Deciphering the mechanism of action of drugs targeting EGFR, by Cisbio's advanced cell-based pathway readout assays.

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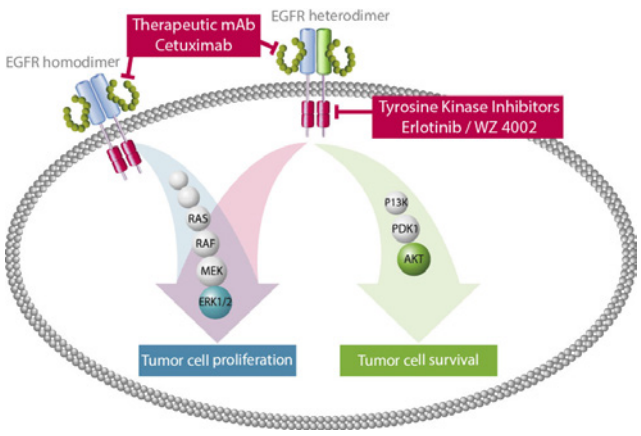
HOW can the discovery of EGFR inhibitors be efficiently improved and their characterization facilitated? Since sufficient knowledge of the drugs' mechanism of action is a key step of drug development, the choice of technology enabling cellular pathway analysis in response to compound treatment is a crucial step towards success.
In this study, we successfully applied Cisbio's fluorescent sandwich immunoassay technology HTRF®, which is based on TR-FRET, to monitor the response of a pancreatic cancer cell line BxPC3, to increasing concentrations of EGFR inhibitors.
The key nodes of the major signaling pathways, MAPK/ERK pathway and PI3K/AKT pathway, were investigated by the detection of the endogenous phosphorylation status of EGFR, ERK and AKT. For this purpose, the reference method Western-blot was compared to Cisbio's HTRF cellular phospho-protein assays.



