HTRF® phospho-assays reveal subtle drug-induced effects in tumor xenografts. A method of choice beyond Western blot

J. Vallaghé[1], D. Monchiet[1], N. Costy[1], T. Chardès[2], C. Larbouret[2], E. Trinquet[1], F. Charrier-Savournin[1]
[1] Cisbio Bioassays, Codolet, France | [2] Institut de Recherche en Cancérologie de Montpellier, INSERM U896, Université Montpellier 1, Institut régional du Cancer Montpellier / Val d'Aurelle, France

INTRODUCTION Cell signaling pathways involving phospho-proteins are often over-activated in cancers, leading to aberrant cell proliferation and survival. Therefore, protein phosphorylation is frequently assessed while developing novel anti-tumor therapeutics, such as tyrosine kinase inhibitors (TKI) or monoclonal antibodies. In preclinical cancer studies, human tumor xenograft-bearing mice are routinely used as predictive models to evaluate the in vivo efficacy of such anticancer drugs. Western blot is the standard technique for analyzing proteins in tumor lysates, but it is time- and labor-intensive.

This poster compares the use of HTRF phospho-assays with traditional Western blot for the analysis of AKT and ERK1/2 in human pancreatic tumor xenografts, after mouse treatment with the anti-EGFR TKI erlotinib (Fig 1). The data demonstrates that HTRF is a more convenient, accurate and sample-saving method than Western blot for assessing protein phosphorylation in tumor xenografts.

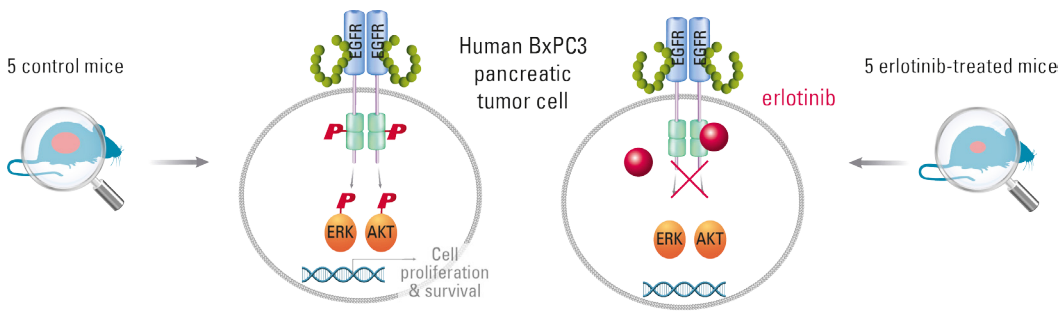


Fig 1: Design of animal experiments
3 x 10^6 tumor cells were injected into the flanks of nude mice. After 40 days, the mice were randomly distributed into two groups: a control group (non-treated) and a group treated for 21 days with a daily oral dose of erlotinib (100 mg/kg).

