-578-T | Claudin low | - | - | - | | 1,58E-05 | wt | wt | wt | wt | wt | wt | wt | p.V157F |
| BT-549 | B | - | - | - | 8,44E-06 | 1,59E-05 | wt | wt | wt | wt | wt | p.V275fs*1 | p.? | p.R249S |
| CAL-120 | B | | | | 1,00E-05 | 1,81E-05 | wt | wt | wt | wt | wt | wt | wt | p.? |
| HCC1937 | B | - | - | - | | 2,00E-05 | wt | wt | wt | wt | wt | wt | p.? | p.R306* |
| MDA-MB-468 | B | - | - | - | 6,47E-06 | 2,01E-05 | wt | wt | wt | wt | p.E545A | p.L70fs*7 | p.? | p.R273H |
| MDA-MB-436 | Claudin low | - | - | - | | 2,03E-05 | wt | wt | wt | wt | wt | wt | wt | p.E204fs*7 |
| MDA-MB-157 | Claudin low | - | - | - | | 2,40E-05 | wt | wt | wt | wt | wt | wt | wt | p.A88fs*52 |
| HCC1806 | B | - | - | - | | 2,88E-05 | wt | wt | wt | wt | wt | wt | wt | p.T256fs*90 |
| HCC1395 | Claudin low | - | - | - | | 3,86E-05 | wt | wt | wt | wt | wt | wt | wt | p.R175H |
| MDA-MB-231 | B | - | - | - | 1,00E-05 | 2,83E-04 | wt | p.G464V | p.?fs | p.G13D | wt | wt | wt | p.R280K |

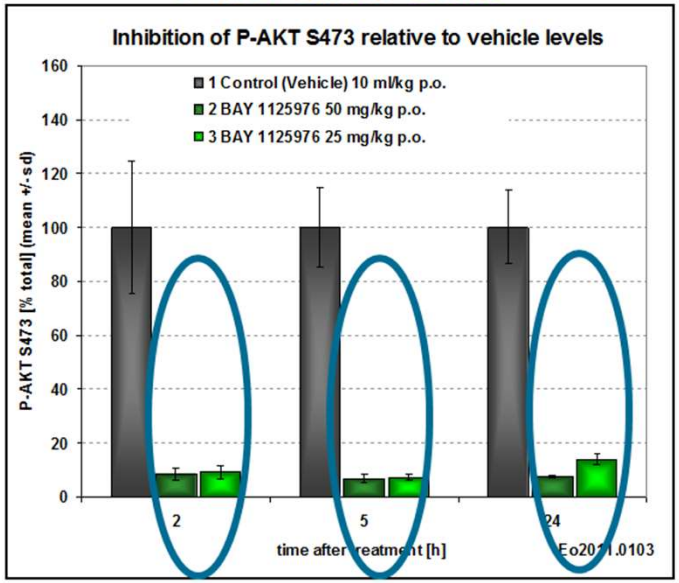



- BAY 1125976 demonstrates potent anti-proliferative efficacy in **luminal and luminal Her2$^{O/E}$** breast cancer lines *in vitro*