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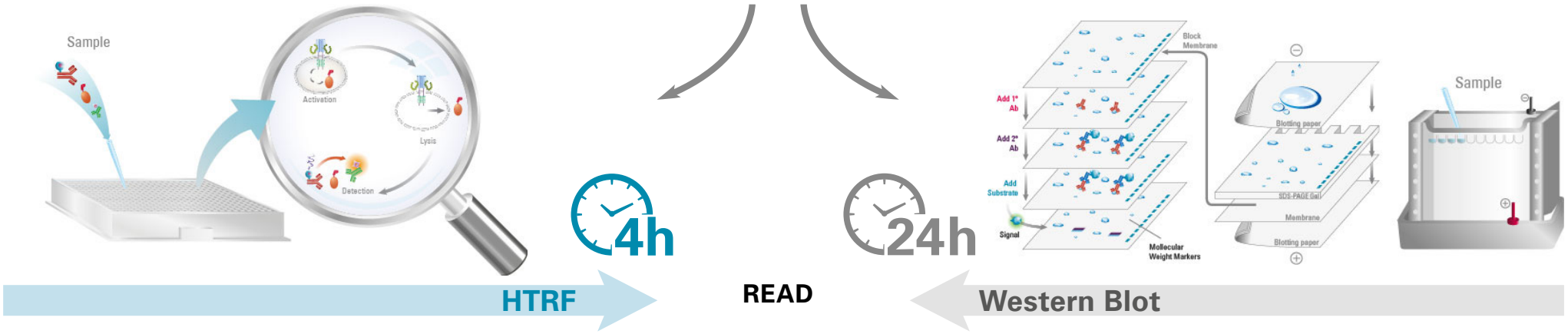
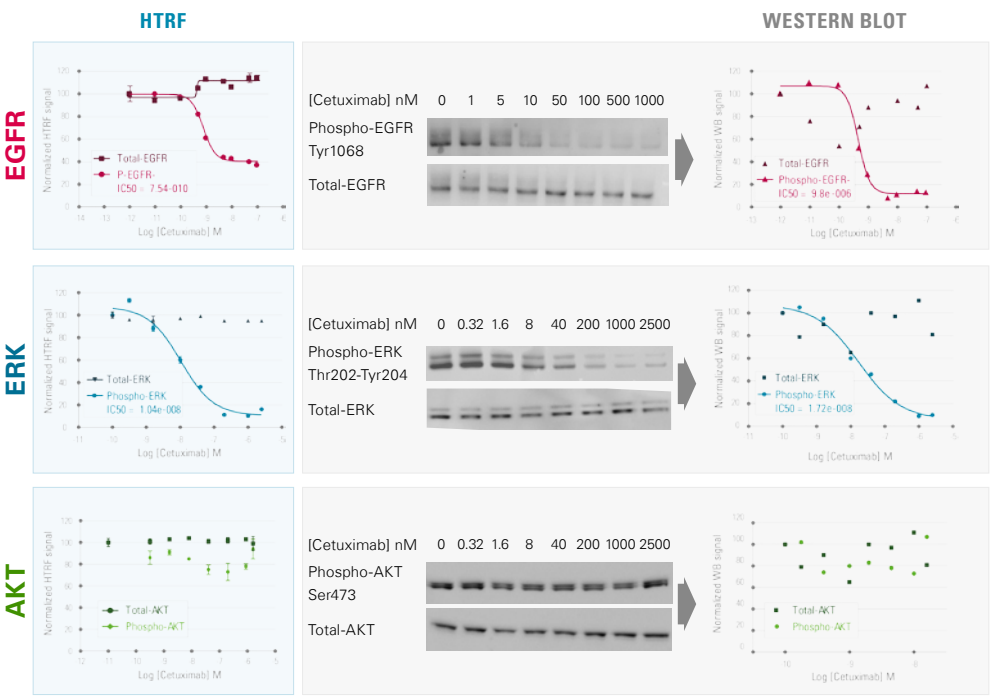
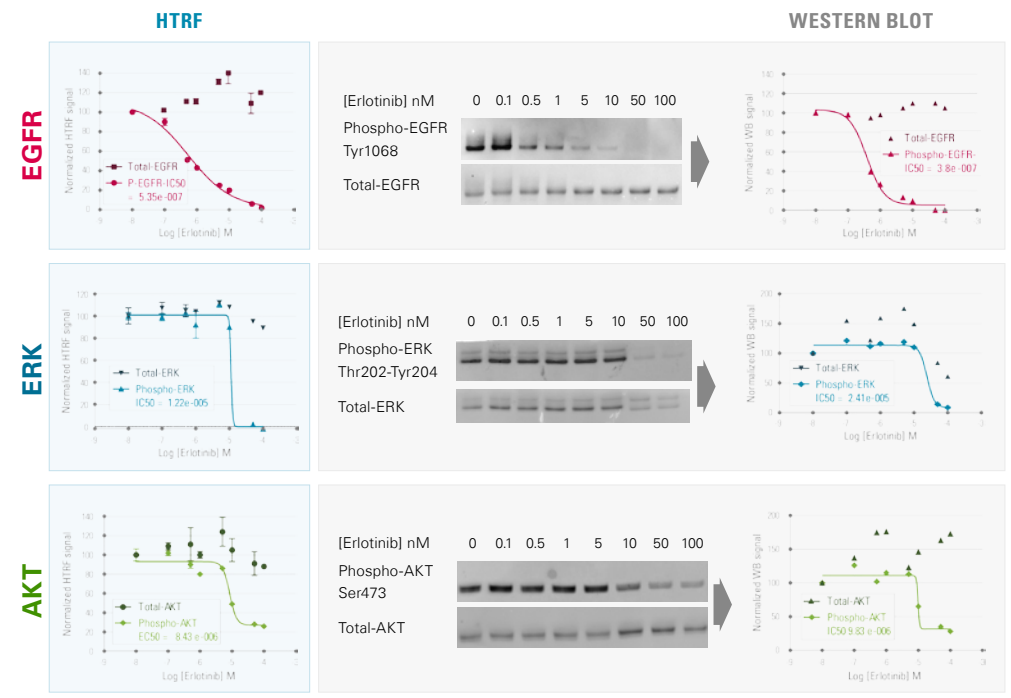


BACKGROUND The epidermal growth factor receptor EGFR and its downstream signaling pathways have been involved in the development and progression of several human tumors, including pancreatic cancer. Therefore this membrane-bound receptor tyrosine kinase EGFR became a key target of therapeutic strategies designed to treat metastatic pancreatic cancer. EGFR inhibitors, when used alone or in combination with cytotoxic drugs or radiation, have been shown to reduce the proliferation of tumor cells and are currently used in pancreatic cancer treatment. These EGFR inhibitors are represented by two major classes: small molecule intracellular inhibitors, mainly ATP-competitive, and monoclonal antibodies directed against the extracellular domain of the receptor.

