# Quantification of PI3K/AKT/mTOR Pathway Inhibition is Predictive of Biological Response

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## Introduction:

More and more targeted therapies are currently approved in the treatment of various cancer types although the success rate of these therapies varies enormously, creating a massive clinical need for predictive biomarkers.
It becomes clear that the presence of a single receptor or mutation does not provide sufficient information to accurately predict response in the majority of patients. Additional biological insight into the tumor growth driving pathways in individual patients is still lacking, which may support accurate response prediction towards specific drugs.
Here, we show that the reduction in pathway activity as measured by InnoSIGN Comprehensive Pathway Analysis (CPA) is predictive for the biological response as measured by cell growth inhibition in various cell lines.

## Methods:

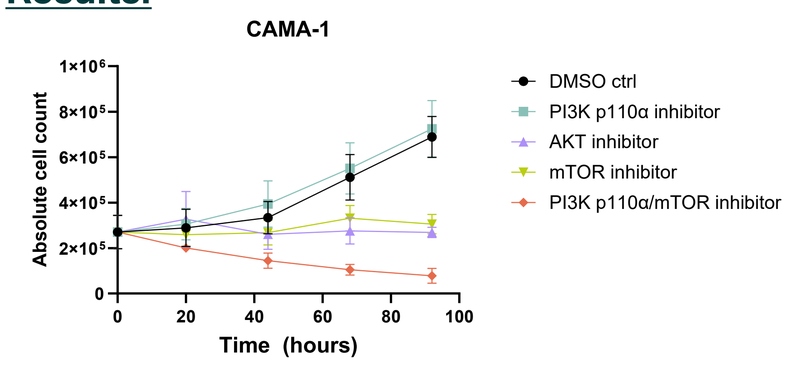
Six different breast cancer cell lines were used with the following characteristics:

| Cell line | ER status | PR status | HER-2 | Mutation |
|---|---|---|---|---|
| MCF-7 | + | + | – | PIK3CA |
| CAMA-1 | + | + | – | PTEN loss |
| HCC 1500 | + | + | – | wt |
| | | | | |
| SK BR 3 | – | – | + | wt |
| BT 20 | – | – | – | PIK3CA |
| HCC 1806 | – | – | – | wt |

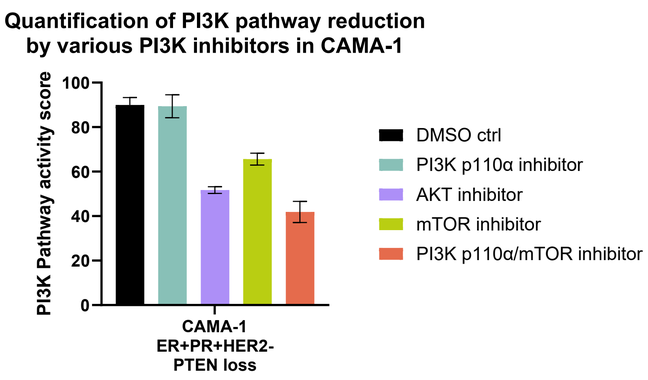
Inhibitors targeting PI3K, Akt, mTOR or both mTOR /PI3K were administered at 1 µM. DMSO served as control.

RNA was collected from treated cells and controls after 20h to measure the pathway activity of PI3K, MAPK, ER, AR, Hh, TGF-beta, and Notch using CPA test. Ki67 mRNA expression levels were also determined. Cell growth was measured (cell counts) by taking samples of the treated and control cells after 20, 44, 68, and 92 hours of drug exposure.

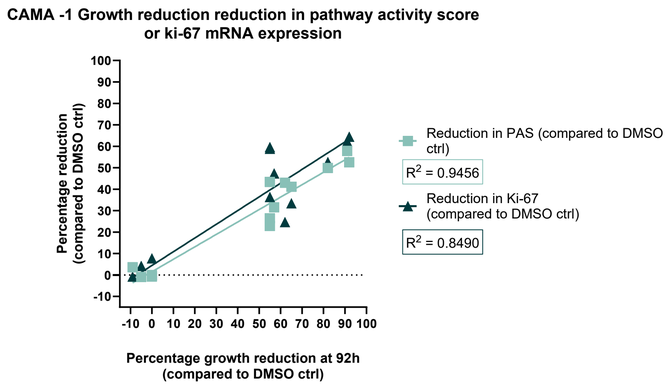
## Results:



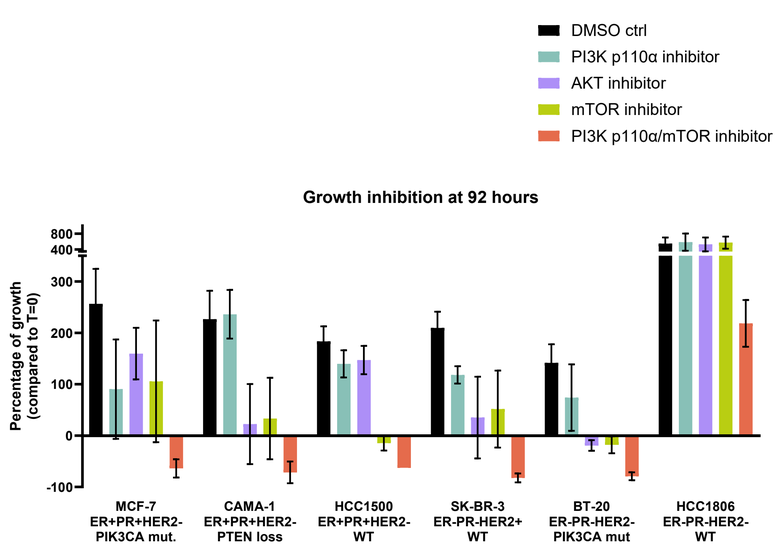

For each of the cell lines a growth curve has been created with the different PI3K inhibitory drugs. The CAMA cell line outcome is shown here as a typical example.