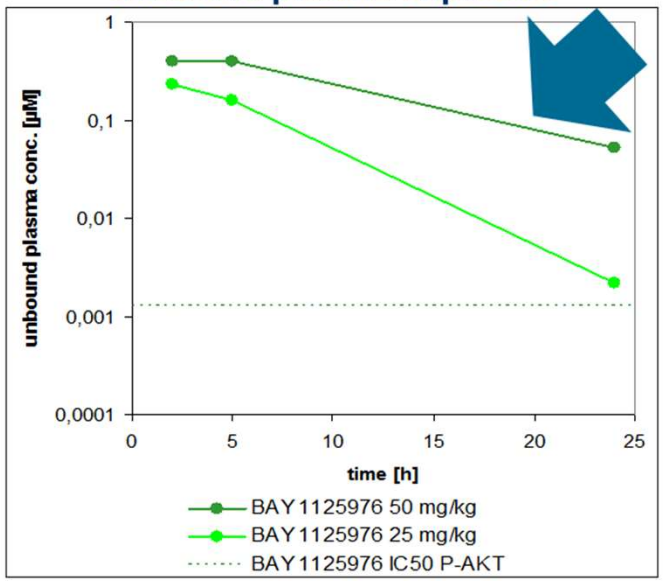
# AKT1/2 Probe BAY1125976

## Mode of Action in vivo KPL-4 model (PIK3CAH1047R, HER2$^{O/E}$)

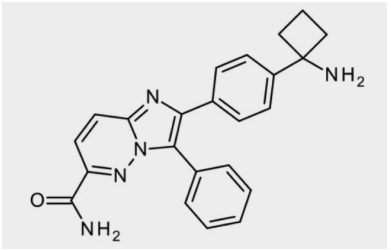
**P-AKT tumor levels**




**Unbound plasma exposure**



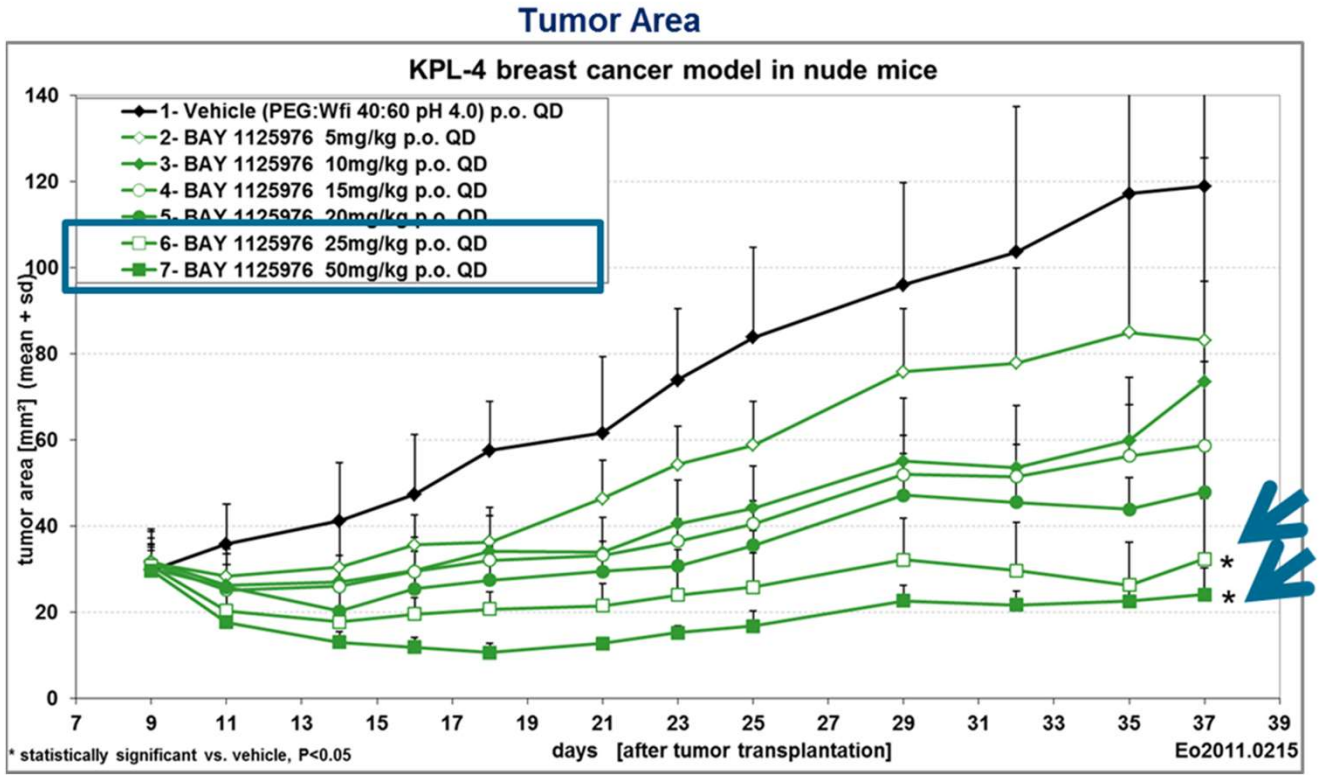

**Study Design:**
- Single oral dose; fast exposure over 24 h
- P-AKT inhibition (S473) measured in tumor extracts

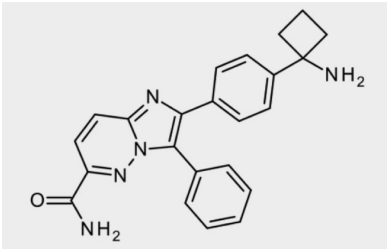



- Strong P-AKT tumor inhibition (>70%)
- Significant unbound plasma exposure up to 24 hours post treatment

# AKT1/2 Probe BAY1125976

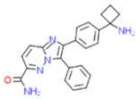
Allo AKT inhibitor BAY 1125976 demonstrates potent anti-tumor efficacy in KPL-4 BC (PIK3CA$^{H1047R}$, HER2$^{O/E}$) *in vivo*




- Continuous QD dosing schedule preferred based on preclinical studies and used in FiM protocol

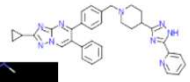
# AKT1/2 Probe BAY1125976

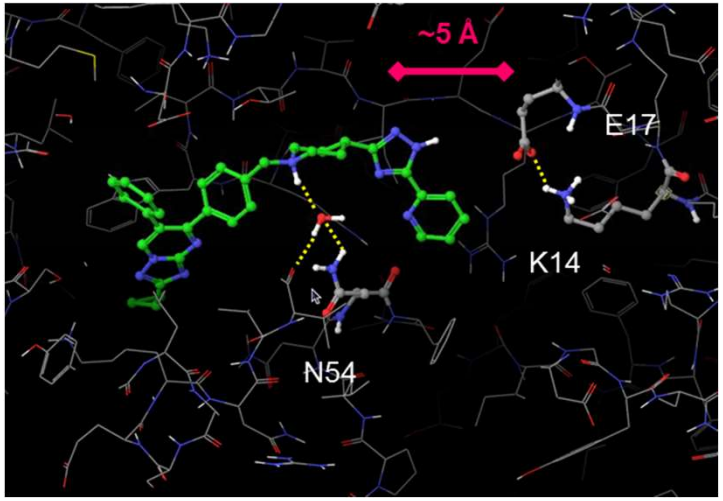


BAY 1125976

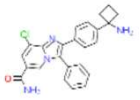
## AKT1-E17K mutation: structural data

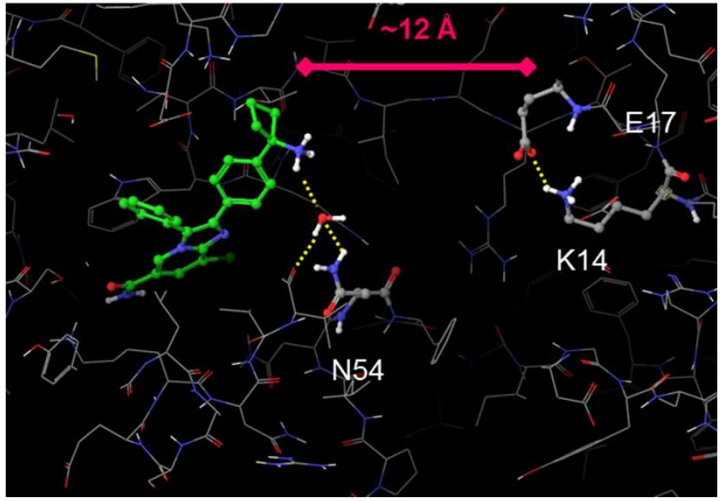
**BAY 1001931**






**BAY 1122171**

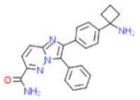





- Docking into allosteric binding pocket formed by kinase and PH domain of AKT1
- Binding site of BAY 1125976 like series (right) is more remote from E17K mutation site than first-generation series (BAY 1001931)
- Thus, binding affinity of current series is probably less affected by E17K mutation
- Both PH as well as kinase domain of AKT1 undergo major conformational change upon complex formation. Impact of E17K mutation on conformational change and ligand binding site is unpredictable