ASSAY PROTOCOL

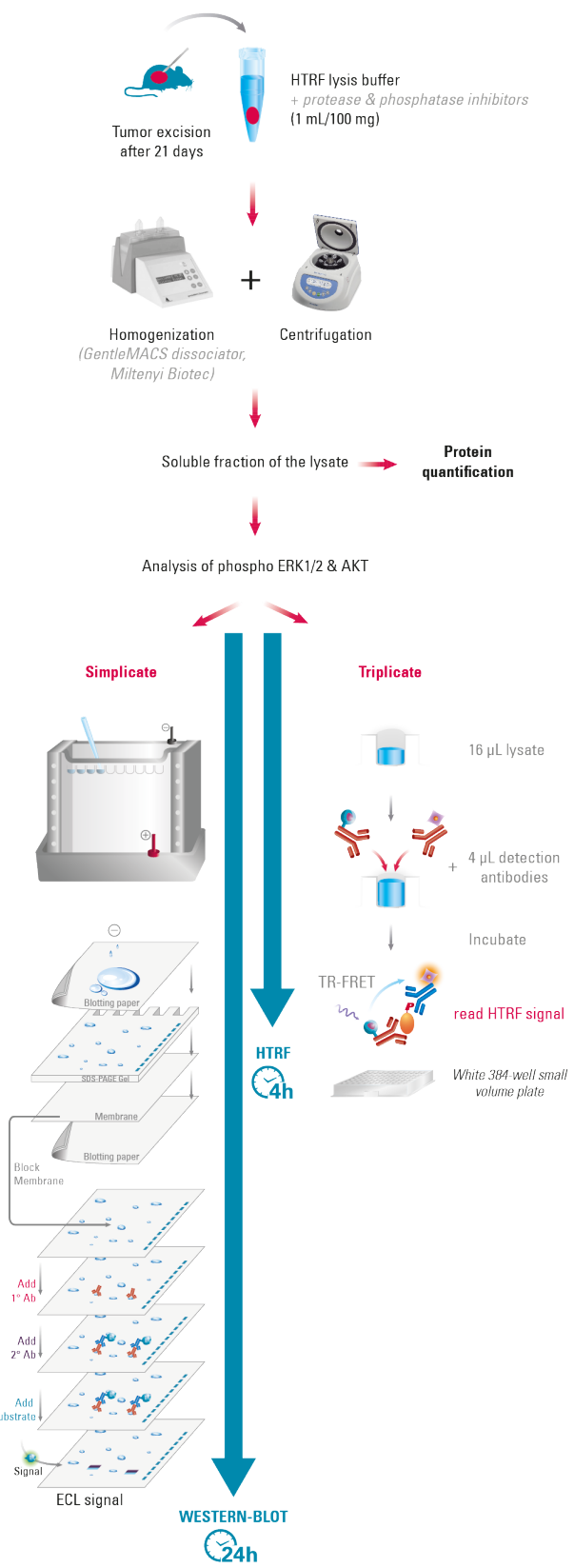


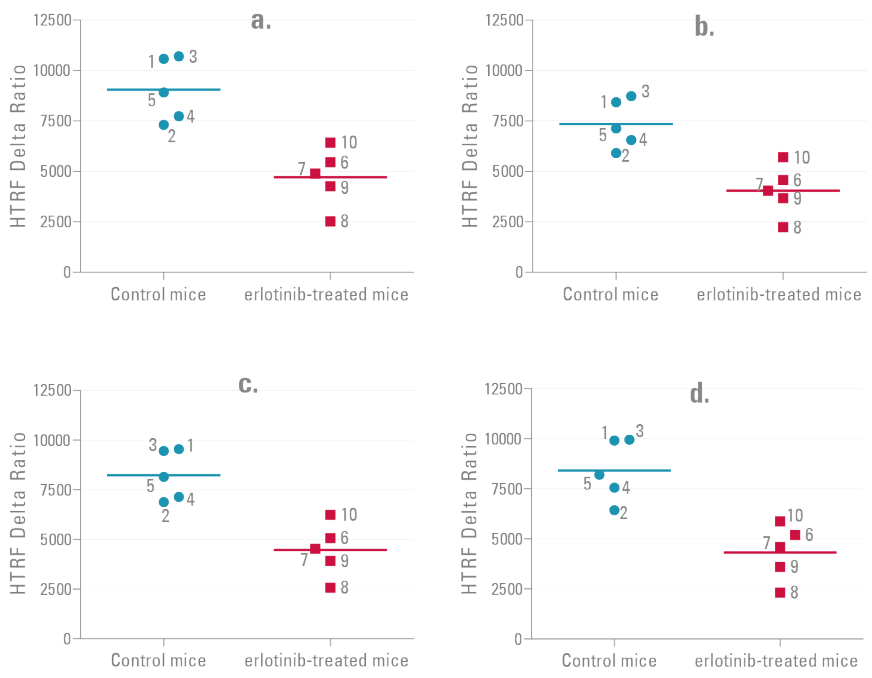
Fig 2: Flow chart of the assay procedures.

For more information on sample preparation, assay protocol and data handling, please refer to the corresponding application note « HTRF® phospho-assays reveal subtle drug-induced effects in tumor xenografts. A method of choice beyond Western blot ».