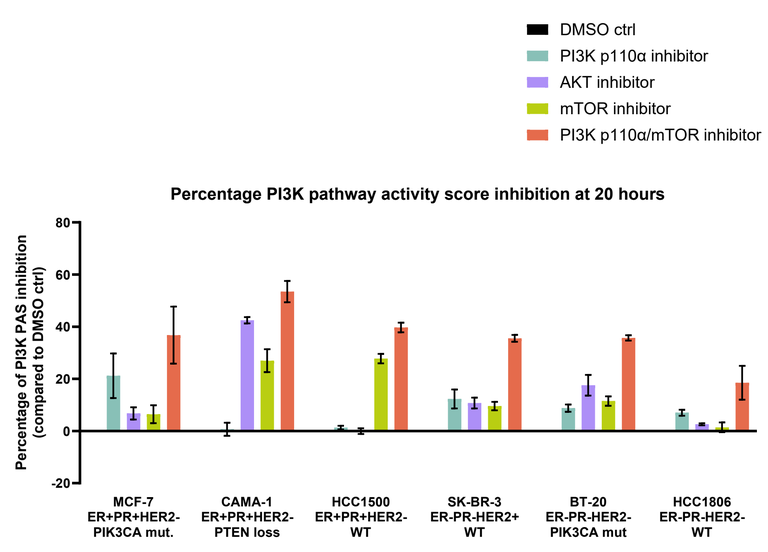

Quantification of the PI3K pathway activity at 20h. The lowest pathway activity relates to the strongest growth inhibition.




PI3K pathway score shows a better correlation to growth inhibition than Ki67 mRNA expression.




Growth inhibition (%) per drug per cell line measured at 92 hours of treatment.



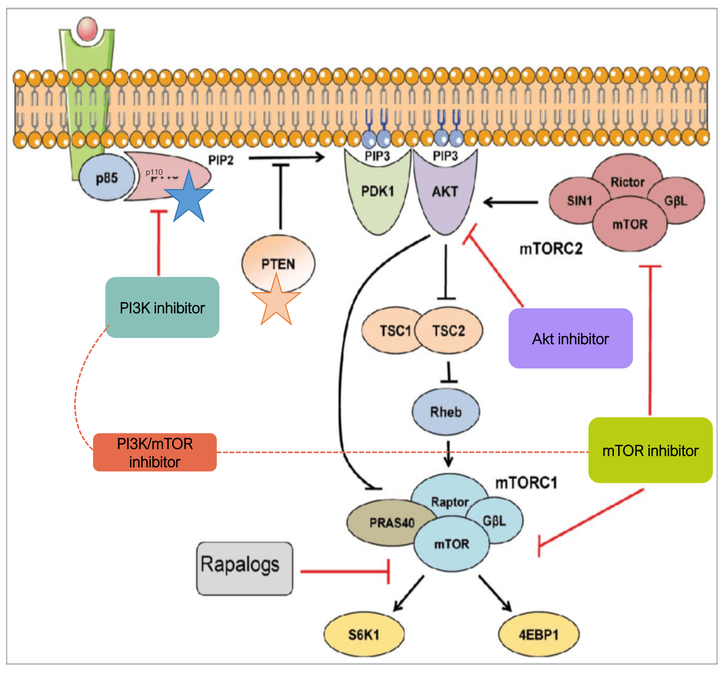

PI3K pathway inhibition (%) per drug per cell line measured at 20 hours of treatment.

It is clearly observed that the different drugs vary in their ability to inhibit growth, and that this is cell line (mutation) dependent.

Cell growth inhibition correlates well with a reduction in PI3K pathway activity.

Drugs with the most efficient inhibitory effect on the PI3K pathway after 92 hours can already be detected after 20h based on pathway activity profiles.

## Discussion:




The four different drugs inhibit the PI3K pathway at different levels.

When there is a mutation and a drug has its effect upstream from that, the inhibition will be less or not effective. The CAMA cell line has a PTEN loss, which means that the PI3K pathway remains active. The used PI3K inhibitor inhibits the PI3K complex upstream from the PTEN loss and therefore has no or limited effect as an efficient inhibitor.

The PI3K/mTOR inhibitor has its mechanism of action both upstream and downstream of the PI3K cascade (PI3K and mTOR level) and shows inhibition in all cell lines, regardless of where the mutations are located.

Interestingly, the HCC1806 cell line (triple negative) only shows a growth reduction under PI3K/mTOR treatment. Under *in-vitro* conditions all the cell lines show a high PI3K and MAPK activity. It could be that especially the triple negative cell line growth is driven by the MAPK pathway and that inhibition of the PI3K pathway has limited effect in these cell lines.

## Conclusion:

Using InnoSIGN Comprehensive Pathway Analysis (CPA) it is possible to quantitatively measure the capacity of drugs to inhibit activity of the pathways (like PI3K) and to correlate its activity directly to biological response. Pathway activity measurement is more predictive for the cell growth inhibition than measurement of Ki-67 mRNA expression. The mechanism of action of drugs, including the efficacy on pathway inhibition can be explored in more detail using CPA and might be used for optimal compound selection and patient stratification.

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