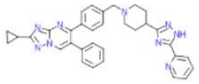
- **Differential docking of selected allosteric AKT inhibitors in wt AKT1**
- **No direct interaction of BAY1125976 like compounds with E17 position**

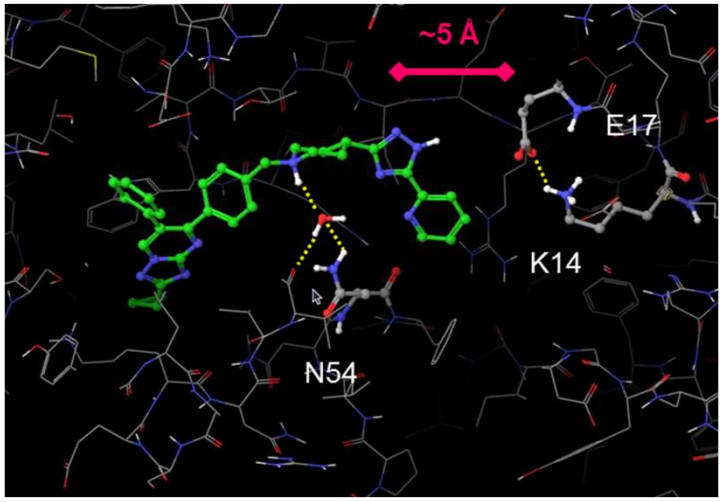
# AKT1/2 Probe BAY1125976



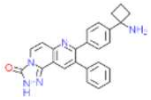

## AKT1-E17K mutation: structural data

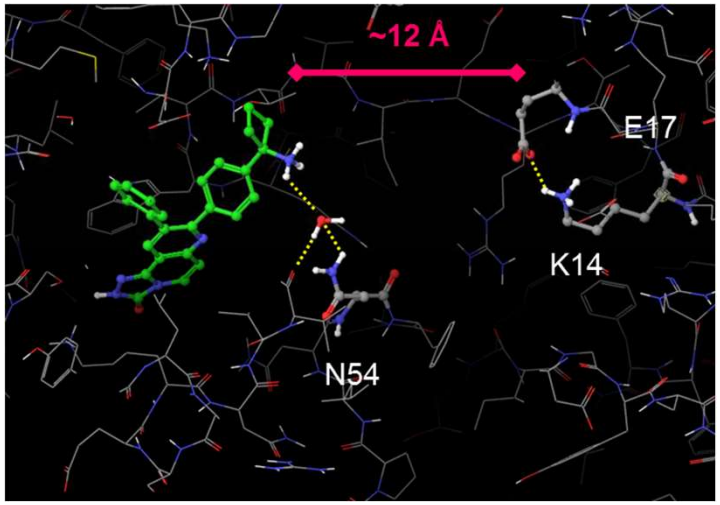
**BAY 1001931**






MK-2206

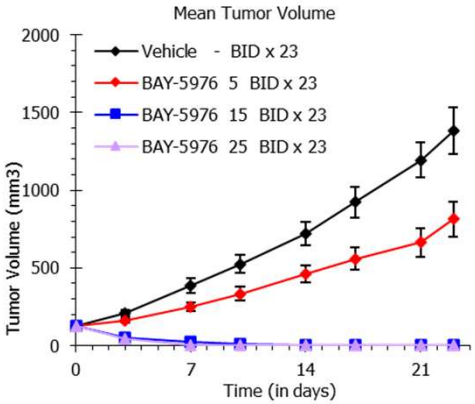


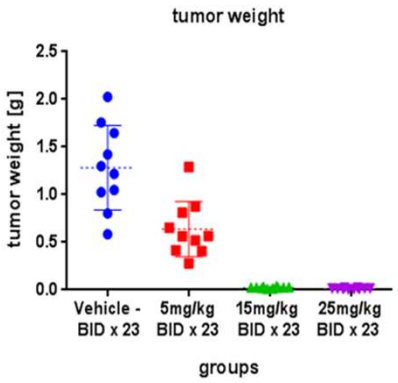


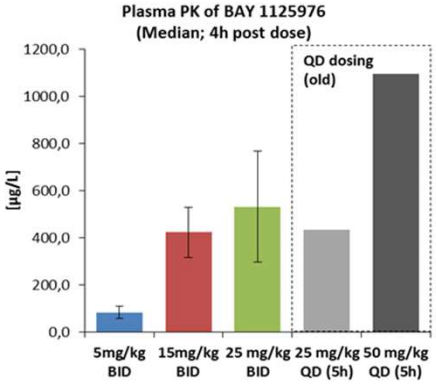

- Docking of Competitor MK2206 into allosteric binding site

# Strong Activity of BAY 1125976 in Breast Cancer Model with the AKT1$^{E17K}$ mutation

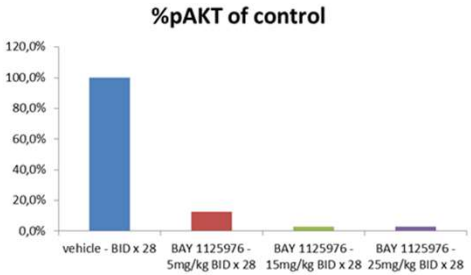
- **BAY 1125976 with high efficacy in an AKT1$^{E17K}$ breast cancer PDx model when dosed in a BID dosing schedule, comparable PK between QD and BID dosing**