PERFORMANCE OF HTRF ASSAYS

HTRF Phospho-AKT (S473) assay

INTRA-ASSAY REPRODUCIBILITY	INTER-ASSAY REPRODUCIBILITY
CV < 7%	CV < 9%



ANALYSIS	P VALUE	SIGNIFICANCE
a.	0.0019	**
b.	0.0029	**
c.	0.0019	**
d.	0.0022	**

Fig 3: Four independent analyses of phospho-AKT (S473) in tumor lysates.

- Same ranking of control and treated mice in each separate HTRF assay (Fig 3)
- Excellent reproducibility of the data
- Same conclusion given by each analysis: erlotinib treatment induces a very significant decrease of AKT phosphorylation in vivo (results in accordance with the literature*)

SIDE-BY-SIDE COMPARISON OF WESTERN BLOT AND HTRF ASSAYS

Western blot phospho-ERK assay

HTRF® Advanced phospho-ERK assay

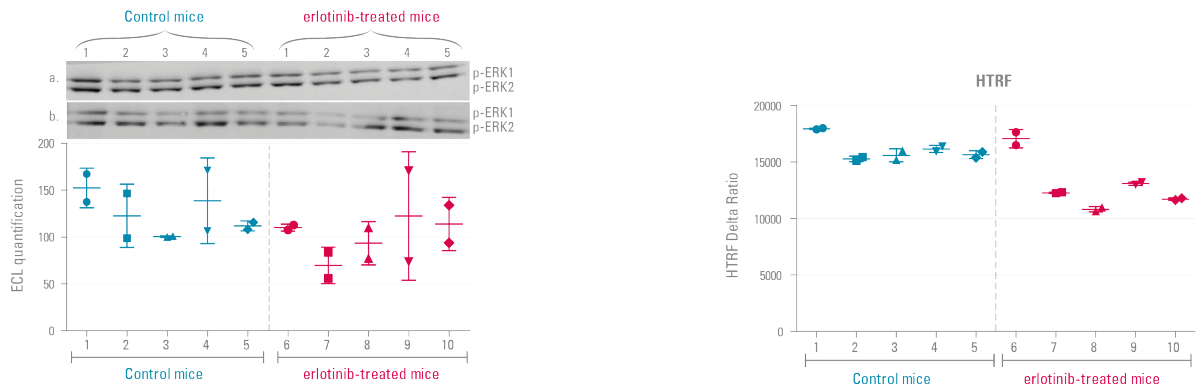


Fig 4: Two independent analyses of phospho-ERK1/2 (T202/Y204) in tumor lysates using Western blot and HTRF. Error bars represent standard deviations.

TECHNOLOGY	ANALYSIS	INTER-ASSAY	P VALUE	SIGNIFICANCE
WESTERN BLOT	a.	0% < CV < 56% Mean CV = 22%	0.0588	NS
	b.		0.3224	NS
HTRF	a.	0% < CV < 5% Mean CV = 2%	0.0396	*
	b.		0.0227	*

- Marked difference in the reproducibility and in the results obtained with the two techniques (Fig 4)
- Western blot results difficult to interpret with confidence due to the high inter-assay variability: numerous additional analyses required to generate statistically significant results, consuming precious samples and time
- HTRF data highly reproducible and highlighting a significant decrease of ERK1/2 phosphorylation by erlotinib (results in accordance with the literature*)

CONCLUSION The data presented here demonstrates that HTRF phospho-assays are completely suitable for revealing subtle drug-induced modulations of protein phosphorylation in tumor xenografts, and represent a more convenient and sample-saving method than Western blot. Most importantly, highly reproducible HTRF assay results rapidly lead to correct data interpretation, essential in evaluating the in vivo efficacy of candidate anti-tumor therapeutics.

*References

Buck E, Eyzaguirre A, Brown E, et al. Rapamycin synergizes with the epidermal growth factor receptor inhibitor erlotinib in non-small-cell lung, pancreatic, colon, and breast tumors. *Mol Cancer Ther* 2006; 5(11): 2676-2684.

Diep CH, Munoz RM, Choudhary A,et al. Synergistic effect between erlotinib and MEK inhibitors in KRAS wild-type human pancreatic cancer cells. *Clin Cancer Res.* 2011; 17(9): 2744-56.

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