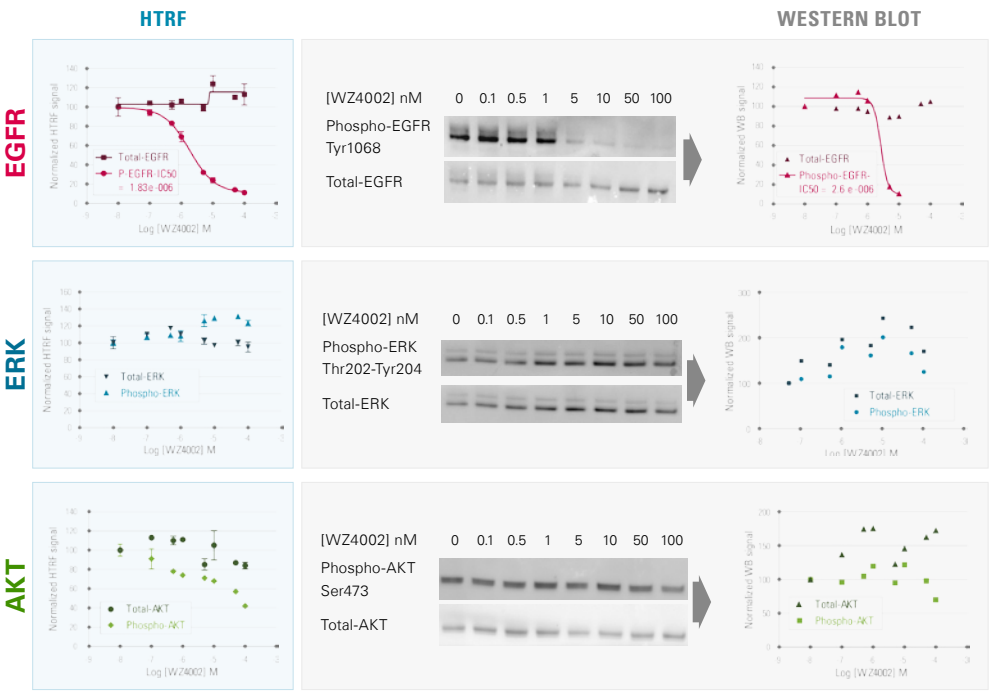
CETUXIMAB MoA on cell signaling



ERLOTINIB MoA on cell signaling



WZ4002 MoA on cell signaling



IC50 CORRELATION	CETUXIMAB		ERLOTINIB		WZ4002	
	HTRF	WB	HTRF	WB	HTRF	WB
P-EGFR	7.54 E-10	4.53 E-10	5.35 E-7	3.85 E-7	1.83 E-6	2.6 E-6
P-ERK	1.04 E-8	1.72 E-8	1.22 E-5	2.41 E-5	-	-
P-AKT	-	-	8.4 E-6	9.8 E-6	-	-

CONCLUSION These experimental results point out differences of the mechanism of action of the 3 classes of EGFR inhibitors assessed in the pancreatic BXPC3 cell line, overexpressing wild-type EGFR.
Cetuximab, Erlotinib and WZ4002 block the EGFR activation with various potencies, as reflected by the IC50 values. Whereas the reversible tyrosine kinase inhibitor Erlotinib abolishes the activation of the MAPK and PI3K/AKT pathways, the irreversible EGFR mutant TKI WZ4002 has no significant effect.
This study shows that HTRF® cell-based phospho-protein and Western-Blot deliver highly correlated results, as indicated by IC50 values.
Due to the "mix and read" protocol of the HTRF technology, the analysis of cell signaling pathways and the biomolecular responses are significantly speeded-up and simplified.
Moreover, the microplate format of HTRF phospho-protein assays facilitates the analysis of replicates, thus providing a higher accuracy of biological and pharmacological characterization.
The quantitative Cisbio pathway readout enables furthermore a precise interpretation of the results, much simpler than semiquantitative protein bands allow.
The reproducibility, the time to results and the simpler handling make Cisbio phospho-protein assays the detection method of choice.

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cisbio_ls_pt_2014_02_FCS-pathway-readout-mechanism-of-action-of-drugs