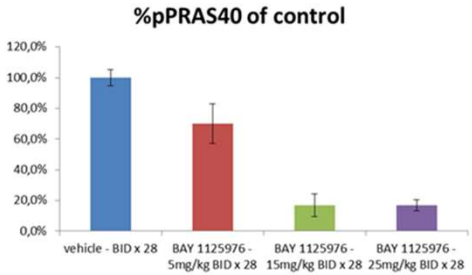



- **Strong inhibition of pAKT and downstream marker pPRAS40**

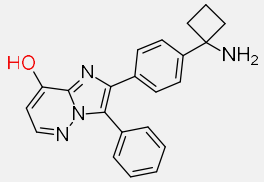



- **pPRAS40 levels correlate with efficacy**
- **pAKT with inhibition also at non-efficacious doses**

# AKT1/2 Probe BAY1125976

## In vitro profile of Negative Control BAY-940




**BAY-940**

| POTENCY ($IC_{50}$ [nM]) | |
|---|---|
| AKT1 (PKB alpha) | 3740 |
| AKT2 (PKB alpha) | 3840 |
| AKT1 (Nano BRET) | 6000 |
| AKT2 (Nano BRET) | 10400 |
| | |

| Properties & Physchem | |
|---|---|
| LogD @ pH 7.5 | 1.60 |
| TPSA [g*mol / Å$^2$] | 76 |
| Sw @ pH 6.5 [mg/L] | 99 |
| MW [g/mol] | 356 |
| Stability (r/h/d plasma, 37°C, 2h) [%] | n.d. |

### *in vitro* DMPK Properties

| | | | | | | |
|---|---|---|---|---|---|---|
| Caco2 Permeability | $P_{app}$(A-B) [nm/s] | | $P_{app}$(B-A) [nm/s] | | efflux ratio | |
| | n.d. | | n.d. | | n.d. | |
| metabolic stability | | | CL [L/h/kg] | | $F_{max}$ [%] | |
| | rat hepatocytes | | n.d. | | n.d. | |
| | dog hepatocytes | | n.d. | | n.d. | |
| | human hepatocytes | | n.d. | | n.d. | |
| CYP inhibition $IC_{50}$ [µM] | 1A2 | 2C8 | 2C9 | 2D6 | 3A4 | 3A4 preinc. |
| | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| CYP Induction | n.d. | | | | | |

### Selectivity

| | |
|---|---|
| In-house kinase panel | n.d. |
| Eurofins @ 1 µM (kinase panel) | n.d. |

### SAFETY

| | |
|---|---|
| Ames | n.d. |
| hERG $IC_{50}$ [µM] | n.d. |

- BAY-940 negative on AKT1 & AKT2
- BAY-940 perfect as negative control to the Chemical Probe

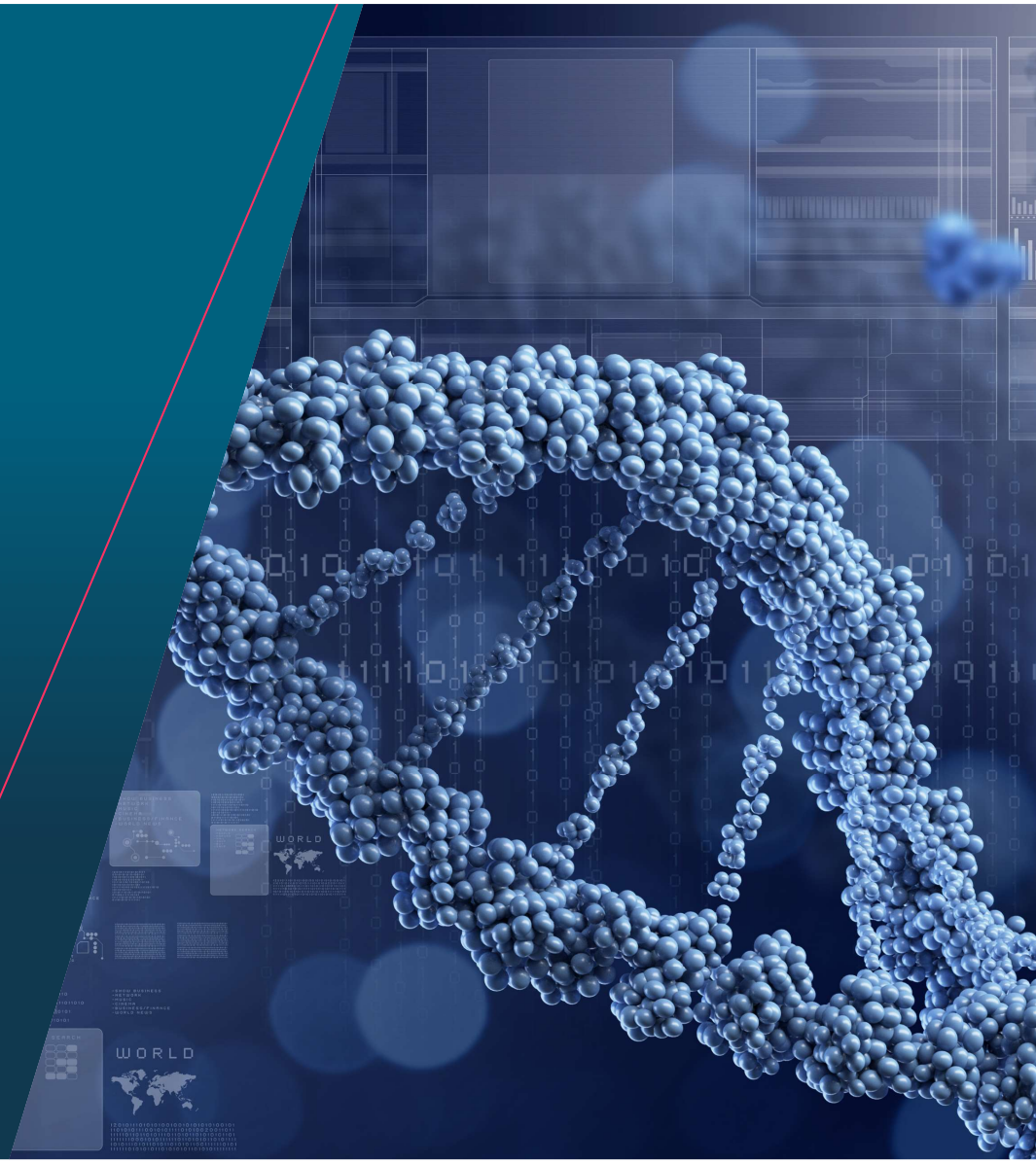
# AKT1/2 Probe BAY1125976

## Summary / Conclusion

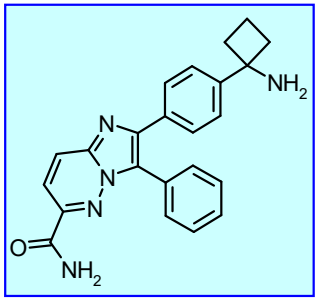
| Probe criteria | |
|---|---|
| **Inhibitor/agonist potency: goal is < 50 nM** ($IC_{50}$, Kd) | **Surpasses criteria** |
| **Selectivity within target family: goal is > 30-fold** | **Surpasses criteria** |
| Selectivity outside target family: describe the off-targets (which may include both binding and functional data) | |
| **On target cell activity for cell-based targets: goal is < 1 µM** $IC_{50}/EC_{50}$ | **Surpasses criteria** |
| On target cell activity for secreted targets: appropriate alternative such as mouse model or other mechanistic biological assay, e.g., explant culture | Additional proof for on target activity: |
| Neg ctrl: *in vitro* potency – > 100 times less; Cell activity – >100 times less potent than the probe | **Surpasses criteria** |

We ask for acceptance of AKT inhibitor BAY1125976 as chemical probe, accompanied by BAY-940 as negative control

# Thank You



# BAY 1125976 – in vitro profile in detail




## Key Activity (IC50)

| | |
|---|---|
| • AKT1/AKT2 (active kinase, median) | 5 / 18 nM |
| • AKT1/AKT2 (active kinase, high ATP) | 44 / 36 nM |
| • AKT3 (Bioduro) | 428 nM |
| • AKT1 (PDK1 activation assay) | 22 nM |
| • ΔPH-AKT1 / ΔPH-AKT2 | >20 / >20 µM |

## Mechanistic Cell Assay (median $IC_{50}$)

| | |
|---|---|
| • p-AKT (S473/T308) Alphascreen, KPL4 | 1.1 / 0.9 nM |
| • p-4E-BP1 (T70) Alphascreen, KPL4 | 35 nM |

## Kinase selectivity (median IC50 M)

| | |
|---|---|
| • Mer (10 µM/ 2 mM ATP) | 1.29E-7 / 5.63E-6 |
| • Flt-4 (10 µM/ 2 mM ATP) | 2.68E-7 / 4.26E-6 |
| • MKNK1 (10 µM/ 2 mM ATP) | 2.76E-7 / 8.86E-7 |
| • Mps1 (10 µM/ 2 mM ATP) | 5.94E-7 / >2.00E-5 |

## Tumour Cell Proliferation (median $IC_{50}$)

| | | |
|---|---|---|
| • LNCaP | prostate cancer | 15 nM |
| • LAPC4 | prostate cancer | 62 nM |
| • MCF7 | breast cancer | 82 nM |
| • KPL4 | breast cancer | 174 nM |

## In vivo

| | |
|---|---|
| • MTD nude mice/ rats: | 50/ 25 mg/kg QD po |
| • MOA 80% P-AKT inhib (25 mg/kg): | >24h |
| • Mouse: KPL4 (25 / 50 mg/kg QD) | T/C 0.22 / 0.16 |
| • Mouse: PC3 (50 mg/kg QD) | T/C 0.13 |
| • Rat: KPL4 (15 / 25 mg/kg QD) | T/C 0.43 / 0.23 |

## Safety / Tox

| | |
|---|---|
| • Cytotoxicity | Category 0 |
| • Ames | negative |
| • hERG ($IC_{50}$) | 8.7 µM |
| • Ricerca Lead Profiler screen | no effects < 10µM |

## DMPK

| | |
|---|---|
| • Microsomal Clint [L/kg/h] | 0.69/0.86/0.30 (h/m/r) |
| Fmax prediction [%] | 48/84/93 (h/m/r) |
| • Hepatocytes CL [L/kg/h] | 0.64/1.2/1.3 (h/d/r) |
| Fmax [%] | 52/43/70 (h/d/r) |
| • Caco2 ap-bas (ratio) | 135 (3.1) |
| • Fu % (h / d / g / r / m ) | 6 / 13 / 17 / 11 / 14 |
| • $CL_{blood}$ (rat) | 1.2 L/h/kg |
| • Vss (rat) | 13 L/kg |
| • $t_{1/2}$ (rat) | 3.7 h |
| • F (rat @ 2 mg/kg) | 65 % |
| • $C_{max\ norm,po}$ ($t_{max}$) @ 2 mg/kg | 0.035 kg/L (4h) |
| • $AUC_{norm,po}$ @ 2 mg/kg | 0.25 kg*h/L |
| • brain/plasma ratio (AUC, $AUC_u$) | 0.41, 0.15 |
| • CYP Inhibition (microsomes) | >20 µM, hint for TDI |
| • 3A4 Cyp Induction, NOEL | No induction up to 62 µg/L |
| • $F_{rel}$ (rat) [%] | 128 |

## Physchem

| | |
|---|---|
| • Sw flask @ pH 6.5 / 4 | 619.4 / 484.6 mg/L (25 °C) |
| • cLogP / logMA | 2.82 / 4.66 |
| • pKa (photometric) | 4 (basic) |
| • Oral PC Score | 0 |
| • PSA | 99.3 |
| • MW | 383.45 |

# AKT1/2 Probe BAY1125976

## Biology of an allosteric inhibitor in blocking activation of AKT/PKB

**Activation of AKT:**

- Switch between **inactive** and active state involves molecular rearrangement of PH-and catalytic domain (PH-in ⇔ PH-out)

Depends on:

1. interaction between PDK1 and AKT
2. Integration into membrane by association with phosphoinositides (PtdIns(3,4,5)$P_3$)

3. Stepwise process with two phosphorylations at T308 (by PDK1)
   S473 (by mTORC2 / DNA-PK)

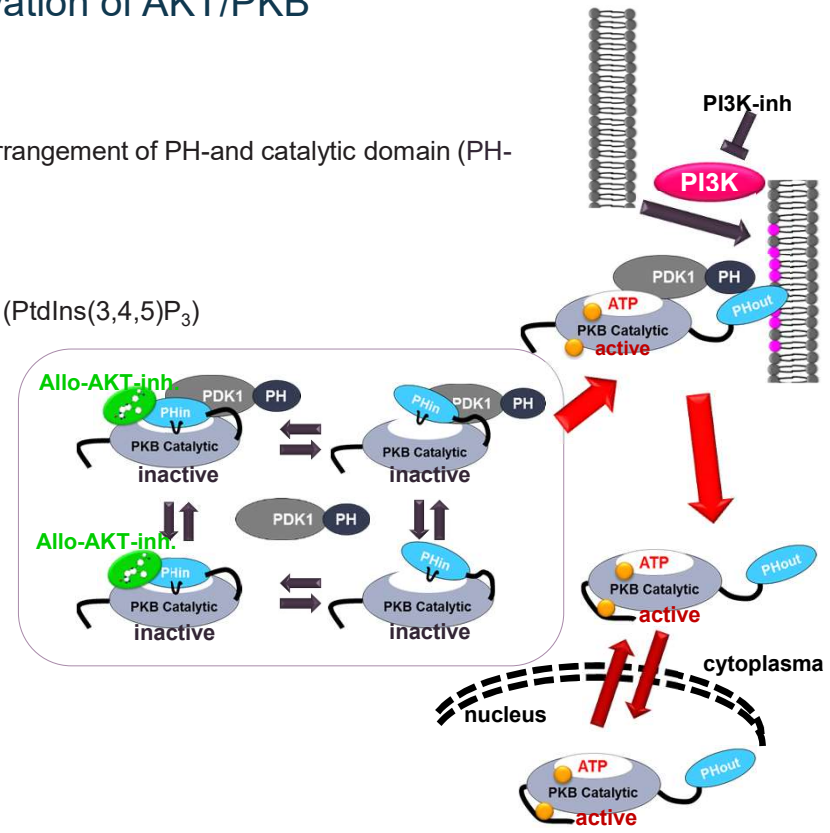
- Allosteric AKT inhibitor stabilize inactive conformation in cytoplasma preventing phosphorylation

Allosteric AKT-inhibitor binding depends on:

1. Interaction of PH and catalytic domain
2. Interaction of PDK1 and AKT

Allosteric AKT-inhibitor binding impaired by:

1. Membrane integration and PI3K activity
2. AKT-phosphorylation at T308 / S473




Modified from: Calleja, V., D. Alcor, et al. (2007). PLoS Biol 5(4): e95.

# AKT1/2 Probe BAY1125976

## Competitors

- Ipatasertib (GDC-0068; Genentech, Inc.) is a potent, selective, ATP-competitive small-molecule inhibitor of all three isoforms of AKT (IC 50 of 5–18 nmol/L
- Capivasertib (AZD5363) is a highly selective ATP-competitive pan-AKT inhibitor
- MK2206: allosteric AKT 1/2/3 inhibitor; well established in the scientific community


Nitulescu, G. M., D. Margina, P. Juzenas, Q. Peng, O. T. Olaru, E. Saloustros, C. Fenga, D. A. Spandidos, M. Libra and A. M. Tsatsakis (2016). "Akt inhibitors in cancer treatment: The long journey from drug discovery to clinical use (Review)." Int J Oncol **48(3): 869-885.**

Nitulescu, G. M., M. Van De Venter, G. Nitulescu, A. Ungurianu, P. Juzenas, Q. Peng, O. T. Olaru, D. Grădinaru, A. Tsatsakis, D. Tsoukalas, D. A. Spandidos and D. Margina (2018). "The Akt pathway in oncology therapy and beyond (Review)." International journal of oncology **53(6): 2319-2331.**


Nitulescu-2016-A
inhibitors in cance

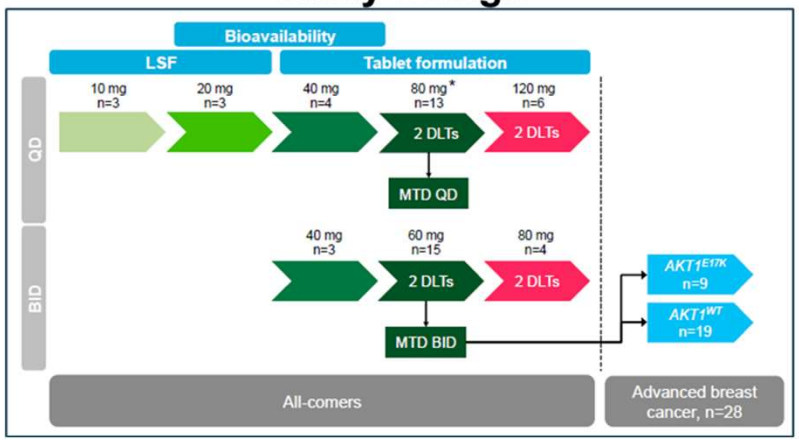
Nitulescu-2018-T
kt pathway in onc

Schneeweiss-2019
ase 1 Dose Escalati

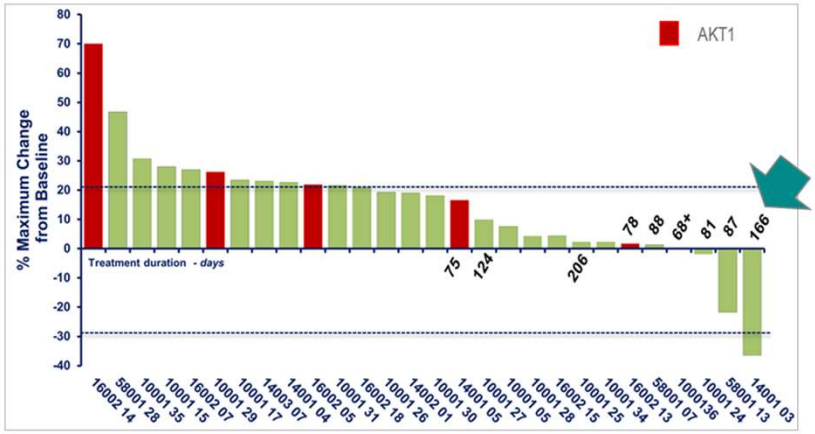
# Phase 1 Dose Escalation Study of the Allosteric AKT Inhibitor BAY 1125976 in Advanced Solid Cancer (NCT01915576)

## Lack of Association between Activating AKT Mutation and AKT Inhibition-Derived Ecacy

### Study Design




### BC Expansion Cohort

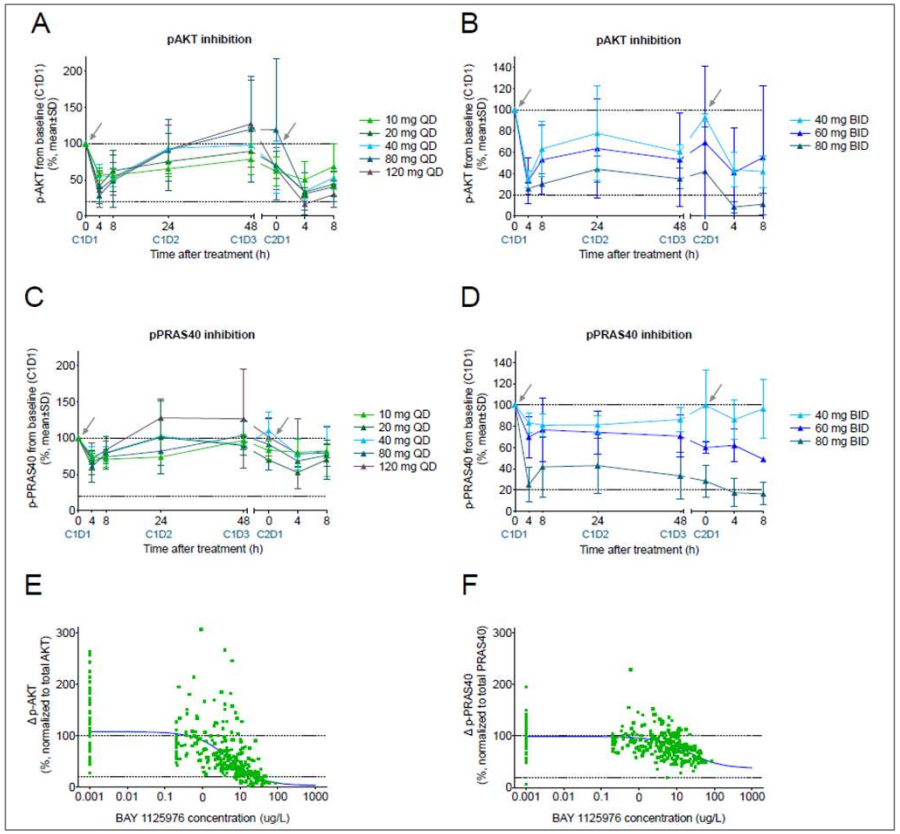



**BC expansion cohort:**

- Best response is 1 confirmed PR in AKT1wt BC pt
- CBR (PR+SD)=29.2%; ORR (PR+SD)=3.5%
- Median SD duration of 129d and ongoing

- evaluate safety, pharmacokinetics, and maximum tolerated dose of BAY 1125976 in patients with advanced solid tumors
- Application of continuous once daily (QD) treatment (21 days/cycle) and a twice daily (BID) schedule
- Dose expansion in 28 patients with hormone receptor-positive metastatic breast cancer, including nine patients harboring the $AKT1^{E17K}$ mutation at the recommended phase 2 dose (R2D) of 60 mg BID.
- Dose-limiting toxicities (Grades 3–4): increased in transaminases, glutamyltransferase (-GT), alkaline phosphatase in four patients in both schedules and stomach pain in one patient

# Biomarker Results from phase 1 study




Schneeweiss A, et al. "Phase 1 Dose Escalation Study of the Allosteric AKT Inhibitor BAY 1125976 in Advanced Solid Cancer-Lack of Association between Activating AKT Mutation and AKT Inhibition-Derived Efficacy." Cancers (Basel). 2019;11(12).

Figure 3. Inhibition of phosphorylation of AKT (A,B) and PRAS40 (C,D) as pharmacodynamic biomarkers in platelet-rich plasma (PRP) from patients in the QD (left column) and BID (right column) dose escalation parts in the BAY 1125976 Phase 1 study. PK/PD analysis on the suppression of p-AKT (E) and p-PRAS40 (F) from thrombin receptor-activating peptide (TRAP)-stimulated platelets across dose intervals relative to baseline (screening, C1D1 pre-dose or C1D-3 pre-dose).

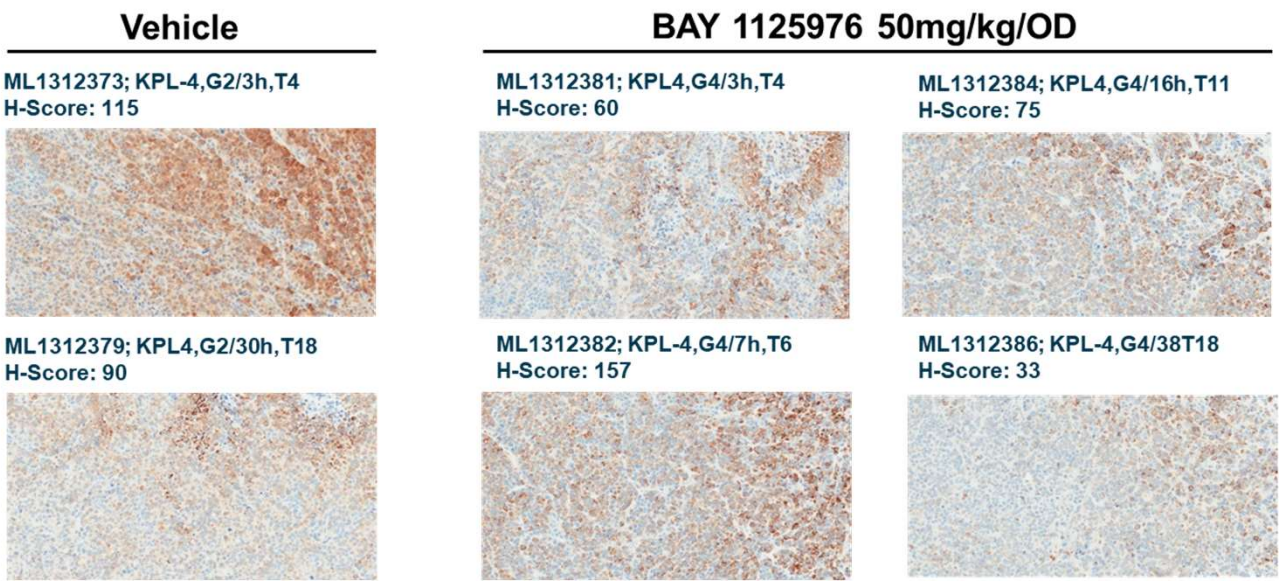
*Values are normalized to total AKT and total PRAS40, respectively. Vertical dotted line represents IC90 for p-AKT based on clinical pharmacokinetic/pharmacodynamic (PK/PD) modeling. Arrows indicate when treatments were started. C, cycle; D, day. PD: progressive disease.*

- dose-dependent inhibition of both pharmacodynamic biomarkers with maximum inhibition detectable at 4 h post-dosing
- rapid return to baseline suggesting a rapid direct effect link between AKT inhibition and biomarker modulation
- QD administration, even at the MTD, was not able to achieve sustained coverage above the in vitro IC50 of p-AKT or p-PRAS40.

# AKT1/2 Probe BAY1125976

## Preclinical data: pAKT staining (BYR040; Mosaic)

KPL-4 model; TRG-exp. 00577; IHC, Mosaic, BYR040, clone: 14-5




pAKT-S437 IHC staining shows no clear treatment effects (potential antibody issue)

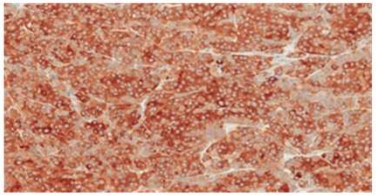
# AKT1/2 Probe BAY1125976

## Preclinical data: pPRAS40 staining (BYR040; Mosaic)

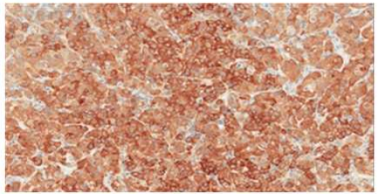
KPL-4 model; TRG-exp. 00577; IHC, Mosaic, BYR040, clone: C77D7

**Vehicle**

ML1312373; KPL-4,G2/3h,T4
H-Score: 257

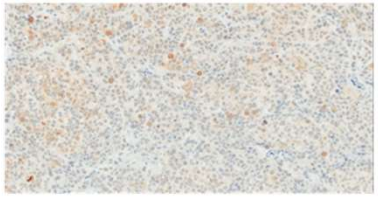


ML1312379; KPL4,G2/30h,T18
H-Score: 235

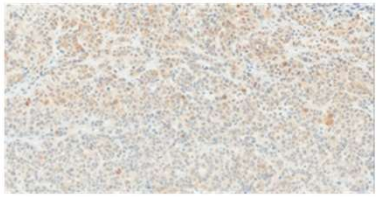


**BAY 1125976 50mg/kg/OD**

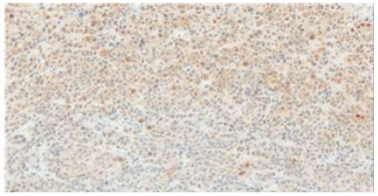
ML1312381; KPL4,G4/3h,T4
H-Score: 37



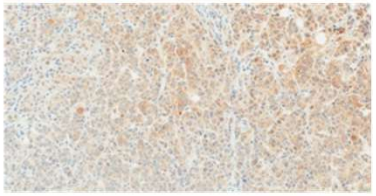
ML1312384; KPL4,G4/16h,T11
H-Score: 20



ML1312382; KPL-4,G4/7h,T6
H-Score: 27



ML1312386; KPL-4,G4/38T18
H-Score: 60



pPRAS40 IHC staining shows clear treatment effects